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INFECTIOUS DISEASES

THIRD EDITION

VOLUME ONE

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Preface to the Third Edition

These are extraordinary times in the fields of microbiology and infectious diseases. The genomes of essentially all the major bacterial and viral pathogens known to infect humankind have now been sequenced and are available on public databases. The human genome project is now complete and whole genome sequencing of the major malaria parasite *Plasmodium falciparum* and the common fungal pathogen *Candida albicans* are now available on line. We have learned an enormous amount of new information about the molecular mechanisms that underlie microbial pathogenesis and the host response to pathogens since the second edition of this book some five years ago. An expanding number of antiviral and antifungal agents are now available to clinicians, and new generations of vaccine constructs and adjuvants are now entering clinical practice.

Despite these advances, progress has been uneven with very little in the developmental pipeline for novel antibacterial agents, antituberculosis drugs, or chemotherapeutic agents against parasitic infection. We find ourselves increasingly on the defensive against a variety of newly emerging and remerging pathogens. The specter of progressive antimicrobial resistance now threatens the long-term viability of the very foundation of our primary treatment approach against bacterial pathogens, including extensively drug resistant tuberculosis (XDR-TB). Our collective vulnerability to airborne pathogens within our highly mobile and crowded global community has become poignantly evident with the H1N1 swine flu pandemic of 2009 and ongoing threats of human dissemination of H5N1 avian influenza strains. Environmental disruption and global warming largely attributable to our ever expanding human population is likely to have adverse health consequences; among them with be the spread of vector-borne, waterborne and airborne pathogens.

In preparing this third edition we have continued the themes that initially inspired the creation of this textbook of Infectious Diseases. The book maintains its tradition of well illustrated and tightly referenced chapters with an emphasis on clinical practicality along with a detailed review of disease pathogenesis and microbiology. Practice points are found throughout the text that highlight common, and not so common, clinical scenarios that require specific and targeted information to provide informed responses. The interactive website, complete with its frequently updated information sources and a downloadable set of illustrations, will continue to support the print version of the text and now includes a number of innovative new functions. The whole text has been carefully reviewed, many chapters have been totally re-written with new figures added, and new authors and editors have been commissioned to ensure that the material is fresh, up to date and relevant.

We are indebted to the superb group of section editors and the extensive collection of highly skilled, international, contributors for each chapter, without whom the third edition of this book would not have been possible. We would especially like to extend our sincere gratitude to Sue Hodgson, Sven Pinczewski, Poppy Garraway and the staff at Elsevier for their unflappable spirit and their attention to detail throughout this considerable undertaking. Finally, we thank the Section Editors from the second edition who have now stood down: Steven Holland, Dennis Maki, Ragnar Norrby, Allan Ronald, Claus Solberg and Jan Verhoef without whom we would never have been in the position of preparing a third edition. We trust that our readers will find the readily accessible knowledge distilled into these pages to have been well worth the effort in generating the Third Edition of Infectious Diseases.

Jon Cohen
William Powderly
Steven Opal

User Guide

Volumes, sections and color coding
Infectious Diseases is divided into two volumes. The book is divided into eight sections, which are color-coded as follows for reference:

Volume 1

Section 1 – Introduction to infectious diseases
Section 2 – Syndromes by body system
Section 3 – Special problems in infectious disease practice
Section 4 – Infections in the immunocompromised host

Volume 2

Section 5 – HIV and AIDS
Section 6 – International medicine
Section 7 – Anti-infective therapy
Section 8 – Clinical microbiology

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Chapter 1

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Nature and pathogenicity of micro-organisms

Human existence would be impossible without the micro-organisms that surround us, as they play critical roles in processes as diverse as photosynthesis, nitrogen fixation, production of vitamins in the human intestine and decomposition of organic matter. They are the sole, true 'recyclers' of our planet. Micro-organisms are also the major driving force behind the evolution of life. They evolved photosynthesis and respiration, which have since been acquired by present-day eukaryotes, and they mediate genome rearrangements in infected host cells.

In a rather simplified view, micro-organisms may be considered to be no more than 'little machines that multiply'. In fact, this is what they do best. We are starting to understand some of the strategies micro-organisms have developed to stay alive, grow and reproduce. The lifestyle of a micro-organism is intimately related to its environment, whether that environment is the human body or a polluted riverbed. Some highly specialized micro-organisms have adapted to the harsh environmental conditions, while others, such as root-colonizing bacteria and our own intestinal flora, have taken advantage of the abundant resources provided by higher organisms.

This chapter focuses on the lifestyle of pathogenic micro-organisms and how they infect us, reproduce and cause disease. We shall use the word 'pathogenicity' to indicate the capacity to cause disease (or damage) in nonimmune individuals. Although the word 'virulence' is often used in the same sense, we mean it to refer to the severity of the illness that is caused. Communicability refers to the transmissibility or infectiousness of micro-organisms.

DEFINITION AND COMPARISON OF INFECTIOUS AGENTS

The definition of an 'infectious agent' was proposed by J Henle in 1840 and put to the test by the German physician Robert Koch. In 1876, Koch reported experiments on mice with *Bacillus anthracis* showing that:

- a single micro-organism could be isolated from all animals suffering from anthrax;
- the disease could be reproduced in an experimental host by infection with a pure culture of this bacterium; and
- the same micro-organism could subsequently be reisolated from the experimental host.

This definition is an oversimplification because many pathogenic microbes have never been cultured (e.g. *Mycobacterium leprae*), others lack a suitable animal host in which the infection can be reproduced (e.g. *Salmonella enterica* serovar Typhi) and some cause disease only under specific conditions.

Infectious agents can be divided into four groups:

- Prions, which consist of only a single protein (PrP). The infectious form (PrP^{TSE})[1] is transmissible as spongiform encephalopathy (see Chapter 22).
- Viruses, which contain proteins and nucleic acids, and virioids which contain only nucleic acid. These characteristically disassemble after cell entry and then assemble their progeny during replication[2] (see Chapters 151 and 152).
- Bacteria, including archaea and eubacteria. Unlike eukaryotes, the DNA genomes of these prokaryotes are not separated from the cell by a membrane. Unlike viruses, they remain enclosed within their own cell envelope throughout their life cycle (see Chapters 165–177).
- Eukaryotes, including fungi (see Chapters 178 and 179), protozoa (see Chapters 180–183) and multicellular parasites (see Chapter 184). These organisms have subcellular compartments, including the nucleus, where DNA transcription occurs.

Table 1.1 compares the properties that define prokaryotes with eukaryotes and Table 1.2 emphasizes the differences between bacteria and fungi, many of which determine the specificity of antimicrobial agents.

GENERAL PROPERTIES AND CLASSIFICATION OF VIRUSES

Taxonomy of viruses

The hierarchical classification system[4] groups viruses in families (-viridae), genera (-virus or -viruses) and species (-virus), emphasizing similarities in the type (DNA or RNA) and nature (single stranded or double stranded, segmented or nonsegmented) of genetic material, and structural features (size, symmetry and presence or absence of a lipid envelope; see Table 1.3). For example, picornaviridae is a family of small, non-enveloped RNA viruses containing the enterovirus genus, which in turn includes poliovirus species of serotypes 1, 2 and 3.[5] The Baltimore classification system[6] emphasizes the relationship of the genetic material of the virus and the viral replication scheme. For example, group IV contains viruses with single-stranded RNA genomes where the mRNA shares the same sense as the viral RNA (+ssRNA), which includes picornaviridae, enterovirus and poliovirus.

Common steps in the replication of viruses

Virus replication involves the following steps.

1. Attachment: Virus particles (virions) must first attach to specific receptor(s) on the surface of a host cell.
2. Penetration: This may proceed via direct fusion with the cell membrane or through endocytosis and pH-mediated fusion.
3. Uncoating: The virion disassembles, freeing nucleic acid and viral proteins needed for replication.

Table 1.1 Comparison of prokaryotes and eukaryotes

Feature	Prokaryotes	Eukaryotes
Chromosome	Single, circular or linear	Yes
Gene organization	Operon-polycistrionic mRNA	Single genes and block of genes
Nucleosomes	No	Yes
Nuclear membrane	No	Yes
Mitosis	No	Yes
Introns in genes	No	Yes
Transcription	Coupled with translation	Separate from translation
mRNA	No terminal polyadenylation (except archaebacteria); polygenic	Terminal polyadenylation; usually monogenic
First amino acid	Unstable formylmethionine (except archaebacteria)	Methionine
Ribosome	70S (30S + 50S)	80S (40S + 60S)
Cell wall	Presence of muramic acid, D-amino acids, peptidoglycan (except archaebacteria and mycoplasma)	No muramic acid, D-amino acids or peptidoglycan
Membrane	No sterols or phosphatidyl-choline (except mycoplasma)	Sterols and phosphatidyl-choline
Endoplasmic reticulum	No	Yes
Mitochondria	No	Yes (*E. histolytica*, *Giardia* and microsporidia have vestigial remnants of mitochondria)
Lysosomes and peroxisomes	No	Yes
Movement	By flagella, composed of a single fiber	Ameboid, by cilia or cilia-like flagella

Table 1.2 Comparison of bacteria and fungi

Characteristics	Bacteria	Fungi
Cell volume (mm^3)	0.6–5.0	Yeast: 20–50; molds: greater than yeast
Nucleus	No membrane	Membrane
Mitochondria	No	Yes
Endoplasmic reticulum	No	Yes
Sterol in cytoplasmic membrane	No (except for mycoplasma)	Yes
Cell wall components	Muramic acids and teichoic acids; no chitin, glucans or mannans	Chitin, glucans and mannans; no muramic acids or teichoic acids
Metabolism	Autotropic or heterotropic	Heterotropic
Sensitivity to polyenes	No	Yes

Adapted from Kobayashi.[3]

4. Replication: Viral proteins and messages are expressed. Intermediates such as viral complementary RNA or integrated proviral DNA may be needed.
5. Assembly: New virions containing viral nucleic acid are formed.
6. Release: New virions are released from the cell via lysis of the cell or intra- or extracellular budding.

Structure of viruses

The virion is designed to protect the viral genome and to mediate the migration of the virus and the invasion of the target host cell. Viruses are small, the smallest being ~25–30 nm in diameter, while the largest (mimivirus, an infectious agent of amebae) are 400 nm or more in size.[7] The viral genome is tightly associated with a nucleoprotein in a highly organized core structure, the nucleocapsid. In some virus families, such as (–) strand RNA viruses and retroviruses, the virion contains enzymes required for early stages of virus replication. The main proteinaceous outer structure of a virus is the capsid or tegument. Clefts or vertices in the assembled capsid, protruding protein spikes or other structures may mediate viral attachment. Alternatively, the capsid is surrounded by an outer lipid membrane (the envelope) derived from membranes of the host cell in a process termed 'budding.' Viral proteins inserted within the envelope then serve as receptors for specific host cell molecules.

The viral genome

Viral genomes consist of DNA or RNA, and range from 1.7 kb to 1.2 Mb in size. There may be only a single gene in the smallest virions, whereas the larger genomes encode hundreds. For example, parvoviridae have only two open reading frames, whereas the vaccinia poxvirus has 263 known genes.

The nucleic acid of all mammalian DNA viruses except the parvoviridae and circoviridae is double stranded (dsDNA, Fig. 1.1). In contrast, the nucleic acid of all mammalian RNA viruses except the reoviruses (e.g. rotavirus, coltivirus) is single stranded (ssRNA). The genome structure may be linear, circular or rod-like, and either non-segmented or segmented. Genome segmentation facilitates genetic exchange between co-infecting virions in a process known as reassortment. Some viral nucleic acid molecules may contain modified nucleotides, which inhibit host cell nucleases and/or mediate recognition by viral polymerase. Linear genomes often contain conserved terminal sequences. When complementary, these allow partial circularization of the genome or formation of panhandle or tube-like structures. Terminal sequences may also allow incomplete replication products to recombine or mediate recognition by proteins that prime transcription or replication. The proviral DNA of retroviruses is flanked by repeat sequences similar to transposable genetic elements.

The genome of ssRNA viruses may have either of two possible polarities. The viral RNA (vRNA) of positive-strand RNA viruses acts directly as mRNA for protein synthesis; they resemble eukaryotic RNAs with a cap at the 5′ end and are polyadenylated at the 3′ end. In contrast, the RNA-dependent RNA polymerase of (–) ssRNA viruses uses the vRNA as a template for mRNA transcription. Negative-strand RNA genomes have neither cap structures nor poly-A tails, often parasitizing cap structures from cellular pre-mRNA or mRNA. Retroviruses synthesize a dsDNA copy of the positive-strand RNA genome, which integrates into the cellular DNA and then expresses viral messages.

Table 1.3 Classification of viruses

Family name	Example	Genome size (kb) polarity (+ or –) and segments	Morphology	Envelope
DNA viruses				
Single-stranded (Class II)				
Parvoviridae	Human parvovirus B19	5 (±) single	Icosahedral	No
Mixed-stranded (Class VII)				
Hepadnaviridae	Hepatitis B	3 (±) single	Icosahedral	Yes
Double-stranded (Class I)				
Papovaviridae	Wart virus	8 (±) single	Icosahedral	No
Polyomaviridae	JC virus	5 (±) single	Icosahedral	No
Adenoviridae	Adenovirus	36–38 (±) single	Icosahedral	No
Herpesviridae	Herpes simplex	120–220 (±) single	Icosahedral	Yes
Poxviridae	Vaccinia	120–280 (±) single	Complex	Yes
RNA viruses				
Positive-sense (Class IV)				
Picornaviridae	Poliovirus	7.2–8.4 (+) single	Icosahedral	No
Togaviridae	Rubella	12 (+) single	Icosahedral	Yes
Flaviviridae	Yellow fever	10 (+) single	Icosahedral	Yes
Coronaviridae	Infectious bronchitis	16–21 (+) single	Helical	Yes
Negative-sense (Class V)				
Rhabdoviridae	Rabies	13–16 (–) single	Helical	Yes
Paramyxoviridae	Measles	16–20 (–) single	Helical	Yes
Orthomyxoviridae	Influenza	14 (–) 8	Helical	Yes
Bunyaviridae	California encephalitis	13–21 (–) 3	Helical	Yes
Arenaviridae	Lassa fever	10–14 (–) 2	Helical	Yes
Filoviridae	Marburg, Ebola	19 (–) single	Helical	Yes
Reverse (Class VI)				
Retroviridae	HIV-1	3–9 (+) diploid	Icosahedral	Yes
Double-stranded (Class III)				
Reoviridae	Rotavirus	16–27 (±) 10-12	Icosahedral	No

The capsid

The viral genome is protected by one or more protein coats, the nucleocapsid and/or capsid. The capsid is made of structures known as capsomeres, which consist of proteins coded by the viral genome, and accounts for a large portion of the viral mass. Papillomavirus produces only two capsid proteins and poliovirus four, but more complex viruses may encode a much larger variety.

Picornaviruses, adenoviruses and papovaviruses have a nucleocapsid structure with icosahedral symmetry. The capsid consists of 20 triangular facets and 12 corners or apices. Influenza, measles and rabies virus form capsids with helical symmetry. The central core is formed by the nucleic acid genome, around which the nucleocapsid proteins are arranged like the steps of a spiral staircase, forming long cylinders (Fig. 1.2).

More complex virion morphologies also exist. Bacteriophages, which use bacteria as hosts, have additional attachment structures fixed to the capsid. The nucleocapsid of orthopoxviruses, such as variola and vaccinia virus, consists of a network of tubules, sometimes surrounded by an envelope, forming a brick-shaped virion.

The envelope

Enveloped viruses contain nucleocapsids of either icosahedral (e.g. herpesviruses, togavirus) or helical symmetry (e.g. influenza). The outer envelope consists of a lipid bilayer derived from host cell membrane in which the viral glycoproteins are embedded. The viral matrix proteins (M proteins) associate with the envelope, playing an important role in the structural organization of the virion by connecting the capsid to the viral glycoprotein inserted in the lipid bilayer. Besides oligosaccharide residues, the glycoproteins contain a membrane anchor and, in many cases, one or two molecules of fatty acid. Glycoproteins play a key role in the attachment of virions to the cell surface and penetration into the cell.

Some glycoproteins have enzymatic activity, such as the influenza virus neuraminidase, which promotes the release of newly formed viral particles from the host cell membrane. Maturation of protein structures and transcription steps may occur after release from the host cell. For example, the typical conical core of the human immunodeficiency virus retrovirus may be incomplete at release and partial reverse transcription occurs within the virion.[9]

Viral gene expression strategies

In the Baltimore classification, seven major viral replication strategies are distinguished (see Figs 1.1, 1.3 and the following).

In positive-strand RNA viruses (Baltimore Class IV), the viral genome serves immediately as mRNA. The first step in viral infection consists of a complete translation of the genome to produce a polyprotein, which is sequentially processed into smaller polypeptides via enzymatic cleavage which is at least partially autocatalytic. In the early phase, processing of viral structural proteins occurs slowly, due to the low concentrations of mature viral proteases. Viral complementary RNA is synthesized, followed by new viral RNA. As the viral proteases accumulate, core proteins are more efficiently processed, assemble and begin to encapsidate viral RNA. This strategy is used by picornaviruses and flaviviridae, including hepatitis C viruses. In other (+)ssRNA virus families (e.g. togaviridae, coronaviridae, caliciviridae and hepatitis E virus), the viral complementary RNA serves not only for production of

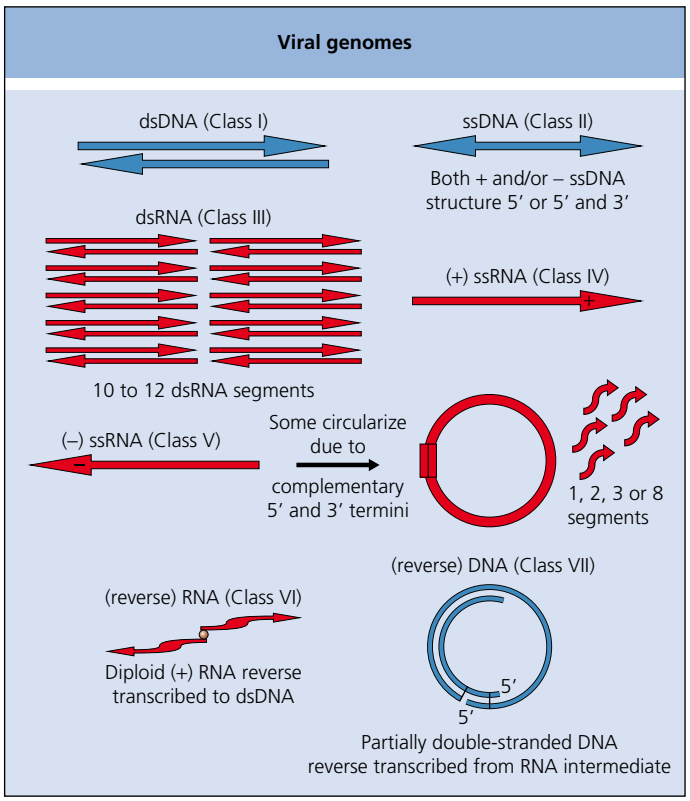


Fig. 1.1 Viral genomes. The schematic genomes are grouped according to the Baltimore classification system. DNA is represented by blue shades, RNA in red shades. Arrows indicate direction of DNA/RNA 5′ to 3′, not necessarily relationship of sense of messenger RNA to that of genome. See Figure 1.2 for a diagram of typical viral structures, Table 1.5 for a list of representative viruses within groups and Figure 1.3 for strategies of viral replication.

full length vRNA, but also subgenomic transcripts encoding structural proteins, allowing regulation of expression.

The RNA-dependent RNA polymerase of (–)ssRNA viruses (Baltimore Class V) must first produce transcripts using the infecting vRNA as a template. Primary transcription may generate a full-length viral complementary RNA (positive-strand) which acts as both mRNA for viral protein synthesis as well as a template for transcription of new viral RNA. This is typical of segmented (–)ssRNA viruses including orthomyxoviridae and most bunyaviridae. Alternatively, the incoming viral RNA may initially be transcribed into mRNA messages for individual genes, which must accumulate before transcription of full-length viral complementary RNA and replication of viral RNA can occur (e.g. paramyxoviridae, rhabdoviridae). The late phases of replication transcription and viral protein synthesis proceed simultaneously.

The arenaviridae and the some bunyaviridae use a somewhat more complicated strategy, termed ambisense. For one or more of the RNA genome segments, both the viral RNA and the viral complementary RNA serve as templates for mRNA transcription by the viral polymerase. This does not result in the formation of complementary double-stranded mRNAs, as different portions of the genome are transcribed from the viral and complementary RNA strands.

Only a few double-stranded RNA viruses (reoviridae, Baltimore Class III) produce human disease. The coltivirus Colorado tick fever virus causes a febrile syndrome with myalgia, headache, rash and occasionally encephalitis. The rotaviruses are important causes of diarrheal illness. Virions have 10–12 dsRNA segments and replication resembles that of (–)ssRNA viruses in that RNA is not infectious, and transcription of segment length mRNAs using the negative strand of the genomic dsRNA must first occur before genome replication. Regulation is accomplished via transcription rate (inverse to segment length) and efficiency of translation (varying up to 100-fold).

In the case of ssDNA viruses (parvoviridae and circoviridae, Baltimore Class II), the incoming genome is first used to express proteins that permit the synthesis of the complementary DNA strand. Double-stranded DNA is a replication intermediate, and DNA replication is dependent on repeats which form DNA structures on one or both ends. These parvoviridae include the B19 erythrovirus that causes erythema infectiosum (fifth disease) in children and exanthem, arthropathy and aplastic crisis in adults. This family also includes the apathogenic adeno-associated dependoviruses (AAV), which serology indicates cause ubiquitous human infection.[10] Parvoviridae have 4.5–5.5 kb ssDNA genomes and only two open reading frames, one of which codes for between two and four nonstructural proteins and the other coats polypeptides. The circoviridae have circular ssDNA genomes. This family includes Torque Teno viruses (TTV, TTMV) which cause widespread human infection without evidence of disease.[11]

Replication of dsDNA viruses (Baltimore Class I) proceeds to lysis or latency depending on cellular conditions. The lytic phase can be subdivided into early and late phases. In the early lytic phase, viral genes alter cellular conditions to allow efficient viral DNA synthesis and transcription, often activating the host cell and inducing cell division. In the late lytic phase, viral proteins accumulate, virions are assembled and released upon death of the cell. In latency, viral gene expression is confined to functions that prevent replication while maintaining the viral genome within the cell, often for the lifetime of the individual. When cellular conditions become favorable, the latent virus can be 'activated' into lytic replication.

There are many dsDNA viruses of medical importance for humans, including:

- herpes simplex viruses (cause of genital and labial herpes, meningitis and encephalitis);
- varicella-zoster virus (the causative agent of chickenpox);
- human cytomegalovirus (frequently causing disease in immunocompromised hosts);
- Epstein–Barr virus (causing infectious mononucleosis);
- adenoviruses (causing respiratory disease and conjunctivitis);

and many others.

In the case of the retroviruses (Baltimore Class VI), the virus enters the cell and uncoats, discharging the preintegration complex, consisting of the polyadenylated, diploid viral RNA genome together with nucleoproteins, the viral reverse transcriptase and the viral integrase. The two RNA genomes are converted to a single, mostly dsDNA copy by reverse transcriptase in a process requiring template switching. The viral integrase then cuts the host genome and inserts the linear viral DNA into the chromosome as a provirus. This process may require cellular activation and/or cell division, though retroviruses can persistent for weeks at stages before integration. Integrated virus may become latent, with limited or no transcription by cellular RNA polymerase II, until conditions allow virus replication.

More complex retroviruses (e.g. spumaviruses, lentiviruses) first transcribe multiply-spliced mRNAs that direct the synthesis of regulatory proteins. As these accumulate, the processing of viral transcripts changes and more singly or unspliced mRNAs coding for viral structural proteins are produced. For example, human immunodeficiency virus (HIV) *Rev* protein, produced from early, multiply-spliced RNA transcripts, prevents splicing and allows nuclear export of singly spliced and unspliced messages.

Hepadnaviruses (Baltimore Class VII), including hepatitis B virus, encode genetic information in DNA but use reverse transcription during infection in the cell to produce the (–) strand of viral DNA, which in turn is used as a template for synthesis of (+) strand viral DNA.

GENERAL PROPERTIES AND CLASSIFICATION OF BACTERIA

Bacteria are small (0.6–4.0 µm) unicellular organisms; 3×10^{12} bacteria weigh in the order of 1 g. Under optimal conditions, a bacterium may divide between two or three times per hour. Theoretically, nearly 300 g of bacterial mass can be produced from a single bacterial cell in

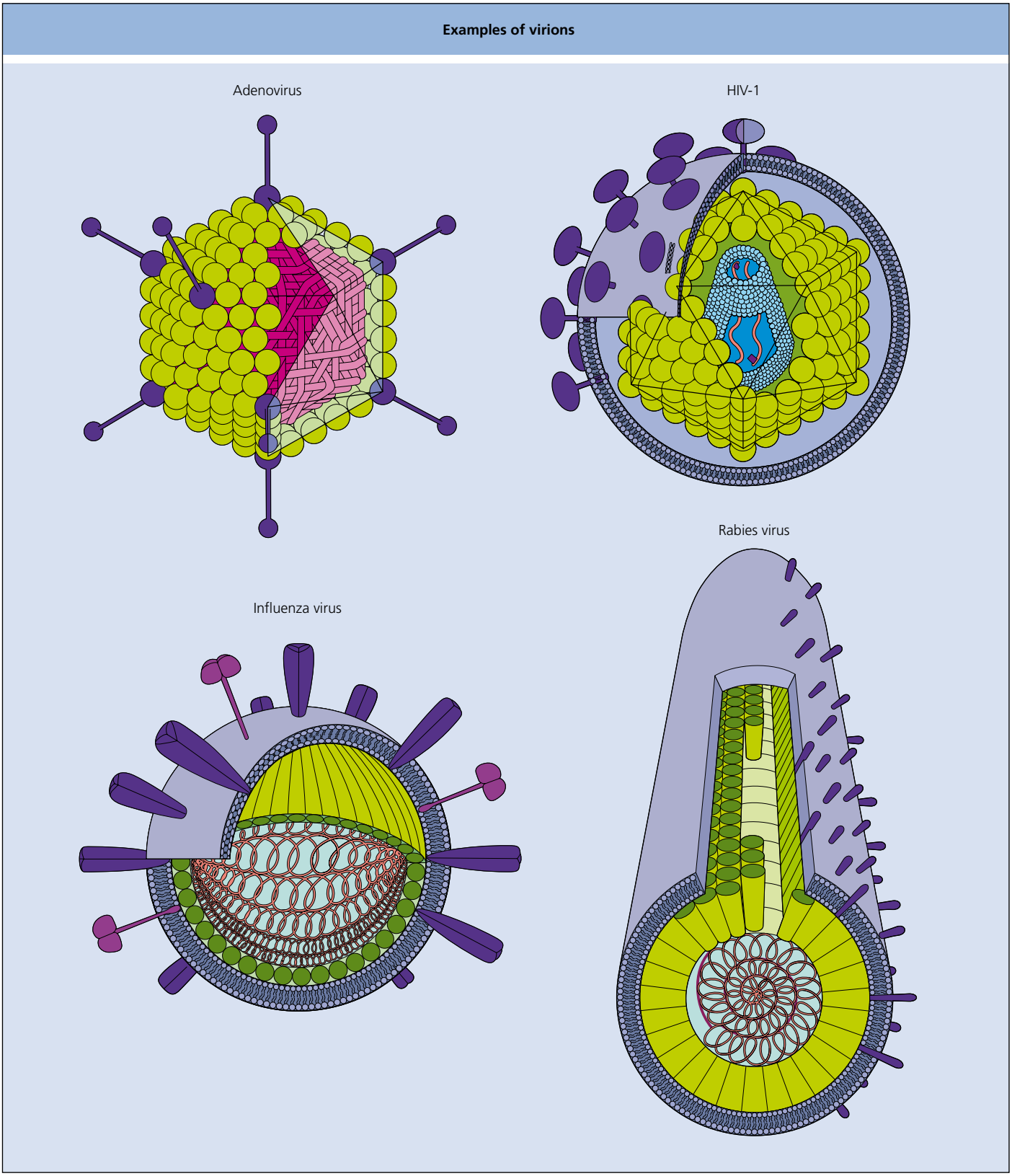


Fig. 1.2 Examples of virions. Adenovirus is an icosahedral DNA virus without an envelope; fibers extend from the 12 points of the icosahedral coat; DNA forms a ribbon-like molecule. Approximate size 8 nm. HIV-1; glycoprotein (GP) molecules protrude through the lipid membrane; the icosahedral capsid encloses a truncated conical nucleocapsid in which the diploid RNA is enclosed. Approximate size 100 nm. Influenza virus is an enveloped RNA virus containing nucleocapsid of helical symmetry; spikes of hemagglutinin and neuraminidase protrude from the lipid bilayer. Approximate size 100–200 nm (variable). Rabies virus is a helical RNA nucleocapsid with a bullet-shaped lipoprotein envelope in which approximately 200 GPs are embedded. Approximate size 150 nm. (The diagram is not to relative scale.) Adapted from Collier and Oxford[8] by permission of Oxford University Press.

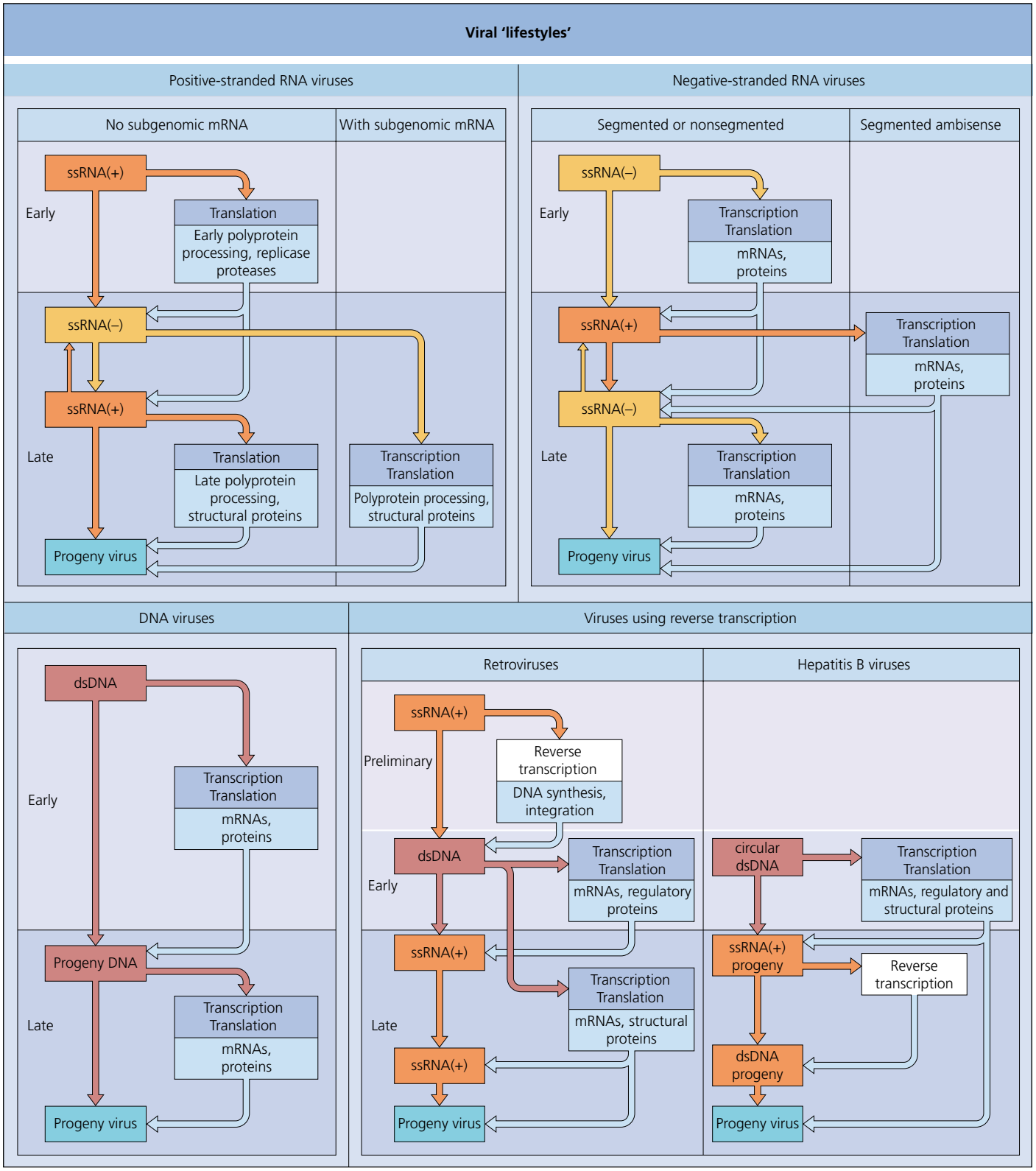
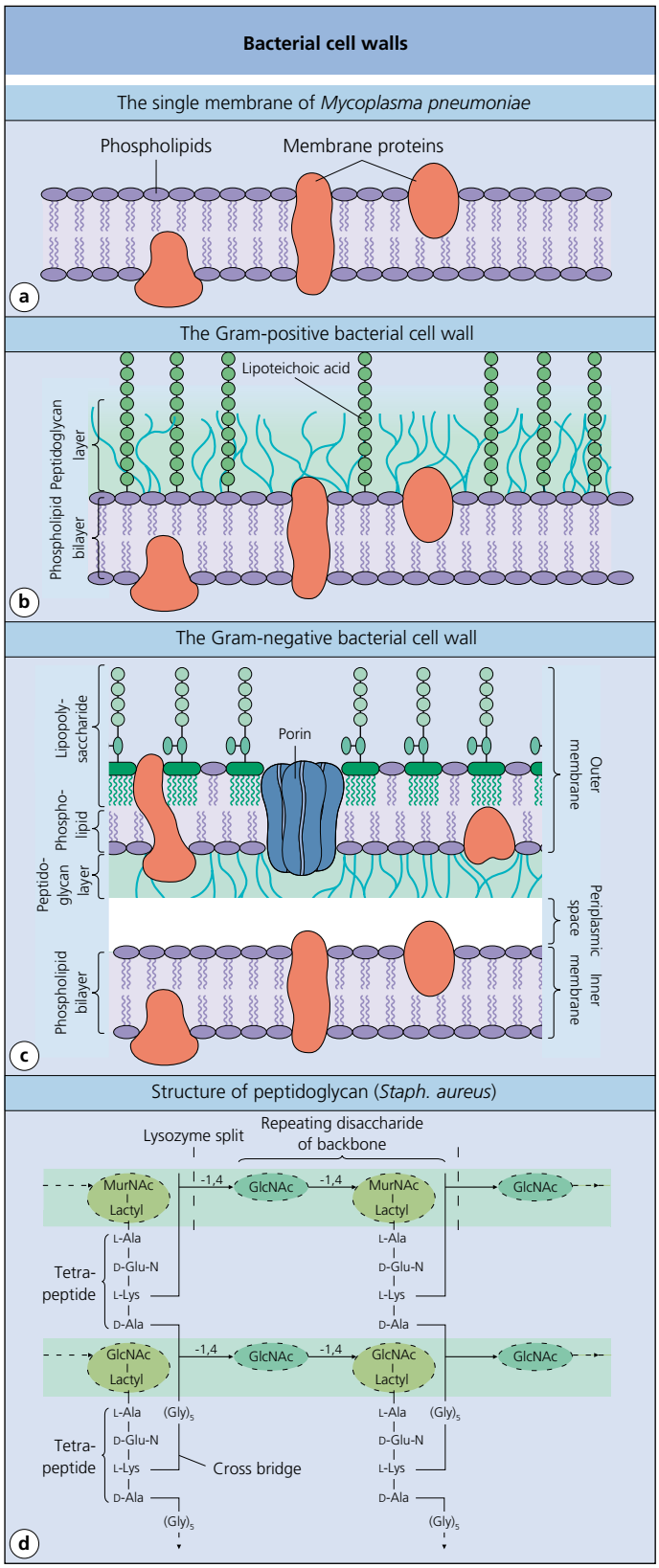


Fig. 1.3 Viral 'lifestyles'. Upper left. Single stranded (+) sense RNA viruses can be subdivided into those which produce subgenomic mRNAs (e.g. togaviridae, coronaviridae, calciviridae), allowing additional regulation of transcription, and those which do not (poliovirus, hepatitis A virus, flaviviridae including hepatitis C virus). Upper right. Negative sense RNA viruses must first transcribe RNA messages from incoming viral RNA (vRNA), as well as making a copy complementary to the viral genomic DNA (vcRNA) to serve as a template for synthesis of new viral RNA. Some (–) ssRNA viruses use both vRNA and vcRNA as templates for transcription of mRNA (arenaviruses, some bunyaviruses). Lower left. Double stranded DNA viruses have early and late lytic phases of replication, and some (e.g. Epstein–Barr herpesvirus) also have latent (usually episomal) phases. Lower right. Viruses using reverse transcription include RNA retroviruses (e.g. HIV), which reverse transcribe a dsDNA copy from diploid (+) ssRNA vRNA, and hepadnaviruses (e.g. HBV) which reverse transcribe the (–) strand of DNA from RNA transcribed from incoming viral DNA. (Courtesy of Menno Kok & Jean-Claude Pechere.)

one day. Such small organisms profit from a favorable cell surface-to-volume ratio, which allows metabolic fluxes largely superior to those attained by the larger eukaryotic cells. Bacteria react very quickly to environmental changes, regulating gene transcription to adapt their physiology.



Bacteria were probably the first cells to appear on earth more than 3.5 billion years ago. They have since developed into an overwhelming diversity representing the bulk of the world's biomass today. Although evolution has not led to bacteria associating into multicellular organisms, they are capable of cell-to-cell communication.[12] By using low molecular weight compounds, bacteria have found a way to sense how dense their local population is and decide whether or not to activate developmental programs such as plasmid conjugation, light production (in association with deep-sea fish) or virulence gene expression.

Different cell morphologies can be observed with light microscopy (e.g. spherical cocci, rod-shaped bacilli, curved vibrios, spiral treponemes). Electron microscopy unveils a distinctive cell wall, a simple nuclear body without a nuclear membrane and the presence in the cytoplasm of ribosomes and mesosomes, sometimes granules of reserve material, but no endoplasmic reticulum and no organelles such as mitochondria or chloroplasts. There frequently are appendages such as flagella that are used for motility, pili and fimbriae that may be used for adhesion or for conjugation.

Bacterial dichotomy revealed by a simple staining technique

In 1884, the Danish bacteriologist Hans-Christian Gram developed a simple staining technique that distinguishes two types of bacteria: the Gram-positive and the Gram-negative bacteria. The distinction is based on the ability of one group of bacteria, the Gram-positives, to retain a crystal violet-iodine dye in the presence of an organic solvent such as alcohol or acetone. The solvent dissolves the dye from Gram-negatives and they can be counterstained with other dyes such as safranin. This simple observation reflects distinctive structures. Gram-positive bacteria characteristically have a thick cell wall made up mainly of a vast molecule of peptidoglycan, with protruding chains of teichoic acids. Surrounding the peptidoglycan skeleton in the periplasm of Gram-negative bacteria is an additional asymmetric outer membrane, the outer layer composed of lipopolysaccharide (endotoxin) (Fig. 1.4). *Escherichia coli* is an example of a Gram-negative bacterium; it is rod shaped and growing cells are between 2 µm and 4 µm long.

The rigid cell wall determines the shape of bacteria and allows them to resist the osmotic pressure caused by the large difference in solute concentration between the cytoplasm and the environment. *Mycoplasma* spp. lack peptidoglycan and thus have neither a rigid wall nor a defined shape.

Fig. 1.4 Bacterial cell walls. (a) *Mycoplasma pneumoniae* has a single membrane, made up of phospholipids and membrane proteins. (b) In Gram-positive organisms the cytoplasmic membrane is covered with a thick layer of peptidoglycan; chains of lipoteichoic acid anchored in the cell membrane protrude outside. Negatively charged teichoic acids are covalently attached to the peptidoglycan. Cell wall proteins also are covalently attached to the peptidoglycan. There is no periplasmic space in Gram-positive bacteria. (c) The cell wall of a Gram-negative rod is more complex. The layers are: the cytoplasmic membrane, the periplasmic space, a layer of peptidoglycan which is thinner than that in Gram-positive bacteria and an asymmetric outer membrane. The inner leaflet of the outer membrane is made of phospholipids. The outer leaflet has lipopolysaccharides as its principal lipids; porins, which are channel-forming proteins often organized as trimers, allow the penetration of hydrophilic molecules through the outer membrane. (d) The peptidoglycan of *Staphylococcus aureus* has polysaccharide chains ('backbone') that are alternating residues of *N*-acetylglucosamine (GlcNAc) and *N*-acetylmuramic acid (MurNAc). Tetrapeptides are attached to MurNAc and are linked together by pentaglycines bridging the L-lysine of each tetrapeptide chain to the D-alanine of the neighboring one. (Courtesy of Menno Kok & Jean-Claude Pechere.)

Organization of the bacterial cell

The bacterial cytoplasm does not contain physically separated compartments. DNA replication, transcription, protein synthesis, central metabolism and respiration all take place in the same environment. Complex biochemical processes may nonetheless be spatially organized in the cell. Transcription of DNA into mRNA and translation of the mRNA into protein are coupled processes. This means that polysomes are linked to the DNA, via the enzyme RNA polymerase (Fig. 1.5). The cytoplasmic membrane not only contains numerous metabolite transport systems, but it is the site of intense enzymatic activity as well. Like eukaryotic cells, bacteria possess efflux systems that allow them to expel unwanted substances from the cytoplasm into the environment. Gram-positive bacteria express many important enzymes and ligands in their cell wall. It is estimated that *Listeria monocytogenes* has 42 different cell wall anchored proteins.

The genetic information is usually stored in a single circular chromosome. Bacterial chromosomes vary considerably in size. The *Haemophilus influenzae* chromosome, the first completely sequenced genome of a cellular life form, is 1.83 million base pairs (Mbp) long and encodes 1703 putative proteins.[13] The chromosome of laboratory strains of *E. coli* K12 is approximately 2.5 times bigger (5 Mbp), though still rather small if compared with the 30 Mbp *Bacillus megaterium* genome that is more than 500 times the length of the cell (Fig. 1.6). A few organisms such as *Vibrio cholera* and *Borrelia* spp. have fragmented genomes, and the *Borrelia* genomes are encoded on linear DNA. The advantage to the organisms of such an arrangement is not known.

The bacterial chromosome codes for polypeptides and stable RNA molecules such as transfer RNA and ribosomal RNA molecules. *E. coli* probably contains well over 1500 different polypeptides with a variety of functions, including maintenance of membrane structure; transport; respiration; degradation of nutrients; synthesis of amino acids, sugars, nucleotides, lipids and vitamins; and production of polymers such as DNA, RNA, proteins and polysaccharides. Mobile genetic elements, such as plasmids, bacteriophages and transposable elements, are important sources of genetic variation. They supply genes that are not essential for bacterial growth but may offer a selective advantage under specific conditions. Virulence factors and antibiotic resistance elements are frequently associated with these mobile DNA structures.

Comparison of the genome sequences of the harmless laboratory strains of *E. coli* shows that naturally occurring *E. coli* isolates can differ by up to 1 Mb, ranging from approximately 4.5 to 5.5 Mb. Thus the commensal *E. coli* K12 has a genome of 4.64 Mb, while the human pathogen *E. coli* O157:H7 has a genome of 5.53 Mb. Several uropathogenic *E. coli* (UPEC) have been sequenced and have genomes varying from 4.94 to 5.23 Mb. The differences in genome sizes are largely due to insertions or deletions of large chromosomal regions, referred to as pathogenicity islands. Genomic islands in UPEC that are not found in commensal *E. coli* account for more than 10% of the genome, emphasizing the importance of lateral gene transfer in the evolution of pathogens. The overall gene order, except for the insertions, remains the same in all *E. coli* and is remarkably similar to the gene order in other Enterobacteriaceae such as *Salmonella enterica*.

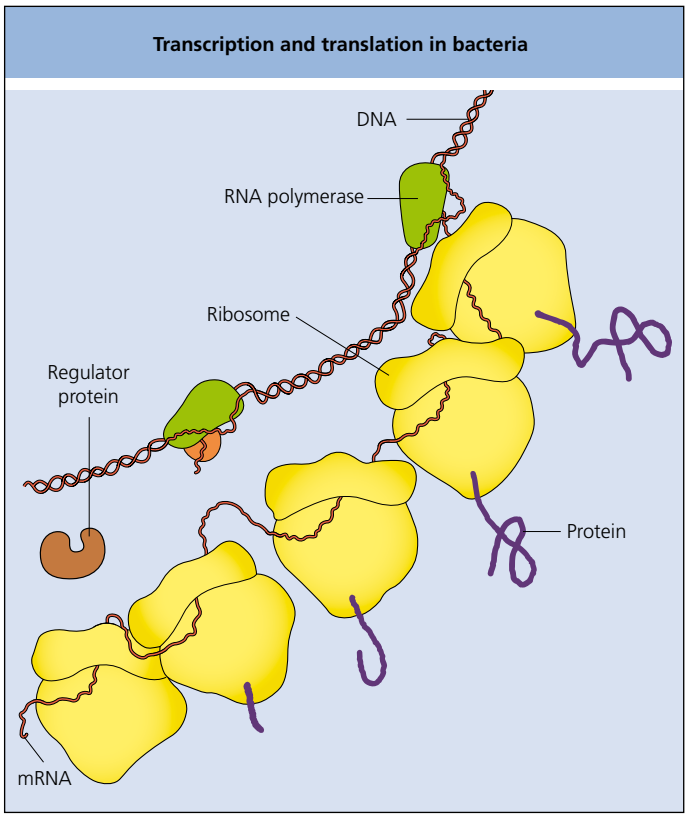


Fig. 1.5 Transcription and translation in bacteria (*Escherichia coli*). (Courtesy of Menno Kok & Jean-Claude Pechere.)

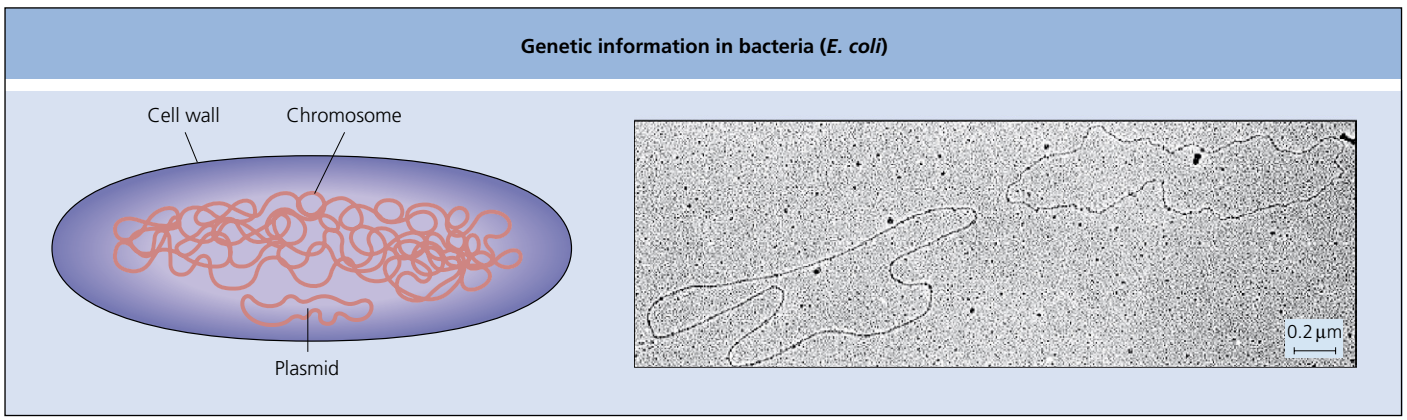


Fig. 1.6 Genetic information in bacteria. This example is *Escherichia coli*. Additional genetic information may be supplied by extrachromosomal elements such as plasmids or bacteriophages. Bacteria may carry a variety of these 'mobile genetic elements', which may transfer readily from one cell to another. The electron micrograph shows a 8.65 kb *E. coli* plasmid that confers sulfonamide and streptomycin resistance (left) and a single-stranded derivative of the plasmid (right). (Courtesy of Menno Kok & Jean-Claude Pechere.)

Transcription and translation in bacteria

Gene expression is usually regulated at the level of transcription initiation by regulator proteins and occasionally by small RNA molecules, which interact with the 'promoter DNA' and with the enzyme RNA polymerase (see Fig. 1.5). The promoter is the site where RNA polymerase opens ('melts') the dsDNA to synthesize an RNA copy of one of the two DNA strands. A sigma factor transiently interacts with the polymerase when it binds the promoter DNA and determines the nucleotide sequence specificity of the enzyme. Bacterial cells produce multiple sigma factors, each controlling the expression of a set of genes, and each expressed under different environmental conditions.

Three types of RNA are produced: regulatory RNA, 'stable' RNA and messenger RNA (mRNA). Stable RNAs include the transfer RNA molecules, which position the amino acids on the ribosomes during protein synthesis and are important structural components of the ribosomes. Messenger RNA molecules are generally quite unstable but are protected from premature degradation by ribosomes, the protein synthesis machines.[14] Regulatory RNAs, such as small RNAs (sRNA) in two component systems, may function in a fashion similar to microRNAs in eukaryotes.[15] Transcription and translation are coupled in bacteria; ribosomes bind the mRNA as soon as it 'leaves' RNA polymerase and start protein synthesis by coupling the initiator amino acid (formyl-methionine) to the second amino acid in the coding sequence and uncoupling it from the tRNA molecule. As mRNA elongation proceeds, more ribosomes bind to the mRNA to form a 'polysome'. The polypeptides that are produced by the ribosomes fold either spontaneously or with the help of molecular chaperones into their native structures. Bacterial mRNAs generally encode more than one protein. The bacterial protein synthesis machinery is an important target for antibiotics.

Motility

Many bacterial species can detect very small variations in concentrations of either valuable or harmful substances in the surrounding environment, guiding movement in a process called chemotaxis.[16] Flagella are the effectors of chemotaxis (Fig. 1.7). By changing the direction of flagellar rotation, micro-organisms swim towards sites favorable to survival and growth and away from noxious stimuli. Amino acids and sugars are powerful chemoattractants. Although many pathogenic species are flagellated, a role for motility in virulence has not been established in many cases.

PATHOGENESIS OF INFECTIOUS DISEASE

The key microbial factors involved in the onset and spread of microbial infection can be identified by carefully analyzing the interaction of the micro-organism with its host (Table 1.4). Insight into the intimate relationship between host and pathogen will help us find the answers to the all-important questions: how can we eliminate the cause of disease and how can we reduce its harmful effects on the human body?

One of the major advances in pathogenic microbiology has been the use of molecular techniques to make targeted mutations in organisms. These mutants can then be tested in appropriate animal or tissue culture models to determine if the loss of the gene affects the virulence of the pathogen without affecting its ability to grow *in vitro* in standard media. Genes that are identified as 'virulence' genes are sometimes considered to be 'accessory' genes because they are not required for replication outside the host. A virulence gene can also be cloned into a genetically related nonpathogenic microbe and tested for its ability to confer a new property in that organism such as adhesion or hemolysis.

Virulence factors can be thought of as falling into one of two functional categories, though there can be overlap in the categories. There are purely defensive functions that help the organism to escape the host's innate immune response. Two examples of this are the polysaccharide capsule made by *Streptococcus pneumoniae* and the golden pigment made by *Staphylococcus aureus*. The former prevents complement from effectively opsonizing the bacteria for ingestion and destruction by neutrophils, while the carotenoid pigment

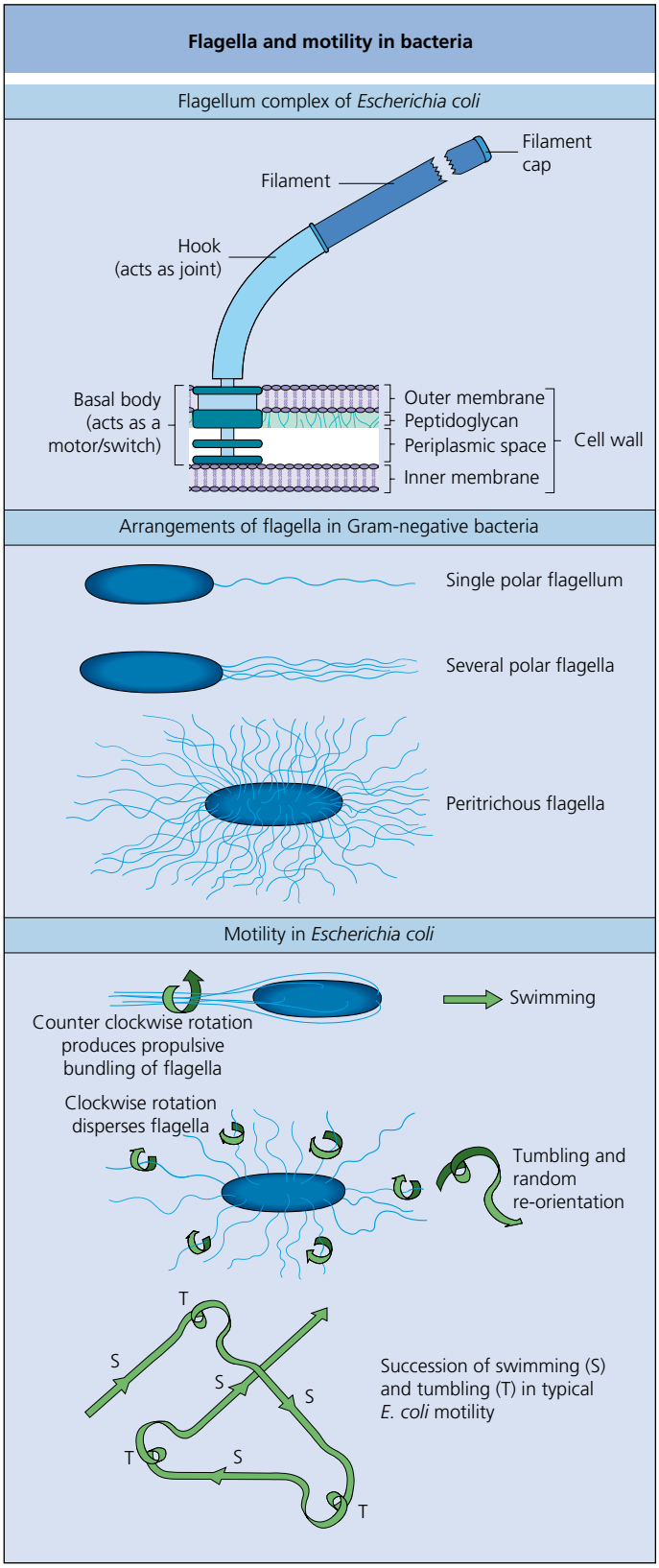


Fig. 1.7 Flagella and motility in bacteria. (Courtesy of Menno Kok & Jean-Claude Pechere.)

Table 1.4 Important steps in microbial pathogenesis

- Encounter
- Attachment to host cells
- Local or general spread in the body (invasion)
- Cell and tissue damage
- Evasion of host defenses
- Shedding from the body

(staphyloxanthin) that gives *Staph. aureus* its name is an antioxidant that helps the bacteria to survive the oxidative damage inflicted by the respiratory burst of phagocytes. In contrast, many exotoxins (e.g. cholera and diphtheria toxins) secreted by the bacteria actively inflict damage on the host and so can be thought of as offensive weapons. Lipopolysaccharides (LPS) serve both functions for the organism. The polysaccharide chains divert the membrane attack component of complement from the inner membrane, making Gram-negative bacilli resistant to the bactericidal action of complement (defensive), while the lipid A end can stimulate exuberant and damaging inflammation by binding to the TLR4/MD-2/CD14 complex.

Because bacteria do not constitutively produce virulence factors that are not necessary for their growth, they transcriptionally regulate expression of virulence genes. Since expression of these regulated virulence genes (or 'accessory genes') may carry a large metabolic cost, organisms regulate their expression in response to environmental signals. Mutation of the regulators often affects the expression of many genes, both positively and negatively, and regulatory mutants are more impaired and less virulent than most single gene mutants. Examples of this include the phoP/Q regulon in *Salmonella enterica* and the agr regulon in *Staph. aureus*. Some regulators sense environmental conditions such as pH or magnesium concentration and others sense bacterial density via quorum sensing.

Viruses face problems similar to bacteria in the host, but must solve them using a limited genetic repertoire. Analogous to bacterial virulence factors, viral genes required for replication and/or pathogenesis in the host that are dispensable for replication in tissue culture are termed accessory genes. For example, simian immunodeficiency virus (SIV) strains lacking the *nef* gene replicate well in certain cell lines but are much less capable of producing disease.[17] Expression of both SIV and HIV *nef* both increases viral infectivity and assists in evasion of adaptive host immunity by downregulation of major histocompatibility complex (MHC) class I, needed for presentation of antigen to allow recognition of infected cells by cytotoxic lymphocytes. The HIV *vif* gene inactivates an innate restrictive factor, APOBEC3G, which otherwise renders progeny virus uninfectious.[18]

Lifestyles and pathogenesis

Each pathogen has its own infection strategy. In the following sections we shall examine the lifestyles of some pathogenic species.

Contamination

In the developed areas of the world, the majority of human infections are caused by pathogens that either belong to the normal microflora of the host (so-called endogenous infections), though there are many exceptions. Infections caused by exogenous micro-organisms have steadily declined over the past century. In contrast, exogenous infections are still prevalent in poorer areas.[19]

Endogenous infections and normal microbial flora of the human host

The fetus in utero is normally sterile but immediately after birth it starts acquiring its indigenous microflora, which will quickly outnumber its own cell content; a normal adult carries more than 10^{14} bacteria, which represents roughly 10 bacteria for each eukaryotic cell. In addition to bacteria, we provide permanent or transient hospitality to an estimated 150 viral species, including numerous remnants of 'endogenous' retroviruses that are inherited in our DNA,[20] to a few fungi, some protozoa and occasionally to worms. The indigenous flora, or 'normal flora' that consists of many species of bacteria and one fungus (*Candida albicans*), is found in any part of the body exposed to the outside environment – the alimentary tract from the mouth to the anus, nose and the oropharynx to the epiglottis, the anterior part of the urethra and the vagina, and the skin (Fig. 1.8). The human microbial population is especially dense in the large intestine; it has been estimated that each gram of stool specimen contains about 10^{12} bacteria. The normal flora is well adapted to its niche and may multiply rapidly under favorable nutritional conditions such as those found in the colon. Although the host's age and physical condition, and especially antibiotic treatment, may induce important variations, the microbial population of the gastrointestinal tract seems to be stable, consisting of more than 99% of obligate anaerobic species. The fecal flora is much more diverse in vegetarians than in omnivores or carnivores, probably reflecting the difficulty of digesting complex carbohydrates found in plants. Facultative anaerobes such as *E. coli*, which are frequently used as markers for environmental pollution with human feces, represent less than 1% of the normal flora.

Transient micro-organisms, ingested with food or water, will normally pass through the proximal small intestine because of the high flow rate and low gastric pH, without being able to penetrate the mucous gel that overlays the intestinal epithelium or to adhere to the epithelial surface. Population levels of the different areas of the gastrointestinal tract are controlled mainly at the level of metabolic competition, the normal flora being well adapted to the low oxidation reduction potentials and tightly adherent to the mucosal epithelium. Pathogens that use the gastrointestinal tract as a portal of entry must find ways of dealing with the fierce microbial competition in the colon, or they target the less densely populated small intestine. Small intestinal pathogens have specific adhesions that allow them to remain attached to epithelial cells or to invade those cells.

The skin is much less densely populated by the indigenous flora. In comparison with the gastrointestinal tract, it supplies a considerably less stable microenvironment and one that is often devoid of water. Nevertheless, there are bacteria within skin appendages in all areas of the skin. Although intact skin is impermeable to bacteria, a number of parasites, among them *Schistosoma mansoni*, which poses a major health threat in developing countries, can penetrate the intact human skin. Skin disruptions due to lacerations or insect bites may allow entry of pathogenic microbes into the body.

The large majority of micro-organisms in the human flora reside on the body surface without creating any damage. This peaceful cohabitation can be called a symbiotic ('both sides win') relationship. Some bacteria find shelter and food in the intestine and, in turn, supply vitamins or digest cellulose. We are just beginning to understand the roles that normal flora play in human nutrition, in maintaining oral tolerance and in developing our innate immune system. If the micro-organisms, rather than the host, derive benefit from the association, those inhabitants of our body are called commensals. True commensals do not invade the host and, therefore, do not elicit an immune response. Parasitism constitutes a third category where the micro-organisms, after invading the host, cause an infection. Some have suggested that chronic infection with highly prevalent viruses, including herpesviruses, may play a protective role against bacterial infection by boosting innate immunity, suggesting a complex, three-way symbiotic relationship.[21]

The separation between parasitism, commensalism and symbiosis is not always clearly defined and the condition of the host may make a big difference. Some micro-organisms, referred to as opportunistic pathogens, are commensals in the majority of people but can cause disease in an immunocompromised host. For instance, *C. albicans* is part of the normal oral flora, but in the absence of adequate numbers of CD4 T cells, as occurs in AIDS, the yeast can proliferate and cause thrush and esophagitis. Similarly, virtually all individuals chronically

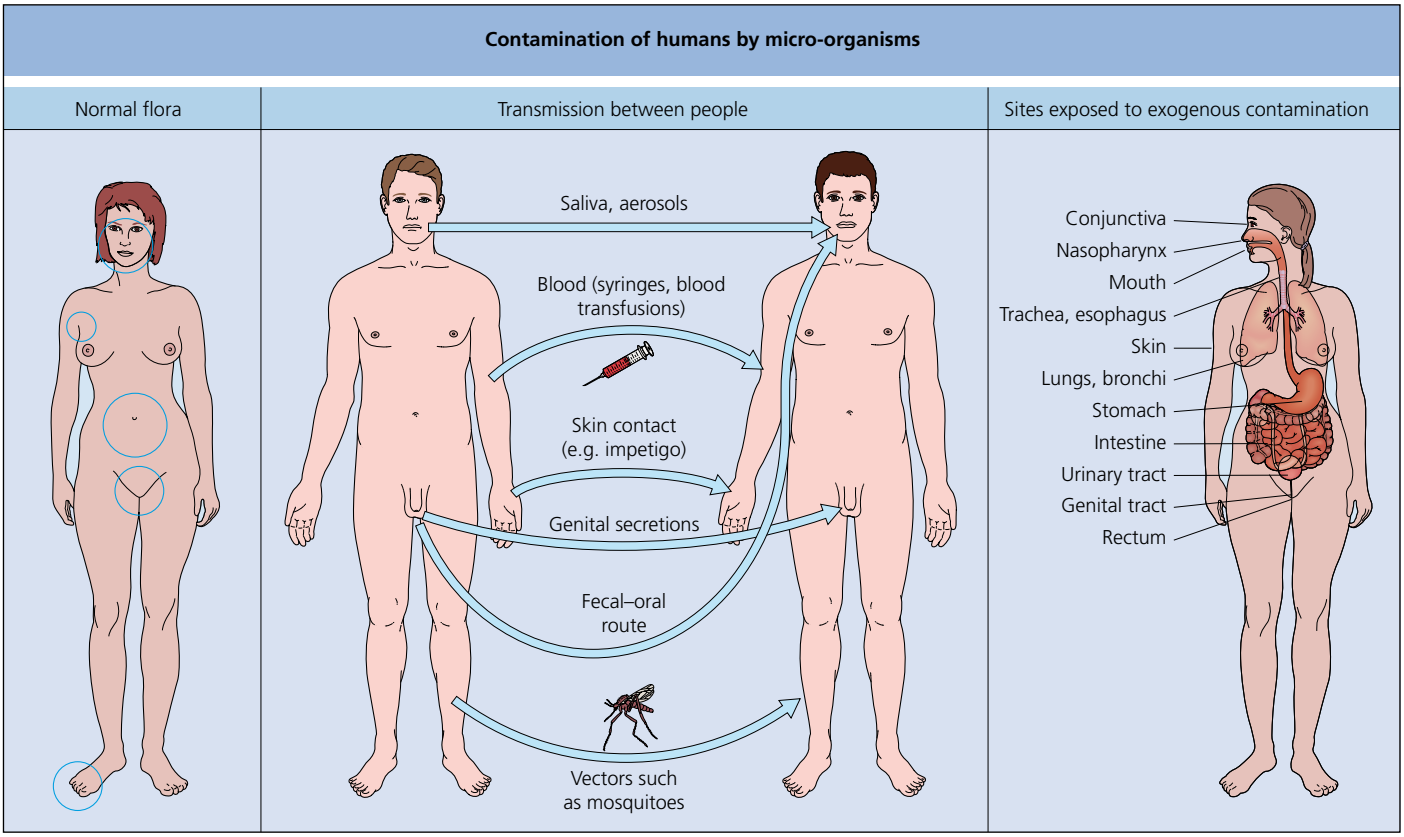


Fig. 1.8 Contamination of humans by micro-organisms. Many parts of the body are colonized by normal flora, which can be the source of endogenous infection. Large numbers of micro-organisms are found in moist areas of the skin (e.g. the groin, between the toes), the upper respiratory tract, the digestive tract (e.g. the mouth, the nasopharynx), the ileum and large intestine, the anterior parts of the urethra, and the vagina. Other routes are interhuman transmission of infections and exposure to exogenous contamination. (Courtesy of Menno Kok & Jean-Claude Pechere.)

infected with human cytomegalovirus (CMV) are asymptomatic, but CMV can cause serious diseases such as colitis and pneumonia when the immune system is suppressed.

The host and its indigenous microflora maintain a delicately balanced relationship that, when disrupted, may lead to the development of infectious disease.

An inevitable consequence of antibiotic treatment is the elimination of susceptible bacteria, which are quickly replaced by antibiotic-resistant species. This phenomenon can cause diseases such as mucosal candidiasis, pseudomembranous colitis or enterococcal superinfection. Even a short course of antibiotics can cause a large change in the composition of the fecal flora.

Probiotics (live micro-organisms) may help to restore the natural flora after antibiotic use, but their usefulness is still not fully established. For example, *Saccharomyces boulardii* may be used to prevent relapses of colitis caused by *Clostridium difficile,* but there are no convincing studies showing efficacy.

Exogenous infections

Exogenous infections occur after direct contamination from microbial populations in the environment.

Humans are continuously in intimate contact with the large exogenous microbial populations in the air, soil and water, which may harbor highly pathogenic bacteria such as *Clostridium tetani* and *Bacillus anthracis*. Important pathogenic species, such as *Salmonella enterica, Staph. aureus, Clostridium perfringens* and *Clostridium botulinum*, may be present in our food and cause food poisoning or gastroenteritis.

Live animals represent another important source of exogenous micro-organisms. Infectious diseases of animals that may be transmitted to humans (called zoonoses) include cat-scratch fever, brucellosis, tularemia, toxoplasmosis and rabies. In addition, microbial pathogens can be transmitted from animals to humans by insect vectors such as flies, mosquitoes and ticks. Plague and Lyme disease are examples of vector-borne zoonotic bacterial infection. Many viruses, such as dengue virus, which is transmitted by Aedes mosquitoes, are transmitted by insect vectors. The Sin Nombre hantavirus, which produces hantavirus pulmonary syndrome, is acquired from rodents by aerosolization of their dried, infected urine. Many protozoan pathogens are transmitted by insect bites, malaria being the most important.

The most important sources of exogenous infections are probably humans themselves (see Fig. 1.8). Well-known examples of human-to-human transmission include AIDS and other sexually transmitted diseases, airborne infections such as varicella, rubella, measles and tuberculosis, and fecal–oral infections such as shigellosis and typhoid fever. Vertical transmission of infections to the fetus or newborn is uncommon but they often have devastating effects. They include toxoplasmosis, CMV, rubella, HIV, listeriosis and syphilis. In contrast, vertical transmission of hepatitis B virus (HBV) occurs at or after parturition, and the infants have a high likelihood of becoming asymptomatically but chronically infected. This is common in many populations in South East Asia where HBV is endemic. Cross-infection in hospitals poses enormous problems, especially in intensive care units, but these infections are usually transmitted on fomites or inadvertently on the hands of hospital personnel rather than by direct contact or by droplets.

Several regions of the body may be exposed to exogenous contamination (see Fig. 1.8). Healthy people may be carriers if they harbor and excrete potentially disease-producing micro-organisms. For instance, people recovering from typhoid fever may retain *Salmonella typhi* in the gallbladder and continue to excrete the pathogen in the feces long after recovery from the disease. These people are chronic carriers, even

though they have recovered from the illness themselves. Certain bacterial respiratory pathogens have no environmental or animal hosts, and are passed by droplets from person to person. These include *Strep. pyogenes, Strep. pneumoniae* and *Neisseria meningitidis*. If newly colonized individuals do not have protective antibodies they are liable to develop symptomatic infections. Other than pre-existing immunity, why some people become ill after they acquire these organisms and others remain asymptomatic is not well understood.

A small number of exogenous pathogens are airborne and establish infection by direct interactions with alveolar macrophages or mucosal dendritic cells. For this to happen the particles must be of a certain size; particles larger than 4 microns in diameter will not reach the terminal airways and very small particles will be trapped in the nasopharynx. Alveolar macrophages are inherently downregulated for inflammatory responses, which is probably necessary to prevent lung damage from the many encounters with particles in the air. However, this makes them ill equipped to kill organisms that they may ingest. *Mycobacterium tuberculosis*, the primary pathogenic fungi such as *Histoplasma capsulatum, Paracoccidioides braziliensis* and *Coccidioides immitis*, and the environmental bacterium *Legionella pneumophila* are examples of airborne pathogens.

Exogenous infections, predominant in the past, have dramatically declined in the developed world thanks to improved hygiene, vaccination programs and infection control programs. They are, however, still prevalent in areas with limited resources. Pneumococcal pneumonia, diarrheal diseases from contaminated food and water, malaria, measles, AIDS and tuberculosis are the main causes of mortality in developing countries, other than malnutrition and trauma. In the 1990s there was a large diphtheria epidemic in Russia as the result of the collapse of the public health infrastructure, demonstrating that pathogenic microbes are still in the environment and can become epidemic even in technologically advanced countries if we relax our efforts to contain them.

The infection process

Three stages in the infection process may be functionally distinguished:

- attachment of the micro-organism to the target cell(s) and, for intracellular pathogens, entry into the host cell;
- development of the infection, local multiplication of the pathogen and spread of the micro-organism to distant sites; and
- shedding of the organism and transfer to a new host.

Attachment to host cells

Only a few pathogens have the capacity to penetrate our body directly through the skin. Examples include the cercariae of various schistosome species, which can invade the skin with the help of their glandular secretions. Many other pathogens enter the body after an insect bite (e.g. *Simulium* blackfly bite for *Onchocercus volvulus*, anopheles mosquito bite for malaria) or iatrogenically from intramuscular or intravenous injection of contaminated medications or blood. This can transmit various blood-borne pathogens such as HBV, hepatitis C virus (HCV), HIV, West Nile virus, syphilis and malaria.

Although 'free' micro-organisms exist in the body (for instance, in the lumen of the intestine or in the saliva), most members of the human flora need to be attached to a cellular surface to avoid being swept away by biologic fluxes such as swallowing or the passage of the alimentary bolus. For many microbial and viral pathogens, adherence to the epithelial surface of the respiratory, digestive or reproductive mucosa is a compulsory step in pathogenesis.

Adherence

The approach of micro-organisms to an epithelial surface is guided by a balance between attractive and repulsive forces. Eventually, multiple high-affinity contacts between the microbe or virion and the cellular surface may establish a virtually irreversible association between the two. Even for viruses, attachment may involve multiple different mechanisms. Such contacts may involve nonspecific interactions, such as those between exposed hydrophobic structures on the microbial cell envelope and lipophilic areas on the cell membrane. Glycocalyx, made essentially of a mixture of polysaccharides and 'slime', produced in particular by *Staph. epidermidis*, may mediate nonspecific adherence between prokaryotic and eukaryotic cells. Interestingly, carbohydrate capsules on respiratory pathogens appear to interfere with epithelial cell adherence and bacteria that progress from epithelial colonization to invasion and need capsules to survive after invasion, downregulate capsule expression in order to adhere and invade epithelial cells, the first step in their pathogenesis.

Specific adherence involves microbial adhesins on the one side and host cell receptors on the other. Although the interaction between adhesins and cell receptors may be highly specific, this is not always the case. The specificity can be tested by artificially blocking adherence with an excess of purified adhesin or receptor or with antibodies directed against one of these two. The specificity accounts for the early observation that many pathogens distinctively infect certain areas or organs of the body and not others. For instance, *Strep. pneumoniae* causes pneumonia but not urethritis, whereas *Neisseria gonorrhoeae* exhibits the opposite pattern of specificity. The receptors for poliovirus, rhinovirus and HIV are expressed only by specific cell types, restricting virus infection accordingly. Different strains of influenza virus adhere via the hemagglutinin to different sialic acids, and this largely determines not only their host range but also their organ tropism. These and many other examples support the notion that adhesins determine the tropism of microbial pathogens. On the other hand, cell receptors for many organisms are ubiquitous and these organisms have no tissue or even host restriction, possibly because they encode many adhesins. *Salmonella enterica* serovar Typhimurium encodes 12 different fimbriae, but the binding specificity is known for only two.

Ubiquitous receptors

Fibrinogen, fibronectin, collagen and heparin-related polysaccharides are major components of the extracellular matrix (ECM) that coats the mucosal surface of epithelial cells. Members of the integrin family are involved in the interaction between the ECM and the underlying epithelium. A number of components of the ECM are used as receptors for microbial adhesins and viral receptor proteins. *Staph. aureus* has cell wall proteins that recognize nearly all ECM proteins including fibronectin, elastin, von Willebrand factor, vitronectin and collagen.

Attachment to fibronectin is also required for *Staph. aureus* to invade nonphagocytic cells. Fibronectin specifically binds fibronectin-binding factors on the cell envelopes of other bacteria including *Strep. pyogenes, Treponema pallidum, Mycobacterium* spp. and *Orientia tsutsugamushi*, the etiologic agent of scrub typhus; fibrinogen binds groups A, C and G streptococci and a member of the integrin family binds the major invasion factor of *Yersinia pseudotuberculosis*. Their abundance and structural conservation among mammalian species make ECM components ideal targets for bacterial adhesins.

Bacterial adhesins

Close contact between micro-organism and host cell represents an essential step in pathogenesis. It optimizes the interaction of microbial virulence factors with the target cell to allow the pathogen to penetrate or cause local cell damage, or both. Other possible functions of adhesins include modulation of the inflammatory response, adhesin-directed degranulation from mast cells and adhesin-mediated bacterial phagocytosis by neutrophils. Bacteria use two general strategies to attach themselves to host cells: fimbrial and afimbrial adhesion (Fig. 1.9).[22]

Pili and fibrillae

Attachment of bacteria to the plasma membrane can be mediated by filamentous structures protruding from the bacterial surface, called

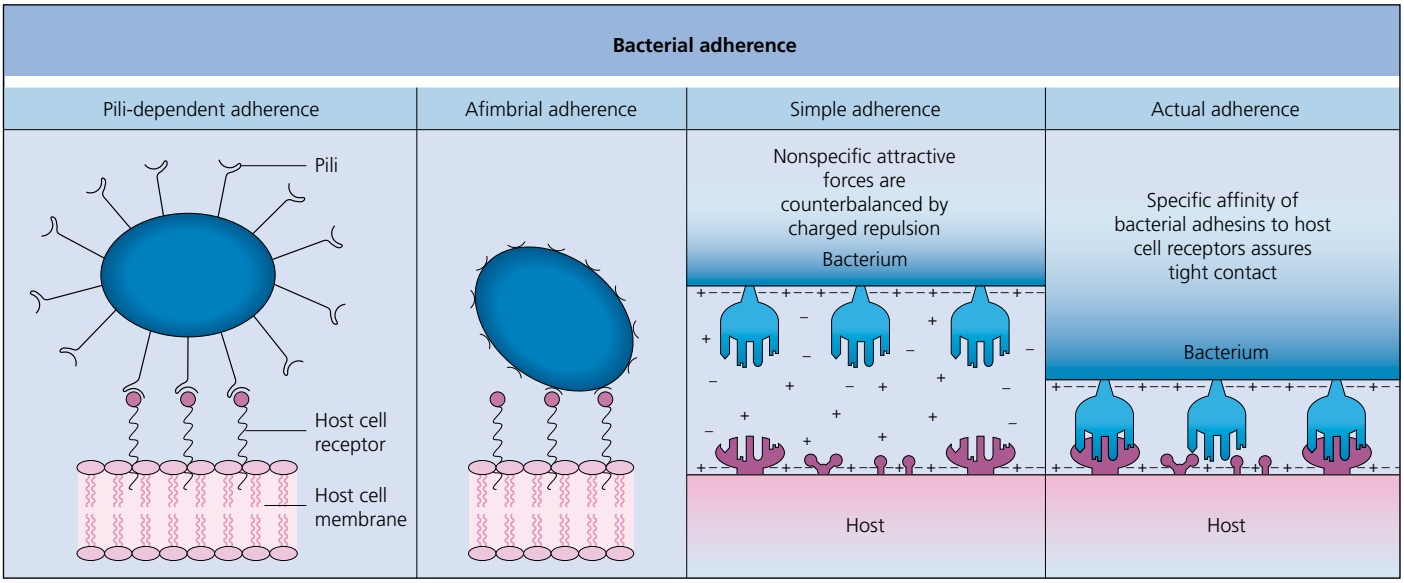


Fig. 1.9 Bacterial adherence. (Courtesy of Menno Kok & Jean-Claude Pechere.)

fimbriae or fibrillae. The classification of these colonization factors is based on morphologic criteria. Fimbriae (or common pili) are rigid hair-like structures with a regular diameter, whereas fibrillae are flexible and have an irregular diameter. These structures are distinct from flagella, which are responsible for bacterial motility (see Fig. 1.7), and sex pili, which are associated with bacterial conjugation.

Twenty different colonization factors have been described for *E. coli*.[23] One of these, the so-called P-pili expressed by uropathogenic *E. coli* strains, mediates adherence of the bacterium to the urinary mucosa to avoid elimination by the urinary flux. P-pili consist of a long and rigid base section attached to an outer membrane scaffold and a short flexible tip (Fig. 1.10).[24] The rigid section is composed of hundreds of pyelonephritis-associated (PapA) pilin subunits arranged in a right-handed helix. The pilus tip is 2 nm in diameter with a 15 nm pitch composed of PapE monomers. The PapG monomer is located at the end of the tip and is the actual adhesin. It recognizes the glycolipid receptor globobiose (α-1–4 linked di-galactose) on the host cell surface.

Afimbrial adhesins

Afimbrial adhesins, such as lectins (carbohydrate-binding proteins), also mediate tight binding between the bacteria and the host cell but, unlike pili, they do not form supramolecular structures. Similar adhesins exist in viruses, fungi and protozoa. Afimbrial binding has been extensively studied in *Strep. pyogenes* (Fig. 1.11). Two surface components are believed to be critical in the colonization of an epithelial surface: lipoteichoic acid and fibronectin-binding protein.

Purified lipoteichoic acid binds to fibronectin and inhibits the binding of *Strep. pyogenes* to oral epithelial cells. The binding properties of *Strep. pyogenes* lipoteichoic acid are confined to the lipid moiety. Similarly, artificially added fibronectin-binding protein inhibits adhesion of *Strep. pyogenes* to epithelial cells even after the streptococci have been depleted of lipoteichoic acid.

The complex surface of this micro-organism also includes the M protein.[26] This protein is a major virulence factor but it does not seem to be involved in adherence to epithelial cells, as was previously assumed. However, the M protein binds fibrinogen in a stoichiometric fashion and exerts an antiphagocytic effect, which may partially explain its role in virulence.

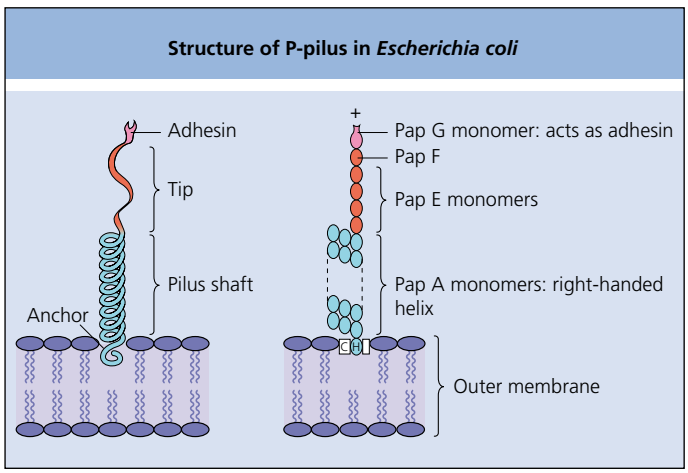


Fig. 1.10 Structure of P-pilus in *Escherichia coli*. (Courtesy of Menno Kok & Jean-Claude Pechere.)

Viral adhesion

Adhesion represents the first in a series of steps that ultimately leads to the delivery of the viral genome to its site of replication. Multiple different viral proteins may be required to mediate attachment, viral fusion and entry into the cell. For example, the HIV gp120 protein first attaches to the CD4 molecule on the cell surface, exposing an area of gp120 that interacts with a seven-loop transmembrane protein co-receptor, finally triggering fusion via a portion of gp41 transmembrane protein. Similarly, different viral proteins in rotavirus interact with membrane carbohydrates, integrins and a heat shock protein to mediate attachment and entry.[27]

For some viruses (typically enveloped viruses, including measles and mumps viruses[28]), attachment proceeds via direct fusion with the cell plasma membrane. These virions have a transmembrane fusion protein that induces contact between the viral and cellular lipid bilayers. Alternatively, attachment may trigger a process of endocytosis, proceeding through clathrin-coated pits, frequently requiring acidification to trigger structural changes in viral proteins that results in escape from the endolysosome into the cytoplasm. This is used by many non-enveloped viruses, including adenovirus, rhinovirus and some other enteroviruses.[29]

Availability of receptors and/or co-receptors on the cell surface determines whether a virus particle will bind. Cell specificity

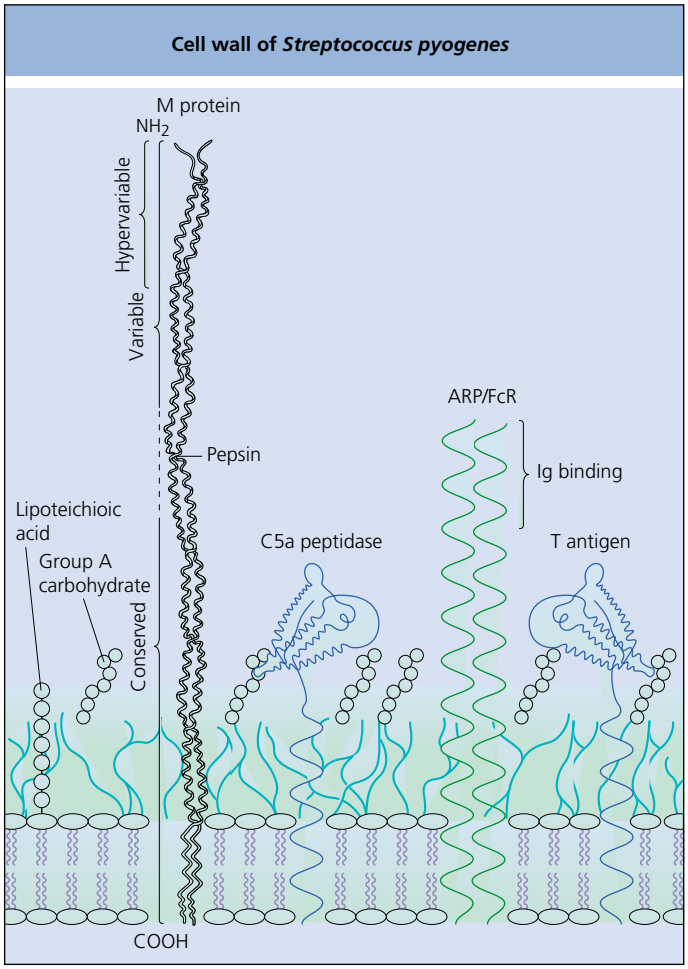


Fig. 1.11 Cell wall of *Streptococcus pyogenes*. The proposed model of the M protein is based on current sequence and structural data. ARP, immunoglobulin A receptor protein; FcR, receptor for the Fc portions of immunoglobulin. Adapted from Kehoe.[25]

('tropism') may be rather relaxed for viruses that use ubiquitous receptors while strongly restricted for viruses requiring two or more cellular receptors. As noted, HIV requires co-expression of CD4 and one of several chemokine receptors for efficient infection, and it principally infects only $CD4^+$ lymphocytes and monocyte/macrophages. In contrast, herpes simplex virus type 1 glycoproteins B and C interact with ubiquitous heparin sulfate present on cell plasma membranes. A second interaction is between glycoprotein D and nectin-1 or herpes virus entry mediator (HVEM) cellular receptors, widely expressed in epithelial cells and some neurons.[30] Herpes simplex virus first infects epithelial cells of the skin and mucosal surfaces, where the initial replication cycles take place, passing into axon termini of neurons. Tropism is not always restricted by surface binding, however. The JC polyoma virus receptor, sialyl (α2–6) Gal, has a wide distribution in human tissues, while virus replication is restricted primarily to oligodendrocytes, urothelial cells and perhaps B lymphocytes.[31]

Viral adherence and invasion can be blocked by neutralizing antibodies, which specifically bind the active site(s) of the adhesin(s). However, many viruses have hidden this region in a protein pocket (or 'canyon'), making it physically inaccessible to potentially neutralizing antibodies, thus escaping humoral immunity. In addition, many viruses produce huge amounts of variation due to relative infidelity of viral polymerases, allowing selection and escape of variants during infection.

INVASION

Invasive and noninvasive micro-organisms

Many micro-organisms, including those of the natural flora, remain at the epithelial surface without invading the underlying tissue (Table 1.5). This type of colonization is usually harmless although it may, in some cases, induce damage to adjacent cells through the production of toxins or elicit a local inflammatory or allergic response. Nonpenetrating micro-organisms include *Corynebacterium diphtheriae*, which cause pharyngitis, *Mycoplasma pneumoniae*, which cause pneumonia, and *Trichomonas vaginalis*, a cause of vaginitis.

Other micro-organisms gain access to deeper tissues only after a physical or chemical injury of the epithelial barrier. *Staph. aureus*, a harmless microbe when on the nasal mucosa, may become a dangerous toxin-producing pathogen once it penetrates the body, or can become locally destructive if it can penetrate beneath the stratified epithelium of the epidermis.

Invasive micro-organisms exhibit the capacity to penetrate the target tissue to which they adhere without the need for local disruption of the protective epithelium. Invasive bacteria have developed the capacity to enter host cells that are not naturally phagocytic. Penetration into these 'nonprofessional' phagocytes is achieved by either engulfment or zippering. *Salmonella enterica* and *Shigella* spp. are examples of bacteria that trigger their own engulfment using type III secretion systems to rearrange the host cell cytoskeleton using enzymes that are injected through the host cell membrane resulting in actin rearrangement so that the bacteria are carried into the cell (Fig. 1.12). The bacteria inject additional enzymes that restore the cytoskeleton to its original shape and restore integrity. In contrast, bacteria such as *Listeria monocytogenes* and *Yersinia pseudotuberculosis* use a 'zipper' mechanism to enter cells that starts with binding to integrins on the cell surface, which leads to cytoskeletal rearrangements. *Listeria* uses a second adhesion factor to enter hepatic cells, attaching to the hepatocyte growth factor receptor, which triggers phosphatidylinositol (PI) 3 kinase activation. Surprisingly, *Listeria* enters all cells in a clathrin-dependent, endocytic manner, except that after clathrin and dynamin are recruited there is cytoskeletal rearrangement necessary for the large particle to enter the cell.

In some cases infection remains confined to the epithelial surface (see Table 1.5), but in others the micro-organism may be transported across the superficial epithelium to be released into subepithelial space. This process is called transcytosis and involves the host cell actin network (see below). After transcytosis, the underlying tissues may be invaded and infected and the infection may eventually spread all over the body (e.g. *N. meningitidis* may cross the pharyngeal epithelium and cause meningitis, and *Salmonella enterica* serovar Typhi may cross the intestinal epithelium and infect the reticuloendothelial system causing typhoid fever). Some pathogens such as *Strep. pyogenes* usually cause disease on an epithelial surface, but they are also capable of invading epithelial cells and causing deep tissue infections. For a more detailed analysis of the mechanisms of invasion, we shall use the example of enteroinvasive pathogens.

Enteroinvasive pathogens and the membranous cell gateway

Acute infectious diarrhea may cause the clinical spectrum ranging from watery diarrhea to dysentery (bloody diarrhea). It occurs when the pathogen invades the intestinal mucosa and causes structural damage to the intestine. The immunologic protection of the intestine is performed by the gut-associated lymphoid tissues, which are separated from the intestinal lumen by epithelium. The follicle-associated epithelium is covered by membranous cells (M cells) that play a prominent role because they are specialized in the transport of antigens. Enteroinvasive viruses, protozoa and bacteria exploit the transport facilities provided by M cells to invade the host. Entry into (and passage through) M cells

Table 1.5 Interaction of micro-organisms with epithelial cells

	Order	Micro-organism	Disease
Generally confined to epithelial surfaces	Bacteria	*Bordetella pertussis*	Pertussis
		Chlamydia trachomatis	Trachoma, urethritis
		Corynebacterium diphtheriae	Diphtheria
		Streptococcus pyogenes	Uncomplicated pharyngitis
		Vibrio cholera	Cholera
		E. coli (EPEC)	Diarrhea
	Viruses	Coronaviruses	Common cold
		Rhinoviruses	Common cold
		Rotaviruses	Diarrhea
	Fungi	*Candida albicans*	Thrush
		Trichophyton spp.	Athlete's foot
	Protozoa	*Giardia lamblia*	Diarrhea
		Trichomonas vaginalis	Vaginitis
Enter through the epithelium	Bacteria	*Shigella* spp.	Bacillary dysentery
		Brucella melitensis	Brucellosis
		Neisseria meningitidis	Meningitis
		Salmonella typhi	Typhoid fever
		Treponema pallidum	Syphilis
		Yersinia pestis	Plague
	Viruses	Measles virus	Measles
		Rubella virus	Rubella
		Varicella	Chickenpox
		Poliovirus	Poliomyelitis
	Fungi	*C. albicans*	Disseminated candidiasis
	Protozoa	*Toxoplasma gondii*	Toxoplasmosis
		Entamoeba histolytica	Liver abscess

by these pathogens is preceded by adherence. While its pathogenicity is uncertain, enteric infection by mammalian reovirus type 1 involves initial entry through M cells mediated by the capsid proteins σ1 and μ1.[32] Infection by poliovirus may proceed by a similar route.[33]

Enteroinvasive bacteria such as *Salmonella*, *Shigella* and *Yersinia* spp. appear to distinguish between different subsets of M cells. Membranous cells produce glycocalyx, which contains a distinctive profile of lectin-binding sites. Diversity in lectin-binding sites between different locations of the gut may account for the tropism of enteric pathogens, such as the preferential colonization of colonic mucosa by *Shigella* spp. rather than *Salmonella* spp., which are more commonly found at the end of the ileum. Following adherence, the interactions with the M cells vary according to the pathogen (Fig. 1.13). Enteroadherent *E. coli* are not internalized and hence are not invasive. *Vibrio cholerae* is taken up and transported by the M cells but rapidly killed thereafter. It is considered to be invasive at the cellular level but not at the clinical level.

The *Salmonella* and *Shigella* spp. genes involved in invasion of the eukaryotic host cell are homologous and have been remarkably well conserved with respect to both the individual coding sequences and their genetic organization (Fig. 1.14).[35] Detailed molecular analyses of virulence factors produced by enteroinvasive *Shigella* spp. have revealed that all virulent species harbor a 220 kb plasmid, of which a 31 kb operon, encoding 32 genes, is both necessary and sufficient for invasion of epithelial cells.[36] The *Salmonella* spp. entry functions are clustered in a 35–40 kb pathogenicity island[37] inserted in the chromosome at centisome 63.[38] Using a needle-like complex,[39] the bacteria translocate a number of effector proteins into the cytosol and the plasma membrane of the target cell.[40] Some of these effector proteins specifically modify the activities of cellular GTPases (see Fig. 1.12), inducing the alterations of the cytoskeleton required for bacterial internalization.

An important difference between the pathogenic lifestyles of these two bacterial species involves the intracellular fate of the bacteria. Once internalized, the bacteria are enclosed by a host cell membrane in an endocytic vesicle, deprived of nutrients. Soon after entry into the epithelial cell, *Shigella* spp. escape from the endosome into the nutritious cytoplasm; however, *Salmonella* spp. have adopted an entirely different strategy. Salmonellae modify the endocytic pathway of the host cell by means of virulence factors encoded largely by pathogenicity islands and, for highly invasive strains, the virulence plasmids, thus avoiding exposure to bactericidal mechanisms of the cell. Although only some of the cellular targets of the translocated bacterial virulence proteins have been identified to date, it is clear that the physiology of the infected cell is profoundly modified to suit bacterial growth and maintenance.

Actin-based intracellular motility of microbial pathogens

Listeria monocytogenes, *Rickettsia* spp., *Shigella* spp. and vaccinia, measles and rabies viruses use active actin modification to move within the cytoplasm of infected cells and to invade neighboring cells. They induce the formation of actin cross-linked filaments, which assemble in characteristic 'comet-like tails' (Fig. 1.15).[41,42] Elongation of the actin filaments generates sufficient force to move the micro-organisms through the cytoplasm at rates of 2–100 mm/min.

The intracellular life cycle of *L. monocytogenes* illustrates this strategy (Fig. 1.16).[43,44] Under natural conditions, *Listeria* first penetrates enterocytes and subsequently spreads through the body to infect a variety of host cells, including endothelial cells, Kupffer cells, hepatocytes, phagocytes and, most importantly, the trophoblasts of the placenta. Entry is facilitated by the products encoded by the internalin (*inl*) family of genes, which seem to confer tropism for different cell types. Once inside the cell, *L. monocytogenes* remains confined to the phagosome for only a short time. Following lysis of the endosomal membrane, it escapes into the cytosol. Membrane lysis is achieved by a production of listeriolysin-O, which attains maximum activity under the acidic conditions of the intravacuolar environment.

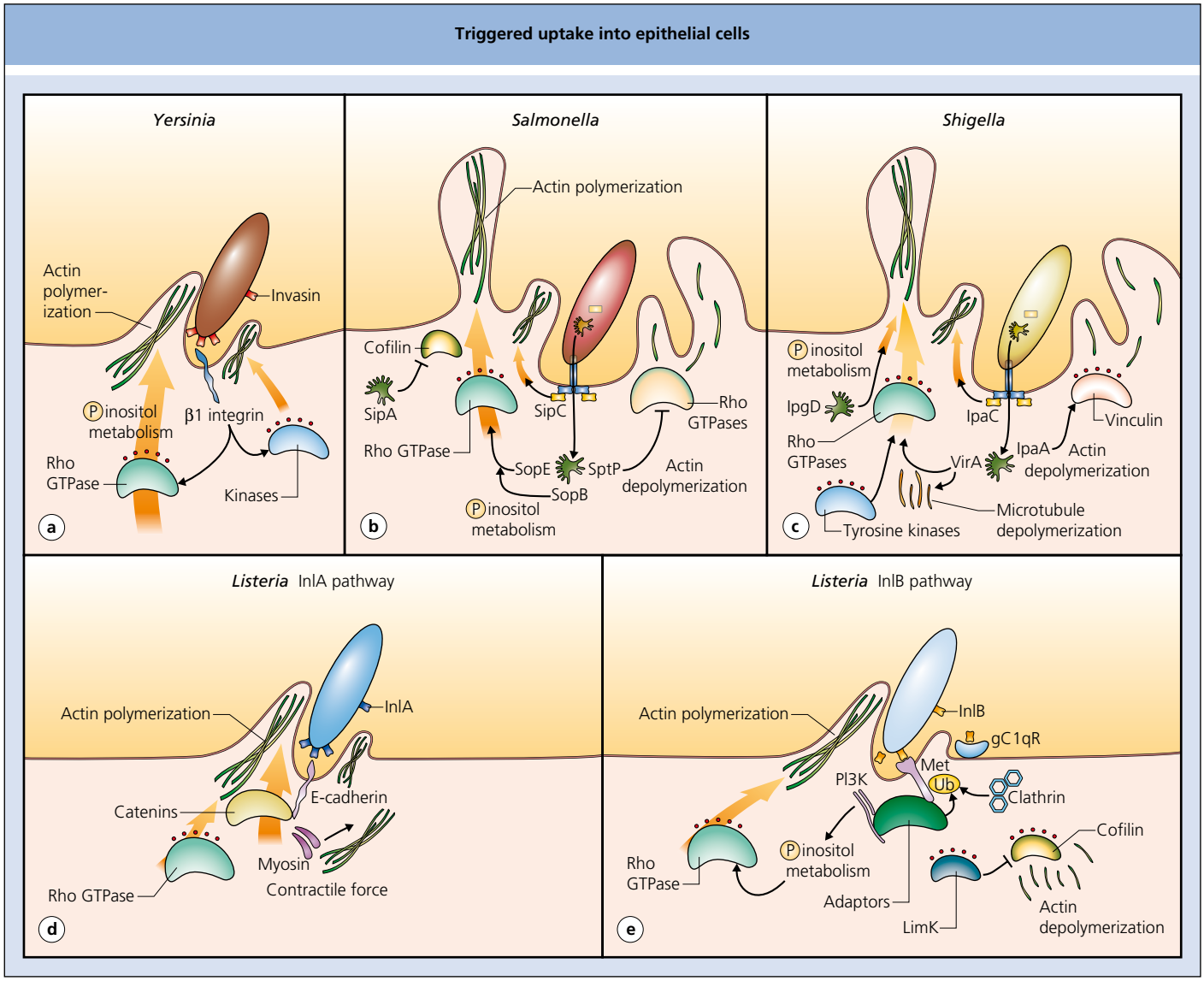


Fig. 1.12 Triggered uptake into epithelial cells. Some invasive bacteria (b and c) express a complex needle-like structure on their cell surface (Type III secretion system) through which they inject proteins that hijack the adjacent cytoskeleton, triggering membrane ruffling that embraces the adjacent bacterium and internalizes it in a vacuole. Others have surface receptors for integrins (a, d and e) which trigger a pinocytic-like response leading to uptake.

Once in the cytosol, the bacteria multiply and migrate towards the plasma membrane by using the actin-based mechanism as described above. Actin polymerization is mediated by the *L. monocytogenes* protein ActA, localized at one end of the bacterium. For spread to neighboring cells, *L. monocytogenes* requires bacterial lecithinase and phospholipase C, which stimulate lysis of the two membranes that separate the bacterium from the cytoplasm of the newly infected cell. Interestingly, most of the virulence genes associated with this process are clustered in a single region of the *L. monocytogenes* chromosome. By spreading in this manner the bacteria are not exposed to human and cellular defenses. However, the intracytoplasmic bacteria stimulate cell innate immune responses via Nod signaling.

Subepithelial invasion and spread through the body

Invasion from the site of infection can only be achieved by micro-organisms that effectively resist or subvert the host defense mechanisms in the subepithelial space, most prominently phagocytosis.

Some organisms take advantage of the normal transport of antigens and are carried by dendritic cells to regional lymph nodes. In the lymph nodes, resident macrophages and polymorphonuclear cells actively fight the invaders. As a result, the first line of lymph nodes is often inflamed. If the invading micro-organism is sufficiently virulent or present in sufficiently large numbers, it may pass into efferent lymphatic vessels to be conducted to the bloodstream. The result is primary bacteremia or viremia.

Some microbes can enter directly into the blood vessels via an injury. A typical example is provided by viridians streptococci, which enter the bloodstream during dental extraction, enabling them to infect an abnormal cardiac valve and produce endocarditis. Insect bites (malaria and arthropod-borne viruses) or damage to the blood vessel wall inflicted during infection with hemorrhagic fever viruses are alternative ways to circumvent the body's first line of defense: the mucosal immune system.

Once in the bloodstream, the micro-organisms may circulate as either an extracellular or an intracellular species. Pathogens have been found in polymorphonuclear cells (*Anaplasma*), lymphocytes (HIV), macrophages (*M. tuberculosis, Histoplasma capsulatum* and CMV) and

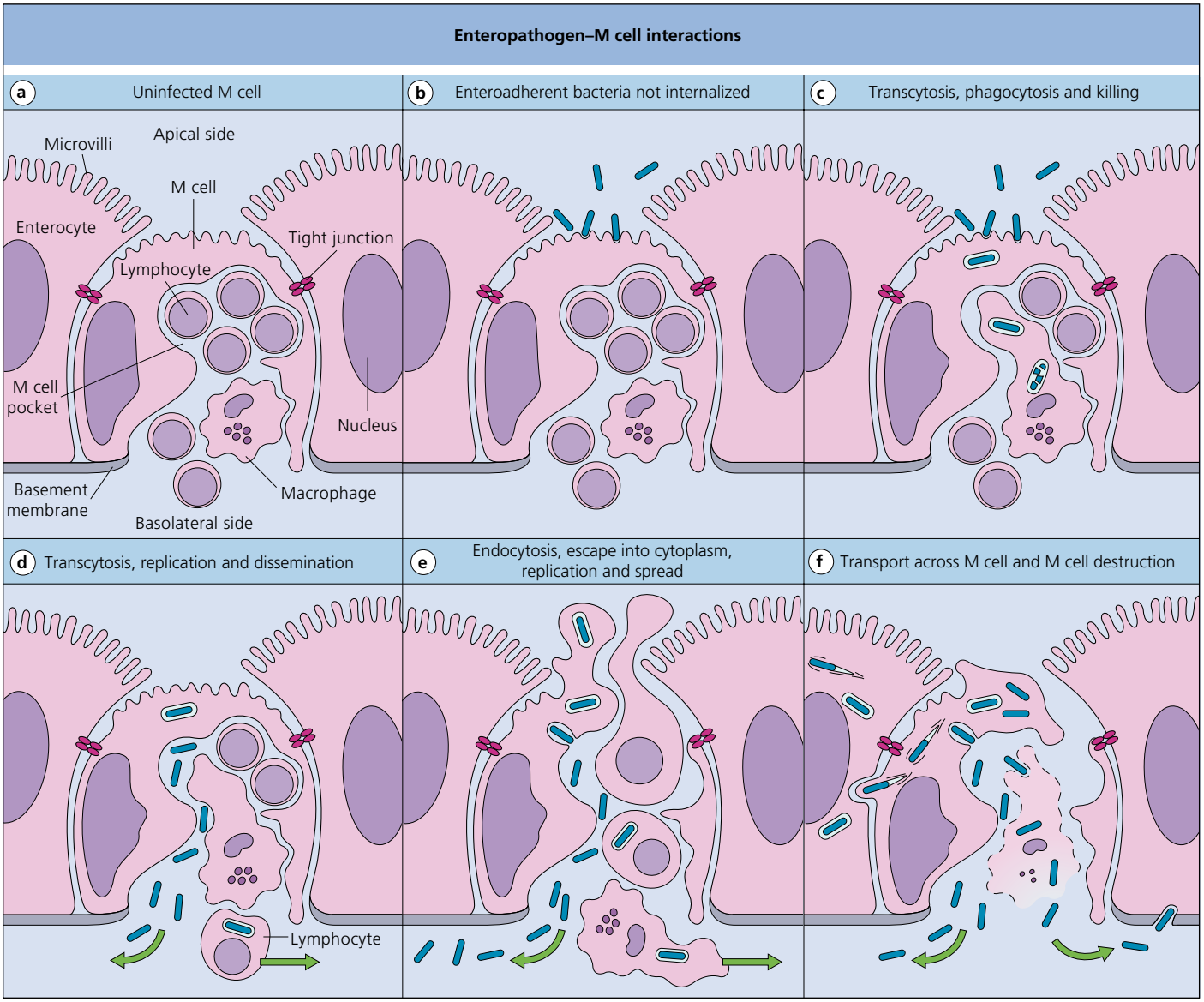


Fig. 1.13 Enteropathogen–M cell interactions. (a) An uninfected M cell, enclosed between two adjacent enterocytes. The basolateral side forms a pocket where lymphocytes and macrophages are located. (b) Enteroadherent *Escherichia coli* forms microcolonies at the M cell surface, but is not internalized. (c) *Vibrio cholerae* undergoes transcytosis but is efficiently phagocytosed in the submucosa. (d) *Campylobacter jejunii* and *Yersinia* spp. undergo transcytosis, replicate in the submucosa and disseminate. (e) *Salmonella* spp. are transported across M cells, leading to destruction of the M cell. (f) *Shigella flexneri* is endocytosed by M cells, escapes into the cytoplasm, replicates, is propelled by actin tails and spreads to adjacent enterocytes. Adapted from Siebers & Finlay.[34]

even in red blood cells (*Plasmodium* spp., *Bartonella bacilliformis*), which provide protection against potent humoral factors in the serum, such as complement.

Infection of distant target organs

Transported by the bloodstream, the invasive micro-organisms can reach distant target organs and create infective metastases throughout the body. Almost any tissue can be reached, but the organs containing abundant capillary and sinusoid networks (e.g. lungs, liver, kidneys, bone marrow) and macrophages that are exposed directly to circulating blood are especially exposed, because blood flows slowly at these sites and transported micro-organisms get the opportunity to adhere and establish an infection. The epiphyses of long bones in children are an important target for certain pathogens such as *Staph. aureus* and *Haemophilus influenzae*. From the target organs, the invaders may produce a secondary bacteremia or viremia, in which microbial counts in the blood are generally higher than during primary infections.

The example of measles virus

Inhaled airborne measles virus recognizes membrane co-factor protein (CD46)[45] and/or the signaling lymphocyte activation molecule (SLAM/CD150)[46] as a receptor on the epithelial surface of the respiratory mucosa. Infection in a nonimmune host proceeds with 2–4 days of limited local replication in the lining of the trachea and bronchi. Pulmonary macrophages carry the virus to the regional lymph nodes, where exponential virus replication causes formation of reticuloendothelial giant cells. Progeny virions enter the bloodstream, causing a primary viremia with spread to the spleen, other lymphatic tissue, the lung, nasopharynx, oral mucosa, thymus, liver, skin and the central nervous system over the next 4–5 days, with secondary replication

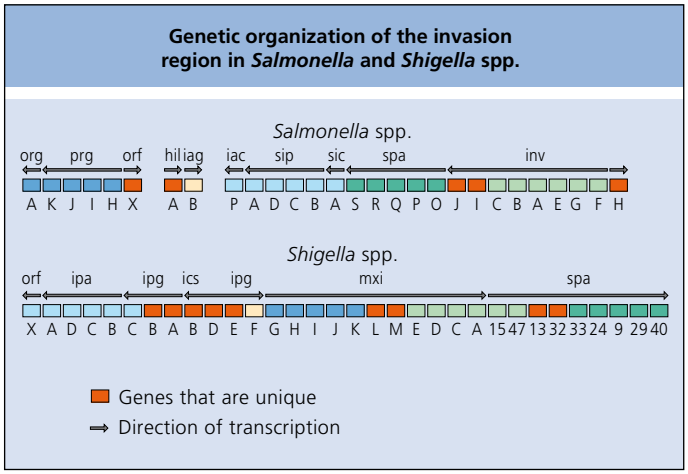


Fig. 1.14 Genetic organization of the invasion region on *Salmonella* and *Shigella* spp. Identical patterns indicate topologically conserved blocks of genes. Each genus has genes that are unique. Despite remarkable genetic similarities, the invasion strategies of the two bacteria are quite different (see Fig. 1.13). Adapted from Galan.[38]

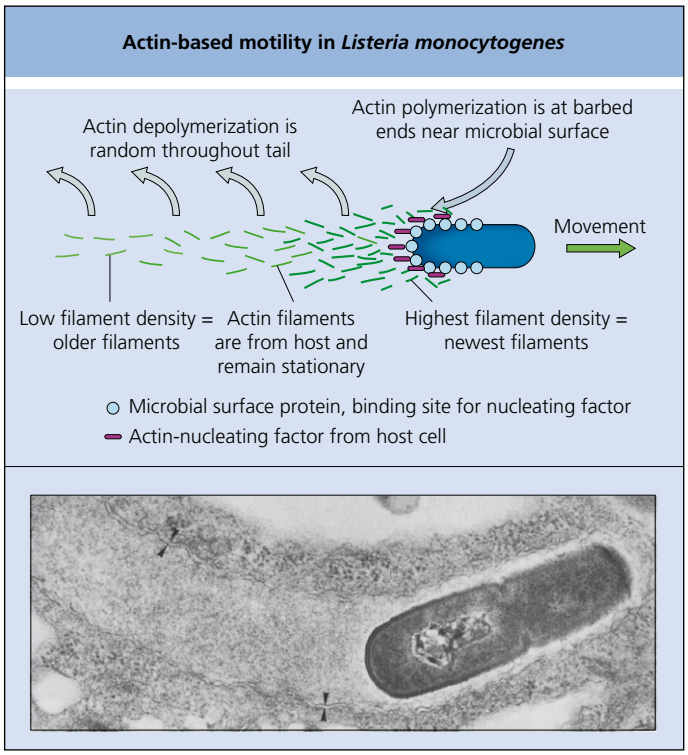


Fig. 1.15 Actin-based motility in *Listeria monocytogenes*. The bacterium moves forwards at the rate of actin-filament growth behind the pathogen. Adapted from Sanders & Theriot.[41] The electron micrograph shows a section of a CaCo-2 cell infected with *Listeria monocytogenes*; the bacterium protrudes into the cytoplasm of an adjacent cell; protrusion is limited by a double membrane (arrowheads).

and increasing viremia. Virus shedding from the nasopharynx begins about 12 days after infection, before symptoms or rash develop, contributing to a secondary attack rate of 80% in susceptible contacts.

These pathogenic steps correspond to different clinical periods. During the 10-day incubation period, infection and primary viremia proceed with no clinical symptoms. Symptoms of fever, malaise, cough and conjunctivitis are concomitant with secondary viremia, followed

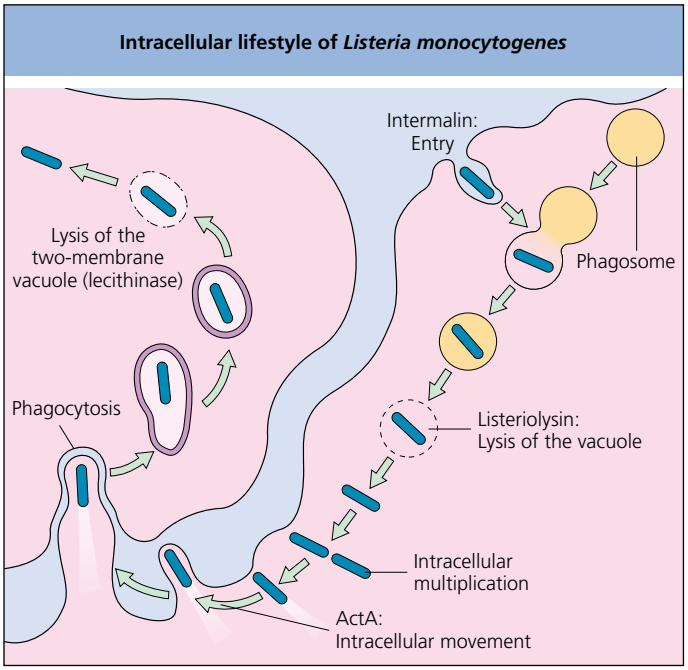


Fig. 1.16 Intracellular life cycle of *Listeria monocytogenes*. (Courtesy of Menno Kok & Jean-Claude Pechere.)

by rash at 12–13 days. The characteristic morbilliform exanthem is associated with a perivascular mononuclear infiltrate, including cytotoxic T cells which migrate to the site of virus infection of dermal endothelial cells and overlying dermis. Virus infection produces epithelial giant cells, but does not directly destroy infected cells in the skin. Leukopenia occurs late in viremia, and immune suppression can be seen from the time of appearance of symptoms until 2–3 weeks after resolution of clinical infection (see Chapter 152).

Serum resistance in *Neisseria gonorrhoeae* and *Salmonella* spp.

Complement is a complex system of circulating proteins that can be activated in three ways:

- by antibodies that interact with C1q;
- by spontaneous hydrolysis of C3 to the enzymatically active C3b that is then stabilized on appropriate surfaces by factors B, D and properdin of the alternative pathway; and
- binding of mannose-binding protein to microbes with subsequent attraction of C1 and mannan-binding lectin-associated serine proteases (MASPs) that proceed to activate C2 and C4.

Complement activation is a major component of the innate immune system, primarily because it can function as an opsonin in people who do not have antibody against the carbohydrates and proteins that form the outer layer of invasive bacteria. Children who are born lacking C3, the central component of all three complement pathways, suffer from repeated bacterial infections and often die in infancy. In contrast, people who are deficient in one of the late complement proteins (C5, 6, 7, 8 or 9) are usually in their teens before they become ill, and the only infections that occur at a higher than expected frequency are disseminated *N. meningitides* and *N. gonorrhoeae*.

In theory, Gram-negative bacteria are susceptible to complement lysis, as the membrane attack unit can insert itself through the outer and inner membranes if C3b binds close enough to the peptidoglycan. However, *Salmonella* and many other Gram-negative bacteria have developed various strategies to prevent complement lysis. These include diverting C3b binding to the ends of the long chain polysaccharide components of LPS that project from the bacterial surface so that the attack component assembles harmlessly at a distance from the inner membrane. Another strategy used by bacteria with short chain

polysaccharides is to use outer membrane proteins to bind host regulatory proteins such as factor H and C4 binding protein to their surface.

Most immunocompetent hosts contracting gonorrhea do not develop a systemic disease because complement can kill bacteria that penetrate through the mucosal barrier. However, the clones of *N. gonorrhoeae* that cause disseminated infections in normal hosts are serum resistant, but often lose that property when passed *in vitro*, as they undergo phase variation. A large number of genes in the *N. gonorrhoeae* genome appear to be phase variable, but which of these is responsible for the invasiveness and complement resistance of disseminated gonococcal infection (DGI) strains is not clear. Another strategy used by pathogenic *Neisseria* to escape complement lysis is stimulation of 'blocking antibodies', which are often IgA (noncomplement binding) that recognize outer membrane proteins and prevent the attachment of bactericidal IgG antibodies and complement to the cell surface.

Cell and tissue damage induced by micro-organisms

Infectious disease is often characterized by cell and tissue damage. Paralysis in poliomyelitis, exanthem in varicella, gastroduodenal ulcers in *Helicobacter pylori* infections and bloody diarrhea in shigellosis all result from damage caused directly or indirectly by micro-organisms. Cell damage can be generated by a variety of different mechanisms (Table 1.6).

Bacterial toxins

Bacteria produce a large diversity of toxins, which have been classified according to their mode of action (Table 1.7, Fig. 1.17). Historically, toxins were defined as soluble substances that alter the normal metabolism of the host cells with deleterious effects on the host. However, as we learn more about the mechanisms of action of exotoxins, the distinction between them and secreted enzymes that play a role in pathogenesis is disappearing. The clostridial exotoxins are good examples of proteases that are exotoxins because they have specific cellular targets within nerve cells. These toxins, which are responsible for tetanus and botulism, are zinc metalloproteases that cleave synaptobrevins or a related protein in the same pathway so that docking and fusion of synaptic vesicles are impaired. The substrate specificity of the proteases and the binding affinity of the heavy chain of the toxins are what determine the different clinical presentations of these diseases. The scalded skin syndrome (SSSS), a blistering skin disorder caused by some strains of *Staph. aureus*, is another example of clinical illness that is the consequence of a secreted protease with a specific target. Cleavage of human desmoglein 1 results in the widespread acantholysis and the flaccid bullous lesions of generalized SSSS.

Cholera toxin ADP ribosylates G_s protein, locking it into the 'on' position, resulting in unregulated activity of adenyl cyclase and high levels of cAMP. The singularity of cholera as an enterotoxin is not due to the specificity of the toxin binding or function but to the location of the pathogen; *V. cholera* is an extracellular mucosal pathogen so only intestinal epithelial cells are exposed to the toxin *in vivo*. Traditionally, exotoxins are said to be excreted toxins. However, some of the so-called exotoxins are actually intracellular and are released into the environment only after cell lysis. The pneumolysin of *Strep. pneumoniae*, for example, is cytoplasmic, the adenylate cyclase of *Bordetella pertussis* is associated with the cytoplasmic membrane and the heat-labile toxin 1 (LT-1) from *E. coli* is periplasmic. The genetic information that encodes bacterial toxins is frequently carried on mobile DNA elements, which may readily pass from one microbial host to another. The toxins associated with diphtheria, cholera, botulism and scarlet fever, as well as Shiga-like toxins in *E. coli*, are encoded by temperate bacteriophages. Genes for LT-1 and methanol-susceptible heat-stable toxin (Sta) of *E. coli* are carried on plasmids.

Toxins deregulate the physiology of the host cell before or during bacterial adhesion and invasion. The bacteria may profit from the induced damage, which compromises the cellular defense against the intruder and release of nutrients from the cytosol. In the case of enteric pathogens, the flux of fluid that characterizes diarrhea makes it more likely that the bacteria will find their way to another host.

The diphtheria toxin as example of an A–B toxin

Diphtheria toxin belongs to the so-called bifunctional A–B toxins (Fig. 1.18). Portion A mediates the enzymatic activity responsible for the toxicity after internalization into the target cell, but cannot penetrate by itself. Portion B is not toxic but binds to a cell receptor localized on the cell surface and mediates the translocation of the A chain into the cytosol. Portion B accounts for the cell specificity of the A–B toxins. The receptor recognized by the B chain of diphtheria toxin is a heparin-binding precursor of epidermal growth factor, an important hormone for growth and differentiation of many different cell types.

Table 1.6 Mechanisms of cell and tissue damage produced by micro-organisms

	Mechanism	Examples
Direct damage by micro-organisms	Production of toxins	See Table 1.7
	Production of enzymes	Proteases, coagulase, DNAses produced by *Staphylococcus aureus*
	Apoptosis	HIV ($CD4^+$ T cells); *Shigella* spp. (macrophages)
	Virus-induced cytopathic effects: Cell enlargement and lysis Formation of syncytium	 Cytomegalovirus Respiratory syncytial virus
	Inclusion bodies: Intracytoplasmic Nuclear	 Rabies Herpesviruses
	Transformation	Human papillomavirus type 16
Damage via the host immune response	Cytotoxic T cells and natural killer lymphocytes	Production of the measles rash, hepatitis A
	Autoimmunity	Acute rheumatic fever
	Immediate hypersensitivity	Rashes associated with helminthic infections
	Cytotoxic hypersensitivity	Cell necrosis induced by hepatitis B
	Immune complexes	Glomerulonephritis in subacute endocarditis
	Delayed type hypersensitivity	Tuberculous granuloma, caseous necrosis

Table 1.7 Examples of bacterial toxins

Toxin type	Example of sources	Toxin	Targets	Mechanisms	Effects
Endotoxin (LPS, lipid A)	Gram-negative bacteria	Endotoxin	Macrophages, neutrophils, B lymphocytes, endothelial cells, plasma components	Activation of target cells via TLR4, complement activation; release of IL-1, TNF, kinins	Fever, septic shock
Membrane-disrupting toxins	*Staphylococcus aureus*	α-Toxin	Many cell types	Formation of pores	Tissue necrosis
	Listeria monocytogenes	Listeriolysin	Many cell types	Formation of pores at acidic pH	Escape from the phagosome
	Clostridium perfringens	Perfringolysin-O	Many cell types	Phospholipase (removes polar head groups from phospholipids)	Gas gangrene
A–B type toxins	*Clostridium tetani*	Tetanospasmin	Synaptic transmission	Inhibits release of inhibitory neurotransmitters	Spastic paralysis
	Clostridium diphtheriae	Diphtheria toxin	Many cell types	ADP ribosylation of EF-2	Myopathy, polyneuropathy
	Vibrio cholerae	Cholera toxin	Intestinal cells	ADP ribosylation of adenylate cyclase, leading to rise cyclic AMP	Profuse watery diarrhea
Superantigen	*Streptococcus pyogenes*	Streptococcal pyogenic exotoxin	T cells, macrophages	T cell stimulation, release of IL-1, IL-2, TNF; possible enhancement of LPS activities	Fever, rash, toxic shock-like syndrome
	Staphylococcus aureus	Toxic shock toxin	T cells, macrophages	Same as streptococcal pyrogenic toxin	Toxic shock syndrome

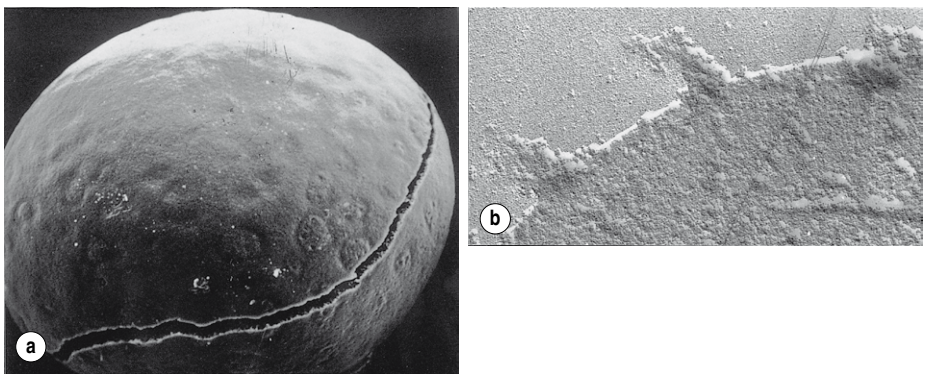


Fig. 1.17 Action of bacterial toxins. (a) *Xenopus* oocyte treated with the cytolytic delta toxin (perfringolysin) of *Clostridium perfringens*. (b) Rabbit erythrocyte exposed to a very small quantity of streptolysin-O, produced by *Streptococcus* A,C,G. Hemoglobin escapes from sites of membrane rupture. (Courtesy of Menno Kok & Jean-Claude Pechere.)

Uptake of diphtheria toxin proceeds via receptor-mediated endocytosis. Acidification of the endocytic vesicle induces a conformational change in the enclosed holotoxin, enabling the A subunit to traverse the membrane and reach its cytoplasmic target. The A subunit of diphtheria toxin catalyzes ADP ribosylation of the elongation factor-2 (EF-2), resulting in its inactivation. The *tox* gene is encoded by a phage and is under the control of the repressor protein DtxR, which forms a complex with iron, DtxR-Fe (Fig. 1.19), binds DNA and represses *tox* expression. Thus diphtheria toxin is only synthesized under low iron conditions, suggesting that it may be produced to stimulate iron release from target cells. Interestingly, the *Pseudomonas aeruginosa* exotoxin A has a very similar structure, but uses a different cell receptor: the α-2 macroglobulin low-density lipoprotein receptor. Like diphtheria toxin, exotoxin enters the cell via receptor-mediated endocytosis but the toxin is released only after passage through the Golgi system.

Hydrolyzing enzymes

Microbial pathogens often secrete hydrolyzing enzymes, such as proteases, hyaluronidases, coagulases and nucleases. As such, these enzymes do not harm the host cells directly and they are therefore not considered to be toxins. However, in the context of an ongoing infection they can facilitate colonization of host tissues by a variety of mechanisms, such as proteolysis of IgA; fluidification of pus; induction of plasma clotting, which may hinder the influx of phagocytes into the focus of infection; and destruction of extracellular DNA nets produced by polymorphonuclear neutrophils (PMNs) as an antimicrobial factor. The release of hydrolytic enzymes by phagocytes damaged by a bacterial toxin may have similar effects.

An example of how one such exoenzyme can nevertheless contribute to the pathogenesis of disease was recently discovered. Host

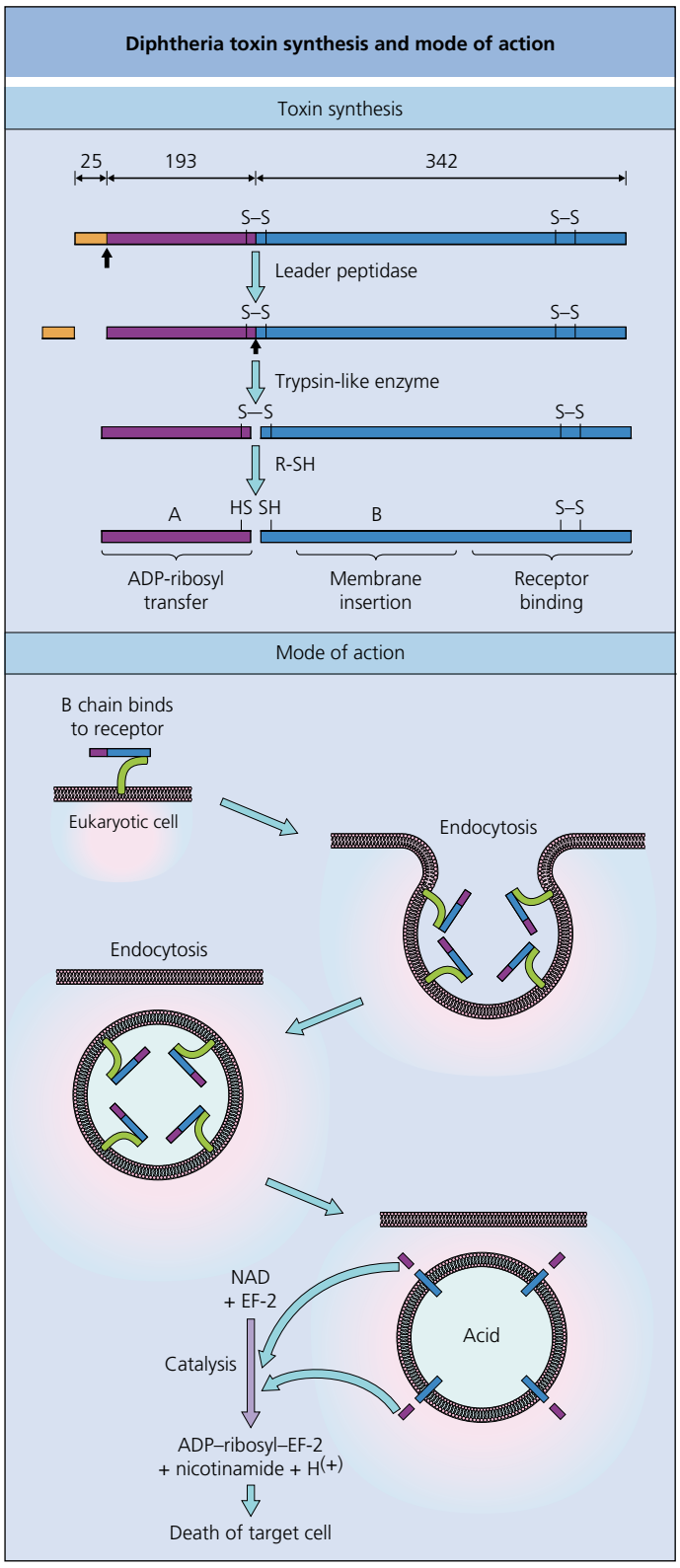


Fig. 1.18 Diphtheria toxin synthesis and mode of action. (Top) The 25-residue leader sequence is cleaved off by the bacterial leader peptidase; the A and B subunits are generated from the precursor protein by a 'trypsin-like enzyme'. Once in the cytoplasm of a targeted eukaryotic cell, the A chain, responsible for ADP-ribosyl transfer, is disconnected from the B chain, responsible for receptor binding and membrane insertion. (Bottom) The B chain binds to a specific receptor on the eukaryotic cell. After endocytosis, acidification in the endosome induces insertion of the B chain into the endosomal membrane and translocation of subunit A into the cytosol, where it catalyzes the ADP ribosylation of EF-2. As a result, protein synthesis is inhibited and the targeted cell dies. (Courtesy of Menno Kok & Jean-Claude Pechere.)

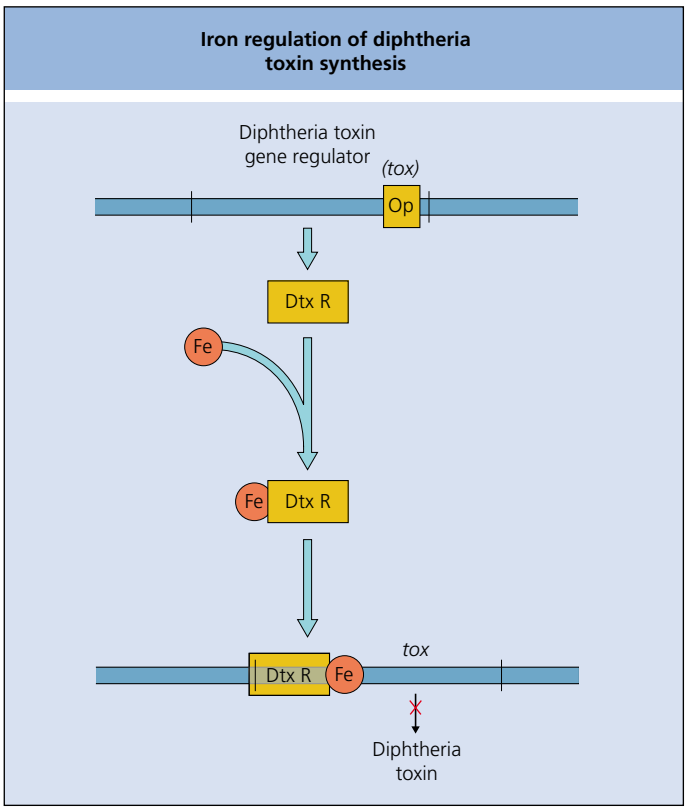


Fig. 1.19 Iron regulation of diphtheria toxin synthesis. High iron concentrations in the environment repress the synthesis of diphtheria toxin. When bound to iron, DtxR-Fe binds to the operator (Op) of the *tox* gene and acts as a transcriptional repressor of the *tox* gene. (Courtesy of Menno Kok & Jean-Claude Pechere.)

proteins that have a sialic acid in a 2,3 linkage to galactose residues at the termini of N- and O-glycan chains are not substrates for the host Ashwell receptor unless the terminal sialic acid is cleaved off. Pathogens such as *Strep. pneumoniae* that secrete a sialidase can remove sialic acids from host platelets that are then cleared from the circulation by the Ashwell receptor. This produces thrombocytopenia but avoids disseminated intravascular coagulation (DIC). Thus, one complication of severe pneumococcal sepsis is due to a secreted enzyme that is not generally considered to be an exotoxin.

Apoptosis

Apoptosis is a process in which the cell activates an intrinsic suicide program. It plays a key role in processes like organ development, tissue repair and maintenance of the dynamic equilibrium of the immune system. These processes critically depend on the generation of self-limiting organized structures through addition of new cells and elimination of 'old' cells. The morphologic changes associated with apoptotic death are a reduction of the volume of the cytosol and nuclear condensation (Fig. 1.20). The genome is fractionated by an endonuclease that cuts the DNA into multiples of 180–200 bp.[47] Finally, the remains of the cell are removed by phagocytosis without triggering an inflammatory response. In necrosis the cell does not participate actively in its own death and the dead cells trigger production of proinflammatory cytokines by macrophages.

Viral infection often triggers apoptosis of infected cells due to interruption of protein synthesis, transcription or signaling. For instance, apoptosis seems to contribute to the depletion of $CD4^+$ T cells, both in cell culture and in HIV-infected people.[48] Several different HIV proteins have been noted to both promote and inhibit apoptotic cell death.[49] Similarly, apoptotic cells have also been observed in infections caused by Epstein–Barr virus (EBV) and adenoviruses, though in latency EBV

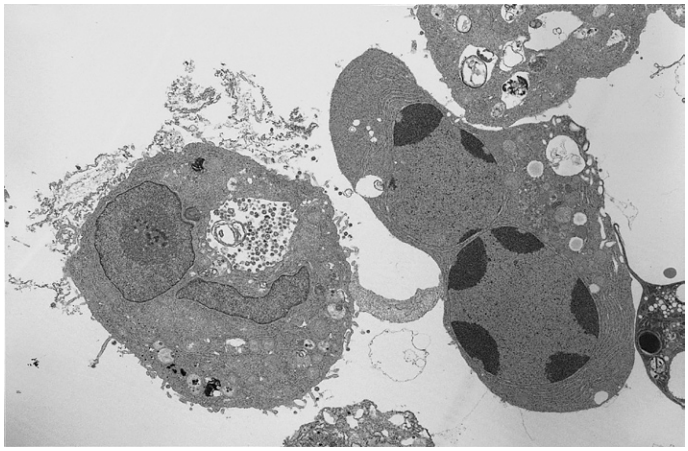


Fig. 1.20 Apoptosis induced by Sendai virus. Morphologic changes in the apoptotic Sendai infected cell (right) include the typical condensation of chromosomal DNA. (Courtesy of Menno Kok & Jean-Claude Pechere.)

latent membrane protein 1 (LMP-1) inhibits apoptosis.[50] Apoptosis can be seen to be beneficial to the host in that it can eliminate a host cell before it has produced a full complement of progeny virus.

Bacteria can also induce apoptosis. *Bordetella pertussis*, the agent of whooping cough, triggers macrophage apoptosis by interfering with cellular regulation at the level of the cytoplasmic second messenger cyclic AMP (cAMP).[51] The bacterium induces high levels of cytoplasmic cAMP, favoring the induction of apoptosis. *Shigella flexneri*, the etiologic agent of dysentery, can kill macrophages by apoptosis. Cell death is induced by invasion plasmid antigen B (IpaB) encoded by the *Shigella* virulence plasmid (see Fig. 1.14).[52] The *Shigella* IpaB protein binds to the host cytoplasmic enzyme interleukin (IL)-1b converting enzyme (caspase-1) and activates it.[53] Caspase-1 activates the proinflammatory cytokines IL-1 and IL-18 by proteolytic cleavage and initiates one of the proapoptotic pathways. In *Salmonella* infection the IpaB homologue SipB similarly activates caspase-1 to stimulate secretion of the proinflammatory cytokine IL-18 and induce apoptosis.[54] Timely induction of apoptosis in dendritic cells may well allow Salmonellae to exploit the mobility of these host cells to migrate away from the intestinal mucosa and establish systemic infection.

Virus-induced cytopathic effect

Many viruses damage the cells they infect, sometimes inducing visible and distinctive cytopathic effects (Fig. 1.21). Cytopathic effects may be mediated by either the presence of the virus or the host immune response. Poliovirus shuts off host cell protein translation through action of viral protease 2A resulting in cleavage of eIF4G needed for recognition of capped mRNA and cellular protein synthesis[55] and Coxsackie B virus protease 2A cleaves dystrophin in cardiac muscle, contributing to myocarditis.[56] In contrast, infection with hepatitis A and hepatitis C viruses produce very little direct killing of hepatocytes, with most liver damage mediated by the cytotoxic lymphocyte response.

Virus infection may also result in the intracellular accumulation or release of small molecules, such as reactive oxygen or nitric oxide, probably via effects on cellular signaling pathways or induction of innate immune responses. These may play important roles in cell destruction, particularly in macrophages. Rotavirus, CMV and HIV infection produce significant increases in intracellular calcium, a common pathway for the development of irreversible cell injury.

Viral fusion proteins mediate characteristic formation of multinucleated giant cells (syncytia). Examples include respiratory syncytial virus, parainfluenza viruses, measles virus, herpesviruses and some retroviruses. Viral infection can also produce eosinophilic or basophilic inclusion bodies in the cytoplasm or the nucleus. Inclusion bodies

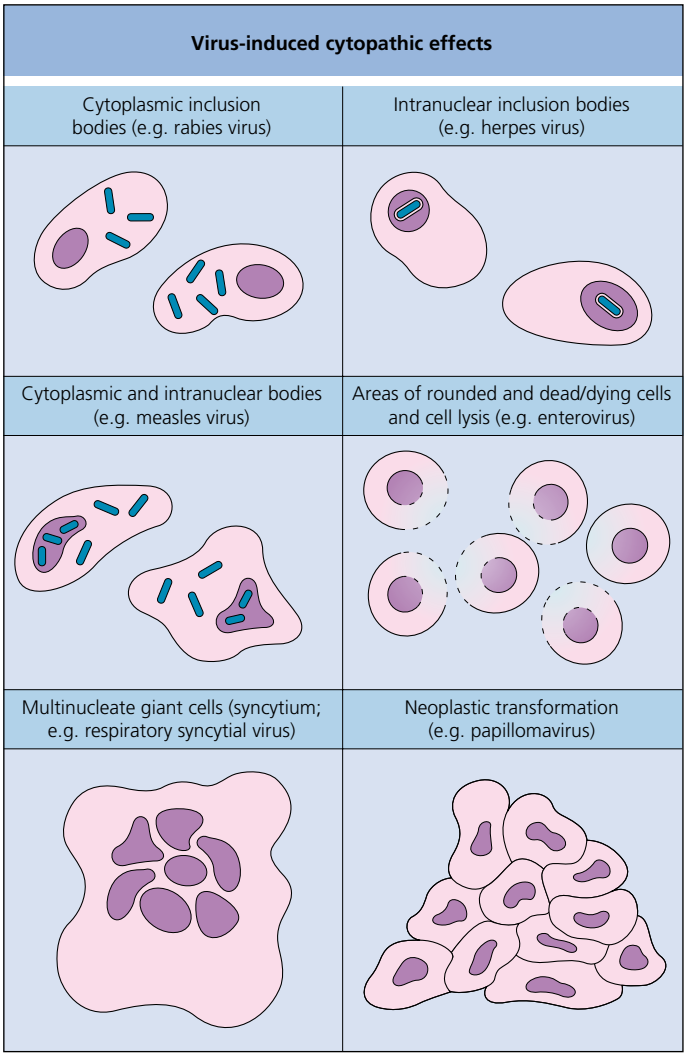


Fig. 1.21 Virus-induced cytopathic effects. (Courtesy of Menno Kok & Jean-Claude Pechere.)

represent aggregations of mature virions, sites of viral replication or assembly, or degenerative changes.

Infection and cancer

Infection can favor development of cancer by producing chronic inflammation, impairing immune surveillance and directly altering cell growth and death, for example:

- chronic *H. pylori* infection is associated with gastric adenocarcinoma and mucosa-associated lymphoid tissue (MALT) lymphoma;
- inflammation associated with *M. tuberculosis* infection may lead to adenocarcinoma of the lung; and
- *Schistosoma haematobium* infestation is associated with bladder cancer.

It is possible that different types of infection may act together in promoting neoplasia. For example, human papillomavirus (HPV) has been found to be present in all bladder tumors associated with schistosomiasis but only the minority without parasitic infestation. Expression of some viral genes (oncogenes) can drive cellular proliferation and impair apoptosis, producing disordered growth (transformation) that may lead to cancer. As examples, Burkitt's type lymphomas and craniopharyngioma are associated with EBV, cervical and anogenital carcinomas are associated with HPV, hepatitis B and C infection is associated with hepatocellular carcinoma, Kaposi's sarcoma-associated herpesvirus produces

Kaposi's sarcoma, and adult T-cell leukemia is caused by human T-cell lymphotropic virus type 1.[57]

In the case of HPV, persistent 'high-risk' infections (e.g. HPV16 and 18) may lead to the development of high levels of viral DNA, progressing to invasive carcinoma.[58] The HPV E2 protein regulates expression of E6 and E7 but is disrupted by viral integration, E5 acts as a viral analogue of the platelet-derived growth factor, E6 functions as a ubiquitin ligase to degrade the p53 anti-oncogene, and E7 binds to retinoblastoma protein (Rb) and cyclins A and E, allowing Rb phosphorylation and release of the E-2F, promoting G1 to S cell cycle transition.[59] With further chromosomal destabilization and mutations promoted by enhanced viral expression following DNA integration, the infected cells transform to a malignant phenotype.

Damage resulting from cytotoxic lymphocytes

The most effective host defense mechanism against most viral infections is mediated by the $CD8^+$ cytotoxic T lymphocytes (CTLs). The CTLs recognize, attack and lyse virus-infected cells that present viral antigens on their surface in the context of MHC class I molecules. In addition to CTLs, natural killer lymphocytes can kill virus-infected cells. The cytotoxic reaction contributes to the pathologic and clinical picture of many viral diseases. The characteristic measles rash is produced by infiltration of lymphocytes including CTLs attacking skin cells infected by the measles virus. This explains why children with defects in cell-mediated immunity do not develop a rash during measles infection. In this disease, rash indeed represents a good immune response by the host, whereas its absence may signal uncontrolled viral growth. As previously noted, hepatitis due to hepatitis A and C may be largely or entirely the consequence of immune attack rather than viral cytopathic effects. It is also believed that lymphocyte-induced cytotoxicity contributes to the pathology associated with persistent virus infections such as the subacute sclerosing panencephalitis caused by defective measles virus within the brain.

Harmful immune responses

The destructive potential of the immune system is considerable. It can damage the host in a variety of ways.

Autoimmunity

Autoimmune reactions break the rules of the 'self versus nonself' dichotomy. Autoimmune reactions, directed against 'self-proteins', may result from partial identity of antigenic determinants of the host and an infective agent or from alterations of self-components caused by infection. Acute rheumatic fever occurring after group A streptococcal pharyngitis has been associated with antibodies against antigens found in the cell wall of the streptococcus that also recognize components of the endocardium and the joint synovial membrane molecules and thus induce an autoimmune response. Mycobacterial infection may give rise to antibodies and T cells that are reactive to both the microbial (nonself) and the host (self) heat shock proteins.

Another example of molecular mimicry is the association between production of antiganglioside antibodies, the Miller-Fisher (MFS) variant of the Guillain–Barré syndrome (GBS), and prior *Campylobacter jejunii* infection. This illness is almost certainly due to cross-reacting antibodies against the sialated LPS of *C. jejunii*.

Hypersensitivity reactions

Hypersensitivity reactions occur if the host immune system seemingly overreacts to microbial infection. Hypersensitivity reactions have been classified by Gell and Coombs into four types.

Type I or immediate hypersensitivity

Type I hypersensitivity occurs within minutes of antigen exposure. It results from antigen binding to mast cell-associated IgE. Vasoactive amines are released and anaphylactic reactions may develop. Certain forms of rash after helminth infections seem to be due to this type of hypersensitivity.

Type II or cytotoxic hypersensitivity

Type II hypersensitivity is a consequence of the binding of specific antibodies to cell surface-associated antigens. Antibody binding mediates cytotoxicity via complement activation or natural killer cells. Thus cells bearing microbial antigens may be lysed via an antibody-dependent mechanism. Such a mechanism has been suggested to account for liver cell necrosis during hepatitis B infection.

Type III or immune complex-mediated hypersensitivity

Type III hypersensitivity is induced by classic complement activation, caused by extracellular antibody–antigen complexes. This causes inflammation and changes in vascular permeability and attracts neutrophils to tissues where the immune complexes are deposited, including the kidneys, joints and small vessels of the skin. Glomerulonephritis in malaria and subacute endocarditis are probably due to this mechanism.

Type IV or delayed-type hypersensitivity

Type IV hypersensitivity typically occurs at least 48 hours after exposure to an antigen. It involves activated T cells, which release cytokines, macrophages attracted by these cytokines, and cytotoxic $CD8^+$ T cells. Delayed-type hypersensitivity and granuloma play a major role in tissue damage observed during infections with slow-growing intracellular organisms, such as *M. tuberculosis* (tuberculosis), *M. leprae* (leprosy) and *Histoplasma capsulatum*. Many of the clinical manifestations of chlamydial disease, in particular trachoma, seem to result from a delayed-type hypersensitivity triggered by chlamydial heat shock proteins. In spite of the involvement of bacterial heat shock proteins, this is not an autoimmune phenomenon, because the unique rather than the conserved portions of these proteins seem to be implicated here.

Superantigens and bacterial components associated with toxic and septic shock

Toxic shock and septic shock are exceptionally impressive syndromes associated with a variety of infectious diseases. Severe hypotension, multiple organ failure and intravascular disseminated coagulopathy occur in the most severe cases. Pathogenesis of these syndromes is complex. Various bacterial components, including LPS, peptidoglycans, lipoteichoic acid and (in some cases) exotoxins acting as superantigens (see Table 1.7) trigger an intense, potentially lethal host response. Macrophages, neutrophils and/or T cells play important roles in the cascade of events leading to this condition (see Chapters 9 and 44) by releasing high levels of inflammatory response mediators, notably tumor necrosis factor and IL-1.

How micro-organisms escape host defense

In spite of the efficacy of host defense mechanisms, microbial pathogens can still infect humans and cause disease. This is in part due to the very potent weapons micro-organisms have (a single gram of crystalline botulinum toxin could potentially kill more than 1 million people) but it is also due to the intricate strategies that micro-organisms use to evade host defenses (Table 1.8).

Surviving the phagocyte and complement attack

Immediately after passage through the epithelial surface, the invading micro-organism encounters the most powerful actors of host defense: phagocytes. The two main types of phagocyte are PMNs and macrophages. These 'professional' phagocytes can bind micro-organisms

Table 1.8 Evasion of host defenses

Mechanism	Examples
Surviving the phagocyte and complement attack	
Inhibition of chemotaxis	C5a peptidase by *Streptococcus pyogenes*
Killing the phagocyte before ingestion	α-Toxin and Panton–Valentine leukocidin by *Staphylococcus aureus*
Avoiding ingestion	Bacterial capsules (e.g. *Streptococcus pneumoniae*) K (capsule) and O (LPS) antigens in Gram-negative rods
Avoid complement lysis	Coating with IgA antibodies (*Neisseria meningitidis*) Porin binding factor H and C4 binding protein (*N. gonorrhoeae*) M protein (*Streptococcus pyogenes*)
Surviving within phagocytes	Inhibition of phagolysosome fusion (*Chlamydia trachomatis*) Escape from phagolysosome (*Listeria monocytogenes*) Inhibit NADPH oxidase fusion with phagosome (*Salmonella typhimurium*) Inhibition of acidification of phagosome due to exclusion of the vacuolar H^+-ATPase (*Mycobacterium tuberculosis*)
Antigenic variations	Shift and drift in influenza A virus, pilin variation in *N. gonorrhoeae*
Tolerance	Prenatal infections
Immunosuppression	
Destroying lymphocytes	Depletion of $CD4^+$ cells by HIV
Proteolysis of antibodies	IgA protease by *Haemophilus influenzae*
Presence in inaccessible sites	Latent infection in dorsal root ganglia (herpes simplex virus)

with a variety of receptors, some of which specifically interact with bacterial surface structure or with antibodies or complement bound to the microbial surface (opsonized micro-organisms). The micro-organisms usually pass into the epithelial cell via phagocytosis or pinocytosis, although some (especially viruses) may enter the cytosol directly.

Bacteria invariably go through an endosomal stage, in which they will be exposed to a multitude of phagocyte defense mechanisms such as acidification, reactive oxygen species, bactericidal peptides, and hydrolytic enzymes released after phagosome–lysosome fusion. In addition, in the endosomal pathway, micro-organisms are deprived of the nutritional wealth of the cytosol. If the pathogens are killed and degraded their microbial antigens may be presented to lymphocytes. However, micro-organisms have developed strategies to avoid, mislead, deregulate or even profit from phagocytes.[60] Organisms like *Salmonella* require acidification of the phagosome and low Ca^{2+}/Mg^{2+} concentrations to trigger the PhoP/PhoQ transcriptional regulatory system that is required for survival inside macrophages. If acidification is prevented, *Salmonella* are killed by macrophages. In contrast, *M. tuberculosis* prevents acidification of the phagosome to varying degrees, and those micro-organisms that reside inside less acidic phagosomes remain metabolically active.

Inhibition of the mobilization of phagocytes

Extracellular micro-organisms can avoid phagocytes by inhibiting chemotaxis or complement activation (see below). A bacterial enzyme that degrades complement protein C5a, a main chemoattractant for phagocytes, has been discovered in *Strep. pyogenes* and *agalactiae*. Pertussis toxin catalyzes ADP ribosylation in neutrophils, which causes a rise in intracellular cAMP levels that ultimately impairs chemotaxis. *Yersinia pestis* employs several secreted enzymes (YOPS) to subvert macrophage phagocytosis and thus remain extracellular.

Killing the phagocytes before being ingested

Many soluble products excreted by bacteria are potentially toxic for phagocytes entering the foci of infection. Streptolysin-O binds to cholesterol in cell membranes, which results in rapid lysis of PMNs. In the process, the lysosomes are also disrupted and release their toxic contents, which may have deleterious effects on the neighboring cells. Other examples of toxins that are directed against phagocytes include the γ toxin of *Clostridium perfringens*, α toxin and Panton–Valentine leukotoxin made by *Staph. aureus*; the latter specifically targets myeloid cells and has species specificity. Many so-called extraintestinal pathogenic *E. coli* are hemolytic because they produce an RTX membrane toxin that damages PMNs, impairing their function in several ways, depending on the local concentration of the toxin. Several toxins from *Clostridium perfringens* produce similar effects. Indeed, pus sampled from gas gangrene may contain numerous Gram-positive rods without any visible PMNs.

'Professional' phagocytes as vectors or refuges

Legionella pneumophila provokes entry in mononuclear phagocytes by accumulating complement factor C3bi on the envelope of the organism. This complement factor is a ligand for the phagocyte receptor CR3, and enhances phagocytosis. Following uptake, *Legionella* remains in the phagosomes, which do not fuse with lysosomes and thus provide protection. Alveolar macrophages are host cells for *M. tuberculosis*.[61] *Salmonella enterica* are ingested by intraepithelial dendritic cells and carried into regional lymph nodes and the systemic circulation by those cells. Many viruses (HIV, dengue virus, measles, etc.) infect and replicate in monocyte macrophages. Infected monocytes may provide HIV with a route through the blood–brain barrier into the CNS[62] and dendritic cells loaded with infectious HIV may activate and infect T cells[63] (see Chapters 120 & 121). *Ehrlichia*, which are small, Gram-negative, obligatory intracellular bacteria, directly infect the cytoplasm of granulocytes or macrophages, depending on the bacterial species. All organisms that have adapted to live inside phagocytic cells have developed mechanisms to escape, disarm or survive the onslaught of antimicrobial factors.

Avoiding ingestion

The surface of numerous pathogenic bacteria is covered with a loose network of polymers, which constitutes the bacterial capsule.[64] Capsular material may be very thin, visible only by electron microscopy, as is the case with the hyaluronate capsule of *Strep. pyogenes*. In some species (*Strep. pneumoniae, Klebsiella pneumoniae*) capsule material is abundant, easily visible with a light microscope and responsible for a mucoid aspect of the bacterial colonies. Most of the capsules are composed of polysaccharides, others are made of proteins or a combination of carbohydrate and protein. Some capsule contents mimic host polysaccharides and are thus recognized as 'self' by the host immune system. Examples are the capsules of *N. meningitidis*, which contain sialic acid, and *Strep. pyogenes*, which contain hyaluronic acid. Proteins that envelop bacteria in S-layers can serve the same function as polysaccharides, as in the cases of *Campylobacter fetus* and *Bacillus anthracis*.

Capsules may protect bacteria from complement activation.[65] As a result, encapsulated bacteria are not immediately recognized as invaders by the phagocytes. Capsulated *Strep. pneumoniae* resist engulfment by macrophages and PMNs and are virulent; however, noncapsulated strains are easily phagocytosed and are avirulent.[66]

Meningococci circulating in the blood are coated with IgA, which is not an activator of the complement cascade. *Schistosoma mansoni* incorporates decay accelerating factors in its membrane; these are host plasma proteins that inhibit deposition of C3 onto host cell membranes. Activation of complement in the blood is thus avoided by the parasite.

Matrix (M) proteins, which form fibrillae (see Fig. 1.11), are considered to be the primary virulence determinants of *Strep. pyogenes*. Matrix protein renders the bacteria resistant to phagocytosis by human neutrophils. Matrix fibrillae are approximately 50–60 nm in length and exhibit a seven-residue periodicity. They exist as stable dimers, arranged in an α-helical, coiled coil configuration, with the carboxyl terminal portion closely associated with the cell wall (see Fig. 1.11). Streptococci that express M proteins on their surface are poorly opsonized by the alternative pathway and resist PMN phagocytosis. In contrast, streptococci that fail to express M protein are readily opsonized and phagocytosed, and are avirulent. Resistance to phagocytosis can be overcome by antibodies directed against type-specific M epitopes.

The mechanism of antiphagocytic activity of M proteins is still unclear. According to one hypothesis, fibrinogen, known to bind to M protein, may hinder access to complement-binding sites on the bacterial surface, disguising the pathogen as 'self'. In another hypothesis, a complement control protein (protein H), which also binds M, may be responsible for the observed complement resistance of virulent *Strep. pyogenes*.

Survival within phagocytes

Once ingested by the phagocyte, the pathogen may survive and grow using a variety of strategies (Fig. 1.22). Some microbes prevent exposure to hydrolytic enzymes by inhibiting fusion of the phagosome and the lysosome, others survive within the phagolysosome because they resist enzymatic degradation or neutralize toxic products to which they are exposed in this compartment. Some bacterial pathogens (such as Salmonellae discussed above) extensively modify endosomes into customized survival vesicles. Certain types of bacteria rapidly escape from the phagolysosome and propagate in the cytoplasm, as described above for *Listeria monocytogenes*. Recent studies suggest that intracellular pathogens, notably *M. tuberculosis*, may inhibit the early host response at the level of host gene expression.

Inhibition of phagolysosomal fusion

Salmonella spp. have developed several strategies to survive and propagate in macrophages; *Salmonella* spp. that lack this capacity to survive in macrophages are avirulent. Several hours after infection *in vitro*, two distinct *Salmonella* populations can be seen in the macrophage. One consists of rapidly dividing bacteria located in large unfused phagosomes. This population may grow rapidly and kill the macrophage, leading to the liberation of intracellular bacteria.[67] *In vivo*, this population may be responsible for the acute stage of salmonellosis.

The second population of *Salmonella* consists of nondividing organisms located in phagolysosomes. This population resists the toxic effect of lysosomal products and is believed to account for the prolonged survival of *Salmonella* spp. in the body. Long-living stromal macrophages of the bone marrow may act as long-term reservoirs and be responsible for the relapses of salmonellosis that are seen in immunosuppressed patients with AIDS. The dormant phase represents a well-regulated physiologic condition associated with nutrient deprivation *in vitro*.

Inactivation of reactive oxygen species

Reactive oxygen species damage DNA and inhibit bacterial oxidative phosphorylation. Bacteria may escape from the damaging effect of reactive oxygen species by rapid detoxification of the bactericidal products and by efficient DNA repair. Several bacterial pathogens produce superoxide dismutase (SOD) and catalase, two enzymes that might eliminate the reactive oxygen species and damage to DNA may be efficiently repaired through a RecA-dependent pathway. In Salmonellae the RecA pathway seems to be more important, as *rec*A mutants are avirulent. However, enzymes that inactivate oxygen radicals are also virulence factors.

A SOD encoded by a bacteriophage that is present in many virulent *Salmonella enterica* is expressed under the control of the sigma factor rpoS when the bacteria are inside phagocytes. In contrast, expression of the chromosomally encoded SOD that is in all strains of *Salmonella enterica* is controlled by another sigma factor and expression is repressed in intracellular bacteria. However, the ability of this bacterial species to modify the endocytic pathway of the host cell seems to be the most important mechanism of resistance to reactive oxygen species. In macrophages, virulent Salmonellae localize in phagosomes devoid of NADPH oxidase, the enzyme that drives the respiratory burst.[68]

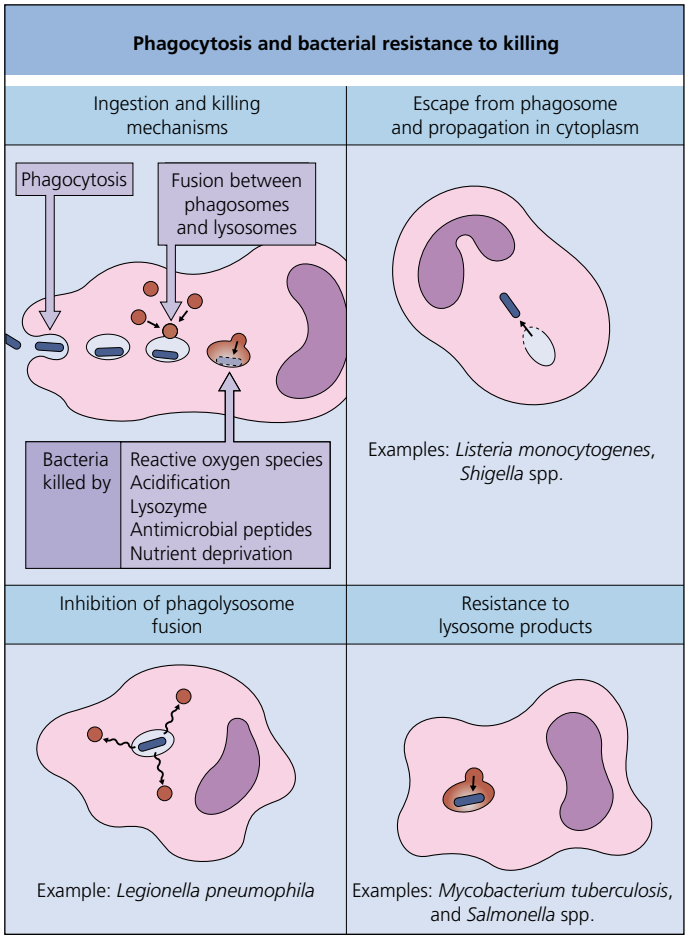


Fig. 1.22 Phagocytosis and bacterial resistance to killing. (Courtesy of Menno Kok & Jean-Claude Pechere.)

Resistance to antimicrobial peptides

Several cationic peptides are produced within the lysosomal granules and are believed to kill intracellular pathogens by forming channels in the bacterial cell wall. *Salmonella* spp. resist these antimicrobial peptides by at least two complementary mechanisms, one of which, encoded by the *sap* locus, is characterized in some detail (Fig. 1.23). It seems that the SapA protein forms a complex with the antimicrobial peptides, reducing the deleterious effect on the bacterial membranes.

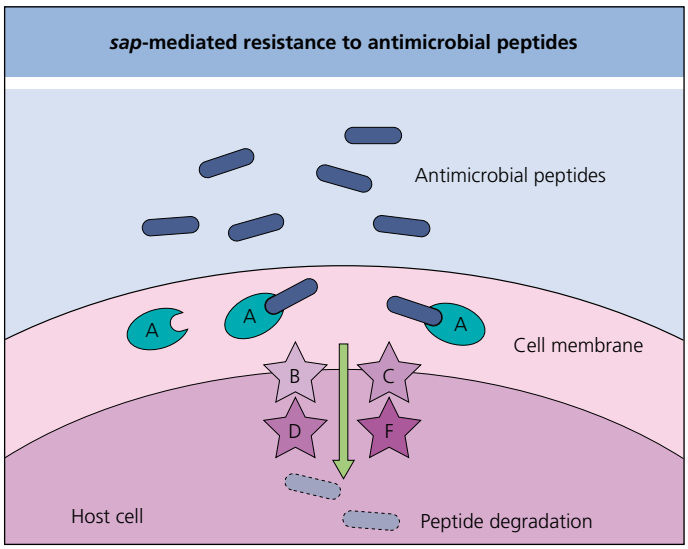


Fig. 1.23 Mechanism of resistance to macrophage antimicrobial peptides by *Salmonella* spp. *Salmonella* produces the SapA (A) peptide, which complexes with host cell antimicrobial peptides. Other proteins encoded by the *sap* locus (SapB, SapC and SapD) are required for the transport of the SapA-antimicrobial peptide complex into the cytosol where the antimicrobial peptide is degraded. (Courtesy of Menno Kok & Jean-Claude Pechere.)

Other proteins encoded by the *sap* locus (SapB, SapC and SapD) allow the transport of the SapA–peptide complex into the cytosol. Within the cytosol, peptidases degrade the antimicrobial peptides. Recently it was shown that pili make group B streptococci resistant to Mouse CRAMP and the human homologue LL-37.

Antigenic and phase variations

A powerful survival strategy for a pathogen would be to mislead the specific host immune response by 'changing appearances'. Three examples of molecular mechanisms used to achieve antigenic variation, one each by a bacterium, a virus and a protozoan, are illustrated below (Table 1.9).

Table 1.9 Examples of antigenic variations

Genetic mechanisms	Examples
Recombination between different copies of pilin genes	Pili in *Neisseria gonorrhoeae*
Phase variation – turning expression of an antigen on or off ('flip-flop')	Flagella in *Salmonella*; pili in *N. gonorrhoeae*
Gene reassortment between two strains infecting the same cell	Influenza virus type A
Mutation of surface antigens	Influenza virus type A, B and C, deletion of flagella in *Shigella* spp. (avoids TLR5 signaling)
Gene switch leading to surface glycoprotein changes	*Trypanosoma brucei*
Gene switch leading to production of nonactivating lipopolysaccharides	*Yersinia pestis* growing at 37°C; *Salmonella enterica* growing inside phagosome (PhoP regulated)

Antigenic variation in *Neisseria gonorrhoeae*

Neisseria gonorrhoeae varies the composition of at least three major components of its outer membrane: the pili, which mediate the initial attachment to host cells; the membrane protein P.II, responsible for closer attachment resulting in phagocytosis; and LPS, described earlier.

Antigenic variations in the major pilin subunit are essentially due to recombination between different copies of *pil* genes scattered over the chromosome (Fig. 1.24). Only one or two of these are expressed (*pil*E, where E denotes 'expressed') at any point in time, but an array of antigenically distinct pili may be produced in response to an antibody challenge. In addition to this mechanism, pili are subject to phase variation (i.e. switches between *pil*-positive and *pil*-negative variants). Phase variation is controlled at the transcriptional level.

The P.II protein is similarly subject to genetic variation. As a consequence, the specific immune response never quite catches up with genetic variation in the bacterial population. The combination of this mechanism, LPS sialylation (see above) and IgA protease production, explains the apparent lack of acquired immunity to gonorrhea and makes vaccine development very difficult.

Shift and drift in influenza A viruses

Every year, during seasonal influenza, vaccination programs are confronted with the problem of antigenic variation. Influenza viruses change through drift and shift. Antigenic drift refers to the gradual accumulation of point mutations during annual circulation of influenza as a consequence of the high error rates associated with RNA-dependent RNA polymerase during virus replication. Influenza A virus mutants with antigenic changes tend to have a selective advantage over the nonmutant viral population. The rapidity with which drift can produce change is illustrated by the dramatic increase in amantadine resistance (from 2–12% to >91%) in influenza A/H3N2 strains, associated with a single mutation at position 31 of the M2 protein from 2005 to 2006. As a consequence of antigenic drift, the composition of the influenza vaccine must be evaluated very carefully and updated on an annual basis in order to offer coverage for the strains likely to be circulating at the time.

Antigenic shift refers to the emergence of a novel influenza virus in humans, due to direct introduction of an avian strain or to a new strain produced by recombination and reassortment of two different influenza viruses. Antigenic shift results in dramatic changes in the antigenic composition of the surface hemagglutinin (which binds the host cell receptor) or the neuraminidase (which modifies these receptors) and can cause devastating worldwide epidemics, or pandemics, in the immunologically unprepared population. Recent influenza A pandemics occurred in 1957 (the H2N2'Asian Flu') and 1968 (the H3N2'Hong Kong Flu'). An outbreak of avian influenza from exposure to infected poultry in Hong Kong in 1997 caused 18 human deaths. A genetically different strain of A/H5N1 circulated in domestic birds throughout Asia, causing 387 cases and 245 deaths between 2003 and 2008, raising concerns that a new pandemic might arise. Instead, in 2009, an unanticipated, novel H1N1 reassortant influenza A virus with origins in circulating seasonal influenza, avian, and both classic and Eurasian swine strains emerged to cause the latest worldwide influenza A pandemic.[70]

Antigenic variations in *Trypanosoma brucei*

African trypanosomes (*Trypanosoma brucei*) are flagellated protozoa, transmitted to humans by several species of *Glossina* (tsetse). The parasite survives in mammalian body fluids thanks to antigenic variation of the variant surface glycoprotein (VSG), which forms a 15 nm thick monolayer covering most of the parasite surface.[69] Within a single generation, most or all of the 10^7 VSG molecules may be replaced by an unrelated species, stemming from a repertoire of an estimated 1000 genomic copies of the gene. The VSG gene is invariably expressed from a polycistronic transcription unit, in the

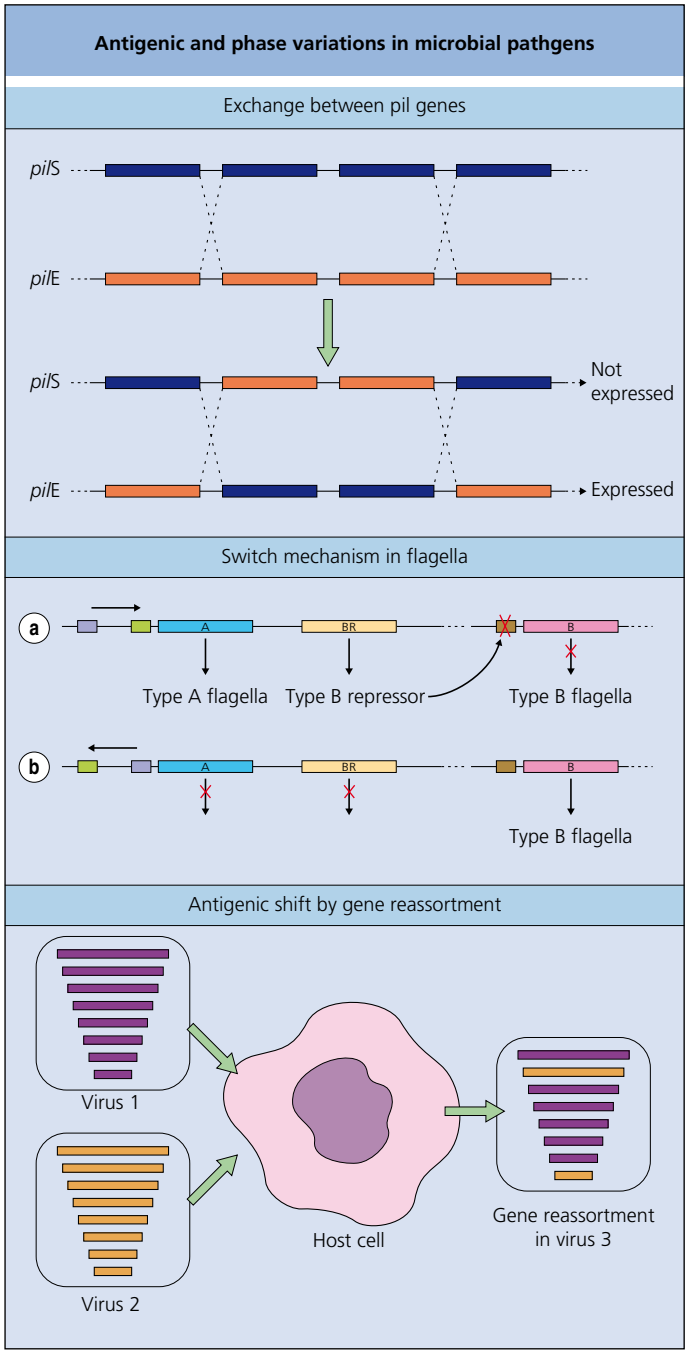


Fig. 1.24 Antigenic and phase variations in microbial pathogens. Three mechanisms are shown. (Top) Exchange of DNA between nonexpressed copies of *pil*S and the expressed gene *pil*E in *Neisseria gonorrhoeae* can change the expressed antigen. (Middle) A switch mechanism is responsible for the (mutually exclusive) production of type A and type B flagella in *Salmonella typhimurium*. Phase variation depends on the orientation of a DNA fragment adjacent to the type A flagella gene. When A is expressed (a) from the promoter in the invertable fragment, the repressor for the type B flagella is expressed at the same time. As a consequence the type B flagella gene is repressed. Inversion of the DNA fragment abolishes expression of the A-repressor gene and the B-repressor gene (b). In this situation type B flagella are produced. (Bottom) Antigenic shift by gene reassortment results from infection of a single cell by two different virions. (Courtesy of Menno Kok & Jean-Claude Pechere.)

so-called telomeric expression site adjacent to the telomeric repeats. During chronic infection, patients experience successive episodes of parasitemia, each episode coinciding with the expression of a new VSG on the surface of the parasite. With this strategy, trypanosomes avoid complete eradication by the specific immune response, while maintaining the pathogenic burden at sublethal levels. The closely related *T. brucei brucei*, which causes the bovine disease nagana, does not spread to humans because it is sensitive to high-density lipoprotein in human serum.

IMMUNOSUPPRESSION

The most illustrative example of immunosuppression induced by microbial infection is provided by HIV. Human immunodeficiency virus circulating in the bloodstream readily infects $CD4^+$ lymphocytes, macrophages and dendritic cells. The destruction of $CD4^+$ T-helper cells is particularly detrimental to the host and accounts for the emergence of a variety of opportunistic infections as soon as the T-cell count drops below a critical level. In addition to its general immunosuppressive effects, HIV-1 preferentially infects HIV-1 specific $CD4^+$ T cells, thereby undermining the ability of the host to mount an effective immune response to the virus itself.[71] It has recently been shown that HIV infection rapidly causes a profound depletion of $CD4^+$ T cells in the gut as well as the lymph nodes and peripheral blood. This local immunosuppression may in turn be instrumental in producing continued T-cell activation due to increased bacterial translocation through the damaged mucosal barrier, increasing susceptibility to additional HIV infection.[72]

Other viruses may produce immunosuppression in a more subtle fashion. Measles virus infects macrophages and both B and T lymphocytes, interfering with the immunocompetence of the host for weeks after resolution of clinical disease. As a consequence, in areas with a high prevalence of tuberculosis, measles epidemics may be followed by outbreaks of tuberculosis. Gonococci, meningococci and *Haemophilus influenzae* produce proteases that hydrolyze secretory IgA1 antibodies. Protease-negative mutants of these bacterial strains are less virulent, suggesting a role for mucosal IgA1 antibodies in host defense against these pathogens.

CONCLUSION

Throughout evolution, humans, like all mammalian species, have maintained an intimate relationship with the microbial world. We have survived thanks to the efficient defense mechanisms we have developed against potentially dangerous micro-organisms. Pathogenic micro-organisms are still here because they have found ways of avoiding elimination by their host or by the microbial competition. 'Successful' pathogens have developed strategies to enter the body and reach and colonize their favorite niche, while defying the powerful human immune system.

In this chapter we have looked into microbial survival strategies. Although some of these have been analyzed in 'molecular detail', a lot remains to be discovered. Future remedies for infectious diseases are likely to be aimed at specific molecular interactions between the pathogenic micro-organism and its host.

REFERENCES

References for this chapter can be found online at http://www.expertconsult.com

Chapter 2

Robert Schooley
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Host responses to infection

OVERVIEW

The host response to potential microbial pathogens is complex and largely successful in preventing microbial invasion and disease. However, a number of organisms have evolved means of evading host immune mechanisms. This interplay between pathogen and host immune response results in a delicate balance, the weight of which can shift in favor of the pathogen in the setting of acquired immune deficiency states associated with immunosuppressive drugs, human immunodeficiency virus type 1 (HIV-1) infection and pregnancy. This chapter will broadly address the mechanisms that humans have developed to prevent microbial invasion as well as those that aim to rid the body of invading pathogens in order to prevent established infection. Lastly, we will address the consequences to the host of a strong inflammatory response, a form of 'friendly fire' in the body's war against infection.

The immune response can be viewed as consisting of two distinct but interconnecting branches, termed innate and adaptive immunity, that differ in their manner and rapidity of response to pathogens (Fig. 2.1). The innate response, the oldest mechanism of host defense, serves as the first line of response against invading microbes. The innate system is primarily mediated by cells that serve a barrier function, such as intestinal epithelium and skin, and phagocytes

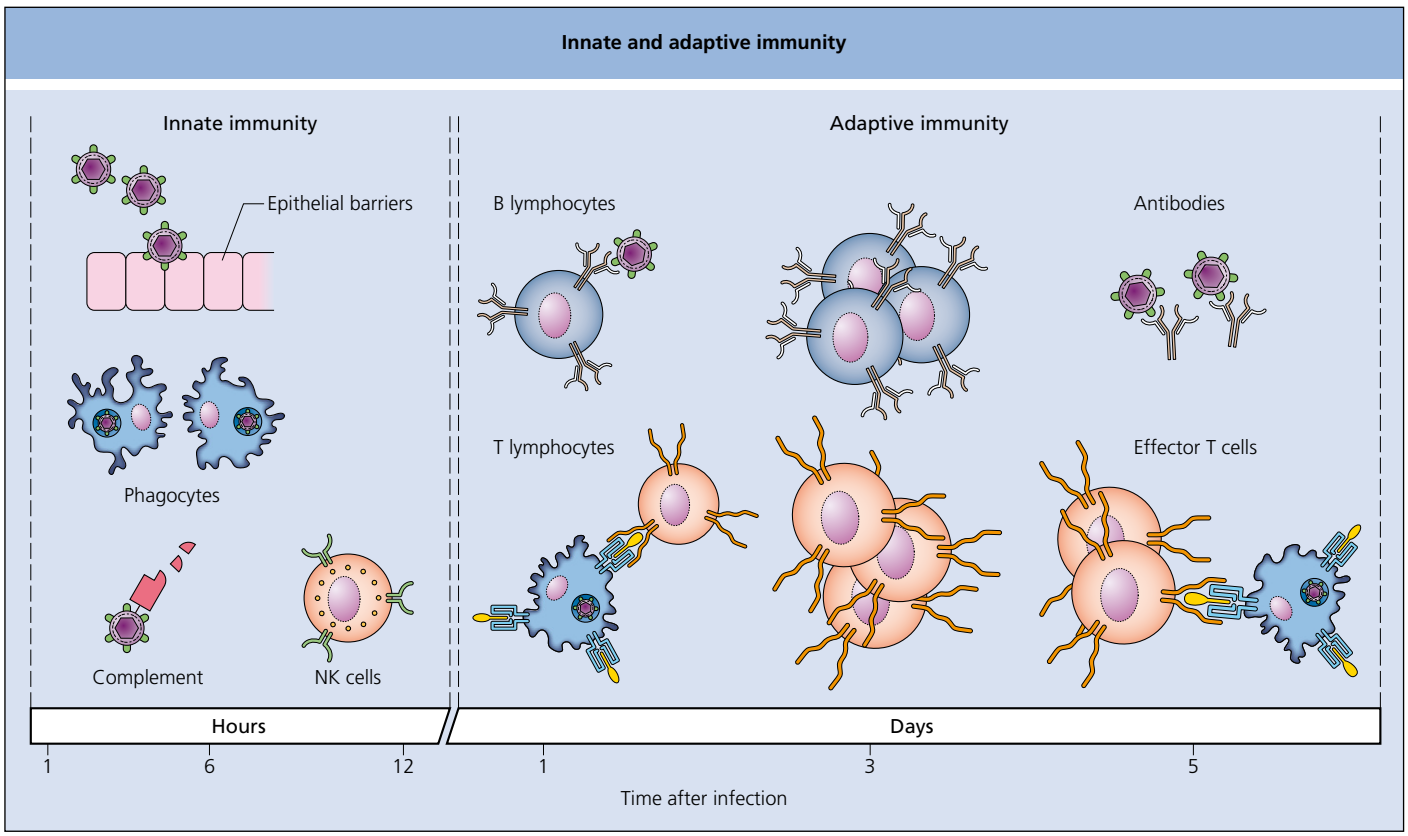


Fig. 2.1 Innate and adaptive immunity. The mechanisms of innate immunity provide the initial defense against infections. Adaptive immune responses develop later and consist of activation of lymphocytes. The kinetics of the innate and adaptive immune responses are approximations and may vary in different infections. From Abbas & Lichtman.[1]

Table 2.1 Innate versus adaptive immunity

	Innate	Adaptive
Components		
Barriers	Mucosal epithelia, skin, antimicrobial peptides	Antibodies secreted at epithelial surfaces, lymphocytes in epithelia
Cells	Phagocytes (neutrophils, macrophages, dendritic cells), natural killer cells	Lymphocytes, antigen-presenting cells (dendritic cells)
Blood proteins	Complement	Antibodies
Antigen receptors	Pattern recognition receptors	T-cell receptors, membrane immunoglobulin
Characteristics		
Specificity	For microbial-associated molecular patterns shared by microbes	For unique linear and conformational determinants (epitopes) of microbial antigens
Diversity	Limited, germline-encoded	Diverse, receptors produced by somatic recombination
Memory	No	Yes

From Abbas & Lichtman.[1] Copyright Elsevier, 2003.

that are recruited early to sites of pathogen invasion. Recognition of pathogens by innate immune cells is mediated by a limited number of germline-encoded pathogen recognition receptors (PRRs) that recognize conserved microbial components called microbial-associated molecular patterns (MAMPs). Conversely, cells of the acquired immune system respond to pathogens at later stages of infection and recognize microbial antigens using highly specific receptors that require gene rearrangement and clonal expansion. Furthermore, acquired immunity is associated with the development of immunologic memory (Table 2.1).

INNATE IMMUNITY

The innate immune response serves several functions in the early response to pathogenic microbes. The first is a barrier function to prevent organisms from invading the body. The second major function is an effector mechanism designed to eliminate microbes that breach the epithelial barriers through recognition, ingestion and killing by phagocytes. A third function is to alert the adaptive immune response to the presence of the pathogen, stimulate the appropriate adaptive effector cells and influence the nature of the adaptive immune response generated based on the nature of the pathogen.

Barrier functions of the innate immune response

Epithelial barriers form the first line of defense against the microbial world surrounding all multicellular organisms. The skin and mucosal surfaces lining the gastrointestinal, respiratory and genitourinary tracts provide both a physical barrier to invasion and also play a critical role in serving as the first geographic location at which interactions between pathogens and host occur.[2] The critical contribution of an intact skin to the prevention of infection is clearly demonstrated by the direct relationship between percent of body surface area disrupted in burn injuries and mortality associated with the event. Disruption of the integrity of the gastrointestinal tract by cytotoxic chemotherapy compounds the risk of enteric pathogen bacteremia during periods of neutropenia during cancer chemotherapy. In the case of mucosal surfaces, additional physical protection is provided by the secretion of mucus and immunoglobulins in which bacteria and other pathogens are aggregated for clearance by cilia, coughing and/or peristalsis depending on the specific mucosal surface (Fig. 2.2).

In addition to the role played by the skin and mucosal surfaces in providing physical separation and clearance, both organs are heavily fortified with both innate and adaptive effector immune mechanisms. Antimicrobial peptides such as defensins, cathelicidins, lysozyme and lactoferrins provide one of the first lines of immunologic defense and are present at integumentary surfaces on a chronic basis. Immunoglobulins are both secreted into luminal cavities and affixed to the surface of cells lining these surfaces. Finally, cellular defense mechanisms abound in and around integumentary surfaces as locations where antigens are processed and presented to the immune response for amplification and recruitment, where pathogens are physically destroyed by effector cells and where they are actively captured and transported to local lymphoid structures for both secondary processing and destruction. These integrated immunologic functions of cutaneous and mucosal surfaces play critical roles in determining the events that follow any encounter with a potential pathogen.

Recognition and effector functions of the innate immune response

Innate immune recognition by pattern recognition receptors

As noted above, PRRs recognize MAMPs, and many of these microbial-derived products are shared by both commensal organisms as well as pathogens (termed pathogen-associated molecular patterns, PAMPs).[3]

Microbial-associated molecular patterns may be derived from a variety of organisms, including bacteria, fungi, parasites and viruses. These microbial products tend to be highly conserved, are essential to micro-organism survival and may contain carbohydrate, lipid, protein or nucleic acid components. Examples of MAMPs include lipopolysaccharide (LPS) from Gram-negative bacteria, peptidoglycan from Gram-positive bacteria, flagellin from flagellated bacteria, fungal mannans, bacterial CpG DNA, and viral DNA or RNA, to name just a few.

Although PRRs do discriminate between self and nonself, certain PRRs have been reported to recognize host self-antigens, such as heat-shock proteins and fibrinogen.[4,5] Each PRR recognizes a specific MAMP or set of related MAMPs, and their binding results in activation of specific signaling pathways that lead to distinct response patterns. As a result of the conserved nature of MAMPs, a given PRR may recognize multiple organisms that share PAMPs. In turn, a given organism may express multiple MAMPs and thus stimulate multiple PRRs. This specificity, combined with redundancy, ensures an appropriate pathogen-tailored innate, and subsequent adaptive, immune response against microbial invasion. The three major classes of PRRs that will be covered in this chapter include Toll-like receptors (TLRs), Nod-like receptors (NLRs) and C-type lectin receptors (CLRs).

Toll-like receptors

Toll-like receptors, originally identified in the antifungal response of *Drosophila*, are a family of transmembrane PRRs that are evolutionarily conserved from worms to mammals.[6,7] Thirteen mammalian TLR family members, including 10 in humans, have been identified to date, and ligand recognition is mediated by TLR homodimers or, in some cases, heterodimers.[8,9] Toll-like receptors have been further divided into subfamilies based on sequence similarities and recognition of related MAMPs. For instance, TLRs 7, 8 and 9 recognize nucleic

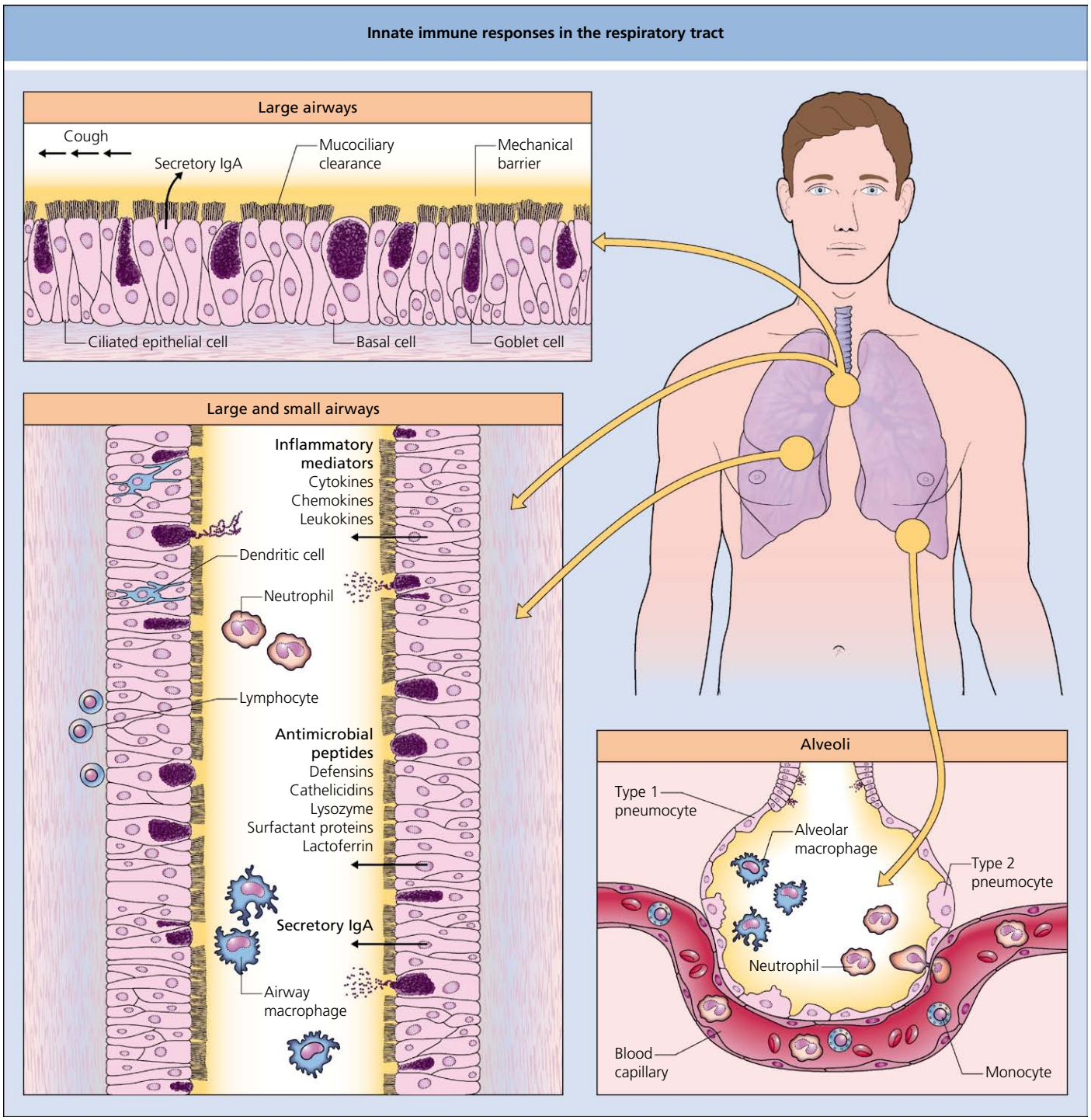


Fig. 2.2 Innate immune responses in the respiratory tract. From Sethi & Murphy.[2] Copyright 2008, Massachusetts Medical Society.

acids, whereas TLRs 1, 2 and 6 recognize lipids. Alternatively, TLR4 recognizes a diverse array of structurally dissimilar ligands.[3]

Toll-like receptors are expressed on both immune and nonimmune cells. On immune cells, TLRs are found on a number of phagocytic and antigen-presenting cells, such as dendritic cells (DCs), macrophages/monocytes and B cells. Toll-like receptors are also reported to be expressed on some subsets of T lymphocytes.[10,11] Among nonimmune cells, TLR expression has been reported on epithelial cells and fibroblasts. Interestingly, TLR expression on intestinal epithelial cells may play an important role in regulating intestinal inflammation.[12] Toll-like receptors may also have differential expression within a cell. For instance, TLRs 1, 2, 4, 5 and 6 are expressed on the cell surface, whereas TLRs 3, 7, 8 and 9 are expressed intracellularly in compartments such as endosomes. Ligands for intracellular TLRs require internalization before signaling can take place.[3,13]

As noted above, TLRs recognize components of multiple organisms, including Gram-positive and Gram-negative bacteria, mycobacteria, fungi, protozoan parasites and a number of both RNA and DNA viruses (Table 2.2). A number of unique cell wall components of bacteria serve as PAMPs and are recognized by TLRs. Lipopolysaccharide (endotoxin) is a component of enteric Gram-negative bacterial cell walls that binds to CD14, then is able to associate with TLR4 to induce

Table 2.2 Toll-like receptor (TLR) recognition of microbial components

Microbial components	Species	TLR usage
Bacteria		
Lipopolysaccharides	Gram-negative bacteria	TLR4
Diacyl lipopeptides	*Mycoplasma*	TLR6/TLR2
Triacyl lipopeptides	Bacteria and mycobacteria	TLR1/TLR2
Lipotechoic acid	Group B *Streptococcus*	TLR6/TLR2
Peptidoglycans	Gram-positive bacteria	TLR2
Porins	*Neisseria*	TLR2
Lipoarabinomannan	Mycobacteria	TLR2
Flagellin	Flagellated bacteria	TLR5
CpG DNA	Bacteria and mycobacteria	TLR9
Not detected	Uropathogenic bacteria	TLR11
Fungi		
Zymosan	*Saccharomyces cerevisiae*	TLR6/TLR2
Phospholipomannan	*Candida albicans*	TLR2
Mannan	*Candida albicans*	TLR4
Glucuronoxylomannan	*Cryptococcus neoformans*	TLR2 and TLR4
Parasites		
tGPI-mutin	*Trypanosoma*	TLR2
Glycoinositol phospholipids	*Trypanosoma*	TLR4
Hemozoin	*Plasmodium*	TLR9
Profilin-like molecule	*Toxoplasma gondii*	TLR11
Viruses		
DNA	Viruses	TLR9
dsRNA	Viruses	TLR3
ssRNA	RNA viruses	TLR7 and TLR8
Envelope proteins	RSV, MMTV	TLR4
Hemagglutinin protein	Measles virus	TLR2
Not detected	HCMV, HSV1	TLR2
Host		
Heat-shock protein 60, 70		TLR4
Fibrinogen		TLR4

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signaling. Lipopolysaccharide preparations from some nonenteric bacteria have been reported to serve as TLR2 agonists.[14] Components of Gram-positive bacteria, such as lipoteichoic acid (LTA) and peptidoglycan (PG) serve as immune activators primarily by binding to TLR2. Flagellin, the major component of flagella used by many microbes for motility, is a potent activator of TLR5. Flagellins from some pathogenic bacteria have been shown to escape this important host defense mechanism.[15]

Innate recognition of mycobacterial bacteria by TLRs is also an important aspect of the host response to these intracellular organisms. Mycobacterial cell wall products have been shown to stimulate TLR2, TLR4 and TLR2/1 heterodimers.[16,17] In addition to proteins, lipoproteins and lipopolysaccharides, bacterial DNA also may induce an innate immune response. This effect is mediated by binding of unmethylated CpG dinucleotides in genomic bacterial DNA (CpG DNA) to TLR9, a TLR that resides intracellularly in endosomes.

TLRs are also critical components of the host response to fungi, protozoan parasites and viruses. Several cell wall components of fungi are recognized by TLRs 2 and 4, and, interestingly, different morphologic forms of a fungus (e.g. hyphae versus conidia) may preferentially stimulate one TLR over another.[18] As will be further discussed below, TLRs function in collaboration with other PRRs, in particular CLRs, to ward of pathogenic fungal infections. Components of protozoan parasites, including those of *Trypanosoma*, *Plasmodium*, *Toxoplasma* and *Leishmania* spp., have been shown to activate innate immune responses through stimulation of TLR2, TLR4 and TLR9.[19]

Moreover, nucleotide sequences from numerous RNA and DNA viruses are recognized by intracellular TLRs, in particular TLR7/8, TLR9 and TLR3.

- Recognition of viral PAMPs by TLR7/8 appears to play a role in several viral infections. TLR7 and TLR8 show a great deal of sequence homology, are expressed in endosomal membranes and are capable of recognizing viral ssRNA.[20] There is some published evidence to suggest that recognition of HIV-1 RNA sequences by TLR7/8 expressed on APCs such as DCs may be in part responsible for the generalized immune activation that characterizes HIV-1 disease and is associated with disease progression.[21–23]
- TLR9 plays an important role in innate host recognition of DNA viruses such as cytomegalovirus antigen-presenting cells and herpes viruses, as these viruses contain genomes rich in CpG-DNA sequences. TLR9 is expressed on a subpopulation of blood DCs and plasmacytoid DCs (pDCs) that are capable of secreting high levels of type I interferons (IFN, an antiviral cytokine) in response to TLR9 ligation and play a major role in the innate antiviral response to such pathogens.[24]
- TLR3 recognizes dsRNA that can be generated as a replication intermediate or by-product during infections with ssRNA or DNA viruses.[25] TLR3 is expressed in conventional DCs but also in a variety of epithelial cells and in astrocytes in the brain. Despite its wide expression, the role of TLR3 in antiviral immunity remains to be clarified, especially since TLR3 knockout mice do not appear to have increased susceptibility to viral infections.[26]

Finally, envelope proteins from several viruses may bind to and activate extracellular TLRs, such as TLR2 and 4, and this activation generally results in the production of inflammatory cytokines but not antiviral IFNs. This innate cytokine response may be in part responsible for the inflammatory complications associated with certain viral infections.[27]

Upon ligand recognition by TLRs, signaling cascades mediated through intracellular adaptor proteins such as myeloid differentiation factor 88 (MyD88) are induced in a similar manner to that seen with interleukin (IL)-1R signaling. Signaling ultimately results in the activation of transcription factors (such as nuclear factor kappa B, NF-κB), enhanced gene expression and the production of inflammatory cytokines and chemokines.[28] The topic of TLR signaling is too complex to be adequately covered in this chapter and a comprehensive review can be found in West *et al.*[9]

The differences in response patterns mediated by different TLR ligands may be in part explained by differential recruitment of adaptor molecules, although multiple factors are involved, including responder cell type and additional signals received. For instance, distinct adaptor molecules mediate the specific signaling pathways that result in the production of type I IFNs versus inflammatory cytokines (Fig. 2.3). As will be discussed further below, the early innate response

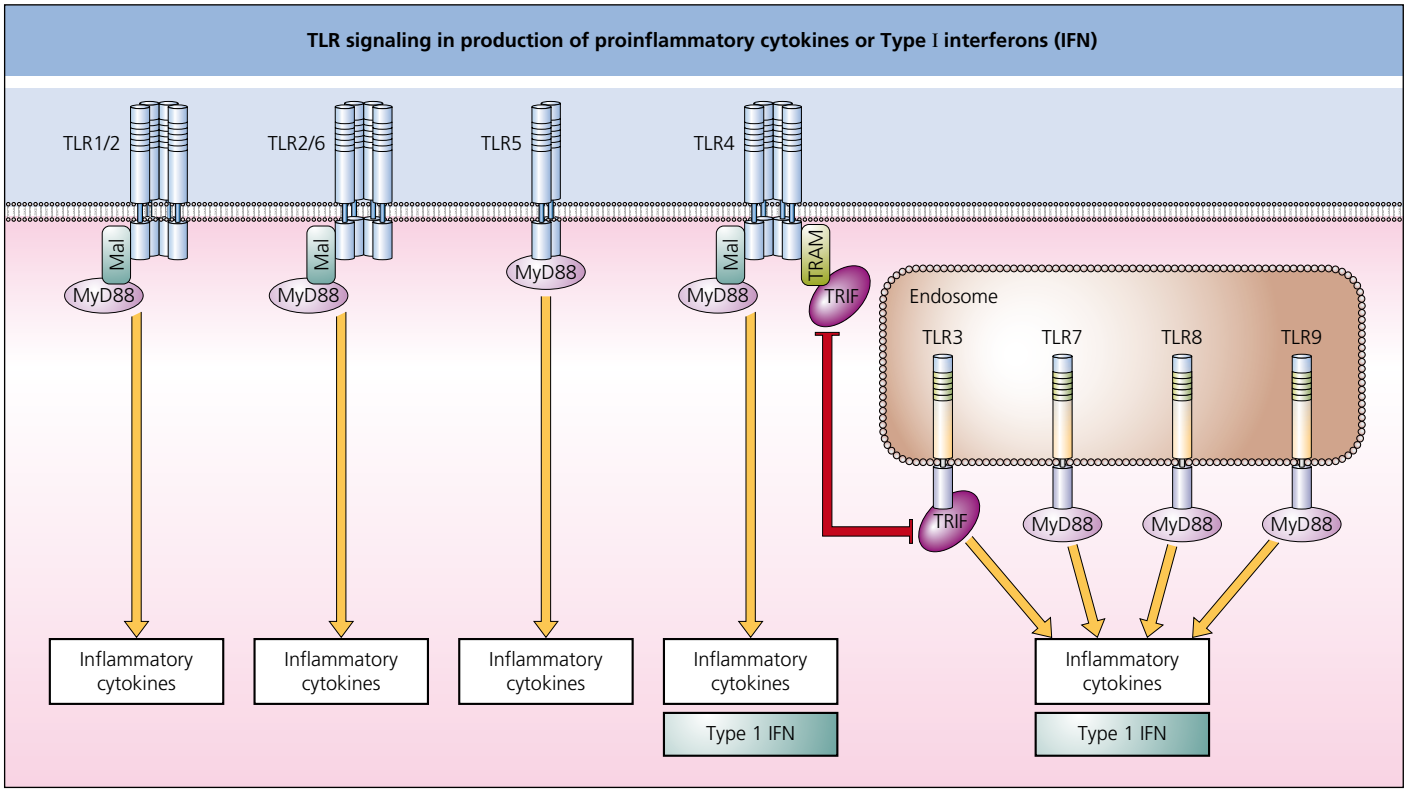


Fig. 2.3 TLR signaling results in production of proinflammatory cytokines or type I interferons (IFNs).

influences the adaptive immune response to a given pathogen that develops later. Thus, the specific early response pattern dictated by the sum of pathogen-specific TLR signals may profoundly influence whether an effective adaptive response is generated.

In addition to the production of cytokines and chemokines, additional programs may be induced upon TLR signaling, depending on the responder cell type. Immature tissue DCs serve as sentinels to sample the environment. Upon appropriate stimulation of DC TLRs by microbial MAMPs, the process of DC maturation is set into motion, ultimately leading to changes in phagocytic/endocytic capacity, secretion of cytokines, enhanced processing and presentation of antigen, upregulation of the major histocompatibility complex (MHC) and co-stimulatory molecules, and migration of DCs to T-cell areas of draining lymph nodes (Fig. 2.4). Mature DCs are then capable of effectively and efficiently presenting antigen to naive T cells. Thus, TLR ligation on DCs serves to link innate and adaptive immunity.[29]

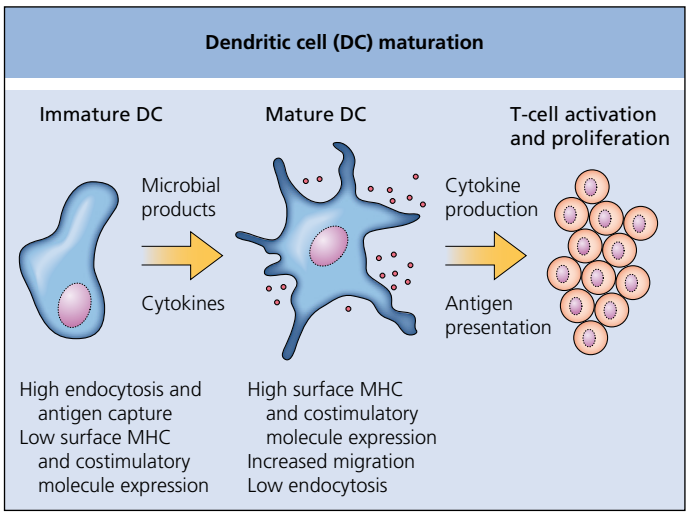


Fig. 2.4 Dendritic cell (DC) maturation.

Nod-like receptors

The nucleotide oligomerization domain (Nod) proteins are another more recently described family of germline-encoded PRRs (NLRs) that respond to microbial 'danger signals' in the form of MAMPs.[30,31] Unlike TLRs, NLRs sense MAMPs in the cytoplasm and are felt to be responsible for responding to bacterial and viral pathogens that replicate intracellularly. In some cases, extracellular pathogens can also be sensed by NLRs, especially if a cell contains machinery for transporting virulence factors (such as muropeptides) into the cytoplasm.[32,33] Upon ligand binding, NLRs oligomerize and recruit several adaptor proteins to activate a number of signaling pathways that result in formation of an 'inflammasome' associated with inflammatory cytokine production, production of antimicrobial peptides and, in some cases, rapid cell death.

One difference between pathogenic and nonpathogenic microbes is their ability to escape intracellular compartments and either replicate in the cytosol or inject microbial products into the cytosol for the purpose of manipulating host cellular machinery.[13] Activation of the NLR pathway may then be seen by the host as a sign of extreme danger, requiring a dramatic response with death of the host cell to prevent the microbe from hiding from host defenses within it.[34] Although much remains to be learned about this complex family of PRRs, there is already evidence to suggest that NLRs play an important role in the host response to organisms such as *Listeria monocytogenes*,[35] *Legionella pneumophila*[36] and *Helicobacter pylori*.[33] Like TLRs, NLRs are also likely involved in driving and directing the pathogen-specific adaptive immune response.

C-type lectin receptors

The last group of PRRs that will be reviewed in this chapter are the large family of C-type lectin receptors (CLRs) which are grouped based on organization of their CLR domains and are diverse in function. They are involved in mediating binding, internalization and potentially killing of micro-organisms, especially fungal pathogens.[37] Many of the CLRs recognize carbohydrate MAMPs, such as fungal cell wall mannans. Examples of CLRs include the mannose receptor (MR), dectins 1 and 2, DC-SIGN (dendritic cell-specific ICAM-3-grabbing nonintegrin) and collectins. CLRs are found on a number of phagocytic cells, such as macrophages, DCs and neutrophils. Some of the CLRS such as dectin are able to mediate intracellular signaling,[38] whereas others such as the MR lack classic signaling motifs in their cytoplasmic tail. Despite lack of signaling motifs, several studies have reported NF-κB activation and inflammatory cytokine production following ligation of the MR,[39,40] although the exact role of the MR in phagocytosis and signaling remains controversial.[41]

Dectin-1 binds to β-glucan ligands and has been shown to recognize numerous fungal species, including *Candida*, *Coccidioides*, *Pneumocystis*, *Aspergillus* and *Saccharomyces*. Dectin-mediated signaling results in phagocytosis, respiratory burst, activation of phospholipase and cyclooxygenase, and production in inflammatory cytokines and chemokines that may influence the adaptive immune response.[42] Dectin-2 is a CLR that is upregulated during inflammation and is important for its recognition of fungal hyphae.[43,44]

DC-SIGN is a CLR reported to internalize pathogens as well as to induce signaling, sometimes resulting in production of the immunosuppressive cytokine IL-10.[45] DC-SIGN has been shown to be involved in the innate response against fungal pathogens as well as viruses, such as HIV-1 and hepatitis C virus (HCV).[46–48]

Although CLRs clearly have a role in the innate immune response against microbial pathogens, their actual functions *in vivo* and their relative contribution to the overall innate response require further evaluation.

Collaborative interactions between PRRs

It is clear from the above discussion that multiple PRRs are capable of recognizing potentially pathogenic microbes and the overall innate response to a given pathogen likely utilizes multiple PRRs from several families simultaneously. Much interest has developed over recent years in determining how the different families of PRRs cooperate *in vivo* to induce optimal immune responses. There are a number of ways in which different PRRs might theoretically interact to benefit the host and there is some evidence that these coordinated interactions, or 'collaborations', are in fact necessary for an effective response to a microbe.[49]

Since NLRs recognize microbial ligands in the cytosol, they rely on mechanisms that make those ligands available to them. Herskovits *et al.*[50] reported that phagocytosis and killing of bacteria can generate PAMPs that become available to NLRs. Likewise, CLRs such as dectin may collaborate with TLRs by triggering phagocytosis, phagosome maturation and reactive oxygen production, and enhancing NF-κB activation.[51–53] Thus, it is likely that innate recognition of many microbes involves surface recognition and binding of the organism via CLRs and surface TLRs (e.g. TLR2, 4, 5), followed by internalization and recognition of PAMPs by intracellular PRRs, such as intracellular TLRs (e.g. TLR7/8 or 9) or NLRs, depending on the ability of the organism itself or its products to escape the endosome and reach the cytosol.

Since ligation of many of these PRRs results in induction of signaling cascades, the ultimate response depends on the various signals received. Several studies now suggest that antimicrobial inflammatory responses can be synergistically activated via ligation of multiple PRRs. For instance, concurrent ligation of TLR4 by bacterial LPS and TLR 7, 8 or 9 results in the synergistic production of IL-12 p70, a cytokine that has a significant impact on directing the adaptive immune response.[54–56] Similarly, there is evidence to suggest that concurrent ligation of TLRs and NLRs results in synergistic production of inflammatory cytokines, such as tumor necrosis factor (TNF) and IL-1β.[57,58]

Additional research is needed to more clearly define these complex receptor networks and the results of such studies could have a major impact on the development of vaccines and vaccine adjuvants.

Phagocytes

Phagocytosis is one of the most basic elements of the immune response with phylogenetic origins in unicellular organisms. Phagocytosis can be undertaken by mononuclear cells, polymorphonuclear cells or natural killer (NK) cells. Although phagocytosis is often thought of as a mechanism by which organisms are merely engulfed and either sequestered or destroyed, the process actually plays the equally important role of providing a mechanism by which organisms are broken down and processed for presentation as antigens to critical elements of the adaptive immune response.[59–63]

Cells of the monocytic lineage are among the most diverse in the immune system and adapt both morphologically and functionally to their geographical location in the body.[64,65] Monocytes originate in the bone marrow and emerge into the peripheral blood as blood monocytes.[62] These cells migrate into tissues and differentiate into cells with a wide variety of morphologies and functions related to host defense (macrophages and dendritic cells), tissue maintenance (glial cells) and structural maintenance (osteoclasts and osteoblasts) (Fig. 2.5).[66–68] Macrophages involved in host defense further differentiate into alveolar macrophages in the lung, Kupffer cells in the liver, Langerhans' cells in the skin and other specialized cells depending on their ultimate

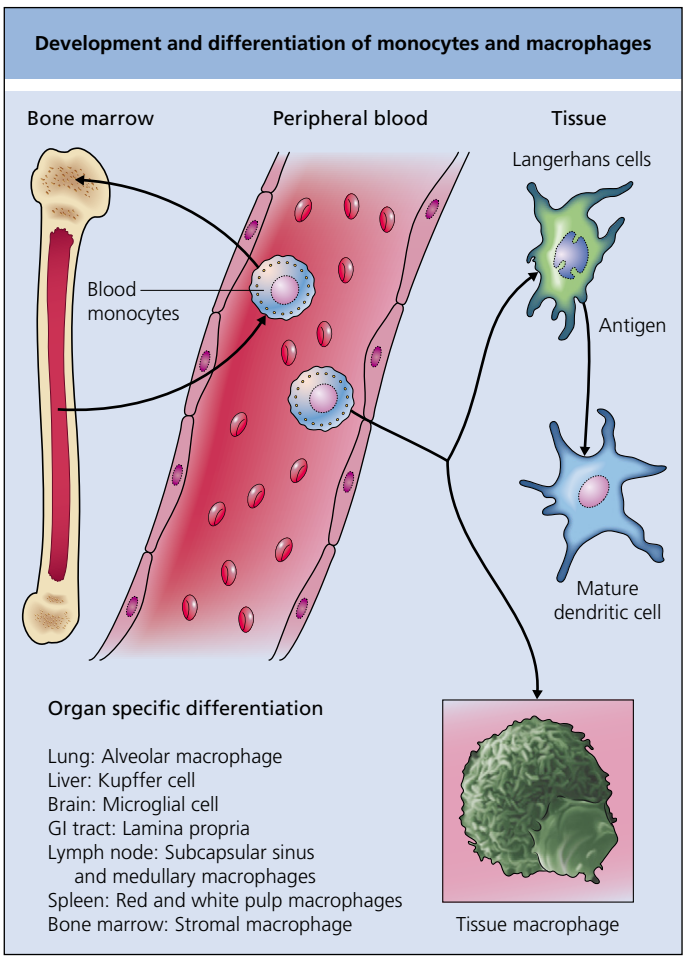


Fig. 2.5 Development and differentiation of monocytes and macrophages.

location. In each of these locations, monocytes adapt functions that are particularly suited to their specific locations. In addition to their stereotypical functions related to phagocytosis of foreign organisms, macrophages play important housekeeping roles related to removal of cellular debris, immune complexes and other cellular and subcellular entities requiring recycling.

Mononuclear cells are both resident in tissues on a chronic basis and respond to a number of well-characterized growth factors, chemoattractants and activators that both attract them to sites of inflammation and activate them for more efficient phagocytosis and intracellular killing. Macrophages interact with these stimuli with several families of surface receptors that provide specific instructions to the macrophage depending on the stimulus and the receptor.[69] Macrophages are dependent on growth factors such as granulocyte colony-stimulating factor (G-CSF) and granulocyte-macrophage colony-stimulating factor (GM-CSF) for growth, differentiation and survival. Chemokines, cytokines and microbial products play important roles in directing migration, secretion and activation. Macrophage functions are further directed by interleukins and interferons produced by lymphocytes and NK cells.

Phagocytes can identify pathogens by the presence of PRRs on the cell surface that recognize PAMPs expressed by the target organism.[69,70] Although phagocytes can engulf micro-organisms and other entities through this PRR–PAMP interaction, the process can also be facilitated by the presence of IgG or C3b on the surface of targets. IgG1 and IgG3 bind to the FcγR1 receptor constitutively produced by monocytes and produced after induction on polymorphonuclear cells. These receptors both serve as opsonins to facilitate recognition and tethering of pathogens by the phagocyte and as activators of the phagocyte oxidative burst through several intracellular pathways.[71,72] Binding of any one of several other surface molecules including TLRs, the IL-1 receptor or CD14 can also activate these intracellular pathways. FcR can either activate or downregulate macrophages depending on whether tyrosine-based activation (ITAM) or inhibitory (ITIM) motifs are formed intracellularly. ITIMs and certain other receptors such as signal regulatory proteins (SIRPs) are important in quenching inflammation to prevent excessive tissue damage as the inflammatory process develops momentum. In addition to surface molecules that regulate macrophage activation, a number of intracellular molecules can also mediate these functions. These include nucleotide oligomerization domains (NOD), caspase-associated recruitment domains (CARD) and leucine-rich repeats. The NOD pathway is particularly important in the gastrointestinal tract.

Once activated, macrophages both secrete additional inflammatory and chemotactic molecules and are primed to kill engulfed organisms within the cell. Inflammatory mediators such as IL-6 and IL-1 act remotely to promote the systemic inflammatory (acute phase) response.[73,74] Excessive production of these inflammatory cytokines can be dangerous to the host, contributing to the pathogenesis of septic shock and the systemic inflammatory response syndrome (SIRS). Other macrophage products such as collagenase and elastase may produce local tissue destruction and participate in deleterious processes including arthritis and pulmonary inflammation.

In addition to cells of the macrophage lineage, polymorphonuclear cells are capable of phagocytosis and intracellular killing. These cells are also ubiquitous in the body and are much shorter lived than cells of the mononuclear lineage. Polymorphonuclear cells share many, but not all, of the surface molecules expressed by macrophages. These cells are particularly efficient at intracellular killing, especially after activation, but are less intimately involved in immunoregulation and 'crosstalk' between the innate and adaptive immune systems than monocytes.

Phagocytes kill organisms by a number of means. Activation of the macrophage greatly extends these killing capabilities. Lysozyme which is constitutively produced by macrophages can destroy the integrity of the cell walls of Gram-positive organisms or collaborate with complement to damage Gram-negative organisms. Macrophages and polymorphonuclear cells also produce a number of short peptides termed 'defensins' that can permeabilize the cell walls of ingested bacteria. The most potent microbicidal functions of phagocytes are mediated by reactive oxygen molecules formed through peroxidase-dependent and -independent pathways and by nitric oxide produced after stimulation by interferon gamma (IFN-γ) or TNF.[75] Genetic or acquired defects in either of these pathways greatly increases susceptibility to pathogens traditionally killed inside the cell such as *Staphylococcus aureus* and *Salmonella*.[76,77]

ADAPTIVE IMMUNITY

As discussed above, whereas innate immunity is an immediate response to conserved microbial products that is limited in scope of recognition by a 'hardwired' set of receptors, the adaptive response is more specific and all-encompassing in its scope of recognition but takes longer to develop. Innate and adaptive responses to a particular microbial pathogen are linked in a complex manner and the inflammatory responses induced by innate activation are often necessary for appropriate activation and direction of the adaptive response.

Adaptive immunity is characterized by several distinctive features that include:[1]

- antigen specificity;
- diversity of responses;
- pathogen-specific memory;
- nonreactivity to self antigens; and
- response self-limitation.

First, the adaptive response is characterized by exquisite specificity for distinct molecules, mediated by a diverse array of surface receptors on B and T lymphocytes. Microbial recognition by the adaptive immune system results in expansion of clones of B and T lymphocytes that express these randomly generated and unique microbe-specific receptors. This specific response is generated upon initial exposure to an infectious pathogen, and the response increases in magnitude and rapidity with each subsequent exposure (termed an 'anamnestic response') to the same pathogen. Thus, the adaptive immune system provides the host with 'memory' for each microbe that is specific, diverse in nature and quick to respond upon reexposure.

Adaptive immunity is comprised of two major types of responses, cell-mediated and humoral immunity (Fig. 2.6). Humoral immunity is mediated by antibodies that are produced by B lymphocytes. Antibodies recognize and bind extracellular microbes and their toxins and neutralize their infectivity. Cell-mediated immunity is primarily mediated by T lymphocytes, with the primary goal of recognizing and fighting intracellular pathogens such as viruses and bacteria that can proliferate inside cells, such as phagocytes. The specific features of both types of response are further described below.

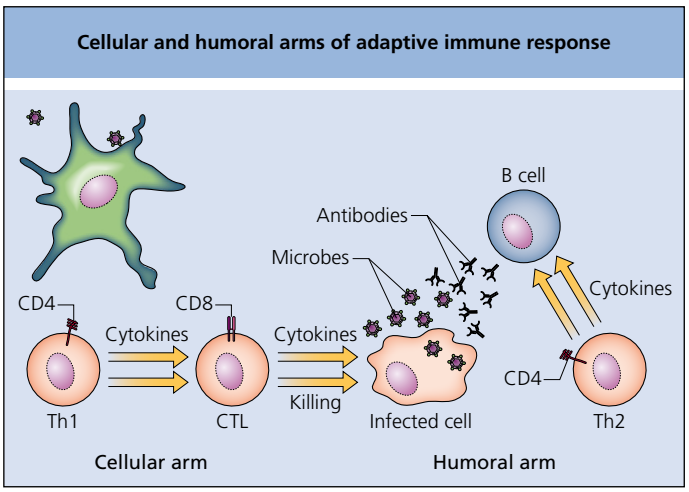


Fig. 2.6 Cellular and humoral arms of adaptive immune response. CTL, cytotoxic T lymphocyte; DC, dendritic cell; Th, T helper cell.

Cellular immune responses

Overview

The components of the cellular immune response include several varieties of T lymphocytes and antigen-presenting cells (APCs), such as DCs and monocyte/macrophages. Antigen-presenting cells capture and process exogenous microbes or process microbes or microbial antigens that are present in the cytoplasm (such as intracellular bacteria or viruses). Processed microbial peptide antigens are expressed in the context of MHC molecules on the surface of APCs and are recognized by surface receptors on T cells (T cell receptors, TCRs).

There are several functionally distinct populations of T cells. Helper T cells express the CD4 molecule, recognize antigen in the context of MHC class II molecules and help to orchestrate the adaptive immune response through the production of cytokines that provide 'help' to B cells and $CD8^+$ T cells. Helper T cells also interact directly with APCs, such as DCs, to enhance their ability to present antigen and stimulate effector CD8 T-cell responses.[78] CD8 T lymphocytes, often called cytotoxic T lymphocytes or CTLs, recognize intracellular antigens presented in the context of widely distributed MHC class I molecules. These effector cells often have cytolytic potential and play an important role in killing infected cells. Another group of T lymphocytes, regulatory T cells, function to inhibit or suppress inflammatory adaptive responses.[79]

The complex interplay between these different cell types is critical to achieve the ultimate goal of cell-mediated immunity, which is to eliminate dangerous microbes without damaging the host through excessive inflammation.

$CD4^+$ T-cell responses

Antigen-specific $CD4^+$ T-cell responses play a central role in the adaptive immune response to microbial pathogens. Naive $CD4^+$ T cells expand in response to antigenic stimulation and differentiate into effector T helper (Th) cells (Fig. 2.7).[80] Two distinct Th cell subsets with differing cytokine profiles and effector functions, Th1 and Th2, were originally described.[81,82]

Th1 cells are involved in mediating inflammatory responses against intracellular pathogens, such as viruses and intracellular bacteria, and produce cytokines such as IFN-γ and IL-2. Activated Th1 cells interact with APCs, such as DCs, to enhance their ability to prime effector $CD8^+$ T cells that recognize intracellular microbial pathogens.[78] They further enhance the expansion of antigen-specific effector $CD8^+$ T cells through the production of IL-2. IFN-γ produced by Th1 cells also serves to activate phagocytic cells, such as macrophages, and enhances their ability to kill ingested organisms. IL-12, a cytokine produced by APCs such as DCs, is important in the development of Th1 cells.[83]

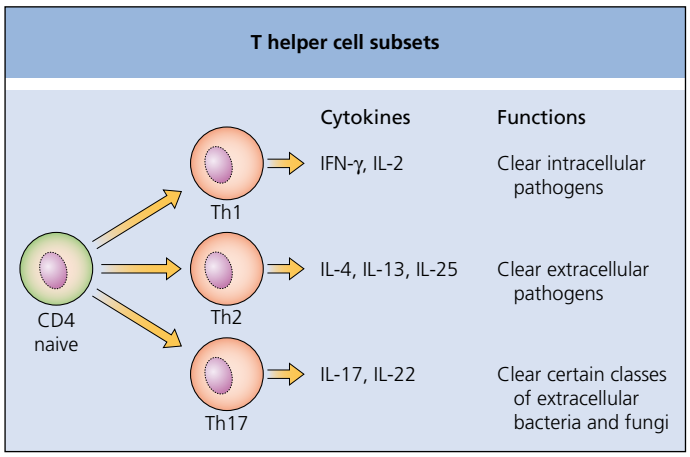


Fig. 2.7 T helper cell subsets. Adapted from Bettelli *et al*.[79]

On the other hand, Th2 cells produce IL-4, IL-5, IL-13 and IL-25, and play an important role in the clearance of extracellular pathogens by providing help to B cells, enabling the production of antibodies. Thus, they have a central role in orchestrating the humoral immune response, as will be described in more detail below. Aberrant or uncontrolled Th1 responses may result in inflammatory disorders or autoimmunity, whereas excessive or misdirected Th2 responses may result in atopy and allergy. Thus, a number of immune checks and balances are in place to ensure that activation and expansion of Th cells are tightly regulated. Anti-inflammatory cytokines, such as IL-10[84] and transforming growth factor beta (TGF-β), and regulatory T cells play a role in maintaining a balance between effective antimicrobial activity and excessive inflammation.

More recently, a third subset of T helper cells, Th17 cells, has been described.[85,86] Th17 cells produce several cytokines in the IL-17 family,[87,88] including IL-17A (IL-17), IL-17F and IL-22, have proinflammatory properties and are felt to play a role in clearance of certain extracellular bacterial (e.g. *Klebsiella pneumonia*, *Mycobacterium tuberculosis*) and fungal (e.g. *Candida*) pathogens.[89–92] The expansion and maintenance of Th17 cells is driven in part by IL-23, a proinflammatory cytokine in the IL-12 family.[85] Th17 cells have been identified in significant numbers in the intestinal mucosae and are postulated to play a key role in intestinal defense against invading microbes.[93–95] Mucosal defense by Th17 cells is likely mediated through a number of mechanisms, including cytokine-enhanced epithelial cell (EC) defensin production and neutrophil recruitment, and by enhancing epithelial integrity by fortifying EC tight junctions.[96,97] In addition to its role in normal host defense, the Th17–IL-23 axis has been implicated in a number of autoimmune diseases as well as in inflammatory bowel disease.[94,95]

$CD8^+$ T-cell responses

$CD8^+$ T cells are considered the primary effector cells responsible for clearance of intracellular pathogens, such as viruses and intracellular bacteria. Naive $CD8^+$ T cells must interact with mature, antigen-loaded DCs in lymphoid tissue in order to differentiate into pathogen-specific effector cells. These activated effector $CD8^+$ T cells are then capable of manifesting both cytolytic and noncytolytic antimicrobial effector functions that include MHC class I-dependent cytolysis of infected target cells and rapid production of β-chemokines and cytokines such as IFN-γ and TNF.[98,99] Cytolytic T lymphocytes (CTLs) proliferate and expand markedly during acute infections, migrate to the sites of infection, then contract into a state of memory. As noted above, CD4 T cells play an important role in the differentiation of $CD8^+$ T cells and perhaps an even more critical role in their memory development.[100] These memory $CD8^+$ T cells form the basis for protective immunity and are capable of rapidly expanding upon future reexposure to the same pathogen.[99]

Although some intracellular pathogens are readily cleared by CTLs, others, such as cytomegalovirus and Epstein–Barr virus, can maintain a state of latency by avoiding such clearance.[101–103] In the case of these chronic infections, ongoing immune surveillance by virus-specific CTLs results in suppression of overt disease, unless the host becomes immunocompromised. In contrast, in the case of pathogens (e.g. HIV-1) with a high mutation rate, escape from detection by $CD8^+$ CTLs is a major mechanism used by the virus to advance its agenda of more active persistence, resulting in chronic progressive disease.[104–106]

Regulatory T-cell responses

Given the inflammatory potential of activated, antigen-specific $CD4^+$ and $CD8^+$ T cells, a number of mechanisms are in place to maintain the balance between the protective and tissue-damaging effects of the adaptive immune response. Regulatory T cells, or T_{reg}, are a heterogeneous category of T cells that suppress cytokine secretion and proliferation of effector T cells. This broad category consists of several populations of $CD4^+$ T_{reg}, including $CD4^+CD25^{high}$ natural T_{reg} cells of thymic origin, peripherally induced, IL-10-producing adaptive type 1

regulatory cells (Tr1) and TGF-β-producing T helper type 3 (Th3) cells. In addition, $CD8^+$ and NK regulatory T cells have been described.[107,108]

One challenge in characterizing T_{reg} populations has been the difficulty in finding phenotype markers specific to these cells. The most specific marker to date for characterizing natural T_{reg} cells is the transcription factor forkhead box P3 (Foxp3). Foxp3 has been shown to be critical for the development and suppressive function of $CD4^+CD25^{high}$ natural T_{reg} cells by controlling expression of multiple regulatory genes.[109,110] In fact, gene mutations that render Foxp3 dysfunctional result in a severe autoimmune disease in humans.[111,112]

The mechanisms by which regulatory T cells exert their suppressive effects on effector T cells, especially in humans, are not entirely clear, although both indirect mechanisms such as secretion of suppressive cytokines (e.g. IL-10, TGF-β) and direct mechanisms such as blocking T-cell activation or degranulation have been postulated.[107] Also, although antigen-specific T_{reg} cells have been reported in the setting of viral infections such as human papillomavirus,[113] T_{reg} cells may also suppress pathogen-specific $CD4^+$ and $CD8^+$ T cells nonspecifically upon activation. In any event, regulatory T cells are postulated to play a key role in modulating the adaptive immune response in the setting of both acute and chronic infections in order to limit inflammation and prevent tissue damage.

Antigen-presenting cells

Dendritic cells are indispensable in the initiation of the adaptive immune response based on their ability to process exogenous antigens and present them in the context of both MHC class I and II molecules to $CD8^+$ and $CD4^+$ T cells. Dendritic cells are the most potent APCs capable of activating naive, antigen-specific T cells and inducing their proliferation and differentiation into T helper and CTL effector cells. The immunostimulatory capacity of DCs arises in part as a result of high level expression of MHC and co-stimulatory molecules, such as CD40 and CD80. More recently, DCs have also been shown to have a role in stimulating memory T cells.[114]

Dendritic cells exist in an immature state in many tissues, where they serve to sample the environment by internalizing microbes and microbial antigens. In many cases, exposure to such antigens results in DC activation, migration to local lymphoid tissues and subsequent interactions with T cells. As noted above (see Fig. 2.4), the process of DC activation or 'maturation' is a complex one that involves changes in phagocytic capacity, upregulation of MHC and co-stimulatory molecules, changes in chemokine receptor expression that facilitate migration to lymphoid tissue, and production of cytokines and chemokines.[115] These changes are necessary to induce antigen-specific T cells; in fact, exposure of T cells to 'immature' DCs may result in their anergy or tolerance. The innate response of DCs to microbes and their antigens are critical to DC maturation and, in particular, the recognition of MAMPs by the many types of PRR expressed by DCs (such as CLRs and TLRs) regulates each step in the maturation process.[116] As a result, DCs are considered to bridge innate and adaptive immunity.

The type of DC activated, its tissue location and the microbe that is sensed all determine the nature of the innate response. The type of innate response generated, and in particular the cytokine profile of the activated DC, then has a major impact on determining the type of adaptive response that is generated in turn. For instance, an activated DC that produces IL-12 or IL-23 may stimulate inflammatory T cells, whereas activated DCs that produce IL-10 may regulate or suppress T-cell inflammation. Thus, the adaptive immune response cannot be fully understood without an understanding of the upstream, innate signals that influence it.

Humoral immune response

By definition, the humoral immune response consists of immunologic effector molecules that are present in soluble form in plasma and other bodily fluids. The two major components of this response consist of immunoglobulins that are produced by specialized cells of the lymphoid lineage (termed B cells) and a family of complement proteins that are primarily synthesized by liver cells. These proteins are capable of interacting with pathogens alone or in concert to reduce their infectivity or, in some cases, to kill them; however, they are most effective when they contribute to a coordinated immune response that includes both cellular and humoral components.

Antibodies

Immunoglobulins are members of a family of molecules that combine variable regions that provide exquisite specificity for binding to target molecules with constant structural motifs that allow well-defined interactions with other components of the immune response. The variable region of the molecule provides the diversity required to identify and to bind to an essentially limitless range of target molecules while the constant region allows the antibody molecule to channel effector activities of other components of the immune response that lack the ability to specifically identify diverse structural targets.

Antibody structure

Immunoglobulin molecules are composed of four protein chains that are joined by a variable number of disulfide bonds. Each molecule consists to two identical heavy chain proteins and two identical light chain proteins (Fig. 2.8). These proteins are joined in the shape of a 'Y', with the heavy chain proteins providing the base of the 'Y' and part of each of the arms. The base of the 'Y' is termed the constant (Fc) region and serves as the portion of the immunoglobulin molecule that binds to other Fc receptors on the surface of specialized immune cells and that provides a locus for binding the initial protein of the complement cascade. Each light chain pairs with a heavy chain to form one of the two arms of the molecule. These arms are termed the 'antibody binding' (Fab) regions of the molecule. These two arms of the molecule are characterized by major molecular diversity and provide the specificity by which each immunoglobulin molecule defines its optimal binding ligand.

Immunoglobulin classes

Antibodies are subdivided into isotypes (or classes) and are termed IgM, IgG, IgA, IgD and IgE antibodies, based on which of the five different heavy chain molecules are synthesized by the B cell producing the antibody (Fig. 2.9, Table 2.3) Two subclasses of IgA (termed IgA1 and IgA2) and four subclasses of IgG (IgG1, IgG2, IgG3 and IgG4) are

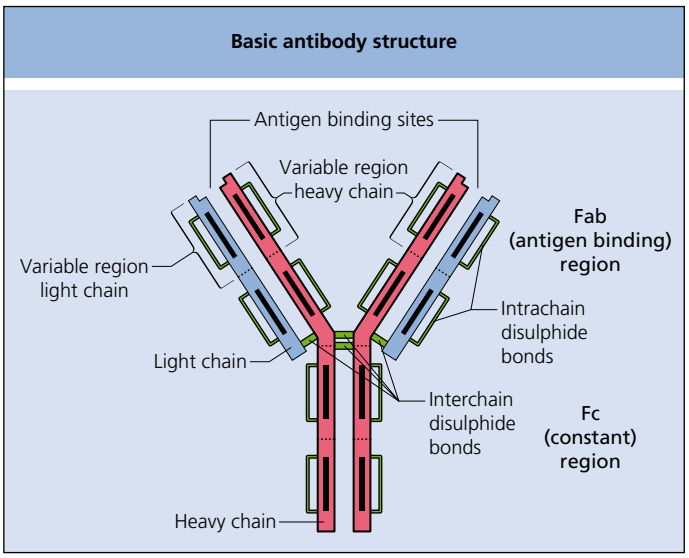


Fig. 2.8 Basic antibody structure.

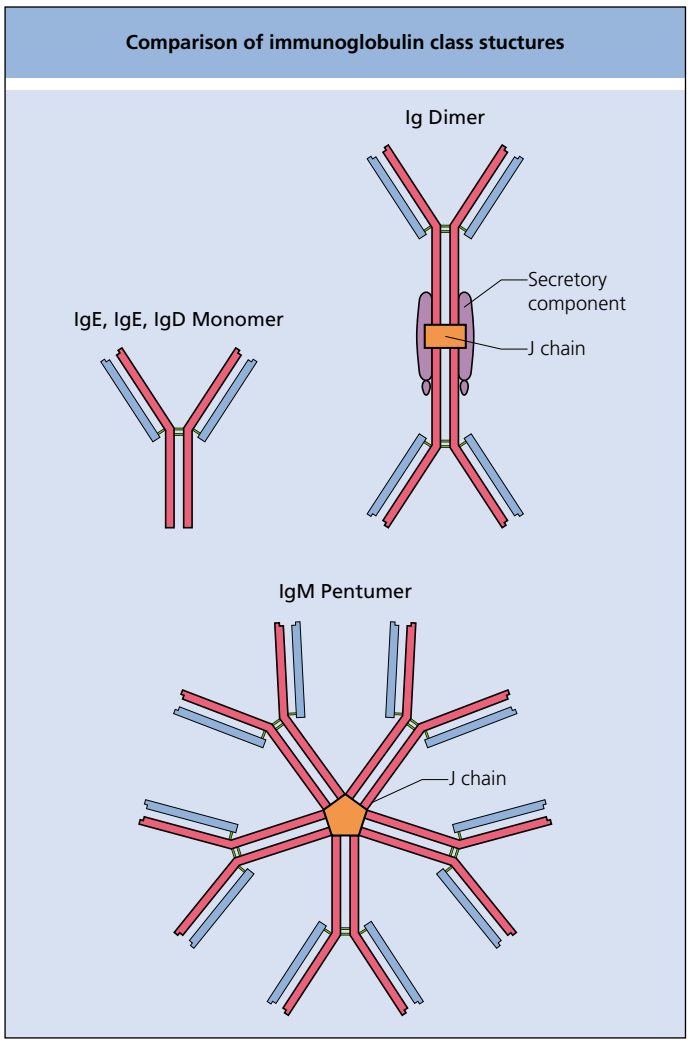


Fig. 2.9 Comparison of immunoglobulin class structures.

present in humans. The specific range of functions that can be mediated by an antibody is defined by its subclass. Except for a short period of time in B-cell development when a B cell can produce both IgM and IgD, each B cell can make only one immunoglobulin subclass at any one time. Isotype switching during B-cell development (described in more detail below) is one of the most interesting biologic processes yet defined. In addition to the production of heavy and light chains, B cells producing IgM and IgA can produce a joining (or 'J') protein that results in multimerization of the antibodies produced.

IgM antibodies are the first antibodies produced in response to an antigenic challenge and may appear within the plasma within a week of initially encountering an antigen. IgM antibodies are of relatively low affinity but are produced with a joining piece that results in five molecules possessing 10 binding sites being produced as a molecular unit. This greatly enhanced valency increases the effectiveness of IgM antibodies and partially compensates for the relatively low binding affinity of each Fab unit. Because of the high molecular weight of IgM antibodies, they do not readily leave the vascular space and are usually found in plasma. IgM antibodies primarily function to prevent binding of pathogens to cells or to aggregate pathogens, thereby enhancing physical clearance of the organism.

The four subclasses of IgG antibodies are present in the highest concentration in the plasma because of their long half-lives (~3 weeks) and high production rates. These antibodies, produced in the second wave of response to an antigenic challenge, evolve to much higher affinities than observed in IgM antibodies and diffuse much more widely throughout the body. Approximately 50% of IgG antibody is found outside the vascular space. IgG antibodies are particularly effective in bringing pathogens into contact with effector cells through binding of the Fc portion of the IgG antibody to Fc receptors on these cells.

IgA antibodies, primarily produced as dimers joined by a J piece by B cells residing in submucosal locations, are present in the greatest concentrations on the mucosal surfaces of the respiratory, gastrointestinal and genitourinary tracts. Antibodies of this class are produced in greater quantity than those of all other classes combined. IgA dimers are linked to a secretory component (SC) protein on the internal surface of epithelial membrane cells and then exported into secretions on the apical side of the membrane. Once within secretions most of the SC molecule is cleaved and IgA dimers cross link infectious agents, thereby both blocking adherence of pathogens to mucosal

Table 2.3 Immunoglobulin classes

Antibody class	Subclasses	Heavy chain isotype	Number of units	Molecular weight (kDa)	Complement fixation	FcR binding	Adult serum half-life (days)	Adult serum concentration (μg/ml)
IgM		μ	5	950	Yes	No	10	600–3500
IgG				150			23	6400–13 500
	IgG1	γ1	1		Yes	Yes		
	IgG2	γ2	1		Yes	No		
	IgG3	γ3	1		Yes	Yes		
	IgG4	γ4	1		No	No		
IgA				400			6	700–3100
	IgA1	α1	2		No	Yes		
	IgA2	α2	2		No	Yes		
IgE		ε	1	190	No	Yes	2	0.04
IgD		δ	1	175	No		NA	NA

surfaces and facilitating physical clearance by cilia and other mechanisms. IgM molecules can also be exported through this pathway. Since most IgA antibodies are secreted through mucosal surfaces, serum levels of IgA are much lower than those of IgG. IgA antibodies do not fix complement or bind to Fc receptors, and thus are relatively poor inducers of inflammatory responses. Because of this property, IgA antibodies are thought to be important in binding potential food allergens and facilitating clearance without sensitization.[117] Individuals who lack IgA antibodies have a higher incidence of food allergies than the general population.

IgE antibodies bind tightly and specifically to mast cells where they reside for a prolonged period of time.[118] When IgE antibodies on the surface of mast cells bind an antigen, they trigger the mast cell to release histamine and other inflammatory mediators resulting in an immediate hypersensitivity response. Although the specific role for IgE antibodies in host defense against multicellular parasites has not been fully delineated, these antibodies seem to be integral to the immune response to this group of pathogens.[119]

IgD antibodies are transiently present on the surface of B cells during B-cell development and play a key role in B-cell maturation and selection.

Generation of antibody diversity and isotype switching

The mechanisms by which the immune response selects for high affinity antibodies, regulates production of the appropriate isotypes during induction of the immune response, and by which antibody production is downmodulated following an immunologic challenge, are among the most elegant regulatory mechanisms in nature. This response features a series of DNA rearrangements that result in the creation of massive antibody diversity followed by an efficient mechanism for selecting for B cells producing antibodies of the highest affinity and eliminating those that produce antibodies that bind to antigens of the host.

Antibody diversity is created by a series of rearrangements of DNA as cells mature from pro-B cells to B cells in the bone marrow (Fig. 2.10). These rearrangements result in the joining of four segments of DNA into the necessary components for the heavy chain of an IgM molecule. These components include a constant μ segment as well as three other segments termed V (variable segment), D (diversity segment) and J (joining segment). These latter segments are selected randomly from 130 possible V segments, 27 possible D segments and 6 possible J segments, allowing for over 20 000 possible combinations of V, D and J segments with each μ segment. The splicing mechanisms responsible for this process are themselves 'sloppy' and allow for the insertion and deletion of bases, thereby greatly increasing potential diversity. Once these arrangements have occurred with the heavy chain, the cell is termed a B cell and an analogous process ensues with the light chain to introduce additional diversity as another rearrangement process develops a DNA segment capable of encoding an intact light chain. In the case of the light chain, only the V, J and constant segments are involved. With the light chain, the B cell can choose one of two different possible constant regions termed kappa or lambda for fusion to the V and J segments. Once a DNA sequence capable of encoding a light chain has been assembled, two identical light and two identical heavy chains are synthesized and unite to form a full IgM molecule which is then displayed on the B-cell surface to allow for expansion of those with the appropriate specificity and elimination of any B cells with self-specificity.

B cells first express surface IgM molecules while still in the bone marrow. Since the bone marrow is a reasonably sequestered location, antigens present in the bone marrow are most likely host antigens rather than foreign ones. If a B cell still resident in the marrow encounters an antigen that binds to its surface IgM molecule, it is signaled to either undergo apoptosis or to become anergic and not expand further. Thus, B cells that recognize self-antigens are prevented from further expansion before leaving the marrow.

As B cells leave the marrow, long RNA transcripts are produced that include both the μ and δ segments as well as the previously selected V, D and J segments. Since this messenger RNA can be spliced to produce either a heavy chain for an IgM antibody or one for an IgD antibody, both are produced and B cells express both IgM and IgD simultaneously as they emerge from the marrow. If a B cell expressing both IgM and IgD encounters cognate antigen, it receives what has been termed the 'first signal' and is subsequently capable of proliferating when stimulated by the 'second signal' which is delivered by an activated T cell. If a B cell does not encounter antigen with which it can bind within a week of leaving the marrow, it becomes anergic and can no longer divide.

At this stage of development, B cells express a molecule known as CD40 that is specifically bound by a molecule on the surface of activated T cells (CD40 ligand, or CD154). This results in B-cell activation and stimulation of antibody production. Additional adhesion molecules are then expressed by T and B cells, resulting in stable contact between the two cells and a channel between them by which T cells can secrete specific cytokines and further direct the B cell to switch its antibody isotype to IgG, IgE or IgA. Once bound by an activated T cell and appropriately stimulated, B cells can undergo three or four divisions per day and produce thousands of daughter cells. By this sequence of events, the immune response is capable of randomly creating immunoglobulin molecules of an almost infinite number of specificities through recombination in the marrow but, at the same time, allows for expansion only of those responding to foreign antigens being actively encountered as B cells emerge from the marrow.

Isotype switching is directed by cytokines produced by T cells in close proximity to IgM producing B cells. IL-6, IL-10 and TGF-β signal B cells to switch to production of IgA antibodies, while IL-4 and IL-5 direct a switch to the IgE isotype. Isotype switching to IgG, IgA and IgE is accomplished by another DNA rearrangement with ligation of the heavy chain V, D and J segments to γ, α or ε segments, respectively. Unlike the IgM to IgD isotype switch which is accomplished by RNA splicing, this final isotype switch involves DNA rearrangement and is a permanent event for the B cell. Progeny of an isotype-switched B cell produce only that isotype from that time forward.

Ongoing production of specific antibodies is dependent on ongoing antigenic stimulation in the face of a regulatory bias to cease production of antibodies of a given specificity and ongoing selection of antibodies of even higher specificity. As B cells undergo division, they express increasing amounts of a surface molecule known as Fas and lower levels of Bcl-2. Bcl-2 is required for ongoing division while ligation of Fas results in death by apoptosis. If a B cell encounters an antigen that binds its surface immunoglobulin, it produces higher levels of Bcl-X_L and survives. As B cells divide, the immunoglobulin gene continues to undergo somatic hypermutation. B cells that generate immunoglobulin with a higher affinity for the antigens of interest outcompete other B cells for this antigen and have a selective advantage, thereby further increasing antibody specificity over time. As antigen density decreases with resolution of an infection, for example, B cells cease producing antibodies and cells that survive become memory B cells that are capable of rapid expansion if a related antigen is subsequently encountered.[120]

Antibody functions

Antibodies serve in a number of different roles, both as freestanding molecules and working in concert with other components of the immune response (Table 2.4).

Neutralization

The most straightforward function of antibodies involves direct binding to microbes or toxins to block binding to host cell receptors or to reduce infectivity through a process termed neutralization. Microbial pathogens often gain entrance into target cells by binding with specific ligands on the cell surface. Antibodies that interfere with this interaction reduce the infectivity of organisms by preventing effective

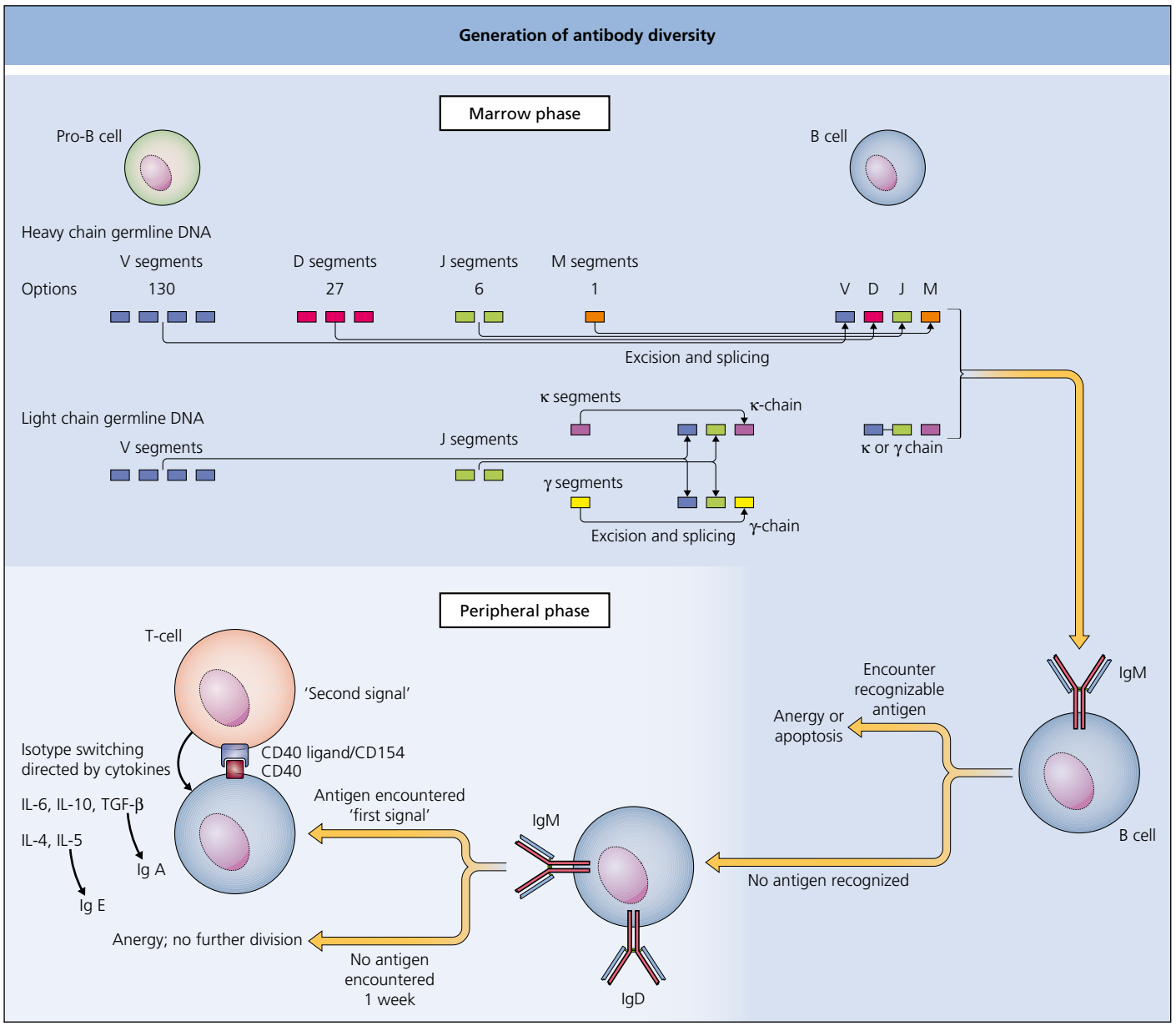


Fig. 2.10 Generation of antibody diversity.

interactions with target cells.[121] Microbial toxins also usually bind to specific molecules on the surface of target cells and interactions with antibodies that interfere with this interaction can markedly reduce the effects of these toxins. Antibodies can also reduce the infectivity of organisms without binding directly to receptor molecules if they interfere with molecular rearrangements involved in infectivity after initial binding has occurred.[122]

Table 2.4 Functions of antibodies

- Cross linking and physical clearance of pathogens
- Neutralization of pathogens
- Initiation and enhancement of complement activity
- Opsonization of pathogens
- Antibody-dependent cellular cytotoxicity
- Modulation of the immune response

Complement activation

Antibodies of the IgM class as well as those of the IgG1, IgG2 and IgG3 subclass can activate complement after binding to the surface of a microbe. IgG1 and IgG3 are usually targeted to protein antigens while antibodies of the IgG2 subclass usually bind to polysaccharide antigens. Individuals with selective IgG2 deficiency exhibit greater susceptibility to encapsulated organisms.[123] As will be described in more detail below, when antibodies activate complement through the classic pathway, the first complement component (C1q) binds to the Fc portions of at least two antibody molecules and sets off a series of molecular interactions with distal components of the complement cascade that culminates in the formation of pores in the wall of the microbe leading to an osmotic death. Complement can also facilitate the formation of large soluble complexes of antibodies and pathogens that can be eliminated from the host. Red blood cells are coated with receptors for complement protein C3b and can transport pathogens coated with this protein to the spleen and liver for ingestion by the reticuloendothelial system.

Opsonization

Antibodies of the IgG1 and IgG3 subclass also target pathogens for ingestion by phagocytic cells of the immune system by binding to the pathogen with the Fab end of the antibody and to a phagocytic cell through Fc receptors on the surface of these cells. In addition to providing a bridging mechanism between the microbe and a phagocytic cell and facilitating phagocytosis, the interaction with the Fc receptor activates the oxidative pathway within the phagocyte and makes it much more effective in killing the pathogen within its phagocytic vacuoles. Pathogens can also be targeted for ingestion by deposition of complement proteins C3b and C4b on the surface of the pathogen.

Antibody-dependent cellular cytotoxicity

Antibodies can also form bridges between pathogens and NK cells, monocytes or granulocytes and activate these cells to secrete microbicidal substances including perforins or granzymes that can kill organisms without requiring that they be ingested. This mechanism is particularly well suited to targets too large for ingestion or for virus producing cells in which these antibodies facilitate destruction of the cell before a full complement of infectious virus can be produced.[124]

Modulation of the immune response

Antibodies are also important for modulation of the immune response. When complexed with antigens in the form of immune complexes, antibodies can downregulate the humoral immune response, thereby modulating ongoing production of antibodies as antigen and immune complexes are cleared.

Complement

The complement system is probably best characterized as a highly regulated series of proteins that play a key role in interfacing the innate and adaptive immune responses.[125,126] The complement system is composed of over 30 interactive proteins that amplify the effects of antibodies, integrate the cellular and humoral responses, assist in the clearance of immune complexes, enhance antigen presentation and can stimulate cellular immunity in the absence of an antibody response. These proteins are highly evolved both in their regulation and in their effector function to provide tightly targeted amplification of the immune response, especially in its earliest phases. Complement proteins are also critical to the cross linking of antibodies and antigens in immune complexes and play a critical role in the clearance of immune complexes.[127] Complement proteins are primarily synthesized by hepatocytes although monocytes and certain epithelial cells can also produce complement components.

Initiation of the complement cascade

The complement cascade can be activated by one of three different types of signal (Fig. 2.11). Once activated by any of these signals, the three signaling pathways converge at an activated protein common to all three pathways, C3b, that then catalyzes the remainder of the cascade to produce a membrane attack complex as well as a family of terminal components with immunoregulatory functions.[126] The classic complement pathway is activated by interactions between pathogens and IgM or IgG.[128,129] The other two pathways do not require antibody for activation and can evolve very early during interactions between pathogen and host. The first of these, termed the 'lectin pathway', is triggered when one or more circulating mannose-binding ligands bind to monosaccharides on the surface of pathogens.[130] The final pathway, termed the 'alternative pathway', is activated when C3b is incorporated into an active complex by a circulating protein known as properdin and stabilized on the surface of a pathogen.[131]

Classic pathway

The classic pathway of complement activation can be triggered when the first component of the complement cascade, C1, interacts with IgM, IgG1, IgG2 or IgG3 molecules that have undergone structural rearrangement following binding to cognate antigen.[128,129] C1 circulates as a huge noncovalently linked polyprotein composed of 22 protein subunits of five different types and is not activated unless it simultaneously encounters Fc regions from at least two immunoglobulin molecules that have bound to an antigenic target. Since IgM circulates as a pentamer, a single IgM molecule can activate C1 after binding to a target antigen. IgG-based activation requires that at least two molecules of antibody bind in close enough proximity to be bridged by the C1 protein. By requiring both the conformational change following antigen binding and simultaneous interaction with more than one Fc region, C1 can freely circulate in its nonactive form and avoid activation by encountering unbound antibody.

Once the C1 molecule is activated by interaction with the appropriate antibody ligands, two of its protein subunits, C1r and C1s, are sequentially activated as proteases. The active C1s serine protease then cleaves another circulating complement protein, C4, to continue the complement cascade. C4 is cleaved into a larger component, C4b, that covalently bonds to the pathogen through either an amide or an ester bond and a smaller soluble component, C4b, that possesses inflammatory activity. When C4b is bound to the membrane of a pathogen, it can, in turn, bind complement protein C2 which then becomes a target for proteolysis by the C1s protease. The larger fragment of this proteolysis, C2a, then gains proteolytic capability and remains bound to C4b and forms a protein dimer that can convert C3 to its active form. C4b binds to circulating C3 and C2a cleaves one of the two chains of the C3 heterodimer. One of these, C3b, binds either to the C4–C2 dimer and forms a three-component complex known as C5 convertase that is capable of cleaving C5 and activating the remainder of the complement cascade, or that can bind directly to the pathogen surface and activate the terminal components of the complement cascade through the alternative pathway.[132]

Alternative pathway

The alternative complement pathway can be activated as a by-product of the classic pathway when the C4–C2 dimer facilitates direct binding of the activated C3 to the pathogen surface as above or when circulating C3 is spontaneously hydrolyzed and acted on by two proteins (factors B and D) to form a free C3 convertase molecule.[131] This molecule can, in turn, activate C3b to be deposited on the pathogen surface where it binds first to factor B and is then trimmed by factor D to form a surface-bound alternative C3b convertase. This molecule forms more activated molecules of C3b and can also be bound by another protein, properdin, and a second activated C3b molecule to form an alternative C5 convertase that activates the terminal components of the complement cascade.

Lectin pathway

Complement can also be deposited on the surface of pathogens displaying certain monosaccharides in a process that is initiated when these polysaccharides bind to one of two multimeric protein complexes composed of either mannose-binding lectin (MBL) or C-reactive protein (CRP).[130,133] In either case, once one of these ligands binds to a monosaccharide on the pathogen surface, proteases analogous to C1r and C1s (MASP1 and MASP2) in the classic pathway are triggered.[134] With MBL the sequence of events is the same as in the classic pathway and results in the deposition of C5 convertase on the surface of the pathogen; with CRP the classic pathway is initiated but the process stops with the formation of C3 convertase, allowing opsonization but not activation of the terminal components of the complement pathway.

Formation of the membrane attack complex

Once the complement cascade has been initiated by one of these three pathways, the process converges on cleavage of C5 into an anaphylotoxin (C5a) and C5b which is bound to the wall of the pathogen by the relevant C5 convertase complex. This process triggers a terminal sequence of binding events for C6 through C9 to form the membrane activation complex.[135] The C9 component of this complex forms

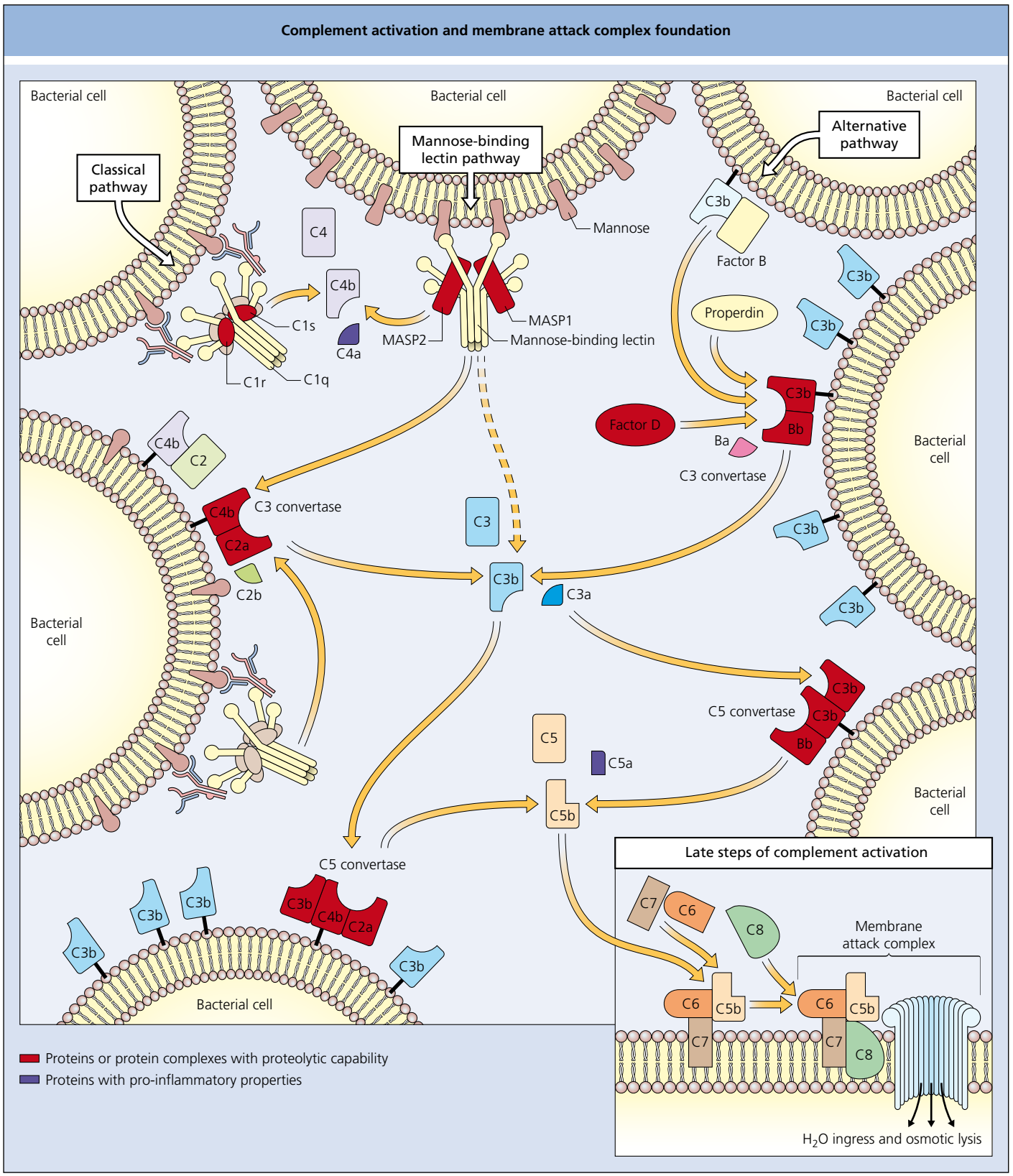


Fig. 2.11 Complement activation and membrane attack complex foundation. Adapted from Walport.[127] Copyright 2001, Massachusetts Medical Society.

a pore in the membrane of the pathogen and allows for the ingress of water, osmotic lysis and death of the target organism.[136]

Disorders of the complement system

As is evident from the above, the complement is a self-amplifying pluripotent system that has evolved to play a central role in amplifying both the innate and adaptive immune responses. From its location in the crossroads of many aspects of the immune response, aberrant or inappropriate activation of complement or an absence of any of several of its key components can have very visible clinical manifestations. Individuals with a deficiency in the terminal components of the complement pathway exhibit an increased susceptibility to disseminated infection with encapsulated organisms, especially organisms such as *Neisseria meningitidis* and *N. gonorrhoeae*.[137–139] On the other hand, deficiencies in components of the complement system that result in inefficient activation of the classic complement pathway may increase the risk for autoimmune diseases such as systemic lupus erythematosus.[140] Disorders in complement proteins responsible for regulation of C3 activation may increase the risk for membranoproliferative glomerulonephritis or paroxysmal nocturnal hemoglobinuria.[126,141] The absence of functional C1 inhibitor is associated with hereditary angioedema.[126]

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Chapter 3

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Vaccines and vaccination

Before vaccines were developed, children died in large numbers from diphtheria, meningitis and pertussis. From time to time, epidemics of smallpox, measles, poliomyelitis, rubella, and other contagions swept across cities and age groups. Many individuals succumbed to tetanus, hepatitis, rabies and septicemia. Fortunately, scientists and clinicians progressively developed an arsenal of vaccines, increasing both life expectancy and the quality of life. Indeed, the prospects for preventing even more diseases have brightened with the development of new vaccines to prevent severe gastroenteritis, cervical cancer and herpes zoster.

THE VALUE OF VACCINATION

The distinction between vaccines (i.e. the products) and vaccination (i.e. the act of administering vaccines) is important. Edward Jenner's recognition of the value of inoculating cowpox to prevent smallpox in 1796 was nothing less than historic. But smallpox was not contained until wide-scale vaccination programs snuffed out the ability of variola virus to spread naturally. Indeed, the disease was eventually eradicated in the late 1970s, by means of a global vaccination program.[1,2]

In the last 50 years, vaccines have tended to be used widely more quickly after invention than in Jenner's case. The plummeting rates of *Haemophilus influenzae* type b, pneumococcal disease, meningococcal serogroup C and rotavirus gastroenteritis among children (Figs 3.1–3.4) within a few years after widespread vaccination programs began, clearly show how quickly the benefits of vaccination can be achieved.[3–5]

In notable contrast, pertussis has not yet been controlled, even though adult-formulation acellular pertussis vaccines were introduced in developed countries in recent years. Adolescents and adults provide a large reservoir for this pathogen; uptake of the new vaccines in these cohorts is still insufficient to reduce disease incidence, either among themselves or in younger contacts.[6,7]

As a matter of biologic science and medicine, vaccines are remarkable accomplishments. As a matter of social science, vaccines are similarly impressive. As the number of vaccinated people in a cohort increases, the less likely it is for a contagious person to be able to transmit that infection to others. Above a certain threshold, a condition of 'herd immunity' or 'community immunity' develops.[8–10] At that point,

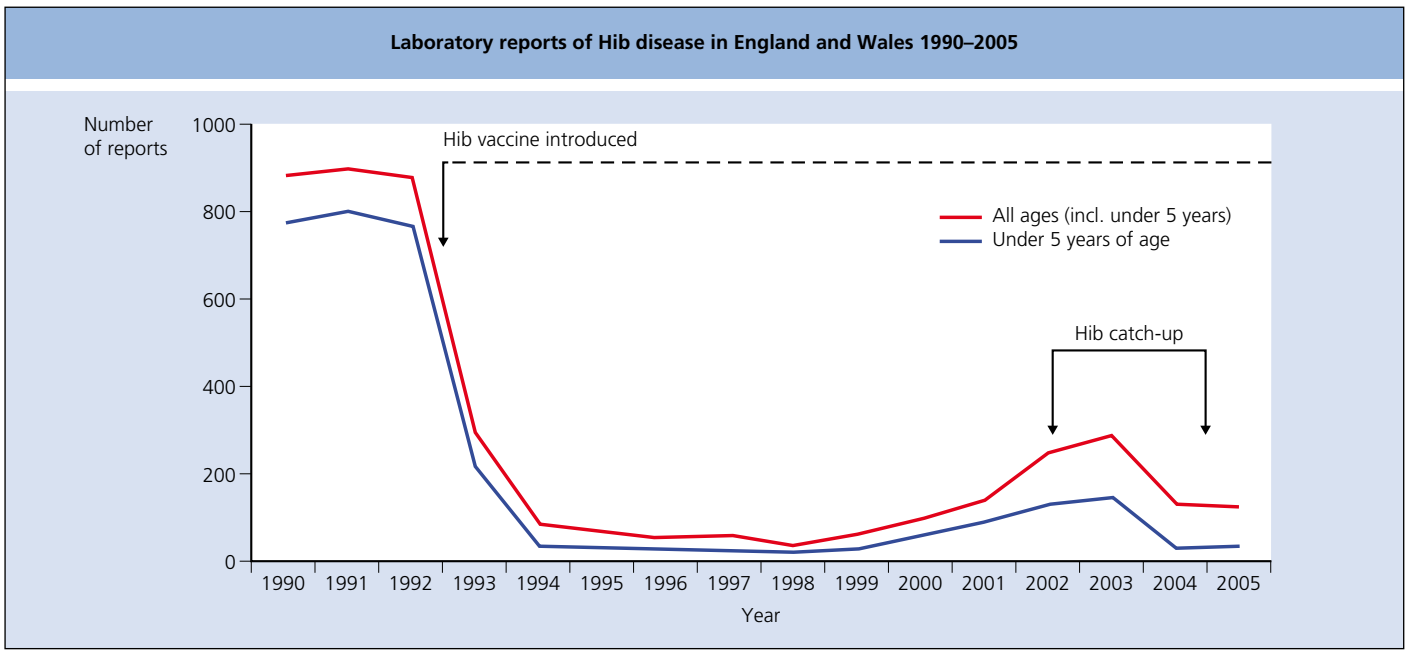


Fig. 3.1 Laboratory reports of Hib disease in England and Wales 1990–2005. Redrawn from Joint Committee on Vaccination and Immunisation.[3]

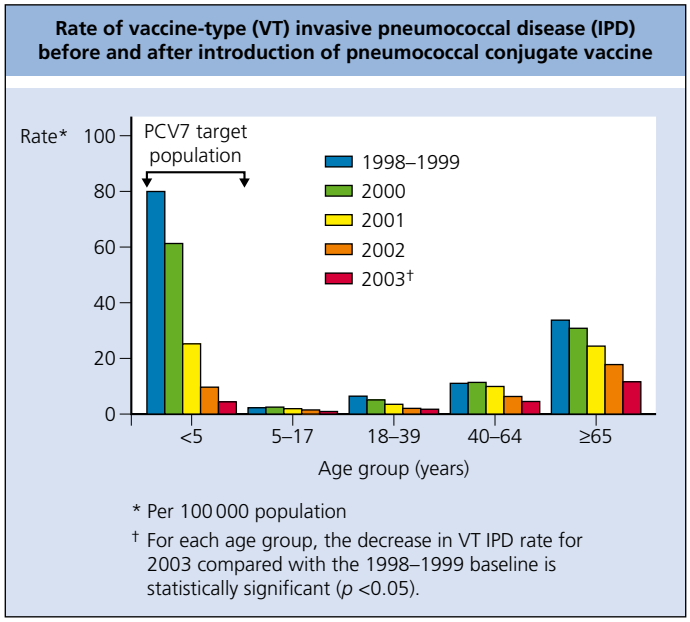


Fig. 3.2 Rate of vaccine-type (VT) invasive pneumococcal disease (IPD) before and after introduction of pneumococcal conjugate vaccine (PCV7), by age group and year. Active Bacterial Core surveillance, United States, 1998–2003. Redrawn from Centers for Disease Control and Prevention.[4]

it is difficult for a chain of transmission to sustain itself, to the advantage of the public's health. In this way, a decision to be vaccinated can offer two distinct forms of benefit:

- the vaccine recipient becomes personally immune to infection; and
- the vaccine recipient helps contribute to the community's collective immunity, making those unable to be vaccinated less vulnerable to infection.

Thus, the vaccine recipient gains personal, direct protection and altruistically helps others at the same time. The threshold to achieve herd or community immunity varies from disease to disease, based on the reproductive and infectious characteristics of the pathogen and the vaccine's efficacy and duration of protection. As two of the most contagious of the childhood diseases, measles and chickenpox require some of the highest proportions of immunity to achieve herd immunity.

In addition to their human benefits through prevention of morbidity and mortality, most vaccination programs are cost-effective. That is, most vaccines either result in net cost savings or correspond to a price per infection or death averted or a quality-adjusted life year gained that is within the range society has been willing to pay for value returned.[11–13] In developed countries, rabies vaccine may be the least cost-effective, at least in some scenarios, with a calculated cost of $2.9 million to $4 billion per life saved.[14]

In stark contrast, preventing 10 diseases among children (i.e. diphtheria, tetanus, pertussis, Hib, poliomyelitis, measles, mumps, rubella, varicella and hepatitis B) averted over 14 million cases of disease and more than 33 500 deaths over the lifetime of the immunized birth cohort of children, saving an estimated $10 billion per year.[15] If indirect economic benefits (e.g. time parents take off from work to care for sick children) are included, the annual savings to society exceed $40 billion. In a ranking of 30 preventive services based on preventable disease burden and cost-effectiveness, childhood vaccination ranked first; the value of increased use of pneumococcal vaccination among those 65 years or older was also noted.[16,17]

With high rates of childhood vaccination in most developed countries, the challenge for contemporary clinical services is to consistently and reliably extend the benefits of vaccination to adolescents and adults. Interest in preventing cervical cancer and genital warts via human papillomavirus (HPV) vaccination or meningitis provides an opportunity to protect against pertussis and other infections as well. Bundling these vaccination efforts for teenagers can provide what is known as the 'adolescent platform' for vaccination.

Efforts to deliver the annual influenza vaccine formulation to the elderly can provide a similar 'adult platform' for assessing vulnerability to pneumococcal disease and herpes zoster ('shingles' or 'zona'). Vaccination assessments before international travel provide an opportunity to assess susceptibilities to both exotic travel-related infections and ubiquitous domestic infections as well. Adults aged from their 30s to their 50s remain perhaps the group least well evaluated for vaccination adequacy, which could often benefit from periodic vaccination check-ups.

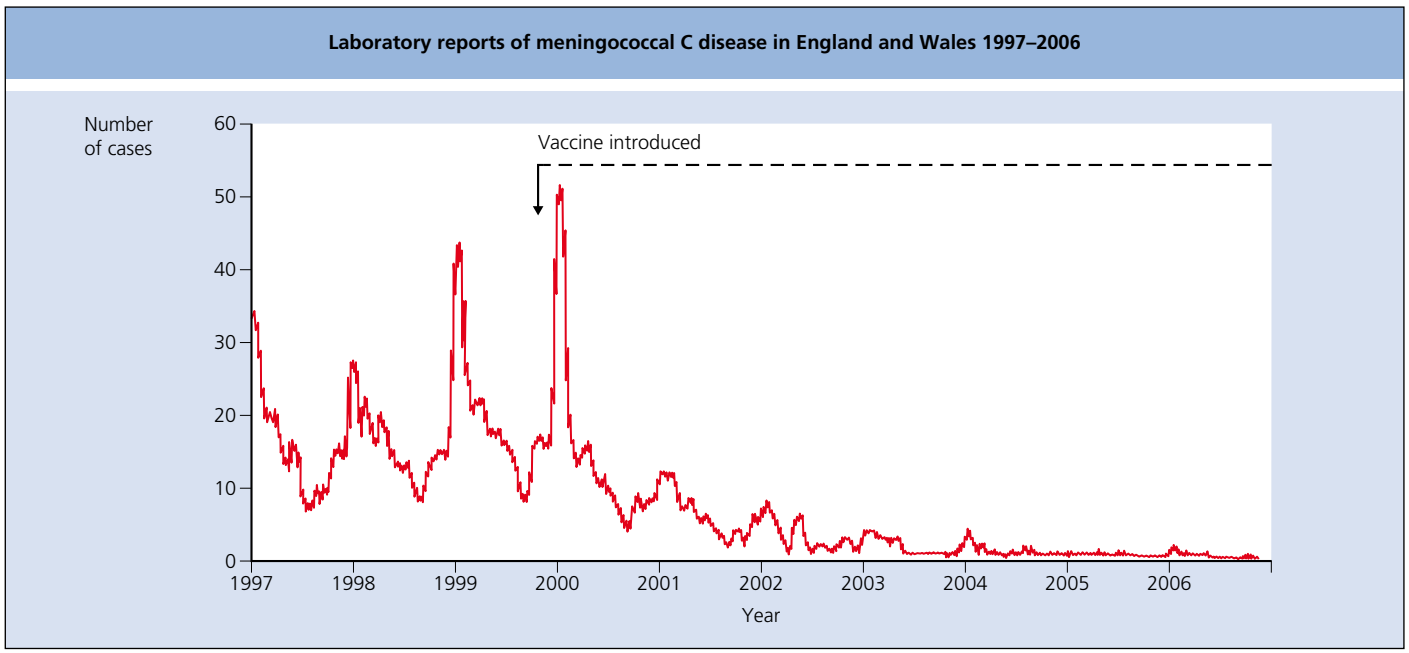


Fig. 3.3 Laboratory reports of meningococcal C disease in England and Wales 1997–2006. Redrawn from Joint Committee on Vaccination and Immunisation.[3]

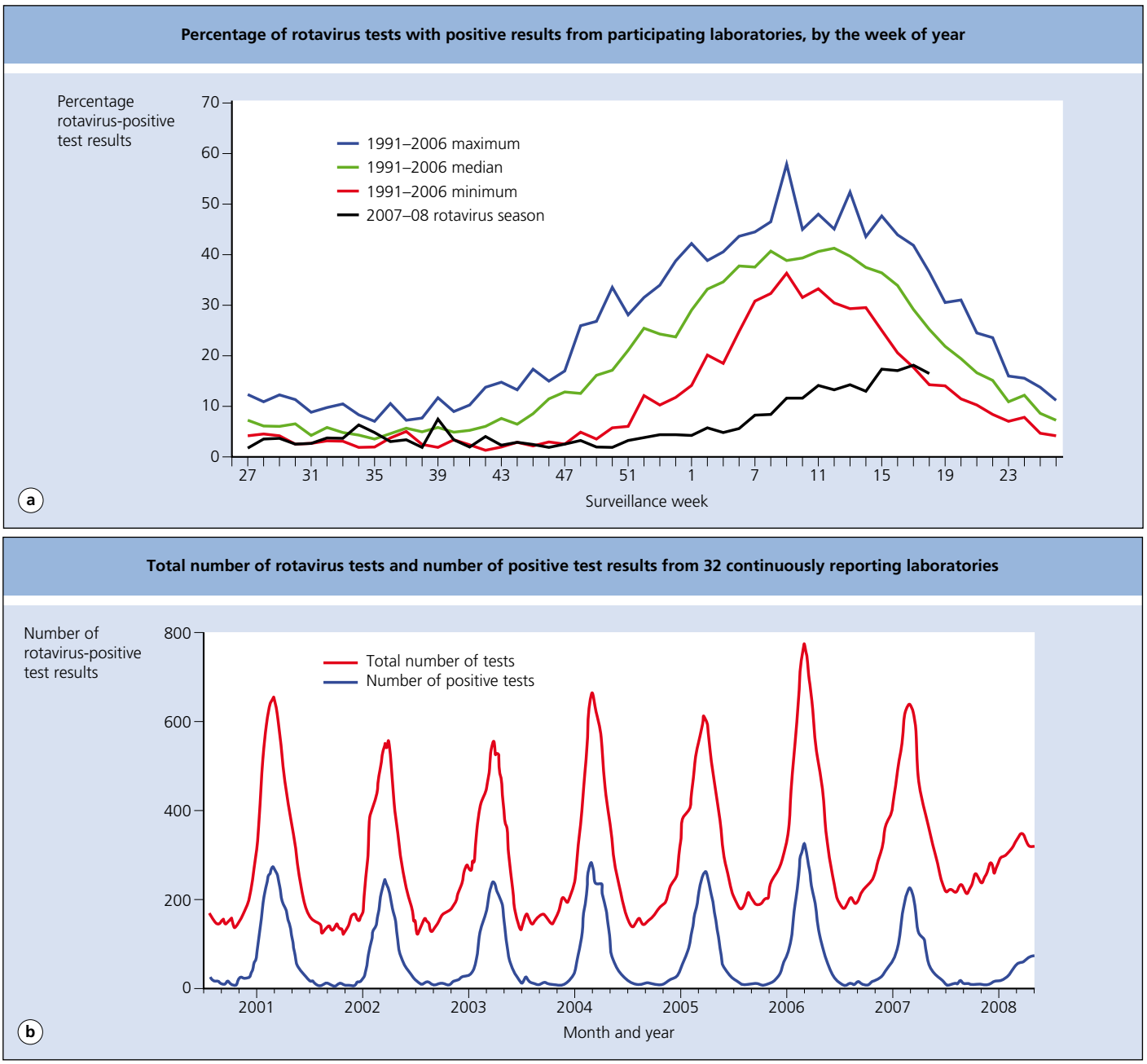


Fig. 3.4 Rotavirus tests. (a) Percentage of rotavirus tests with positive results from participating laboratories, by week of year – National Respiratory and Enteric Virus Surveillance System, United States, 1991–2006 rotavirus seasons and 2007–08 rotavirus season. (b) Total number of rotavirus tests and number of positive test results from 32 continuously reporting laboratories – National Respiratory and Enteric Virus Surveillance System, United States, July 2, 2000–May 3, 2008. Redrawn from Centers for Disease Control and Prevention.[5]

TYPES OF VACCINES, TYPES OF IMMUNITY

A vaccine is a medication used to induce active immunity. Vaccines typically consist of several ingredients, of which antigens (or immunogens) are the principal active ingredients. The specific three-dimensional sites on antigens that bind with antibodies are called epitopes. Vaccines also contain excipients, sometimes called inactive ingredients, necessary to manufacture the vaccine components, stabilize and suspend those components, preserve them against bacterial contamination, or other purposes. Some vaccines contain adjuvants (from the Latin *adjuvare*, to help) or carrier proteins to enhance the immune response to the primary antigens.[18,19]

Vaccines do not protect directly. Rather, they stimulate the immune system to develop a specific state of immunity by means of T lymphocytes, B cells (i.e. plasma cells), antibodies and immunologic mediators (e.g. interferons, interleukins). Vaccines evoke a state of 'active immunity', protection that typically lasts for years or decades. Although their protection tends to be durable, vaccines typically require a period of 10–14 days or more after each dose to elicit an enhanced state of immunity against specific pathogens or diseases.[18]

Most vaccines elicit an immune response similar to what would prevent re-infection in people who survive natural infection. Most pivotal clinical trials of new vaccines evaluate a vaccine's ability to prevent or accelerate clearance of an infection. Once a body of knowledge for a vaccine-disease relationship is established, later vaccines may be

evaluated in a simpler manner by comparing antibody responses or serologic or clinical 'correlates of immunity'.

Vaccines can be divided into four major categories, quadrants based on whether the pathogen is a virus or a bacterium and whether the antigen is viable or not (Table 3.1).[18]

Most inactivated whole-microbe vaccines, toxoids and subunit vaccines evoke relatively simple antibody responses, with meager CD8 T-cell responses. In such cases, the humoral (i.e. antibody) response is likely the principal or only protective mechanism. An antibody that neutralizes a circulating toxin is the paragon of this type of vaccine-induced benefit.

Table 3.1 Diseases that can be prevented with vaccines licensed in Europe or the USA, categorized by microbial type and antigenic type*

	'INACTIVATED' VACCINES				
	Live vaccines	**Whole microbe**	**Subunit**	**Other**	**Typical primary populations**
Bacterial diseases					
Anthrax			Cell filtrate		Occupational
Cholera	Live (oral)	Whole			Endemic areas, travelers
Diphtheria				Toxoid	Throughout life
Haemophilus influenzae type b				Protein-conjugated polysaccharide	Infants, children
Meningococcal, some or all of serogroups A, C, Y, and W135			Polysaccharide, polyvalent	Protein-conjugated polysaccharide, polyvalent	Varies by country; children, adolescents, travelers
Pertussis			Multiple acellular components		Throughout life
Pneumococcal			Polysaccharide, polyvalent	Protein-conjugated polysaccharide, polyvalent	Infants, children, elderly, plus underlying chronic diseases
Tetanus				Toxoid	Throughout life
Typhoid	Live (oral)		Vi polysaccharide		Endemic areas, travelers
Tuberculosis (BCG)	Live				Varies by country; children, selected other groups
Viral diseases					
Hepatitis A		Whole			Varies by country; infants, children, adolescents, occupational, travelers
Hepatitis B			Surface antigen		Varies by country; infants, children, adolescents, occupational, travelers
Influenza A and B			Split virus		Elderly, plus other groups, such as children, adolescents, occupational
Japanese encephalitis		Whole			Endemic areas, travelers
Measles	Whole				Infants, children
Mumps	Whole				Infants, children
Papillomavirus, types 6, 11, 16, 18			Virus-like particles		Adolescents, adults
Poliovirus	Whole	Whole			Infants, children
Rabies		Whole			Occupational, postexposure
Rotavirus (mono- or pentavalent)	Whole				Infants
Rubella	Whole				Infants, children
Tick-borne encephalitis		Whole			
Vaccinia (to prevent smallpox)	Whole				Occupational
Varicella	Whole				Infants, children
Yellow fever	Whole				Endemic areas, travelers
Zoster	Whole				Elderly

*Multiple entries within a row indicate availability of more than one type of vaccine for that disease.

In contrast, live-microbe vaccines can induce both humoral and cellular (i.e. specialized lymphocyte) immune responses against the pathogen; however, in most such cases, the actual mechanisms of protection elicited by either natural infection or vaccination are not understood in detail.

In the course of investigating diphtheria and tetanus in 1890, Emil Behring and Shibasaburo Kitasato identified protective substances that they could transfer from one animal to another.[1] These substances were eventually recognized to be IgG antibodies, found in human serum as well as in other animals. Because these antibodies neutralize toxins, they warrant designation as antitoxins or antisera. These pathogen-specific antibodies offer the ability to provide 'passive immunity', the donation of one person's immunity to another person. Such a benefit provides prompt protection. Unfortunately, the protection from these antibodies is relatively short-lived, generally measured in weeks.

In many cases (e.g. diphtheria, tetanus) the quantity of circulating antibody and memory B cells present at the time of infection is very important in predicting protection. In other cases, such as the relatively long incubation period with hepatitis B infection, people who achieve a sufficient level of antibody response, even if their antibody concentration later falls below detectable limits, may remain protected from infection. This strongly indicates a cellular component to their immunity. While some may believe simplistically that vaccines induce 'sterilizing immunity' (i.e. blocking the pathogen from infecting even one cell), this is clearly not the case with several vaccines (e.g. poliovirus, rotavirus, pertussis).

SPECIFIC VACCINE ADVANCES

In 1885, a few years before Behring's achievement, Louis Pasteur did not understand he was attenuating the rabies virus as he dried rabbit tissue and transformed it into the material for a set of vaccinations of increasing potency. But his novel approach was dramatically successful. Although neither Jenner nor Pasteur understood viruses as we understand them today, their preparations contained whole, viable viruses.[1]

Antibodies developed by Behring and his contemporaries (e.g. Paul Ehrlich) relieved suffering and helped reduce case-fatality ratios. But the immunity induced was too short to much affect community transmission of infections. So Gaston Ramon and Alexander Glenny separately developed modified bacterial toxins ('toxoids') in the 1920s that induced active immunization against diphtheria and tetanus.[1]

A few years later, Ramon, Glenny and their respective teams added a variety of compounds to these toxoids, searching for combinations that would make immune responses to vaccination stronger and more durable. Eventually, these researchers focused on aluminum salts, still the most common adjuvant used worldwide. Their adjuvanted products of the 1920s worked quite well. But, as with smallpox vaccine, it was only with vaccination of large segments of populations in the 1950s that diphtheria and tetanus came under control in developed countries.[1] It would take several decades longer for the Expanded Programme on Vaccination to make prevention of diphtheria, tetanus and several other common infections commonplace in developing countries.[20,21]

In this era, most bacteria (unlike viruses) were readily visible via microscopes and thus the main topics of vaccine development. Vaccine researchers like Waldemar Haffkine, Almroth Wright, Wilhelm Kolle and Richard Pfeiffer and their colleagues, between 1890 and 1910, developed the first bacterial vaccines by cultivating the microbes (e.g. those causing plague, cholera, typhoid), inactivating the bacteria with chemicals and/or heat, and then formulating these products into vaccines for administration.[1] These products contained whole bacteria.

Gradually, through the 20th century, additional whole-microbe vaccines were developed and introduced into clinical practice. But slowly subunit vaccines came into being, as Michael Heidelberger and Oswald Avery identified the capsular antigens of pneumococcal bacteria in the 1920s. Colin MacLeod showed the ability of pneumococcal polysaccharide vaccines to protect against pneumococcal diseases in the 1940s.[1]

Malcolm Artenstein, Irving Goldschneider and Emil Gotschlich developed analogous meningococcal polysaccharide vaccines in the 1960s. Then David Smith and Porter Anderson, Jr, developed the first *Haemophilus influenzae* type b (Hib) polysaccharide vaccine in 1973, subsequently licensed in 1985.[18,22,23]

Unfortunately, polysaccharides do not elicit much of an immune response in young children. John Robbins and Rachel Schneerson solved this problem by developing the technique for conjugating polysaccharides to protein carriers in 1980. This technology was applied first to Hib, then pneumococcal, and finally meningococcal vaccines in succeeding decades.[18,24]

Although whole-cell vaccines were the norm among bacterial vaccines through most of the 20th century, inactivated whole-cell vaccines were entirely replaced by subunit vaccines by 2000 in the USA, and now largely replaced in most developed countries.[18] This transition resulted in fewer injection-site reactions than whole-cell vaccination and more specific immune responses. Whole-inactivated and subunit bacterial vaccines are typically administered with an adjuvant (e.g. an aluminum salt) to enhance a typically modest immunogenicity, relative to live vaccines.

Sometimes research teams approached the goal of vaccine development from very different angles. Perhaps the best-known example was Jonas Salk's injectable, inactivated poliovirus vaccine, developed in 1954, and Albert Sabin's oral, attenuated poliovirus vaccine, developed around 1960.[1,18]

One of the major advances in vaccinology was the discovery by John Enders and his team of how to grow viruses in cell culture in 1954.[1] This breakthrough enabled vaccines against measles, mumps, rubella, varicella and other viruses in the decades that followed. Indeed, most live vaccines in use today were derived by serially propagating initially pathogenic organisms in nonhuman cell culture or at nonphysiologic temperatures for prolonged periods. These conditions allowed evolutionary selection for variants that grow poorly in humans and do not cause clinical symptoms.

Effective vaccines must strike a balance between degree of attenuation and degree of immunogenicity. Modern live vaccines tend to be highly efficacious, with prolonged duration of efficacy. Such vaccines reflect an ability to replicate within vaccinees and expose the immune system to antigens in a manner resembling the nature, anatomic location and effects of natural infection. Because live attenuated vaccines replicate within the vaccinee, they can induce cellular (e.g. CD4, CD8), humoral (B cell) and innate responses and immunologic memory.

In the case of hepatitis B vaccine, the initial vaccines were developed by Maurice Hilleman, Philippe Maupas, and their teams in the mid-1970s.[25,26] These vaccines contained hepatitis B surface antigen (HBsAg) harvested from the serum of chronic carriers. The next step was to harness recombinant DNA technology in the 1980s to develop yeast cells that expressed the same single protein, HBsAg. Such technology is now used to manufacture other vaccines (e.g. human papillomavirus, HPV). HPV vaccines are notable because they consist of virus-like particles (VLPs) that are polymeric spheres built of multiple copies of a single protein.[18]

Most of today's live vaccines are viral vaccines. But there are several remarkable exceptions involving bacteria. In 1921, Albert Calmette and Camille Guérin developed a tuberculosis vaccine, whose common name evokes their memory: bacille Calmette-Guérin (BCG). More recently, live oral vaccines to prevent typhoid or cholera have been licensed (or registered) in various countries.[1,18]

Before closing this section, it is important to note that a vaccination involves far more than the antigen(s) that comprise the active ingredient(s) of a vaccine. The vaccination of one person is the sum of the effects achieved by the antigens, the other components of the vaccine formulation (e.g. adjuvant, carrier proteins), the route of administration, and the number and timing of the vaccine doses. Even then,

the product administered is affected by the recipient's genetic characteristics (e.g. major histocompatibility complex polymorphisms) and physiologic condition (e.g. age, nutrition, gender, stress, immune competence). The net effect of all these parameters determines the degree to which a vaccine confers or evokes immunity in that one person. And, as mentioned above, the real societal benefits come when effective vaccines are used on a broad scale.

Considerations in vaccine use

In contrast to drugs, which are typically prescribed to patients to treat existing diseases, vaccines are generally administered to healthy persons with the objective of preventing disease. With the notable exception of communicable diseases, drugs confer benefit only to the patient who is treated. Vaccines benefit both the vaccine recipient as well as society, and are considered one of the major public health resources to improve the health of the general population. These attributes account for several distinct features in vaccine prescribing by health-care practitioners.

While clinical guidelines are relatively recent as a tool in therapeutics, and represent discretionary suggestions, clinical guidelines have long been integral to vaccine use. Examples of common policies for smallpox and diphtheria vaccines date to the early 20th century, although nationwide policies on vaccine use more commonly arose with the introduction of poliovirus and measles vaccines in the 1950s and 1960s.

Typically, vaccine use is guided by recommendations issued by professional bodies or government agencies, which are often coordinated on a national level (Table 3.2). One of the oldest is the Advisory Committee on Immunization Practices (ACIP), which advises the US Centers for Disease Control and Prevention (CDC). Its working structure is analogous to many similar groups around the globe. ACIP interdisciplinary work groups, consisting of technical and program experts drawn from academia, government and professional associations, evaluate the potential population-based value of new vaccines. Their review incorporates evidence derived from preclinical and clinical safety and efficacy data, as well as public health considerations (e.g. disease burden, populations at risk, ease of integration of the vaccine into the health-care system) and a formal health economic analysis. Although ACIP recommendations are nonbinding, they are considered critical peer-reviewed information for professional associations and individual clinicians, as well as governmental and private payers.[27]

ACIP recommendations are summarized in immunization schedules published periodically in the *Morbidity and Mortality Weekly Report* (http://www.cdc.gov/mmwr). The ACIP monitors licensed vaccines throughout their period of availability, which may last for multiple decades, and periodically revises its recommendations as additional information (e.g. safety data, clinical efficacy in populations not studied at licensure, duration of protection) becomes available.

The approach to developing vaccine recommendations in the European Union (EU) is somewhat more complicated, because of the multiple sovereign countries involved. While efforts are underway to coordinate vaccine registration or licensure, there are to date no European regulations to harmonize vaccine policy making. At present, vaccines are evaluated for registration through a centralized procedure by the European Medicines Evaluation Agency (EMEA) and its Committee for Medicinal Products for Human Use (CHMP). Vaccines are reviewed by a country representing the EU as a whole, and ultimately registered through a mutual recognition procedure based upon endorsement by concerned member states, or (occasionally) by individual countries. In contrast, for vaccine policy making, each member country retains its own national advisory committee with responsibility for national recommendations. As a result, there is significant heterogeneity in vaccine recommendations in the EU.[28] With the continuing integration of the EU and increasing ease of travel among populations, the differences in vaccine schedules may create complexity for practitioners (e.g. providing for infants of immigrants immunized under varying standards) as well as for public health officials. An example of this latter difficulty includes providing for people freely traveling from countries with low hepatitis B endemicity (where hepatitis B vaccination may not be included in the national guidelines) to countries with high hepatitis B endemnicity.

Table 3.2 Selected national or multinational vaccine policy bodies

Australia	Australian Technical Advisory Group on Immunisation (ATAGI)
Canada	National Advisory Committee on Immunization (NACI); Canadian Immunization Committee (CIC)
European Union	European Technical Advisory Committee of Experts (ETAGE)
France	Conseil supérieur d'hygène publique de France (CSHPF); Commité technique des vaccinations (CTV); Institute de Veille Sanitaire (InVS)
Germany	Ständige Impfkommission (STIKO, Standing Advisory Committee on Vaccinations)
Ireland, Republic of	National Immunisation Advisory Committee (NIAC) of the Royal College of Physicians of Ireland
Korea, Republic of	Korea Advisory Committee on Immunization Practice (KACIP)
Mexico	Consejo Nacional de Vacunación (CONAVA)
Netherlands	National Vaccine Institute (NVI); National Institute for Public Health and the Environment (RNVI)
New Zealand	Immunisation Technical Working Group (ITWG)
Poland	Sera-Vaccine Evaluation Committee
Switzerland	Eidgenössische Kommission für Impffragen (also called Commission fédèrale pour les vaccinations, Commissione federale per le vaccinazioni)
Taiwan	Taiwan Advisory Committee for Immunization Practice (ACIP)
United Kingdom	Joint Committee on Vaccination and Immunisation (JCVI)
United States	Advisory Committee on Immunization Practices (ACIP)
World Health Organization	Strategic Advisory Group of Experts (SAGE)

Many developing and mid-income countries lack the regulatory infrastructure and technical expertise to conduct their own formal review of new vaccines. These countries may rely upon assessments made by the World Health Organization (WHO) through its prequalification procedure.[29] The prequalification procedure assesses a candidate vaccine, according to prespecified criteria of quality, safety and efficacy of vaccines, and is often used as a criterion by third-party organizations (e.g. UNICEF) that endorse (and may purchase) vaccines for developing countries. Manufacturers may request review of a vaccine, which involves not only review of safety and efficacy data, but also an inspection by WHO officials of the manufacturing facilities. In addition to the preceding efforts related to product quality, the WHO Strategic Advisory Group of Experts (SAGE) provides vaccine-use recommendations analogous to that of national bodies (see Table 3.2).

Recently, the EMEA recognized an unmet need to evaluate vaccines intended for endemic diseases of the developing world and which may never be licensed (registered) in the EU. A new approach

has been introduced, which provides an expert assessment (in contrast to a licensure evaluation) of such vaccines (as well as therapeutic products).[30]

VACCINE SCHEDULES

Vaccination is a cornerstone of care of infants and small children, and is carefully integrated into routine pediatric care. With the introduction of an increasing number of vaccines (usually administered by injection), manufacturers and public health authorities continue to try to simplify the approach to childhood vaccination. The primary approaches involve combination vaccines (i.e. a single dosage form containing components targeted against more than one disease) as well as through administration of multiple vaccines during a minimal number of visits (consistent with safety and efficacy evidence).

To assure safety and efficacy, vaccines are evaluated when given in combination. The timing of pediatric, adolescent and adult vaccinations is typically summarized in composite vaccine schedules, such as those published by the ACIP (Figs 3.5–3.7).[31,32] With the recent introduction of new vaccines (e.g. human papillomavirus, meningococcal conjugate vaccines) and revised recommendations for longstanding vaccines (e.g. pertussis), there is a growing need for a practical approach to vaccinate adolescents and young adults. Because this population receives less routine scheduled clinical care than infants and young children, new approaches are needed to ensure adequate vaccination. This is also true for older adults, for whom coverage with existing vaccines (e.g. influenza, pneumococcal) has long lagged behind that of pediatric coverage with their respective recommended vaccines. Integration of regular reviews of the immune status of adolescents and adults is essential to avoid vaccine-preventable diseases in these cohorts. A variety of approaches are being tested in various countries to enhance vaccination of adolescents and adults. These include alternate delivery sites (e.g. schools, workplaces, pharmacies), more assertive marketing or outreach campaigns, use of standing orders to empower nurses and pharmacists to attend to needed vaccination, and use of electronic health records to help identify those unvaccinated.

Vaccine safety

Because vaccines are usually administered to healthy individuals to prevent disease, both public health authorities and vaccine manufacturers take extraordinary efforts to ensure safety in the development, manufacture and use of vaccines. Because many vaccines include components derived from biologically active material (e.g. pneumococcal vaccine antigens consist of capsular polysaccharides harvested from bacterial culture), there is a necessary emphasis on assuring that vaccines are manufactured in a consistent and defined manner to promote patient safety. Failure to do so could result in tragedy that compromises both patient health and confidence in public health policies.

This situation was exemplified in the so-called Cutter incident of 1955, in which several lots of inactivated poliovirus vaccine (IPV) were inadequately treated, resulting in poliomyelitis in 79 vaccine recipients, as well as 125 family and community contacts.[33,34] Regulators responded to the incident with more intensive requirements for quality control. In following decades, these quality requirements have become more intricate and specific.

Vaccine development follows a parallel pathway to that used for pharmaceutical products. While the details vary for individual vaccines, in general, preclinical research is followed by three phases of human clinical trials. Phase I studies involve safety, immunogenicity and dose ranging in a few dozen healthy volunteers. Phase II studies add safety and immunogenicity information from a few hundred people with the demographic characteristics expected of those intended to receive the final vaccine, as well as an initial assessment of efficacy. Phase III studies are the large-scale efficacy and safety studies in several thousand people that form the pivotal basis for licensure (registration).[18]

Vaccine registration is like that of chemically defined pharmaceuticals in that preclinical and clinical data are submitted to regulators, but the detailed technical specifications describing the process of vaccine manufacture are more extensive than that of pharmaceuticals. To limit the possibility of contamination, vaccine facilities are typically constructed as dedicated plants designated to manufacture a specific vaccine. Following licensure, vaccine manufacture continues to be actively monitored: a sample of each manufactured vaccine lot is

Recommended immunization schedule for persons aged 0–6 years

Vaccine \ Age	Birth	1 month	2 months	4 months	6 months	12 months	15 months	18 months	19–23 months	2–3 years	4–6 years
Hepatitis B[1]	HepB	HepB		see footnote 1	HepB						
Rotavirus[2]			RV	RV	*RV*[3]						
Diphtheria, tetanus, pertussis[3]			DTaP	DTaP	DTaP	see footnote 3	DTaP				DTaP
Haemophilus influenzae type b[4]			Hib	Hib	*Hib*[4]	Hib					
Pneumococcal[5]			PCV	PCV	PCV	PCV				PPSV	
Inactivated poliovirus			IPV	IPV	IPV						IPV
Influenza[6]					Influenza (yearly)						
Measles, mumps, rubella[7]						MMR		see footnote 7			MMR
Varicella[8]						Varicella		see footnote 8			Varicella
Hepatitis A[9]						HepA (2 doses)				HepA Series	
Meningococcal[10]										MCV	

Range of recommended ages

Certain high-risk groups

Fig. 3.5 Recommended immunization schedule for persons aged 0–6 years – United States, 2009. Redrawn from Advisory Committee on Immunization Practices.[31] (See reference for detailed footnotes and ancillary information.)

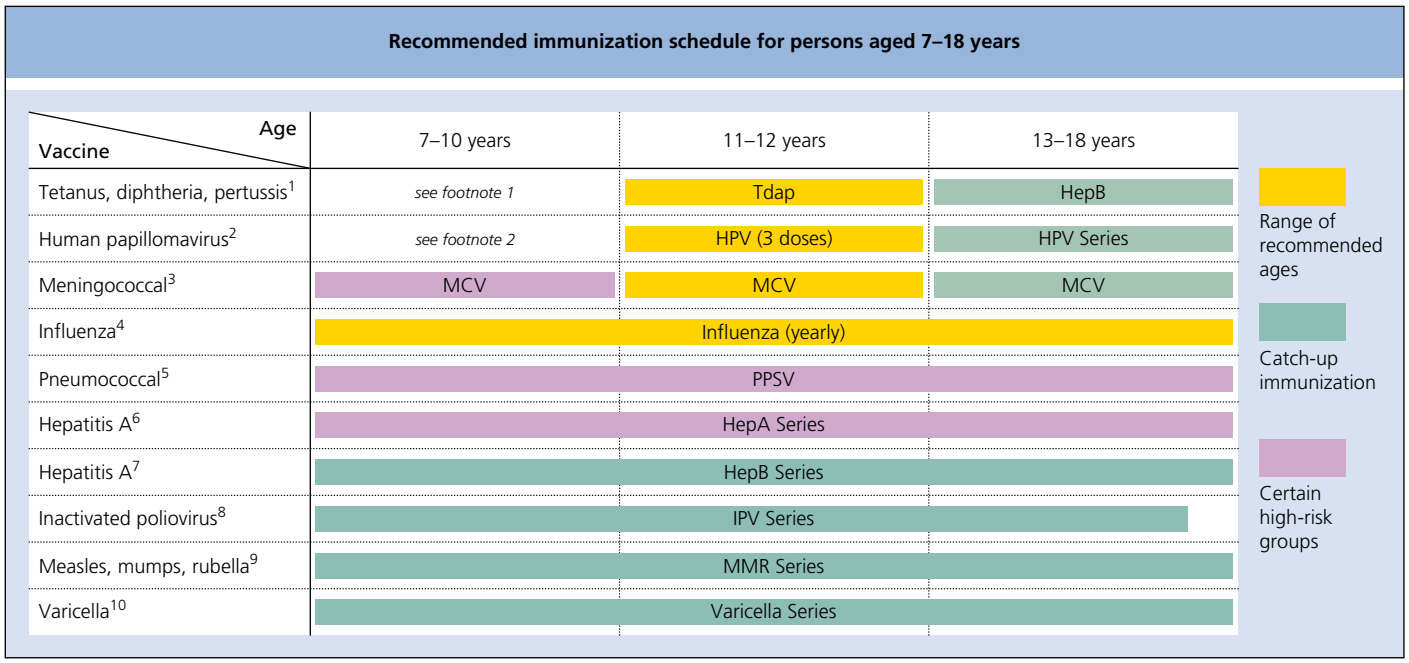


Fig. 3.6 Recommended immunization schedule for persons 7–18 years – United States, 2009. Redrawn from Advisory Committee on Immunization Practices.[31] (See reference for detailed footnotes and ancillary information.)

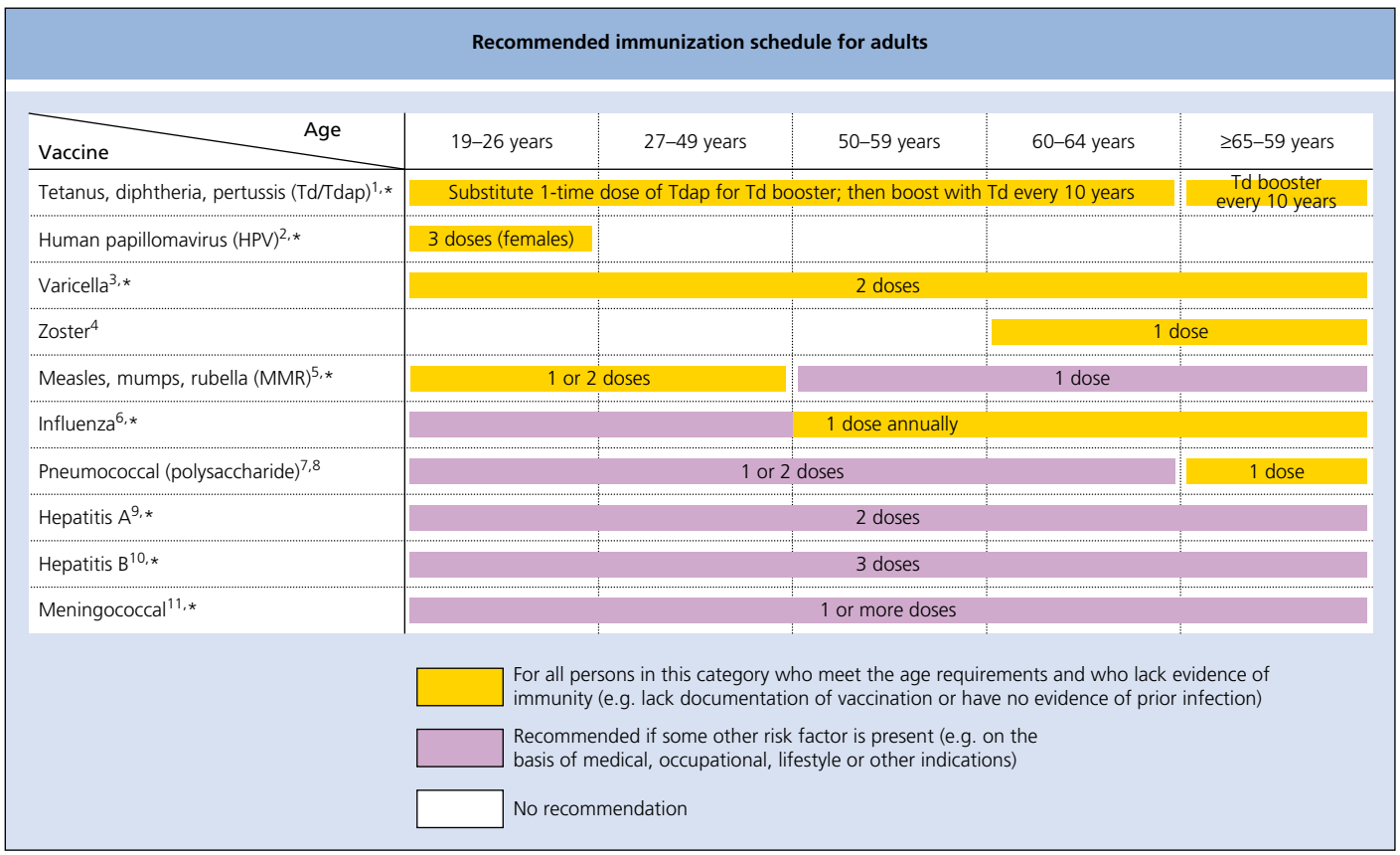


Fig. 3.7 Recommended immunization schedule for adults – United States, 2009. Redrawn from Advisory Committee on Immunization Practices.[32] (See reference for detailed footnotes and ancillary information.)

typically provided to the US Food and Drug Administration (FDA) for review. Any change in protocol/manufacture requires formal regulatory review.

Licensed (registered) vaccines undergo extensive safety testing, which may include clinical trials enrolling tens of thousands of individuals. But clinical trials can never be statistically powered to reliably identify extremely rare adverse events. Several post-licensure measures exist to ensure that adverse events associated with vaccine use are identified promptly and completely. Manufacturers are required to promptly communicate to regulators those adverse events reported by providers and consumers, as well those identified in post-marketing studies, along with relevant publications.

In the USA, a spontaneous vaccine adverse event reporting system (VAERS, http://www.vaers.hhs.gov) is administered jointly by the FDA and the CDC. The corresponding system in Canada is called the Canadian Adverse Events Following Immunization Surveillance System (CAEFISS). These surveillance systems and their counterparts worldwide collect vaccine safety information from consumers, providers and manufacturers.

Passive surveillance systems can identify extremely rare events after vaccination (e.g. Guillain-Barré syndrome) and are most appropriately considered a mechanism for signal detection. These systems do not collect information on overall vaccine use and cannot provide quantitative estimates of risk (i.e. strength of association) or of incidence.

Once a potential safety signal is identified, formal epidemiologic studies may be employed to assess strength of association. One mechanism for this type of research is the Vaccine Safety Datalink (VSD) coordinated by the CDC. A network of US managed-care organizations conduct controlled epidemiologic studies to evaluate safety signals. The RotaShield (quadrivalent rhesus-human reassortant rotavirus vaccine) experience provides an example of the manner in which these safety systems operate in a complementary manner. During clinical trials of this vaccine designed to prevent pediatric rotavirus infection, a small but not statistically significant number of cases of intussusception was noted, but considered to occur at background rate. This potential safety concern was noted in the ACIP recommendation. Some months following introduction of RotaShield, the VAERS system became concerned that the number of cases of intussusception occurring in vaccine recipients was larger than expected by background rates and that vaccine use should be temporarily suspended. Several CDC epidemiologic studies then quantified the risk of intussusception in vaccinees, and concluded that it exceeded the expected rate. RotaShield was withdrawn from the market.[35–37] Subsequent rotavirus vaccines were subjected to larger safety studies with special focus on intussusception before they were licensed, with additional post-licensure studies after registration.

Vaccine use in special populations

Pregnancy

Most clinical studies leading to vaccine licensure do not routinely enroll pregnant women; indeed, pregnancy is a common exclusion criterion. As a result, information on vaccine safety in pregnant women is commonly based upon empiric evidence derived from other sources:

- women enrolled in vaccine trials who inadvertently become pregnant during the course of study;
- data made available through passive reporting systems (e.g. VAERS); and
- specific registries (often sponsored by vaccine manufacturers) that collect data prospectively on women seen in clinical practice who happen to become vaccinated while pregnant.[38–41]

Due to concerns regarding teratogenicity, live viral vaccines (e.g. rubella, vaccinia, varicella) are typically contraindicated during pregnancy. There is no convincing evidence of harm resulting from vaccinating pregnant women with inactivated viral or bacterial vaccines, or with toxoids. The risk of hospitalization due to influenza infection is elevated in pregnancy; thus, women pregnant during the influenza season are recommended for vaccination with inactivated influenza vaccine.[42,43] Because neonatal tetanus is associated with severe infant outcomes, and maternal antibodies to tetanus have been shown to be protective in infants, incompletely vaccinated pregnant women are also recommended for tetanus toxoid vaccination.

Vaccines for international travel

The increasingly globalized society is associated with unprecedented international travel, traveling far from their places of residence, and risking exposure to diseases that are either well controlled or not found in their homeland. In addition, travelers may import infectious diseases back to their homeland. The key principles of vaccination for travel are to ensure that patients are up to date on their routine ('domestic') vaccinations (e.g. tetanus, measles, influenza, and to provide vaccinations against vaccine-preventable diseases in those countries that they will visit. Several websites summarize vaccination recommendations by country (http://www.cdc.gov/travel; http://www.who.int/ith/en). While the decision regarding appropriate vaccination fundamentally belongs to patients and providers, certain vaccines may be required to enter specific countries (e.g. yellow fever) under international health regulations. Evidence of vaccination is provided in standard travel documents recognized by the WHO (International Certificate of Vaccination, http://www.who.int/csr/ihr/icvp/en/print.html).

Immunocompromised populations (see also Chapter 83)

Immunocompromised patients are at increased susceptibility to infection, may not respond to vaccination with adequate immunogenicity, and could face added risk of adverse events after live vaccinations. Increased risk for infection is a sine qua non for immunosuppression. Vaccine recommendations in these populations balance the estimated risk for specific vaccine-preventable infections against known (based usually on empiric data) safety and efficacy information. In general, individuals who will, through medical intervention (e.g. autologous bone marrow transplantation), become immune suppressed, are vaccinated in a timely manner to optimize their vaccine immune response in advance of the period of immune suppression. Live (self-replicating) vaccines may pose a safety concern and are not generally administered to immune-suppressed persons. Killed (or subunit) vaccines are considered safer than live vaccines for these cohorts. Hepatitis B vaccine is of particular interest due to the concern of the risk of infection in a seronegative patient recipient of hepatitis B virus transmitted by solid organ transplant or by transfusion. These patients should be vaccinated well before transplantation to allow induction of immunity. HIV infection is associated with progressive immunodeficiency; recommendations for vaccination are based upon the stage of disease (and by patient age). The reader is referred to specialized texts for detailed information on vaccination in this population.[44–55]

FUTURE PROSPECTS FOR VACCINES

While vaccines provide some of the greatest contributions to improving the health of individuals and populations, tremendous unmet public health opportunities remain. These include both the development of new vaccines for infectious diseases that cannot yet be prevented effectively and programs to meet the needs of cohorts who do not have reliable access to existing vaccines.

Not only will the future bring vaccines to prevent currently uncontrolled diseases, it will likely harness new technologies to do so. Promising new advances may allow DNA or viral-vector vaccines to take their places on shelves next to vaccines consisting of whole microbes, subunits and toxoids. DNA vaccination is accomplished by injecting

genetically engineered DNA (in the form of circular plasmids) into the body, where the gene expresses the corresponding immunogenic protein. With viral-vectored vaccines, a suitable virus (e.g. vaccinia, one of the adenoviruses) is genetically engineered to convey genetic material into cells, where it can be expressed. With each of these approaches, intracellular expression can elicit T-cell immunity. Practical challenges to overcome include induction of sufficient immune responses to confer immunity, overcoming pre-existing immunity to the viral vector, and questions about product safety. These approaches are also limited to protein epitopes.

Several resources track the development of new vaccines targeted against outstanding infectious diseases.

The US National Institute of Allergy and Infectious Diseases (http://www3.niaid.nih.gov) publishes *The Jordan Report: Accelerated Development of Vaccines*, to periodically summarize global vaccine development.[56] First issued in 1982, the 2007 edition complements a review of investigational vaccines with a discussion of programmatic issues in vaccine implementation (e.g. development of an approach to vaccinate adolescents).

The World Health Organization also provides a periodic update on the status of vaccines in development, along with its assessment of the outstanding public health need for those vaccines.[57] The American Society for Microbiology does likewise.[58]

In 2000, the Institute of Medicine (IOM, a component of the US National Academy of Sciences) issued a report entitled *Vaccines for the 21st Century: A Tool for Decision Making*.[59] This publication focused upon US public health needs and prioritized 26 candidate vaccine targets (ranked by levels; level I refers to those considered most attractive) based on their public health relevance and the estimated likelihood that they might be licensed within 20 years. In addition, the reviewers included vaccine program implementation considerations, along with a health economic assessment, in their recommendations (Table 3.3).

Notably, the IOM panel did not consider HIV/AIDS, for which a preventive vaccine would be of extraordinary public health value. Efforts to develop a successful HIV vaccine have bedeviled researchers for almost 20 years.[60] The recent failure of an adenovirus vector-based HIV-1 vaccine intended to induce a cell-mediated immunity response, speaks to the scientific uncertainty and technical complexity of successful vaccine development against this pathogen.[61–63] Decisions to develop vaccines are sometimes subject to pressures outside of those considered by expert panels (or by market forces), including specific ethical, legal and regulatory hurdles.[64]

The IOM report also included proposed vaccines for conditions not generally considered infectious (e.g. multiple myeloma), highlighting expectations that future vaccines will expand their scope beyond the traditional limits of infectious diseases. It is worth noting that some current vaccines have public health value beyond the prevention of an infection. Two widely used licensed vaccines help prevent cancer: the hepatitis B vaccines (i.e. to prevent hepatocellular carcinoma) and human papillomavirus vaccines (i.e. to prevent cervical, vaginal and other cancers). Widespread use of hepatitis B vaccine has been associated with a decline in liver cancer deaths.[65,66] In the controlled trials supporting licensure, one of the recently introduced human papillomavirus vaccines has already demonstrated a decrease in the incidence of cervical cancer precursor lesions;[67] however, the long natural history of HPV infection leading to cervical cancer makes it likely that some additional time will be needed for population-based declines in cervical cancer in vaccinated populations to become apparent. Research is underway with therapeutic cancer vaccines, and the reader is referred to specialized texts for additional information.[68]

One of the great and unfortunate paradoxes in public health is that many vaccines have not been readily available or routinely utilized by populations who bear the greatest burden of a disease. While widespread adoption of newly recommended vaccines in industrialized countries usually occurs within a few years, there has historically been a delay in common use in developing countries where vaccine-preventable diseases are often endemic, and where access to therapeutic care is typically hindered by lack of medical resources.

Hepatitis B virus vaccines and *Haemophilus influenzae* type b vaccines, each of which targets important pathogens common in the developing world, only began to be widely introduced there 10–15 years after their licensure in the USA.[69] However, it is possible that global access to new vaccines is beginning to improve.

In affluent countries, rotavirus infection is almost universal, yet mortality is rare.[70,71] In contrast, the WHO estimates that more than 500 000 children die annually due to this infection, with a majority of the deaths concentrated in South and South East Asia.[72] The rotavirus vaccine licensed in the USA in 2006 was associated with a decline in domestic rotavirus cases within just 2 years of widespread use[5] (see Fig. 3.4). Although rotavirus vaccines are not yet widely used in developing countries, clinical trials are presently underway in some developing countries with a high rotavirus burden of illness, and efforts are in place to provide funding and access to this vaccine in a timely fashion.

These programs include networks of organizations, institutions and companies working in vaccine development and use, such as the Global Alliance for Vaccines and Immunization (GAVI), large private foundation contributions (e.g. Bill and Melinda Gates Foundation) and government-supported 'push' mechanisms to encourage development of vaccines specifically for developing world use (e.g. advance market commitments).[73] Initiatives such as these are considered critical to overcome the significant costs (vaccine purchase, delivery and surveillance) that have so far impeded the use of vaccines to reduce the enormous burden of vaccine-preventable diseases in the developing world.[74,75]

Beyond issues of immunology and vaccine technology, those interested in preventing disease through vaccination will need to address society's needs for thorough safety evaluation. The last 20 years have witnessed several concerns about vaccine safety become widely debated (e.g. autism, diabetes, sudden infant death syndrome). Those questions have been answered through epidemiologic and other studies, which

Table 3.3 Priorities for vaccine development, US Institute of Medicine[59]

Level I (highest priority)	Level II	Level III	Level IV
Cytomegalovirus (CMV)	*Chlamydia, Helicobacter pylori*	Parainfluenza	*Borrelia burgdorferi*
Influenza	Hepatitis C virus	Rotavirus	*Coccidioides immitis*
Insulin-dependent diabetes mellitus*	Herpes simplex virus	Group A streptococcus	Enterotoxigenic
Multiple sclerosis*	Human papillomavirus	Group B streptococcus	*Escherichia coli*
Rheumatoid arthritis*	Melanoma*		Epstein–Barr virus
Group B streptococcus	*Mycobacterium tuberculosis*		*Histoplasma capsulatum*
Streptococcus pneumoniae	*Neisseria gonorrhoeae*		*Neisseria meningitidis* type b
Diabetes mellitus	Respiratory syncytial virus		Shigella

Note: HIV/AIDS was omitted from consideration.
*Not usually considered infectious in origin.

now provide a substantial body of scientific evidence. With the success of vaccines in making so many now-preventable diseases recede from public attention, specific efforts are needed to explain what vaccines have accomplished and why continued vaccination is needed to keep these diseases suppressed. Vaccines have improved many millions of lives around the world. Expanded use of existing vaccines and development of new vaccines are likely to add substantially to this remarkable record of achievement.

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Chapter 4

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Emerging and re-emerging pathogens and diseases

INTRODUCTION

Humans and microbes have engaged in an epic struggle for survival since human life began. Rapid microbial evolution and adaptation allow bacteria, viruses and parasites to overcome human defenses (e.g. physiologic mechanisms and manmade drugs) and exploit human behaviors (e.g. sexual practices and methods of food production and preparation). Some zoonotic microbes have 'jumped' from animals to humans to become major human pathogens. The ancestors of smallpox virus and malaria parasites, for example, probably became human pathogens 10 000 years ago when humans began to domesticate animals in settlements that were large enough to sustain human-to-human transmission. In recent times the AIDS virus, one of the most destructive pathogens in human history, evolved from a virus carried by a nonhuman primate.[1]

Human evolution, though far slower and more difficult to observe, has in turn been influenced by microbes. Some pathogens have apparently been sufficiently virulent and widespread over the course of human history to affect the make-up of the human genome. The best evidence for this concerns malaria parasites, which are less likely to kill individuals who have globin gene alleles that make their red blood cells poor hosts for intracellular parasites (e.g. sickle hemoglobin and α- and β-thalassemias).[2] Moreover, most or all components of the human immune system probably evolved to prevent disease agents from co-opting human cellular machinery for microbial gene expression and reproduction. In addition, the human body benefits from the presence of nonpathogenic, symbiotic microbes, such as bacteria in the human gut that synthesize components of the vitamin B complex (folic acid and biotin).

Today, the increasing complexity of human behavior coupled with our ability to change our natural environment has hastened the pace of disease emergence and re-emergence (Fig. 4.1). Many modern human activities facilitate microbial transmission (e.g. air travel and the globalization of the food supply; see Table 4.1),[3–5] while others have decreased it (e.g. improvements in sanitation, antimicrobial drugs, vaccines and disease control and eradication programs). Looking ahead, the most significant risk factor for disease emergence in the 21st century is likely to be population growth and urbanization, leading to the creation of megacities (a city with 10 million inhabitants)[6] throughout the developing world. According to a recent United Nations report (http://www.unfpa.org/swp/2007/english/introduction.html), more than half the world's population will be living in urban areas by the end of 2008, and by 2025 there are likely to be eight new megacities, most of them in the developing world, bringing the total to 28. By 2050, 6.4 billion people will be living in urban areas, out of a total projected population of 9.2 billion. These population increases will be associated with further stresses on resources (e.g. clean air, water and food) and natural habitats (e.g. higher temperatures caused by global warming that allow disease-carrying mosquitoes to move into new areas). Moreover, as human cities expand into forested areas, increased contact between humans and wildlife may lead to human infection with previously unknown animal pathogens that can cause human disease. Intensified livestock production – both traditional and modern – to meet increasing demand may also facilitate the spread of new and re-emerging zoonotic diseases (http://www.ifpri.org/2020/dp/dp28.pdf).

New and re-emerging infectious diseases

Over the past 40 years, more than 40 new human pathogens have been identified and characterized, often by using serologic and molecular methods (Table 4.2). Some of these microbes cause diseases of global importance (e.g. HIV/AIDS, hepatitis C, rotavirus diarrheal disease; and the (H1N1) 2009 influenza pandemic). Others appear to be limited (thus far) to particular countries or continents (e.g. Ebola hemorrhagic fever in central Africa (although simian Ebola has also been identified in the Philippines); Argentinian, Bolivian, Venezuelan and Sabia-associated hemorrhagic fevers in South America; hantavirus pulmonary syndrome in the Americas; and new variant Creutzfeldt–Jakob disease in Europe, Japan, Canada, and Saudi Arabia). A recent example is the 2003 outbreak of severe acute respiratory syndrome (SARS), caused by a novel coronavirus, which spread overnight from Hong Kong to Canada and several other countries by airplane (Fig. 4.2; see also below). In addition to these newly identified diseases, several 'old' threats have re-emerged in new or drug-resistant forms (Table 4.3). These include highly virulent epidemic strains of *Mycobacterium tuberculosis* (see below) and *Clostridium difficile*.[10,11]

Selected examples of major outbreaks since 1993 are listed in Table 4.4.

The essential role of the clinician in disease detection and surveillance

Physicians are in the best possible position to observe and report unusual illnesses, syndromes and disease risk factors. In 1993, for example, an Indian Health Service physician reported a cluster of fatal cases of unexplained respiratory disease in the southwestern United States that proved to be caused by a previously unrecognized hantavirus. In 1999, the first US outbreak of West Nile encephalitis was identified when an infectious disease physician reported unusual neurologic disease in three elderly people who lived in the same area (Fig. 4.3).[12] West Nile encephalitis – whose causative agent is carried by migratory birds in Africa, the Middle East, and Europe and transmitted to humans by mosquito bite – had never before been reported in the Western hemisphere.[13] In 2000, physicians in New York, London and Toronto helped identify an international outbreak

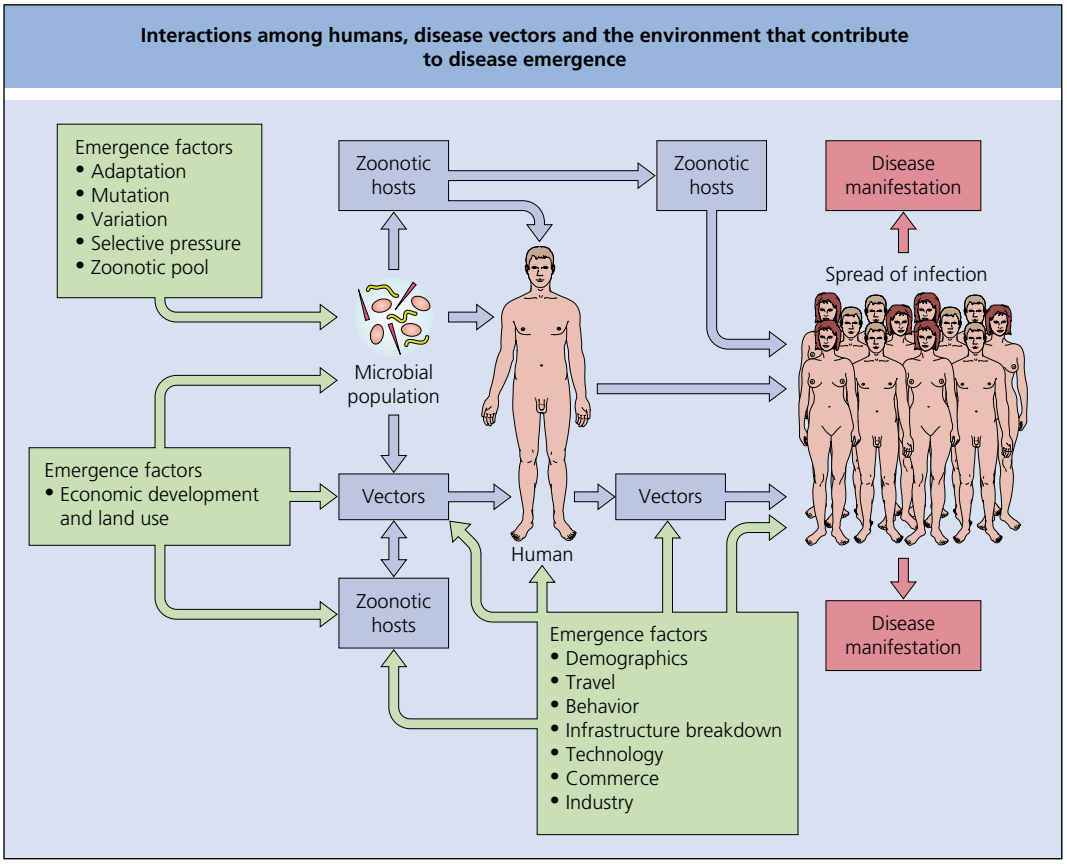


Fig. 4.1 Interactions among humans, disease vectors and the environment that contribute to disease emergence. *Source*: Institute of Medicine. Emerging infections: microbial threats to health in the United States. Washington, DC: National Academy Press; 1992.[3] (In March 2003, the Institute of Medicine published a reassessment: Microbial threats to health: emergence, detection, and response. Washington DC: National Academy Press; 2003.[4])

of leptospirosis among athletes returning home from a competition in Malaysian Borneo. These physicians reported their findings to Geosentinel, the surveillance network of the International Society of Travel Medicine (http://www.istm.org/geosentinel/main.html), which alerted travel clinics in 11 countries. An astute clinician was also responsible for reporting the first cases of a multistate outbreak of *Cyclospora* infection that was associated with imported raspberries.[14] Later cases were detected in 20 states, the District of Columbia and two Canadian provinces.

Table 4.1 Factors in the emergence of infectious diseases

Modern demographic, environmental, and behavioral factors that favor the spread of infectious diseases include:

- global travel
- globalization of the food supply and centralized processing of food
- population growth and increased urbanization and crowding
- population movements due to civil wars, famines and other manmade or natural disasters
- irrigation, deforestation and reforestation projects that alter the habitats of disease-carrying insects and animals
- human behaviors, such as intravenous drug use and risky sexual behavior
- increased use of antimicrobial agents and pesticides, hastening the development of resistance
- increased human contact with tropical rainforests and other wilderness habitats that are reservoirs for insects and animals that harbor unknown infectious agents
- deteriorating public health infrastructures in many parts of the world
- changes in climate and weather patterns
- lack of political will to institute disease control measures
- intentional release of pathogenic agents by terrorists.

Adapted from references[3–5].

The US anthrax attacks in autumn 2001 also highlighted the crucial role of clinicians in monitoring unusual and dangerous diseases. The first case of anthrax (an inhalational case) was recognized in a hospitalized patient by an infectious disease physician in Palm Beach County, Florida, and six of eight anthrax cases detected in New York City (one inhalational and seven cutaneous) were reported to city health authorities by alert physicians. Three were diagnosed by infectious disease physicians (including the inhalational case), one by a dermatologist, one by a public health physician and one by an emergency room physician. Physicians also reported anthrax cases in Washington DC and New Jersey.

As these examples illustrate, the vigilance of physicians and other health-care providers remains the most important factor in infectious disease surveillance and control and in recognition of new and emerging infectious diseases.

Linkages between animal and human disease surveillance

Between 60% and 75% of all emerging and many re-emerging infectious diseases of human health importance are zoonotic.[15] The intimate relationship between animal and human health is illustrated by the emergence of the pandemic (H1N1) 2009 influenza virus, which includes gene segments from humans, birds, and pigs. Other examples include the 1999 outbreak of West Nile encephalitis, which was preceded by die-offs

Table 4.2 Newly identified microbial pathogens

	Agent	Type	Disease
1972	Calicivirus (Norwalk agent)	Virus	Acute gastroenteritis
1973	Rotavirus	Virus	Major cause of infantile diarrhea worldwide
1975	Parvovirus B19	Virus	Aplastic crisis in chronic hemolytic anemia
1976	*Cryptosporidium parvum*	Parasite	Acute and chronic diarrhea
1977	Ebola virus	Virus	Ebola hemorrhagic fever
1977	Hantaan virus	Virus	Hemorrhagic fever with renal syndrome (HFRS)
1977	*Legionella pneumophila*	Bacterium	Legionnaires' disease
1977	*Campylobacter jejuni*	Bacterium	Enteric pathogens distributed globally
1980	Human T-lymphotropic virus type 1 (HTLV-1)	Virus	T-cell lymphoma-leukemia
1981	Toxin producing strains of *Staphylococcus aureus*	Bacterium	Toxic shock syndrome
1982	*Escherichia coli* O157:H7	Bacterium	Hemorrhagic colitis; hemolytic uremic syndrome
1982	Human T-lymphotropic virus type 2 (HTLV-2)	Virus	Hairy cell leukemia
1982	*Borrelia burgdorferi*	Bacterium	Lyme disease
1983	Human immunodeficiency virus (HIV)	Virus	Acquired immunodeficiency syndrome (AIDS)
1985	*Helicobacter pylori*	Bacterium	Peptic ulcer disease
1985	*Enterocytozoon bieneusi*	Parasite	Persistent diarrhea
1986	*Cyclospora cayetanensis*	Parasite	Persistent diarrhea
1988	Human herpesvirus 6 (HHV-6)	Virus	Exanthema subitum
1988	Hepatitis E	Virus	Enterically transmitted non-A, non-B hepatitis
1989	*Ehrlichia chafeensis*	Bacterium	Human ehrlichiosis
1989	Hepatitis C	Virus	Parenterally transmitted non-A, non-B hepatitis
1991	Guanarito virus	Virus	Venezuelan hemorrhagic fever
1991	*Encephalitozoon hellem*	Parasite	Conjunctivitis, disseminated disease
1991	New species of *Babesia*	Parasite	Atypical babesiosis
1992	*Bartonella henselae*	Bacterium	Cat-scratch disease; bacillary angiomatosis
1992	*Tropheryma whipplei*	Bacterium	Impaired absorption of nutrients, weight loss, joint pain, and anemia
1992	*Vibrio cholerae* O139	Bacterium	Cholera with wide-spectrum drug resistance
1993	Sin Nombre virus	Virus	Hantavirus pulmonary syndrome (HPS)
1993	*Encephalitozoon cuniculi*	Parasite	Disseminated disease
1994	Sabia virus	Virus	Brazilian hemorrhagic fever
1994	Henipaviruses (Hendra)	Virus	Encephalitic disease carried by fruit bats that can be transmitted from horses to humans (Hendra)
1995	Human herpesvirus 8 (HHV-8)	Virus	Associated with Kaposi's sarcoma in AIDS patients
1996	New variant Creutzfeldt–Jakob disease agent	Prion	Progressive degenerative neurologic disease
1997	H5N1 strain of avian influenza	Virus	Influenza transmitted from chickens to humans; often fatal
1999	Henipaviruses (Nipah)	Virus	Encephalitic disease carried by fruit bats that can be transmitted from pigs to humans
2001	Human metapneumovirus	Virus	Acute respiratory infections
2002	Vancomycin-resistant *Staphylococcus aureus*	Bacterium	First vancomycin-resistant *S. aureus* strain identified in the USA
2003	SARS coronavirus	Virus	Severe acute respiratory syndrome (SARS)
2003	*Clostridium difficile*, strain NAPI/027	Bacteria	Pseudomembranous colitis

Table 4.2 Newly identified microbial pathogens—cont'd

	Agent	Type	Disease
2005	Bocavirus	Virus	Associated with lower respiratory tract infections in children; discovered through molecular screening of respiratory tract samples
2007	New strain of Ebola (the fifth one identified)	Virus	Hemorrhagic fever
2008	Transplant-associated arenavirus related to lymphocytic choriomeningitis virus	Virus	Severe febrile illness
2008	*Plasmodium knowlesi* as a human pathogen	Parasite	Previously classified as a cause of simian malaria, this parasite is now known to be a cause of human malaria in Malaysia, sometimes misdiagnosed as *Plasmodium malariae*, a milder form of malaria
2009	Pandemic (H1N1) 2009 virus	Virus	Pandemic influenza (see Chapter 161)

Updated from World Health Organization.[7]

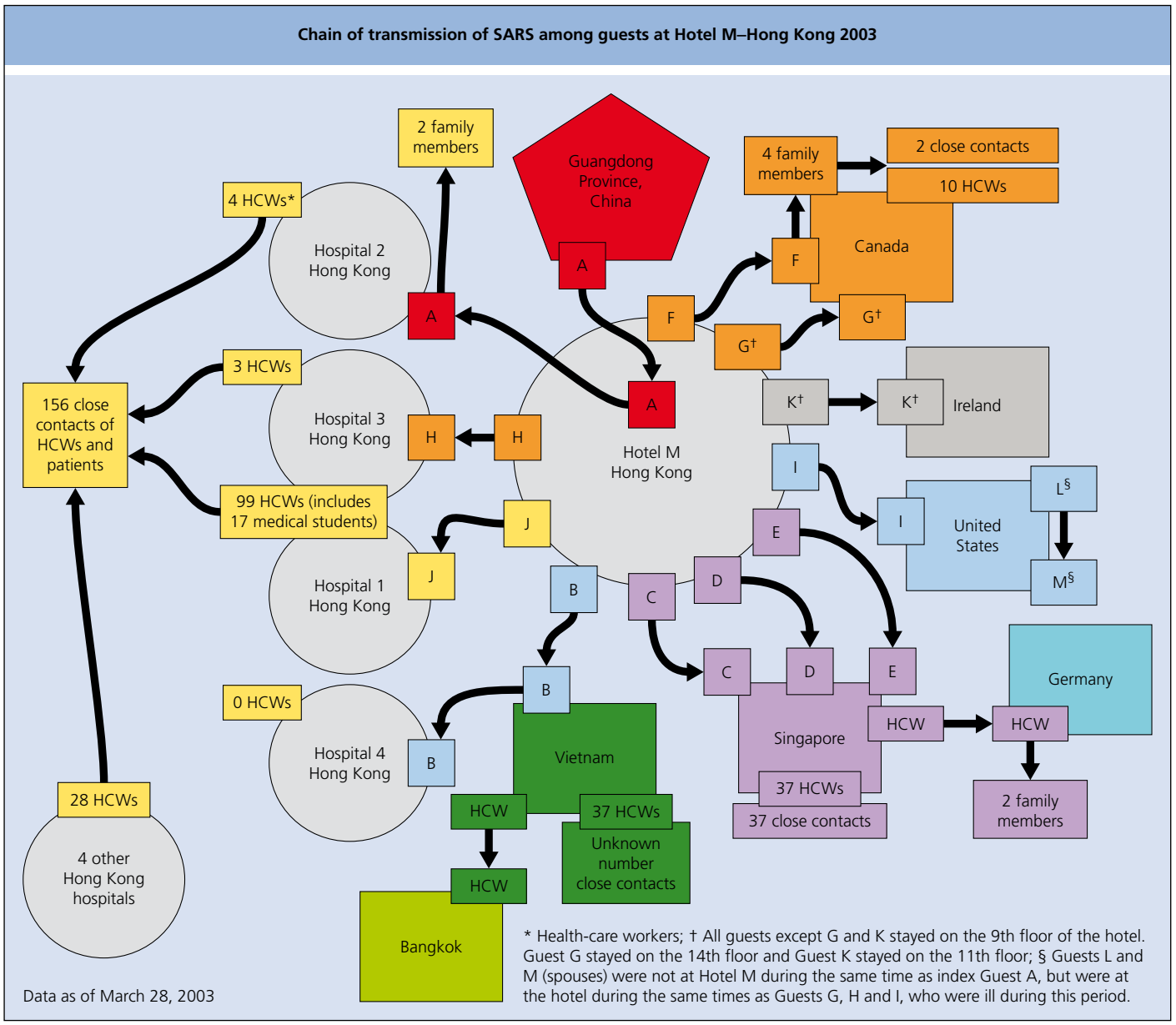


Fig. 4.2 Chain of transmission of SARS among guests at Hotel M – Hong Kong, 2003. Adapted from Centers for Disease Control and Prevention.[8]

Table 4.3 Resurging diseases

Disease or agent	Factors in re-emergence
Viral	
Rabies	Breakdown in public health measures; changes in land use; travel
Dengue and dengue hemorrhagic fever	Transportation; travel and migration; urbanization. Many countries reported high numbers of dengue infections in 2007; this trend continued in 2008, with a large outbreak of 120 570 cases reported in Brazil, including 75 399 cases in Rio de Janeiro
Yellow fever	Favorable conditions for growth of the mosquito vector. In June 2008, Paraguay reported the first identified yellow fever cases in more than 30 years, some of them near the capital city, Asuncion. The apparent re-emergence of urban yellow fever, which can spread rapidly through susceptible populations, has raised public health concerns in other countries in the region
Rift Valley fever	Infected humans, animals or mosquitoes that have traveled across East Africa to Saudi Arabia and Yemen
Polio	Cessation in vaccination campaigns or decline in average vaccine coverage
Chikungunya fever	A strain of chikungunya virus isolated during a 2005–2006 outbreak on Reunion Island (an island in the Pacific Ocean) has a mutation[1,2] that may facilitate transmission by *Aedes albopictus* (the Tiger mosquito), which is native to South East Asia, but is now present on all continents except Antarctica. Disease spread via travelers who visited chikungunya-affected areas in India, Mauritius or other islands in the Pacific Ocean apparently led to the first cases of local transmission in Italy (in 2007) and in Australia, Singapore and Malaysia (in 2008)
Parasitic	
Malaria	Drug and insecticide resistance; civil strife; lack of economic resources
Schistosomiasis	Dam construction, improved irrigation and ecologic changes favorable to the snail host
Neurocysticercosis	Immigration; agricultural practices
Acanthamebiasis	Introduction of soft contact lenses
Visceral leishmaniasis	War; population displacement; immigrations; habitat changes favorable to insect vector and increase in immunocompromised human hosts
Toxoplasmosis	Increase in immunocompromised human hosts
Giardiasis	Increased use of child-care facilities
Echinococcosis	Ecologic changes that affect the habitats of the immediate (animal) hosts
Trypanosomiasis (African sleeping sickness)	Breakdown in public health infrastructure
Bacterial	
Tuberculosis	Human demographics and behavior; international commerce and travel; breakdown of public health measures; microbial adaptation (i.e. multidrug-resistant tuberculosis [MDR-TB] and extensively drug-resistant tuberculosis [XDR-TB]); the HIV/AIDS pandemic
Trench fever	Breakdown of public health measures
Plague	Economic development; land use
Diphtheria	Interruption of immunization programs due to political changes
Pertussis	Refusal to vaccinate in some countries because of belief that pertussis vaccines are not safe
Salmonella	Industry and technology; human demographics and behavior; microbial adaptation; changes in food production
Pneumococcus	Human demographics; microbial adaptation; international travel and commerce; misuse and overuse of antibiotics
Cholera	International travel; long-term increases in sea surface temperatures and sea levels that may lead to higher concentrations of *Vibrio cholerae,* which grow on zooplankton that flourish in warm water

[1]Martin E. Epidemiology: tropical disease follows mosquitoes to Europe. Science 2007; 317(5844):1485.
[2]Tsetsarkin KA, Vanlandingham DL, McGee CE, Higgs S. A single mutation in Chikungunya virus affects vector specificity and epidemic potential. PLoS Pathog 2007;3(12):e201.
Adapted and updated from Lorber.[9]

Table 4.4 Selected infectious disease challenges, 1993–2008

1993	Hantavirus pulmonary syndrome (USA)
1994	Plague (India)
1995	Ebola fever (Zaire)
1996	New variant Creutzfeldt–Jakob disease (UK)
1997	H5N1 influenza (Hong Kong); vancomycin-intermediate resistant *Staphylococcus aureus* (Japan, USA)
1998	Nipah virus encephalitis (Malaysia, Singapore)
1999	West Nile encephalitis (Russia, USA)
2000	Rift Valley fever (Kenya, Saudi Arabia, Yemen); Ebola fever (Uganda)
2001	Anthrax (USA); foot and mouth disease (UK)
2002	Vancomycin-resistant *Staphylococcus aureus* (USA)
2003	Severe acute respiratory syndrome (SARS)
2003	Re-emergence of H5N1 influenza (beginning in South East Asia and spreading internationally)
2003	Monkeypox (USA)
2003	Highly virulent strain of *Clostridium difficile* (Canada, the Netherlands, UK, USA)
2005–2006	Multistate outbreak of mumps in the USA, involving 11 states (The widespread use of a second dose of mumps vaccine among US schoolchildren beginning in 1990 was followed by historically low reports of mumps cases, and it was hoped that the disease might be eliminated by 2010. However, a multistate outbreak of mumps – the largest US outbreak in two decades – occurred in 2006)
2004	Chikungunya fever outbreak associated with high fever and severe protracted joint pain in Lamu Island, Kenya
2007	New strain of Ebola (the fifth one identified), responsible for large outbreaks in Uganda and the Congo
2008	Measles outbreaks in Europe (Switzerland, Austria, Ireland and Britain), Israel and the USA (San Diego, California), associated with declining rates of measles vaccination
2008	Outbreak of Zika fever (a mosquito-borne illness similar to dengue) in the Yap Islands, Federated States of Micronesia
2009	Pandemic (H1N1) influenza. See Chapter 161.

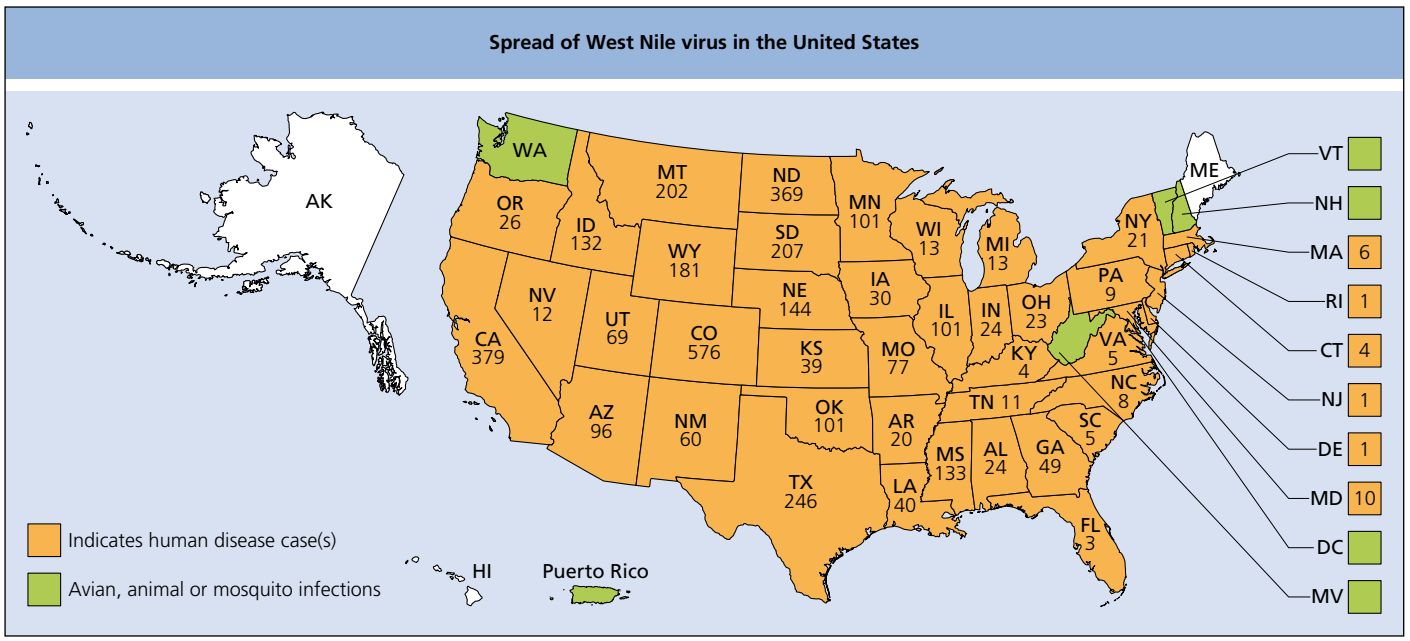


Fig. 4.3 Spread of West Nile virus in the United States. In the 8 years since West Nile virus was first reported in the USA (in New York City), human infections have been reported in 47 states. West Nile activity from January 1, 2007, to February 5, 2008, is indicated above. *Source:*Centers for Disease Control and Prevention. Adapted from: http://www.cdc.gov/ncidod/dvbid/westnile/Mapsactivity/surv&control107Maps.htm

of crows; the 1999 Nipah encephalitis outbreak, which was preceded by illness in pigs; and the 1996 outbreak of new variant Creutzfeldt–Jakob disease, which was preceded by the recognition of an epidemic of bovine spongiform encephalopathy (BSE) among cows.

Formal mechanisms have been developed to link public health disease monitoring with surveillance for diseases in agricultural animals, migrating birds and wild animals. They include the US-government-sponsored Global Avian Influenza Network for Surveillance (http://www.gains.org), which was established in 2006 in co-operation with the Wildlife Conservation Society (WCS) to monitor high pathogenicity avian influenza (HPAI) in wild birds. Within the USA, ArboNet tracks nationally notifiable arboviral diseases (including West Nile fever; see Fig. 4.3), and the National Antimicrobial Resistance Monitoring System (NARMS; http://www.cdc.gov/narms) continues to monitor the emergence of antimicrobial resistance in zoonotic bacteria (e.g. *Salmonella* and *Campylobacter*) that infect livestock. In addition, the federal, state and local public health laboratories are also linked with agricultural, wildlife and military reference laboratories through the Laboratory Response Network (LRN), which was founded in 1998 by the Centers for Disease Control and Prevention (CDC) in partnership with the Association of Public Health Laboratories and the Federal Bureau of Investigation (FBI).

In addition to protecting human health, rapid detection and control of animal diseases will benefit agriculture, help preserve wildlife species and avert economic losses due to expensive disease control measures (Box 4.1). The special challenges of understanding the ecologic factors that favor the emergence and spread of zoonotic diseases are discussed below.

Molecular diagnostics and outbreak detection

To ensure optimal medical care and disease control strategies, clinical diagnosis of unusual diseases must be confirmed by laboratory tests. Today's advances in microbial genomics, molecular immunology and bioinformatics are facilitating the development of accurate, rapid and sensitive diagnostics based on detection of microbe-specific or strain-specific nucleic acids or proteins. Molecular testing that involves amplification of genetic sequences using the polymerase chain reaction (PCR) technique is especially useful when unidentified pathogens are difficult to grow in the laboratory, making other types of testing difficult or impossible. In the future, advances in human genomics may also allow the identification of individuals with increased genetic susceptibility to certain diseases or to severe manifestations of those diseases (Table 4.5).[16,17]

Molecular testing has the potential not only to confirm clinical diagnoses but also to facilitate the detection of outbreaks. This function may be especially important when individual cases are not clustered geographically or by other easily identifiable risk factors. Examples include:

- food-borne pathogens transmitted simultaneously to many localities when a contaminated product is shipped to supermarkets or restaurants in several states or countries;
- disease agents dispersed by international travelers, such as the 2000 leptospirosis outbreak in Malaysian Borneo and the 2003 outbreak of SARS; and
- an unannounced and undetected (covert) release of a bioterrorist agent.

Public health tools are already in place in Europe and the USA to identify and trace the source of geographically dispersed food-borne

Box 4.1 Agricultural costs of controlling diseases carried by food animals

When a dangerous animal-borne disease threatens human health or food safety, a government may be forced to slaughter large numbers of food animals as a control measure, despite considerable economic costs. Recent examples include two zoonotic diseases (Nipah encephalitis and H5N1 influenza) and two veterinary diseases (foot and mouth disease and bovine spongiform encephalopathy (BSE; also called mad cow disease)). Ingestion of beef containing the causative agent of BSE (a prion) may result in the development of a fatal human neurodegenerative illness (new variant Creutzfeldt–Jakob disease) many years later.

Between 1997 and 2007, the culling of animals to control outbreaks of Nipah encephalitis, avian influenza, foot and mouth disease, and BSE cost the agricultural sector billions of dollars.

- *Nipah encephalitis*. In 1999 Malaysian health authorities were faced with an outbreak of encephalitis among farm workers, which had a nearly 50% mortality rate. The cause was a previously unknown paramyxovirus called the Nipah virus, which is carried by bats. To control the outbreak, approximately 1.1 million pigs were culled within a few weeks, severely harming the Malaysian meat industry.
- *Avian influenza (strain H5N1)*. In 1997, a similar precautionary measure was taken by the government of Hong Kong, which arranged the culling of all 1.6 million chickens on Hong Kong Island and the New Territories to prevent chicken-to-human transmission of a virulent avian form of influenza.

The 2003 re-emergence of avian influenza A[H5N1] in South East Asia – and its consequent spread to Central Asia, Africa, Europe and the Middle East – has also had a significant economic impact. In South East Asia, for example, bird deaths due to disease and to bird cullings have caused severe losses to farmers in Vietnam and Thailand (whose poultry stocks declined by 15–20%), as well as to farmers in Indonesia, China, Cambodia and Laos. According to the World Bank, economic impacts in these countries have also affected poultry traders, feed mills and breeding farms (http://web.worldbank.org/WBSITE/EXTERNAL/COUNTRIES/EASTASIAPACIFICEXT/EXTEAPHALFYEARLYUPDATE/0,contentMDK:20708543~menuPK:550232~pagePK:64168445~piPK:64168309~theSitePK:550226,00.html).

- *Bovine spongiform encephalopathy*. In 2006, the European Union lifted a 10-year ban on the export of live cattle and cattle products (other than milk) from the UK. Control measures, including the slaughter of affected cows, cost the UK government an estimated £3.5 billion sterling (about US$5 billion). The export ban cost the UK agricultural sector about £675 million a year.
- *Foot and mouth disease*. The costs of controlling an outbreak of foot and mouth disease in the UK and continental Europe in 2001 – estimated at £8.5 billion – dwarfed those of the BSE outbreak and devastated the centuries-old British livestock industry. Foot and mouth disease does not infect humans but can be spread by travelers who have contaminated soil on their shoes or clothing or who carry contaminated food products. The St Patrick's Day parade in Ireland was cancelled due to concerns about spreading the virus, and the British army was mobilized to help bury the carcasses of 7 million animals slaughtered because of potential exposure to the disease.

In August 2007, the European Union imposed a temporary ban on export of livestock, meat and milk from the UK, and the UK halted the internal movement of farm animals nationwide, to minimize the risk of spreading foot and mouth disease after cases were detected in Surrey. The outbreak, which affected about eight farms, was traced to an accidental release of an experimental vaccine strain from a high-security animal laboratory in rural England.

Table 4.5 Examples of genetic factors that influence susceptibility to disease or disease progression

- Alleles of the chemokine receptor gene CCR5 confer partial protection against HIV infection and the development of AIDS[1]
- Globin gene alleles (e.g. sickle globin and α- and β-thalassemias) confer partial protection against malaria[2]
- Lack of the Duffy blood group on red cells (due to a mutation in a chemokine receptor gene) confers complete protection against *Plasmodium vivax* malaria[3]
- Some vitamin D receptor (VDR) genotypes may reduce the risk of developing clinical tuberculosis[4]
- Blood group O is associated with severe cholera[5]
- Mutations in the cystic fibrosis gene may confer some protection against cholera[6]
- HLA alleles may influence susceptibility to – or course of infection of – HIV/AIDS, hepatitis B, measles, hantavirus pulmonary syndrome, malaria, tuberculosis, human papillomavirus infection and coccidioidomycosis[7]
- Mutations in the regulatory region of the PARK2 gene – the cause of early onset Parkinson's disease – and the co-regulated gene PARKCG are associated with increased susceptibility to leprosy[8]
- Heterozygosity at codon 129 of the prion protein gene might provide a measure of protection against sporadic and iatrogenic infection with Creutzfeldt–Jakob disease (CJD)[9]
- The apolipoprotein E, type ε4 allele (ApoEε4) – which is a marker for cognitive decline with Alzheimer's and cardiovascular diseases later in life – might play a protective role in the cognitive and physical development of children with heavy burdens of diarrhea in early childhood[10]

[1]Reiche EMV, Bonametti AM, Voltarelli JC, Morimoto HK, Watanabe MAE. Genetic polymorphisms in the chemokine and chemokine receptors: impact on clinical course and therapy of the human immunodeficiency virus type 1 infection (HIV-1). Curr Med Chem 2008;14(12):1325–34.
[2]Weatherall DJ. Thalassaemia and malaria, revisited. Ann Trop Med Parasitol 1997;91(7):885–90.
[3]Langhi DM Jr, Bordin JO. Duffy blood group and malaria. Hematology 2006;11(5):389–98.
[4]Roth DE, Soto G, Arenas F, *et al*. Association between vitamin D receptor gene polymorphisms and response to treatment of pulmonary tuberculosis. J Infect Dis 2004;190(5):920–7.
[5]Harris JB, Khan AI, LaRocque RC, *et al*. Blood group, immunity, and risk of infection with *Vibrio cholerae* in an area of endemicity. Infect Immun 2005;73(11):7422–7.
[6]Gabriel SE, Brigman KN, Koller BH, Boucher RC, Stutts MJ. Cystic fibrosis heterozygote resistance to cholera toxin in the cystic fibrosis mouse model. Science 1994;266(5182):107–9.
[7]Nikolich-Zugich J, Fremont DH, Miley MJ, Messaoudi I. The role of mhc polymorphism in anti-microbial resistance. Microbes Infect 2004;6(5):501–12.
[8]Mira MT, Alcaïs A, Nguyen VT, *et al*. Susceptibility to leprosy is associated with PARK2 and PACRG. Nature 2004;427(6975):636–40.
[9]Riemenschneider M, Klopp N, Xiang W, *et al*. Prion protein codon 129 polymorphism and risk of Alzheimer disease. Neurology 2004;63(2):364–6.
[10]Oriá RB, Patrick PD, Blackman JA, Lima AA, Guerrant RL. Role of apolipoprotein E4 in protecting children against early childhood diarrhea outcomes and implications for later development. Med Hypotheses 2007;68(5):1099–107.

outbreaks by comparing molecular fingerprinting data from clinical and public health laboratories. This is the operating principle behind PulseNet,[18] a molecular subtyping network that has used the pulse-field gel electrophoresis (PFGE) technique to detect several major multistate food-borne outbreaks. For example, PulseNet was used to detect an outbreak of *Escherichia coli* O157:H7 transmitted by fresh spinach sold in 26 states (in 2006) and an outbreak of *Salmonella* St Paul associated with jalapeno peppers sold in more than 40 states (in 2008). This technology is in use in many countries all over the world (http://www.pulsenetinternational.org). In the future, advances in genome sequencing and bioinformatics may lead to the development of increasingly sophisticated typing methods that will facilitate discovery of novel disease risk factors for hundreds of pathogens and help health authorities identify linkages among geographically dispersed cases of disease. For example, the CDC has proposed the development of an international database called Microbe. Net that could potentially match a pathogen isolated from an ill person in one country with an environmental microbe isolated in another.

Being prepared for the unexpected

Infectious pathogens are extraordinarily resilient and have a remarkable ability to evolve, adapt and develop resistance to drugs in an unpredictable and dynamic fashion. Because we do not know what new diseases will arise, we must always be prepared for the unexpected (see Table 4.4). In 2002, the first vancomycin-resistant *Staphylococcus aureus* strain was identified in the USA.[19] In 2003, SARS emerged from an animal reservoir in Guangdong province in China and quickly spread via travelers through South-East Asia and then to Toronto, Canada. Later in the same year, more than 30 people in the midwestern United States contracted monkeypox – a disease that resembles a mild case of smallpox – from rodents imported as pets that carried an orthopox virus known to infect people who live in remote villages near tropical rainforests in Central and Western Africa (http://www.cdc.gov/MMWR/preview/mmwrhtml/mm5226a5.htm). In 2009, a new strain of pandemic influenza – the pandemic (H1N1) 2009 virus – emerged in North America and spread rapidly around the world (see later).

The unexpected may include intentional as well as naturally occurring outbreaks. In 2001, the USA experienced a multistate outbreak of anthrax that necessitated antibiotic prophylaxis for more than 30 000 people. Rapid medical, public health and law enforcement action limited the outbreak to 11 inhalational cases, 11 cutaneous cases and five deaths.

These examples of unforeseen outbreaks underscore the need to maintain a strong public health system that is supported by a well-informed and vigilant medical community.

Shifts in perspectives on infectious disease in the past 50 years

Over the past 50 years, there have been significant shifts in how infectious diseases are viewed by the medical and scientific world and by the public. In the years following the Second World War, it was widely believed that humans were winning the war against infectious microbes. Vaccines and antibiotics, coupled with earlier improvements in sanitation and water quality, had dramatically lowered the incidence of infectious diseases. Therefore, it became possible to imagine a world in which infectious pathogens would no longer prey upon humanity. In 1962, the Australian Nobel prize winner Frank Macfarlane Burnet stated:[20]

> *One can think of the middle of the twentieth century as the end of one of the most important social revolutions in history, the virtual elimination of the infectious disease as an important factor in social life.*

Five years later, Surgeon General William H Stewart expressed the views of many US doctors and health experts when he stated that it was time to 'close the book on infectious illnesses', as long as we continue to prevent disease through vaccination'.[21] He encouraged the public health

community to turn its attention to chronic diseases. Over the following years, biomedical research in the USA became increasingly focused on heart disease, stroke and the 'war on cancer', which was declared by President Richard Nixon in 1971. Local and federal programs aimed at monitoring and studying infectious diseases were greatly reduced or abolished.

New diseases continue to emerge and re-emerge

In spite of optimistic predictions, infectious diseases continued to cause serious problems. As early as the 1940s and 1950s, certain bacteria, such as *Staphylococcus aureus*, began to develop resistance to penicillin. In 1957 and 1968, new strains of influenza emerged in China and Hong Kong, respectively, and spread rapidly around the globe. In the 1970s there was a resurgence of sexually transmitted diseases in the USA (perhaps due in part to changes in human sexual behaviors and to the importation of antibiotic-resistant strains of gonorrhea by infected soldiers returning from Vietnam). The final blows to our complacent attitude toward infectious diseases came in the 1980s, with the appearance of AIDS and the re-emergence of tuberculosis, including multidrug-resistant strains.

By the early 1990s, many health experts no longer believed that the threat of infectious diseases was receding in the developed world. Growing concern about the threat of emerging infectious diseases was cogently expressed in a 1992 report issued by the Institute of Medicine (IOM) of the National Academies. The report, *Emerging Infections: Microbial Threats to Health in the United States*, emphasized the intimate links between US health and international health.[3] It described the major factors that contribute to disease emergence, including societal changes and microbial evolution (see Table 4.1). The report concluded that emerging infectious diseases are a major threat to US health, and it challenged the US government to take action.

In 1994, the CDC answered the challenge by launching a national effort to revitalize the US capacity to protect the public from infectious diseases. This ongoing effort is described in *Addressing Emerging Infectious Disease Threats: A Prevention Strategy for the United States* (http://www.cdc.gov/mmwr/PDF/rr/rr4305.pdf) and an updated version published in 1998 (http://www.cdc.gov/mmwr/preview/mmwrhtml/00054779.htm). In view of the public health importance of vector-borne and zoonotic diseases – which account for more than half of all emerging diseases[15] – CDC is working with medical partners (e.g. the American Medical Association [AMA]) and animal health partners (e.g. the World Organization for Animal Health [OIE], the American Veterinary Medical Association [AVMA] and the Wildlife Conservation Society [WCS]); see below to study ecologic aspects of disease emergence and develop prevention strategies that take into account human, veterinary, and environmental factors (see Fig. 4.1).

Looking back to the 1950s and 1960s it is useful to remember that very little was then known about how microbes evolve or develop drug resistance. In the 1970s and 1980s, with the development of molecular biology, biologists learned how resistance genes are carried on plasmids and transmitted from one bacterium to another. They also learned how quickly viruses can evolve by generating mutations with each round of replication, as well as by reassorting gene segments or jumping species barriers. As molecular tools became available, many new viruses (pathogenic and nonpathogenic) were discovered in humans, animals and plants.

CATEGORIES OF EMERGING AND RE-EMERGING INFECTIOUS DISEASES

For the purposes of this discussion, emerging and re-emerging threats may be grouped into five (sometimes overlapping) categories:

- drug-resistant diseases;
- food-borne and water-borne diseases;
- zoonotic and vector-borne diseases;
- diseases transmitted through blood transfusions or blood products; and
- chronic diseases caused by infectious agents.

Drug-resistant diseases

Drug-resistant pathogens are a growing menace to all people, regardless of age, sex or socioeconomic background. They endanger people in affluent, industrial societies such as the USA, as well as in less developed nations. Examples of clinically important microbes that are rapidly developing resistance to available antimicrobial agents include bacteria that cause pneumonia, ear infections and meningitis (e.g. *Streptococcus pneumoniae*), skin, bone and bloodstream infections (e.g. *Staphylococcus aureus*), urinary tract infections (e.g. *E. coli*), food-borne infections (e.g. *Salmonella*) and infections transmitted in health-care settings (e.g. enterococci) (Table 4.6).

Special concerns include health-care-associated and community-acquired methicillin-resistant *Staphylococcus aureus* (HA-MRSA and CA-MRSA) and extensively drug-resistant tuberculosis (XDR-TB; see below). A large study led by the CDC estimates that HA-MRSA was responsible for 94 360 serious infections and associated with 18 650 hospital stay-related deaths in the USA in 2005, making it the cause of more deaths in the USA each year than HIV/AIDS.[22]

Until recently, most MRSA cases were seen in hospitals. However, clusters of CA-MRSA skin infections are increasingly reported among athletes, military recruits, prisoners and groups of people who live in crowded conditions. Athletes at highest risk include those who participate in contact sports such as wrestling, football and rugby, probably because most MRSA infections begin with a cut, scratch or bruise.[23] However, MRSA infections have also been diagnosed among athletes in other sports such as soccer, basketball, field hockey, volleyball, rowing, martial arts, fencing and baseball. Risk factors for

Table 4.6 Examples of emerging resistance in bacterial pathogens

Gram-positive cocci	Methicillin-resistant and vancomycin-resistant *Staphylococcus aureus* Coagulase-negative staphylococci Penicillin-resistant pneumococci Macrolide-resistant streptococci Vancomycin-resistant enterococci
Gram-negative cocci	Penicillin-resistant meningococci Quinolone-resistant gonococci
Gram-negative bacilli	*Enterobacter* spp. and other Enterobacteriaceae with chromosomal β-lactamases Multidrug-resistant *Pseudomonas aeruginosa* *Stenotrophomonas maltophila* *Acinetobacter* spp. with novel β-lactamases, aminoglycoside-modifying enzymes and other resistance mechanisms *Enterobacteriaceae* with extended-spectrum β-lactamases Multidrug-resistant diarrheal pathogens (*Shigella* spp., *Salmonella* spp., *Escherichia coli*, *Campylobacter* spp.)
Acid-fast bacilli	Multidrug-resistant *Mycobacterium tuberculosis* (MDR-TB) Extensively drug-resistant *Mycobacterium tuberculosis* (XDR-TB) Multidrug-resistant *Mycobacterium avium* complex

these athletes may include contact with contaminated objects such as towels and sports equipment. It is also possible that other strains could emerge in farms where animals are fed antibiotics as growth promoters.[24]

Many other pathogens – including the bacteria that cause gonorrhea; the virus that causes AIDS; the fungi that cause *Candida* infections; and the parasites that cause malaria – are also becoming resistant to standard therapies. In the absence of effective action to address the problem of antimicrobial resistance, drug choices for the treatment of common infections will become increasingly limited, expensive and, in some cases, unavailable.

Reasons for the rapid development of antibiotic resistance include the ability of organisms to mutate and share genetic material. However, this process has been facilitated by:

- inappropriate prescription practices by physicians and veterinarians;
- administration of antibiotics to agricultural animals as growth promoters;
- unrealistic patient expectations that lead to requests for antibiotic treatment of nonbacterial infections;
- the economics of pharmaceutical sales; and
- increased use of sophisticated medical interventions that result in the administration of large quantities of antibiotics (e.g. transplant surgery and immunosuppressive and cytotoxic drug therapy).

Growing antibiotic resistance poses a substantial threat to modern gains in infectious disease control. About 70% of bacteria that cause infections in hospitals in many countries, including the US, are resistant to at least one of the drugs most commonly used to treat infections. The medical community must therefore join with patients and members of the agricultural and pharmaceutical industries in a common effort to promote appropriate use of antibiotics to safeguard their effectiveness for future generations. Steps to be taken by the European Union and the US government to facilitate this process are outlined in the *Copenhagen Recommendations* (http://www.im.dk/publikationer/micro98/recommen.htm) and *A Public Health Action Plan to Combat Antimicrobial Resistance*, respectively (http://www.cdc.gov/drugresistance/actionplan). In addition, the Infectious Diseases Society of America (IDSA) has issued *Bad Bugs, No Drugs* (http://www.idsociety.org/Content.aspx?id=5558) to call attention to the lack of new antibiotics in the pharmaceutical development pipeline.

Food-borne and water-borne diseases

Twentieth-century improvements in sanitation, food sterilization and processing, and water treatment have greatly reduced the burden of food-borne and water-borne illnesses in developed countries, nearly eliminating many diseases that remain major killers in the developing world (e.g. typhoid fever, cholera and dysentery). Now, in the 21st century, however, there is growing evidence that modern factors such as centralized food processing may pose challenges to food safety and water quality.

Food-borne diseases

Food-borne diseases cause an estimated 76 million illnesses, 325 000 hospitalizations and 5000 deaths in the USA each year.[25] As noted above, food-borne pathogens such as *Salmonella*, *Shigella*, *Cyclospora*, *Campylobacter* and *E. coli* O157:H7 can be transmitted via commercial products that are processed in large quantities and shipped to different states or nations. Although food processing techniques are very advanced, when contamination does occur it can affect many people in many different localities (see Table 4.5). A recent example is an outbreak of *E. coli* O157:H7 (transmitted via frozen hamburger patties) that affected people in eight US states and led to the recall of 21.7 million pounds of ground beef products. Six days after the recall, the company that marketed the beef went out of business (http://www.cdc.gov/ecoli/2007/october/100207.html).

Water-borne diseases

Microbial contamination of water can occur when animal or human sewage contaminates source water that is not adequately treated by filtration, chlorination or other methods. In addition, some parasites are resistant to routine water treatment methods. These include *Cryptosporidium*, the causative agent of a major disease outbreak in the Milwaukee drinking water system in 1993 that affected more than 400 000 people.[26] In 2006, series of articles reviewing current research on water-borne diseases was published in a special issue of the *Journal of Water and Health* (http://www.epa.gov/nheerl/articles/2006/waterborne_disease.html). This research was mandated by the Safe Drinking Water Act Amendments of 1996 and supported by the US Environmental Protection Agency (EPA) and the CDC. A workshop of experts from academia, the EPA and the CDC made recommendations for additional health studies related to microbial exposures in drinking water. Although associations between illness and water are often difficult to evaluate – since most people drink water every day, often from more than one source – the EPA estimates that there may be as many as 16.4 million cases of water-borne illness in the USA each year (http://www.epa.gov/nheerl/articles/2006/waterborne_disease/national_estimate.pdf).

Worldwide, approximately 1 billion people lack access to safe water and 2.6 billion lack access to basic sanitation. Water-borne diarrheal diseases cause 1.8 million deaths every year, most of them in children in developing countries with unsafe water supplies. In 2007, the World Health Organization (WHO) issued *Combating Waterborne Disease at the Household Level* (http://www.who.int/household_water/advocacy/combating_disease/en/index.html), which advocates the use of inexpensive point-of-use water quality interventions that can be used in the home in areas that lack access to safe drinking water.

Zoonotic diseases

Many of the novel human pathogens identified over the past decade are carried by animals. For example, Sin Nombre virus, which is carried by the deer mouse, was identified in 1993 in the USA as the cause of hantavirus pulmonary syndrome, and Hendra virus, carried by fruit bats, was identified in Australia in 1994 as a cause of encephalitis in humans and horses.[27] Nipah virus, which is also carried by fruit bats, was identified in 1999 in Malaysia as a cause of encephalitis in humans and swine and has since been reported in Bangladesh and India.[28–30] Like Sin Nombre virus, the Nipah and Hendra viruses (henipaviruses) are highly virulent, and there are as yet no drugs or vaccines for their treatment or prevention. Other examples include Marburg hemorrhagic fever virus, which is apparently maintained in cave-dwelling African fruit bats,[31,32] and the SARS coronavirus, which is associated with Chinese horseshoe bats.[33]

Zoonotic agents can become established in any geographic area that has a suitable animal reservoir. The arenavirus that causes lymphocytic choriomeningitis (first isolated in 1933) was probably introduced into the New World at the same time as its vector, *Mus musculus*, the common house mouse. Plague (which is both rodent-borne and vector-borne) was introduced into the USA in the early 1900s, via infected rats and fleas in ships that arrived at port cities. It quickly became established in the North American prairie ecosystem, infecting a wide range of animals, including native rodents and their fleas, which have been the most frequent sources of human infection. Like plague, newly emergent disease agents that are able to infect many animal species (e.g. Nipah and Hendra viruses and monkeypox virus) have the potential to spread worldwide.

Disease dispersion via insect vectors

Diseases that are carried by insect vectors (i.e. mosquitoes, fleas, ticks and other blood-sucking arthropods) can also spread into new geographic areas and infect new human populations. In 1999, for example, mosquito-borne transmission of three nonendemic diseases – malaria, dengue and West Nile fever – was reported in the USA. West Nile

encephalitis may have entered New York City via an imported or migrating bird or a mosquito that 'hitch-hiked' on an airplane or (less likely) an infected traveler; dengue fever most likely arrived in Texas via both people and mosquitoes.

Asian tiger mosquitoes (*Aedes albopictus*) that can transmit dengue and Chikungunya fever arrived in the USA in Houston in 1987 in imported used-tire casings[34] and appeared more recently in California in commercial shipments of a Chinese ornamental indoor plant called 'lucky bamboo'.[35] During the 1990s, *A. albopictus* became established in Italy after mosquito eggs traveled to Italy in imported tires.[36] In 2007, when a traveler who had contracted Chikungunya in Kerala, India, fell ill after arriving in Ravenna, Italy, his disease was transmitted to others via the now-indigenous Italian *A. albopictus* mosquitoes, causing an outbreak that affected at least 204 people.[37]

While many cases of 'airport malaria' have been reported in Europe and North America (presumably involving small numbers of traveling mosquitoes), locally acquired malaria cases have also been identified that apparently involved at least one cycle of human-to-mosquito transmission. For example, a cluster of non-airport malaria cases was detected in Virginia in 2002 in a community that included immigrants from malarious countries who might have had asymptomatic malaria infections.[38] Another cluster was detected in 2003 in Palm Beach County, Florida.[39]

Another example of vector-borne disease spread is the recent identification of Rift Valley fever (previously seen only in Africa) in the Middle East.[40] Within a few years, Rift Valley fever (RVF) virus traveled from sub-Saharan Africa to northern Africa (Egypt and the Sudan) and then to Saudi Arabia and Yemen. Competent mosquito vectors for RVF, a febrile hepatitis associated with encephalitis, retinitis and hemorrhagic fever (among both humans and animals), are present throughout the world. Importation of RVF into the USA would constitute a major threat to the US livestock industry and to human public health.

Diseases transmitted through blood transfusions, blood products, or transplanted organs or tissues

Improvements in donor screening, serologic testing and transfusion practices have made the US blood supply one of the safest in the world, despite its size and complexity. However, because blood is a human tissue, it is a natural vehicle for transmission of infectious agents. During the 1980s, HIV was transmitted through clotting factor and blood transfusions, and during the 1990s, hepatitis C virus was transmitted by intravenous immunoglobulin. More recently, transmission of Chagas disease trypanosomes,[41] West Nile virus,[42] rabies virus,[43] Creutzfeldt–Jakob disease prions,[44] tuberculosis,[45] a new arenavirus related to lymphocytic choriomeningitis virus[46] and HIV virus (for the first time in the USA since 1986[47]) has been reported in patients who received organ transplants, grafts or blood from infected persons.

In recent years, there has been renewed interest in xenotransplantation – the transplantation of animal organs and tissues to humans – because of the shortage of human organs and tissues. The US Food and Drug Administration (FDA) is currently developing a comprehensive approach to the regulation of xenotransplantation to address public health concerns about potential infection of recipients with recognized or unknown zoonotic pathogens and possible subsequent transmission of these pathogens to close contacts and into the general population (http://www.fda.gov/cber/xap/xap.htm).

Infectious diseases also have implications for the availability of blood and blood products, because people who have traveled in countries where they may have been exposed to blood-borne diseases are often excluded as blood donors. Examples include individuals who have recently visited malaria-endemic countries and people who have stayed for 6 months or more in the UK, where they might have ingested beef from cows with BSE and acquired the prion that causes new variant Creutzfeldt–Jakob disease.

Chronic diseases caused or exacerbated by infectious agents

Several chronic diseases once attributed to lifestyle or environmental factors (such as some forms of cancer, diabetes, heart disease, arthritis and ulcers) are actually caused by or exacerbated by an infectious agent.[48–50] Three of the six major causes of cancer death in the world are caused by infectious agents: hepatocellular carcinoma by hepatitis B and C viruses, cervical cancer by human papillomavirus and gastric cancer by *Helicobacter pylori* bacteria (see below). Hepatitis B and C are also major causes of cirrhosis and end-stage liver disease, while *H. pylori* causes peptic ulcer disease in addition to stomach cancer. Moreover, Epstein–Barr virus is associated with nasopharyngeal carcinoma, Burkitt's lymphoma, B-cell lymphoma and post-transplant lymphoproliferative disease.

Current research suggests that some chronic cardiovascular, intestinal and pulmonary diseases may also have an infectious etiology. For example, Whipple's disease is caused by a bacterial infection (*Tropheryma whipplei*)[51,52] and tropical spastic paraparesis is caused by a viral infection (HTLV-1).[53] Potential associations are being investigated for many other illnesses and syndromes, including coronary artery disease (*Chlamydia* infection),[54] Paget's disease (paramyxoviridae infection),[55,56] Crohn's disease (mycobacterial infection)[57] and bronchiectasis (respiratory infections during early childhood).[58] There are also preliminary data suggesting that Wegener's granulomatosis responds to antibiotic therapy. These findings raise the possibility that some chronic conditions, including asthma, arthritis and heart disease, may someday be treated with antimicrobial drugs or prevented by vaccines.

INFECTIOUS DISEASES AND THE GLOBAL VILLAGE

As stressed in the Institute of Medicine reports in 1992 and 2003, US health and global health are inextricably linked.[3,4] Modern factors that connect us culturally, commercially and physically such as air travel and the globalization of the food supply (see Table 4.5) put us at risk of exposure to microbes that are endemic in other countries, whether we live in large cities or small rural hamlets. As the HIV/AIDS epidemic has illustrated, a disease that emerges or re-emerges anywhere in the world can spread far and wide. This concern is reflected in the most recent (2005) version of the WHO International Health Regulations (http://www.who.int/csr/ihr/en), which require reporting not only of some specific diseases, as in the 1969 version, but also of 'all events that may constitute public health emergencies of international concern' – including outbreaks caused by previously unidentified microbes, by microbes that appear in new, drug-resistant forms, or by bioterrorism. The list of specific notifiable diseases (which used to consist of cholera, plague and yellow fever) now includes a single case of smallpox, polio due to wild-type poliovirus, human influenza due to a new subtype and SARS.

Several diseases of global public health importance – including epidemic-prone diseases, newly emerging or re-emerging diseases, vaccine-preventable diseases and diseases slated for regional elimination or worldwide eradication (e.g. polio) – have resurged in recent years, spreading across countries and (sometimes) continents (see Table 4.3). HIV/AIDS, tuberculosis, malaria and measles continue to be major infectious causes of death worldwide, along with acute lower respiratory infections and diarrheal diseases (Fig. 4.4). Globally important diseases that continue to be of special domestic concern to the USA and other developed countries include HIV/AIDS, tuberculosis and pandemic influenza.

HIV/AIDS

HIV/AIDS incidence in the USA increased rapidly through the 1980s and early 1990s, with the peak of new diagnoses occurring after the expansion of the AIDS surveillance case definition in 1993.[59]

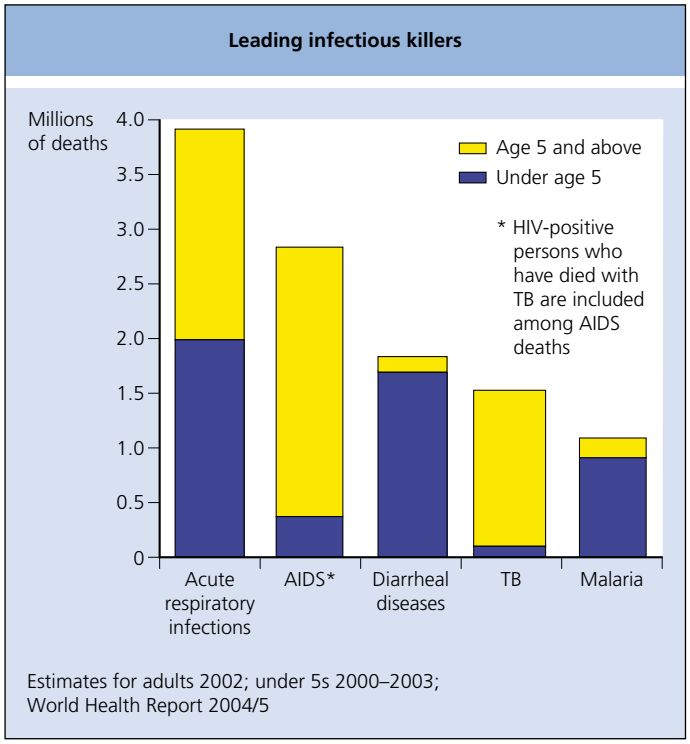


Fig. 4.4 Leading infectious killers.

After 1996, with the introduction of effective combination antiretroviral therapies, sharp declines were reported in AIDS incidence and deaths, while AIDS prevalence continued to increase. Currently, US disease prevention efforts are targeted primarily to young men who have sex with men, the group that currently exhibits the highest incidence nationally.[60,61]

Worldwide, an estimated 33.2 million people are living with HIV/AIDS (Fig. 4.5). In 2007, there were 2.1 million HIV-related deaths and approximately 2.5 million new infections.

Malaria

Forty-one percent of the world's population lives in areas where malaria is transmitted (e.g. parts of Africa, Asia, the Middle East, Central and South America, Hispaniola and Oceania) and 350–500 million cases occur annually. More than one million people die of malaria each year, most of them young children in sub-Saharan Africa.

Malaria prevention efforts include treatment with artemisinin-containing drugs, mosquito control (insecticide-treated bed nets and/or indoor residual spraying) and prophylactic treatment of vulnerable populations such as pregnant women and children.

Tuberculosis

Tuberculosis is the attributable cause of one-third of all adult deaths in developing nations.[62] Once thought to be controlled in Western countries, tuberculosis has re-emerged in Europe and the USA, where multidrug-resistant *Mycobacterium tuberculosis* (MDR-TB) has been reported in 45 of the 50 states,[63] and extensively drug-resistant *Mycobacterium tuberculosis* (XDR-TB) is present in 4% of cases. MDR-TB bacteria are defined as resistant at least to the two first-line drugs against TB (isoniazid and rifampin (rifampicin)), while XDR-TB bacteria are defined as resistant not only to isoniazid and rifampin but also to any fluoroquinolone and at least one of three injectable second-line drugs (capreomycin, kanamycin and amikacin).[64] Since 1998, the percentage of US-born patients with MDR-TB has remained at less than 0.7%. However, the frequency of resistant infections in foreign-born persons increased from 25% (103 of 407) in 1993 to 80% (73 of 91) in 2006 (http://www.cdc.gov/tb/statistics/reports/2007/default.htm).

HIV infection confers the greatest known risk for the development of TB, stimulating both the activation of latent infection and rapid progression to primary disease. UNAIDS estimates that approximately 30% of all AIDS deaths result directly from tuberculosis.[65]

Special challenges

Climate change and infectious diseases

Understanding the effects of climate change on specific diseases – especially those of major concern to developing countries, such as malaria, dengue and cholera – is currently an active area of research,

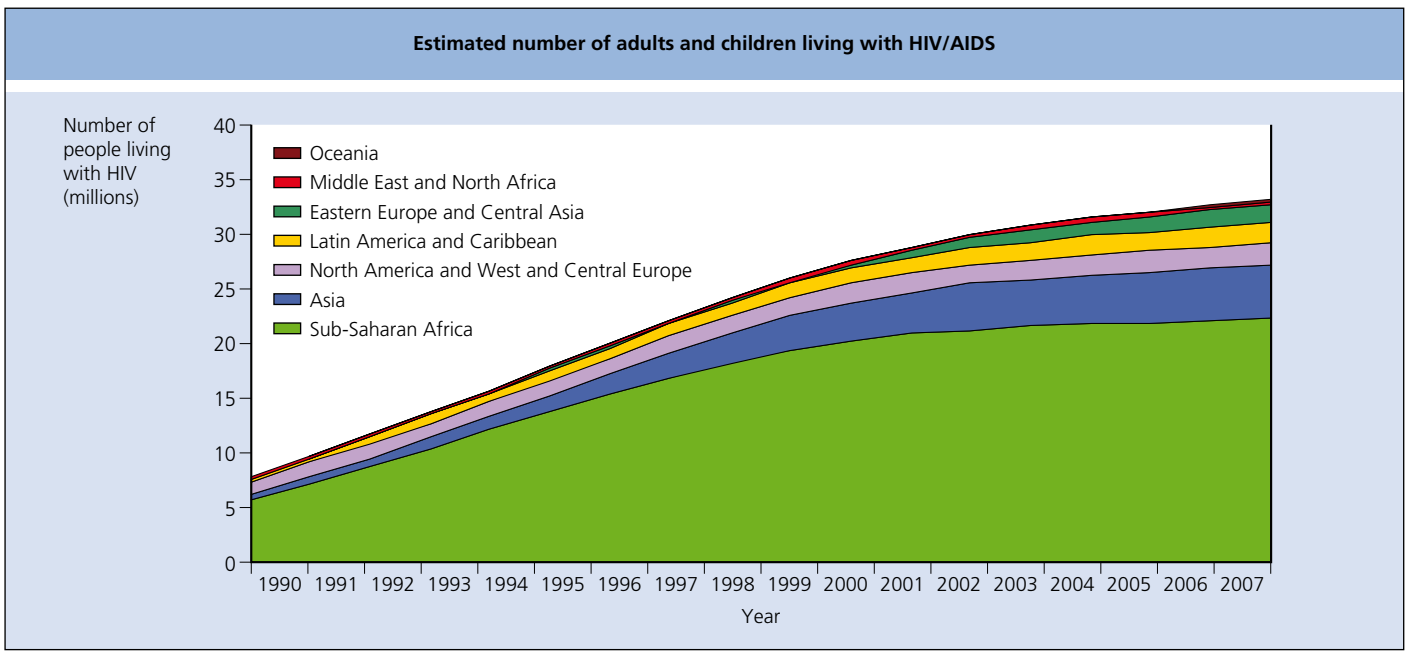


Fig. 4.5 Estimated number of adults and children living with HIV/AIDS, by region, 1990–2007.

which involves separating weather effects from other environmental factors that influence disease spread, such as land use, water management, urbanization and human behavior. From a public health point of view, the major challenge is to enhance surveillance for disease vectors and reservoirs, as well as for the diseases they carry, and to address all the factors that facilitate disease transmission. The potential introduction of disease vectors and reservoirs into new areas as weather patterns change is another reason why we need to be prepared for the unexpected. See Practice Point 1 for a further discussion on the relationship between health and climate change.

An ecologic approach to prevention and control of zoonotic and vector-borne diseases

The development of public health strategies to prevent and control zoonotic and vector-borne diseases – which include the great majority of new and re-emerging diseases[15] – requires an ecologic approach that takes into account the human, animal, microbial and environmental facts that influence disease emergence (see Fig. 4.1). In recognition of this fact, the Wildlife Conservation Society (WCS) has organized a series of *One World – One Health* symposia in New York, Thailand, China and Brazil to focus world attention on the 'essential link between human, domestic animal, and wildlife health' and the threat that animal diseases pose to people, food supplies and national economies.

A comprehensive approach to addressing these diseases would involve:

- new partnerships among experts in animal and human health, conservation biology, law and public policy;
- expansion of disease surveillance systems that integrate animal and public health data;
- routine availability of veterinary and entomologic expertise at public health departments; and
- development of an interdisciplinary research agenda to identify ecologic factors and interactions among animals, humans, and insects and their environments that influence disease emergence.

A better understanding of the ecologic factors that influence disease emergence will facilitate the development of innovative strategies for detection, control and prevention of zoonotic and vector-borne diseases such as the One Health Initiative (http://www.onehealthinitiative.com), as well as the creation of predictive models that indicate when outbreaks are likely to occur.

Infectious diseases as national security threat

From the end of the Cold War until 2001, US concerns about the impact of health issues on national security were focused primarily on events overseas[67] (Table 4.7). During the 1990s, security experts expressed particular concern about the destabilizing effects of HIV/AIDS in poor countries where high death rates among young adults have damaged economic, social, political, military and educational infrastructures and created vast numbers of orphans. In July 2000, the Group of Eight Industrialized Nations pledged to reduce deaths from HIV/AIDS, malaria, tuberculosis and vaccine-preventable diseases by supporting global health initiatives launched by the World Health Organization and other groups.[69] Combating HIV/AIDS and malaria was adopted by 189 nations as a Millennium Development Goal in 2000 (http://www.un.org/millenniumgoals), and the Global Fund to Fight AIDS, Tuberculosis and Malaria was established by the G8 in 2001 (http://www.theglobalfund.org/en). These ongoing international efforts reflect a growing consensus that global health security must be a shared responsibility.

Since the autumn of 2001, domestic security concerns have taken center stage, as they did in the 1950s, when the Epidemic Intelligence Service (EIS), the US training program for epidemiologists, was established in response to the Cold War threat of biologic warfare.[70] The anthrax attacks illustrated that the USA is vulnerable to bioterrorism, stimulating renewed bioterrorism preparedness and response efforts. Of the 15 National Planning Scenarios developed by the White House Homeland Security Council to guide emergency preparedness exercises, five involve infectious disease threats (anthrax, pandemic influenza, plague, food contamination and disease of agricultural animals).

Table 4.7 Biologic national security issues

- Diseases that spread across borders, crossing countries and continents (e.g. cholera, bacterial meningitis, measles, and pandemic influenza)
- The emergence of new and antibiotic-resistant diseases that arise in one region and spread throughout the world (e.g. AIDS and drug-resistant TB)
- Environmental issues that may have global effects (e.g. pollution, loss of biodiversity and global warming, which may affect the growth rate of insect vectors of disease)
- Population overgrowth that may lead to disease, war, famine, large-scale migration, and political instability
- Bioterrorism: the deliberate release of infectious agents by a terrorist or rogue nation

Adapted from Goldberg.[68]

Physicians are one of the three cornerstones of the 'golden triangle' for bioterrorism preparedness, along with the health-care delivery system and public health officials.[71] All clinicians, regardless of their specialty, must have basic information about the clinical manifestations of such potential bioterrorist agents as variola virus (smallpox), *Yersinia pestis* (plague) and *Bacillus anthracis* (anthrax). They must have a high index of suspicion and know how to recognize unusual diseases and report them to local public health and law enforcement officials. The expertise and full engagement of the medical community are essential to the national effort to preserve health security. For further discussion of issues related to bioterrorism, see Chapter 71.

Pandemic influenza

New strains of influenza viruses can emerge unpredictably from animal reservoirs and spread rapidly and pervasively through susceptible populations, sometimes causing worldwide epidemics. This is due in large part to two features of the influenza virus: its ability to exchange genetic information between strains and its ability to occasionally 'jump' species barriers between avian and porcine (or other mammalian) hosts. Influenza pandemics typically cause major morbidity and mortality, with significant societal and economic impacts.[46]

The sudden and unpredictable emergence of a potential pandemic strain of influenza was illustrated in 1997 by an outbreak of avian influenza A (H5N1) in Hong Kong, which raised the specter of a worldwide epidemic similar to the one that killed 20–50 million people (including 500 000 Americans) in 1918. Epidemiologic studies suggested that the H5N1 virus was transmitted from chickens to people and only poorly (if at all) from person to person. Nevertheless, it was feared that the virus might reassort with a human influenza virus during the winter influenza season, creating a virulent strain capable of air-borne human-to-human transmission. To ensure that the H5N1 virus would have no opportunity to evolve, the Hong Kong government authorized the culling of all 1.6 million chickens in Hong Kong (see Box 4.1). Six years later (in 2003) influenza A (H5N1) re-merged in poultry in South East Asia and then spread to Central Asia, Africa, Europe and the Middle East, leading to a worldwide pandemic alert (http://www.who.int/csr/disease/avian_influenza/phase/en/index.html).

Unexpectedly, however, a different virus – unrelated to avian influenza A (H5N1) – has emerged as the cause of a new pandemic officially declared by WHO in June 2009. First detected in the United

States and Mexico, the pandemic (H1N1) 2009 virus was originally referred to as 'swine flu' virus because laboratory tests indicated that it contained genes similar to those of influenza viruses in North American pigs. However, further study has shown that this new virus is actually a 'quadruple reassortant' virus, which also contains gene segments from human, avian and Eurasian swine influenza viruses.

As of January 2010, the pandemic (H1N1) 2009 virus was the predominant influenza virus in circulation worldwide. Disease severity appeared to be generally similar to the severity of disease during recent influenza seasons, although different age groups have been predominantly affected, with most cases and most severe cases occurring in older children and adults less than 65 years of age (http://www.cdc.gov/h1n1flu/).

CONCLUSION

Now more than ever, global health and well-being depend on the vigilance of concerned and well-informed clinicians. Each and every clinician plays a critical role in the public health early warning system for unusual infectious diseases, whether they are new, rare, zoonotic, vector-borne, drug-resistant or intentionally caused. These efforts are essential to maintaining a strong and effective public health system.

REFERENCES



References for this chapter can be found online at http://www.expertconsult.com

Chapter 5

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Mathematical models in infectious disease epidemiology

The first mathematical model of infectious disease transmission was constructed by Bernoulli in 1760[1] to determine the effectiveness of variolation, a crude form of smallpox vaccination. Specifically he sought to calculate life tables for actuarial purposes assuming an eradication of smallpox in the population (for a review of Bernoulli's life and work, see Dietz & Heesterbeek[2]). Mathematical systems have been used extensively not only in the study of pandemic viral diseases such as AIDS, severe acute respiratory syndrome (SARS), influenza, smallpox and polio, but have also been successful in modeling transmission of bacterial pathogens including sexually transmitted infections (STIs) and methicillin-resistant *Staphylococcus aureus*, vector-borne diseases including malaria and diseases caused by macroparasites such as helminths.

Modern infectious disease epidemiology relies heavily on mathematical modeling to characterize the complex interactions between the biology and environment of human or animal hosts, the pathogens responsible for disease and, where present, vector species. Transmission models are vital for understanding the dynamics present in the spread of infectious agents in populations and for assessing the likely impact of public health interventions such as isolation, vaccination and chemotherapy. The defining characteristic of infectious diseases – that they are transmissible – means that an individual's risk of acquiring infection changes dynamically as levels of infection rise and fall in the population. Therefore vaccinating individuals or treating infectious individuals benefits not only the individual patient directly but also benefits others in the population indirectly by reducing their risk of acquiring infection through the reduction in levels of infection in the population. This was demonstrated by the effect of vaccination against pneumococcus: the incidence of infection with 'vaccine' serotypes fell in those who were older than the target group for vaccination who did not receive the vaccine, as well as those who were vaccinated.[3]

However, 'indirect' effects are not always beneficial and may even be harmful.[4] For example, whilst reducing levels of infection in the population through vaccination protects those who are not vaccinated as well as those who are vaccinated by reducing the overall rate of infection, those who do still get infected are older on average when they get infected and can suffer more severe disease outcomes, so vaccination can increase the overall rate of disease for a period, until infection is eliminated from the population.[4] Mathematical models can be constructed that can help predict these effects and aid the design of strategies to mitigate them.

Mathematical models of infectious disease transmission are increasingly being used to guide public health policy in the areas of naturally disseminated pathogens such as viruses, bacteria and fungi and in developing response measures to mitigate the human and environmental costs caused by the deliberate release of infectious agents, i.e. bioterrorism.[5] Examples include the control of an epidemic of foot-and-mouth disease in the UK in 2001,[6,7] the SARS outbreak of 2003,[8,9] planning control strategies for TB, HIV and STIs,[10–13] and planning for pandemic influenza,[14,15] as well as examining general principles of disease control.[16]

Importantly for infectious diseases, there is typically a complex nonlinear relationship between the size of an intervention and the benefits. This is due to the 'indirect' effects interventions have on those who are not treated by changing levels of infection in the population, which affects the risk of acquiring infection.

As the scale of an intervention (e.g. vaccination coverage or provision of treatment) increases from a low level, the benefits – reductions in levels of disease – increase faster than the costs, until disease has been reduced to a low level or even eliminated. This means that when health economic analyses are performed to determine the cost-effectiveness of interventions, it is essential that the models take account of the transmissible nature of infectious diseases and the 'indirect' effects of interventions.[17] One example is vaccination, where vaccinating only a small proportion of the population mostly benefits only those who receive the vaccine because it does little to interrupt transmission, i.e. the indirect effect is small. Vaccinating a large proportion of the population can prevent epidemics, providing a large indirect benefit to those who were not vaccinated in addition to the direct benefit. Another example is in the control of curable infections (e.g. STIs) through treatment: if treatment capacity is inadequate then there is a 'vicious circle' where failing to control transmission in the present results in more infections in the future, maintaining the inadequacy of treatment capacity.[13] Conversely, making a concerted effort to increase capacity can break this vicious circle and change it to a virtuous circle, where promptly treating a large enough proportion of infections reduces transmission rates, allowing a more intense focus on remaining disease and reducing future need for treatment, leading to significant cost savings.[13]

Infectious disease epidemiology is inherently multidisciplinary because the transmission of infection within a population is affected not just by the biologic characteristics of the infectious agent and its human host, but also by the patterns of contact between individuals, their use of health services, their response to public health interventions, etc. Mathematical models enable information from diverse sources, including social sciences, to be integrated. Infectious disease modeling is not a purely technical, 'mathematical' exercise – many modelers come from biologic or clinical backgrounds.

EPIDEMIOLOGIC DATA

The fundamental measures of the epidemiology of a pathogen in a population are the incidence and prevalence, which are sometimes confused in the nonspecialist literature. Incidence is the number of new infections arising per unit time, and is usually expressed at x% per year or x cases per 1000 per year or x cases per 100 000 per year.

Prevalence is the proportion of the population (usually expressed as a percentage) that is infected at a point in time and is measured by cross-sectional surveys. Incidence can be measured directly in longitudinal cohort studies, where a group of subjects is followed through time, or can be calculated from a series of cross-sectional prevalence surveys.[18] Case notifications are often used as a proxy measure for incidence; long-term datasets are available for a large number of infectious agents due to mandatory (notifiable) disease surveillance schemes in developed countries. Historical datasets containing valuable information on causes of death and age, in some cases going back for hundreds of years, are also available. These datasets are rather subjective but have been useful in examining epidemics such as the Black Death (plague) and smallpox.

Table 5.1 lists 30 disease agents that are currently notifiable by clinicians to the Health Protection Agency (HPA) in the UK and similar arrangements are in place with the Centers for Disease Control and Prevention (CDC) in the USA. In developed countries demographic and clinical information is collected by clinicians when examining patients and raw incidence data can therefore usually be stratified by sex, age, ethnicity, spatial location and other factors such as tobacco and alcohol consumption. This is important because incidence can vary markedly across different groups of people. Such stratified longitudinal studies, where populations are placed into discrete classes, are extremely useful in examining trends of infection rates. In many of these datasets the impact of vaccination on childhood infectious diseases is striking.

Models are tools used throughout science and medicine – they are used to derive diagnoses from observed signs and symptoms and test results. Formulating models mathematically facilitates rigorous analysis and allows quantitative predictions to be made of trends in disease burden and the impact of interventions. The benefit of quantitative analysis in research is that one can determine if a putative cause for an observed effect would have been strong enough to cause the effect – for example, a mathematical modeling analysis of the HIV epidemic in Uganda found that several modes of behavior change (delaying sexual debut, reducing numbers of sexual partners, increasing condom use) must have occurred to explain the observed decline in prevalence. This is because no single change was sufficient to account for the reduced disease burden observed.[19]

Crucially, models allow evaluation of the impact of interventions that have been implemented by allowing comparison with what would have happened in the absence of the intervention. Epidemics have 'natural dynamics', with incidence typically rising to a peak then declining in the absence of any intervention. Therefore, simply observing a decline in incidence following an intervention is not sufficient evidence to demonstrate its effectiveness.[19] Indeed, in some circumstances it is even possible to observe incidence continue to rise despite an effective intervention, due to an increase in the prevalence of infection.[20]

Another important use of models is in setting priorities for empiric research by determining the importance of different 'gaps' in knowledge. This is done by testing the 'sensitivity' of a model's behavior to changes in the value of parameters that are poorly estimated by current data – for example, there is uncertainty in the amount of protection that bacille Calmette–Guérin (BCG) vaccination offers against TB acquisition and against progression to disease in those who are infected. By testing how much varying these parameters affects a model's behavior we can determine how important it is to obtain more precise estimates of each parameter.

Table 5.1 Infectious diseases notifiable to the United Kingdom Health Protection Agency

- Acute encephalitis
- Acute poliomyelitis
- Anthrax
- Cholera
- Diphtheria
- Dysentery
- Food poisoning
- Leprosy
- Leptospirosis
- Malaria
- Measles
- Meningitis
- Meningococcal septicemia (without meningitis)
- Mumps
- Ophthalmia neonatorum
- Paratyphoid fever
- Plague
- Rabies
- Relapsing fever
- Rubella
- Scarlet fever
- Smallpox
- Tetanus
- Tuberculosis
- Typhoid fever
- Typhus fever
- Viral hemorrhagic fever
- Viral hepatitis
- Whooping cough
- Yellow fever

DYNAMICS OF INFECTIOUS DISEASE TRANSMISSION

The transmissibility of infectious diseases means that there is dynamic feedback between the prevalence of infection (or, more precisely, of infectious individuals) and the incidence of infection. This is why dynamic models are required for infectious diseases.

In a typical epidemic, the prevalence of infection rises initially as infection spreads. This causes an increase in the incidence of infection, which in turn causes prevalence to increase even faster – so the epidemic accelerates. Consequently, the supply of susceptible individuals becomes depleted (by their becoming infected) and the incidence falls, even though prevalence may continue to rise for a time. Eventually, the fall in incidence leads to a fall in prevalence because infections are 'lost' from the population (due to recovery, death or emigration) faster than they are replaced by the spreading of infection. In the longer term, the infectious agent may be able to persist in the population (i.e. become endemic) if there is a high enough rate of resupply of susceptible individuals due to birth, immigration, recovery from infection (if there is no lasting immunity) or waning of immunity (if applicable); otherwise the infectious agent will go extinct locally.

The key measure of an infectious agent's ability to spread in a population is the reproduction number (sometimes called the net reproduction number or effective reproduction number), $R(t)$, which is the mean number of new infections caused by a single infectious individual in the population of interest. (Note that '(t)' indicates that the value can change with time – see below.) A related quantity is the basic reproduction number R_0, which is defined as the mean number of new infections caused by a single infectious individual in a population of wholly susceptible individuals, i.e. the basic reproduction number is what the value of the reproduction number would be if the population were totally susceptible. It is important to understand that the reproduction number is specific to the particular infectious agent in the particular population at the particular time, and can be changed by interventions. The value of the reproduction number depends upon the average rate of transmission from an infectious individual and the average duration of infectiousness. An epidemic requires that $R(t)$ be greater than 1, so that the prevalence of infection increases because more than one new infection arises from the

average infected person before that person is 'lost' from the infected population. In the typical epidemic described above, depletion of the 'supply' of susceptible individuals causes $R(t)$ to fall, even though R_0 was not changing. In fact, $R(t)$ falls even as incidence rises; the increase in incidence is driven by the increase in prevalence, which initially 'overcomes' the effect of $R(t)$ falling.

Public health interventions aim to reduce and maintain $R(t)$ below 1, which may be achieved by reducing the average infectious period (e.g. through treatment or isolation) or the transmission rate (e.g. by closing schools and workplaces to combat SARS or influenza, or promoting condom use and reductions in numbers of sexual partners to combat STIs) or vaccinating people to remove them from the susceptible population. The higher the value of R_0, the harder an infection will be to control. In a homogeneous population (one where everyone has the same average risk of acquiring and transmitting infection) the relationship between R_0 and $R(t)$ is $R(t) = R_0 \times s$ where s is the proportion of the population that is susceptible. To prevent an epidemic by vaccination requires that s be reduced so that $R(t) < 1$ (i.e. that s be reduced below $1/R_0$), thus the greater the value of R_0 the smaller s must be. The critical vaccination threshold is the proportion of the population that must be successfully immunized to prevent an epidemic; for childhood infections such as measles which have high typical R_0 values this is typically >90% or even >95%.

It is important to realize that R_0 alone does not provide complete information on the transmission dynamics of an infectious agent. A highly infectious agent that spreads rapidly but which has a short infectious period could have the same R_0 value as another infectious agent that is much less infectious but which has a longer infectious period – the latter agent would spread more slowly but for longer.

COMPARTMENTAL MODELS OF INFECTION

The most common approach used in mathematical modeling of disease transmission is to divide or compartmentalize the study population with regard to their infection status (Fig. 5.1). Note that the structure of the model depends upon the natural history of the infection and so differs amongst infections. Important characteristics of the natural history of an infection are the incubation period (the time from the point of infection until the appearance of symptoms) and the latent period (the time from infection to becoming infectious). These periods vary greatly (from days to years, depending upon the infection) and either can be longer than the other. For HIV and influenza the latent period is shorter than the incubation period, with people becoming infectious before they become unwell, but for pulmonary TB they can be the same, with people becoming infectious at the time they become unwell.

In modeling there is a trade-off between complexity/realism and the ability to understand the model's behavior. Since even simple models can have complex dynamics it is important to make the model as simple as possible, whilst still capturing the essential features of the infection. For example, for gonorrhea the incubation period is often omitted from models[13] because it is short relative to the infectious period – and so has little effect on the dynamics of infection – while HIV's incubation period is long compared with the symptomatic late-stage period and so it is usually incorporated into models.[21] In the case of TB, most people with infection never develop infectious disease (they remain latently infected) and so models distinguish between these states.[22]

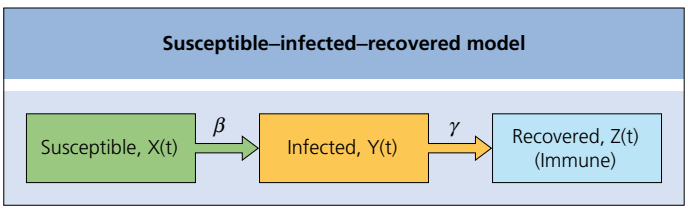


Fig. 5.1 The population is divided into three compartments according to whether they are Susceptible to infection, Infected (and infectious) or have Recovered from infection and are immune. Individuals who become infected move from the Susceptible compartment to the Infected compartment; the process of recovery subsequently moves them from the Infected compartment to the Recovered compartment. The parameters β and γ affect the rate of transmission of infection and the rate of recovery, respectively.

For a directly transmitted pathogen such as influenza, where acquired immunity (to a particular strain) is lifelong, the host population can be represented by three compartments containing the number of susceptible, infected (and infectious) and recovered (immune, non-infectious) individuals. In this example, the latent period is ignored, so individuals become infectious as soon as they become infected. This so-called called 'Susceptible–Infected–Recovered' (or 'SIR') model approach was first developed by Kermack & McKendrick in 1927,[23] elaborated upon more recently by Anderson & May,[4] and now forms the basis for many modern-day models of epidemics.

We present a simple example of an SIR-type model (see Fig. 5.1), which we apply to data from an outbreak of influenza in a boarding school in England.[24] Since the time period of the outbreak is short, we effectively have a 'closed' population: no one enters or leaves the population and there was no mortality due to infection, which simplifies our analysis. (Usually, one has to consider people entering the population through immigration and birth and leaving through emigration and death – and if the infection being modeled causes mortality then infected individuals have an additional disease-induced mortality rate which must be considered.)

Each compartment has a state variable that 'keeps track' of the number of individuals in that compartment, and how that number changes through time. In this case, the state variables are $X(t)$ for the Susceptible individuals, $Y(t)$ for the Infected individuals and $Z(t)$ for the Recovered individuals, where '(t)' indicates that the values can change with time. The total population size is $N(t)$, where $N(t) = X(t) + Y(t) + Z(t)$. (In this particular example, $N(t)$ does not change because the population is closed and there is no mortality.) The model consists of a set of differential equations which describe the rates that individuals flow between different compartments as they become infected, recover, die (if applicable), etc. The net rate of change in $X(t)$ is described by the differential equation $dX(t)/dt$, the net rate of change in $Y(t)$ is described by the differential equation $dY(t)/dt$, etc. In this example, there are two processes: infection and recovery.

The rate of infection (the number of people becoming infected per day) in the population depends upon the force of infection, the risk per susceptible individual of acquiring infection per unit time; and the number of susceptible individuals available to become infected, $X(t)$. The force of infection depends upon the prevalence of infection, $Y(t)/N(t)$, and the transmission parameter, β, which is a combination of the rate of contact between people in the population and the probability of transmission upon contact between an infected person and a susceptible person. Therefore, the force of infection is $\beta Y(t)/N(t)$ and the transmission rate is $X(t) \times \beta Y(t)/N(t)$, which is conventionally written as $\beta X(t)Y(t)/N(t)$. Since infection transfers people from the susceptible compartment ($X(t)$) to the infected compartment ($Y(t)$), the term $\beta X(t)Y(t)/N(t)$ appears negatively in $dX(t)/dt$ and positively in $dY(t)/dt$. (Note that the transmission parameter, β, does not change with time; changes in the infection rate are due to changes in $Y(t)/N(t)$ and $X(t)$.)

The rate of recovery (the number of people recovering per day) depends upon the per capita rate of recovery, γ, and the number of people who are infected, $Y(t)$, and is $\gamma Y(t)$. Since recovery transfers people from the Infected compartment ($Y(t)$) to the Recovered compartment ($Z(t)$), the term $\gamma Y(t)$ appears negatively in $dY(t)/dt$ and positively in $dZ(t)/dt$. (Note that the per capita rate of recovery, γ, does not change with time; changes in the recovery rate are due to changes in $Y(t)$.)

The equations of the model are:

$$\frac{dX(t)}{dt} = \frac{-\beta X(t)Y(t)}{N(t)}$$

$$\frac{dY(t)}{dt} = \frac{\beta X(t)Y(t)}{N(t)} - \gamma Y(t)$$

$$\frac{dZ(t)}{dt} = \gamma Y(t)$$

$$N(t) = X(t) + Y(t) + Z(t)$$

Note that this model is deterministic, i.e. random (stochastic) events are not considered. This is a common simplification that makes it much easier to gain insight into the fundamental dynamics of transmission because the effects of random chance, which cause fluctuations in the graph, are omitted. This model was fitted to data from an outbreak of influenza in a boarding school in England[24] (Fig. 5.2) by estimating values of β and γ.

The algebraic expression for R_0 depends upon the particular model. For this model, it can be derived simply. R_0 is the mathematical product of the transmission rate from a single infected individual in a wholly susceptible population and the average infectious period. The rate of transmission from a single infected individual when the population is wholly susceptible (i.e. when $Y(t) = 1$ and $X(t) = N(t)$; we ignore the fact that really $X(t) = N(t) - 1$ because one person is infected, because we assume that $N(t)$ is large) is:

$$\frac{\beta X(t)Y(t)}{N(t)} = \frac{\beta N(t).1}{N(t)} = \beta$$

The average infectious period is the reciprocal of the average recovery rate (the faster people recover, the shorter their infectious period), which is $1/\gamma$. Therefore $R_0 = \beta/\gamma$. The estimated values of β and γ obtained by fitting to the data were $\beta = 1.97$ per day, $\gamma = 0.47$ per day (corresponding to a mean infectious period of 2.12 days). Therefore the estimated R_0 value was 1.97 day^{-1}/0.47 day^{-1} = 4.18.

Note that there are other types of model used to represent infectious disease transmission, including stochastic compartmental models, individual-based network simulation models and spatial metapopulation models.[25]

EXAMPLES

Below we discuss SARS and influenza. Recent reviews of modeling of STIs and HIV[10,26,27] and TB[22] can be found elsewhere.

Example output of a 'SIR' (Susceptible–Infected–Recovered) model

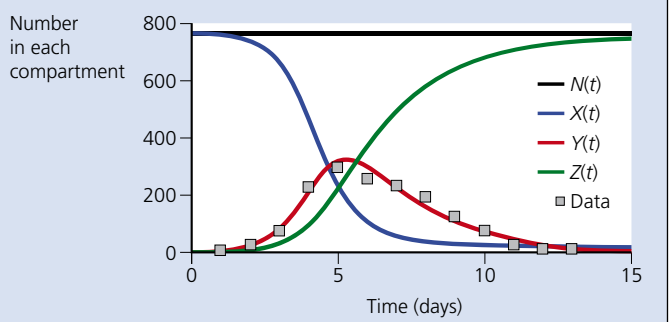


Fig. 5.2 Example output of a Susceptible–Infected–Recovered (SIR) model applied to data from an outbreak of influenza. Model parameters were adjusted to fit the number of Infected individuals, $Y(t)$, to the observed data.

Severe acute respiratory syndrome

The huge rise in the volume of international travel and the huge growth in population densities in many cities, especially in Asia, offer new challenges in controlling the spread of new epidemics. The first such epidemic, 'the first severe and readily transmitted disease of the 21st century' was SARS.[28] On November 16, 2002 the first human case of SARS was identified in Guangdong province in China. From here the disease is known to have spread quickly to other parts of Asia, then to Europe, the Americas and elsewhere, infecting >8000 individuals in 29 countries and killing at least 774 people.[8,28] SARS is caused by a coronavirus (SARS-CoV) normally found in wild animals such as the palm civet cat and Chinese ferret badger.[29] Early cases are thought to have involved zoonotic infection from animal reservoirs, a jumping of the species barrier, but genetic changes in the coronavirus enabled human-to-human transmission which accounted for the vast majority of cases in the global pandemic of 2002/3.

The causative agent of SARS was identified at an early stage of the outbreak and its genome was sequenced in a timely effort involving a multinational collaborative effort.[30] The sequence, published on May 30, 2003, showed it to be a single-stranded RNA virus containing 11 presumptive genes (open reading frames). Other RNA viruses include influenza and HIV and these are thought to be particularly prone to mutation as they are not proofread by DNA polymerases before transcription. The WHO global alert of March 2003 alerted countries to the spread of SARS and accurate case definitions were communicated to identify symptomatic carriers worldwide which led to the rapid control of the disease. This effective collaboration between health-care professionals in different countries and the sharing of patient and demographic data informed public health policy which limited the scale of the epidemic so that more than half of the countries affected reported fewer than 10 cases.

Sophisticated mathematical models of the SARS epidemic of 2002/3 have been developed, providing estimates of the key epidemiologic quantities and showing how disease transmission was controlled by effective intervention. The mean incubation period of SARS was reported to be between 4 and 6 days in most patients with a generation time of 8–12 days. The number of reported cases in the epidemic with time were used to estimate the initial growth rate (r, the rate of exponential increase in new cases at the start of an epidemic) which is related to the basic reproduction number (R_0) and the generation time (Tg, the mean period of time from a host becoming infected to infecting another individual) by the equation $R_0 = rTg + 1$. Using data from the initial period (before control measures were introduced) of the SARS epidemic in Hong Kong the epidemic growth rate was shown to be approximately 0.15 cases per day[31] which, when used in the above equation with $Tg = 10$, gives an R_0 value of 2.5, an estimate close to that made by analyses of data from Singapore by Lipsitch *et al.*[32] of between 2.2 and 3.6 days. Wallinga & Teunis[33] analyzed incidence data from four countries before and after the WHO global alert and subsequent control measures of March 2003. Before and after the alert, average $Z(t)$ values for Hong Kong, Vietnam, Singapore and Canada were 3.6 before the alert (0.7 after), 2.4 (0.3), 3.1 (0.7) and 2.7 (1), respectively. The reduction in the reproductive number in each country reflects the effectiveness of control measures such as quarantine and travel restrictions in curbing the epidemic.

SARS transmission was linked to close contact with another case and most of these were hospital-acquired infections of health-care workers or patients.[8,34] The mortality due to SARS calculated from WHO figures of 8098 probable SARS cases and 774 deaths gives a crude case fatality rate (CFR) of approximately 10.5%; however, the actual CFR is strongly positively correlated with age, with mortality in those over 65 years old exceeding 50% in a number of studies reviewed in Donnelly *et al.*[8] Estimating the case fatality rate of newly emerged pathogens is difficult as defining true cases can be problematic. The CFR may be overestimated if many subclinical infections go

uncounted. Alternatively, in epidemics where patients are hospitalized for lengthy periods, the CFR may be underestimated as patients can be recorded as cases before their outcome is known. In the 2003 SARS epidemic estimates of the CFR became more accurate as the fate of more patients became known but at the time this increasing CFR was wrongly taken as evidence that the virus was increasing in virulence.[8]

Influenza

In common with SARS, influenza is a disease that has been extensively modeled using data from past pandemics to predict the effectiveness of particular interventions in different disease scenarios. Influenza A is divided into subtypes based on differences in hemagglutinin (H) and neuraminidase (N) proteins. The 'Spanish flu' of the 1918 pandemic was an H1N1 lineage that was estimated to have caused up to 80 million deaths – many more than were killed in the First and Second World Wars combined. Small alterations to the influenza A genome occur by a process known as antigenic drift, where mutations increase in prevalence in the population driven by the selection of mutations in the viral genome in proteins exposed to the host immune system. These small changes lead to the differing severity of seasonal flu epidemics.

From a public health perspective the rarer phenomenon of antigenic shift is much more worrying. This results from the recombination of influenza genes to give novel combinations of virulence genes such as strains of avian flu of the H5N1 subtype that is widely feared to be the cause of the next pandemic wave of influenza. Past pandemic strains of influenza A are thought to have emerged from animal reservoirs following antigenic shifts that enabled them to become extremely pathogenic to humans, with surface proteins to which the host had little acquired immunity. Pandemic influenza differs from seasonal epidemic strains not only in the severity of infection but in other ways also. It is not restricted to the 'flu season' of the winter months and it tends to be most lethal in young children who presumably lack the immune memory of older patients.

In 2008 an epidemic of H5N1 influenza amongst Asian and African wild bird and poultry populations led to 385 cases of human disease resulting in 243 deaths – a case fatality rate of 63%.[35] These cases were overwhelmingly in individuals from Indonesia and Vietnam who had been in contact with poultry later shown to be infected with H5N1 strains. Control measures to destroy birds infected with H5N1 have been effective in limiting the number of human cases thus far but experts fear that a strain of highly pathogenic H5N1 will emerge that will acquire the ability to transmit between humans at high frequency which would lead to a global pandemic.

The 1918 influenza pandemic claimed between 50 and 100 million lives worldwide,[36] even although effective infection control procedures were in place in some areas (e.g. in some US cities). Bootsma & Ferguson[37] modeled the impact of infection control measures (such as the banning of mass gatherings, isolation, and improved hygiene and disinfection procedures) on transmission of the H1N1 epidemic in 16 US cities using historical datasets. The found that R_0 was reduced in cities with the most effective control measures (introduced at an early stage), which increased the length of the epidemic but reduced the overall and peak mortality. These data indicate that control measures may have a significant impact on a future H5N1 pandemic if introduced early in the course of the pandemic – in this case reducing mortality by 30–40%. The authors of this study caution against extrapolating data from the study too precisely on modern cities as family units and workplaces nowadays contain fewer people who are generally healthier. However, an H5N1 virus causing death in 14–33% of individuals infected would be expected to kill more individuals globally. This mortality could be mitigated by the rapid deployment of an effective H5N1 vaccine and the use of antiviral drugs such as neuraminidase and influenza A protein M2 inhibitors.[38]

Modeling pandemic influenza

A case fatality rate of 63% for avian influenza (from the number of cases and fatalities reported by WHO above) will be higher than the CFR during a pandemic as the number of cases reported so far will be an underestimate due to the non-inclusion of many nonfatal, mild and asymptomatic cases which will not be entered on the WHO reporting system. Additionally, some authors maintain that changes in the viral genome resulting in high rates of human-to-human transmission may cause a reduction in virulence in humans. From historical data the CFR for pandemic influenza A was calculated at between 0.1% (1957 and 1968 pandemics) and 2.5% (1918).[39] However, a recent article by Li *et al.*[39] suggests that a CFR of between 14% and 33% may be more realistic for a human transmissible H5N1 strain derived from an avian reservoir.

The examination of the SARS epidemic using mathematical models demonstrates some key qualities that enabled it to be contained effectively; however, when we compare these to features of past influenza epidemics and pandemics it appears that containing a future H5N1 pandemic will be much more difficult using similar containment/control measures. The generation time for influenza (4–6 days)[40] is much shorter than for SARS (8–12 days)[9,32] which means that influenza will spread much quicker than SARS given an overwhelmingly naive population (e.g. an H5N1 genotype epidemic). This will make a human transmissible H5N1 epidemic much harder to control than SARS. The R_0 of SARS of ~2.5 is similar if not higher than that estimated from reanalyses of pandemic influenza of 1.4–3.0,[40,41] but with a much higher expected CFR. In younger age groups in particular the impact of H5N1 would be expected to be much more costly not only in terms of disease but also from an economic viewpoint as a larger proportion and number of working-age individuals will be removed from the workforce by influenza.

One caveat about H5N1 that should be mentioned is that the factor that has primarily restricted transmission from birds to humans is that the sialic acid linkage favoured for binding to respiratory epithelium by highly pathogenic H5N1 is found primarily in the lower respiratory tracts of humans. This is one of the reasons that respiratory distress is such a common cause of death. The relative lack of these receptors in the upper respiratory epithelium causes much lower titers of virus to grow in nasal mucosa; hence, the virus is more pathogenic but much less transmissible by infected humans. In order to maintain its pathogenicity, the virus would need to maintain its tropism for the lower respiratory epithelial sialic acid receptor linkage and to acquire the ability to simultaneously bind to sialic acid linkages present in upper respiratory epithelium. It is quite possible (indeed likely) that the virus would have to compromise and be less pathogenic if it becomes more transmissible. Thus, a more highly transmissible H5N1 might be less transmissible than SARS and currently circulating strains of influenza A and less pathogenic than the strains that have to date been acquired directly from birds.

FUTURE RESEARCH

There is increasing integration between infectious disease modeling and empiric research in the field and laboratory. As noted above, models can be used to help set research priorities by determining which gaps in knowledge are most important epidemiologically. Increases in computing power make it possible to develop increasingly sophisticated simulation models and to use them in real-time to analyze outbreaks to determine whether interventions are

working and to guide policymakers in their response. DNA fingerprinting techniques are being used to identify clusters of transmission between individuals[42,43] and there is currently a lot of interest in synthesizing analysis of evolution and transmission dynamics, termed 'phylodynamics'.[44] Another area of research is in characterizing contact patterns between individuals in more detail[45] since this has important consequences for patterns of transmission. Finally, the use of modeling in planning and evaluating clinical trials has been advocated.[46]

REFERENCES

References for this chapter can be found online at http://www.expertconsult.com

Chapter 6

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History of infection prevention and control

In the United States, the hospital discipline of infection control was established in the 1950s in response to a nationwide epidemic of nosocomial *Staphylococcus aureus* and the recognition of the need for nosocomial infection surveillance.[1] As a concept, however, the epidemiology and prevention of infection has its roots in a time prior to the understanding of the germ theory of disease. In 1846 Semmelweis, a Hungarian physician, noted that the mortality from childbed fever among women who had babies delivered by midwives was lower than among mothers with babies delivered by physicians. After in-depth analysis of differences between the groups, Semmelweis concluded that the high rate of childbed fever was caused by cadaverous materials on the hands of medical students who came to the obstetric clinic directly from the autopsy chamber. A policy of hand washing in chlorinated lime solution before maternal contact was instituted, and the mortality rate among mothers cared for by physicians dropped.[2] John Snow, a British physician, applied statistics and epidemiologic approaches to determine and eradicate the source of a cholera outbreak in 1854 in London. The theories underlying the findings of both Semmelweis and Snow – that disease could be spread by hand contamination and fecal–oral transmission – were soundly rejected by the medical community in favor of the miasma or 'bad air' theory of disease causation. These two examples highlight not only some of the conceptual framework for medical epidemiology and infection control but also some of the challenges that face public health and infection control practitioners.

TRENDS AND COMPLEXITY OF CURRENT HEALTH CARE IN DEVELOPED COUNTRIES

Health-care-associated infections (HAIs) are a significant cause of morbidity and mortality in developed countries. It is estimated that between 5% and 10% of patients admitted to acute care hospitals acquire one or more infections. Using a multistep approach and three data sources, the Division of Healthcare Quality Promotion of the Centers for Disease Control and Prevention (CDC) estimated that the number of HAIs in US hospitals in 2002 was approximately 1.7 million. Thirty-two percent of all HAIs were urinary tract infections, 22% surgical site infections, 15% pneumonia and 14% bloodstream infections. The estimated number of deaths associated with HAIs in US hospitals in this study was approximately 99 000. The total number of deaths associated with HAIs by major site was highest for pneumonia (35 967) and bloodstream infection (30 655). The additional cost of patient care attributable to these infections was estimated to be $4.5–5.7 billion per year.[3,4] In the UK in 2000, it was estimated that 100 000 cases of hospital-acquired infection occurred in England with 5000 deaths, costing the National Health Services (NHS) as much as £1bn ($1.4bn) a year.[5]

In addition to the challenges posed by the numbers of HAIs, the complexity of these HAIs and the measures required to prevent them have become more complicated. Additional challenges that come under the umbrella of hospital infection prevention also continue to be identified. Such challenges include:

- controlling antimicrobial resistance and spread of multidrug-resistant pathogens;
- addressing emerging infections such as severe acute respiratory syndrome (SARS) and avian flu;
- providing constantly updated data for an increasingly sophisticated public;
- attempting to modernize surveillance and reporting systems, often with limited resources available;
- addressing the infectious consequences of ever more complicated medical procedures, with special populations such as highly immunosuppressed transplant patients, gene therapy, xenotransplantation; and
- maintaining a safe workplace in an ever more complex medical system.

ORGANIZATION OF INFECTION PREVENTION AND CONTROL

Infection prevention and control is a discipline in which epidemiologic and statistical principles are used in order to prevent or control the incidence and prevalence of infection. The primary role of an infection prevention and control program (IPCP) is to reduce the risk of acquisition of hospital-acquired infection, thereby protecting both patients and staff from adverse infection-related outcomes. In order to ensure that an infection control program is successful, the appropriate infrastructure and institutional support, both material and administrative, needs to be made available to hospital epidemiology staff.

The functions and structure of a hospital epidemiology program may vary between institutions. The critical functions that often fall under the umbrella of a hospital epidemiology program are listed in Table 6.1.[1,6,7]

Managing critical data and information

Developing, implementing and monitoring surveillance based upon an institution-specific risk assessment

The importance of surveillance as a part of hospital infection control programs was established by the 1976 Study on the Efficacy of Nosocomial Infection Control (SENIC). SENIC found that hospitals reduced their nosocomial infection rates by about 32% if their surveillance and control plan included the following components:

Table 6.1 Critical functions often managed by hospital epidemiology

- Managing critical data and information
 - Developing, implementing and monitoring surveillance based upon an institution-specific risk assessment
 - Reporting of surveillance results/infection rates to monitoring services, administration and regulatory bodies
- Developing and implementing policies and procedures to prevent or minimize infection risk (e.g. isolation precaution policies, etc.)
- Intervening to prevent disease transmission
 - Outbreak investigation and control
 - Education and training
- Collaborating with other programs to achieve common goals
 - Occupational and employee health
 - Postexposure prophylaxis in the health-care setting
 - Management of the infected health-care worker
 - Environmental health and safety
 - Construction infection control
 - Infectious waste management
 - Environmental cleaning service
 - Air and water handling
 - Respiratory protection
 - Disinfection and sterilization
 - Microbiology laboratory
 - Monitoring for isolation of sentinel organisms
 - Monitoring antibiotic resistance profiles
 - Pharmacy and therapeutics
 - Antibiotic utilization
 - Safety, quality and public reporting
 - Disaster preparedness committee
 - Bioterrorism preparedness

appropriate emphasis on surveillance activities and control efforts; appropriate staffing of the infection control program; and, for surgical site infections, feedback of wound infection rates to practicing surgeons.[8] Surveillance for nosocomial infection is critical for infection control programs, but must be paired with appropriate risk assessment at any given institution or setting, assessment of the need for intervention and strategies for implementation of control measures.[9]

Hospital infection surveillance should be a systematic, ongoing process to monitor identifiable events (such as surgical site infection) in a defined population. This will initially require a risk stratification to determine what the critical targets of surveillance should be. In the USA and other developed countries, many surveillance activities will be mandated by local or federal authorities, such as the Centers for Disease Control and Prevention (CDC), the American Hospital Association and the regulatory efforts of the Joint Commission (TJC). Other surveillance activities will vary, based on an understanding of the epidemiology and risk at a particular institution. For instance, surveillance for invasive aspergillosis in an institution undergoing new construction and with a large compromised host population might be rated a higher priority than the long-term monitoring of *Legionella* in an institution where *Legionella* has not been identified for years. Each hospital must tailor its surveillance activities based on risk assessment of the population as well as the available resources within the infection control team and hospital. Such 'targeted' surveillance should be defined for each hospital.[10]

A number of components are critical for an effective surveillance system.

1. Clear and uniform definitions of the infection or other outcome should be developed. Often, standardized definitions such as those defined by the CDC are the most useful so that comparisons can be made both within the system and with other institutions.[11]
2. Surveillance should be an active process that includes review of microbiologic data, clinical and nursing records, pharmacologic and pathologic data, readmission and reoperation data following surgery for selected procedures, etc. These data are made more easily accessible by computer-based patient records and other electronic systems for data retrieval and coordination. Indeed, automated surveillance systems for infection control may provide a sensitive, specific, time-efficient and cost-effective mechanism for surveillance in many institutions.[12,13] The surveillance methodology should rely on metrics that are objective, standardized and risk adjusted. Case validation by the practitioners of the procedural area under evaluation should be avoided; in this setting the process may be prone to bias and lose objectivity, especially if financial incentives are involved. On the other hand, periodic review of the case definitions and feedback on the surveillance should include members of the practice team in order to provide insights not necessarily within the skill set of the infection control team and to devise corrective quality improvement actions adapted to that specific practice.
3. Whatever the system of surveillance is, both numerator and denominator data must be available for review. For instance, central line-associated bloodstream infections (CLABSI) are generally expressed as number of CLABSI/number of central line days × 1000. This allows the surveillance to be expressed as a rate such that trends can be tracked and compared within and between institutions.[14]
4. Appropriate benchmarking should be sought. Increasingly, health-care systems are being asked to compare their rates of events to other institutions. In the USA between 1992 and 2004 the National Nosocomial Infections Surveillance (NNIS) System collected data regarding targeted infections from participating tertiary care hospitals; this system was reorganized in 2005 under the National Health and Safety Network (NHSN). Data provided from this network can be used for benchmarking infections between hospitals. However, although standardized data may be useful for identifying areas deserving more intensive interventions within a given institution, differences in hospital size, patient mix and risk adjustment introduce complexity when comparing rates across the spectrum from smaller community hospitals to tertiary care and specialty hospitals.[15,16]
5. Reports describing the surveillance activities and findings should be prepared (using appropriate statistical analysis) and distributed to the appropriate groups, which should include the services associated with the monitoring process and the administrative liaison affiliated with the service.
6. After feedback to the particular service is provided, that service (generally in conjunction with the IPCP) should develop an action plan for process improvement, if needed. This plan, after approval by all relevant parties, should be implemented. If possible, the next surveillance cycle should be used to evaluate if improvements occurred associated with the action plan.

Develop and implement policies and procedures to prevent or minimize infection risk (e.g. isolation precaution policies, etc.)

Another critical role for the infection control unit within a health-care facility is to develop and implement evidence-based policies and procedures that are aimed at preventing HAIs. These policies need to be practicable and available as a written or online resource to users. In general, these policies will be adapted to institutional needs using resources available from the following:

- relevant published literature;
- Healthcare Infection Control Practices Advisory Committee (HICPAC), Society for Healthcare Epidemiology of America (SHEA) and Association for Professionals in Infection Control and Epidemiology (APIC) guidelines;
- professional practice guidelines;
- state and federal regulatory bodies;
- Occupational Safety and Health Administration (OSHA);
- Environmental Protection Agency (EPA), etc.

Institutional policies and procedures should be regularly reviewed and updated.

Intervene to prevent disease transmission

Outbreak investigation and control

An outbreak can be defined as an increase in the incidence of a disease/infection above the background rate in a given population. In a health-care setting, the 'background' rate may be provided by ongoing surveillance activities as described above. In the health-care setting, prompt identification of an outbreak and intervention on the part of the IPCP is critical in preventing adverse outcomes and accruing costs. Whether the outbreak under investigation is SARS or methicillin-resistant *Staphylococcus aureus* (MRSA), or an increase in the baseline rate of hip prosthesis infection, the same basic components of outbreak investigation are followed, as outlined in Table 6.2.[17,18] An example of a functional surveillance program laying the groundwork for an outbreak investigation would be as follows:

> *Hospital X performs a large number of hip joint replacements, and this is a procedure monitored by IPCP (Fig. 6.1). Standardized NHSN criteria are used to define surgical site infections (SSIs) for hip prosthesis. Hospital X's surveillance for hip prosthesis involves review of all microbiology data for all hip replacements done at the institution plus readmission data after hip replacement, as well as antibiotic utilization data for patients with hip replacement. Charts are then reviewed to evaluate if a hip infection occurred and at what level (superficial, deep or organ space, by NHSN criteria).*
>
> *It is noted that in July, there was a large increase in the rate of hip infections that was two standard deviations above the institutional mean and over the 75th percentile for NHSN hip infection rate. Charts are reviewed to confirm, and an epidemic curve is generated suggesting that the increase in infection started in mid July. This information is reported back to Orthopedics as well as Hospital Administration. Patient data review indicates that the infections are with multiple different organisms, with procedures performed with multiple different surgeries in different ORs.*
>
> *It is noted by one of the health-care workers interviewed that a new surgical scrub was put into place in late June in the orthopedic ORs, and the concern is raised that the increased infection rate may be associated with this. A review reveals that the new scrub is not being used per recommendations. A plan to develop and implement an educational module regarding surgical scrub is enacted, and by September infection rates in this procedure are back to baseline.*

The role of the microbiology laboratory

The microbiology laboratory plays a critical role in both surveillance and outbreak investigation. Traditional roles have included detection, identification and susceptibility testing of microbes causing hospital infection. Rapid detection and reporting of key organisms with high potential to cause outbreaks such as *Clostridium difficile* or *Mycobacterium tuberculosis* are critical components of infection prevention, leading to appropriate implementation of control measures and reducing the risk of secondary spread.[19] For instance, at our institution, when a *C. difficile* toxin is identified in the microbiology laboratory, a report goes out simultaneously to the ward where the patient resides, so that the appropriate isolation can be ensured, including signage; to the infection control group to ensure that the isolation is logged in centrally; and to environmental services to ensure use of bleach cleaning for that room.

The development of an institutional antibiogram is a critical function that often results from collaboration between different groups, as will be discussed below.

Understanding pathogen distribution and relatedness in the hospital is an important component of both surveillance and outbreak investigation. Typing of microbial pathogens can help determine if isolates that appear to be epidemiologically linked are in fact genetically related and thus likely to have originated from the same strain. Typing can help distinguish extent and pattern of spread of 'epidemic' clones; it can also help assess the source of an outbreak (environmental, personnel, etc.). The incorporation of molecular typing methodologies along with traditional epidemiologic surveillance has been shown in a number of studies to reduce the number of HAIs and to be cost-effective.[20] Typing can be done using phenotypic methods (such as biotyping and serotyping) or genotypic/molecular methods, such as pulsed field gel electrophoresis, plasmid analysis, southern blotting or various forms of polymerase chain reaction (PCR). Newer modalities, such as sequence-based molecular epidemiologic analysis (SLST or MLST), have been used for the evaluation and typing of some pathogens.

Table 6.2 Steps in the investigation and control of a potential outbreak

1. Establish case definition(s).
2. Confirm that the cases are 'real' (case confirmation).
3. Establish the background rate of disease (in order to confirm the outbreak and determine the scope of the outbreak geographically and temporally).
4. Case finding.
5. Examine the descriptive epidemiology of the cases (e.g. define the age, sex, home/overseas travel, occupation, attendance at events) and plot an 'epidemic curve' of time of onset of disease.
6. Generate a hypothesis regarding the source and route of exposure.
7. Test the hypothesis by case control, cohort or intervention studies and by epidemiologic typing of representative samples if indicated and if possible.
8. Collect and test potential sources of infection such as environmental surfaces, patients, personnel, iv fluids, etc. as indicated; consider epidemiologic typing to establish an epidemiologic link to cases.
9. Devise and implement control measures.
10. Review results of investigation or report on ongoing investigations to administration and staff; consider consultation with local public health officials.
11. Follow-up surveillance to evaluate efficacy of control measures; generate reports for administration and staff.

Education and training

One of the most critical functions of the IPCP is to provide education and training. Education for health-care providers includes instruction on isolation precautions, aseptic practice, prevention of blood and body fluid exposures, and appropriate usage of personal protective equipment and safety devices. Teaching of policies and procedures should be simple, reproducible and, if possible, innovative (e.g. combinations of computer technology and live) in order to make an impact and reach the greatest number of health-care workers.

An important component of teaching involves the measurement and subsequent feedback of infection control data to the staff. As an example, performance and infection rate feedback in a neonatal unit has been effective in sustaining hand hygiene compliance improvement and in reducing infection risk in neonates.[21]

Collaboration with other programs to achieve common goals

Occupational and employee health

An active employee health service and IPCP collaboration is critical in the protection of health-care workers and the control of hospital-acquired infections. Joint objectives generally include:

- education of personnel about the principles and importance of infection control;
- prompt diagnosis and appropriate management of transmissible diseases in health-care workers, such as respiratory syncytial virus or pertussis;
- assessing and investigating potential exposures and outbreaks among personnel;

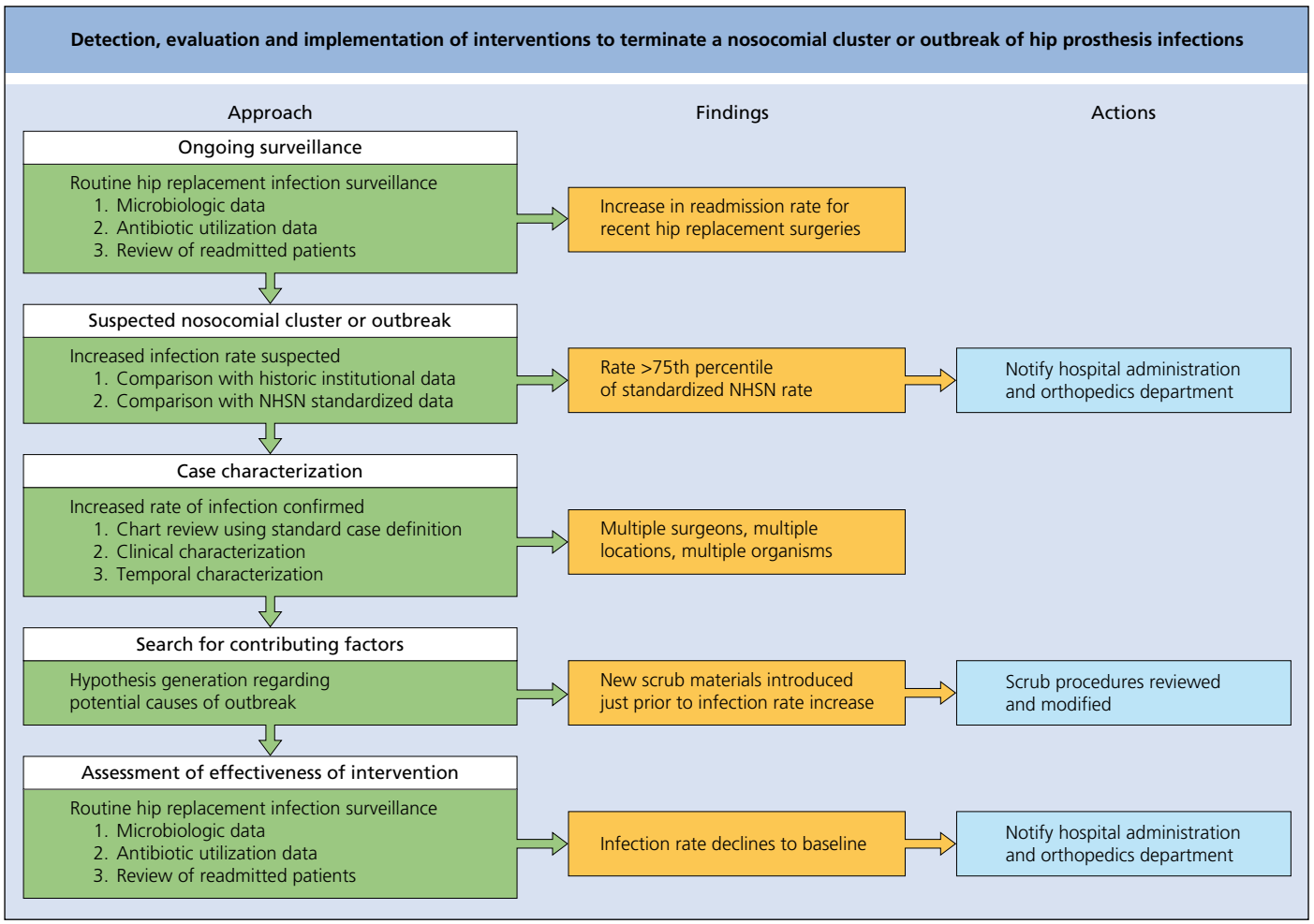


Fig. 6.1 Flow diagram of the detection, evaluation and implementation of interventions to terminate a nosocomial cluster or outbreak of hip prosthesis infections.

- identification and vaccination of workers susceptible to vaccine-preventable diseases;
- identifying work-related infection risks and instituting preventive measures; and
- surveillance of health-care workers for diseases such as tuberculosis.[22]

Detailed discussion of the role of employee health services is beyond the scope of this chapter. The CDC has published extensive guidelines and recommendations on immunization of health-care workers, occupational health guidelines and protection of health-care workers from blood-borne pathogens, including postexposure prophylaxis guidelines.[23–25] These are updated on a regular basis at http://www.cdc.gov.

Environmental health and safety and environmental services

The health-care facility environment is not commonly associated with disease transmission in competent hosts. Environmental Health and Safety and IPCP work together to ensure environmental safety and prevent exposure of patients and staff to environmental and airborne pathogens. The combination of infection control and environmental engineering strategies can help prevent such occurrences. These control measures include:

- adherence to ventilation standards for specialized care environments (e.g. airborne infection isolation rooms, protective environments or operating rooms) and to water-quality standards, including for hemodialysis;
- appropriate infectious waste management;
- appropriate use of cleaners and disinfectants; and
- appropriate use of precautions during construction.

Environmental Safety is often responsible, with Employee Health, for monitoring of pressure-negative airborne isolation rooms and N95 respirator fit testing.[26]

In this era of antibiotic resistant pathogens, the importance of environmental cleaning cannot be overstated. Environmental contamination of floors, beds, tables, faucets, doorknobs, blood pressure cuffs, thermometers, gowns, stethoscopes and computer terminals has all been well documented.[26,27] Among other factors associated with transmission, acquisition of drug-resistant organisms such as vancomycin-resistant *Enterococcus* (VRE) and MRSA may depend on room contamination, and the odds of acquiring antibiotic-resistant bacteria are increased by patient admission to a room previously occupied by a patient harboring the resistant organism.[27]

The cleaning and disinfection of all patient-care areas is important for frequently touched surfaces, especially those closest to the patient. Increased frequency of cleaning may be needed for compromised patients in a protective environment to minimize dust accumulation or in situations where environmental contamination is more likely (e.g. incontinent patients). During a suspected or proven outbreak where an environmental reservoir is suspected, cleaning procedures should be assessed and adherence should be monitored and reinforced.

In general, use of a US Environmental Protection Agency (EPA)-registered detergent/disinfectant (used according to the manufacturer's recommendations for amount, dilution and contact time) is sufficient

to remove pathogens from surfaces of rooms of colonized or infected individuals. Certain pathogens (e.g. rotavirus, noroviruses, *C. difficile*) may be resistant to some routinely used hospital disinfectants. Since levels of spore production for *C. difficile* may be increased when exposed to nonchlorine-based cleaning agents, and the spores are quite resistant to commonly used surface disinfectants, many investigators have recommended the use of a 1:10 dilution of 5.25% sodium hypochlorite (household bleach) and water for routine environmental disinfection of rooms of patients with *C. difficile*. Many institutions also recommend bleach cleaning when faced with outbreaks of norovirus or rotavirus as well.

General and specific recommendations for disinfection and sterilization may be found in the CDC's *Guidelines for Environmental Infection Control in Health-Care Facilities*.[26]

Disinfection and sterilization

Numerous reports detailing infection outbreaks secondary to faulty or inadequately disinfected medical instruments highlights the critical importance of sterilization and disinfection of such items.[28] IPCP collaborates with sterile processing to help prevent such problems.

Medical equipment and instruments/devices must be cleaned and maintained according to the manufacturers' instructions to prevent patient-to-patient transmission of infectious agents. Cleaning to remove organic material must always precede high-level disinfection (a process that eliminates many or all pathogenic organisms except bacterial spores) and sterilization (complete elimination or destruction of all microbial life). Disinfection may be accomplished using physical, chemical or physiochemical strategies to denature proteins. Sterilization is most commonly performed by steam/heat sterilization; ethylene oxide or hydrogen peroxide gas or prolonged liquid sterilization is also sometimes used.

Noncritical equipment, such as commodes, intravenous pumps and ventilators, computers used in patient care, etc., must be thoroughly cleaned and low-level disinfected before use on another patient. Providing patients who are on transmission-based precautions with dedicated noncritical medical equipment (e.g. stethoscope, blood pressure cuff, electronic thermometer) may prevent pathogen transmission. If this is not possible, disinfection after use is recommended. Semicritical items come in contact with mucous membranes and intact skin. This includes respiratory therapy and anesthesia equipment. High-level disinfection after cleaning is an appropriate standard of treatment for heat-sensitive, semicritical medical instruments (e.g. flexible, fiberoptic endoscopes).[28] This process inactivates all vegetative bacteria, mycobacteria, viruses, fungi and some bacterial spores. Critical items are objects that enter sterile tissue or the vascular system and pose a high risk of infection if contaminated with micro-organisms. This includes surgical instruments, various catheters, implants, etc. These items should either be purchased sterile or undergo heat-based sterilization after cleaning prior to patient use.

Information detailing the specific agents and processing used in disinfection and sterilization of equipment has been extensively reviewed elsewhere[26,29] and is beyond the scope of this chapter.

Pharmacy and therapeutics

Infection with antibiotic-resistant bacteria has been associated with increased morbidity, mortality and costs of health care. The goals of an effective antimicrobial stewardship program (ASP) include optimizing clinical outcomes while minimizing both toxicity associated with antibiotic use and the emergence of resistance, and reduction of health-care costs while maintaining or improving quality of care. The Infectious Diseases Society of America recommends a multidisciplinary approach to an ASP, with an infectious disease physician and a clinical pharmacist with infectious diseases training as core members of the team. Collaboration with a clinical microbiologist, an information systems specialist, an infection control professional and hospital epidemiologist is critical. Because such ASPs are important patient safety initiatives, they should function under the umbrella of quality assurance and patient safety and receive hospital administrative and fiscal support.[30–32] The ASP may also work with microbiology, pharmacy and the IPCP to create an institutional and unit-specific antibiogram, which can be accessible to all antibiotic prescribers in the health-care system.

Safety, quality and public reporting

Health-care-associated infections are one of the most common complications affecting hospitalized patients and are considered to be one of the more accurate indicators of the quality of patient care. Thus, the process and outcome data generated by infection control and other practitioners is relevant to patient safety and quality of care at the level of the institution, across institutions and extending to credentialing and governmental regulatory boards such as the Joint Commission (formerly JCAHO) and OSHA.[33] Ensuring the quality of care requires input from many groups, including infection control, quality improvement, risk safety and other committees.

Since 2002, seven states in the USA have enacted legislation that requires health-care organizations to publicly disclose HAI rates, and many others have submitted similar legislation for review. In the UK, mandatory health-care organization-based surveillance and public reporting of MRSA bloodstream infections have been in place since 2001.[34] Despite this movement toward public reporting of HAIs, little is known about its effectiveness in improving health-care performance. The CDC published consensus recommendations for public reporting in 2005,[35] which emphasize choosing standardized epidemiologic methodologies, promoting thoughtful choices of process and outcome measures depending on the nature of the institution, ensuring feedback to health-care providers and providing adequate infrastructure support. They recommended consideration of several process measures to evaluate, such as central line insertion practices, surgical antibiotic prophylaxis and influenza vaccination coverage of health-care workers and patients. The two outcome measures considered most appropriate for some hospitals to consider were rates of central line infections and surgical site infections for selected operations.

Disaster and bioterrorism preparedness

The anthrax letters in 2001, the SARS outbreak in 2002 and the continued concern about an avian influenza pandemic have all heightened awareness of the importance of disaster (natural or bioterrorism related) preparedness. Infection control plays an integral role in such a committee, in order to develop plans to minimize exposure of staff and the potential for nosocomial transmission (see isolation guidelines).

ISOLATION PRECAUTIONS

Standard and transmission-based precautions

Standard precautions

Standard precautions constitute a system of barrier precautions designed to be used by all health-care personnel on all patients, regardless of diagnosis, to reduce the risk of transmission of microorganisms from both recognized and unrecognized sources. These sources include blood, all body fluids, secretions, excretions, intact and non-intact skin, mucous membranes, equipment and environmental surfaces. Standard precautions are part of the standard of care for all patients.

The use of barriers is determined by the care provider's 'interaction' with the patient and the level of potential contact with body substances. It is the responsibility of the individual to comply with

all isolation precautions. Ongoing education concerning standard precaution principles will be given to newly hired employees involved directly or indirectly in patient care and as needed for dissemination of new information or for reinforcement of consistent practices.

Elements of standard precautions include hand hygiene and the banning of artificial nails. This is because most infections in the health-care environment are transmitted through contact with contaminated hands of the health-care workers. In 2002, the CDC published guidelines for hand hygiene.[36] These guidelines were adopted by the Joint Commission on Accreditation of Healthcare Organizations (JCAHO) in 2004 as part of the new National Patient Safety Goal 7A.[37]

Whenever possible and available, alcohol-based products will be the primary method used for decontaminating hands. Alcohol-based products are more effective for reducing microbes on the skin than soap or antimicrobial soaps and water, and should be the routine method for decontaminating hands if hands are not visibly soiled. Visibly soiled hands should be washed with soap or antimicrobial soap and water for 15 seconds.

Artificial nails are not permissible for personnel with any direct patient contact or with patient supplies, equipment or food. Studies have shown that long fingernails, both artificial and natural, are more likely than short natural nails to harbor bacteria that cause health-care acquired infections. The natural nails of health-care workers must be kept neatly manicured and should not extend 5 mm past the fingertips. Besides artificial nails, other nail enhancements must not be worn. This includes but is not limited to tips, wraps, appliqués, acrylics, gels and any additional items applied to the nail surface. Nail polish, provided it is not chipped, is the only enhancement that should be permitted on short natural nails.

Hand washing and hand antisepsis must occur before any direct patient contact and between patients, between tasks/procedures on the same patient to prevent cross-contamination of body sites, before donning gloves and performing an invasive procedure, after contact with patient's intact skin (e.g. taking a pulse, blood pressure or lifting a patient), after removing gloves or other personal protective equipment (PPE), after contact with body substances or articles/surfaces contaminated with body substances, and before preparing or eating food. Hands should be washed with soap and water after 7–10 applications of an alcohol-based product.

In the presence of *Clostridium* spores alcohol hand products should not be used because they are not killed by alcohol. In these situations, hands should be washed with soap and water. In addition, hands should be washed with soap and water after covering a sneeze, nose blowing or using the bathroom, all of which may contaminate and soil hands.

Gloves, masks, eye protection and face shields, aprons, gowns and other protective body equipment

Disposable gloves must be worn for anticipated contact with moist body substances, mucous membranes, tissue and non-intact skin of all patients, for contact with surfaces and articles visibly soiled or contaminated by body substances, during venous blood draws or other vascular access procedures (starting a venous line or blood draws) – in other words, in any situation where contamination of hands is anticipated. If the use of gloves is needed, gloves should be donned immediately prior to the task. Torn, punctured or otherwise damaged gloves must be replaced immediately. Gloves should be removed and disposed of after every task involving body substance contact and before leaving the bedside. Gloves should not be worn away from the bedside or laboratory bench, at the nursing station, to handle charts, when touching clean linen, clean equipment or patient care supplies, or in hallways or elevators. Hands have to be washed as soon as possible after glove removal or removal of other protective equipment. Gloves are not to be washed or decontaminated for reuse. An exception to this rule are utility gloves (not for direct patient care) used by house keepers, plumbers, etc. In this situation, gloves may be decontaminated and reused provided the integrity of the glove is not compromised.

Masks, in combination with eye protection devices (goggles or glasses with side shields) or chin-length face shields, should be worn during procedures or other close contact that are likely to generate droplets, spray or splash of body substances to prevent exposure to mucous membranes of the mouth, nose and eyes. This is particularly relevant in situations known to increase the risk of splash or splatter. Nonexhaustive examples are surgery, trauma care, newborn delivery, intubation and extubation, and suctioning, bronchoscopy and endoscopy, emptying bedpans and suction canisters into hopper or a toilet. Masks and eye protection devices should be used if caring for a coughing patient with suspected infection.

Plastic aprons or gowns and other protective body clothing are used during patient care procedures to prevent contamination of clothing and protect the skin of personnel from blood or body fluid exposure. In laboratory settings, laboratory coats should be used.

Additional protective equipment, including surgical caps, hoods and shoe covers or boots, may be used in surgical or autopsy areas. All protective body clothing should be removed immediately before leaving the work area.[26,36]

Transmission-based precautions

Transmission-based precautions are to be used in addition to standard precautions, in patients with documented or suspected infection or who are colonized with an organism that is transmissible and/or that is of epidemiologic significance. There are three types of transmission-based precautions: contact, droplet and airborne. A sign with the type of transmission-based precautions should be placed outside the room of the patient. In the USA, to comply with the Health Insurance Portability and Accountability Act (HIPAA), enacted by the US Congress in 1996, the name of the infecting organisms may *not* be written on the sign.

Waste disposal, spill management, linen and food trays should be handled in the same way for all patients, regardless of precaution category. Isolation trays are not required. After patient use, both linen and food trays are sent directly for cleaning and disinfection.[26,38,39]

Contact precautions

Contact precautions are initiated and maintained to interrupt the transmission of epidemiologically significant micro-organisms known to be spread by contact. These precautions are intended to reduce the colony count of bacteria on horizontal surfaces and in the immediate vicinity of the patient.

Contact precautions are to be instituted on a case-by-case basis at the discretion of the IPCP staff, infectious disease staff and/or medical or nursing staff. Examples of situations in which contact precautions are to be initiated are:

- when a patient is colonized and/or infected with multidrug-resistant organisms or organisms that are not treatable with the usual antibiotics, i.e. multidrug resistant Gram-negative rods, MRSA and VRE; and
- when a particular organism is identified as being potentially hazardous because of its pathogenicity, virulence or epidemiologic characteristics, e.g. rotavirus, *C. difficile*, *Salmonella* spp. and *Shigella* spp.

After hand hygiene, the key element of contact precautions is personal protective equipment (PPE). Upon entering the room of a patient placed in contact precautions, gown and gloves should be worn at a minimum. All PPE must be removed before leaving the room and hand hygiene must be done. Disposable gowns should be used at all times when entering the patient's room. Gowns may be worn one time only, and then should be disposed of in the regular (nonbiohazardous) waste before leaving the room.

The patient should be placed in a private room whenever possible. When a private room is not available, cohorting of patients with the same confirmed micro-organism (but with no other infection) is acceptable but IPCP should be notified. Because a negative air pressure room is not required, the door may remain open. When neither a private room is available nor cohorting is achievable, a space

separation of at least 3 feet should be present between the infected patient and other patients or visitors.

To minimize contamination, equipment should not be shared (unless it is disinfected properly) between patients. Examples of dedicated equipment include, but are not limited to, electronic thermometer, blood pressure cuff, manometer, stethoscope, intravenous pole, wheelchair or gurney. For pediatric patients with fecal pathogens such as VRE or rotavirus and who require weighing, a dedicated scale should be placed in the room.

In the USA, hospital staff should use an EPA-approved detergent/disinfectant to wipe down high-touch (e.g. door knobs, bed rails) and horizontal surfaces (e.g. over bed table, night stand) as needed and at a minimum once a day. This cleaning should also include the surfaces of electronic equipment, respiratory therapy equipment and other items that come in physical contact with the patient.

In critical care units or units where there is a high endemic rate of the organism the wipe down should be repeated as needed and at minimum each shift. Cleaning cloths used in the room should not be used to clean other patients' rooms and equipment.

Traffic into the patient's room should be limited only to essential staff/visitors. All visitors shall be instructed in proper hand hygiene technique. Visitors that participate in direct patient care shall be instructed in gowning and gloving, if the patient is incontinent, diapered or has diarrhea or a draining wound. Visitors may be referred to infection control or given written educational material.

Droplet precautions

Droplet precautions are required when a patient is suspected or known to have a serious illness transmitted by large particle droplets or direct contact with respiratory secretions. Droplets are often 30–50 microns in size compared to aerosolized droplet nuclei which are less than 5 microns in size. They are often generated by a patient coughing, sneezing or talking, or during suctioning while in close contact with the patient. Droplet precautions include the use of barriers to prevent contact between infectious droplets and the mucous membrane of health-care providers and visitors. Organisms and diseases that require droplet precautions are listed in Table 6.3. After hand hygiene, the key element of droplet precautions is the use of a surgical mask with face shield or 'surgical masks with eye protection' for face to face contact within three feet of a symptomatic patient to prevent self-inoculation. A surgical mask should be donned upon entering the room. All PPE must be removed before leaving the room and hand hygiene must be done.

The patient should be placed in a private room whenever possible. Because a negative air pressure room is not required, the door may remain open. When neither a private room is available nor cohorting is achievable, a space separation of at least 3 feet should be present between the infected patient and other patients or visitors.

Patient movement should be limited to essential needs outside of room. Patients must wear a surgical mask while outside of room.

Visitors should be limited. If visitors are susceptible, they must wear a surgical mask with face shield. Visitors with upper respiratory symptoms should not visit, but special consideration may be given to close family members. Nursing staff must instruct family and visitors to wash hands after contact with patient secretions or contact with the immediate patient environment.

Airborne precautions

Airborne precautions are required when a patient is suspected or known to have a disease transmitted by airborne droplet nuclei. The evaporated droplets contain micro-organisms that remain suspended in the air and can be widely dispersed by air currents within a room or over a long distance. The diseases or infections requiring airborne precautions are listed in Table 6.4.

Strict hand hygiene after contact with patient or items contaminated with respiratory secretions is required. An OSHA-approved mask for tuberculosis, such as the N95 respirator that has been fit-tested or a powered air purifying respirator (PAPR), should be worn by health-care personnel.

In the USA, the patient should be placed in a designated private room with monitored negative air pressure in relation to surrounding areas, with a minimum of 12 air exchanges per hour for new construction and renovation and six air exchanges per hour for existing facilities. Air from the room must be discharged directly outdoors or recirculated through high-efficiency particulate air (HEPA) filters before being circulated to other areas in the hospital. The windows and the door to the patient's room must remain closed except for entry/exit. The patient is confined to the room unless a procedure outside the room is necessary. The patient must wear a tight-fitting surgical mask outside of the room when transported to another department and personnel accompanying the patient should wear an N95 respirator or a PAPR during transport. Patients who are discharged from the hospital but are still considered contagious must be instructed about the need to wear a surgical mask. Visitors should be limited to strictly necessary at all times. Visitors must wear a surgical mask that is secured and snugly fitted. Symptomatic household or other contacts of the patient should not visit until medically cleared. If a symptomatic contact must visit, a mask must be donned before entering the hospital and worn continuously while in the facility.

Table 6.4 Organisms and conditions requiring airborne transmission-based precautions

- Hemorrhagic fevers
- Lassa fever
- Marburg virus disease
- Mycobacteria, tuberculous
- Pneumonia
- SARS (coronavirus)
- Smallpox (variola)
- Smallpox vaccine (vaccinia) from UCSF
- Tuberculosis (TB) including multidrug-resistant tuberculosis (MDR-TB)
- Vaccinia

Table 6.3 Organisms requiring droplet transmission-based precautions

- Adenovirus infection
- Anthrax pneumonia
- Coronavirus infection, respiratory
- Croup (laryngotracheobronchitis)
- Diphtheria
- Ebola virus infection
- German measles (rubella)
- Herpes simplex
- Influenza
- Meningitis
- Meningococcal pneumonia
- Meningococcemia
- Mumps (infectious parotitis)
- *Mycoplasma* infections
- Parainfluenza
- Parvovirus B19
- Pertussis (whooping cough)
- Plague
- Rabies
- Respiratory infectious disease, acute (if not covered elsewhere)
- Respiratory syncytial virus (RSV) infection
- Rhinovirus infection, respiratory
- Rubella (German measles)
- Scarlet fever
- Streptococcus: Group A
- Whooping cough (pertussis)

Table 6.5 Organisms requiring airborne non-acid-fast bacillus transmission-based precautions

- Chickenpox (varicella)
- Herpes zoster (disseminated)
- Herpes zoster (shingles in immunocompromised)
- Measles (rubeola)
- Rubeola (measles)
- Varicella (chickenpox)

Vacating an airborne precautions patient room

If the patient is being ruled out for TB or diagnosed with TB and was in a room without negative pressure, the room must not be used for 1 hour after the patient has been discharged. If the patient is being ruled out for TB or is diagnosed with TB and was in a negative-pressure room, the room must not be used for 30 minutes after the patient has been discharged.

Airborne precautions are also required for patients with diseases that are highly communicable by the airborne route. Examples of diseases that fall into this category of precaution are listed in Table 6.5. Nonimmune staff or visitors are not allowed to enter the patient's room or provide care. Non immunity means either no history of the specific disease or no vaccination against that disease. Respiratory protection is not needed for immune healthcare workers.

HEALTH-CARE AND DEVICE-ASSOCIATED INFECTIONS

Health-care-associated infections (HAIs) are infections occurring as a result of treatment and after exposure to the health-care environment. Infections can be acquired in all health-care settings – ambulatory, inpatient or during emergency room visits. Duration and frequency of exposure appear to increase the risk of infection as does decreased immunity due to co-morbidities or treatments. Infections are considered health-care associated if they manifest 48 hours or more after admission to a hospital, within 30 days of discharge from a health-care facility or if a patient visited an outpatient medical facility within the past 6–12 months.[40,41]

Health-care-associated infections include those with hospital onset, which are diagnosed 48 hours or more after admission to the facility and those diagnosed with community onset in patients with previous health-care encounters. In contrast, community-associated infections are defined as infections manifesting and diagnosed within 48 hours of admission in patients without any previous encounter with health care. In this section, we will address HAIs.

A recent CDC report updated previous estimates of HAIs and related deaths in US hospitals.[42] This report was based on HAI surveillance outcome data submitted to the NNIS System. The NNIS was a voluntary collaborative network of 283 US hospitals with 100 or more beds performing HAI surveillance using standardized CDC definitions and methodologies.[43]

This report estimated that, in 2002, HAI accounted for 1.7 million infections and that 98 987 deaths were either associated or were caused by HAI. While the majority (1 195 142) of HAIs in adults and children occurred outside of an intensive care unit (ICU), 394 288 (23%) were among patients in an ICU, which were also associated with the highest rates of associated deaths, and 52 328 infections were in newborns residing in either a high risk or a well baby nursery. The calculated rates were 9.3 infections per 1000 patient days or 4.5 infections per 100 admissions in 2002.

In addition to the deaths, the morbidity associated with HAI is significant. Studies have demonstrated that HAI results in excess length of stay (LOS) and costs.[6] For example, the mean attributable cost of a catheter-associated bloodstream infection was $18 432 with a mean excess LOS of 12 days.[6] The cumulative excess cost of HAI based on the 2002 CDC data was recently estimated at $4.5–6.5 billion.[44]

Table 6.6 Hospital-acquired infection (HAI) groups

- Device-related infections
 - Central line-associated bloodstream infection (CLABSI)
 - Ventilator-associated pneumonia (VAP)
 - Foley catheter urinary tract infection (UTI)
 - Infection of a prosthetic device
- Nondevice-related infections
 - Health-care-associated pneumonia (HAP) other than VAP
 - Infections due to multidrug-resistant organisms (MDRO)
 - *Clostridium difficile* colitis
 - Methicillin-resistant *Staphylococcus aureus* (MRSA)
 - Vancomycin-resistant *Enterococcus* (VRE)
 - Gram-negative rods with MDRO pattern or extended spectrum β-lactamase (ESBL) producing
- Procedure-related infections
 - Transplant-associated infections
 - Surgical site infections (SSI)
 - Bloodstream infections
 - Septicemia

Of all HAIs with an identifiable source, urinary tract infections (UTI) were the most frequent at 32%, followed by surgical site infections (SSI) at 22% (2% of all surgeries performed), pneumonias (PNA) at 15%, followed by bloodstream infections (BSI) at 14%. In the ICUs, 20–25% of all infections were caused by a UTI, PNA or a BSI. Outside the ICUs, 35% of HAIs were UTI, 11% were either BSI or PNA and 20% SSI. Thirty-six percent of deaths were attributed to PNA, 31% to BSI, 13% to UTI and 8% to SSI.[44]

Since the SENIC study,[45,46] HAI rates have decreased following implementation of infection prevention initiatives, but the proportion of truly preventable infections remains unclear. Based on a meta-analysis of infection control intervention studies, Harbarth and colleagues estimated this proportion to be between 10% and 70%, depending on preintervention HAI rate, hospital setting, study design, types of intervention and infection.[47] Interventions associated with the greatest reduction in a particular HAI have consistently been those aimed at prevention of central line-associated bloodstream infections (CLABSI).

HAIs can be divided into three broad, sometimes overlapping groups: device related, nondevice related and procedure related (Table 6.6).

Device-related HAI

Central line-associated bloodstream infections

Of all device-related HAIs, central line-associated bloodstream infections (CLABSI) are among the best studied. Vascular access is an essential part of care of patients and often extends beyond the inpatient stay into ambulatory care. Colonization of the device around the insertion site by bacteria or fungi on the skin are thought to constitute the most frequent first step of a central line infection. However, for invasion into the bloodstream to occur, bacteria have to adhere and incorporate into the biofilm,[48] multiply and then invade. Infection of the catheter hub and invasion of the lumen by coagulase-negative staphylococci is a known independent major risk factor for a central line infection that has been extensively researched.[49,50]

Bacteremia and septicemia secondary to contamination of the infusate occur much less frequently but are a recognized source of clusters or outbreaks of bloodstream infections with Gram-negative organisms.[51–53]

Risk factors for CLABSI include host factors (severity of illness, lack of skin integrity, type of immunosuppression), factors related to

the device (catheter insertion and maintenance processes, type and size of catheter, number of lumens, insertion site) and finally factors related to the purpose of the catheter (function of catheter, duration of placement).[54,55]

CLABSI prevention initiatives and surveillance have been standardized internationally, have well-established definitions and methodologies and therefore can be easily linked to measurable process and outcome measures. Unlike other quality and safety measures, surveillance of CLABSI has proven very helpful in the objective evaluation of the efficacy of performance improvement initiatives.[4,36,56–59]

The significant reductions in CLABSI following adherence to simple infection control principles and surveillance observed in the SENIC study in the late 1970s have continued, with median CLABSI rates in adult critical care units now ranging from 0.6 to 4.0 CLABSI per 1000 line days.[4,60]

In 2002, a working group published guidelines for the prevention of intravascular device-related bloodstream infections. Among the key evidence-based recommendations were education and standardization of insertion and maintenance processes, the use of maximal sterile barrier precautions upon insertion, chlorhexidine skin preparation, antiseptic/antibiotic-impregnated central venous catheters for short-term use only when rates of infection are high, avoiding routine replacement of the line for the purpose of line-infection prevention and using standardized process metrics to measure compliance with these guidelines. However, it was not until the Institute for Healthcare Improvement (IHI) launched the '100 Thousand Lives' central line-associated bloodstream infection prevention initiative that these recommendations were widely adopted by health-care facilities in the USA in the ICU setting (http://www.ihi.org/IHI/Programs/Campaign). Following implementation of the IHI campaign, CLABSI rates have seen substantial and sustained drops not only in the ICU setting but also on acute care wards.[4]

Ventilator-associated pneumonia

Ventilator-associated pneumonia (VAP) develops in 9–27% of ICU patients who require mechanical ventilation.[61–63] To meet the criteria for VAP, the pneumonia has to manifest more than 48 hours after intubation.

Ventilator-associated pneumonia significantly increases the time on ventilator, the overall costs of care ($23 000–40 000) and length of stay in and after discharge from the ICU. The average length of stay is increased by 9.6 days in a patient who develops VAP.[6,64,65]

Moreover, VAP is the leading cause of death among HAIs and is associated with a doubling of mortality compared to ventilated patients with similar characteristics who do not develop VAP.[42,64,66–70]

Infection control/infectious diseases and critical care specialists have not come to full agreement on the definitions and methodology to be used for the diagnosis of VAP.[71,72] Diagnosis of VAP is challenging because patients requiring mechanical ventilation have underlying complex diseases and co-morbidities with similar and confounding symptoms and signs.[19,73]

Despite the lack of an uncontested gold standard for the diagnosis of VAP, the American Thoracic Society and the Infectious Diseases Society of America published guidelines in 2005 recommending that clinical signs and quantitative cultures of the bronchoalveolar lavage (BAL) fluid be used to diagnose and treat VAP.[74] In 2006, a large randomized trial of 740 patients on mechanical ventilation in 28 ICUs across the USA and Canada did not demonstrate any difference in outcomes and in antibiotic use between quantitative cultures of BAL and nonquantitative cultures of the endotracheal aspirate.[72] The development of institution-specific collaborative guidelines for the diagnosis and management of VAP have led to shorter antibiotic duration and improved antibiotic choice without affecting overall mortality.[75]

VAP prevention process measures are now better established and many are supported by randomized controlled trials. Preventive strategies are aimed at avoiding unnecessary intubation, decreasing the duration of ventilation, preventing aspiration, and minimizing inoculation and colonization of the lower respiratory tract with mouth, gastrointestinal and upper respiratory tract flora. When implemented fully, these measures have resulted in better patient outcomes and are cost-effective.[76,77]

Multidrug-resistant organisms

Many organisms can be potentially acquired in the health-care setting (Table 6.7).

As care has evolved and become more complex, new antimicrobials have increased antibiotic pressure and thus selection of drug-resistant mutants. As a result, organisms resistant to multiple classes of drugs have emerged worldwide.[65] Infections due to multidrug-resistant organisms (MDRO) represent a significant proportion of the both the HAI burden and the day-to-day work of the IPCP. A nonexhaustive list of MDRO associated with HAI is shown in Table 6.8.

Guidelines for metrics to be used to monitor, and processes to prevent, MDRO in health-care settings have just been published.[78–80]

While resistance definitions for Gram-positive organisms are well established, there is no standard definition for most Gram-negative

Table 6.7 Organisms that may be acquired in the health-care setting

- *Acinetobacter* species
- Blood-borne pathogens
- *Burkholderia cepacia*
- Chickenpox (varicella)
- *Clostridium difficile*
- *Clostridium sordellii*
- Creutzfeldt–Jakob disease (CJD)
- Ebola (viral hemorrhagic fever)
- Gastrointestinal infections
- HIV/AIDs
- Influenza
- Methicillin-resistant *Staphylococcus aureus* (MRSA)
- Mumps
- Norovirus
- Parvovirus
- Poliovirus
- Rubella
- Severe acute respiratory syndrome (SARS)
- *Streptococcus pneumoniae* (drug resistant)
- Tuberculosis
- Vancomycin-intermediate *Staphylococcus aureus* (VISA)
- Vancomycin-resistant *Enterococcus* (VRE)

Table 6.8 Multidrug-resistant organisms acquired in the health-care setting

- Drug-resistant *Staphylococcus aureus*
 - Methicillin-resistant *Staphylococcus aureus* (MRSA)
 - Healthcare-associated: acquired in hospital or outpatient health-care facilities
 - Community-associated: acquired in the community
 - Vancomycin-intermediate/resistant *Staphylococcus aureus* (VISA/VRSA)
- Other drug-resistant organisms
 - Vancomycin-resistant *Enterococcus* (VRE)
 - Gram-negative organisms with multidrug resistance (MDR) or extended spectrum β-lactamase (ESBL) producing
 - MDR *Acinetobacter* spp.
 - Penicillin-resistant *Streptococcus pneumoniae*
 - MDR *Pseudomonas* spp.
 - MDR *Mycobacterium tuberculosis*
- *Clostridium difficile*-associated disease (CDAD)

MDRO.[81,82] For the purpose of this chapter, Gram-negative MDRO are defined as organisms resistant to one or more classes of antimicrobial agent.[83]

Infections caused by MDRO are particularly prevalent in intensive care, transplant and human immunodeficiency virus units, where patients are most susceptible to invasion by colonizing organisms because of the acuity of the primary disease and the comorbidities with multiple potential portals of entry and high exposure to broad-spectrum antibiotics. In this setting, the inability to perform personal hygiene to decrease the bioburden and the forced immobilization exponentially increase the potential for acquiring pathogens from the contaminated environment or the health-care worker's hands.

New guidelines for the prevention of MDRO in the health-care setting underscore the importance of well-described evidenced-based infection prevention measures and coordinated antimicrobial stewardship programs.[78]

REFERENCES



References for this chapter can be found online at http://www.expertconsult.com

Chapter 7

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Bacterial genomes

SUMMARY

Within just a decade, genomics has become an irreplaceable source of insight into the genetic diversity, pathogenesis, evolution, diagnosis and treatment of bacteria. To date, 883 genomes have been completed, including those of all major human pathogens, and the development of high-throughput sequencing methods in parallel with improved analysis tools should boost this field, as highlighted by more than 2600 announced projects. Herein, we summarize the current achievements in bacterial genomics, with emphasis on their applications to clinical microbiology and infectious diseases.

INTRODUCTION

Two decades ago, the availability of a complete genome sequence was a dream. In 1995, scientists at The Institute for Genomic Research (TIGR) opened the road of bacterial genomics by sequencing the *Haemophilus influenzae* genome.[1] This pioneering work generated major enthusiasm from scientists worldwide and bacterial genome sequencing subsequently experienced considerable development, with the annual number of sequenced genomes nearly doubling every 2 years since 2000 (Fig. 7.1).

Initially, genome sequences were mostly used to address fundamental questions in research, notably for studying bacterial evolution. However, the development of high-throughput sequencing technologies,[2] computational assembly of sequences and functional inferences gave access to a tremendous source of information and revolutionized basic aspects of microbiology. In particular, genome size boundaries between viruses, bacteria and eukaryotes are less clear than initially thought. As a matter of fact, *Mimivirus*, the largest known virus to date, has a genome of 1.18 Mb, larger than those of 54 bacterial and two eukaryotic genomes, the latter two genomes, from protists, being smaller than all but four bacterial genomes.

Genome analysis, also known as genome mining or in-silico analysis, now constitutes an irreplaceable research tool for various aspects of microbiology. In particular, the availability of genomes from virtually all bacterial human pathogens has opened perspectives in the fields of diagnosis, epidemiology, pathophysiology and treatment.[3]

A major advantage of genome sequences over phenotypic methods is that data can be deposited in online databases and thus are easily comparable among laboratories. The main three databases are the

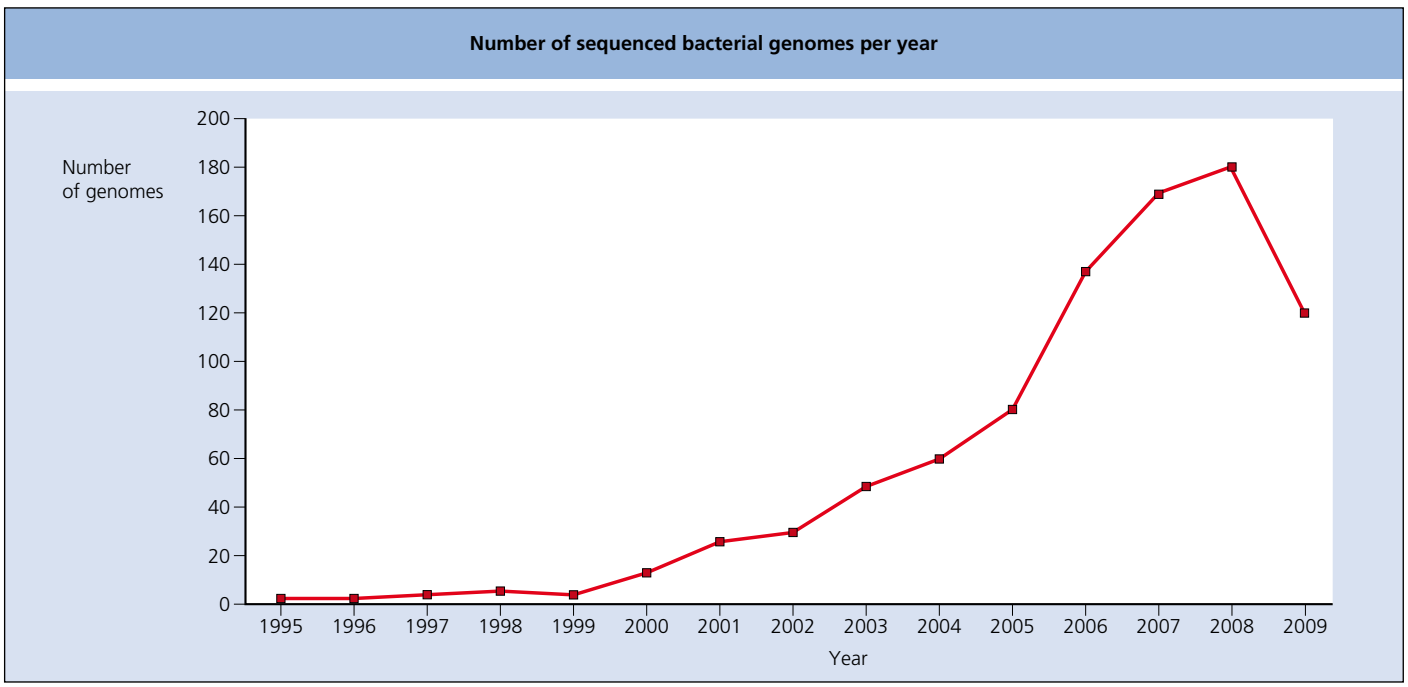


Fig. 7.1 Number of sequenced bacterial genomes per year. (For 2009, we indicate the number of genomes sequenced until July 28.)

National Center for Biotechnology Information (NCBI, http://www.ncbi.nlm.nih.gov/), the DNA Data Bank of Japan (DDBJ, http://www.ddbj.nig.ac.jp/) and the European Bioinformatics Institute (EBI, http://www.ebi.ac.uk/). In addition to offering complete microbial genome sequences with links to corresponding publications, these databases provide online tools for analyzing genome sequences. As of July 28, 2009, 883 genome sequences from 573 bacterial species are available online (http://www.genomesonline.org/, http://www.ncbi.nlm.nih.gov/genomes/static/eub_g.html). The discrepancy between the number of genome sequences and bacterial species is explained by the fact that genomes from several individual strains have been sequenced for 68 species. Sequenced genomes include almost every significant human bacterial pathogen, covering all the phylogenetic domains of bacteria. In addition, more than 2600 sequencing projects are ongoing (http://www.genomesonline.org/). Moreover, new sequencing technologies are making possible the sequencing of random community DNA and single cells of bacteria without the need for cloning or cultivation.

There are multiple applications for genome sequences:

- They may be used as target sources for molecular detection or genotyping.
- Following the critical step of genome annotation which consists of identifying gene functions by analogy with other bacteria and is facilitated by comparison to databases such as Clusters of Orthologous Groups (http://www.ncbi.nlm.nih.gov/COG/) or Kyoto Encyclopedia of Genes and Genomes (http://www.genome.ad.jp/kegg/), specific phenotypic traits may be deduced from the genotype. Resulting applications include development of specific culture media, identification of antibiotic resistance mechanisms and virulence factors, and exploration of host–pathogen interactions.
- Within the deduced proteome, antigens may be selected for serologic applications, development of monoclonal antibodies or development of vaccines (Fig. 7.2).

SEQUENCING STRATEGIES

With the development of ultrahigh-throughput applications of genomics, such as metagenomics, conventional capillary sequencing, also named Sanger sequencing, is no longer the technology of choice. Over recent years, new high-throughput sequencing methods, termed 'next generation sequencing', 'flow cell sequencing' or 'massively parallel sequencing', have opened new perspectives in genomic studies (Table 7.1).

Sanger sequencing

In 1977, Sanger *et al.*[4] developed a DNA sequencing method using di-deoxynucleotides as chain terminators; this became the reference sequencing method worldwide for the next 27 years. Most recent developments of this method use capillary sequencers, with a maximum output of 0.44 Mb every 7 hours. The Sanger technology, used for sequencing most of the 883 bacterial genomes available to date, produces 650–800 bp reads.

Pyrosequencing

In 2004, the Roche (454) company commercialized a high-throughput pyrosequencer, the GS-20, later upgraded as GS-FLX. Each of the strands obtained by shearing the template DNA is hybridized to the surface of a distinct agarose bead and then amplified by emulsion polymerase chain reaction (PCR). The sequence of the fragment from each individual bead is subsequently obtained by iterative pyrosequencing, whereby the pyrophosphate molecule released on nucleotide incorporation by DNA polymerase initiates a set of reactions that ultimately produces light from the cleavage of oxyluciferin by luciferase[5] (an explanation of the technology is available at http://www.roche-applied-science.com/publications/multimedia/genome_sequencer/flx_multimedia/wbt.htm). The GS-FLX instrument produces an average read length of ~250 bp per sample, with a combined throughput of ~100 Mb of sequence data per 7-hour run.

Sequencing by synthesis

Commercialized by Illumina (Solexa), the 1G analyzer uses a glass substrate with covalently attached oligonucleotides that are complementary to specific adapters previously ligated onto DNA library fragments. *In-situ* amplification is obtained using an isothermal polymerase and sequencing proceeds by synthesis using Sanger-like but reversible four-color fluorescence terminator nucleotides. An explanation of the technology is available at http://www.illumina.com/media.ilmn?Title=Sequencing-By-Synthesis%20Demo&Cap=&Img=spacer.gif&PageName=illumina%20sequencing%20technology&PageURL=203&Media=1. Sequencing by synthesis produces ~40–50 million sequence reads of 32–40 bp simultaneously.

Ligation-based sequencing

The Supported Oligonucleotide Ligation and Detection (SOLID) instrument by Applied Biosystems uses a sequencing process catalyzed by DNA ligase. In this technology, oligonucleotide adapter-linked DNA fragments are coupled with magnetic microbeads that are covered with complementary oligonucleotides. Each bead-bound DNA fragment is amplified by emulsion PCR and ligation-based sequencing is obtained using a thermostable ligase and four color dye-labeled oligonucleotides. The method includes a quality check in which each base sequenced is verified twice, which provides greater overall accuracy. An explanation of the technology is available at http://marketing.appliedbiosystems.com/images/Product/Solid_Knowledge/flash/102207/solid.html. Sequencing by ligation produces 3–4 Gb of sequence data with an average read length of 25–35 bp.

Advantages, drawbacks

Sanger sequencing suffers major drawbacks, including its financial cost and the need for a substantial quantity of DNA (several micrograms), and requires that DNA fragment libraries be clonable in *Escherichia coli*. The latter constraint is particularly limiting because some bacterial genes were demonstrated to be highly unclonable in *E. coli*.[6]

In contrast, next-generation sequencing methods, although readily outperforming capillary sequencing in terms of higher output and lesser DNA quantity required and cost, see their applications limited by short read lengths (25–250 bp), small numbers of samples that can be run simultaneously, limited paired-end and targeted sequencing, and difficult sequence assembly, especially to disambiguate repeat regions. In addition, the Roche technology, although producing 250-bp reads, is impaired by homopolymers in the template DNA, in particular those made of more than four nucleotides. Other issues include misincorporation of excess nucleotides that are incompletely eliminated after a previous cycle, beads with mixed templates and multiple copies of the same template on different beads. The Illumina and Applied Biosystems technologies only produce 25–40 bp reads. The Applied Biosystems technology requires that reference genome sequences be converted into 'color space' format prior to analysis.

As a consequence, next-generation sequencing may currently be preferred for genome resequencing than for de novo assembly, although a combination of Sanger sequencing and next-generation sequencing has been proposed as a valuable alternative for this latter purpose.

Single cell genome sequencing

A recently developed technique allows genomic DNA sequencing from single microbial cells. This strategy is especially valuable for microorganisms that cannot be cultivated. Prior to sequencing, genomic

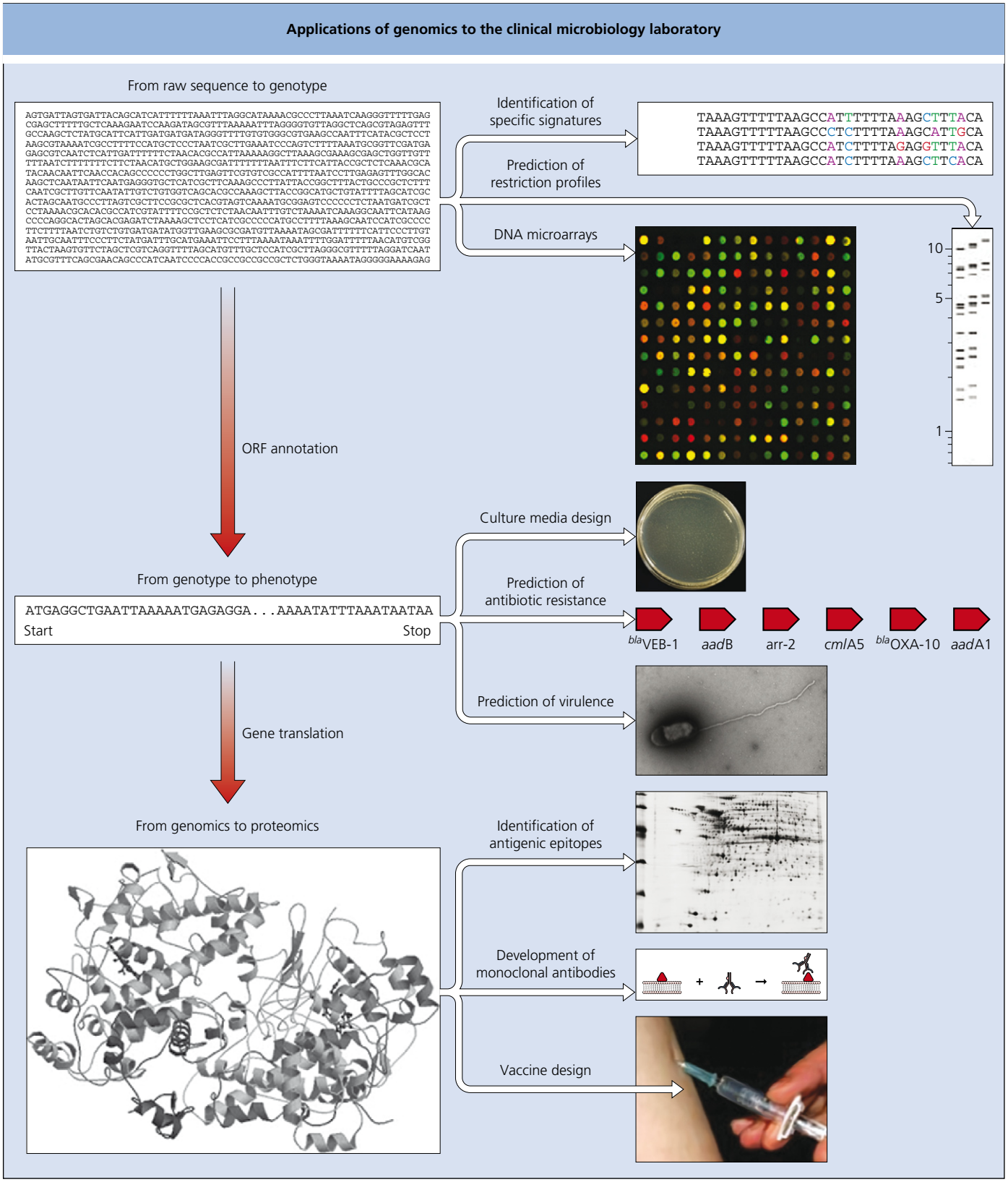


Fig. 7.2 Applications of genomics to the clinical microbiology laboratory.

DNA is amplified from a single bacterial cell, using three potential methods. These include primer extension preamplification, degenerate oligonucleotide primed PCR and multiple displacement amplification (MDA).[7] The best method, MDA, is based on isothermal strand displacement synthesis, i.e. a highly processive DNA polymerase repeatedly extends random primers on template DNA and concurrently displaces previously synthesized copies. MDA was used successfully for *E. coli* and *Bacillus subtilis*[8] and may be of particular interest for complex floras. However, to date, MDA may not recover the entire genome from a single bacterial cell, and some DNA rearrangements may occur

Table 7.1 Currently available sequencing methods

	Sanger di-deoxy nucleotide sequencing	Pyrosequencing	Sequencing by synthesis	Ligation-based sequencing
Platform	Capillary sequencers	Roche (454) GS-FLX	Illumina genome analyzer	Applied Biosystems SOLID
Mb/run	0.44	100	1300	3000
Time/run	7 hours	7 hours	4 days	5 days
Read length (bp)	650–800	250	30–40	35
Limits	Cost	Limited paired-end and targeted sequencing, difficult sequence assembly, especially to disambiguate repeat regions		
	Need for high DNA quantity Cloning step	Low sensitivity in homopolymer sequencing Misincorporation of excess nucleotides Beads with mixed templates Redundancy		Specific sequence format

during the DNA amplification reaction; rather, a complete genome sequence may be obtained from the combination of data from several single cell MDA reactions.

Following genome amplification, sequencing may be obtained by any of the above-described methods, although an upcoming single molecule platform, the HeliScope by Helicos, should help solve single molecule sequencing challenges.

ANALYSIS OF GENOME SEQUENCES

Data analysis

The huge number of sequences produced at low cost by new sequencing methods created an increasing need for computational hardware and expertise. In particular, the assembly challenge posed by the short read length suggests that the software developed for Sanger sequencing cannot be applied to new sequencing methods.

Another major issue of genomics is sequence annotation.[9] In fact, the results from computational studies of hundreds of genomes depend directly on annotation quality. Genome annotation may be obtained using sequence similarity searches, calculated directly from the predicted gene and protein sequences or derived from comparisons of gene order between species, with some annotation systems being fully automated.[10] However, genomic annotations available in the above-cited databases may vary in several features (gene designation, level of detail, operons, repeats, etc.). To date, there is no consensus on annotation method and standardization of genomic data. Such an effort will be necessary to facilitate future studies.

Genetic diversity

A bacterial genome is a dynamic structure influenced by several events, including gene acquisition, duplication or loss, and/or genome reduction or rearrangement. Genome sequence comparison enabled the identification of many biologic patterns such as gene fusions, pseudogenes or non-coding RNAs, and ORFans. Such findings suggested that the mosaic of bacterial genomes would be more complex than expected. Indeed, the availability of several hundreds of bacterial genome sequences shed light on the great genetic diversity of the bacterial world, with genome sizes ranging from 0.159 Mb for *Candidatus* 'Carsonella ruddii' to 13 Mb for *Sorangium cellulosum*. For example, among bacteria, members of the order Rickettsiales experienced a strict intracellular association with eukaryotic cells. This particular lifestyle paralleled a reductive genome evolution marked by the paradoxic expansion of genetic elements such as plasmids, repeats and gene duplications.[11] An extreme example of repeat expansion is the *Orientia tsutsugamushi* genome that contains the highest concentration of repeats (46.7%) for a bacterial genome.[12]

The study of the genetic diversity of bacteria using genomic sequences might also include phylogeny. Such an approach may limit the risk of biases linked to the selection of specific genes. The problem is to define adequate tools that would enable comparison of such large amounts of sequences. Recently, 8362 signature genes were identified that may served as targets for genome-based bacterial phylogeny.[13]

Pan-genome

Although a genome sequence gives access to a tremendous amount of information for a given strain, it may not reflect the genetic diversity at the species level. Recent studies have shown that intraspecies diversity in gene content may vary greatly. Therefore, the gene pool of some bacterial species may be far larger than that of a single genome, in part because of the frequency of homologous recombination and lateral gene transfer.[14] For *E. coli*, *Streptococcus agalactiae* and *Strep. pyogenes* it was estimated that an average 441, 33 and 27 new genes, respectively, would be unveiled by every new sequenced genome.[14] Such intraspecies variations might be linked to niche adaptation. Bacteria living in niches with limited access to the global microbial gene pool, such as *Bacillus anthracis*, *Chlamydia trachomatis* and *Mycobacterium tuberculosis*, have a much smaller intraspecies genomic diversity. The most extreme example of genome homogeneity is *Buchnera aphidicola* which experienced neither genome rearrangement nor gene duplication or transfer.[15]

The availability of several genomes for a given species led to the concepts of 'universal' minimal genome and species being challenged. Each bacterial species is composed of a 'core genome' containing genes present in all strains, a 'dispensable genome' made of genes present in two or more strains and genes unique to single strains. According to their structural and metabolic requirements, distinct species might have distinct minimal gene sets that would be different from the set of shared orthologs.

Metagenomics

Direct cloning and sequencing of DNA from complex floras, or metagenomics, has provided a unique access to the microbial species and genes present in these environments. Initially, metagenomic sequencing was performed using a random shotgun method following purification of any DNA. Metagenomic studies have been greatly facilitated by the development of new sequencing methods (described above) that enable the sequencing of random community DNA without the need for cloning or cultivation. In recent years, metagenomics nearly doubled the number of identified proteins and unveiled community-wide patterns in gene and taxa distributions among various habitats. In addition, when compared to the 883 bacterial genomes sequenced to date, metagenomic studies have demonstrated that the majority of the biologic diversity in the bacterial world remains unexplored.

To date, the metagenomic approach has been used to study the human microbiome, in particular the oral, lower intestinal and vaginal floras. In the gastrointestinal tract, 395 distinct bacteria were demonstrated; 62% of these were not previously known and 80% were uncultivated.[16] In addition, microbial genes outnumbered human genes 100 times, suggesting that the human microbiome may play a major role in human physiology.[16] Unexpectedly, this study demonstrated great intersubject variability and significant differences between stool and mucosal community compositions. Subsequently, other metagenomic studies allowed the identification of an imbalance in the distal gut flora of obese individuals compared to lean subjects, thus demonstrating that metagenomics may have medical applications.[17]

Aside from the human body, metagenomic studies are ongoing in several important ecosystems, including global soil, deep mines and the ocean. Ten grams of soil sample were estimated to contain 10^7 distinct bacterial species out of approximately 10^{10} cells.[18] In contrast, when applied to extremely simple microbial communities, metagenomic sequencing may enable genome reconstruction of their predominant members.

USING GENOME SEQUENCES

DNA sequences

The main limitation of molecular detection and sequence-based identification techniques is selection of target sequences, which can alter the reliability of results in terms of sensitivity and specificity of detection, as well as ability to discriminate between species and strains. Genome sequences offer the possibility of rational selection of targets depending on the desired objective. Their use has been demonstrated to be extremely valuable for fastidious pathogens that have poorly characterized genetic systems.

Detection of bacterial pathogens

Initially, the choice of PCR primers was empirical. 16S rRNA, the first 'universal' target gene, was widely used and enabled the first identification of the agents of bacillary angiomatosis[19] and Whipple's disease.[20] However, use of this gene was limited by a high risk of contamination, usage difficulties associated with polymicrobial specimens and insufficient discriminatory power.

The availability of genome sequences enables a rational choice of DNA signatures that may serve as PCR targets, according to the objective and degree of specificity required.[21] On the one hand, a PCR assay targeting a gene common to all bacteria may allow detection of any species. On the other hand, if the objective is to detect a unique or limited number of bacteria, it is possible by comparison of phylogenetically close genomes to identify DNA fragments that are specific for a genus, a species or a group of strains that exhibit, for example, specific virulence or antimicrobial resistance phenotypes. Such an approach was used to identify species-specific targets for *Borrelia burgdorferi* for example.[22] However, the specificity of in-silico identified genomic targets should always be verified by comparison with databases, in particular GenBank (http://www.ncbi.nlm.nih.gov/Genbank).

In addition, the study of genomic sequences enabled the optimization of the sensitivity of detection, either by selecting a gene or fragment of noncoding DNA present as several copies in the genome[23] or by designing nested PCR assays with a reduced risk of contamination such as 'suicide PCR'.[24]

Molecular genotyping

Various genotyping methods may benefit from the study of genomic sequences.

- First, nonsequence-based methods that include in-silico design of macro restriction profiles for rare cutter enzymes may serve for restriction fragment length polymorphism (RFLP)-based analyses. Genomes may also be screened for single nucleotide polymorphisms (SNPs) or for tandem repeats, whose number and length are used in the variable number of tandem repeats (VNTR) method.[25]
- Second, several sequence-based genotyping methods have incorporated data from genome sequences or been designed on the basis of genome sequences. These include multilocus sequence typing (MLST) and multispacer typing (MST). MLST is based on sequencing several housekeeping genes, and genome sequences offer the possibility to select better candidate genes to be used in MLST approaches. MST relies on the assumption that intergenic spacers, undergoing less evolutionary pressure than coding sequences, are more variable between strains than genes. This is the first typing method to be entirely designed based on genomic data.
- Third, DNA microarrays have the advantage of allowing a simultaneous comparison of strains at the whole genome level with the sensitivity to detect subtle differences that are not detected by other methods. Another advantage of microarray technology is its ability to be facilitated by the development of automation. Chips may comprise up to 20 000 300–800 bp shotgun fragments or PCR products of one or several whole genome(s), specific sets of genes or as many as 600 000 50–70 bp oligonucleotides.[26] DNA microarrays have been used for genotyping bacterial strains in molecular epidemiologic studies.[25] DNA microarrays may also be used to detect and identify microorganisms in complex flora. Microarray-based typing methods can potentially identify new genes by including sequences derived from related bacteria or identifying polymorphisms that would still be detectable by oligonucleotide hybridization. In addition to genotyping applications, DNA microarrays may be used for detection of pathogens in clinical specimens, identification of vaccine candidates and detection of virulence factors or antibiotic resistance.[25]

Phenotype prediction

Development of specific culture media

Cultivation of the causative agent remains a priority during the diagnosis of bacterial infections. Genomic sequences thus constitute a unique source to identify incomplete metabolic pathways as well as the essential nutrients that a bacterium is unable to produce. It is thus theoretically possible to compose specific media by incorporating metabolites that bacteria cannot produce. Websites such as KEGG allow identification of anomalous pathways by comparison with previously analyzed genomes (http://www.genome.ad.jp/kegg/).

The first 'uncultivable' pathogenic bacterium whose genome analysis permitted axenic culture was *Tropheryma whipplei*, the causative agent of Whipple's disease.[27] However, the counter-example of *M. leprae* that cannot be grown axenically, despite the identification of many important lacking metabolic activities, highlighted the fact that the genomic identification of deficient metabolic pathways may not always provide all the clues to the growth of fastidious bacteria.

Detection of resistance to antimicrobials

The rapid acquisition of antibiotic resistance by pathogenic bacteria is a major public health problem. Genome sequencing has the potential to identify the various resistance determinants of a bacterium and allow for discovery of new arrangements of known resistance genes or new putative resistance markers. Genomic findings have identified resistance-causing genes or mutations that may be easily detected by PCR of clinical isolates and may serve as targets for routine detection tools.[28]

Another situation demonstrating the value of genome sequencing is during identification of resistance mechanisms in fastidious bacteria, for which phenotypic testing of antibiotic resistance is difficult or impossible, as in *Tropheryma whipplei* where the genome revealed the presence of mutated *gyrA* and *parC* genes, which explains resistance to fluoroquinolones.[29] Genome sequencing may also allow the identification of the mechanism of action and target genes of new antimicrobial compounds.

Identification of virulence factors

Virulence genes are potential targets for risk assessment and intervention strategies. The identification of virulence genes may lead to the development of rapid screening tests in order to proceed with effective isolation measures in hospitalized patients or delay hospitalization after carriage decontamination. Comparative sequence analyses provide insight into pathogenic mechanisms of bacteria, allowing identification of known virulence proteins with conserved sequences or motifs, as well as putative new virulence proteins.

There are three main methods for identifying virulence genes in a genome sequence:

- method 1 is based on homology searches of known virulence factor-encoding genes in the same or other species;
- method 2 identifies genes that are likely to result from lateral gene transfer (such genes are often localized in pathogenicity islands);
- method 3 identifies virulence genes by comparing the genomes of strains with different pathogenicity profiles.

Significant advances in understanding the pathogenic mechanisms of bacteria have been facilitated by genome sequence analysis, in particular by comparison of closely related species or strains with different virulence traits. For example, genome comparison allowed identification of pathogenicity islands in *H. influenzae*, *E. coli*, *Salmonella enterica* serovar Typhimurium and *Staphylococcus aureus*. In addition, various virulence mechanisms were found by genome comparison in *Bacillus cereus*, *Campylobacter upsaliensis*, *Chlamydia trachomatis*, *Francisella tularensis*, *Helicobacter pylori*, *Listeria monocytogenes*, *M. tuberculosis*, *Strep. pyogenes*, *Strep. agalactiae*, *Treponema pallidum* and *Yersinia pestis*.

In addition to allowing a better understanding of bacterial pathogenesis, identification of virulence factors in genomes may also allow the design of new potential antimicrobials in addition to allowing the identification of antimicrobial targets.

Exploration of host–pathogen interactions

The potential of genome sequence analysis for the exploration of host–pathogen interactions was recently demonstrated in *Strep. pyogenes*, for which an M3 protein variant was shown to alter the phagocytosis of human leukocytes, and *Neisseria meningitidis* for which a vaccine candidate protein was identified.

Proteome prediction

Development of serologic tools

Genome analysis offers the possibility of identifying all putative protein-encoding genes of a given bacterium. This exhaustive approach may be completed by expression of the corresponding proteome, testing immunoreactive characteristics of selected proteins, and use of the best antigens for the development of serologic tools. This strategy allowed the identification of a representative panel of antigens for *Treponema pallidum* and *M. leprae*. Conversely, the genome may serve to identify antigens that have been detected within the proteome of a bacterium by immunoblotting and mass spectrometry. This strategy was used for *Tropheryma whipplei* and resulted in identification of 17 proteins specifically reacting with patients' antibodies.

Development of specific monoclonal antibodies

Monoclonal antibodies are widely used for the diagnosis of infectious diseases, with many applications in serology and pathology. However, the usual strategy of monoclonal antibody production relies on the use of the entire bacterium or proteins selected empirically, which makes it difficult and expensive. In contrast, genomic analysis allows for identification of all potential antigenic proteins of a bacterium. One can, then, limit the number of candidate antigens by selecting those that are genus-, species- or strain-specific. In addition, it is possible to focus selection on protein fragments large enough to contain valuable epitopes but small enough for expression in *E. coli*. The genomic approach of monoclonal antibody production has been used successfully for *M. tuberculosis*, *N. meningitidis* and *Treponema pallidum*.

Vaccine design

The availability of complete bacterial genome sequences offers unprecedented access to its complete proteome, with the possibility of rational selection of vaccine candidates rather than empiric testing of antigens one at a time. This strategy, named reverse vaccinology, may be completed by functional immunomics for optimal epitope prediction and may result in DNA vaccines. It has been used successfully to identify potential vaccine targets for *Bacillus anthracis*, *Chlamydophila pneumoniae*, *Leptospira interrogans*, *M. tuberculosis*, *N. meningitidis*, *Porphyromonas gingivalis*, *Rickettsia prowazekii*, *Strep. agalactiae*, *Strep. pneumoniae* and *Strep. pyogenes*. However, the major drawback of reverse vaccinology for vaccine development is that the strain under investigation does not represent the genetic diversity of its species. This risk has been highlighted by the comparison of genome sequences from several strains of *Strep. agalactiae*. In this species, the core genome is constituted by only approximately 80% of genes, with each new genome exhibiting ~18% new genes. This finding motivated the design of a 'universal anti-*S. agalactiae*' vaccine made up of four antigens, none of which was present in all strains but the combination of which was protective against all strains.[30]

This example, together with accumulating evidence that a single genomic sequence may not be sufficient to represent the variability of bacterial populations within a species, suggests that genome sequences from multiple strains of a species might be necessary to identify an efficient vaccine formulation.

CONCLUSIONS AND PERSPECTIVES

In 1995, the outcome of bacterial genome sequencing promised breakthroughs in microbiology and infectious disease research. Since then, 883 bacterial genomes have been sequenced. The multiplication of genome sequencing projects, together with extensive metagenomic studies, will provide a much more complete picture of the bacterial world. As we move towards cataloging the sequences of all bacterial genomes, we are facing new potential but also new challenges. The potential of this inestimable source of information has already allowed scientists to reconsider the fields of bacterial virulence, host–bacteria interactions, microbiologic diagnosis and human microbial ecology. However, among the challenges is the need to develop new programs able to handle the specific characteristics of new sequencing technologies and the continuously growing number of genomic sequences, with a need in calculation capacity following an exponential growth, the difficulty of sequencing the genomes of uncultivated bacteria identified by metagenomics, and the large proportion of genes that still need a function assignment, which warrant wide open future research. The ability to sequence DNA from individual cells is also likely to have a significant impact on microbiology.

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Practice point | 1 |

Paul R Epstein

Health consequences of a changing climate

Climate change has direct and indirect consequences for human health. Heat waves affect health directly and are projected to take an increasing toll in developed and developing nations. Climate constrains the range of infectious disease vectors and agents, while weather affects the timing and intensity of outbreaks.

INTRODUCTION

In 1989 Alexander Leaf, in his prescient *New England Journal of Medicine* paper, explored the pantheon of ills that climate change portends[1]. Twelve years later, the Third Assessment Report of the United Nations' Intergovernmental Panel on Climate Change[2] – the world's most ambitious international scientific collaboration to date, involving over 2000 scientists from 100 nations – concluded:

- climate is changing;
- humans are contributing;
- weather patterns have become more extreme; and
- biologic systems on all continents are responding to warming.

The combustion of fossil fuels – coal, oil and natural gas – and deforestation are the principal producers of heat-trapping greenhouse gases, above all carbon dioxide (CO_2).

Since 2001 we've learned a great deal more:

- the pace of atmospheric accumulation of CO_2 and warming is quickening;[2]
- polar and alpine ice sheets are melting at rates not thought possible just several years ago;[3]
- global warming is retarding the repair of the 'ozone shield'; and, most ominously
- circumpolar westerly winds are accelerating in both hemispheres.[2]

The underlying physics of warming is driving changes in climate, i.e. changes in global temperatures and weather patterns. The ocean is the repository of the last century's global warming, and having warmed 22 times more (as measured in exajoules) as has the atmosphere.[4,5] Warming of the world ocean accelerates Earth's hydrologic cycle: water is warming, ice is melting and water vapor is rising. Enhanced evapotranspiration over land parches some regions, while tropical-like downpours fall elsewhere. When drought is followed by heavy rainfall, the dry, cracked, less-absorbent soil increases the potential for flooding.

Heavier rainfalls are becoming more frequent. In the USA, from the 1970s to the 1990s, rainfall increased 7%, while 2-inch a day rains increased 14%, and 4-inch a day rains increased 20%. Indeed, 6-inch a day precipitation has increased 27%[6] and more winter precipitation is falling as rain at high latitudes.

Signs of climate instability are most evident in the ice. West Antarctica's 'rivers of ice' are flowing faster towards the sea and, in Greenland, melt water is seeping through crevasses to lubricate the base of outlet glaciers, accelerating their slippage. One large outlet glacier on the western shelf – the Jakobshavn Isbrae – was slipping at a rate of 12 km a year in 2006, double the speed of the previous decade.

While loss of the entire West Antarctic or Greenland ice sheets are not projected to occur any time soon (each would raise sea levels 7 meters), slippage of portions of ice sheets at either pole is becoming more likely. The sudden collapse of West Antarctic Peninsula ice shelves (floating) in 2005 demonstrated that ice sheets can disintegrate precipitously.

As ice cover retreats (decreasing reflectivity or albedo), open seas absorb more heat (decreasing albedo), opening more water to absorb more heat, etc. The loss of albedo is therefore a 'positive feedback', amplifying warming. Moreover, the change in albedo could reach a threshold or tipping point, triggering an abrupt phase-state change in the climate.[7]

The observed changes in climate and biologic responses have occurred with only 1°F warming, while temperatures are projected to increase by 3–12°F (1.4–5.8°C) – or even up to 52°F (11°C) by some simulations – over this century (depending, primarily, on society's response).[2] Earth has experienced neither the current level of CO_2 nor the projected temperature changes since *Homo sapiens* evolved. We have truly entered uncharted seas.

EMERGING INFECTIOUS DISEASES

For interconnected sets of social and environmental reasons, we are in an era of the emergence, resurgence and redistribution of infectious diseases on a global scale. Since 1976, 40 diseases new to medicine have emerged.[8] Approximately half of these are antibiotic-resistant microbes associated with medical and farming practices. Changing social conditions, such as the growth of 'mega-cities' and widespread ecologic change, overwhelming Earth's carrying capacity (i.e. exhausting Earth's finite resources and generating wastes at rates beyond the ability of biogeochemical cycles to recycle them), are also contributing to the spread of infectious diseases. As climate changes, the warming and associated weather extremes are playing a growing role in the generation and spread of emerging infectious diseases.

For communicable diseases, land-use changes, species extinctions, global transport, wars and widening social inequities have conspired to unleash a barrage of emerging infectious diseases in the last three decades,[9] afflicting humans, wildlife, livestock, crops, forests and marine life. Multiple disturbances are contributing to this renegotiation between microbial and 'macrobial' communities: as climate changes, warming and wider weather fluctuations are playing expanding roles in the emergence, re-emergence and redistribution of infectious disease.

CLIMATE CHANGE AND EMERGING INFECTIOUS DISEASE PREVALENCE

Temperature constrains the range of vector-borne diseases (VBDs), while weather affects the timing, intensity and location of outbreaks.

Most insects are highly sensitive to temperature change: ants even run faster in warmer weather. Mosquitoes are especially sensitive to changes in temperature: warming (within their viable range) increases their activity, biting and reproductive rates, prolongs the breeding season and shortens the maturation of microbes (the extrinsic incubation rate or EIP) within them. Provided there is adequate moisture, warming can increase the populations of insects, although excessive heat decreases their survival. Between the limits of too hot and too cold there lies an optimum viability range in which warmth enhances metabolism, growth and the opportunities for disease transmission.

Findings from paleoclimatic (fossil) studies demonstrate that changes in temperature (and especially in winter temperatures; temperature minimums or TMINs) were closely correlated with geographic shifts of beetles near the end of the last glacial maximum about 10 000 years ago. Indeed, fossil records indicate that when changes in climate occur, insects shift their range far more rapidly than do grasses, shrubs and forests, and move to more favorable latitudes and elevations hundreds of years ahead of larger animals: 'Beetles', concluded one climatologist, 'are better paleo-thermometers than bears.'

THE VARIABLES

Three outcome variables are central to the question of changes in infectious disease range with warming: (1) shifts in altitude; (2) shifts in latitude; and (3) changes in seasonality. While models can project the changing envelope of *conditions conducive* to these shifts, their occurrence is a function of temperature and additional factors (e.g. previous exposure and immunity, drug-resistance, forest cover and preventive measures).

Another climate-related variable is the impact of extreme weather events on clusters of outbreaks and impacts on human nutrition.

Assessing range change

Mountain ranges are sentinel regions for monitoring global climate change and the biologic responses. Precisely because of their verticality, one can move quickly from desert to tropical to polar ecosystems. Adaptive shifts for plants and animals responding to warming (or cooling) occur much faster with regard to elevation than they do with respect to latitude. Due to the 'adiabatic lapse rate' (~1°F cooling for every 200 feet rise in elevation) the ratio is 1/600 for comparable cooling as one moves north or south.[10] For example, a plant moving 1 km up a mountain in response to warming would have to move 600 km north or south to adjust to the same shift in freezing isotherms – the line depicting the altitude or latitude at which the ground is permanently frozen all year round.

There are several concurrent changes occurring at high altitudes: (1) accelerating melt of alpine glaciers; (2) upward migration of plant communities; (3) appearance of disease vectors and/or diseases at high altitudes; and (4) the upward shift in the freezing isotherm as measured with weather balloons.[11]

Some examples of the shifts in disease vectors and diseases follow.

- Dengue fever – previously limited to about 1000 m in elevation in the tropics by the 10°C winter isotherm – has appeared at 1700 m in Mexico.
- *Aedes aegypti* mosquitoes – vectors for yellow, dengue, Rift Valley and chikungunya fevers – have been reported at 2200 m in the Andes, where temperature once restricted them to 1000 m.
- Dengue fever is now found in highland regions of Vietnam.
- Since the 1980s high-elevation malaria outbreaks have occurred in:
 - Rwanda at 1700 m
 - Ethiopia, Uganda and Kenya[12]
 - Nairobi, a mile-high city, where malaria is now circulating
 - Zimbabwe, where roughly 45% of the population is currently at risk for malaria (with warming, the area suitable for transmission will envelop the nation later this century)
 - the highlands of Papua New Guinea
 - high elevations in Bolivia.

Changes in the ranges of disease vectors in some areas may, in part, be attributable to local changes in habitat, vector ecology, pathogen and insect resistance. Climate affects not only the movement of animal populations, but can also provoke humans to migrate. Meanwhile Lafferty[13] argues that the belt of VBD transmission may shift but not expand due to excess heat and changes in hydrologic regimes. However, the disproportionate warming at night and winter, and the exaggeration of this rise in TMINs as one moves toward the poles, means that conditions at the southern end of vector ranges are not changing as fast as those at northern boundaries. Thus the conditions conducive to changes in the potential range of many vectors are expanding. Tick populations have not only shifted north in Sweden[14] with each warm winter, they have become more abundant in the areas they previously inhabited (the Stockholm Archipelago).

In addition, weather extremes can create conditions conducive to large outbreaks, and this may have as much or more to do with the location of outbreaks as the warming itself. Moreover, the changes are often nonlinear: in Honduras conditions in the south (around Choluteca) have become so hot and dry (due to climatic change, deforestation and wetland destruction that have altered the regional hydrologic cycle) that malaria has died out and agriculture has suffered greatly. Thus, people have also out-migrated, settling in the forested north where malaria is still rife.

The global pattern of physical, zoologic and botanical data is consistent with model projections, constituting an internally consistent 'fingerprint' of climate change. The set of changes occurring in the Americas (Andes, Sierra Nevada), the East African highlands, European Alps and in Asia (India and Nepal, Papua New Guinea and Irian Jaya) provides a consistent picture of changing conditions conducive to the transmission of VBDs at high altitudes.

Changes in latitude

On land, with spring arriving 1–2 weeks earlier in the Northern Hemisphere (depending on latitude) than in the 1961–1990 baseline period, birds are arriving and laying eggs earlier, butterflies are shifting in latitude and altitude, some pollinators are arriving (too) early, and plants are flowering and shedding pollen and seeds earlier.[2] In Antarctica – where the West Antarctic Peninsula has warmed 6°C since 1950 – a wholesale shift of the sub-Antarctic ecosystems poleward has occurred (as indicated by shifts in fish, penguin and seal populations), replacing polar ecosystems.

Today, ticks in Sweden are moving north with each warm winter and models project a similar shift in the USA and Canada. Meanwhile, declining predators of deer and competitors of mice, plus living patterns that fragment habitat, also contribute heavily to the propagation of Lyme disease.[15]

Computer models of global greenhouse warming project increased temperatures that will, in turn, favor the spread of VBDs into more temperate latitudes. While 42% of the globe presently offers conditions that can sustain the transmission of malaria, the fraction could rise to 60% with a global increase of a few degrees centigrade (Fig. PP1.1).

In the marine environment, the outbreak of *Vibrio parahaemolyticus* gastroenteritis from oysters harvested in warmed Alaskan waters (the mean daily water temperatures exceeding the threshold of 63°F/15°C) extended the northernmost documented source of oyster consumption causing *V. parahaemolyticus* illness by 1000 km (600 miles).

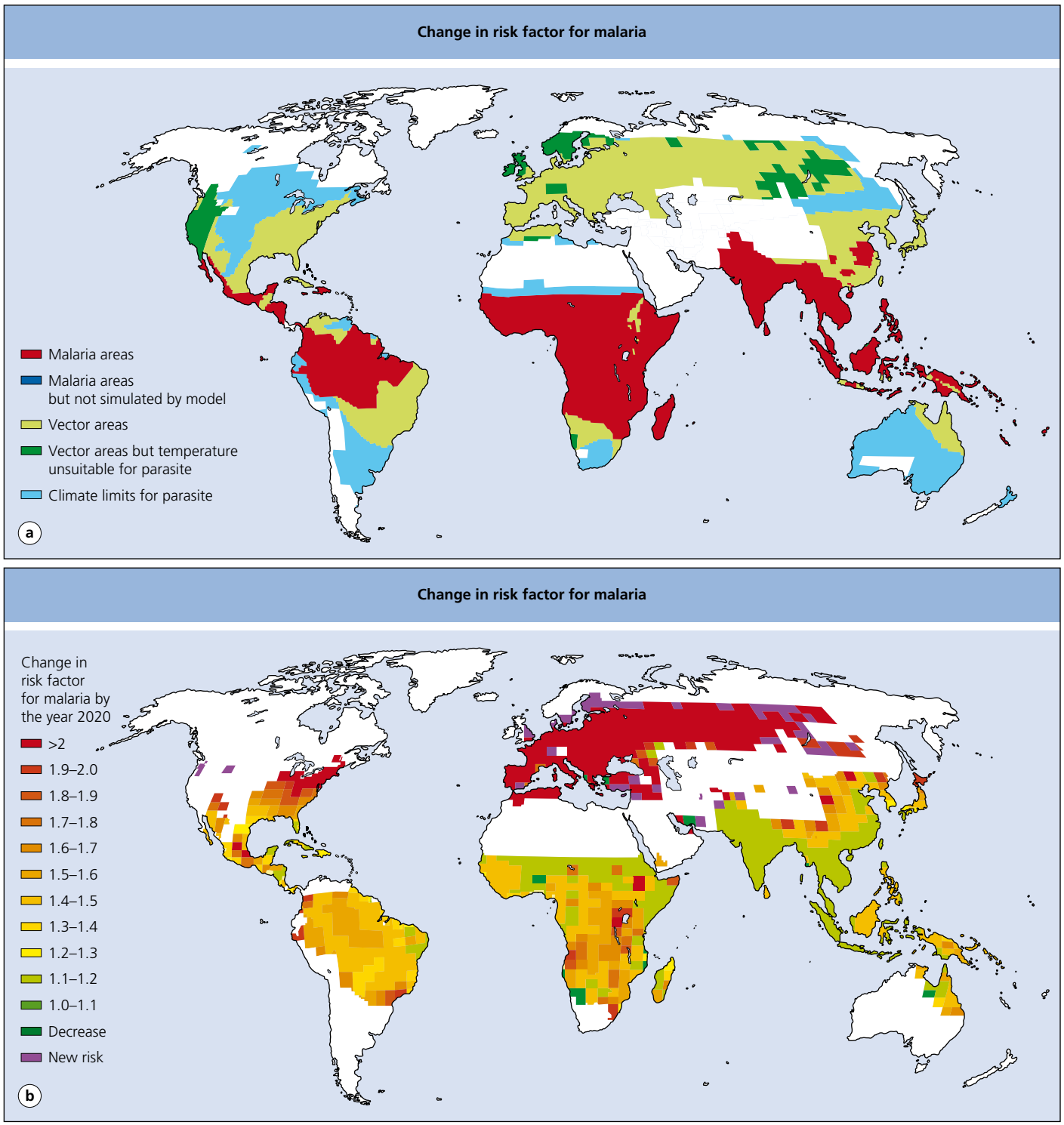


Fig. PP1.1 Projected change in risk factor for malaria by the year 2020 (1999 forecast). Reproduced with permission from Martens.[16]

Projected changes in seasonality

Models for conditions in sub-Saharan Africa project longer seasons (as measured in person-months) during which malaria may circulate. This variable needs monitoring in endemic areas.

A new variable: variability

Although weather always varies, increased variability, with rapid temperature fluctuations, altered precipitation patterns and wide swings in weather are characteristics of an unstable climate. Variability is most often considered the 'noise' about a trend; something to be cancelled out by using multi-year running averages. But for ecosystems (and humans) increased variability can be a disturbance. In the study of global climate change, variability is itself a signal – an outcome variable with multiple implications for health.

Increased variability can influence health via several pathways:

- increased seasonal and daily variability may have consequences for travel and ambulatory risks (see Health consequences of extreme weather events, below);

- increased seasonal and daily variability can affect winter cold and summer heat mortality, perhaps by weakening host defenses;
- climate instability can increase ecologic instability, altering the ratios of predators and prey (that can be pests) and populations of competitors (e.g. mice), constituting biodiversity that can 'dilute' pathogens:[15]
 - opportunistic *r*-strategists (e.g. micro-organisms, insects and rodents) rapidly proliferate with disturbances (pests and pathogens tend to be generalists and *r*-strategists)
 - specialist *K*-strategists reflect the carrying capacity of the environment (the resources used and wastes generated) and fare well under stable environmental conditions (large predators tend to be *K*-strategists)
 - increased climate variability (along with habitat fragmentation and excess chemicals) can alter *K*-to-*r* ratios, allowing the prey (insects, rodents) to escape their predators (bats, birds, damselflies, owls and kestrels) to become pests that can carry pathogens.

Increased interannual variability has been observed,[2] and the trend toward heavier precipitation events[2] (see below) and more winter precipitation falling as rain at high latitudes are indicative of increased seasonal and day-to-day variability.

Health consequences of extreme weather events

Floods frequently lead to disease 'clusters'. Heavy rains drive rodents from burrows, create mosquito-breeding sites, foster fungi and flush pathogens, nutrients and chemicals into waterways.[17] In the USA, water-borne disease outbreaks are highly correlated with heavy rains and flooding. Milwaukee's *Cryptosporidium* outbreak followed the 1993 Mississippi floods and outbreaks of *Escherichia coli* O157:H7 have most often occurred following intense rains, with sewage contaminating fresh water supplies. Floods in Europe have also been associated with outbreaks of noroviral, rotaviral and *Campylobacter* infections. Warming and extreme events can both contribute to diarrheal illness in underdeveloped nations.

Heavy rainfall along coasts can flush nutrients (chemicals and micro-organisms) into rivers, estuaries and bays, and trigger harmful algal blooms ('red tides') which can be toxic, harbor pathogens (e.g. *Vibrio* spp.) and contribute to the over 350 hypoxic 'dead zones' that affect marine coastal zones worldwide.

Flooding can also create breeding grounds for mosquitoes. The enormous outbreak of Rift Valley fever (RVF) in East Africa in 1998 occurred in the wake of heavy rains, in which the impact of the strongest El Niño event of the past century was compounded by exceptionally warm Indian Ocean sea surface temperatures; RVF also resurged in 2007 in the wake of intense East African flooding. Rift Valley fever causes a viral hemorrhagic fever in humans and its impact on animals affects nutrition, trade and livelihoods. Remote sensing and climate forecasting can generate health early warning systems for RVF,[18] facilitating timely veterinary vaccination programs.

Prolonged droughts can have severe health consequences. Droughts promote wildfires, leading to injuries, burns, respiratory illness and the loss of life. Drought can also set the conditions for infectious disease outbreaks. Water-borne diseases can stem from inadequate water supplies and hygiene, and stored water about houses can become breeding grounds for *Aedes aegypti* mosquitoes, carriers of dengue, yellow and chikungunya fevers. The recent Indian Ocean outbreak of over 500 000 cases of CHIK fever in Mauritius, the Comoros, Reunion and coastal Kenyan cities was associated with drought.

The outbreak of chikungunya fever in Italy was transmitted from an Indian traveler by *Aedes albopictus*, the cold-hardy Asian tiger mosquito introduced into Europe and the USA in old tires. This outbreak of a tropical disease in a temperate, developed region has been suggested to be a sign of global warming, and warmth may have played a role in the proliferation of mosquitoes and acceleration of the EIP of the chikungunya virus within them. On the other hand, the explosive appearance and spread of chikungunya in nations ringing the overheated Indian Ocean may have more to do with the extremes of temperatures and recurrent drought accompanying global warming.

Droughts also favor epidemics of meningococcal meningitis in the Sahel (Sahel rainfall has already fallen 25% in the past 30 years). The transmission of this person-to-person infection is presumably favored when dried mucous membranes allow colonized organisms to invade through weakened respiratory tracts. The 'meningitis belt' spans 14 nations: Ethiopia, Kenya, Sudan, Uganda, Burkina-Faso, Democratic Republic of Congo, Niger, Mali, Chad, Nigeria, Côte d'Ivoire, Ghana, Togo and Benin.

For Africa, the climate prognosis is dire. A United Nations report projects continued drying of the continent, with increasing consequences for health, crop yields, livelihoods, refugees and conflict.

The Institute of Medicine/National Academy of Sciences report *Global Climate Change and Extreme Weather Events: Understanding the Contributions to Infectious Disease Emergence* (2008) provides an excellent introduction to the impacts of weather extremes on pests and pathogens threatening humans, livestock, wildlife, forests, crops and coastal marine life.[19]

Case studies

West Nile virus

The means by which West Nile virus (WNV) was introduced into New York City in 1999 remains unknown; however, based on analysis of outbreaks of St Louis encephalitis (SLE) in the USA and WNV in Europe, some meteorologic conditions appear conducive to large epidemics. SLE first appeared in the US midwest during the Dust Bowl and outbreaks for the next four decades were associated with drought. (Once established in wildlife, rains and warmth can increase populations of 'bridge vectors'.) Outbreaks of WNV in Russia and Romania in the 1990s were also associated with drought.

The New York City epidemic occurred following a prolonged spring and summer drought along the Atlantic seaboard, and a 3-week heat wave in July. Urban, drain-dwelling, bird-biting *Culex pipiens* mosquitoes survive in warm winters and thrive in shallow pools of foul water that collect during spring droughts. Meanwhile, warm temperatures shorten the EIP and accelerate viral development, helping to boost the mosquito–avian and viral transmission cycle. Drought eventually yielded to heavy rains at the end of August (flooding New York subways and leading to an outbreak of *E. coli* O157:H7 at an upper New York State Fair) and Hurricane Floyd in mid-September.

During the hot, dry summer of 2002 (absent snow pack in the Rockies, thus no spring runoff) WNV raced across the North American continent, stopping in 44 states and reaching California and five Canadian provinces, resulting in the infection of 230 species of animals, including 37 species of birds.

Rodent-borne ills

The emergence of hantavirus pulmonary syndrome (HPS) and the explosive debut of WNV in the USA were both influenced by extreme weather. In the case of HPS, 6 years of drought in the American southwest is reported to have reduced rodent predators (owls, snakes and coyotes), while early downpours in 1993 produced a bounty of piñon nuts and grasshoppers, food for the rodents. The resulting mismatch hatched legions of white-footed mice, precipitating the explosive appearance of HPS in the Americas.

NATURE'S ILLS

A still greater threat to human health comes from the rash of illnesses stalking natural systems. The Millennium Ecosystem Assessment, involving 1300 scientists worldwide, found that 60% of the 'ecological services' they examined were being degraded or used unsustainably. The resulting biologic impoverishment has profound consequences for the control of pests and pathogens and for the quality of air, food and water.

Wildlife and livestock are developing new diseases and are increasingly suffering from old ones. Blue tongue disease, carried by *Culicoides* midges, has increased its range in Europe, threatening sheep and other ruminants.

The victims of WNV include raptors, such as owls, hawks, eagles, accipiters and kestrels. Improved monitoring of population-level impacts is needed, since these birds of prey help rein in rodent populations that can spread hantaviruses, arenaviruses, plague, *Leptospira* and Lyme-infected ticks. Most emerging infectious diseases are zoonoses.

Climate change, crop pests, nutrition and food security

Crops face a number of growing stresses, including vanishing pollinators, more volatile weather and the proliferation of agricultural pests, pathogens and weeds.[20] One fungal disease, Asian soybean rust (ASR) – believed to have entered the USA on the wings of Hurricane Ivan (one of the four to batter Florida in 2004) – is now present in over a dozen US states. Warm, moist conditions will favor the spread of ASR, a disease already widespread in Brazil and China, the other major producers of soybeans, a major staple used as food for humans and feed for animals.

- Climate change poses risks for food security and the reliability of crops used for feed, fiber and fuel.
- Warmer winters, more extreme weather events, and alterations in the timing, location and intensity of precipitation have already begun to reduce yields globally.
- Today 42% of growing and stored crops are lost annually due to pests, pathogens and weeds (equaling losses of ~$300 billion/year).
- Warming and extremes are conducive to crop pests and pathogens:
 - warming allows their overwintering and potential range;
 - floods foster fungi, the primary cause of crop blights; and
 - droughts favor aphids, geminivirus-injecting whiteflies and locusts.
- Agricultural weeds – like ragweed – are strengthened by rising CO_2 levels.
- Cassava plants grown in elevated CO_2 have increased cyanide and decreased yields (R Gleadow, Monash University, Australia, personal communication, 2009):
 - cyanide poisoning from cassava consumption during droughts has caused permanent neuropathy in children in Mozambique, Uganda and several West African nations.
- Higher atmospheric levels of CO_2 also change carbon-to-nitrogen ratios, forcing leaf-eating pests to consume more biomass (increasing herbivory) to obtain the nitrogen they need for growth.
- More pests, pathogens and weeds will, in turn, require higher use of pesticides, fungicides and herbicides that harm insectivores (spiders, ladybugs and birds), and can be carcinogenic, neurotoxic and harmful to reproductive health.
- Together, climate change and weather extremes, pests, pathogens and weeds may have 'nonlinear' impacts on agricultural yields, i.e. sudden, widespread losses.
- The potential for such extensive losses goes beyond the direct effects of changing growing seasons and shifting ecotones, with implications for nutritional health, biofuel reliability and political stability.

Climate change and resource scarcity can exacerbate strife, increasing refugees, malnutrition and disease.

Water

In many regions of the globe, ground water systems are being overdrawn and underfed. Shifting weather patterns further jeopardize water quality and quantity, and most montane ice fields will disappear this century – removing a primary source of water for humans, livestock, crops and hydropower for many nations.

In the last half of the 20th century the hydrologic cycle in the western USA changed significantly, affecting this arid region with a large and growing population. Barnett and colleagues find that warming winters, changes in precipitation and river flow, and diminished snow pack – 60% of which is attributable to anthropogenic greenhouse gas-induced warming – 'portend … a coming crisis in water supply for the western United States'.[21]

With current usage levels, an increase in environmentally displaced persons and a changing water cycle, the number of people suffering water stress and scarcity today is projected to triple in two decades.

PUBLIC HEALTH RECOMMENDATIONS

Climate forecasting and satellite images can provide early warnings of conditions conducive to disease outbreaks. During El Niño events, extreme weather conditions occur often and in relatively predictable geographic locations. Given scarce resources, the alerts can help communities target timely, environmentally friendly, health-protecting measures to the most vulnerable areas and populations. The measures include:

- distribution of antimalarials, impregnated bed nets and insecticides;
- breeding site (source) reduction;
- introducing the Western mosquito-fish (*Gambusia affinis*) into ponds;
- vaccination campaigns for bacterial meningitis; and
- preparation of oral rehydration therapy solutions and facilities for cholera.

Clean energy and development

Consistent energy supplies are necessary for development. Where grids are inadequate, stand-alone power generators – using solar, wind, geothermal and human power (hand- and bicycle-driven power) – can desalinate, decontaminate and pump water; irrigate land; power clinics; light homes; and drive small-scale enterprises. Clean distributed generation is necessary for meeting the Millennium Development Goals,[22] including adequate health care, agricultural productivity and provision of livelihoods.

Ultimately, however, governments must provide the infrastructure and financial incentives to enable large shifts in private investments, and the level of international cooperation needed to drive the clean energy transformation and preservation of the world's forests is unprecedented.

CONCLUSION

Just as we underestimated the rate at which climate would change, we have underestimated the breadth of biologic responses to those changes. Treating climate-related ills will require preparation, and early warning systems forecasting weather extremes can help to reduce casualties and disease spread. But primary prevention involves halting the extraction, mining, transport, refining and combustion of fossil fuels – a transformation that carries innumerable health and environmental benefits and is necessary to stabilize the climate.

Health professionals helped curb nuclear proliferation and focused world attention on the health implications of stratospheric ozone depletion. Now the public health community must focus its attention on the proposed energy solutions to ensure that safe technologies and practices are chosen.

The good news is that we may have also underestimated the economic benefits of the challenge ahead. With properly aligned rewards, rules and regulations, the clean energy transformation can become the driving engine for the international economy in the 21st century, laying the basis for a healthier, more equitable and sustainable future.

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Chapter 8

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Viral exanthems

INTRODUCTION

An exanthem or rash in a patient with fever can offer the clinician an additional clue to the etiology of the disease. The age, the immune state of the patient, geographic location and season will narrow the list of this broad differential diagnosis. A systematic history of immunizations, past childhood illnesses, exposure to other patients, animals including insects, sexual contacts, travel history and use of medications is essential. The appearance of the rash including the characteristics of a single lesion, the distribution on the skin and mucosa, the appearance and progression in relation to the development of the fever in combination with the medical history will guide the clinician. In this chapter we will focus on the viral diseases frequently accompanied by an exanthem. For an extensive review of the clinical presentation, pathogenesis, complications and treatment of the individual infections the reader is referred to the specific chapters elsewhere in this book. Viral causes of rash are outlined in Table 8.1.

Classic Viral Exanthems

VARICELLA

Varicella is an exanthematic disease with a characteristic vesicular appearance. It is caused by varicella-zoster virus (VZV), which is a member of the Alphaherpesvirinae with an enveloped linear, double-stranded DNA genome. Like other herpesviruses, it causes a primary infection (varicella or chickenpox) with seroconversion and subsequent lifelong latency. Reactivation causes localized neurologic disease with an associated skin eruption (herpes zoster or shingles). VZV is transmitted by droplets and by direct contact with the contents of the vesicles and is highly contagious. Virus shedding starts 2 days before the onset of the rash. The incubation period is 11–20 days. The peak age of infection is between 1 and 8 years.[1]

Table 8.1 Viral exanthems

Erythematous maculopapulous	Papulovesiculous	Vesiculobullous	Petechial and purpuric
Morbillivirus	Hepatitis B virus	Varicella-zoster virus	Cytomegalovirus (congenital)
Rubella	Gianotti–Crosti syndrome	Herpes simplex virus	
Human herpesvirus 6		Variola virus	Rubella (congenital)
Human herpesvirus 7	Asymmetric periflexural exanthem	Vaccinia virus	Enterovirus
Parvovirus B19		Monkeypox virus	PPGSS†
Epstein–Barr virus		Cowpox virus	Flaviviruses
Cytomegalovirus		Enteroviruses	Viral hemorrhagic fevers
Enteroviruses			
HIV			
Alphaviruses*			
Flaviviruses			
Menangle virus			

*Alphaviruses: Chikungunya virus, O'nyong-nyong virus, Sindbis virus, Ross River virus, Mayaro virus.
†Papular–purpuric gloves and socks syndrome

Clinical features

The lesions of varicella begin as papules, but progress within hours to clear vesicles surrounded by a variable halo of erythema. Vesicles are often oval, with the long axis parallel to skin creases, and are commonly pruritic (Fig. 8.1). New lesions appear progressively over 5–7 days. The head and upper trunk are affected first and most densely, whereas the limbs have fewer lesions and these appear later. The vesicular fluid opacifies, and in 2–3 days a central dimple appears. A crust then forms from this center outward, and falls off after about 5 days. Prodromal symptoms of malaise, headache and loss of appetite are mild and more common in adults. Unless secondary infection of the skin has occurred, scarring is limited to faint, pale outlines. Indicators of severe disease include confluence of the rash, multiple lesions in the mouth, pharynx, esophagus, trachea and mucosa of the genital tract.

Herpes zoster is heralded by pain in the dermatome served by the affected sensory root. Groups of papules then appear at the sites where the cutaneous nerves reach the skin. The skin eruption will be restricted to one dermatome unilaterally in the immunocompetent host; in the immunocompromised patient multidermatomal skin eruptions or generalization may occur. The papules progress to vesicles, pustules and crustae but, unlike varicella, lesions may become confluent and form large, flaccid bullae that rupture to leave weeping bare areas (Fig. 8.2). Uncomplicated lesions can heal in 4–6 days, but severe rashes may take 3–5 weeks. Nevertheless, skin depigmentation is often the only sequel. Scarring is rare.



Fig. 8.2 The rash of herpes zoster. It can be seen that the lesions occur in groups, with coalescence of lesions in the larger groups. Courtesy of Barbara A Bannister, MD.

Complications

Secondary bacterial skin infection is the commonest complication of varicella infection and occurs in 5–10% of the patients. The usual pathogens are *Streptococcus pyogenes* or *Staphylococcus aureus* (Fig. 8.3). Bacterial infections of individual spots cause pain, induration and often abscess formation. Local extension can cause cellulitis or erysipelas; necrotizing fasciitis is rare. Children are particularly prone to staphylococcal or streptococcal toxic shock syndrome. Secondary bacterial infection can also complicate herpes zoster, especially when severe and in the elderly. Varicella pneumonia which occurs in immunocompromised patients and pregnant women is a complication which is beyond the scope of this chapter (see Chapters 75 and 155.).

Postherpetic neuralgia is defined by persisting pain after an episode of herpes zoster and older patients are at risk of developing postherpetic neuralgia. Average duration in those aged over 60 years is 60 days, but may persist much longer.

Management

Antiviral treatment has minimal impact on mild childhood infections and is not recommended. In adolescents and adults, aciclovir shortens the duration of VZV infections and may reduce the risk of complications. Valaciclovir, a prodrug of aciclovir, has an enhanced bioavailability and is the preferred oral antiviral drug. Intravenous aciclovir (10 mg/kg q8h) is the treatment of choice for VZV infections in immunocompromised patients or for severe and complicated VZV infections.

In herpes zoster, antiviral treatment started in the first 48 hours after the onset of the skin eruption reduces the duration of the rash and ameliorates zoster-associated pain, but does not prevent postherpetic neuralgia.[2,3] Prevention of herpes zoster and postherpetic neuralgia in elderly persons has been shown by immunization with the live attenuated Oka VZV vaccine. A reduction in the incidence of herpes zoster



Fig. 8.1 The lesions of varicella (chickenpox). At the same time papules, vesicles and pustules, some of which are beginning to crust from the center, are seen.



Fig. 8.3 Secondary staphylococcal infection of varicella lesions. Staphylococcal pyrogenic exotoxin has caused a 'scalded skin' type of lesion surrounding the infected spots. Courtesy of Dr MG Brook.



Fig. 8.4 The acute rash of measles. Marked conjunctivitis accompanied the maculopapular skin lesion in this unimmunized adult. Courtesy of Barbara A Bannister, MD.

and consequently the development of postherpetic neuralgia was seen by 61.1% and 66.5%, respectively, in adults more than 60 years of age.[4] Secondary bacterial skin infections should be treated promptly with antibiotics with activity against *Strep. pyogenes* and *Staph. aureus*. Postherpetic neuralgia responds poorly to analgesics. Amitriptyline or antiepileptic drugs may give some relief in patients with persistent postherpetic neuralgia.

MORBILLI

Morbilli is an exanthematic disease caused by the measles virus, genus Morbillivirus, member of the Paramyxoviridae, a family of enveloped, negative, single-stranded RNA viruses.

In most populations the majority of adults and children are immune due to exposure to the measles virus or vaccination. In resource-poor countries, where the vaccination coverage is low, measles is still a significant cause of morbidity and mortality. In countries where measles vaccination is included in the pediatric vaccination program, measles has become a rare disease and is often diagnosed as a disease imported by nonimmune individuals from endemic countries. Furthermore, sporadic epidemics of measles occur in religious communities where vaccination is not accepted. Humans are the only known reservoir of measles. The infection is highly contagious and is spread airborne, by droplets. The virus replicates in the respiratory mucosa and spreads (primary viremia) in leukocytes to endothelial and epithelial cells, monocytes and macrophages, which will release large amounts of virus several days after the first viremia, resulting in a secondary viremia. The asymptomatic incubation period of measles lasts 10–14 days. During the second viremia, symptoms of malaise, fever, anorexia, followed by conjunctivitis, coryza and cough, occur. This prodromal phase lasts for 2–3 days, sometimes up to 8 days. The period of contagion ranges from 5 days before to 4 days after the appearance of the rash. Maximum contagion is probably during the late prodromal phase, when the patient develops fever and respiratory symptoms.[5]

Clinical features

During the late prodromal phase, patients may develop an enanthem known as Koplik's spots. Koplik's spots are 1–3 mm grayish or bluish elevations with an erythematous base, seen on the buccal mucosa usually opposite the molars or sometimes on the labial mucosa. They have been described as 'salt grains on a red background'. Koplik's spots



Fig. 8.5 The generalized confluent maculopapular rash as seen in measles.

occur approximately 48 hours before the measles exanthem occurs. Koplik's spots disappear by the second day of the exanthem. The characteristic rash is maculopapular and blanches, beginning on the face and spreading to the neck, upper trunk, lower trunk and extremities (Figs 8.4, 8.5). The lesions may become confluent, especially in areas such as the face. The palms and soles are rarely involved. The cranial-to-caudal progression of the measles rash is characteristic. Unlike Koplik's spots, the rash of measles is not pathognomonic. The rash begins to fade 3–4 days after it first appears, and changes to a purplish brownish color which is sometimes followed by fine desquamation. Clinical improvement ensues within 48 hours of the appearance of the rash.

The differential diagnosis of the measles exanthem includes human herpesvirus 6 (HHV-6) infection, rubella, infectious mononucleosis, scarlet fever, Kawasaki disease, toxic shock syndrome, dengue, Rocky Mountain spotted fever and drug allergy. Measles



Fig. 8.6 Secondary infections complicating measles. Perioral infection and paranasal herpes simplex lesion in a 2-year-old girl. Courtesy of Barbara A Bannister, MD.

can usually be distinguished clinically from rubella, erythema infectiosum (parvovirus B19 infection), roseola and enteroviral infection by the intensity of the measles rash, its brownish coloration and the combination with other physical findings (e.g. coryza and conjunctivitis).

Complications

When poor hygiene, crowding or malnourishment exist, severe infection of macerated perioral or perinasal skin (cancrum oris) may be caused by pyogenic organisms, often accompanied by herpes simplex and/or anaerobic mouth flora (Fig. 8.6). Postinfectious encephalitis and subacute sclerosing panencephalitis are rare complications that are beyond the scope of this chapter.

Management

After introduction of the live attenuated measles vaccine in the 1960s this epidemic disease was rapidly controlled. Due to the inverse relationship between serum retinol concentrations and measles mortality, oral vitamin A is advised at diagnosis in areas where health care and nutrition are suboptimal. There is no specific treatment.

RUBELLA

Rubella, also known as German measles, is caused by the rubella virus, a member of the Togaviridae, which is a single-stranded RNA virus. The virus is transmitted from person to person by respiratory secretions. The infectious period starts 7 days before the onset of the rash and continues for another 2 days. Most shedding of infectious virus occurs before the onset of the rash. The incubation period is 15–20 days. In susceptible populations, the peak age of infection is between 5 and 9 years.[6]

Clinical features

Prodromal symptoms such as malaise are minimal in children, although the lymph nodes begin to enlarge 3–4 days before the onset of the rash. Large tender lymph nodes high in the occipital region are typical of rubella. Adults may suffer 3–4 days' prodrome with low-grade fever, muscle pains and headache. Conjunctivitis and coryza, if present, are usually minimal and accompany the rash. The rash presents with pink-red macules and papules on the face and spreads to the chest and peripheries over 1–2 days (Fig. 8.7). The exanthem begins to fade after 1–2 days in order of appearance and disappears completely in 2–3 days. There is often an enanthem with punctate erythematous spots scattered over the hard and soft palate and uvula, called the Forchheimmer sign. It mimics the rashes of parvovirus infection, echovirus infections and mild scarlet fever or early Kawasaki disease, although it does not desquamate on healing.

Rubella in pregnant women may lead to the congenital rubella syndrome with a wide range of anomalies, and ongoing virus replication and inflammation after birth (see Chapter 52). Newborns with



Fig. 8.7 Presenting rash with pink-red macules and papules on the face and neck as seen in rubella.



Fig. 8.8 Congenital rubella syndrome. Characteristic rash called 'blueberry muffin spots' in a newborn with congenital rubella.

congenital rubella syndrome show a completely different exanthem. The rash consists of petechial/purpuric-like lesions scattered over the skin (Fig. 8.8). Due to its appearance this characteristic exanthem is referred to as 'blueberry muffin spots'. These spots are thought to be the consequence of extramedullary dermal erythropoiesis.[7,8] Congenital cytomegalovirus (CMV) infection should be in the differential diagnosis when confronted with a neonate with 'blueberry muffin spots'.

Management

The illness is mild and self-limiting; specific treatment is not available. An excellent live attenuated rubella vaccine has been available since 1968 and is used in many parts of the world in childhood immunization programs. The efficacy of the rubella vaccine has been shown by a marked reduction in the prevalence of congenital rubella syndrome.

INFECTION BY PARVOVIRUS B19 (ERYTHEMA INFECTIOSUM)

Erythema infectiosum is caused by human parvovirus B19, which belongs to the Erythrovirus genus, and is the only member of the Parvoviridae family pathogenic to humans. The Parvoviridae are very small, nonenveloped, single-stranded DNA viruses. Parvovirus B19 infection occurs worldwide. Cases can be sporadic or can occur in clustered outbreaks. Most individuals become infected during their school years. More than 70% of adults have parvovirus B19-specific IgG antibodies. Approximately 25% of the infections are asymptomatic, 50% will have nonspecific flu-like symptoms and 25% will present with the classic symptoms of parvovirus B19 infection including rash and/or arthralgia. Parvovirus B19 infections are transmitted from person to person via respiratory secretions. It has an incubation period of 4–14 days. Furthermore, vertical transmission, resulting in congenital infection, and transmission by the administration of blood or blood products containing parvovirus B19 may occur.[9]

Clinical features

The childhood infection usually manifests itself as erythema infectiosum (fifth disease or slapped cheek syndrome), a mild febrile illness with rash.

In the first week after infection the viremia is accompanied by a nonspecific flu-like illness, with symptoms of fever, malaise, myalgia, headache and pruritus. Between 2 and 5 days later (i.e. 1–2 weeks after infection) the classic erythematous malar rash appears (the so-called slapped cheek rash) with circumoral pallor (Fig. 8.9). This facial rash is often followed by a maculopapular exanthem on the trunk and extremities, which confluences into a lacy, reticular pattern. The rash in adults is less characteristic than the rash in children. By the time the rash develops, viremia and symptoms have resolved. The rash disappears within a week.

Parvovirus B19 infection has been associated with other types of rash including morbilliform, confluent and vesicular. Moreover, parvovirus B19 has been linked in more than half of the reported cases of papular–purpuric gloves and socks syndrome (see below).

Complications

Severe outcomes may occur in immunocompromised patients and patients with hemoglobinopathies. Infection in these patient groups can cause a serious prolonged chronic anemia (pure red cell aplasia) owing to persistent lysis of red blood cell precursors.

Parvovirus B19 infection during pregnancy can result in fetal complications including miscarriage, intrauterine fetal death and/or nonimmune hydrops fetalis.

Management

Treatment of parvovirus B19 infection is symptomatic.

HUMAN HERPESVIRUS 6 INFECTION (ROSEOLA INFANTUM)

Roseola infantum is caused by human herpesvirus 6 (HHV-6), a double-stranded DNA virus, a member of the (beta) Herpesviridae family, genus Roseolovirus.[10] Worldwide the HHV-6 seroprevalence rates in adults vary from 20% to 100%; in industrialized countries rates of seroprevalence range from 72% to 95%. Ninety percent of cases occur in children younger than 2 years with a peak between 7 and 13 months of age. The most common route of HHV-6 transmission appears to be via saliva from mother to child. Perinatal transmission is also possible; approximately 2% of pregnant women shed low levels of virus in genital secretions. The incubation period is estimated at 5–15 days.

Clinical features

The classic manifestation of HHV-6 infection in children (roseola infantum, exanthema subitum, sixth disease) is an abrupt onset of fever that may exceed 104 °F (40 °C) and will lasts for 3–5 days, often accompanied by irritability, although most children appear well. Furthermore, malaise, palpebral conjunctivitis, edematous eyelids, uvulo-palatoglossal junctional macules or ulcers (sometimes called Nagayama spots), upper and lower respiratory symptoms, vomiting,



Fig. 8.9 Parvovirus B19 infections. Characteristic skin abnormalities of the 'slapped cheeks', circumoral pallor and a maculopapular exanthem on the trunk.

diarrhea and a bulging fontanel may be present. As the fever subsides, a blanching macular or maculopapular nonpruritic rash develops, starting on the neck and trunk and spreading to the face and proximal extremities. Sometimes this rash is vesicular. The rash usually persists for 1–2 days, but can disappear within several hours. Acute HHV-6 infection can also present as a febrile illness without a rash.

Cervical, postauricular and/or occipital lymphadenopathy are also common but later findings. Primary infection with HHV-6 in adults is rare. However, a mononucleosis-like syndrome of varying severity (with or without an exanthem) with prolonged lymphadenopathy has been described in adults.

The rash can be easily misdiagnosed as a drug allergy. Furthermore, the differential diagnosis of the exanthem as seen in roseola infantum includes rubella (rash and fever occur simultaneously), rubeola (rash and fever occur simultaneously), erythema infectiosum (slapped cheeks in school-aged children) and scarlet fever (rash may be confluent, which may be preceded by pharyngitis).

Complications

HHV-6 infections have been associated with the Gianotti–Crosti syndrome (see below) and the papular–purpuric gloves and socks syndrome (see below). HHV-6 infections have been associated with seizures, either secondary to the high fever or to the infection itself. Panencephalitis of variable severity can occur as the primary manifestation of HHV-6. HHV-6 remains latent in host cells after primary infection and can be reactivated in immunocompromised patients such as after solid-organ or hematopoietic stem cell transplantation. The majority of HHV-6 infections in these patients are asymptomatic. However, clinical syndromes associated with HHV-6 reactivation include unexplained fever, rash, hepatitis, pneumonitis, encephalitis and bone marrow suppression.

Management

Most cases are benign and self-limiting. Treatment is supportive.

Nonclassic Viral Exanthems

EPSTEIN–BARR VIRUS INFECTION (INFECTIOUS MONONUCLEOSIS) (SEE CHAPTER 155)

Infectious mononucleosis is caused by Epstein–Barr virus (EBV) or human herpesvirus 4, a member of the Herpesviridae family. Primary infection results from exposure to the oral secretions of seropositive individuals. EBV shares the properties of lifelong latency and persistence with other members of the herpesvirus family. The incubation period is 2–8 weeks in adolescents and young adults but probably shorter in younger children.

Clinical features

Classic infectious mononucleosis is characterized by fever (mean duration of 2 weeks), angina with tonsillitis, malaise and cervical lymphadenopathy. The age of the patient has a profound influence on the clinical presentation. In children, primary infection is often asymptomatic. A rash, which may be macular, petechial, scarlatiniform, urticarial or erythema multiforme-like, is present in about 10% of the cases between days 4 and 6 of the illness and is localized mainly on the trunk and upper extremities. The administration of penicillins (especially ampicillin and amoxicillin) produces a pruritic maculopapular to morbilliform eruption in 90–100% of the patients.[11,12] This rash has a prolonged duration (up to 10 days). Streptococcal pharyngitis/tonsillitis, CMV infection, thrombocytopenic purpura, lupus erythematosus and malignancies might give a comparable clinical picture to that seen in infectious mononucleosis.

Complications

Epstein–Barr virus infections are also associated with the Gianotti–Crosti syndrome, Sweet's syndrome, Stevens–Johnson syndrome and persistent erythema multiforme.[13–15]

Management

Specific treatment is not available.

ACUTE CYTOMEGALOVIRUS INFECTION

Cytomegalovirus, belonging to the Herpesviridae family, is a beta-herpes virus and is the largest virus infecting human beings. Infection is common in all human populations. Disease in humans is varied, ranging from no disease in normal hosts to life-threatening infections in immunocompromised patients. Secondary infection represents activation of latent infection or re-infection in a seropositive immune person. Congenital infection of the neonate is almost always the result of primary infection of the mother during pregnancy.

Clinical features

Most primary infections are subclinical during childhood although in young adults an infectious mononucleosis syndrome with fever and lymphadenopathy can be seen. A maculopapular and rubelliform rash may occur in the setting of CMV mononucleosis.[16] These rashes may develop after administration of penicillins and are thought to result from immunologic reactions to cellular antigens that are uncovered or expressed in association with acute CMV infection. CMV mononucleosis needs to be differentiated from EBV infection and malignancies.

Congenital infections in newborns can present with a petechial/purpuric rash, comparable to the so-called 'blueberry muffin spots' seen in congenital rubella syndrome.[7]

Complications

Skin manifestations of acute CMV infections are usually mild but can occasionally be severe, ranging from erythema multiforme to epidermolysis.[17,18]

Management

CMV infections in immunocompetent patients are self-limiting and specific treatment is not indicated. In contrast, CMV infections in immunocompromised patients can be life-threatening and need to be treated with (val)ganciclovir (see Chapter 52). Congenital CMV infections can be treated in the neonate with ganciclovir for 6 weeks and has been shown to be effective in preventing hearing loss[19] (see Chapter 52).

ACUTE HIV INFECTION

Acute HIV infection may present with a skin rash (see Chapter 89). HIV is a single-stranded RNA virus, a member of the Retroviridae family, genus Lentivirus.

HIV is transmitted via unprotected sexual intercourse, vertical transmission and incidents with blood–blood contact, such as needlestick incidents. Due to screening of blood and blood-derived products, transmission via transfusion is now extremely rare.



Fig. 8.10 The lesions in HIV are characteristically small (5–10 mm), well-circumscribed, oval or round, pink to deeply red colored macules or maculopapules.

Clinical features

The majority of patients with an acute HIV infection appear to be symptomatic (40–90%).[20] The usual time from HIV exposure to the development of symptoms is 2–4 weeks. Acute HIV infection is characterized by an abrupt onset of symptoms; the clinical syndrome develops within 24–48 hours and lasts 1.5–2 weeks. The most common findings are fever (range 100–104°F/38–40°C), lymphadenopathy, mild hepatosplenomegaly, sore throat, painful mucocutaneous ulceration (mouth, esophagus, penis, anus), myalgia/arthralgia, diarrhea, headache, nausea/vomiting, weight loss, dry cough and hepatitis. The presence of mucocutaneous ulcers is suggestive of the diagnosis.

The exanthem, occurring in 40–80% of symptomatic patients, typically occurs 48–72 hours after the onset of fever and persists for 5–8 days. The upper thorax, collar region and face are most often involved though the scalp and extremities, including the palms and soles, may be affected. The lesions are characteristically small (5–10 mm), well-circumscribed, oval or round, pink to deeply red colored macules or maculopapules (Fig. 8.10). Vesicular, pustular and urticarial eruptions have also been reported.

The differential diagnosis of acute HIV infection includes mononucleosis due to EBV or CMV, toxoplasmosis, rubella, syphilis, viral hepatitis and other viral infections.

Management

There is ongoing debate as to whether starting antiretroviral therapy during an acute HIV infection will improve prognosis by preserving the HIV-specific immune response. At the moment, no consensus has been reached on whether patients with primary HIV infection should be treated (see Chapter 89).

VIRAL HEMORRAGHIC FEVERS

Viral hemorrhagic fevers are discussed in detail in Chapter 126.

HAND, FOOT AND MOUTH DISEASE

Hand, foot and mouth disease (HFMD) is caused by enteroviruses, members of the picornavirus group (single-stranded RNA, nonenveloped) and is most commonly associated with Coxsackie virus A16 or enterovirus 71. This disease was described for the first time in 1957 in Toronto. The UK shows an epidemic course every 3 years. These epidemics seem to be associated with the warmer seasons (summer and early fall). Sporadic cases caused by Coxsackie viruses A4–7, A9, A10, B1–3 and B5 have been reported.

HFMD is highly contagious and is spread by oral–oral and fecal–oral routes. Fecal shedding may last for 4–6 weeks but its relevance in transmission is unknown. It typically affects children under 10 years of age. The incubation period is 3–6 days.[21]

Clinical features

A brief 12–36-hour prodrome of low-grade fever, malaise, cough, anorexia, abdominal pain and sore mouth occurs. Patients may present with either exanthem or enanthem but most manifest both. Painful ulcerative lesions in the oral cavity are most commonly found on the hard palate, tongue and buccal mucosa. The enanthem begins as 2–8 mm erythematous macules and papules, which progress through a short vesicular stage to form a yellow-gray ulcer with an erythematous halo. Lesions may coalesce, the tongue may become red and edematous (Fig. 8.11). Pain may interfere with oral intake. The oral lesions resolve spontaneously in 5–7 days.

The skin rash is characterized by 2–3 mm erythematous macules or papules with a central gray vesicle; these usually appear shortly after the oral lesions, with the hands more commonly involved than the feet (Figs 8.12, 8.13). The sides of the fingers and dorsal surfaces are more often involved than the palms and soles. Lesions appear elliptical, with the long axis running parallel to skin lines, and may be asymptomatic or painful. They crust and gradually disappear over 5–10 days without scarring. In addition, one-third of the cases show a maculopapular exanthem on the nates.

Stomatitis caused by herpes simplex virus is clinically indistinguishable from the oral lesions seen in HFMD, although the former is not followed by an exanthem. Other differential diagnostic considerations are herpangina, EBV infections, morbus Behçet and syphilis in adults.

Management

The illness is mild and self-limiting. Specific treatment is not available, but the lesions can be painful or tender and simple analgesics may help this.



Fig. 8.11 Hand, foot and mouth disease. Painful ulcerative lesions on the tongue and buccal mucosa.



Fig. 8.12 Hand, foot and mouth disease. The skin rash is characterized by erythematous macules or papules, with the hands more commonly involved than the feet.



Fig. 8.13 Hand, foot and mouth disease. Typical vesicles are seen on the foot of a 3-year-old child. Courtesy of Barbara A Bannister, MD.

ACROPAPULOVESICULAR SYNDROME

Papular acrodermatitis of childhood or acropapulovesicular syndrome is a papular or papulovesicular skin eruption localized on the limbs. This syndrome was described for the first time by Gianotti in Milan, Italy and is also named Gianotti–Crosti syndrome. Initially, this syndrome was diagnosed in association with anicteric hepatitis B infection. It is well known that several viruses and some bacteria can cause this syndrome, and a number of reports suggest that it may also be associated with immunizations (Table 8.2).[22–24] The pathogenesis is still unclear. It mainly affects children between 2 and 6 years of age.

Clinical features

The rash is characterized by a monomorphic exanthem consisting of skin colored to erythematous papules and papulovesicles with a diameter of 2–4 mm distributed symmetrically on the cheeks, nates and the extensor side of the limbs (Fig. 8.14). Larger plaques of confluent papules can be seen on the elbows and knees. The nonitching exanthem develops within a week and disappears in 2–4 weeks; however, cases have been described in which the skin abnormalities lasted for 4 months. General symptoms are usually mild. Malaise, lymphadenopathy, hepatosplenomegaly and low-grade fever may accompany the rash.[25]

Henoch–Schönlein purpura, erythema multiforme, hand, foot and mouth disease, pityriasis lichenoides and asymmetric periflexural exanthem of childhood may mimic the Gianotti–Crosti syndrome. Papular urticaria can also give a comparable exanthem.

Table 8.2 Viral and bacterial infections and immunizations associated with the Gianotti–Crosti syndrome

Viral infections	Hepatitis A, B and C virus, Epstein–Barr virus, cytomegalovirus, human herpes virus 6, Coxsackie virus (A16, B4, B5), adenovirus, rotavirus, parvovirus B19, respiratory syncytial virus, parainfluenza virus, rubella virus
Bacterial infections	Group A streptococci, *Mycobacterium avium intracellulare* (HIV+), *Mycoplasma pneumoniae*
Immunizations	Measles–mumps–rubella, polio, influenza, diphtheria, pertussis, hepatitis B



Fig. 8.14 Acropapulovesicular syndrome. Generalized monomorphic exanthem consisting of erythematous papules and papulovesicles distributed symmetrically.

Management

Specific treatment is not indicated. Symptomatic treatment of the pruritus with antihistamine drugs may give some relief. Due to the association (although sporadic) of hepatitis B virus infections, it is recommended to perform serology to exclude this disease.

UNILATERAL LATEROTHORACIC EXANTHEM OF CHILDHOOD

In 1962 a new localized exanthem was reported[26] and in 1992 was described as laterothoracic exanthem.[27] Because this exanthem is not only laterothoracically located, the term asymmetric periflexural exanthem is preferred. It is most likely caused by a virus, with a human herpesvirus considered to be the most likely cause. The peak age of infection is 2 years.

Clinical features

The rash consists of an erythematous papular, sometimes vesicular or squamous exanthem with a mild pruritus, localized around a skin fold such as the axilla or groin (Fig. 8.15). The skin fold itself is not affected. The exanthem consists of 1 mm erythematous pap-



Fig. 8.15 Gianotti–Crosti syndrome. Mild papulovesicular rash on the lower extremities in a young boy. (Illustration kindly provided by the Department of Dermatology, Radboud University Nijmegen Medical Center, the Netherlands.)

ules that are often surrounded by a pale halo, followed by eczematous patches separated by normal skin. Centrifugal progression of the lesions can be seen for 8–15 days, with spontaneous recovery within 4 weeks. A mild upper respiratory illness or prodrome is often associated with the rash. Lymphadenopathy can be found but is not obligatory.[28]

The exanthem has to be differentiated from allergic contact eczema, miliaria, scarlet fever, pityriasis rosea (affecting mainly adolescents) and Gianotti–Crosti syndrome.

Management

Symptomatic treatment for the pruritus may be necessary. Complications are not known.

PAPULAR–PURPURIC GLOVES AND SOCKS SYNDROME

It has been suggested that papular–purpuric gloves and socks syndrome (PPGSS) is a manifestation of an underlying immunologic mechanism that may be induced by viral or drug-related antigens. Reported etiologies include parvovirus B19 (more than half of the cases),[29] measles virus, EBV, CMV, HHV-6, Coxsackie B6 and hepatitis B. PPGSS occurs most commonly in young adults during the spring and summer.[30]

Clinical features

Symmetric erythema and edema of the hands and feet progress to petechial and purpuric macules and papules that are followed by desquamation (Fig. 8.16). A sharp demarcation is seen at the wrists and ankles. Very seldom the rash extends to nonacral sites. Occasionally, complaints of pruritus or pain may accompany the skin eruption.[31] A polymorphous enanthem, including hyperemia, aphthae, petechiae and erosions on the palate, pharynx and tongue, are seen in the majority of patients.

The differential diagnosis includes erythema multiforme, HFMD, Gianotti–Crosti syndrome, Kawasaki disease and Rocky Mountain spotted fever, although the defined acral distribution generally allows differentiation from other exanthems.

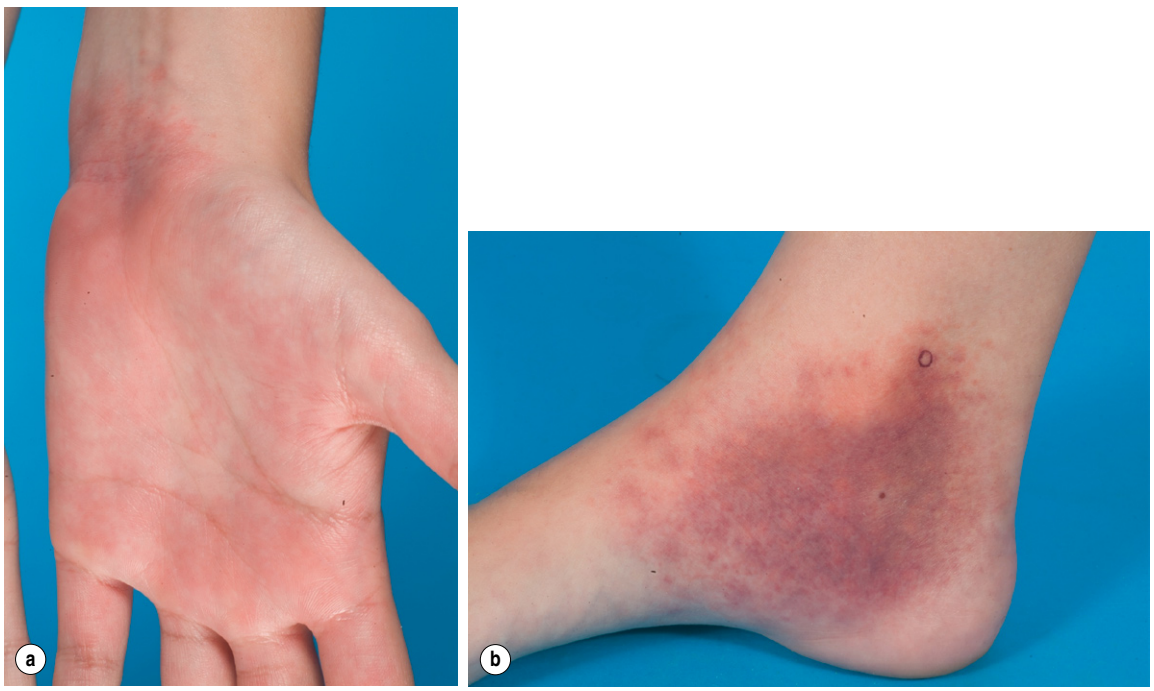


Fig. 8.16 Papular–purpuric gloves and socks syndrome. Symmetric erythema and edema of the hands and feet progress to petechial and purpuric macules and papules.

Complications

The rash resolves spontaneously in 1–2 weeks without any known sequelae.

Management

Symptomatic treatment with moisturizers and antihistamines may be indicated.

REFERENCES

References for this chapter can be found online at http://www.expertconsult.com

Chapter 9

Dennis L Stevens

Cellulitis, pyoderma, abscesses and other skin and subcutaneous infections

INTRODUCTION

Infections of the skin and/or subcutaneous tissues are highly diverse in respect to etiologic organisms, incidence, clinical manifestations, severity and complications. They may occur as single or recurrent episodes. Many cases are mild or self-limited, but some progress to cause scarring, loss of digits or limbs, or even death.

The terminology can be confusing because several different names, which are often not precisely defined, may be used to describe the same condition. Nomenclature for the most common infections is summarized in Table 9.1.

When a patient presents with soft tissue infection, the clinician faces the challenge of establishing a specific diagnosis and prescribing definitive treatment. Important points in diagnosis are:

- the patient's symptoms;
- the general appearance of the infected site;
- historic clues such as contact with insects or animals, especially involving bites, travel to specific geographic areas, occupation or use of a hot tub (see Folliculitis, furuncles and carbuncles, below);
- the immune status of the host;
- chronicity; and
- anatomic distribution.

If the diagnosis cannot be established based upon the history, symptoms and signs, then needle aspiration, biopsy or surgical exploration may be necessary to obtain specimens for appropriate staining and culture.

As the antimicrobial susceptibility of these microbes varies greatly, treatment (particularly for severe infections) should be based upon the results of microscopy, Gram stain and culture whenever possible.

Table 9.1 Nomenclature, location and etiology of some common skin and subcutaneous infections

Terminology	Subgroups	Location	Etiology
Pyoderma	Impetigo (impetigo contagiosa)	Skin	*Streptococcus pyogenes*, *Staphylococcus aureus*
	Bullous impetigo	Skin	*Stap. aureus* with group II phage
	Folliculitis (pustulosis)	Skin, hair follicles	*Staph. aureus*
	Folliculitis (sycosis) barbae	Skin, hair follicles of the beard	*Strep. pyogenes, Staph. aureus*
	Hot tub folliculitis	Skin	*Pseudomonas aeruginosa*
Abscesses	Furuncle (boil, subcutaneous abscess)	Subcutaneous tissue	*Staph. aureus*
	Hydradenitis suppurativa	Multiple furuncles in sweat glands: axilla, groins	*Staph. aureus* and other bacteria, including Gram-negative bacilli and anaerobes
	Carbuncle	Dense group of furuncles in areas of thick skin: back of neck, shoulders, buttocks	*Staph. aureus*
Cellulitis		Skin and subcutaneous tissue	*Staph. aureus, Strep. pyogenes,* group C and G streptococci, *P. aeruginosa, Haemophilus influenzae* or Gram-negative bacilli; fungi can cause cellulitis in immunocompromised hosts
	Erysipelas	Skin	*Strep. pyogenes*
Ecthyma		Skin and subcutaneous tissue	*Strep. pyogenes, Staph aureus* or both; other bacteria *P. aeruginosa*
	Ecthyma gangrenosum	Skin and subcutaneous tissue in neutropenic patients	

EPIDEMIOLOGY

Although the exact incidence of these infections in the general population is unknown, they are among the most common infections occurring in all age groups. Some are age related; for example, impetigo is more common in children, erysipelas is more common in older adults.

Infections of the skin and soft tissues can be caused by bacteria (including rickettsiae), fungi, viruses, parasites and spirochetes. Although there are hundreds of possible etiologic agents (Tables 9.1–9.4), two common species of Gram-positive cocci are the predominant causes of skin and soft tissue infections – *Staphylococcus aureus* and *Streptococcus pyogenes*. Skin and soft tissue infections caused by newly recognized or previously rarely encountered microbes are continually being described in immunocompromised patients, especially those who have AIDS.

Several noninfectious diseases can mimic infection of the soft tissues. For example, patients who have contact dermatitis, pyoderma gangrenosum, gout, psoriatic arthritis with distal dactylitis, Reiter's syndrome, relapsing polychondritis or mixed cryoglobulinemia secondary to immune complex disease from chronic hepatitis C or B virus infection may present with erythematous rashes, with or without fever.

PATHOGENESIS

The integument is an organ that reacts to noxious, infectious, external and internal stimuli in a limited number of ways. It is therefore not surprising that infection can be mimicked by noninfectious inflammatory conditions. The rich plexus of capillaries beneath the dermal papillae provides nutrition to the stratum germinativum and the dermatocytes, which are bound together by tight junctions and form the barrier to microbial invasion. Once microbes have penetrated this barrier through a hair follicle, cut or bite, the dermal plexus of capillaries delivers the components of the host's defense – oxygen, complement, immunoglobulins, macrophages, lymphocytes and granulocytes – to the site of infection.

Table 9.3 Differential diagnosis of bullous skin lesions

Clinical condition	Etiology
Bullous impetigo	*Staphylococcus aureus* carrying group II phage
Erysipelas	*Streptococcus pyogenes*
Staphylococcal scalded skin syndrome	*Staph. aureus* producing exfoliative toxin
Necrotizing fasciitis	Type I: mixed aerobic and anaerobic bacteria Type II: *Strep. pyogenes*
Gas gangrene	*Clostridium perfringens, C. septicum*
Halophilic vibrio sepsis	*Vibrio vulnificus*
Pemphigoid	Immune-mediated
Toxic epidermal necrolysis	Drug-induced

Table 9.4 Differential diagnosis of crusted skin lesions

Clinical condition	Etiology
Impetigo	*Staphylococcus aureus, Streptococcus pyogenes* or both
Ringworm	Dermatophytic fungi (e.g. *Tinea rubrum*)
Systemic fungal infections	*Histoplasma capsulatum* *Coccidioides immitis* *Blastomyces dermatitidis*
Cutaneous mycobacterial infection	*Mycobacterium tuberculosis* *M. marinum*
Cutaneous leishmaniasis	*Leishmania tropica*
Nocardiosis	*Nocardia asteroides*

Table 9.2 Probable etiology of soft tissue infections associated with some specific risk factor or setting

Risk factor or setting	Likely etiologic agent
Cat bite	*Pasteurella multocida*
Dog bite	*P. multocida, Capnocytophaga canimorsus (DF-2), Staphylococcus intermedius*
Tick bite followed by erythema chronicum migrans rash	*Borrelia burgdorferi*
Hot tub exposure	*Pseudomonas aeruginosa*
Diabetes mellitus or peripheral vascular disease	Group B streptococci
Periorbital cellulitis (children)	*Haemophilus influenzae*
Saphenous vein donor site cellulitis	Groups C and G streptococci
Fresh water laceration	*Aeromonas hydrophila*
Sea water exposure, cirrhosis, raw oysters	*Vibrio vulnificus*
Cellulitis associated with stasis dermatitis	Groups A, C and G streptococci
Lymphedema	Groups A, C and G streptococci
Cat scratch	*Bartonella henselae, B. quintana*
HIV-positive patient with bacillary angiomatosis	*B. henselae, B. quintana*
Fishmongering, bone rendering	*Erysipelothrix rhusiopathiae*
Fish tank exposure	*Mycobacterium marinum*
Compromised host with ecthyma gangrenosum	*P. aeruginosa*
Agammaglobulinemic patient with 'erysipelas'	*Campylobacter jejuni*
Human bite	*Eikenella corrodens, Fusobacterium* spp., *Prevotella* spp., *Porphorymonas* spp., *Streptococcus pyogenes*

Perhaps the first clue available to the immune system to the presence of foreign material in the deep tissues is provided by the organisms themselves. Virtually all bacteria are comprised of proteins whose *N*-terminal amino acid sequence begins with an *N*-formyl-methionine group and these proteins are chemoattractive to phagocytes such as macrophages and granulocytes. Other microbial cell wall components such as zymosan of yeast, endotoxins of Gram-negative bacteria and peptidoglycans of Gram-positive bacteria activate the alternative complement pathway,[1] yielding serum-derived chemotactic factors. Chemotactic factors are therefore promptly produced at the site of infection by multiple mechanisms.

The efflux or diapedesis of phagocytes through endothelial cell junctions is dependent upon the orchestrated sequential expression of adherence molecules on the surface of the polymorphonuclear leukocyte (PMNL)[2,3] such as L-selectin and CD11b/CD18 in association with counter-receptors (adhesins) on the endothelial surface.[4] *In vivo*, surface expression of these molecules results first in 'rolling' of PMNLs along the endothelial surface, followed by tethering, and finally firm adhesion of the PMNLs onto the surface of endothelial cells. Phagocytes actually leave the capillary through endothelial cell interstices bound by peripheral endothelial cell adherence molecules, which are found only at these junctional sites.

Once diapedesis has occurred, the PMNL follows the gradient of chemotactic factors derived from the bacteria and serum to the site of active infection. Recent studies suggest that the activated endothelial cells also produce chemotactic cytokines, such as interleukin (IL)-8. Finally, activated granulocytes synthesize leukotriene B_4 from arachidonic acid, and this too is a potent chemoattractant for leukocytes.

Production of proinflammatory cytokines such as IL-1, IL-6 and tumor necrosis factor results in an augmentation of the immune functions described above. These cytokines induce fever, prime neutrophils, and increase antibody production and the synthesis of acute phase reactants, such as C-reactive protein.[5,6] Cytokine-driven stimulation of endothelial cells also results in the generation of nitric oxide and prostaglandins, both of which cause vasodilatation. The net physiologic effect is greater blood flow to the tissue. These processes result in the cardinal manifestations of inflammation:

- heat;
- swelling;
- erythema; and
- tenderness or pain.

At some locations, factors such as pressure, thrombosis or drugs may reduce or stop blood flow, resulting in inadequate oxygenation. Compounds such as corticosteroids, which inhibit phospholipase A_2 activity (necessary for releasing arachidonic acid from cell membranes), and nonsteroidal anti-inflammatory agents, which inhibit cyclo-oxygenase (the endogenous enzyme necessary for the synthesis of prostaglandins from arachidonic acid), reduce local blood flow to tissues. These drugs are therefore useful in the treatment of noninfectious inflammatory conditions because they reduce pain and swelling. However, if the inflammation is secondary to undiagnosed bacterial infection, these drugs may predispose the patient to more severe infection or mask the clinical signs, so delaying the correct diagnosis.

If tissue perfusion is moderately attenuated, tissues may remain viable, but the threshold for progression of infection may be lowered. Predisposing conditions in this category include:

- peripheral vascular disease affecting large arteries;
- diabetes mellitus causing microvascular disease; and
- chronic venous stasis causing postcapillary obstruction.

Necrosis of the skin and deeper tissue may occur if there is severe hypoxia. Two examples are:

- pressure necrosis resulting in decubitus ulcers; and
- compartment syndromes resulting in hypoxia and then necrosis in muscles confined within tight fascial bundles.

When the host is physiologically, structurally and immunologically normal, only certain pathogens such as *Staph. aureus* and group A streptococci are able to cause disease by virtue of their potent virulence factors, such as toxins, capsules or dermonecrotic enzymes, which confer ability to withstand the barrage of host defenses and to induce clinical disease. This statement is supported by the observation that normal skin, although constantly exposed to many indigenous and exogenous microbes, rarely becomes infected.

In contrast, patients who have compromised skin integrity (e.g. patients with burns), vascular defects (e.g. those who have diabetes mellitus or pressure ulceration) or immunologic deficits may become infected with either virulent organisms (e.g. staphylococci or streptococci) or microbes that are usually saprophytic, such as *Pseudomonas aeruginosa*, *Escherichia coli*, enterococci or *Fusarium* spp. Defects such as neutropenia attenuate the host response and once the invading pathogen has breached the skin could predispose to worse outcomes.

PREVENTION

Avoidance of cuts, scratches and other forms of trauma that disrupt the natural barrier function of the skin helps to prevent skin and soft tissue infections. For example, stopping shaving may prevent recurrent folliculitis in the beard area (sycosis barbae). Prompt cleansing, debridement and disinfection of such lesions are important for preventing infection, particularly in the case of bite wounds. Treatment of eczema reduces the risk of secondary bacterial infection.

Prevention of recurrent folliculitis or furunculosis is difficult to achieve, but there has been some success using intranasal applications of bacitracin or mupirocin ointment. Chlorhexidine scrub may be tried to eliminate or reduce staphylococcal carriage in adults.[7] Prophylaxis with systemic antibiotics is of doubtful efficacy and can result in the emergence of resistant strains; it should be tried only for severe cases (see Folliculitis, furuncles and carbuncles, below).

Recurrent bacterial cellulitis of the lower extremities can often be prevented by topical antifungal treatment for dermatophyte infections such as tinea pedis because even minor or inapparent superficial fungal infection can serve as a portal of entry for Gram-positive cocci.

CLINICAL FEATURES, DIAGNOSIS AND MANAGEMENT OF SPECIFIC SOFT TISSUE INFECTIONS

Folliculitis, furuncles and carbuncles

Pustules or abscesses can develop when organisms permanently or transiently resident on the skin surfaces are introduced into deeper tissues (Fig. 9.1). Pathogens can also seed the skin from hematogenous sources such as bacteremias, for example associated with staphylococcal endocarditis (Fig. 9.2) or by contiguous spread from infectious foci in the lung or gastrointestinal tract. Most commonly,



Fig. 9.1 Cutaneous infection at the previous insertion site of an intravenous catheter. Organisms from the skin were likely introduced into the dermis and subcutaneous tissue at the time of catheter insertion. Many of these infections remain superficial, but in this patient suppurative thrombophlebitis with bacteremia ensued.



Fig. 9.2 Diffuse skin involvement. Petechial lesions in a patient with *Staphylococcus aureus* bacteremia, endocarditis and acute aortic insufficiency.

small focal abscesses develop in the superficial layers of the skin, where hair follicles serve as the portal of entry. Such lesions are called folliculitis. *Staphylococcus aureus* accounts for most of these infections, but many different bacterial species can occasionally cause localized folliculitis.

Folliculitis can progress to form subcutaneous abscesses, called furuncles or boils, which usually drain and resolve spontaneously, but may progress to form a large, exquisitely painful group of contiguous furuncles, called a carbuncle (Fig. 9.3). Carbuncles require surgical drainage as well as antibiotic treatment.[6]

Recurrent furunculosis

Certain individuals seem to be predisposed to recurrent *Staph. aureus* skin infections (recurrent furunculosis). Although it has been suggested that diabetic patients are especially prone to boils and carbuncles, few data support this concept. In contrast, it is well established that patients with Job's syndrome, who have high levels of serum IgE antibody and often a congenital defect of the STAT3 signaling pathway, are strongly predisposed to these focal *Staph. aureus* infections. However, most patients do not have immunologic or metabolic abnormalities, and the greatest predisposing factor has been the colonization of the anterior nares with *Staph. aureus* (Fig. 9.4). Thus, touching the nose or nasal secretions and then rubbing or scratching the skin results in autoinoculation and abscess formation. Breaking the cycle can be useful to prevent recurrences; however, it is important to document nasal colonization by appropriate techniques.

Administration of intranasal mupirocin or bacitracin ointment for the first 5 days of each month has been shown to decrease colonization and reduce the frequency of recurrent infection by about 50%.[8] Treatment of recurrent furunculosis may also require surgical incision and drainage as well as antistaphylococcal antibiotics such as oral dicloxacillin or parenteral nafcillin. In general, surgical drainage alone is successful, unless the patient has fever, leukocytosis or the lesions are greater than 5 cm in diameter. Community-acquired methicillin-resistant *Staph. aureus* (CA-MRSA) which harbors Panton–Valentine leukocidin (PVL) has been associated with epidemics of these types of infection among prisoners, athletes and children in day-care centers.



Fig. 9.3 Carbuncle of the buttock caused by *Staphylococcus aureus*. This large carbuncle developed over the course of 7–10 days and required surgical drainage plus treatment with antibiotics. The patient had previously experienced numerous episodes of *Staph. aureus* cutaneous abscesses. He carried the staphylococci in his anterior nares.

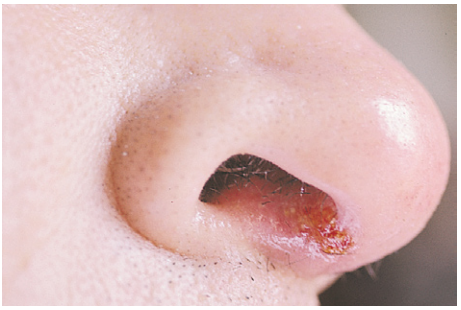


Fig. 9.4 Staphylococcal nasal carriage. This patient had a small staphylococcal abscess beneath the mucosa of the nose, illustrating how *Staphylococcus aureus*, which colonizes the nares, can infect skin and submucosa. Intact mucosa is highly resistant to infection; such infections usually occur as a result of defects in the mucosal membranes or via hair follicles inside the nose.

Predisposing factors

Superficial dermal trauma such as insect bites or abrasions can result in cutaneous abscesses. Eczema may also serve as a portal of entry. Superinfected eczema may be difficult to distinguish from eczema itself because both result in crusted lesions, exudation and cutaneous erythema. The presence of lymphangitis, pustules or fever suggests infection. Because *Staph. aureus* is the most common cause of infected eczema, treatment with an oral antistaphylococcal antibiotic such as dicloxacillin or trimethoprim–sulfamethoxazole (for CA-MRSA) is warranted.

Sebaceous glands empty into hair follicles; if the ducts become blocked they form sebaceous cysts, which may resemble staphylococcal abscess or become secondarily infected. Chronic folliculitis is uncommon except in acne vulgaris in which normal flora (e.g. *Propionibacterium acnes*) may play a role.

Recurrent folliculitis is most common in black males and associated with trauma from shaving (folliculitis barbae). Hidradenitis suppurativa occurs in either acute or chronic forms and can lead to recurrent axillary, periareolar, perineal or pudendal abscesses. Although the exact pathogenesis of recurrent hidradenitis is not understood, it is considered to be due to abnormal apocrine sweat glands.

Diffuse folliculitis

Diffuse folliculitis occurs in two distinct settings. The first – 'hot tub folliculitis' – is associated with water maintained at a temperature between 98.6 and 104°F (37 and 40°C) that is insufficiently chlorinated and is caused by *P. aeruginosa*. The infection is usually self-limited, although serious complications of bacteremia and shock have occasionally been reported.

The second form of diffuse folliculitis – swimmer's itch (Fig. 9.5) – occurs when the skin is exposed to water infected with avian freshwater schistosomes. Warm water temperatures and alkaline pH are suitable for mollusks, which are the intermediate host between birds and humans. Free-swimming cercariae readily penetrate human hair follicles or pores, but quickly die. This triggers a brisk allergic reaction, causing intense itching and erythema. The infestation is self-limited, secondary infection is uncommon and antipruritics and topical corticosteroid cream promptly relieve the symptoms.

Staphylococcal scalded skin syndrome

Staphylococcal scalded skin syndrome has been described in all age groups, but it is usually seen in children under 5 years of age,



Fig. 9.5 Swimmer's itch. Diffuse folliculitis can be caused by *Pseudomonas aeruginosa* (hot tub folliculitis), schistosomes (swimmer's itch) or *Staphylococcus aureus* (folliculitis). This young man had been fishing in an alkaline lake in the western part of the USA. He had been fishing from a 'float tube' and had exposed only his hands and arms to the water. The rash was associated with severe itching. Although his white blood count was not elevated 35% of the white cells were eosinophils.



Fig. 9.6 Staphylococcal scalded skin syndrome. Flaccid bullae occur as single or multiple lesions. Examination of a frozen tissue section reveals that the cleavage plane is at the stratum corneum. This disease must be distinguished from toxic epidermal necrolysis (see Fig. 9.7).

including neonates.[9] The characteristic features are a faint erythematous rash with the formation of flaccid bullae (Fig. 9.6). *Staphylococcus aureus* of phage group II is the causative organism. These organisms produce the toxin exfoliatin, which appears to affect the cell junctions of young dermal cells. Specifically, there is intraepidermal cleavage at the level of the stratum corneum. A classic clinical feature is Nikolsky's sign, in which lateral pressure on the skin results in shearing off of the top layer of skin (Fig. 9.7). The mortality of staphylococcal scalded skin syndrome is low, and fluid loss from the skin is minimal. Appropriate antibiotic therapy is the main component of treatment.

Staphylococcal scalded skin syndrome must be distinguished from toxic epidermal necrolysis, a condition that is more common in adults, is usually secondary to a drug reaction and is associated with a high mortality rate (see Fig. 9.7). Frozen section examination of a punch biopsy readily distinguishes these two entities:

- staphylococcal scalded skin syndrome shows a cleavage at the level of the stratum corneum; and
- toxic epidermal necrolysis shows deeper cleavage at the stratum germinativum.



Fig. 9.7 Toxic epidermal necrolysis. This picture shows a skin slough (Nikolsky's sign), which resulted when lateral pressure was applied by the thumb in a plane parallel to the skin surface. This disorder is more common in adults, has a high mortality rate and is usually caused by medications.

Impetigo

Impetigo contagiosa is a form of superficial pyoderma caused by streptococci and/or staphylococci. Currently, about half of impetigo cases are caused by *Staph. aureus*, including CA-MRSA.[9] Staphylococci and group A streptococci can be co-isolated from impetiginous lesions in many cases. Group A streptococci alone currently cause less than half the cases. Staphylococcal impetigo tends to occur sporadically, whereas epidemics of impetigo caused by group A streptococci have been well described. Epidemics occur throughout the year in tropical areas or during the summer months in more temperate climates. Impetigo caused by group A streptococci is sometimes complicated by the development of poststreptococcal glomerulonephritis. This important nonsuppurative complication is more likely to occur during epidemics of impetigo caused by certain M types such as M type 49 (see Chapter 166).

Impetigo is characterized by thick-crusted lesions with rounded or irregular margins (Fig. 9.8a), often located on the face.[10] Streptococcal pyoderma frequently has a golden brown or honey color, resembling a plaque of dried serum. Children between 2 and 10 years of age are most commonly infected. Impetigo is often associated with poor socioeconomic conditions and poor hygiene.

Initially, colonization of unbroken skin occurs either exogenously from other infected persons (hence the term impetigo contagiosa) or endogenously by contamination of the skin with organisms carried in the anterior nares or oropharynx. The development of impetiginous lesions takes 10–14 days and likely is initiated through lesions such as minor abrasions and insect bites, which serve as a means of intradermal inoculation. Initially, impetigo may appear as vesicular lesions, which then evolve into crusts (Fig. 9.8b).

Patients with group A streptococcal impetigo should receive penicillin treatment, particularly when numerous sites of the skin are involved, although treatment may not prevent poststreptococcal glomerulonephritis. Topical treatment with an agent effective against Gram-positive bacteria, such as bacitracin or mupirocin, is also effective.

Bullous impetigo

Bullous impetigo is caused by strains of *Staph. aureus* harboring a group II bacteriophage that contains genetic elements coding for a toxin, which causes cleavage in the epidermis. This results in separation of the cellular planes at the level of the stratum corneum and this is responsible for the superficial flaccid bullae that characterize this condition. Several of these lesions may coalesce and spread to form large reddish plaques, usually involving the neck, face or chin. The superficial flaccid bullae are easily ruptured and may not be apparent at the time when the patient is first seen. Because of the superficial

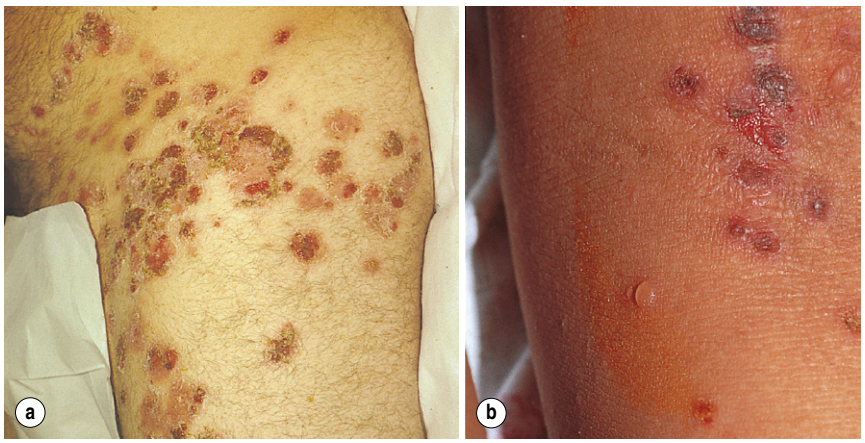


Fig. 9.8 Impetigo. (a) Impetigo in a homeless man. Both *Staphylococcus aureus* and group A streptococci were cultured from these lesions. (b) Impetigo, with initial vesicles changing to crusts.

nature of these infections, scarring does not occur. Appropriate treatment is an antistaphylococcal antibiotic, which may be given orally.

Ecthyma, paronychia and blistering distal dactylitis

Ecthyma, like impetigo, is characterized by dry crusted lesions of the skin and may be caused by *Staph. aureus*, group A streptococci or both. Unlike impetigo, this lesion extends into the dermis and may therefore lead to post-treatment scarring.[9]

Paronychia is an infection between the nail plate of a digit and the cuticle. It is associated with sucking of the fingers and occupations or hobbies involving prolonged immersion of the hands in water. Herpetic whitlow (see below) may resemble paronychia. Paronychia may occur in some immunocompromised patients. Staphylococci are the most common etiologic agents, although oral anaerobes and streptococci may also be isolated. *Candida albicans* and fungi such as *Fusarium* spp. may be isolated from paronychias in immunocompromised patients. Drainage is best accomplished between the nail plate and the cuticle. Antimicrobial agents are rarely needed in otherwise healthy individuals.

Blistering distal dactylitis is characterized by painful blisters on the fingerpads of digits. It is most common in children. *Streptococcus pyogenes* is the most common organism isolated, although *Staph. aureus* can cause a similar lesion. Incision and drainage may be useful, in conjunction with an antibiotic appropriate for *Staph. aureus* or *Strep. pyogenes*.

Erysipelas

Erysipelas is a specific variant of cellulitis caused by a toxin of *Strep. pyogenes,* and occasionally by streptococci of groups B, C and D.[11,12] It is characterized by an abrupt onset of fiery red swelling of the face or extremities. Distinctive features are well-defined margins, particularly along the nasolabial fold, rapid progression and intense pain (Fig. 9.9). Flaccid superficial bullae may develop during the second to third day of the illness, but extension to deeper soft tissues is rare. Since it is a toxic reaction in the skin, cultures of skin biopsies are generally negative.

Surgical debridement is rarely necessary, and treatment with penicillin is effective. Swelling may progress for a time despite appropriate treatment, even while fever, pain and the intense red color are diminishing. Desquamation of the involved skin occurs after 5–10 days.

Erysipelas is most common in elderly adults, and the severity of systemic toxicity can vary from region to region. Local lymphedema (e.g. after mastectomy) predisposes to recurrent erysipelas. It seems to be less common and less severe now than in the past. A rare form of erysipelas is caused by *Campylobacter jejuni* and *Campylobacter fetus*, especially in patients with agammaglobulinemia and occasionally in patients with AIDS.



Fig. 9.9 Erysipelas. This form of cellulitis is caused by *Streptococcus pyogenes* and is most common in the elderly. Unique characteristics include a fiery red or salmon color, well-demarcated edges, desquamation after 5–7 days and location on the face or lower extremities. This picture was taken 48 hours after treatment with penicillin when the brilliant red salmon color had evolved to a reddish blue color. On the second day of treatment patients usually have less pain and fever subsides, but swelling may be more extensive.

Cellulitis

The term 'cellulitis' is commonly used by physicians, but is not well defined in the literature. It is a localized area of soft tissue inflammation characterized by:

- leukocytic infiltration of the dermis;
- capillary dilatation; and
- proliferation of bacteria.

Clinically cellulitis is recognized as an acute inflammatory condition of the skin characterized by localized pain, erythema, swelling and heat.[6] The area of erythema is a paler pink than the flaming red of erysipelas, and has indistinct margins (Fig. 9.10).

Cellulitis caused by *Staphylococcus aureus* and *Streptococcus pyogenes*

Cellulitis is most commonly caused by indigenous flora such as *Staph. aureus* and *Strep. pyogenes* which colonize the skin and appendages. Bacteria may gain access to the epidermis through cracks in the skin, abrasions, cuts, burns, insect bites, surgical incisions and intravenous catheters.

Cellulitis caused by *Staph. aureus* spreads centripetally from a central localized infection such as an abscess (Figs 9.11, 9.12), folliculitis or foreign body (e.g. a sliver, prosthetic device or intravascular catheter).

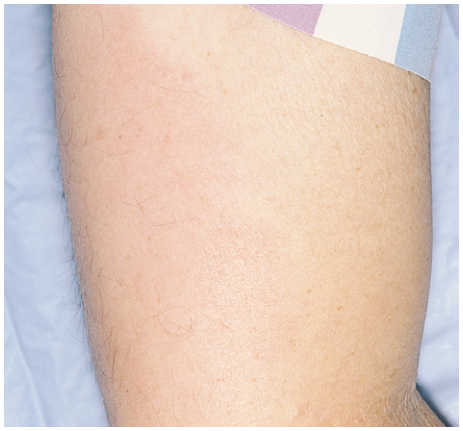


Fig. 9.10 Cellulitis. In contrast to erysipelas, cellulitis is a pink color rather than brilliant red and has indistinct margins. *Staphylococcus aureus* and group A, C and G streptococci are the most common etiologies. Many other bacteria may cause cellulitis (see Table 9.1).

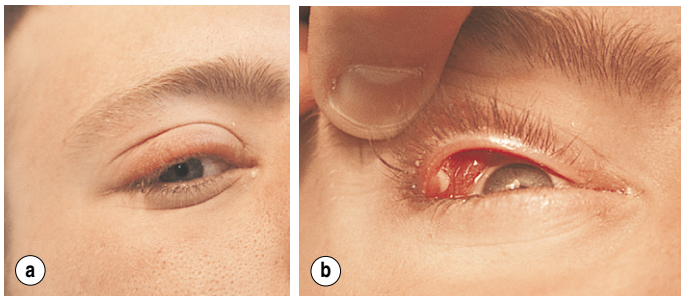


Fig. 9.11 Cellulitis. (a) This case was caused by *Staphylococcus aureus* and is spreading centripetally from a central localized focus of infection. The redness and swelling characteristic of cellulitis are apparent over the upper eyelid. (b) The cellulitis has developed from a localized staphylococcal abscess formed in a meibomian gland (chalazion).



Fig. 9.12 *Staphylococcus aureus* cellulitis of the nose. The focal lesion began in a hair follicle inside the nose, with redness, swelling and pain. Rarely, such lesions on the nose are complicated by extension into the cavernous sinus via veins draining the central part of the face.

In contrast, cellulitis due to *Strep. pyogenes* is a more rapidly spreading diffuse process, frequently associated with lymphangitis (Fig. 9.13) and fever.[13]

Recurrent cellulitis

Recurrent streptococcal cellulitis of the lower extremities may be caused by group A, C or G streptococci in association with skin lesions such as chronic venous stasis (Fig. 9.14), saphenous venectomy for coronary artery bypass surgery[14] or healed burns, especially if the skin is colonized by dermatophyte fungi. Streptococci also cause recurrent cellulitis/erysipelas among patients with chronic lymphedema resulting from irradiation, lymph node dissection, Milroy's disease or elephantiasis.



Fig. 9.13 Lymphangitis. Cellulitis caused by group A streptococci began below the knee and rapidly spread; about 4 hours later lymphangitis had spread up the inner aspect of the thigh.



Fig. 9.14 Cellulitis of the lower leg associated with chronic venous insufficiency. Streptococci of groups A, B, C and G are the most common isolates. Group B streptococci seldom cause cellulitis in previously healthy hosts, but should be considered in people who have peripheral vascular disease or diabetes mellitus.

Recurrent staphylococcal cutaneous infections occur in individuals who have Job's syndrome (see above) and among chronic nasal carriers of staphylococci.

Cellulitis associated with predisposing conditions

A number of other conditions predispose to infection by endogenous or exogenous pathogens (see Table 9.2). For example:

- *Streptococcus agalactiae* cellulitis occurs in patients who have diabetes mellitus or peripheral vascular disease; and[15]
- *Haemophilus influenzae* causes periorbital cellulitis in children in association with sinusitis, otitis media or epiglottitis and will presumably become less common, as has *Haemophilus* meningitis, due to the impressive efficacy of the *H. influenzae* type b vaccine.

Cellulitis associated with bites

Many other species of bacteria can cause cellulitis. These often occur in special settings, and the history can provide useful clues to the diagnosis (see Table 9.2). Bites of various types may introduce specific organisms into the deeper tissues, resulting in soft tissue infections. For example, cellulitis associated with cat bites and, to a lesser degree, dog bites is commonly caused by *Pasteurella multocida*, although in the latter case *Staphylococcus intermedius* and *Capnocytophaga canimorsus* (DF-2) must also be considered. Cellulitis and abscesses associated with dog and human bites also contain a variety of anaerobic organisms.[16] *Pasteurella multocida* is resistant to dicloxacillin and nafcillin, but sensitive to all other β-lactam antimicrobials as well as quinolones, tetracycline and erythromycin. Ampicillin–clavulanate, ampicillin–sulbactam or cefoxitin are good choices for treating animal or human bite infections.

Soft tissue infections may result from the bites of mosquitoes, horse flies and spiders; usually they cause only local allergic reactions with itching, swelling and erythema. Similarly, brown recluse spider bites may resemble acute infection at first, but later there is primary tissue destruction and central necrosis due to the action of dermonecrotic toxins. These infections may resemble pyoderma gangrenosum or may become secondarily infected with skin organisms. Mosquito bites may serve as portals of entry for skin organisms such as *Staph. aureus* or *Strep. pyogenes*. Such infections are not uncommon in clinical practice, but given the number of individuals bitten by insects, infection is a relatively rare complication.

Cellulitis associated with water exposure and exposure to fish

Aeromonas hydrophila causes a highly aggressive form of cellulitis in tissues surrounding lacerations that were sustained in fresh water lakes, rivers and streams. This organism is sensitive to aminoglycosides, fluoroquinolones, chloramphenicol, trimethoprim–sulfamethoxazole (co-trimoxazole) and third-generation cephalosporins, but is resistant to ampicillin.

Vibrio vulnificus can cause cellulitis or necrotizing fasciitis and is associated with swimming in the Gulf of Mexico or South Atlantic Ocean. In addition, patients who have cirrhosis of the liver may develop severe soft tissue infection after ingestion of oysters from these areas.

Streptococcus iniae is a newly recognized cause of cellulitis among workers handling tilapia fish.

Fish food containing the water fleas of the genus *Daphnia* can be contaminated with *Mycobacterium marinum*, which may cause cellulitis or granulomas on skin surfaces exposed to the water in aquariums or following injuries in swimming pools. Rifampin (rifampicin) plus ethambutol has been an effective treatment for some, although no comprehensive studies have been carried out. In addition, some strains of *M. marinum* are susceptible to tetracycline or trimethoprim–sulfamethoxazole.

There are four types of soft tissue cellulitis caused by *P. aeruginosa* and other Gram-negative bacteria:

- ecthyma gangrenosum in neutropenic patients;
- hot tub folliculitis;
- burn wound sepsis; and
- cellulitis following penetrating injury.

In the last of these *P. aeruginosa* is often introduced into the deep tissues by stepping on a nail, a scenario referred to as the 'sweaty tennis shoe syndrome'.

Treatment includes surgical inspection and drainage, particularly if the injury also involves bone or joint capsule. Choices for empiric treatment pending antimicrobial susceptibility data include aminoglycosides, third-generation cephalosporins such as ceftazidime, cefoperazone or cefotaxime, semisynthetic penicillins such as ticarcillin, mezlocillin or piperacillin, or fluoroquinolones. (The quinolones are not approved in children under 13 years of age.)

Cellulitis caused by Gram-negative bacilli, including *P. aeruginosa* as described above, is most common in hospitalized immunocompromised hosts. Recently, *Stenotrophomonas maltophilia* has emerged as an important cause of nosocomial cellulitis in patients who have cancer.[17] The bacterium has been isolated from incubators, nebulizers, humidifiers and tap water in hospitals. The cellulitis may be related to intravenous catheters and in some circumstances may be metastatic via the bloodstream.

Trimethoprim–sulfamethoxazole, or ticarcillin–clavulanic acid, with or without ciprofloxacin are reasonable treatment choices, although cultures and sensitivities are important because of the high prevalence of antibiotic-resistant organisms in the health-care environment.

Other causes of cellulitis

The Gram-positive aerobic rod, *Erysipelothrix rhusiopathiae*, which causes cellulitis in bone renderers and fishmongers, remains susceptible to erythromycin, clindamycin, tetracycline and cephalosporins, but is resistant to sulfonamides and chloramphenicol.

Differential diagnosis

The etiology of cellulitis can be suspected on the basis of the epidemiologic data supplied above. If there is drainage, an open wound or an obvious portal of entry, Gram stain and culture can often provide a definitive diagnosis (Table 9.5). In the absence of these findings, the bacterial etiology of cellulitis may be difficult to establish. Even with needle aspiration from the leading edge or punch biopsy of the cellulitis itself, cultures are positive in only 20% of cases.[18] This suggests that relatively low numbers of bacteria may cause cellulitis and that the expanding area of erythema within the skin may be the direct result of extracellular toxins or the soluble mediators of inflammation elicited by the host.

Antibiotic treatment

Because many different microbes can cause cellulitis, the choice of initial empiric antibiotic therapy depends upon the clinical features described above. Once cultures and sensitivities are available, the choice is easier and more specific. The physician must first decide whether the patient's illness is severe enough to require parenteral treatment, either in hospital or on an outpatient basis. Because of the virtual epidemic of MRSA infections worldwide, severe soft tissue infections should be treated with agents that have a high level activity against these strains. Local antibiograms are thus crucial for rational treatment.

Presumed streptococcal or staphylococcal cellulitis

For presumed streptococcal or staphylococcal cellulitis, nafcillin, cephalothin, cefuroxime, vancomycin and erythromycin are good choices. Cefazolin and ceftriaxone have less activity against *Staph. aureus* than cephalothin, although clinical trials have shown a high degree of efficacy. Ceftriaxone is a useful choice for outpatient treatment because it can be given once daily. Similarly, teicoplanin, like vancomycin, has excellent activity against *Strep. pyogenes* and both *Staph. aureus* and *Staph. epidermidis* and may be given once daily by intravenous or intramuscular injection. Because MRSA has recently increased in prevalence throughout much of the world, vancomycin, tigecycline, daptomycin or linezolid should be used empirically in patients with severe soft tissue infections who are toxic or in those who have recently been hospitalized or received antibiotics.[19]

For patients being treated with oral antibiotics, dicloxacillin, cefuroxime axetil, cefpodoxime, erythromycin, clarithromycin and azithromycin are all effective treatments.

For known group A, B, C or G streptococcal infections, penicillin or erythromycin should be used orally or parenterally. For serious group A streptococcal infections such as necrotizing fasciitis or streptococcal toxic shock syndrome, clindamycin is more efficacious than penicillin.[20]

Table 9.5 Differential diagnosis of ulcerative skin lesions

Clinical condition	Etiology
Anthrax	*Bacillus anthracis*
Cutaneous diphtheria	*Corynebacterium diphtheriae*
Ulceroglandular tularemia	*Francisella tularensis*
Bubonic plague	*Yersinia pestis*
Buruli ulcer	*Mycobacterium ulcerans*
Primary syphilis	*Treponema pallidum*
Chancroid	*Haemophilus ducreyi*
Lucio's phenomenon	*Mycobacterium leprae*
Decubitus (pressure) ulcer	Mixed aerobic and anaerobic bacteria
Leishmaniasis	*Leishmania tropica*
Ecthyma gangrenosum	*Pseudomonas aeruginosa*
Tropical ulcer	Idiopathic and nonspecific; mixed bacterial species

This is probably because in this type of infection where there are large numbers of bacteria, streptococci are in a stationary growth phase and do not express a full complement of penicillin-binding proteins.[21] In contrast, the activity of clindamycin is not affected by inoculum size or growth phase. In addition, clindamycin suppresses the synthesis of many streptococcal exotoxins and surface proteins.[22,23]

Other types of cellulitis

For cellulitis associated with *Eikenella corrodens* useful antibiotics are penicillin, ceftriaxone, trimethoprim–sulfamethoxazole, tetracyclines and fluoroquinolones. Interestingly, this organism is resistant to oxacillin, cefazolin, clindamycin and erythromycin.

Cellulitis associated with cat bites may fail to respond to treatment with oral cephalosporins, erythromycins and dicloxacillin. Reasons for failure include resistance of *P. multocida* to oxacillin and dicloxacillin and the inadequate serum and tissue levels attained with older oral cephalosporins and erythromycins.

Erysipelas-like skin lesions caused by *Campylobacter* spp. in patients with agammaglobulinemia may not respond to antibiotics alone; supplementation with IgM (by infusion of fresh plasma) may be necessary to obtain bactericidal serum.[24]

Cutaneous ulcers

Infectious ulceration of the skin results from either:

- direct destruction of dermal cells by bacterial products; or
- an intense inflammatory reaction.

Cutaneous anthrax

This is an example of direct destruction of dermal cells by toxins produced by *Bacillus anthracis* (see Table 9.5). This disease is traditionally contracted by direct inoculation of the skin of animal handlers, especially goat and sheep herders or hide processors,[25] but recent cases have occurred as the result of deliberate bioterrorism (see Chapter 71). The lesion begins as a papule, which evolves into a bulla and then ulcerates (Fig. 9.15). Sepsis may occur. The diagnosis is established by aspiration of the leading edge of the lesion, Gram stain and culture. Penicillin is appropriate therapy.

Cutaneous diphtheria

Since 1980 cutaneous diphtheria has been recognized in homeless individuals who present with chronic nonhealing ulcers with an overlying dirty gray membrane. These lesions may mimic those of psoriasis, eczema or impetigo, but have a deeper base. Appropriate cultures of the ulcer are mandatory because organisms growing from routine cultures may be misidentified as diphtheroids.[26]

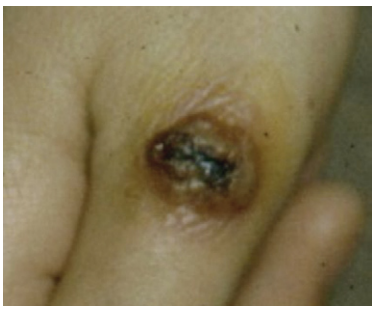


Fig. 9.15 Cutaneous anthrax lesion on the thumb of a 23-year-old Turkish woman. The lesion developed after wounding herself on the teeth of a slaughtered sheep. Microbiologic tests yielded *Bacillus anthracis*. She recovered after treatment with penicillin. Courtesy of IC Gyssens and D Weyns.

Cutaneous tularemia (ulceroglandular tularemia)

Cutaneous tularemia occurs following a tick bite or handling of infected rodents or lagomorphs (rabbits). It most commonly presents with regional lymphadenopathy associated with suppuration and fever, although pneumonic, oculoglandular, oropharyngeal and typhoidal forms have also been described. The characteristic lesion is a small ulceration with an eschar, which develops 2–10 days after exposure. Treatment with streptomycin or gentamicin has been successful, and doxycycline, chloramphenicol or a fluoroquinolone are alternatives.

Buruli ulcer

Buruli ulcer is caused by *Mycobacterium ulcerans*. It presents as a shallow ulcer, which slowly expands centripetally. It is uncommon in the USA and Europe, but is endemic in tropical climates, particularly Africa. Diagnosis is easily established by biopsy, acid-fast staining or culture. The organism is susceptible to isoniazid, rifampin and *para*-aminosalicylic acid (PAS). Oral treatment with isoniazid and rifampin for 2–3 months is usually successful.

Leishmaniasis

Leishmaniasis also presents as shallow ulcers with an expanding margin (Fig. 9.16). Diagnosis should be suspected in patients residing in or returning from Central or South America. A biopsy from the raised edge stained with Giemsa or Wright's stain demonstrates the amastigote stage of *Leishmania tropica*. Treatment with antimony compounds is effective, but requires prolonged administration over 4–6 months.

Other causes of cutaneous ulcers

The differential diagnosis of cutaneous ulcers in genital areas should include:

- syphilis;
- chancroid;
- lymphogranuloma venereum; and
- herpes simplex virus infection.

Noninfectious causes of cutaneous ulceration include:

- Behçet's syndrome;
- cutaneous vasculitis, including lupus erythematosus;
- toxic epidermal necrolysis;
- pressure necrosis; and
- brown recluse spider bites.

Solitary shallow ulcers of skin and mucous membranes have also been described in disseminated histoplasmosis.



Fig. 9.16 Leishmaniasis. Typical crusted edge with central necrosis suggesting leishmaniasis. A history of travel to Central or South America or Iraq should be ascertained. Biopsy of the ulcer crater with Giemsa staining revealed amastigotes of *Leishmania tropica*.Courtesy of M Keuter.

Herpes simplex can cause primary or recurrent cutaneous infections of the digits (herpetic whitlow) or head and neck. This viral infection is often misdiagnosed and mistreated as a bacterial condition, and occurs in those who are exposed to inoculation of the skin from oral secretions, such as from dentists, dental hygienists, nurses, anesthesiologists and wrestlers.

Orf is caused by a DNA virus similar to smallpox. It causes development of shallow ulcers (in general only one lesion) on the digits of animal handlers working with sheep or goats that harbor open mucous membrane lesions.[25]

Bacillary angiomatosis (see also Chapter 96)

Bacillary angiomatosis is a primary infection of endothelial cells that has important cutaneous manifestations.[27] The lesions may appear as purple nodules resembling Kaposi's sarcoma. They may also appear as scaly or ulcerated lesions and may have the appearance of superficial pink papules or plaques in black people. This disease usually occurs in people who have HIV infection and is caused by *Bartonella henselae* or *B. quintana*. The organisms can be acquired from cat bites and scratches or from cat fleas. The course and extent of infection is highly variable and depends upon the host's immune status.

Histopathology reveals capillary proliferation. The organisms can be visualized using Warthin–Starry silver stain or electron microscopy. Bacteriologic identification requires a special culture technique: lysed blood centrifugate or digested tissue is plated onto chocolate or Columbia agar and incubated for 10–14 days at 95°F (35°C) in 5–7% carbon dioxide. Small dry adherent oxidase-negative colonies of Gram-negative curved rods with twitching motility can be identified as *Bartonella* spp. by fluorescent antibody, gas–liquid chromatography or biochemical tests.[28]

Resistance to penicillin, cephalosporins, sulfonamides and vancomycin has been described. The recommended therapy is erythromycin 500 mg q6h.[23]

Cutaneous manifestations of infections of deep soft tissues

Staphylococcal infections of deeper tissues may also cause superficial redness, warmth and swelling of the skin, even though the skin itself is not infected (Fig. 9.17). Examples include olecranon bursitis (Fig. 9.18), septic arthritis, osteomyelitis, staphylococcal parotitis and other deep infections of the head and neck, such as anaerobic infections, actinomycosis and tooth abscesses (Fig. 9.19).

Further information regarding the diagnosis and treatment of common and uncommon skin and soft tissue infections, including those in compromised hosts can be found in 'Practice guidelines for diagnosis and management of skin and soft-tissue infections'.[29]

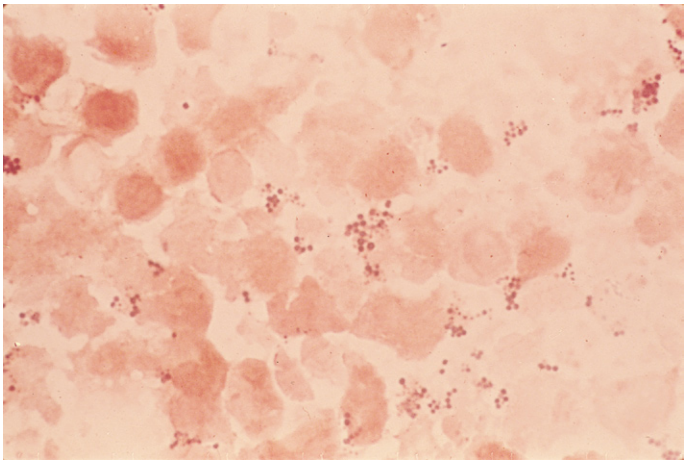


Fig. 9.17 Gram stain of purulent material demonstrating *Staphylococcus aureus*. The microbial etiology of cellulitis may be suspected based upon signs, symptoms and history; however, definitive diagnosis requires Gram stain and culture. If there is no portal of entry, aspiration or even punch biopsy of cellulitic skin yields a positive culture in only 20% of cases.

REFERENCES

References for this chapter can be found online at http://www.expertconsult.com

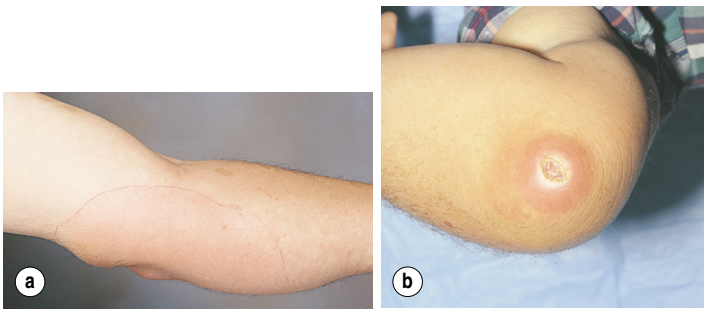


Fig. 9.18 Cellulitis at the elbow associated with olecranon bursitis. (a) Pale pink erythema on the inner aspect of the elbow. (b) Careful inspection demonstrates a focal infection over the point of the elbow. Fluid aspirated from the olecranon bursa yielded a pure culture of *Staphylococcus aureus*.

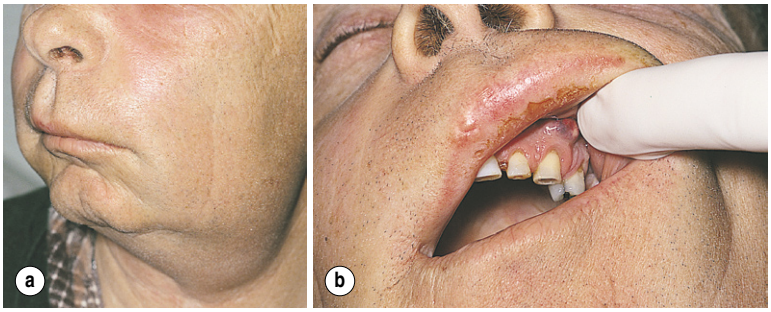


Fig. 9.19 Erythema and swelling of the face due to a tooth abscess. (a) Swelling of the face, on inspection resembling periorbital cellulitis. (b) Further inspection reveals a gingival abscess above the patient's left upper canine tooth.

Chapter 10

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Necrotizing fasciitis, gas gangrene, myositis and myonecrosis

INTRODUCTION

The spectrum of infections of the deep soft tissues ranges from localized bacterial, viral and parasitic lesions to rapidly spreading, tissue-destructive infections such as necrotizing fasciitis and myonecrosis. For example, pyomyositis, which is common in the tropics but rare in temperate zones, is a focal infection of skeletal muscle that is usually caused by *Staphylococcus aureus*; it generally remains localized and rarely causes systemic complications. In contrast, necrotizing fasciitis and myonecrosis may be caused by single or multiple pathogens and often give rise to extensive tissue loss, bacteremia, organ failure, shock and death. Even the experienced clinician may have difficulty distinguishing between the different forms of deep soft tissue infection during the early stages. Finally, despite early diagnosis and appropriate treatment, some patients will lose tissue, even limbs, whereas others will succumb to systemic complications. This chapter emphasizes the clinical clues that help to make early, specific diagnoses.

EPIDEMIOLOGY

Until the middle of the 20th century, wartime injuries were commonly complicated by gas gangrene caused by *Clostridium* spp. During the Civil War in the USA, nearly 50% of soldiers who sustained gunshot wounds developed infection and many of these developed gas gangrene. Clostridial gangrene is typically a sporadic infection but during the Civil War apparent epidemics of 'hospital gangrene' were described. Contributing factors included severe trauma, grossly contaminated wounds, crowded and dirty conditions, application of soiled dressings (often recycled from patients who had just died of infection) and primitive surgical techniques for debridement and fixation of open fractures. Group A streptococci undoubtedly caused some of these infections but other major bacterial pathogens, including *Clostridium perfringens*, Gram-negative bacteria and mixed aerobic–anaerobic bacteria, also contributed.

Gas gangrene was also common during the First World War, particularly in the European theater, where the soil was rich and well fertilized with animal feces containing large numbers of vegetative spores of clostridia. In contrast, in North Africa, cases of gangrene following gunshot wounds were far less common, presumably because the desert sand contained few clostridial spores.[1] Gas gangrene has become uncommon in modern warfare because wounded soldiers are evacuated rapidly to well-equipped hospitals for surgical intervention, arterial reconstruction and antibiotic treatment, all of which have greatly reduced the prevalence of this feared disease.

In modern times, these serious deep soft tissue infections have become less common. Sporadic cases in the general population most often occur as occasional complications of penetrating trauma, compound fractures or septic abortions. For the first time in history, spontaneous gas gangrene caused by *Clostridium septicum* may be more common than trauma-associated gas gangrene caused by *C. perfringens, C. histolyticum* or other *Clostridium* spp. (see Chapter 173). Recently, severe soft tissue infections caused by *C. perfringens, C. sordellii* and *C. novyi* have been described among intradermal ('skin popping') and intravenous drug users.[2,3]

Necrotizing fasciitis is a life-threatening form of soft tissue infection. It can occur in association with gas gangrene as a part of generalized tissue necrosis or as a separate clinical entity.[4] Two types of necrotizing fasciitis are recognized. Type I occurs in patients who have diabetes mellitus or severe peripheral vascular disease, or both;[5] it is usually caused by mixed aerobic and anaerobic bacteria. Although the risk for an individual diabetic patient is low, this type of necrotizing fasciitis is most common in the general population, because the total number of people who have diabetes is very large.

Type II necrotizing fasciitis, formerly called streptococcal gangrene, is caused by group A streptococci. Since the mid-1980s, this disease has been recognized with increasing frequency in many parts of the world, at a current annual incidence of 3–3.5 cases per 100 000.[6]

Morbidity and mortality

Before the availability of antibiotics, gas gangrene was usually fatal. Since then, mortality rates from gas gangrene caused by *C. perfringens* have improved, owing to aggressive antibiotic therapy, aggressive surgical therapy employing better surgical techniques and, possibly, hyperbaric oxygen therapy. The most important factors in improving outcome, reducing the need for amputations and preventing shock have been earlier recognition and aggressive treatment.

The mortality and morbidity of group A streptococcal necrotizing fasciitis have evolved differently. In the pre-antibiotic era this infection carried a mortality rate of about 25% when treated with surgery (such as 'bear claw' fasciotomies) alone.[7] In modern times, mortality due to group A streptococcal necrotizing fasciitis has not decreased and continues to range from 30% to 70% despite antibiotics, appropriate surgical debridement and intensive supportive care. This suggests that more virulent strains have emerged.

CLINICAL FEATURES

Pain, either generalized or localized, is the most common reason patients with deep-seated infection seek medical care (Fig. 10.1). Early in the course of necrotizing fasciitis caused by group A streptococci, patients may have a viral-like prodrome with nausea, vomiting, diarrhea and fever; however, later in the course of the disease, patients seek medical assistance because of increasingly severe localized pain with continuing fever.

Differential diagnosis of infections involving muscle and fascia

Clinical feature	Necrotizing fasciitis type I	Necrotizing fasciitis type II	Gas gangrene	Pyomyositis	Myositis due to viruses or parasites
Fever					
Diffuse pain					Note 1
Localized pain		Note 2			
Systemic toxicity					
Gas in tissue					
Obvious portal of entry		Note 3	Note 4		
Diabetes mellitus					

Note 1: Pain with influenza is diffuse myalgia. Pleurodynia may be associated with severe localized pain (i.e. devil's grip). Pain with trichinosis may be severe and localized.
Note 2: Severe pain is present in necrotizing fasciitis associated with group A streptococcal necrotizing fasciitis. Necrotizing fasciitis type I is commonly seen in people who have diabetes mellitus who have peripheral neuropathy; hence the pain may not be severe.
Note 3: Fifty percent of patients who have necrotizing fasciitis caused by group A streptococci may not have an obvious portal of entry.
Note 4: Gas gangrene associated with trauma may be caused by *Clostridium perfringens*, *Clostridium septicum* and *Clostridium histolyticum* and there is always an obvious portal of entry. Spontaneous gas gangrene caused by *Clostridium septicum* is usually not associated with an obvious portal of entry. Organisms lodge in tissue as a result of bacteremia originating from a bowel portal of entry.

Fig. 10.1 Differential diagnosis of infections involving muscle and fascia. Red, severe; orange, moderate; yellow, mild-to-moderate; blue, mild; white, none.

A portal of entry can be defined in the majority of cases of deep bacterial soft tissue infections such as type I necrotizing fasciitis and traumatic gas gangrene. In type I necrotizing fasciitis, infection begins at the site of a surgical incision, at a mucosal tear or at sites of skin breakdown in patients who have diabetes mellitus or peripheral vascular disease. Similarly, traumatic gas gangrene occurs at the site of major trauma such as crush or penetrating injuries severe enough to cause arterial damage. In these cases, the clinician has reason to suspect infection as a cause of fever or increasing pain. In contrast, patients who have either type II necrotizing fasciitis or spontaneous gas gangrene may have no apparent portal of entry.[8] Early in the course of infection in such patients, the only physical signs of infection may be fever and localized pain. Fever and localized pain are the cardinal clues to this diagnosis but in some cases clinical evidence of localized infection may not become apparent until after the development of systemic signs such as hypotension or organ failure.

Early in the course of type II necrotizing fasciitis associated with a defined portal of entry there is generally evidence of localized inflammation such as swelling, redness, warmth and tenderness. At that point the process may resemble simple cellulitis, which can be caused by any of a multitude of bacteria (see Chapter 9). In type I necrotizing fasciitis there is generally gas in the tissue, as there is in gas gangrene. Where there is no apparent portal of entry, the leading diagnoses to be considered are infection with group A streptococci (type II necrotizing fasciitis), *C. septicum* (spontaneous gangrene) or *Vibrio vulnificus*. The presence of gas in the tissue favors clostridial infection.

Gas may be detected by physical examination (crepitus) or by imaging (radiography, MRI or CT scan). Group A streptococci should be suspected if there is fever and severe pain and a history of blunt trauma or muscle strain. *Vibrio vulnificus* should be suspected in a patient who has cirrhosis of the liver, a history of ingestion of raw oysters or exposure to seawater.[9] Later, erythema is superseded by violaceous bullous lesions, massive local swelling becomes apparent and signs of systemic toxicity develop rapidly (see Chapter 171).

SPECIFIC TYPES OF DEEP SOFT TISSUE INFECTION

Necrotizing infections

Necrotizing infections of the skin and underlying soft tissues share the features of fulminant destruction of tissue and severe systemic signs of toxicity associated with high mortality (see Fig 10.1). Many different and confusing names have been used to describe infectious processes that share common pathologic features:

- extensive tissue destruction;
- thrombosis of blood vessels;
- abundant bacteria spreading along fascial planes; and
- relatively few acute inflammatory cells, although small collections of polymorphonuclear leukocytes or microabscesses have been described (Fig. 10.2).

For patients who have evidence of an aggressive localized soft tissue infection, prompt surgical exploration of that site is of extreme importance to determine whether a necrotizing process is present. The same is true for patients who have milder local features associated with severe systemic toxicity. In addition, although the clinical entity referred to as necrotizing fasciitis may occur alone, there is commonly also evidence of necrosis extending up to the dermis and down to underlying muscle (myonecrosis). Despite these common features, it is worth reviewing the many different types of necrotizing soft tissue infections that have been described in the literature because this may point to clinical clues leading to earlier surgical intervention and therefore an earlier diagnosis.

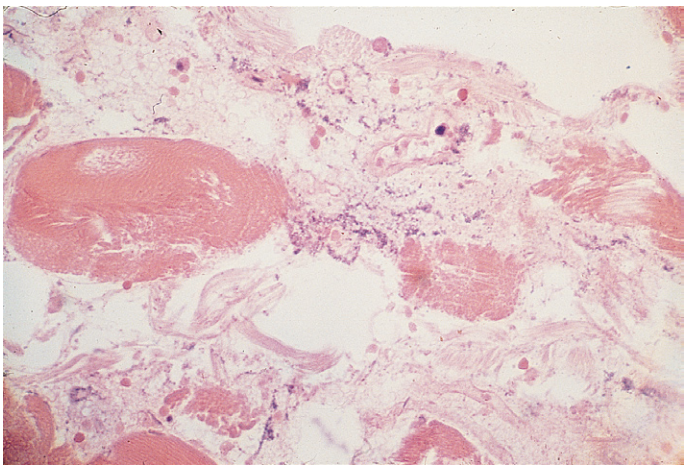


Fig. 10.2 Histopathologic examination of tissue from a patient who has necrotizing fasciitis with extension into the underlying musculature. Note the absence of acute inflammatory cells in the area of muscle necrosis. When present, infiltrating granulocytes can be seen at the interface between normal and necrotic tissue and are often massed within small postcapillary venules.

Necrotizing fasciitis

Necrotizing fasciitis is a deep-seated infection of the subcutaneous tissue that results in progressive destruction of fascia and fat, although it may spare the skin itself.[10] Two clinical types exist.

Type I necrotizing fasciitis

Type I necrotizing fasciitis is a mixed infection caused by aerobic and anaerobic bacteria. It occurs most commonly after surgical procedures, in diabetic patients or in those who have peripheral vascular disease (see Fig. 10.1). Nonclostridial anaerobic cellulitis and synergistic necrotizing cellulitis are both variants of the same syndrome. It may not be important to distinguish these entities from one another because all occur in diabetic patients and are caused by mixed anaerobic and aerobic bacteria.

Clinical features

These infections most commonly occur on or about the feet, with rapid extension along the fascia into the leg. Although cellulitis also occurs commonly in diabetic patients, necrotizing fasciitis should be considered in those who have cellulitis and systemic signs of infection such as tachycardia, leukocytosis, acidosis or marked hyperglycemia. In addition to its spontaneous occurrence in diabetic patients, type I necrotizing fasciitis may also develop as a result of a breach in the integrity of mucous membranes from surgery or instrumentation. In the head and neck region, bacterial penetration into the fascial compartments can result in a related syndrome known as Ludwig's angina (Fig. 10.3) or it may develop into necrotizing fasciitis. Group A streptococci (but also anaerobes such as *Fusobacterium necrophorum*) may cause necrotizing fasciitis or a peritonsillar abscess, which can extend into the deep structures of the neck (Fig. 10.4).

Diagnostic tests

The first goal of management is to determine the depth and extent of the infection. MRI or CT scans are invaluable in this regard to determine whether the infection is localized or spreading along fascial planes. The second goal is to determine whether surgical intervention is necessary. Because of the proximity to vital structures of the neck, surgical consultation is of major importance because exploration, drainage and debridement may be necessary to prevent airway obstruction, to determine the level of soft tissue involvement and to establish which bacteria are involved.



Fig. 10.3 Ludwig's angina. Infection begins with a break in the mucosal lining in the oropharynx; oral bacterial flora invade the soft tissues at the base of the tongue and penetrate through the floor of the mouth and into soft tissue of the neck. The floor of the mouth is elevated and patients talk as though they have a 'hot potato' in their mouth. Potential airway obstruction is a major concern. Although patients usually respond to penicillin, surgical consultation should be obtained and CT or MRI scans are useful for determining whether a necrotizing process is present.

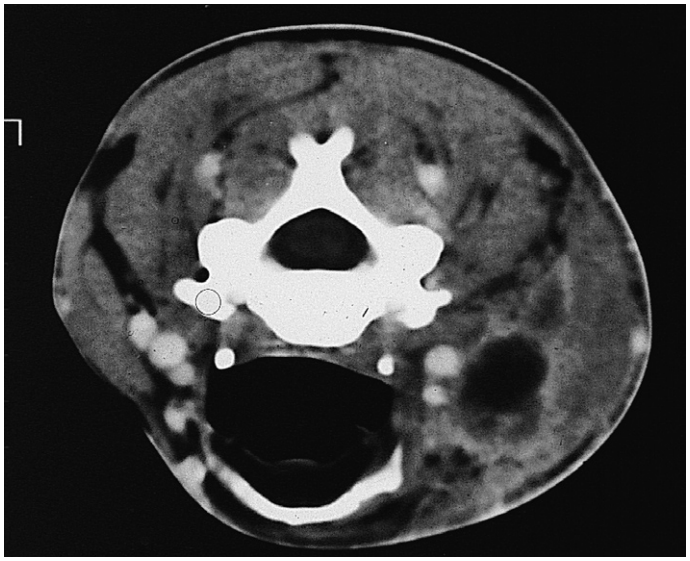


Fig. 10.4 CT scan of a soft tissue infection of the neck. This infection is caused by group A streptococci, which invaded as a rare complication of a previous 'strep throat'. Surgical drainage yielded a pure culture of group A streptococci and established a diagnosis. The patient was treated with intravenous penicillin for 10 days and made a good recovery.

Treatment

Both Ludwig's angina and necrotizing fasciitis of the head and neck are usually caused by mouth anaerobes such as *Fusobacterium* spp., anaerobic streptococci, *Bacteroides* spp. and spirochetes. Either penicillin or clindamycin is effective treatment, largely because the Gram-positive aerobic cocci and anaerobes of the oropharynx are generally susceptible to both. In contrast, type I necrotizing fasciitis below the diaphragm requires ampicillin plus clindamycin and a fluoroquinolone to cover the *Bacteroides* spp. and Enterobacteriaceae.

Type II necrotizing fasciitis

Type II necrotizing fasciitis is caused by group A streptococci and was previously called streptococcal gangrene.[5] In recent years, there has been a dramatic increase in the number of invasive infections, including necrotizing fasciitis, caused by group A streptococci. In contrast to type I necrotizing fasciitis, type II may occur in any age group and among patients who do not have complicated medical illnesses. Predisposing factors include:

- a history of blunt trauma;
- muscle strain;
- childbirth;
- chickenpox;
- nonsteroidal anti-inflammatory agents;[11]
- intravenous drug abuse; or
- penetrating injury such as caused by a laceration or a surgical procedure.

In penetrating injuries, it is the skin rather than the mucous membranes that serves as the portal of entry for the streptococci. In contrast, among patients who do not have a defined portal of entry, hematogenous translocation of group A streptococci from the throat (asymptomatic or symptomatic pharyngitis) to the site of blunt trauma or muscle strain probably occurs.[12] Another, highly conjectural possibility is that group A streptococci reside in a dormant state in the deep tissue and trauma of various types reactivates their growth.

Group A streptococci are contagious microbes that have caused epidemics of pharyngitis and scarlet fever in schools, rheumatic fever in military recruits and surgical wound infections in hospitalized patients. Thus, close contacts of a patient who has type II necrotizing fasciitis have a high likelihood of becoming colonized with a virulent strain.

Clearly, the risk of developing a secondary case of fulminant necrotizing fasciitis is very low, but it is probably 50-fold higher than it is in the general population. In evaluating the risk to family members and hospital workers, the physician should consider the degree of exposure and the susceptibility of the host. Those contacts with conditions such as open wounds or chickenpox, as well as those family members and health-care workers with frequent or continuous contact with a case of necrotizing streptococcal infection, should be treated with an agent to which the strain is sensitive (e.g. penicillin).

Pathogenesis

Pyrogenic exotoxins can bind simultaneously to the major histocompatibility complex (MHC) class II portion of antigen-presenting cells, and specific Vβ segments of the T-cell receptor in the absence of classic antigen processing.[13] Thus, pyrogenic exotoxins are superantigens that can cause rapid proliferation of T cells bearing specific Vβ repertoires (see Chapter 2). This is associated with production of both monokines [tumor necrosis factor (TNF), interleukin (IL)-1 and IL-6] and lymphokines (IL-2, interferon and TNF).[14] Production of these cytokines *in vivo* probably contributes to shock, organ failure and tissue destruction.[15]

Clinical features

Necrotizing fasciitis exhibits a remarkably rapid progression from an inapparent process to one associated with extensive destruction of tissue, systemic toxicity, loss of limb or death.[8,12,16] Unexplained pain that increases rapidly over time may be the first manifestation of infection.[8,12] The early signs and symptoms of infection may not be apparent, particularly in patients who have postsurgical infection, gunshot or knife wounds, or diabetes. In patients who have diabetes, the absence of pain may be related to neuropathy and anesthesia at the site of infection. In surgical patients, patients who have traumatic injuries and postpartum patients, the increasing pain may be assumed to be part of the normal convalescence rather than to be due to acute infection. Such a delay in diagnosis may allow the disease to progress to later stages before appropriate antibiotics and surgical invention are initiated.

In addition to pain, there may also be fever, malaise, myalgias, diarrhea and anorexia during the first 24 hours; erythema, which may be diffuse or local, may also be present. However, in most patients excruciating pain in the absence of any cutaneous findings may be the only clue of infection. Within 24–48 hours, erythema may develop or darken to a reddish-purple color, frequently with associated blisters and bullae. Conversely, erythema may be absent and the characteristic bullae may develop in skin of normal appearance. The bullae are initially filled with clear fluid and rapidly take on a blue or maroon appearance (Fig. 10.5). When the bullous stage is observed, there is already extensive necrotizing fasciitis (Fig. 10.5) and patients usually exhibit fever and systemic toxicity.

Although many different M-types of group A streptococci have been associated with necrotizing fasciitis in the past, M types 1 and 3 have been the strains most commonly isolated from patients throughout the world.[12] These strains can produce one or more of the pyrogenic exotoxins A, B and C.[8,17] Necrotizing fasciitis caused by these strains is frequently associated with 'streptococcal toxic-shock syndrome'.[8] The hallmarks of this syndrome are the early onset of shock and multiple organ failure (see Chapter 166).

Diagnosis

Laboratory tests such as creatine phosphokinase, aspartate aminotransferase and serum creatinine are usually elevated, and, together with leukocytosis with marked left shift, these findings should be sufficient to prompt surgical exploration.[8] Some experts have advocated punch biopsy and frozen section to establish the diagnosis; however, there may be false-negative findings if the deep tissue is not adequately sampled. Routine soft tissue radiographs, MRI and CT scans show soft tissue swelling.[18] Gas is not present and abscess formation is not apparent. These radiographic abnormalities are not unusual in uninfected patients who have trauma or in postsurgical or postpartum patients (Fig. 10.6). Surgeons may not be interested in surgical exploration with such imaging findings, yet a toxic patient with the above laboratory abnormalities should provide the impetus to at least inspect the deep tissue. Direct surgical exploration will usually demonstrate that necrotizing fasciitis is present. At the same time, thorough debridement of necrotic tissue and suitable material for Gram stain and culture can be obtained.

Management

The three main themes in treatment are surgical debridement, appropriate antibiotics and intensive supportive care. Some patients require mechanical ventilation and others need hemodialysis. Because of intractable hypotension and diffuse capillary leak, massive amounts of intravenous fluids (10–20 liters per day) are often necessary, although anasarca is a common complication. In some patients blood pressure

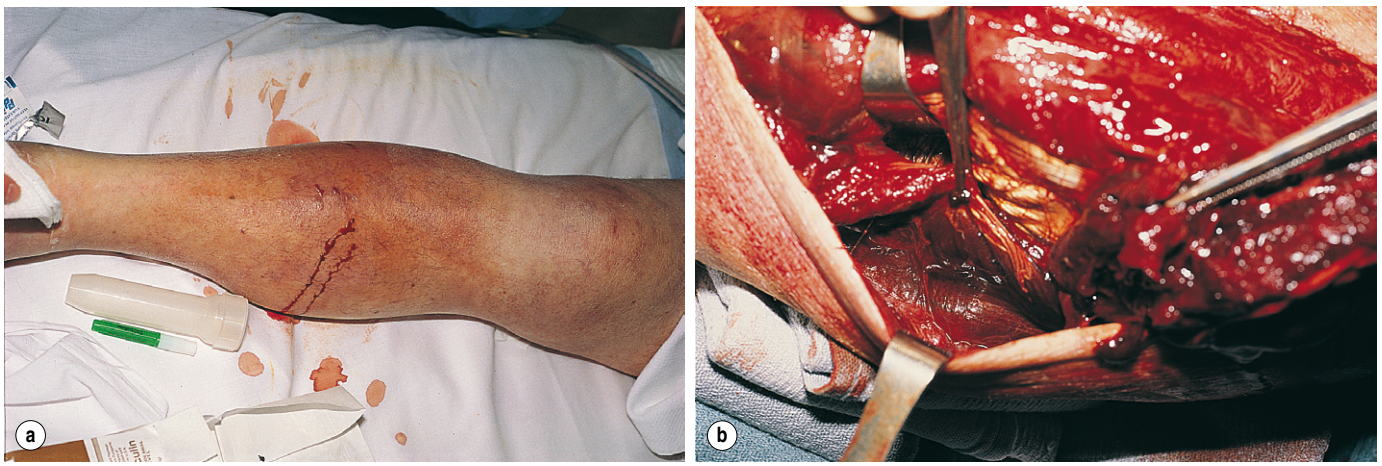


Fig. 10.5 Type II necrotizing fasciitis caused by group A streptococci. (a) This patient was a 60-year-old man who had type 2 diabetes mellitus and who had a 3-day history of malaise, diffuse myalgia and low-grade fever. Over the course of 2–3 hours the pain became excruciating and was localized to the calf. During this time the calf swelled. Note that the skin over the anterior shin looks relatively normal, but that two small purple bullae are present. (b) Extensive necrotizing fasciitis was present on surgical exploration. In addition, myonecrosis was present beneath the fascia. The patient developed profound hypotension, acute respiratory distress syndrome and renal failure. He died despite aggressive surgical and medical management. There was no definable portal of entry, yet group A streptococci were grown from deep cultures and from blood.



Fig. 10.6 Postpartum sepsis due to group A streptococci. The patient was a 24-year-old woman who delivered a normal child. Thirty-six hours after delivery she developed fever, leukocytosis with marked left shift and increasing low abdominal pain. This MRI demonstrates swelling of the uterus, although not out of proportion for a recent delivery. There was no gas in the tissue. An emergency laparotomy revealed necrosis of the mucosa of the uterus, necrotizing fasciitis and myonecrosis of the uterus.

improves with intravenous fluid alone. Pressors such as dopamine may be useful, but there is little controlled information from clinical or experimental studies in this specific infection. Although potent vasoconstrictors such as adrenaline (epinephrine) may improve blood pressure, symmetric gangrene may ensue, partly as a result of the drug and partly as a result of poor perfusion caused by the bacteria, toxins and endogenous mediators.

Antibiotic selection is difficult in patients who have rapidly progressing infection. Recent studies suggest that clindamycin is superior to penicillin for treatment of experimental necrotizing fasciitis or myonecrosis caused by group A streptococci.[19] It seems likely that penicillin failure is due to the reduced expression of critical penicillin-binding proteins during the stationary phase of bacterial growth.[20] Clindamycin may be more efficacious because:

- it is not affected by inoculum size or stage of growth;
- it suppresses toxin production;
- it facilitates phagocytosis of *Streptococcus pyogenes* by inhibiting M-protein synthesis;
- it suppresses the production of regulatory elements that control cell wall synthesis; and
- it has a long post-antibiotic effect.[21]

Neutralization of circulating streptococcal toxins is a desirable therapeutic goal and is advocated by some experts.[22–25] There seems little question that some batches of intravenous gammaglobulin contain neutralizing antibodies against some streptococcal toxins.[24] On the basis of two case reports[22,23] and a report of a nonrandomized clinical trial, there is a suggestion that this treatment may affect the mortality and morbidity of this fulminant infection.[25]

Fournier's gangrene

In the perineal area, penetration of the gastrointestinal or urethral mucosa by bacteria may cause 'Fournier's gangrene', an aggressive infection caused by aerobic Gram-negative bacteria, enterococci and anaerobic bacteria such as *Bacteroides* spp. and peptostreptococci. These infections begin abruptly with severe pain and may spread rapidly to the anterior abdominal wall and the gluteal muscles; in males, infection frequently extends to the scrotum and penis (Fig. 10.7).

Surgical inspection, placement of drains and appropriate surgical debridement are necessary for both diagnosis and treatment. Antibiotic treatment should be based upon Gram stain, culture and sensitivity information when available. An appropriate empiric regimen would be either ampicillin or ampicillin and sulbactam combined with either clindamycin or metronidazole. Broader Gram-negative coverage might be advisable if the patient has had prior hospitalization or if antibiotics have been used recently. This could be accomplished by substituting ticarcillin–clavulanic acid or piperacillin–tazobactam for ampicillin or by adding a fluorinated quinolone or an aminoglycoside.

Meleney's synergistic gangrene

This rare variant occurs in postsurgical patients. The lesion is a slowly expanding, indolent ulceration that is confined to the superficial fascia. It results from a synergistic interaction between *Staph. aureus* and microaerophilic streptococci. As in other forms of necrotizing infection, antibiotic therapy, together with surgical debridement, are the mainstays of treatment.

Nonclostridial anaerobic cellulitis

In nonclostridial anaerobic cellulitis, infection is associated with mixed anaerobic and aerobic organisms that produce gas in tissues. Unlike clostridial cellulitis, this type of infection is usually associated with diabetes mellitus and it often produces a foul odor. Surgical exploration is required to distinguish this condition from necrotizing cellulitis, myonecrosis and necrotizing fasciitis by *Clostridium* spp.

Clostridial cellulitis

In clostridial cellulitis, infection is usually preceded by local trauma or recent surgery. *Clostridium perfringens* is the most common species causing this entity. Gas is invariably found in the skin; the fascia and deep muscle are spared. Although clostridial cellulitis differs from

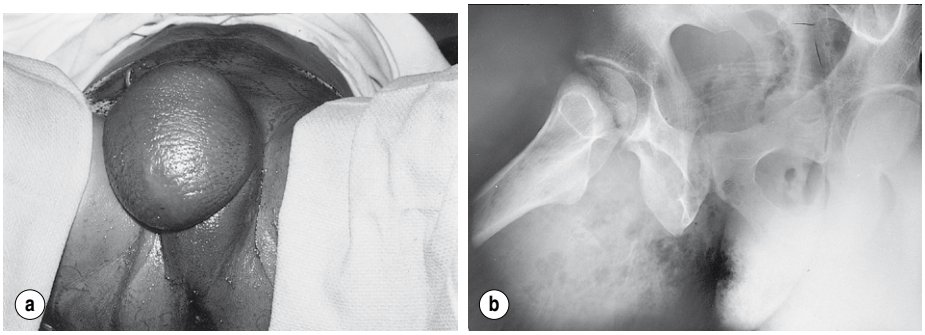


Fig. 10.7 Type I necrotizing fasciitis. A 24-year-old man had been in good health but was awakened with severe perineal pain. (a) This photograph was taken 3 hours later. Note the massive swelling of the scrotum. (b) Soft tissue radiograph shows gas in the tissues of the thigh, buttocks, scrotum and anterior abdominal wall. Surgical inspection revealed brownish fluid in the scrotum, with gray, dull-colored, friable fascia but normal underlying musculature. Cultures grew *Enterococcus faecalis, Bacteroides fragilis, Escherichia coli* and anaerobic streptococci. The patient was treated with ampicillin, clindamycin and gentamicin for 3 weeks and surgical drains were placed in the scrotum, buttocks, thigh and anterior abdominal wall. There was an excellent clinical response. In some cases, surgery of a more radical nature may be necessary.

clostridial myonecrosis in that there is less systemic toxicity, it is mandatory that thorough surgical exploration and debridement be performed to distinguish these entities. MRI or CT scans as well as a serum creatinine phosphokinase assay may also be useful for determining whether muscle tissue is involved. Treatment is discussed below under gas gangrene.

Clostridial gas gangrene

Three types of clostridial soft tissue infections have been defined:[1]

- simple wound contamination or colonization;
- anaerobic cellulitis; and
- clostridial gas gangrene.

The first type, simple wound contamination or colonization, does not progress to true infection for various reasons (e.g. there may be insufficient devitalized tissue to promote infection or there may be effective host responses or effective medical and surgical management). Contamination is a very common occurrence; 30–80% of open traumatic wounds contain clostridial species.[26]

The second type, anaerobic cellulitis, occurs when there is devitalized tissue in a wound, sufficient for growth of *C. perfringens* or other strains. Although gas is produced locally and extends along fascial planes, bacteremia and invasion of healthy tissue do not occur. Appropriate medical and surgical management, including prompt removal of the devitalized tissue, is all that is necessary for cure and mortality is generally nil.[1]

The third type is clostridial gas gangrene or myonecrosis. This is defined as an acute invasion of healthy living muscle that is undamaged by previous trauma or ischemia.[26] It is divided into three different subtypes:

- traumatic gas gangrene;
- spontaneous or nontraumatic gas gangrene; and
- recurrent gas gangrene caused by *C. perfringens*.

Traumatic gas gangrene is the most common subtype. It develops when a deep, penetrating injury that compromises the blood supply (e.g. knife or gunshot wounds, crush injury or car accident) creates an anaerobic environment that is ideal for clostridial proliferation. This type of trauma accounts for about 70% of cases of gas gangrene. *Clostridium perfringens* is found in about 80% of such infections;[1] the remaining cases are caused by *C. septicum*, *C. novyi*, *C. histolyticum*, *C. bifermentans*, *C. tertium* and *C. fallax*. Other conditions associated with traumatic gas gangrene are bowel and biliary tract surgery, intramuscular injection of adrenaline (epinephrine), illegal abortion, retained placenta, prolonged rupture of the membranes and intrauterine fetal demise or missed abortion in postpartum patients.

Spontaneous or nontraumatic gas gangrene is less common. This is often caused by the more aerotolerant species *C. septicum*. As described below, most of these cases occur in patients who have a gastrointestinal portal of entry such as adenocarcinoma.

Third, and least common, is recurrent gas gangrene caused by *C. perfringens*. This has been described in people who have nonpenetrating injuries at sites of previous gas gangrene; residual spores of *C. perfringens* may remain quiescent in tissue for periods of up to 20 years, and then germinate when minor trauma provides anaerobic conditions suitable for growth.[27]

Traumatic gas gangrene

Pathogenesis

The initiating trauma introduces organisms (either vegetative forms or spores) into the deep tissues and produces an anaerobic niche with a sufficiently low redox potential and acid pH for optimal clostridial growth.[1,26] Necrosis progresses within hours. At the junction of necrotic and normal tissues few polymorphonuclear leukocytes are present, yet pavementing of these cells along the endothelium is apparent within capillaries and in small arterioles and postcapillary venules.[28,29] Later in the course of the illness there is leukostasis within larger vessels. Thus, the histopathology of clostridial gas gangrene is opposite to that seen in soft tissue infections caused by pyogenic organisms such as *Staph. aureus*, in which an early luxuriant influx of polymorphonuclear leukocytes localizes the infection without adjacent tissue or vascular destruction. Recent studies suggest that theta toxin (Fig. 10.8), when elaborated in high concentrations at the site of infection, destroys host tissues and inflammatory cells.[30] As the toxin diffuses into surrounding tissues or enters the systemic circulation, theta toxin promotes dysregulated adhesive interactions between polymorphonuclear leukocytes and endothelial cells and primes leukocytes for increased respiratory burst activity.[30]

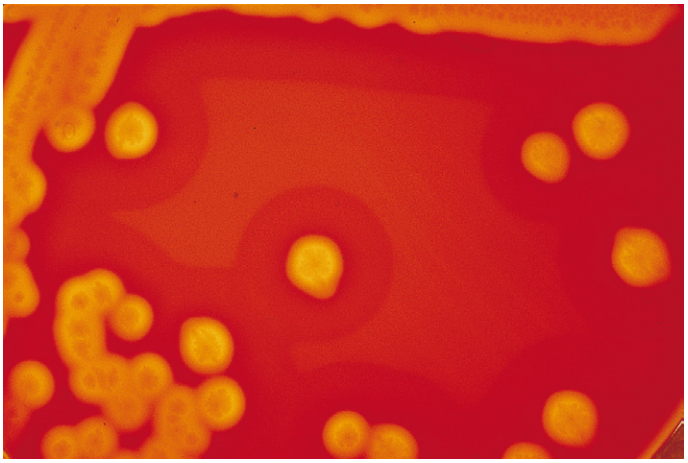


Fig. 10.8 Colonies of *Clostridium perfringens* growing on an anaerobic blood agar plate. Theta toxin causes the clear zone of hemolysis closest to the colony. A second area of partial hemolysis is caused by α-toxin, an enzyme with phospholipase C activity.

Alpha toxin (see Fig. 10.8), a phospholipase C, directly suppresses myocardial contractility *ex vivo*[31] and may contribute to profound hypotension via a sudden reduction in cardiac output.[32] Experimentally, alpha toxin induces a profound and irreversible defect in perfusion as measured by laser Doppler blood flow.[33,34] Simultaneously, there is rapid appearance of circulation aggregates of platelets and neutrophils bound together by the platelet surface receptor GPIIb/IIIa.[33] These actions lead to vascular leukostasis, endothelial cell injury and regional tissue hypoxia. Such perfusion deficits expand the anaerobic environment and contribute to the rapidly advancing margins of tissue destruction that are characteristic of clostridial gangrene.[1] In experimental models, theta toxin (a cholesterol-binding cytolysin) causes 'warm shock', defined as a markedly reduced systemic vascular resistance combined with a markedly increased cardiac output.[31,32] It is clear that theta toxin accomplishes this indirectly by inducing endogenous mediators that cause relaxation of blood vessel wall tension, such as the lipid autacoids prostacyclin or platelet-activating factor.[35] Reduced vascular tone develops rapidly and, in order to maintain adequate tissue perfusion, a compensatory host response is required; this either increases cardiac output or rapidly expands the intravascular blood volume. Patients who have Gram-negative sepsis compensate for hypotension by markedly increasing cardiac output; however, this adaptive mechanism may not be possible in shock induced by *C. perfringens* due to direct suppression of myocardial contractility by alpha toxin.[31] The role of other endogenous mediators such as cytokines (e.g. TNF, IL-1, IL-6) as well as the potent endogenous vasodilator bradykinin have not been elucidated.

Prevention

Aggressive debridement of devitalized tissue and rapid repair of compromised vascular supply greatly reduce the frequency of gas gangrene in contaminated deep wounds. Intramuscular adrenaline (epinephrine), prolonged application of tourniquets and surgical closure of traumatic wounds should be avoided. Patients who have compound

fractures are at particular risk of gas gangrene if the wound is surgically closed. Patients who have contaminated wounds should receive prophylactic antibiotics.

Clinical findings

The first symptom is usually the sudden onset of severe pain at the site of recent surgery or trauma.[2,24] The mean incubation period is less than 24 hours, but it ranges from 6–8 hours to several days, probably depending on the degree of soil contamination or bowel contents spillage and the extent of vascular compromise.

The skin may initially appear pale, but it quickly changes to bronze then purplish red, becoming tense and exquisitely tender (Fig. 10.9). Bullae develop; they may be clear, red, blue or purple.

Gas in tissue may be obvious from physical examination, soft tissue radiographs, CT scan or MRI. None of these radiographic procedures has proved to be more specific or more sensitive than the physical finding of crepitus in the soft tissue.[10] However, radiographic procedures are particularly helpful for demonstrating gas in deeper tissue such as the uterus.

Signs of systemic toxicity develop rapidly; these include tachycardia, fever and diaphoresis, followed by shock and multiple organ failure. Shock is present in 50% of patients at the time they present to the hospital.[36] Bacteremia occurs in 15% of patients and may be associated with brisk hemolysis. In one patient, the hematocrit fell from 37% to 0% over a 24-hour period.[37] Subsequently, despite transfusion with 10 units of packed red blood cells over 4 hours, the hematocrit never exceeded 7.2%.[37] Alpha and theta toxins contribute to this marked intravascular hemolysis. Not all cases of *C. perfringens* bacteremia have been associated with gas gangrene[38] but 90% of *C. perfringens* and 100% of *C. septicum* isolates from blood were associated with clinically significant infection.[39]

Additional complications of clostridial myonecrosis include jaundice, renal failure, hypotension and liver necrosis. Renal failure is largely due to hemoglobinuria and myoglobinuria, but it may be a result of acute tubular necrosis caused by hypotension. Renal tubular cells are probably directly affected by toxins.

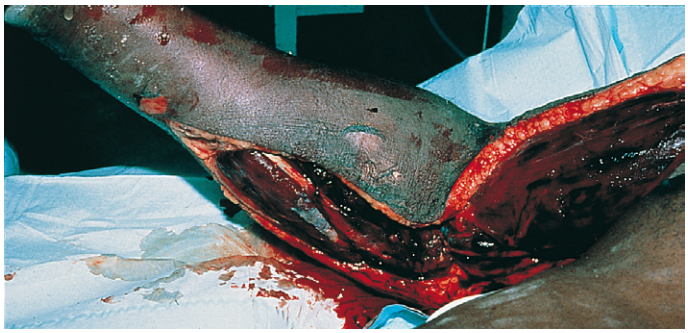


Fig. 10.9 Extensive gas gangrene of the arm due to *Clostridium perfringens*. A 35-year-old man sustained a knife wound to the forearm. He did not seek medical care, but 36 hours later experienced severe pain in the upper arm and came to the emergency room. There was extreme tenderness of the arm and crepitus was easily demonstrated. A radiograph also demonstrated gas in the deep soft tissues. Surgical debridement and antibiotics were instituted, but later amputation at the level of the shoulder was necessary. A pure culture of *C. perfringens* was grown from the deep tissues.

Diagnosis

Increasing pain at a site of previous injury or surgery, together with signs of systemic toxicity and gas in the tissues, support the diagnosis. Definitive diagnosis rests on demonstrating large, Gram-variable rods at the affected site (Fig. 10.10). Note that although clostridia stain Gram-positive when obtained from bacteriologic media, when visualized from infected tissues they often appear both Gram-positive and Gram-negative. In fresh specimens *C. perfringens* may appear to be encapsulated.[40,41]

The affected muscles appear edematous, may be an abnormal reddish-blue to black color and do not bleed or contract when stimulated. Usually, some degree of necrotizing fasciitis and cutaneous necrosis is also present. Microscopic evaluation of biopsy material (see Fig. 10.10) demonstrates organisms among degenerating muscle bundles and characteristically an absence of acute inflammatory cells.[26,42]

Management

Penicillin, clindamycin, tetracycline, chloramphenicol, metronidazole and a number of cephalosporins have excellent *in vitro* activity against *C. perfringens* and other clostridia. No controlled clinical trials have ever been conducted to compare the efficacy of these agents in humans. Based strictly on *in-vitro* susceptibility data, most textbooks state that penicillin is the drug of choice.[43,44] However, experimental studies in mice suggest that clindamycin has the greatest efficacy and penicillin the least.[45,46] Other agents with greater efficacy than penicillin include erythromycin, rifampin (rifampicin), tetracycline, chloramphenicol and metronidazole.[45,46] Slightly greater survival was observed in animals receiving both

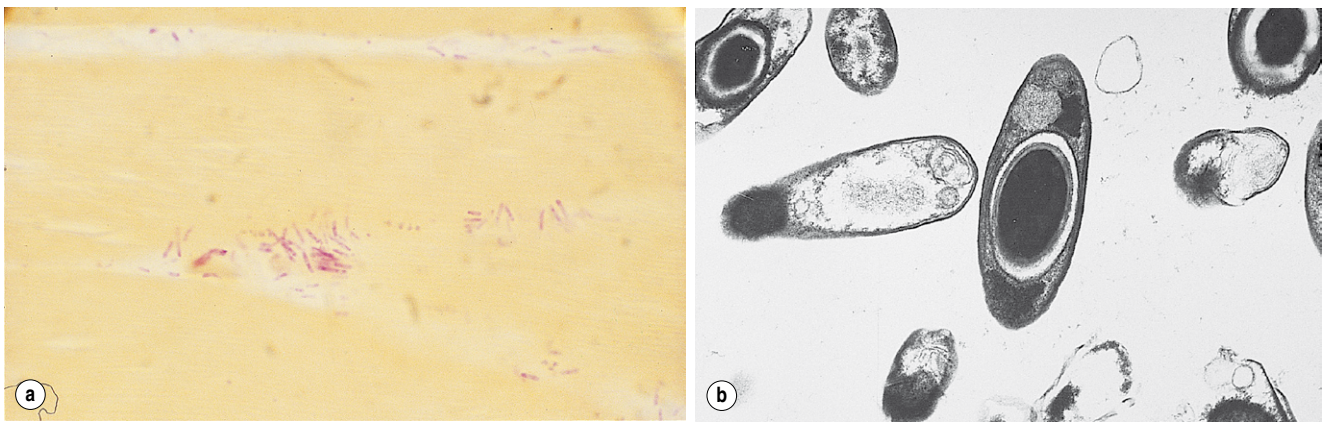


Fig. 10.10 *Clostridium perfringens* in a patient who has extensive gas gangrene. (a) Gram stain of tissue removed from the arm of the patient described in Figure 10.9. Note that the bacteria are rod shaped but Gram variable. Note also that there are few if any acute inflammatory cells at the site of infection. (b) Transmission electron micrograph of *C. perfringens*. Note the endospores.

clindamycin and penicillin; in contrast, antagonism was observed with penicillin plus metronidazole.[46] Because between 2% and 5% of strains are resistant to clindamycin, a combination of penicillin and clindamycin is warranted. Based on his experimental studies and his vast clinical experience with gas gangrene, the late Dr William Altemeier recommended tetracycline and penicillin.[47] Thus, given an absence of efficacy data from a clinical trial in humans, the best treatment would appear to be clindamycin or tetracycline combined with penicillin. The failure of penicillin in experimental clostridial myonecrosis may be related to continued toxin production and filament formation rather than lysis.[48] In contrast, the efficacy of clindamycin and tetracycline may be related to their ability to inhibit toxin synthesis rapidly.[48]

Aggressive and thorough surgical debridement is mandatory to improve survival, preserve limbs and prevent complications.[43,44] The use of hyperbaric oxygen (HBO) is controversial, although some non-randomized studies have reported good results with HBO therapy when combined with antibiotics and surgical debridement.[36,49,50] Experimental studies in animals have demonstrated that HBO alone can be effective treatment if the inoculum is small and treatment is begun immediately.[51] In contrast, other studies have demonstrated that HBO was only of slight benefit when combined with penicillin.[52] However, survival was better with clindamycin alone than with either HBO alone, penicillin alone or HBO plus penicillin together.[52] The benefit of HBO, at least theoretically, is to inhibit bacterial growth,[53] to preserve marginally perfused tissue and to inhibit toxin production.[54] Interestingly, Altemeier did not use HBO and was able to realize a mortality rate of less than 15% using surgical debridement and antibiotics (tetracycline plus penicillin) alone.[47]

Specific antitoxin antibodies for adjunctive treatment are no longer available. Future strategies may target endogenous proadhesive molecules such that toxin-induced vascular leukostasis and resultant tissue injury are attenuated.

Prognosis

Patients presenting with gas gangrene of an extremity have a better prognosis than those who have truncal or intra-abdominal gas gangrene, largely because it is difficult to debride such lesions adequately.[43,44,55] Hyperbaric oxygen could be useful in such patients. In addition to truncal gangrene, patients who have associated bacteremia and intravascular hemolysis have the greatest likelihood of progressing to shock and death. In one study, of those patients who developed shock at some point in their hospitalization, 40% died, compared with 20% mortality in the group as a whole.[36] In another study, those who were in shock at the time of diagnosis had the highest mortality.[55]

Spontaneous, non-traumatic gas gangrene due to *Clostridium septicum*

Pathogenesis

Predisposing factors include:[55–57]

- colonic carcinoma;
- diverticulitis;
- gastrointestinal surgery;
- leukemia;
- lymphoproliferative disorders;
- cancer chemotherapy;
- radiation therapy; and
- AIDS.

Cyclic or other forms of neutropenia are also associated with spontaneous gas gangrene due to *C. septicum* and in such cases necrotizing enterocolitis, cecitis or distal ileitis are commonly found. These gastrointestinal pathologies permit bacterial access to the bloodstream; consequently, the aerotolerant *C. septicum* can become established in normal tissues.[1]

Clostridium septicum produces four toxins:

- α-toxin (lethal, hemolytic, necrotizing activity);
- β-toxin (deoxyribonuclease);
- γ-toxin (hyaluronidase); and
- δ-toxin (septicolysin, an oxygen-labile hemolysin).

Clostridium septicum also produces a protease and a neuraminidase.[1]

The *C. septicum* α-toxin does not possess phospholipase activity and is thus distinct from the α-toxin of *C. perfringens*. Active immunization against α-toxin significantly protects against challenge with viable *C. septicum*.[58] The mechanism by which α-toxin contributes to *C. septicum* pathogenesis is unknown; however, the recent cloning and sequencing of this toxin should facilitate studies in this area (see Chapter 173).

Clinical features

The onset of disease is abrupt, often with excruciating pain, although the patient may sense only heaviness or numbness.[1,28,55–57] The first symptom may be confusion or malaise. Extremely rapid progression of gangrene follows. Swelling advances and bullae appear; these are filled with clear, cloudy, hemorrhagic or purplish fluid. The skin around such bullae also has a purple hue (Fig. 10.11), perhaps reflecting vascular compromise resulting from bacterial toxins diffusing into surrounding tissues.[55] Histopathology of muscle and connective tissues includes cell lysis and gas formation; inflammatory cells are notably absent.[55]

Diagnosis

Unlike the situation in traumatic gas gangrene, bacteremia precedes cutaneous manifestations by several hours. In the absence of the usual cutaneous manifestations of gas gangrene, other causes of fever and extremity pain such as deep vein thrombophlebitis or cellulitis are naturally considered first, delaying appropriate diagnosis and treatment, and, as a consequence, increasing mortality.

Management

No comparative human trials have evaluated the efficacy of antibiotics or HBO for treating clinical cases of spontaneous gas gangrene. *In-vitro* data indicate that *C. septicum* is uniformly susceptible to penicillin, tetracycline, erythromycin, clindamycin, chloramphenicol and metronidazole. The aerotolerance of *C. septicum* may reduce the likelihood that HBO therapy would be effective.[53]



Fig. 10.11 Spontaneous necrotizing fasciitis due to *Clostridium septicum*. This patient developed sudden onset of severe pain in the forearm. Swelling rapidly ensued and he sought medical treatment. Crepitus was present on physical examination and gas in the soft tissue was verified with routine radiographs. Immediate surgical debridement revealed necrotizing fasciitis but sparing of the muscle. Note the purple-violaceous appearance of the skin. See also Figure 10.12.

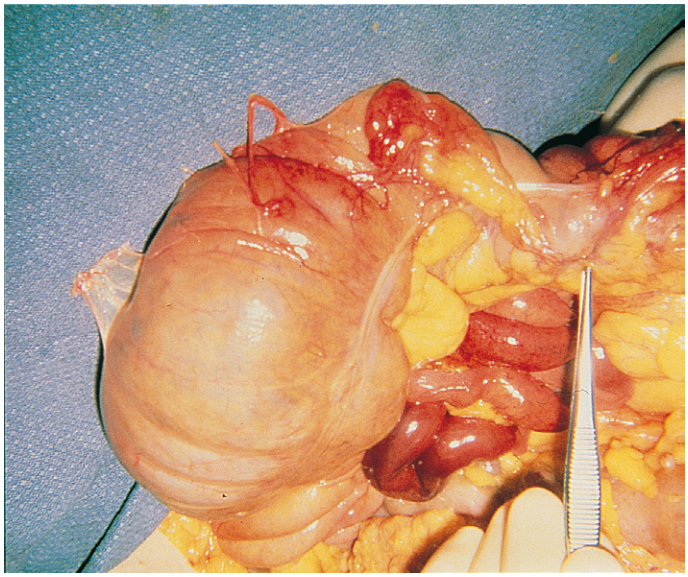


Fig. 10.12 Colonic carcinoma in a patient who has spontaneous gas gangrene caused by *Clostridium septicum*. The patient described in Figure 10.11 was found to have a mass in the colon. Surgical resection revealed an adenocarcinoma, which probably served as a portal of entry for the *C. septicum* bacillus. Hematogenous seeding of the forearm resulted in spontaneous gas gangrene.

Prognosis

The mortality of spontaneous clinical gangrene ranges from 67% to 100%, with the majority of deaths occurring within 24 hours of onset. Unfavorable factors include underlying malignancy and compromised immune status. All patients who survive bacteremia or spontaneous gangrene caused by *C. septicum* should have appropriate diagnostic studies of the gastrointestinal tract (Fig. 10.12). Occasionally, this has led to detection and cure of an unsuspected malignancy that might otherwise have been fatal.[55]

Clostridium sordellii infections

Patients who have *C. sordellii* infection present with unique clinical features including edema, absence of fever, leukemoid reaction, hemoconcentration and later shock and multiple organ failure.[45] Often *C. sordellii* infections develop after childbirth or after gynecologic procedures,[59,60] and most represent endometrial infection. Rarely, other cases have occurred at sites of minor trauma such as lacerations of the soft tissues of an extremity. Recently, outbreaks of *C. sordellii* and *C. novyi* infections have been described among intravenous drug users in Scotland, Ireland and England. Patients have presented with severe soft tissue infections with shock with a case fatality rate of 20–30%.[3] Interestingly, the mortality rate of women who acquire postpartum *C. sordelli* has been 100%.[61]

Unlike *C. perfringens* and *C. septicum* infections, pain may not be a prominent feature of *C. sordellii* infections. The absence of fever and the paucity of signs and symptoms of local infection make early diagnosis difficult.[44] The mechanisms of diffuse capillary leak, massive edema and hemoconcentration are not well established but clearly are related to elaboration of a potent toxin or toxins. Aldape recently demonstrated that a crude toxin preparation caused leakiness of endothelial cells in tissue culture.[62] Hematocrits of 75–80% have been described and leukocytosis of 50 000–100 000 cells/mm^3 with a left shift is common.[42,59]

Recent studies have demonstrated that a neuroaminadase of *C. sordellii* is responsible for proliferation of bone marrow progenitor cells that likely contributes to the leukemoid reaction.[61]

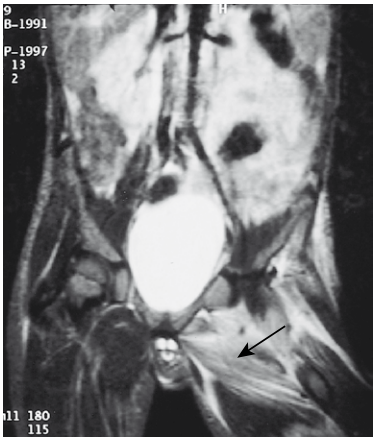


Fig. 10.13 MRI scan showing high-signal STIR sequence (consistent with marked edema) in the adductor muscles of the left leg in a patient with *Staphylococcus aureus* bacteremia (arrow). At operation, necrotic and infected muscle was decompressed and debrided. This represents the 'woody' stage, prior to muscle liquefaction and the formation of frank abscesses.

Clostridium tertium infections

Clostridium tertium has been associated with spontaneous myonecrosis; however, it more commonly causes bacteremia in compromised hosts who have received long courses of antibiotics. Bacteremia probably arises from bowel sources, and the presence of the organism in the bowel may be partly related to its relative resistance to penicillin, cephalosporins and clindamycin. *Clostridium tertium* is, however, usually quite sensitive to chloramphenicol, vancomycin and metronidazole. Because this organism can grow aerobically, it may be mistakenly disregarded as a contaminant such as a diphtheroid or a *Bacillus* sp.[43,44]

Pyomyositis

Most cases of pyomyositis occur in tropical areas; however, recent reports of pyomyositis have documented a large increase in cases in temperate climates caused by methicillin-resistant *Staph. aureus* (MRSA).[62] Local trauma, muscle strain and overuse are common predisposing factors. Initially, seeding of traumatized muscle occurs and physical findings are not usually helpful. Within 10–20 days, fever, chills, muscle pain and tenderness are manifest (see Fig. 10.1). Most patients seek medical care at this stage and a diagnosis can be established by appropriate imaging studies (Fig. 10.13), needle aspiration or exploration. Patients in whom a diagnosis has not been made may progress to shock and organ failure, though these complications are uncommon. *Staphylococcus aureus* is the most common cause of pyomyositis in tropical and nontropical areas, and among HIV-positive patients. Hospitalized immunocompromised patients who are HIV negative occasionally develop pyomyositis caused by Gram-negative bacteria.

Surgical drainage of the abscess and empiric administration of parenteral antibiotics such as nafcillin or cephalosporins are reasonable treatments since most cases are caused by *Staph. aureus*. Definitive treatment can then be established based on cultures and sensitivities. Due to an increase in the prevalence of MRSA, it may be necessary to use vancomycin or linezolid empirically pending sensitivity results.[63]

REFERENCES

References for this chapter can be found online at http://www.expertconsult.com

Chapter 11

Peter Moss
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Arthropods and ectoparasites

Arthropods

INTRODUCTION

The phylum Arthropoda, which includes the insects and the arachnids, is the largest and most varied of all the animal phyla, containing more than one million described species. Although only a small number of these directly or indirectly affect human health, arthropod species are responsible for considerable morbidity and mortality (Table 11.1). Arthropods can cause illness in a number of different ways. These include parasitization (usually as ectoparasites), envenomation, vesication, mechanical transmission of infection, acting as biologic vectors, and as agents of a variety of allergic respiratory and dermatologic conditions. In addition, arthropod parasites of domestic animals may contribute to food shortages and enforced population movement. This section concentrates on the clinical problems caused by arthropods which are *not* true parasites of man.

ENVENOMATION

A variety of ectoparasite orders and species are responsible for envenomation of humans, either through bites or stings. These attacks are usually part of the self-defense behavior of the animal, and are distinct from the feeding bites inflicted by true parasites of man (see below). The severity of the reaction to envenomation varies enormously depending on the nature of the toxin, the location of the wound and the individual host response, which may itself depend in part on previous exposure.[1,2] Even relatively harmless toxins, which produce transient local symptoms in most people, may cause severe systemic reactions (including anaphylaxis) in highly sensitized individuals.[3–5]

Bees, wasps and ants

These social insects, members of the order Hymenoptera, usually attack humans to defend either themselves or their nests. Bees and wasps inject venom using a modified ovipositor, while some species of ant, notably the fire ant and the bulldog ant, have evolved a venomous sting in addition to their bite.[3,6] The resulting envenomation usually causes relatively minor and transient local inflammation.

Scorpions

Scorpions use their sting to inject a neurotoxic venom, which is primarily intended for hunting prey but may also be used in self-defense. Many are relatively harmless, causing symptoms similar to a bee sting, but some species have a far more dangerous toxin which may even be fatal. Scorpion stings kill more than 1000 people each year (mainly children) in Africa, Europe, and the Middle East.[7]

Spiders

Spiders also use their venom mainly for hunting, injecting it through fang-like chelicerae in their mouth parts. Fortunately, although most spiders are venomous, few have chelicerae capable of penetrating human skin. Even those that can bite humans (such as tarantulas and wolf spiders) usually cause no more than transient local pain and swelling. However, a few species can cause much more serious illness, due to production of powerful neurotoxins or venom which triggers serious tissue necrosis.

Widow spiders (genus *Latrodectus*) inject a potent neurotoxin which is capable of causing myalgia, paralysis, convulsions and occasionally death. Mortality rates are reported to be between <1% and 6%, depending to some extent upon what treatment is available, but deaths due to widow spider bites are rare in developed countries. There are no accurate figures for the annual number of deaths in less developed areas. *Latrodectus* species are found worldwide, with *L. mactans* (the black widow) and *L. geometricus* (the brown widow) the best known. The females of all species are distinguished by a characteristic red or orange hourglass-shaped marking on the back (Fig. 11.1). Other potentially dangerous producers of neurotoxins include the funnel web spider (genus *Atrax*) in Australia and the armed spider (genus *Phoneutria*) in South America.[2,8]

Necrotic arachnidism occurs worldwide, and is most commonly due to the bite of one of the recluse spiders (genus *Loxosceles*). These small brown spiders have a dark, fiddle-shaped marking on their back (hence the common names fiddleback or violin spiders), and tend to lurk in quiet undisturbed areas. The bite is initially painless, but after a few hours the area becomes red, swollen and painful. Necrotic enzymes in the venom cause progressive tissue death and sloughing over the subsequent days, and occasionally lead to systemic toxicity including hemolysis and renal failure. The skin lesions can be very slow to heal, and are prone to secondary infection.

Other spider genera implicated in producing necrotic arachnidism include the hobo spider *Tegenaria agrestis* in Europe and the Pacific Northwest of North America, crab spiders (genus *Sicarius*) in Africa and sac spiders (genus *Chiracanthium*), which are found worldwide.[1]

Other envenoming arthropods

A number of other types of arthropod produce defensive toxins which may cause minor symptoms in humans. Centipedes inoculate venom through their pincers, and the larger species may be capable of penetrating human skin to cause local tissue reactions. Some caterpillars

Table 11.1 Arthropods and their medical importance

Class	Subclass	Order	Pathogenic potential
Insecta (insects)		Anoplura (sucking lice)	Biting pests Local hypersensitivity Biologic vectors
		Siphonaptera (fleas)	Biting pests Local hypersensitivity Biologic vectors Superficial tissue invasion (*Tunga penetrans*)
		Dictyoptera (cockroaches)	Mechanical vectors Allergies
		Hemiptera (bedbugs, kissing bugs)	Biting pests Local and rarely systemic hypersensitivity Biologic vector (kissing bugs)
		Hymenoptera (bees, wasps, ants)	Envenomation Local and systemic hypersensitivity including anaphylaxis
		Coleoptera (beetles)	Mechanical and biologic vectors Urticating hairs (larvae), vesicating fluids (adults)
		Lepidoptera (moths, butterflies, caterpillars)	Urticating hairs and venomous spines (larvae) Urticating hairs and scales (adults)
		Diptera (flies, mosquitoes, biting midges)	Biting pests Local hypersensitivity Mechanical and biologic vectors Tissue invasion (myiasis)
Arachnida (arachnids)	Scorpiones (scorpions) Araneae (spiders) Acari (ticks, mites)		Envenomation with neurotoxins Envenomation with neurotoxins and necrotoxins Biting pests Local and systemic hypersensitivity Allergies (mites) Biologic vectors Superficial tissue invasion (*Sarcoptes scabiei*) Neurotoxins (ticks)
Diplopoda (millipedes)			Vesicating fluid
Chilopoda (centipedes)			Envenomation Local and rarely systemic hypersensitivity
Crustacea (crustaceans)		Copepoda (copepods, water fleas) Decapoda (crabs, crayfish)	Biologic vectors Biologic vectors
Pentastomida			Tissue and body cavity infection



Fig. 11.1 Female *Latrodectus mactans*, the black widow spider. Note the characteristic red hourglass marking on the underside of the abdomen. With permission from New England Journal of Medicine 1994;331:777.

(larvae of moths and butterflies) possess hairs which inject a toxin when touched. In the vast majority of cases these cause minor local symptoms, but severe systemic reactions have been reported.[8]

Management

Most envenomation injuries require no specific treatment, although local symptoms may be alleviated with antihistamines. Anaphylaxis is life threatening, requiring immediate treatment with adrenaline (epinephrine) and/or bronchodilators. It may in some cases be possible to desensitize people with severe hypersensitivity reactions using very low doses of venom, but the success of this technique is variable. Specific antitoxins are available for certain spider and scorpion venoms.[9]

VESICATION

Some arthropods are capable of producing an irritant, blister-inducing (vesicating) chemical which is secreted or sprayed from specialized glands. As with envenomation, vesication is usually a self-defense reaction. The most common culprits are the so-called blister beetles

and some species of millipede. Although beetles are best known as pests of agricultural crops, some species can give a painful bite, and others, especially the blister beetles, can exude vesicating fluids, including cantharidin, that cause dermatitis or blister formation. Cantharidin is the chief component of the aphrodisiac known as 'Spanish Fly'. The local irritation caused by these chemicals does not usually require specific treatment.[10]

MECHANICAL TRANSMISSION OF INFECTION

Arthropod species have been implicated as mechanical vectors of infection, carrying micro-organisms on their bodies and mouthparts. Flies play a major part in the transmission of trachoma (*Chlamydia trachomatis*) and can also spread a wide variety of gastrointestinal infections. Cat and dog fleas, which may be swallowed inadvertently, are intermediate hosts for the tapeworm *Dipylidium caninum* and less frequently for *Hymenolepis diminuta* and *H. nana*. The role of biting insects in the mechanical transmission of blood-borne viruses is considered below.

OTHER DERMATOLOGIC AND RESPIRATORY CONDITIONS

In addition to envenomation, vesication and parasitization, arthropods can cause skin problems through other mechanisms. So-called storage mites are small predatory arachnids which live in stored products such as grain, straw, flour, dried fruit, and cheese. They may migrate to humans who come into contact with their habitat, crawling onto exposed skin and getting underneath the horny layer of the skin. Here they can cause an acute dermatitis, with erythema and small vesicles: this condition, which is often related to occupation, is known by a variety of descriptive names such as 'grocer's itch', 'barley itch', and 'grain shoveler's itch'.[11]

Cheyletiellid mites are ubiquitous tissue-feeding parasites of domestic dogs and cats. Bites follow close contact with infected animals and lesions are usually seen on the thighs and abdomen after a pet has sat on someone's lap. Itchy papules similar to other arthropod bites result and the diagnosis is made by finding mites on the animal. Some hematophagous animal mites also attack humans, causing typical lesions in areas that depend on the form of contact with the animal. Poultry, cage birds, wild and domestic rodents and snakes have all been incriminated. In all these cases management is by removal or treatment of the principal animal host, with environmental acaricide if necessary.[11]

Demodex folliculorum, the follicle mite, is a tiny organism that lives in the pilosebaceous glands where it feeds on cell contents. The mites are found in areas of high sebum production: the forehead, cheeks, nose, and nasolabial folds. The role of follicle mites in skin disease is controversial: they are very common, and unless they are present in large numbers they do not appear to cause problems. However, they have been described as a cause of papulopustular eruptions in children and immunocompromised subjects, and have been implicated in the pathogenesis of skin conditions such as pityriasis folliculorum and rosacea.[12]

House dust mites (*Dermatophagoides* spp.) are cosmopolitan free-living mites that feed principally on human and animal skin detritus. Their main medical importance is as a cause of allergic rhinitis and bronchospasm.

MISCELLANEOUS

Pentastomes

Pentastomes or tongue worms are arthropods of uncertain affinities that possess few distinctive morphologic characteristics. Larval stages resemble mites and have occasionally been reported to cause liver and lung infections in humans in Asia and Africa. Adult stages are worm-like organisms that live in the nasal passages of certain predatory reptiles, birds and mammals; they have been recovered from the nasopharynx of individuals from the Middle East and Africa where they are responsible for an obstructive condition known as halzoun.[13]

Crustaceans

Crustaceans, most notably crabs, crayfish and microscopic copepods, are of medical importance by serving as hosts and vectors for larval stages of several different helminths (see Table 11.2).

Ectoparasites

INTRODUCTION

Parasites depend on their host for sustenance; an ectoparasite is a parasite that lives or feeds on the surface of that host. Most ectoparasites are hematophagous, but a few feed on living skin cells and tissue. Some spend their entire life on one host, while others move from host to host as they develop, and many simply alight on the host in order to feed. In this chapter the definition of 'ectoparasite' is extended to include those parasites that burrow into the epidermis as well as those that remain on the surface. The vast majority of ectoparasites are members of the phylum Arthropoda.

Humans are the preferred or only hosts of some ectoparasites, but the majority are less specific in their choice, or turn to humans only when their primary host is unavailable. Ectoparasites can cause human disease in a number of different ways. Local skin disease can be the direct result of the parasite bite, or may arise from secondary infection or hypersensitivity. Allergens or toxins introduced during feeding can cause systemic illness, and transient ectoparasites can act as vectors for a wide range of viral, bacterial, and parasitic infections. This section reviews the clinical syndromes caused by ectoparasites, outlining the key features associated with each.

SKIN PROBLEMS

Transient ectoparasites

Clinical and pathologic features

The most common feature of ectoparasite infection is local reaction to the bite of a blood-feeding arthropod. Numerous species of insects and arachnids rely on blood meals from vertebrate hosts. Although the behavior and feeding methods differ by species, most uncomplicated arthropod bites produce a similar local reaction.

Initial contact in an unsensitized person may produce little or no response. After repeated bites, typical raised pruritic lesions (papular urticaria) appear within 24–48 hours (Fig. 11.2), although delays of more than 1 week have been reported. Occasionally, these lesions are bullous. With further exposure and increased sensitization, an immediate wheal skin response may be seen, followed after some hours by papular urticaria. Some hypersensitive people may develop a pronounced immediate reaction with large areas of superficial edema. After prolonged and frequent biting, the delayed reaction often diminishes and eventually there is no response to further bites. The term papular urticaria is also used to describe a condition (usually seen in children) in which widespread papular lesions occur distant from but temporally related to insect bites; this may represent reactivation of previously sensitized bite sites.

The lesions of papular urticaria consist of pruritic papules, often with a central puncture marking the site of the bite. Superficial erosion and ulceration resulting from scratching is common. Unlike



Fig. 11.2 Typical lesions of papular urticaria, caused in this case by bedbug bites.



Fig. 11.3 Mosquito bites with secondary staphylococcal and streptococcal infection.

most other types of urticaria, papular lesions may persist for several days, although this period tends to decrease with regular exposure. Histologically there is intense inflammatory infiltrate with a predominance of T cells; the response is thought to be a type 1 IgE-mediated reaction.[14] A variety of vasodilators, anticoagulants and other substances have been isolated from arthropod saliva; in some species a specific allergen has been identified as the cause of local hypersensitivity, but in others the stimulus remains obscure.

Treatment of uncomplicated bites is usually unnecessary, although severe pruritus may be relieved by systemic antihistamines. Caution is needed with terfenadine and astemizole, which can cause prolongation of the cardiographic QT interval and have a synergistic cardiotoxic effect with certain antimalarial drugs. Secondary bacterial infection is common in hot moist climates (Fig. 11.3) and this may require antibiotic therapy; infection is usually due to gram-positive cocci. Local lesions caused by various blood-feeding arthropods are usually indistinguishable, and diagnosis of the precise cause relies either on epidemiologic knowledge or on detection and identification of the parasite. This may sometimes be necessary in order to eradicate the source of infection.

The major types of transient-feeding ectoparasites responsible for this type of bite are discussed below.

Flies

Insects of the order Diptera are characterized by the presence of a single pair of membranous wings. They vary enormously in size and appearance. Of all the arthropods, the flies are responsible for the greatest share of human disease as a result of blood feeding, transmission of infection, and myiasis (which is discussed below). Mosquitoes, blackflies, horseflies, sandflies, and midges are ubiquitous, whereas tsetse flies are essentially tropical. Only certain species of each family tend to attack humans, and in all except the tsetse fly only the females are blood feeders. Culicid mosquitoes usually feed by inserting their proboscis directly into the capillary, whereas other species feed by inflicting local tissue damage and sucking the resultant blood (pool feeding). This may explain the relatively more severe irritation that complicates bites by some species, although host factors also play a significant part. Some of the biting flies are important biologic vectors of infection (Table 11.2).

Fleas

Fleas (order Siphonaptera) are small (1–2 mm), laterally compressed, wingless insects with muscular back legs which enable them to jump great distances. They include both transient blood-sucking pests (some of which are also important vectors of infection; see Table 11.2), and the locally invasive jigger flea. Fleas occur worldwide and are a common cause of pruritic bites in humans. They are free living, only approaching their host in order to feed. The human flea, *Pulex irritans*, is found mainly in crowded and unhygienic living conditions and is relatively uncommon in the developed world. However, many species of animal and bird fleas will also feed on humans, and household infestations with cat and dog fleas (*Ctenocephalides felis* and *C. canis*) are increasing in frequency (Fig. 11.4).

Flea bites provoke typical papular urticaria, although there may be a more severe reaction with bulla formation. The distribution of the lesions reflects the source of the infestation; for example, bites on the lower leg are usually due to cat and dog fleas, at least in adults. Diagnosis can be difficult; fleas are rarely seen on the human victim and excoriation may mask the original nature of the lesions. If flea bites are suspected, careful inspection of the home environment is needed, although other sources of exposure (e.g. school, the workplace) should be considered. Household pets and their bedding should be checked thoroughly for fleas and droppings. Flea bites need no specific treatment unless there is secondary infection. Prevention depends on identifying the source of the infestation; in the case of pets, insecticides must be applied to both the animal and its environment. Because larvae of these species often develop in an animal's bedding or in carpets and furniture, eradication may require fumigation and cleaning of these articles.

Bugs

The bugs, or Hemiptera, are one of the largest and most diverse orders of insect. Although a number of bug species are blood feeders, only two families include significant ectoparasites of humans. The Reduviidae (kissing bugs) are large (1–3 cm) insects with a cone-shaped head and often conspicuous body markings. The bite of the kissing bug is relatively painless, although hypersensitivity can develop with repeated exposure. They are important principally as the vector of South American trypanosomiasis. Cimicidae (bedbugs) are flattish, wing-

Table 11.2 Important ectoparasite vectors of human infection

Ectoparasite	Vector genus/species	Infective organism	Disease	Distribution
Flies (Diptera)				
Mosquitoes	*Anopheles* spp. *Anopheles* spp. *Culex* spp. *Aedes* spp. *Anopheles* spp. *Mansonia* spp. *Aedes* spp. *Aedes* spp. *Culex* spp. *Anopheles* spp.	*Plasmodium* spp. *Wuchereria bancrofti* *Brugia* spp. Arboviruses	Malaria Lymphatic filariasis Lymphatic filariasis Dengue, yellow fever, Japanese encephalitis, West Nile virus, St Louis encephalitis, etc.	Tropics and sub-tropics Tropics Southern Asia Specific to each disease
Blackflies	Simuliidae	*Onchocerca volvulus* *Mansonella ozzardi*	Onchocerciasis Mansonelliasis	Sub-Saharan Africa, Central and South America Amazon basin
Sandflies	*Phlebotomus* spp. *Lutzomyia* spp.	*Leishmania* spp. Bunyaviridae *Leishmania* spp. *Bartonella bacilliformis*	Visceral and cutaneous leishmaniasis Sandfly fever Visceral and cutaneous leishmaniasis Bartonellosis (Oroya fever, Carrion's disease, verruga peruana)	Sub-Saharan Africa, Asia, Mediterranean Europe Central Asia, Mediterranean, North Africa Central and South America South America
Biting midges	*Culicoides* spp.	*Mansonella perstans* and *Mansonella ozzardi*	Mansonelliasis	Sub-Saharan Africa and Amazon basin
Tsetse flies	Glossinidae	*Trypanosoma* spp.	African trypanosomiasis	Sub-Saharan Africa
Deer flies	*Chrysops* spp.	*Loa loa* *Francisella tularensis*	Loiasis Tularaemia	West Africa Western USA
Fleas (Siphonaptera)				
E.g. tropical rat flea	Various species (notably *Xenopsylla*) Various species	*Yersinia pestis* *Rickettsia typhi* (*mooseri*)	Plague Murine typhus	Worldwide (patchy) Worldwide (patchy)
Bugs (Hemiptera)				
Kissing bug	Reduviidae	*Trypanosoma cruzi*	South American trypanosomiasis	Central and South America
Lice				
Body louse Body louse Head louse	*Pediculus humanus corporis* *P. h. corporis* *P. h. capitis*	*Rickettsia prowazeki* *Bartonella quintana* *Borrelia recurrentis*	Epidemic typhus Trench fever Louse-borne relapsing fever	Worldwide (patchy) Worldwide Worldwide (patchy)
Ticks				
Hard ticks (Ixodidae)	Numerous hard tick (ixodid) species	*Rickettsia* spp. *Borrelia burgdorferi* *Francisella tularensis* *Babesia* spp. Arboviruses *Ehrlichia* spp.	Tick typhus and various rickettsial spotted fevers Lyme disease Tularaemia Babesiosis Tick-borne encephalitis, Congo–Crimea hemorrhagic fever, etc. Ehrlichiosis	Worldwide USA, Europe, Far East, Australia USA, Europe, Japan USA, Europe Specific to each disease USA, Europe, North Africa, Far East

Table 11.2 Important ectoparasite vectors of human infection—cont'd

Ectoparasite	Vector genus/species	Infective organism	Disease	Distribution
Soft ticks (Argasidae)	*Ornithodoros* spp.	*Borrelia* spp.	Tick-borne relapsing fever	Africa, Central Asia, North and South America
Mites				
	Leptotrombidium spp.	*Orientia tsutsugamushi*	Scrub typhus	South East Asia, Pacific and (rarely) West Africa
	Liponyssoides sanguineus	*Rickettsia akari*	Rickettsial pox	USA, South Africa, Far East
Crustacea				
Crabs, crayfish	Decapods	*Paragonimus* spp.	Paragonimiasis	Asia, Sub-Saharan Africa
Copepods	*Cyclops* spp.	*Dracunculus medinensis*	Guinea worm	Sudan, Ghana
		Gnathostoma spinigerum	Gnathostomiasis	Worldwide (patchy)



Fig. 11.4 Adult *Ctenocephalides canis*, the dog flea. Note the strong muscular hind legs. Courtesy of Thomas R Fritsche.



Fig. 11.5 Adult bedbug *Cimex lectularius*. With permission from ASM Press. Courtesy of Thomas R Fritshe.

less insects about 5 mm in length (Fig. 11.5). Bedbugs live and reproduce in crevices in walls and furniture, only approaching the host to feed (usually at night). They are found worldwide, the tropical bedbug (*Cimex hemipterus*) parasitizing humans in hot climates and the common bedbug (*C. lectularius*) in other areas. Bites cause papular lesions similar to those of other arthropods, but in unhygienic conditions infestations can be very heavy and secondary iron deficiency anemia has been reported. Personal insect repellants and permethrin-impregnated bed nets provide some protection, but decontamination of the environment with residual insecticides may be necessary.

The role of bedbugs as potential vectors of infection remains controversial. Viable infective hepatitis B virus and other pathogens have been found in the gut of bedbugs weeks after their last blood meal. However, community-based studies have failed to confirm that bedbugs have a significant role in hepatitis B transmission,[15] and attempts at transmitting infection among a group of chimpanzees using bedbugs were unsuccessful when the bugs fed normally.[16] Although HIV has been isolated from bedbugs up to 8 days after feeding on heavily infected blood under experimental conditions, there is no evidence to suggest transmission to humans by this route.[17]

Ticks

Ticks, which are arachnids rather than insects, are cosmopolitan ectoparasites of mammals, birds and reptiles. They are hugely important in the transmission of animal as well as human disease. Ticks pass through four stages of development – egg, larva, nymph, and adult – with a blood meal required for progression. All stages of tick attach to vertebrate hosts in order to feed: some spend their entire life cycle on a single animal (one-host ticks), while others drop off before molting and then seek out a new host (multihost ticks). When not attached to feed, ticks are usually found in scrub or grassland in close proximity to their preferred hosts, or in the burrow or nest. Although ticks are relatively host-specific, most will attack humans in appropriate circumstances: this usually occurs when humans stray into the tick's home environment. Adult ticks are usually 2–5 mm in length: larvae and nymphs are smaller, and are easily missed on the skin. All stages swell enormously as they feed. Hard ticks (family Ixodidae) have a hard plate or scutum covering their back (Fig. 11.6), while soft ticks (family Argasidae) have a soft, leathery body (Fig. 11.7).

Most adult and immature ixodid ticks remain attached to the host for several days unless removed; argasid ticks feed more rapidly and may detach themselves after a few hours. The bite is often not irritating, and may pass unnoticed, especially in the early stages of attachment, which is an important factor in the role of the tick as a vector. In some cases, presumably when the host has been presensitized, tick bites can cause itchy papular lesions with evidence of a type 1 hypersensitivity reaction. Elevated levels of specific IgE against tick saliva can be demonstrated in such people.[18] Local skin reactions may occur



Fig. 11.6 Non-engorged adult female *Ixodes scapularis*, the black-legged tick or deer tick, of the family Ixodidae (hard ticks). With permission from Northwest Infectious Disease Consultants. Courtesy of Thomas R Fritshe.

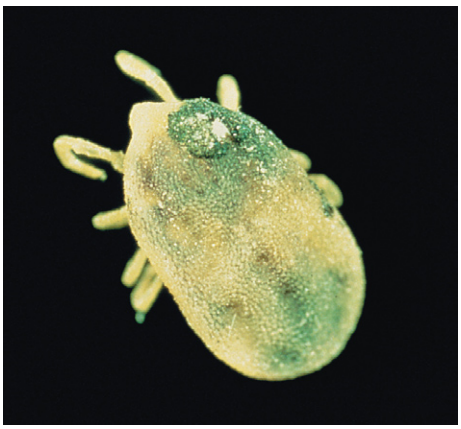


Fig. 11.7 Non-engorged adult *Ornithodoros hermsi*, of the family Argasidae (soft ticks). With permission from Northwest Infectious Disease Consultants. Courtesy of Thomas R Fritshe.



Fig. 11.8 Engorged tick after feeding on the host for several days.



Fig. 11.9 Tick-bite eschar associated with African tick typhus.

when tick attachment is prolonged (Fig. 11.8). Hypersensitive people may experience more generalized urticarial skin reactions and even systemic features of anaphylaxis (see below).

A characteristic necrotic lesion may be seen when the tick bite is associated with the transmission of certain rickettsial infections – the so-called eschar or *tâche noire* (Fig. 11.9). Ticks are biologic vectors of a number of viral and bacterial infections (see Table 11.2).

Avoidance and management of tick bites

Ticks usually prefer certain specific types of vegetation and most species are more commonly found during the spring and summer. In adults, ticks are generally found on the lower body, while children are often bitten on the head, neck and axillae. Some species of tick also have preferential sites of attachment. A knowledge of the epidemiology of local tick species, as well as the wearing of long trousers and the use of permethrin-impregnated clothing, can decrease the incidence of tick bites and their complications (see below).

Most ectoparasite vectors of infection feed rapidly, and length of time on the host probably does not influence the likelihood of infection. Ixodid ticks, by contrast, can spend many days feeding and the risk of transmission of some infections appears to be directly related to the duration of the bite. Tick paralysis is also dependent on prolonged feeding and both infectious agents and toxins may be inoculated into the host by careless removal, which can also leave the barbed mouthparts embedded in the skin. As well as predisposing to bacterial infection this can also generate a chronic granulomatous response; in some cases local surgical excision is needed to relieve the symptoms. Early detection and appropriate detachment of ticks is therefore essential, especially in regions where tick-borne diseases are endemic. The ideal method of removal is to grasp the tick mouthparts as close to the skin as possible with fine forceps or tweezers and gently lever the creature off. The body should not be squeezed, in order to prevent further inoculation. Any retained fragments should not be dug out, but the site cleaned and antiseptic applied. This method of removal is associated with a significantly lower incidence of rickettsial and borrelial infection following tick bites.[19,20]

In the particular case of Lyme disease, early antibiotic therapy after infection with *Borrelia burgdorferi* may provide some protection against the development of disease (see also Chapter 43.). Tick bites are very common, and even in areas where the majority of vector species are infected, the rate of human infection is very low.[20] However, risk of infection rises dramatically once the tick has been feeding for more than 72 hours (from about 1% at less than 72 hours to 20% at 72 hours or more).[21] The approximate duration of attachment of the tick can be estimated from an index of tick engorgement (the scutal index). There is some evidence that antibiotic prophylaxis following a tick bite in hyperendemic areas may decrease the incidence of clinical Lyme disease:[22] this is particularly likely to be justified in cases where the tick has been feeding for more than 72 hours. Other attempts at predicting high-risk groups following tick bites (e.g. by testing the tick for *B. burgdorferi* infection) have not proved successful.[21] There is no evidence that empiric antibiotic prophylaxis following tick bites has a role in the prevention of rickettsial infections such as Rocky Mountain spotted fever.[23]

Mites

Mites are tiny (≤1 mm) arachnids, most of which are free living in the environment. Many feed on other insects and larvae, but some parasitize larger animals including humans. The most important of these are trombiculid mites ('chiggers'), which are found worldwide. The adult and nymphal stages are free-living predators, but the larvae parasitize many animals, including humans. The mites inhabit areas of transitional vegetation, hence the alternative name of 'scrub mite', often forming localized 'mite islands' in areas inhabited by a host species. Larval mites climb onto a human host who is passing through the vegetation and crawl over the host's body to find a suitable area of skin to bite, such as the axillae or groins or areas of skin that are constricted by clothing. They feed for several days if undisturbed and then drop to the ground. The resulting lesions are similar to those caused by tick bites; the methods of prevention are also similar.

In certain parts of the world (principally Asia) trombiculid mites are the vector of scrub typhus.[24] Scabies and other resident parasitic mites are described below.

Infestations of resident hematophagous parasites

Most blood-feeding arthropods that attack humans are transient ectoparasites, alighting to feed and then moving on. A few stay on or close to the human host for their entire life cycle, living and breeding in the hair or clothing, and feeding frequently and repeatedly in the same area. The lifestyle of these resident parasites can cause a different and more chronic pattern of disease.

Lice

The sucking lice (order Anaplura) are dorsoventrally flattened wingless insects with characteristic claws at the end of each leg, allowing them to cling to hair or clothing. All species suck blood, and although single bites will cause typical papular lesions, repetitive feeding combined with chronic scratching and exposure to louse saliva and feces can cause a more generalized dermatitis. Head lice (*Pediculus humanus capitis*) and body lice (*P. humanus corporis*) are morphologically almost indistinguishable (Fig. 11.10), but they each tend to keep to their own territory on the host, and only the latter acts as a biologic vector of infection. Body lice cling to, and deposit eggs on, clothing fibers rather than hair shafts. They parasitize those who do not wash or change clothing, unlike the head louse which is not associated with poor hygiene alone. Pubic lice are distinctly different from the other species, and are usually confined to the pubic region.

Head lice

Children are more commonly infected with head lice than adults, and women are more commonly affected than men, at least in Western cultures. This is because the vast majority of infections are acquired by direct head-to-head contact, which is more likely in these groups. Overcrowding is also an important risk factor, but hair length is not. Head lice are more common in late summer, which probably reflects the need for high ambient temperature to hatch eggs. The role of fomites in transmission is controversial; there is little evidence that hats and brushes are important. The main feature of head louse infestation is scalp itching, although secondary bacterial infection is not uncommon, and louse infection should be looked for in cases of scalp impetigo. Chronic infection can produce cervical lymphadenopathy. The definitive diagnosis can only be made by finding live adult lice in the hair. The presence of empty egg-cases ('nits') stuck to the base of hair follicles indicates recent infection, but nits may still be seen in successfully treated cases (Fig. 11.11). In heavy infestations, developing eggs, nymphs and adult lice may all be seen: the highest concentration of eggs is usually in the occipital and parietal regions.



Fig. 11.10 Adult *Pediculus humanus corporis* (the body louse) feeding. Courtesy of Dr H Lieske. With permission from Peters W, Pasvol G. Tropical medicine and parasitology, 5th ed. London: Mosby-Wolfe; 2002.

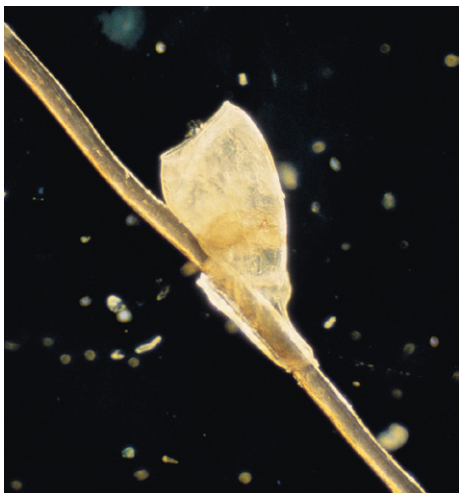


Fig. 11.11 Egg or nit of human head louse attached to a hair shaft. Note that the operculum is missing indicating previous emergence of the larval louse. Courtesy of Thomas R Fritsche.

Traditional combing methods (either wet or dry) can be effective, but they are very time consuming and require good technique and several repetitions to be of any benefit. Several insecticides are available for the treatment of head lice (Table 11.3).[25,26] The acetylcholinesterase inhibitors malathion and carbaryl both have reasonable ovicidal and pediculicidal activity, although some resistance has been seen. Malathion, unlike carbaryl, has a residual action of several weeks, but two applications 10 days apart are necessary whichever agent is used. The synthetic pyrethroids, such as permethrin, also have good activity, although there are concerns about the rapid development of resistance to these compounds. Topical ivermectin has also been used with good effect, although there is little evidence to suggest that it is preferable to other agents. Dimeticone kills lice by physically coating them rather than by a toxic effect, and is therefore not susceptible to the development of resistance.[27] It appears to have greater efficacy and fewer adverse events than malathion.[28]

Malathion and carbaryl need to remain on the scalp for 8–12 hours before they are washed off and they are degraded by high temperatures (e.g. as occurs during the use of a hair dryer). Pyrethroids need only a short application. Lotion or mousse preparations are generally preferable to shampoos, although some commercial preparations contain inadequate concentrations of active compound and are not recommended.[26] There are numerous trials comparing different drugs and preparations, but it is very difficult to draw general conclusions.[29,30] Several of the available treatments have reasonable efficacy and safety profiles,[31] but current British guidelines recommend using one of three agents: dimeticone, phenothrin or malathion.[26] The practice of rotating the locally recommended treatment to prevent resistance is not effective and should no longer be used. Systemic therapy has occasionally been used to treat head lice. Trimethoprim–sulfamethoxazole and ivermectin have been reported to be effective, although repeated doses are necessary because they affect only the feeding stages of the parasite. In most cases topical treatment should be adequate. Older drugs, such as organochlorines and mercury-based preparations, are less effective and more toxic.

Table 11.3 Commonly used topical preparations for the treatment of ectoparasite infestations

Class	Agent	Uses	Toxicity	Comments
Acetylcholinesterase inhibitors	Malathion	Scabies Head lice* Pubic lice		Good residual protection Recommended for pubic lice infestation of eyelashes Safe in pregnancy
	Carbaryl	Head lice Pubic lice	Carcinogenic in animals; minimal risk to humans in therapeutic doses	Prescription only in the UK
Organochlorines	Lindane	Head lice Pubic lice	Neurotoxic (potential for systemic absorption)	Increasing resistance No longer available in the UK
Natural pyrethroids	Pyrethrin	Head lice Pubic lice		Less evidence of efficacy than synthetic pyrethroids
	Phenothrin	Head lice* Pubic lice		
Synthetic pyrethroids	Permethrin	Scabies Head lice Pubic lice	Rarely rash and local edema	Probably safe in pregnancy and breast-feeding (limited data)
Others	Dimeticone	Head lice*		Physically coats lice rather than toxin More effective than malathion in randomized controlled trial No resistance
	Benzyl benzoate	Scabies	Skin irritation	Limited safety data in pregnancy; avoid in breast-feeding
	Ivermectin	Head lice Scabies		Available for topical and systemic use
	Crotamiton	Head lice Scabies		Relatively poor efficacy Avoid in pregnancy
	Mercury preparations	Head lice	Contact dermatitis Systemic toxicity	Available over the counter in some European countries
	Monosulfiram	Scabies	'Antabuse' effect (alcohol should be avoided)	No longer available in the UK
	Sulfur ointment	Scabies	Skin irritation	Cheap, safe, reasonably effective

*Recommended treatments for head lice in the United Kingdom.[26]

Body lice

Body lice are associated with poor hygiene and unwashed clothing and are principally parasites of vagrants and refugees. They can be found worldwide, but are more common in cooler climates where clothing is rarely removed. Transmission is by direct body-to-body spread or by sharing infected clothing. Initially the bites are similar to those of other hematophagous ectoparasites, but the prolonged and persistent nature of the infestation leads to widespread excoriation and secondary infection, and eventually to the hyperpigmented chronic skin condition known as 'vagabond's disease' or morbus errorum.

Body lice are important vectors of infection (see Table 11.2), and have been responsible for major outbreaks of disease such as typhus amongst refugees, soldiers and displaced populations. The diagnosis is made by finding lice and eggs in the clothing (particularly along seams), and treatment should be directed at the clothing rather than the patient. High-temperature washing, tumble-drying and malathion dusting powder are all effective at clearing garments of lice; permethrin-treated clothing may be protective for those at risk of infestation.

Pubic lice

Pthirus pubis (often written as *Phthirus pubis*) is morphologically different from *Pediculus humanus* and is unique to humans (Fig. 11.12).



Fig. 11.12 The 'crab louse', *Phthirus pubis*, infests not only the pubic region but also other sites, including the eyelashes. Courtesy of Dr H Lieske. With permission from Peters W, Pasvol G. Tropical medicine and parasitology, 5th ed. London: Mosby-Wolfe; 2002.

Infection is usually confined to the pubic region, although lice and eggs are sometimes found in axillary and facial hair, eyelashes, eyebrows and (especially in children) the scalp. Infection is transmitted by close, usually sexual, contact and may be associated with other sexually transmitted diseases.

The main symptom is itching of the affected area, especially at night, although there may be few visible skin lesions. Close inspection may reveal eggs that are attached to the hair shafts, with adult lice clinging on close to the skin. Treatment is with the same insecticides as for head lice, with the proviso that alcohol-based preparations may irritate sensitive skin and mucous membranes. This is particularly true for the eyes, and eyelash infestations should always be treated with an aqueous formulation. It is usually advisable to treat the whole trunk and limbs in view of the possible spread to other hairy areas; where appropriate, sexual contacts should also be treated.[32]

Locally invasive ectoparasites

Strictly speaking the term 'ectoparasite' is reserved for those organisms which stay on the surface of the skin. However, there are a few arthropod parasites which spend some or all of their life cycle burrowing within or beneath the superficial layers of the skin, and the definition can usefully be stretched to include these locally invasive species. In some cases (e.g. the myiatic flies), only a specific and limited part of the life cycle is spent as a parasite, while in others such as the scabies mite the entire life cycle takes place within the host skin.

Myiatic flies

The house fly, *Musca domestica*, has no requirement for developing in mammalian tissue, but is occasionally found in dead tissue or under plaster casts. Facultative myiasis is most often caused by blowflies and flesh flies, which ordinarily feed on dead tissues, but may move into adjacent viable tissues or infest exposed ulcers or traumatic wounds. Risk factors in developed countries include homelessness, alcoholism and peripheral vascular disease,[33] and myiasis may occasionally complicate chronic otitis media in children[34] and neglected oral lesions.[35] The beneficial effects of larval therapy have been known to battlefield surgeons for centuries. Deliberate use of such 'biosurgery' is becoming increasingly acceptable to clean difficult wounds, including those that are complicated by multidrug-resistant bacterial infection. The flies most often used are the facultative calliphorids, with the greenbottle blowfly (*Lucilia sericata*) being the most widely used species.[36]

A few species of fly are specifically adapted to parasitize living flesh. These myiatic flies are confined to specific geographic locations, mainly in the tropics, and are quite often found in travelers returning from these areas.[37] Myiatic flies are of major veterinary importance and cause significant mortality in domestic livestock.

There are two main species of fly causing invasive human myiasis: the Tumbu or mango fly (*Cordylobia anthropophaga*) which is extensively distributed in sub-Saharan Africa, and the human botfly (*Dermatobia hominis*) which is found in Central and South America. Various other species which are primarily animal parasites may occasionally infect humans, such as the screwworm, *Cochliomyia hominivorax*. The modes of infection for the two common species are different. The eggs of *C. anthropophaga* are laid on the ground, or sometimes on bedding or clothing that has been left on the ground. Once hatched, the larvae seek out warm skin which they can rapidly penetrate. Infection can thus be avoided by not lying on, or laying out clothing on, potentially contaminated ground, or by ironing clothes that have been exposed. In contrast, the eggs of *D. hominis* are laid on the body of certain species of biting fly, and infection occurs when the host takes a blood meal: prevention of infection is thus more difficult.

The clinical features of furuncular myiasis are similar for all species. A small red papule develops within about 24 hours of infection, and gradually swells over the next few days as the larva develops. A dermal cavity containing the maggot is surrounded by an intense inflammatory response, which may be very itchy and painful. A hole appears at the centre of the swelling, through which the posterior tip of the larva (including its breathing spiracle) can be seen. After about 2 weeks in the case of *C. anthropophaga*, but up to 4 or more weeks in *D. hominis*, the mature larva forces its way out and drops to the ground. If there is no secondary infection the wound rapidly heals.

The diagnosis of myiasis is clinical, based on the appearance and history. Traditional methods of removal include blocking the surface hole with petroleum jelly, pork fat or nail varnish (which is useful in difficult to reach areas such as the scalp) and then grabbing the parasite as it pushes its spiracle out of the hole (Fig. 11.13). This is more effective for *C. anthropophaga*, but less successful for botfly infections, as the larva is flask-shaped and difficult to extrude. Minor surgery under local anesthetic is often needed to remove these (Fig. 11.14). Ivermectin has been used in refractory cases.[35] Secondary infection may necessitate antibiotic treatment. Tetanus has occasionally been reported as a complication of myiasis.

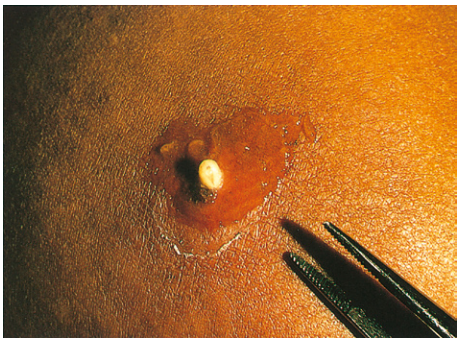


Fig. 11.13 Extracting a larva of *Cordylobia anthropophaga* after covering it with paraffin. The pair of black spiracles can just be seen in the center of the posterior tip of the larva. Courtesy of Professor A Bryceson. With permission from Peters W, Pasvol G. Tropical medicine and parasitology, 5th ed. London: Mosby-Wolfe; 2002.



Fig. 11.14 Larva of the botfly *Dermatobia hominis* after surgical removal from the leg of a patient who had visited Belize (preserved specimens). Note flask shape of maggot, with bulbous portion at base of wound.

Jigger fleas

The jigger (or chigoe) flea, *Tunga penetrans*, has a wide distribution in Central and South America, Africa and parts of the Indian subcontinent. Unlike all other species of flea, the female jigger flea incubates her eggs while living within the dermis of a mammalian host. The gravid flea burrows into the skin until only the posterior end, bearing the respiratory spiracles, is protruding. While the flea feeds and grows the egg sac develops and gradually releases its contents back onto the ground.

Infection with *T. penetrans* results in small painful nodules, most commonly found on the sole of the foot or under a toenail. Multiple lesions are common. A small black spot can often be seen at the centre of the swelling: this is the site of the projecting spiracles. Secondary bacterial infection is common, and tetanus is a particular hazard. The diagnosis is made on the basis of appearance and epidemiology, although the lesions may sometimes be mistaken for plantar warts. The fleas can sometimes be shelled out carefully with a moderately sharp instrument such as a toothpick, but care should be taken not to disrupt the flea, and surgical excision under local anesthetic may be required (Fig. 11.15).

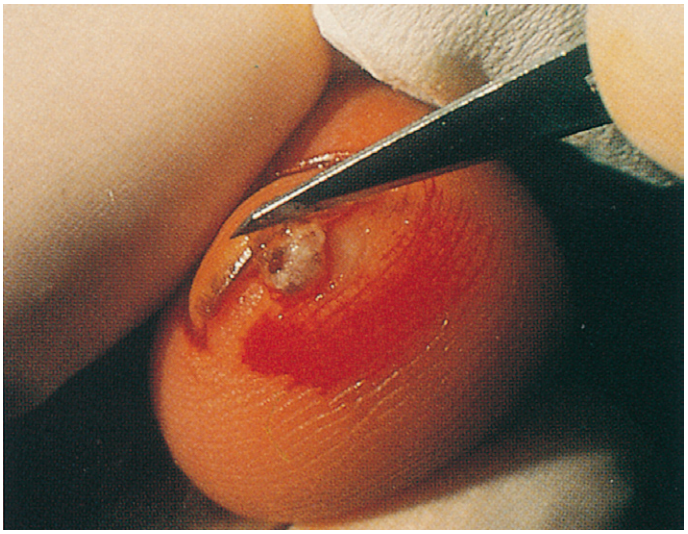


Fig. 11.15 Surgical extraction of a gravid female *Tunga penetrans*. Care must be taken not to disrupt the abdomen and release the eggs. Courtesy of Professor C Curtis. With permission from Peters W. A colour atlas of arthropods in clinical medicine. London: Wolfe Publishing Ltd; 1992.

Scabies and pseudoscabies

Some infesting arthropod ectoparasites do not feed on blood at all, but cause disease in other ways. The most important of these is *Sarcoptes scabiei*, the human scabies mite, which is found worldwide in conditions of poor hygiene (Fig. 11.16). This skin-burrowing mite is not a blood feeder, but ingests predigested dermal cells as it tunnels through the epithelium. Female mites live in small burrows in the skin, which they extend by 2–3 mm daily, leaving a trail of eggs and feces behind them. The burrows are intensely itchy and are responsible for the features of scabies. They are usually found around the wrists, web spaces, toes and genitalia, although they may be more widespread; small papules are sometimes seen at the distal end adjacent to the female mite. Infestations are usually relatively light, with an average of 12 adult females found on an infected patient. Crusted scabies (also known as Norwegian scabies), in which there is heavy mite infestation and gross hyperkeratosis, may be seen in the elderly and in immunocompromised and institutionalized patients (Fig. 11.17). (See also Chapter 95.) Conversely, symptoms of scabies may be masked in some patients taking steroids.[38,39]



Fig. 11.16 Adult *Sarcoptes scabiei*, the human itch or mange mite, seen in skin scrapings. With permission from New England Journal of Medicine 1994;331:777.

Diagnosis based on history and clinical features alone can be difficult and should be confirmed whenever possible by identification of mites. This is usually done by extracting parasites with a needle or in skin scrapings, but these techniques require operator experience and visible burrows. Newer methods of diagnosis include high-magnification videodermatoscopy, epiluminescence microscopy and polymerase chain reaction but none of these is widely available.[40] Treatment should be given if scabies is suspected, even if the diagnosis cannot be confirmed.

Scabies is usually treated with topical acaricides (see Table 11.3). Comparative trials suggest that 5% permethrin is the most effective treatment, with 0.5% malathion the second choice; permethrin is considerably more expensive.[32,41] Oral ivermectin has been increasingly used in recent years, especially in crusted or recurrent infections. It is well tolerated and appears to be highly effective, although a second dose may be needed 1 week after the first. Although unlicensed, oral ivermectin is probably the treatment of choice in recurrent cases, institutional outbreaks, and crusted scabies; combining systemic treatment with a topical preparation may give the best results in hyperkeratotic disease.[42,43] There is clinical and molecular evidence of emerging resistance to ivermectin and 5% permethrin, with renewed interest in the role of traditional remedies such as tea tree oil and other alternatives.[40] Crusted scabies is highly contagious, and can cause large nosocomial outbreaks of infection in staff and other patients. Inpatients should be nursed in isolation, and contact with other immunosuppressed people should be avoided. Bedding and clothing should be boil-washed or treated.



Fig. 11.17 Crusted Norwegian scabies in a patient who has AIDS. With permission from New England Journal of Medicine 1994;331:777.

Sarcoptid mites are relatively host-specific and mites acquired from animals do not usually cause prolonged infestations of humans. However, people in close contact with infected livestock (notably pigs, cows and dogs) may acquire temporary infestation, causing pruritic lesions but lacking the typical burrows of human scabies. In most cases no treatment is required except for avoidance of the source of infection.

NONINFECTIVE SYSTEMIC PROBLEMS

Tick paralysis/toxicosis

Paralysis and death of domestic animals and occasionally humans following tick bites has been documented in Australia and South Africa since the end of the 19th century and more recently it has been observed in North America and Europe. This syndrome is not, as once thought, an infective process, but is caused by a toxin in the saliva of the pregnant females of certain ixodid species. Children are more often affected than adults and the condition is most commonly seen in spring and early summer.

Tick paralysis almost always results from bites on the head, typically behind or within the ear, where the tick may not be noticed. Symptoms appear only after the tick has been feeding for at least 48 hours. The usual presentation is an ascending flaccid paralysis, frequently with cranial nerve involvement, although atypical features such as isolated nerve lesions and cerebellar signs are occasionally seen. There is no sensory loss or decrease in consciousness; however, if the condition is untreated, death from respiratory failure can supervene. In the North American form the paralysis starts to improve within a few hours of removing the tick, but in the Australian form of the disease deterioration may continue for a further 24–48 hours.[44]

The neurotoxin involved probably varies between different tick species. It has not been fully characterized, but there are many clinical and neurophysiologic similarities to the effects of botulinum toxin.[44] The essential treatment is to remove the tick carefully to prevent further transfer of toxin, followed by supportive care until the neurologic signs resolve. Although rare, the diagnosis should be considered in any progressive flaccid paralysis in an endemic area.[45]

Tick anaphylaxis

The local allergic urticarial reactions to tick bites have already been discussed. Rarely, in hypersensitive people, anaphylaxis can follow a bite, with bronchospasm, edema, and hypotension. This is an IgE-mediated response to tick salivary antigens, which can occur soon after the tick first bites or following careless removal.[46] Treatment is as for any form of anaphylactic reaction and sensitive people in tick-infested areas may benefit from carrying adrenaline (epinephrine) for inhalation or self-injection.

Delusional states

Patients with delusions of parasitosis present a difficult challenge. These people believe they are infested with a variety of bugs, and often give graphic descriptions of sensations of things crawling under their skin and/or emerging from their body. They appear in the clinic with a sample of the offending item, which invariably turns out to be fibers, hairs, pieces of tissue paper or bits of skin.[47,48] This is a monodelusional state that has a poor prognosis and may continue for years. Chronically affected patients often have multiple lesions of nodular prurigo, sparing areas of the skin that they cannot reach. A variant of this syndrome has acquired quasi-respectability under the name Morgellon's disease, in which the patient describes small fibers extruding from the skin.[49]

Management requires a sympathetic approach, with careful microscopic examination of the supposed ectoparasites and explanation that they are not genuine parasites. However, it is extremely difficult to persuade patients that they are not infested, and multiple consultations with successive specialists are common. Treatment with psychotropic agents such as risperidone or pimozide (under careful supervision) is often effective, but this approach is rarely acceptable to the patient.[50]

ECTOPARASITES AS VECTORS OF INFECTION

Although arthropod ectoparasites directly cause a wide variety of local and systemic illnesses, their principal medical importance is as biologic vectors of infection. Through blood feeding (and occasionally other routes) ectoparasites can transmit a range of viral, bacterial and protozoan parasites, including some of the most globally significant infectious diseases of humans: these are listed in Table 11.2. Details of the infections themselves, and of the biologic role of the parasite in their transmission, will be found in the relevant chapters.

PREVENTION OF BITES

Principles of prevention are directed at limiting skin and mucous membrane exposure to crawling and flying arthropods, by avoiding locations serving as their habitat, such as gardens, fields, orchards or unclean picnic areas that attract bees, wasps, and yellowjackets; crawl spaces, wood sheds or outdoor privies, which may harbor venomous spiders or scorpions; domiciles conducive to cockroach, bedbug, or kissing bug habitation; and outdoor environments known to be a source of ticks and biting diptera.

Remaining indoors during evening hours lessens the risks associated with exposure to night-biting mosquitoes. Light-colored clothing, especially long trousers, long-sleeved shirts and closed-toed shoes when working outdoors or pursuing outdoor recreation is less attractive than dark clothing, short pants, short-sleeved shirts and opened-toed shoes to flying insects, and allows the wearer to more readily recognize the presence of ticks and other crawling arthropods. In tick-infested areas, trousers should be tucked into socks to reduce tick access, and can be dusted with pesticides.

A variety of chemical repellents are available.[51] Those containing DEET (*N,N*-diethyl-M-toluamide) are among the most widely used and come in a variety of preparations for use on clothing and skin in concentrations varying from about 10% to 50%. DEET repellents should not be used on babies younger than 2 months. Fifty per cent strength DEET repellents can be used by pregnant or breast-feeding women, and on babies and children older than 2 months. Alternative repellents using synthetic compounds, such as picaridin and natural ingredients such as lemon eucalyptus oil are available for those who prefer not to use DEET products. In all cases, the user should read the manufacturer's instructions and follow them carefully. There is no evidence to support the use of garlic or vitamin B.

The introduction of permethrin-impregnated bed nets has revolutionized the control of malaria in both highly endemic and moderately endemic areas of the world and is a major feature of current control programs.[52,53] This has been shown to reduce maternal and infant mortality[54] and also reduces the prevalence of other nuisance arthropods such as bedbugs.[55] Permethrin also kills sandflies, which are small enough to get through the mesh of bed nets. Knock-down sprays and mosquito coils are also useful to kill mosquitoes in the room, but electronic buzzers are useless.[56]

Practical, detailed advice on bite avoidance measures for travelers is readily available from several travel health websites.[57,58]

REFERENCES

References for this chapter can be found online at http://www.expertconsult.com

Chapter 12

Anthony C Chu
Danielle T Greenblatt

Dermatologic manifestations of systemic infections

INTRODUCTION

The skin is the largest and most visible organ of the body. In addition to its role as a barrier separating the body from the external environment and its role in temperature regulation, the skin has a complex immune system that recognizes and attacks foreign antigens and microbes.

The skin may be affected by systemic infections in three ways:

- by direct involvement by the infectious agent;
- by specific reaction to an infection; and
- by nonspecific reaction to an infection.

In addition, there are a number of inflammatory dermatoses that can mimic skin infections.

DIRECT INVOLVEMENT OF THE SKIN BY AN INFECTIOUS AGENT DURING A SYSTEMIC INFECTION

Viral infections

Chickenpox

Viral infection of the skin as part of a systemic infection is well demonstrated by chickenpox. After an incubation period of 14–21 days the patient develops 1–2 days of fever and malaise. This is followed by crops of unilocular vesicles, which quickly become pustular, appearing over 2–4 days. After the acute infection the virus persists in dorsal root nerve ganglion cells and on reactivation of the residual latent virus, herpes zoster or shingles develops.

Hand, foot and mouth disease

Hand, foot and mouth disease is caused most commonly by coxsackievirus A16 and enterovirus 71.[1] It occurs predominantly in children in both sporadic and epidemic forms. After an incubation period of 5–7 days the patient develops painful stomatitis with oral vesicles that ulcerate. Small, thin-walled vesicles may later develop on the fingers and toes. Onychomadesis is a rare complication due to nail matrix arrest leading to nail shedding from the proximal portion.[2] Disease related to coxsackievirus infection is generally mild and self-limiting; however, cardiopulmonary failure and neurologic sequelae may follow enterovirus 71 infection.[3] Viral particles can be identified in the vesicles on electron microscopy.

Bacterial infections

Gonococcal infection

In disseminated gonococcal infection caused by *Neisseria gonorrhoeae*, characteristic skin lesions (called septic gonococcal dermatitis) may be observed. One or more crops of three or four macules or papules develop often over the extremities; these then become pustular or bullous. Occasionally, gonococci can be cultured from the skin lesions.

Tuberculosis

In tuberculosis, skin involvement may occur as the result of contiguous involvement of the skin from underlying lymph nodes, joints or bones, a condition called scrofuloderma.[4] A bluish-red nodule develops over the affected bone, joint (Fig. 12.1) or lymph node and multiple fistulae develop. Diagnosis must be confirmed by biopsy, which shows tuberculous granulation tissue. *Mycobacterium tuberculosis* can often be cultured from involved tissue.

Cutaneous involvement in tuberculosis may also occur secondary to hematogenous dissemination, so-called tuberculosis cutis miliaris disseminata.[5] In miliary tuberculosis, hematogenous dissemination of bacilli to the skin can produce profuse crops of bluish papules, which may become vesiculopustular and finally necrotic, leading to ulceration.



Fig. 12.1 Scrofuloderma in a 60-year-old patient. A biopsy confirmed tuberculoid granulation tissue and the patient responded to antituberculous therapy.

Chronic hematogenous dissemination of tubercle bacilli in patients with moderate or high degrees of immunity may present as one of the tuberculides, which are regarded as localized hypersensitivity reactions to *Mycobacterium tuberculosis*:

- papulonecrotic tuberculid, in which there are symmetric crops of necrotic papules predominantly affecting the extremities;
- lichen scrofulosum, in which minute lichenoid papules appear predominantly on the trunk rather than on the limbs;
- erythema induratum (or Bazin's disease), in which persistent or recurrent nodular lesions appear in the calves of the legs and may lead to ulceration (Fig. 12.2); and
- nodular tuberculid, a more recently described form in which nonulcerating red or bluish nodules present on the lower limbs. This form is considered a hybrid between papulonecrotic tuberculid and erythema induratum with distinctive histologic features.[6]

The tuberculides respond rapidly to antituberculous therapy.

Spirochetal infections

Disease caused by spirochetes tends to affect the skin as part of the primary manifestation, but it may also involve the skin during subsequent, disseminated disease.

Syphilis

In syphilis, the primary lesion or chancre is cutaneous or mucosal, occurring at the site of inoculation. Secondary syphilis starts approximately 3 months after the primary infection and gives rise to nonirritating, coppery red symmetric lesions, which start as macules and become papular. Secondary syphilis is the 'great pretender'[7] and lesions of secondary syphilis can mimic acne, psoriasis and a number of other nonspecific dermatoses. Characteristically, the palms and soles are affected. When mucosal surfaces are involved, 'snail track' ulcers may develop. Later, condylomata may occur perianally and on the vulva or penis. Patchy hair loss is a characteristic sign of secondary syphilis, giving rise to a moth-eaten appearance of the scalp.

Late or tertiary syphilis occurs after a latent period of up to 20 years. Both skin and mucous membranes may be affected. Nodular syphilides present as nodular subcutaneous lesions appearing in groups and tending to develop a circinate arrangement. These are more common on the extensor surfaces of the arms, the back and the face (Fig. 12.3), but they may occur in the oral cavity. Gummas are masses of syphilitic granulomatous tissue; they may originate in the subcutis, underlying bone or muscle. These masses ulcerate to produce punched-out cutaneous defects.



Fig. 12.3 Tertiary syphilis on the face of a 56-year-old woman.

Yaws

Yaws is caused by the spirochete *Treponema pertenue*. The primary lesion of yaws produces a cutaneous erythematous papule, which becomes papillomatous and resembles a raspberry, giving rise to its name, 'frambesia'. After 2–4 months the secondary eruption of yaws occurs, with multiple small papules developing into exudative papillomas (Fig. 12.4). Mucosal involvement does not occur in yaws. After 6 months to 3 years, tertiary yaws occurs; this is characterized by ulcerated nodular and tuberous cutaneous lesions and keratoderma of the palms and soles.

Pinta

Pinta is caused by the spirochete *Treponema carateum*. The initial eruption starts in the skin as multiple erythematous papules and plaques. This is followed after months or years by generalized cutaneous lesions, where the skin becomes pale, pigmented or erythematosquamous. The late phase occurs 2–5 years after primary infection with irregular pigmentation, which can be grayish, steely or bluish in color. Areas of leukoderma, particularly around the elbows, knees, ankles and wrists, may develop. Hyperkeratosis occurs, particularly on the legs and arms, and is associated with areas of atrophy, particularly around the large joints.

Lyme disease

In infections with *Borrelia burgdorferi*, the primary lesion occurs at the site of the Ixodes tick bite, with a characteristic eruption: erythema migrans.[8] The macular erythema starts up to 36 days after the bite and



Fig. 12.2 Erythema induratum on the back of the leg of a 45-year-old woman.



Fig. 12.4 Secondary yaws showing papular and vegetative lesions on the anterior chest wall.

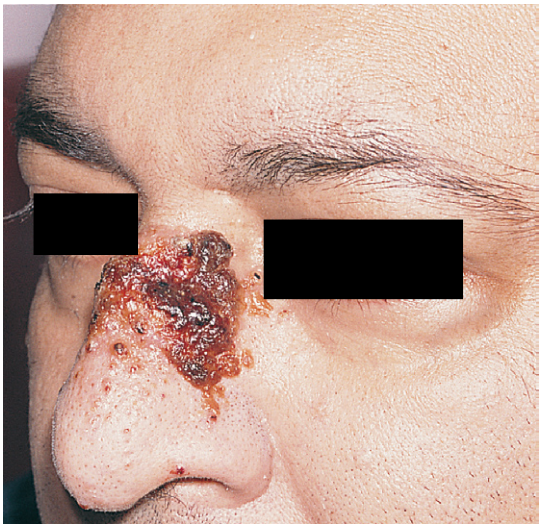


Fig. 12.5 Cutaneous cryptococcosis in a renal transplant patient. These lesions started as nodules that then rapidly ulcerated. CT scan of the patient's brain showed no abnormality but *Cryptococcus neoformans* was isolated from cerebrospinal fluid.

slowly increases in size by several centimeters each week. Subsequent dissemination in untreated patients leads to Lyme disease with involvement of the nervous system, heart and joints.[9]

One year or longer after the original infection, a late cutaneous manifestation may occur: acrodermatitis chronica atrophicans.[10] As the name suggests, this typically affects the hands and feet, but the elbows and knees may also be affected. Erythematous plaques develop, slowly enlarge and become atrophic. *B. burgdorferi* may be cultured from skin biopsies in this condition, though often with difficulty. Diagnosis therefore relies on clinical features supported by serologic testing and detection of *B. burgdorferi*-specific DNA in lesional skin biopsies by polymerase chain reaction.[8]

Fungal infections

A number of deep fungal infections may have cutaneous involvement in the course of systemic disease. These include blastomycosis, coccidioidomycosis, paracoccidioidomycosis, histoplasmosis, cryptococcosis and disseminated candidiasis.

Cutaneous manifestations tend to be nonspecific, with papules, nodules and ulcers developing on different parts of the skin. In disseminated blastomycosis cutaneous lesions start as papules or nodules, which then ulcerate and evolve into serpiginous lesions with raised warty borders. Disseminated coccidioidomycosis may result in cutaneous abscesses, granulomas and discharging sinuses, often on the face.

Disseminated cutaneous cryptococcosis is observed in immunocompromised patients, particularly those with AIDS. It presents as erythematous papules and nodules, which become exudative and eventually ulcerate (Fig. 12.5). Molluscum contagiosum-like lesions are a recognized feature of this disease in AIDS patients. These typically occur around the nose and mouth and punched-out ulcers with rolled edges may develop.

SPECIFIC SKIN REACTIONS RESULTING FROM SYSTEMIC INFECTIONS

Systemic infections with viruses and bacteria can occasionally cause specific cutaneous reactions. These skin reactions can establish the diagnosis of the specific systemic infections and it is thus very important to recognize them.

Viruses

Roseola infantum and pityriasis rosea

Infection with human herpesvirus 6 (HHV-6) and HHV-7, usually in the first 3 years of life, gives rise to a specific dermatitis: roseola infantum (exanthem subitum).[11,12] After an incubation period of 10–15 days, fever starts abruptly and lasts for 3–5 days. Ulcers at the uvulopalatoglossal junction may be an early sign of roseola infantum.[13] As the fever subsides, a maculopapular eruption, which is characteristically rose pink in color, develops on the neck and trunk. This later spreads to the proximal extremities and face. The skin eruption subsides after 1–2 days, leaving no pigmentation or scaling of the skin. In common with other herpesviridae, latency is established. Reactivation in the immunocompromised host is linked to various diseases, including encephalitis.

A similar eruption, known as pityriasis rosea, is observed in adults. There is generally no prodromal syndrome but patients develop a single erythematous macular lesion, which may reach several centimeters in diameter, most commonly on the trunk, thigh or upper arm. The macule has a characteristic collarette of fine scale. This herald patch is followed after 5–15 days by a widespread eruption of small erythematous, scaly macules, which typically form a Christmas-tree pattern on the trunk and eventually spread down the limbs, usually resolving after 6 weeks. A viral etiology is suspected in this condition, although the roles of HHV-7 and to a lesser extent HHV-6 remain controversial.[14]

Cutaneous changes associated with HIV infection are discussed in Chapter 95.

Bacterial infections

Specific bacterial infections may also cause a variety of cutaneous syndromes as a result of toxin production.

Scarlet fever

Scarlet fever complicates acute infections by group A β-hemolytic streptococci that produce pyrogenic exotoxins. Production of the exotoxin depends on the presence of a temperate bacteriophage and is exclusive to group A streptococci. Three antigenically distinct exotoxins can be produced: types A, B and C. After an incubation period of 2–5 days, fever develops with localized signs at the portal of entry (e.g. tonsillitis and lymphadenopathy or tenderness at a wound site).

The eruption of scarlet fever occurs on the second day of infection. It begins on the upper trunk with punctuate erythema that becomes generalized over a few hours to 3–4 days. A characteristic sign, known as Pastia's lines, results from capillary damage and is characterized by transverse red streaks at the sites of skin folds. The face is erythematous but with a characteristic perioral pallor. After 7–10 days the eruption subsides with desquamation of the palms and soles.

Staphylococcal scalded skin syndrome

Staphylococcal scalded skin syndrome is a blistering skin disorder caused by epidermolytic toxins produced by *Staphylococcus aureus*. Two major exfoliative toxin serotypes (ETA and ETB) have been identified.[15] Studies have shown that exfoliative toxins cleave desmoglein 1, resulting in loss of keratinocyte cell to cell adhesion within the epidermis.[16] Histologically, the epidermis is cleaved below the granular cell layer, resulting in the typical scalded skin appearance. The eruption usually starts suddenly with erythema and tenderness of the skin. Flaccid blistering occurs, which often rubs off, leaving raw exudative areas. This syndrome usually carries an excellent prognosis with resolution in 7–14 days following antibiotics.

The disease is one of infants and children. In the few reports of adults who have staphylococcal scaled skin syndrome there is generally an underlying medical problem, such as renal failure or immunosuppression, and it is thought that reduced clearance of the toxin by the kidneys in these patients may be important in the development of the disease.

Toxic shock syndrome

The toxic shock syndrome is recognized as a complication of toxic shock syndrome toxin-1 (TSST-1) production by selected strains of *S. aureus*. Cases have been reported following tampon use, and postcontamination of surgical or traumatic skin wounds and burns. Involvement of the skin, oral mucosa and conjunctiva is common. Dermatologic manifestations include diffuse erythema, punctate lesions and petechiae with subsequent palmoplantar desquamation and occasional nail loss.

Rheumatic fever

Rheumatic fever is caused by an abnormal immunologic reaction to previous infection with group A β-hemolytic streptococci. A specific (although now rare) cutaneous manifestation of rheumatic fever is erythema marginatum rheumaticum.[17] This consists of rings or arcs of pale, dull red erythema, which are either macular or slightly thickened. The rings make up a discrete or enlarged polycyclic pattern. These rings characteristically fade over a few hours or days and appear in recurrent crops, usually at different sites, over many weeks.

Sepsis

Sepsis caused by a variety of bacteria can cause disseminated intravascular coagulation. This results in hemorrhagic skin lesions, particularly of dependent areas, followed by purpura and cutaneous necrosis. In acute meningococcal sepsis, so-called purpura fulminans predicts poor outcome and signifies rapidly progressive infection.[18] Septicemia and purpura fulminans may also uncommonly be caused by infection with *Capnocytophaga canimorsus* following a dog bite. This Gram-negative bacterium is part of the normal oral flora of dogs and septicemia in adults most often occurs in the setting of immunocompromise – usually because of previous splenectomy, alcoholism or glucocorticoid use[19] (Fig. 12.6).

Henoch–Schönlein purpura

Henoch–Schönlein purpura (HSP) is an acute small vessel vasculitis, seen mainly in children and often following an upper respiratory tract infection. Although most cases are not directly linked to streptococcal infection, a substantial minority of patients with HSP have coexistent or previous streptococcal infection, as evidenced by positive throat culture (up to 30%) and raised anti-streptolysin O (up to 50%).[20] Henoch–Schönlein purpura has a characteristic appearance, with palpable purpuric papules developing on the lower legs and buttocks. This may be associated with arthritis, gastrointestinal syndromes and renal disease. Histologically, there is a leukocytoclastic vasculitis and characteristic deposition of IgA within the walls of affected blood vessels.

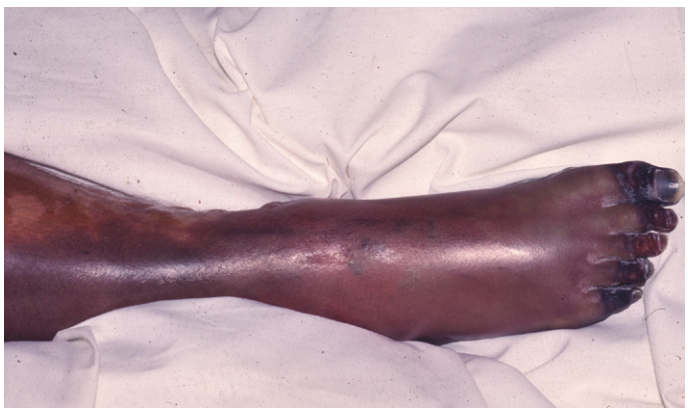


Fig. 12.6 Purpura fulminans associated with *Capnocytophaga canimorsus* infection. Courtesy of Jos WM Van der Meer.

Leprosy

Mycobacterium leprae affects the skin in all forms of leprosy. In tuberculoid leprosy (paucibacillary) the skin lesions tend to be few or solitary. They are often hypopigmented but they may have an indurated coppery color or a purple border. These patches tend to be hypoesthetic and dry with loss of hairs. In lepromatous leprosy (multibacillary) multiple small macules, papules and nodules develop in a symmetric manner in all sites apart from the hairy scalp, the axillae and the groin, where the temperature tends to be higher. Patients often develop leonine facies because of diffuse involvement of the facial skin (Fig. 12.7). Borderline types of leprosy have clinical features between these extremes of immunologic reaction to the causative agent.

Two types of immunologically mediated inflammatory reactions can occur during the course of leprosy. Type 1 leprae (reversal) reactions occur in borderline disease and are associated with upgrading of cell-mediated immunity. Existing skin lesions may become more inflamed, ulcerated and new lesions may appear. This is associated with acute or insidious pain and tenderness of affected nerves. Type 2 leprae reactions occur in patients who have lepromatous leprosy and borderline leprosy. Immune complexes form and a systemic inflammatory reaction ensues. The most common cutaneous manifestation is that of erythema nodosum leprosum, in which painful red nodules occur in the skin, most commonly on the face and extensor surfaces of the limbs.[21] Individual lesions may ultimately ulcerate. Erythema nodosum leprosum may be accompanied by uveitis, myositis, lymphadenitis, neuritis, dactylitis, arthritis and orchitis.

The Lucio phenomenon is another type 2 leprae reaction. Here, a deep cutaneous necrotizing vasculitis leads to infarction of overlying skin. Irregular erythematous patches develop and these may necrose to leave deep painful ulcers.[22]

Ecthyma gangrenosum

Ecthyma gangrenosum is an uncommon cutaneous manifestation of *Pseudomonas aeruginosa* septicemia, usually occurring in immunocompromised or debilitated patients. Erythematous or purple macules initially develop on extremities or the anogenital region. Lesions subsequently become bullous and hemorrhagic, and rupture to leave a



Fig. 12.7 Lepromatous leprosy, with multiple symmetric lesions on the face, causing a leonine facies.



Fig. 12.8 Ecthyma gangrenosum in the setting of *Pseudomonas aeruginosa* septicemia. Courtesy of WM van der Meer.

gangrenous ulcer with central black eschar (Fig. 12.8). The surrounding tissue is painful and inflamed. Histologically, a necrotizing hemorrhagic vasculitis is present and the Gram-negative organism may be seen within the walls of deeper vessels. When the diagnosis is suspected, culture and biopsy of skin lesions is essential, together with blood and urine cultures. Immediate treatment directed towards *P. aeruginosa* is indicated.

NONSPECIFIC CUTANEOUS SIGNS OF SYSTEMIC INFECTIONS

Erythema multiforme

Erythema multiforme (EM) may occur at any age although young adults are most commonly affected. The most common trigger for EM is herpes simplex infection but a variety of other infectious agents, including *Mycoplasma pneumoniae*, have also been implicated (Table 12.1).

The lesions are usually asymptomatic and start as dull red macules and papules that occur on acral sites (particularly the hands) and then spread more centrally. Typical target lesions occur; these have a central area of damaged skin (dusky-hued, blistering or eroded) and a raised edematous border (Fig. 12.9). Less commonly, the feet, elbows, knees, face, neck and trunk are affected. Oral lesions may be present and severe involvement of the lips may occasionally mimic the crusting seen in Stevens–Johnson syndrome, although these conditions are now considered distinct in adult patients.[23]

Table 12.1 Infections and vaccines associated with erythema multiforme

Viral	Herpes simplex virus Human immunodeficiency virus Hepatitis B and C viruses Cytomegalovirus Epstein–Barr virus Coxsackie virus Poxviruses – including orf, milker's nodules Varicella-zoster virus Adenovirus Poliomyelitis virus
Bacterial	*Mycoplasma pneumoniae* *Treponema pallidum* *Legionella* spp. *Mycobacterium tuberculosis* *Rickettsia* spp.
Fungal	*Histoplasmosis capsulatum* *Coccidioides immitis* Dermatophytes
Vaccines	Diphtheria–tetanus–pertussis Hepatitis B Smallpox



Fig. 12.9 Erythema multiforme showing target lesions and bullous lesions on the palms of the hands.

Erythema nodosum

Erythema nodosum is a type IV delayed hypersensitivity reaction to a number of different stimuli. The skin and subcutaneous fat are affected with a septal panniculitis. Clinically, there may be a short prodrome of mild fever, myalgia and malaise. Erythematous nodules develop on the shins and more rarely on the arms, face and neck; these may extend up to several centimeters in diameter (Fig. 12.10). The hallmark of erythema nodosum is pain and exquisite tenderness of the lesions. Initially, lesions are bright red but as they subside over the next 3 weeks or so, they undergo a bruise-like change, becoming dusky in color with mild scaling of the skin.

A number of infectious agents have been implicated in the etiology of erythema nodosum (Table 12.2).[24] These vary with the age of the patient and the country of residence. Streptococcal infections (particularly upper respiratory tract) are the commonest infectious cause in both adults and children, and tuberculosis is still a common cause in endemic areas. Infections with *Chlamydia psittaci* have been responsible for small outbreaks of erythema nodosum in adults in the UK, where contact with birds and poultry may be an important clue in



Fig. 12.10 Erythema nodosum on the lower legs.

Table 12.2 Infections associated with erythema nodosum	
Bacterial	Streptococci *Mycobacterium tuberculosis* *Yersinia* spp. *Mycoplasma pneumoniae* *Chlamydia* spp. *Campylobacter* spp. Rickettsiae *Salmonella* spp. *Bartonella* spp. Treponema pallidum *Leptospira* spp. *Neisseria gonorrhoeae* *Franciscella tularensis*
Fungal	*Histoplasma capsulatum* *Coccidioides immitis* Blastomycoses
Viral	Herpes simplex virus Epstein–Barr virus Hepatitis B and C viruses Human immunodeficiency virus Parvovirus B19
Parasitic	*Entamoeba histolytica* *Giardia lamblia*

source identification. Infection with *Yersinia* spp. has been reported to cause erythema nodosum in France and Finland but is rare in other countries. Gonorrhea and varicella have been reported as rare causes of erythema nodosum in Singapore.[25]

Erythema nodosum may be related to inflammatory tinea capitis in children, particularly in association with kerion formation.[26] Deep fungal infection, particularly coccidioidomycosis, blastomycosis and histoplasmosis, has been associated with erythema nodosum. More rarely, erythema nodosum has been reported in association with tularemia, salmonellosis, *Campylobacter* spp. infection and leptospirosis.

Cutaneous vasculitis

The clinical features of cutaneous vasculitis depend on the size of the blood vessels affected and on whether the vasculitis is acute or chronic. Acute leukocytoclastic vasculitis is caused primarily by immune complex deposition in cutaneous blood vessels with complement fixation and damage caused by neutrophil infiltration and activation. This vessel wall injury is highly regulated by adhesion molecules and selectins, which are upregulated at sites of vasculitis.[27] The targeted blood vessels tend to be small and superficial, and the clinical signs are of a purpuric macular or papular eruption on the lower legs and dependent areas; these eruptions may become bullous and ulcerate (Fig. 12.11).

Cutaneous vasculitis is associated with underlying infection in approximately 15–20% of cases.[28] A variety of infectious agents including bacteria, viruses, fungi, protozoa and helminths have been associated with vasculitis (Table 12.3). Hepatitis B and C viruses may cause both cutaneous leukocytoclastic vasculitis and specific systemic vasculitis syndromes; hepatitis B virus is associated with polyarteritis nodosa and hepatitis C with cryglobulinemic vasculitis.[29]

Gianotti–Crosti syndrome

Gianotti–Crosti syndrome (GCS) is a self-limited cutaneous dermatosis, characteristically seen in children aged between 6 months and 12 years. It was first described in Europe associated with hepatitis B virus (HBV) infection; however, Epstein–Barr virus now appears to be the commonest cause.[30] This may relate to the increased use of anti-HBV immunizations worldwide. Many other viruses have been linked to GCS including cytomegalovirus, coxsackieviruses A16, B4 and B5, human herpesvirus 6, echovirus, rotavirus, respiratory syncytial virus, hepatitis A or C viruses and parainfluenza virus. There are rare reports of bacteria triggering GCS.

The eruption presents acutely with dull red papules of 5–10 mm diameter. These develop symmetrically over 3–4 days, starting on the buttocks and thighs and spreading to the arms and face. The papules may become vesicular or purpuric and are often mildly itchy. Axillary and inguinal lymphadenopathy is often present and may persist for several months after the eruption has settled, which generally occurs within 2–8 weeks to leave mild scaling but no scarring.



Fig. 12.11 Acute leukocytoclastic vasculitis showing bullous lesions on the lower leg. This patient was found to have a high antistreptolysin titer.

Table 12.3 Infections associated with cutaneous vasculitis	
Viral	Hepatitis virus A, B and C Human immunodeficiency virus Herpes simplex virus Varicella-zoster virus Hantavirus Cytomegalovirus Human T-cell lymphotrophic virus (HTLV-1)
Bacterial	Streptococci Staphylococci *Chlamydia* spp. *Mycobacterium* spp. *Treponema pallidum* *Neisseria* spp. *Rickettsia rickettsii* *Brucella* spp. Salmonellae
Fungal	*Candida* spp. *Cryptococcus neoformans*
Protozoal	*Acanthamoeba* *Plasmodia*

Kawasaki disease

Kawasaki disease (KD) is an acute vasculitis affecting medium-sized vessels, including the coronary arteries. Generally, children between 6 months and 4 years are affected. Although the etiology remains unknown, there is epidemiologic evidence that KD may be triggered by a response to an infectious agent.[31] Proposed but unproven causes of KD include adenovirus, herpesvirus, Epstein–Barr virus, streptococci, staphylococci and *Rickettsia* spp., a newly described human coronavirus [32] and human bocavirus.[33] In most cases no agent is identified. The incidence is highest in Japan although it has been reported worldwide.

The disease is acute in onset with fever lasting more than 5 days. The conjunctivae become injected and the lips and tongue are red. At the onset of fever, a generalized polymorphic eruption develops on the trunk and proximal limbs; this is associated with redness and induration of the palms and soles. Cervical lymphadenopathy develops in about three-quarters of children. As the fever subsides, the skin scales and the patient may develop arthralgia and arthropathy. Coronary artery aneurysms develop in up to 30% of untreated patients and myocardial infarction occurs in approximately 2% of those with coronary lesions.[31]

INFLAMMATORY DERMATOSES MIMICKING INFECTION

Acute febrile neutrophilic dermatosis (Sweet's syndrome)

Acute febrile neutrophilic dermatosis was first described by Sweet in 1964.[34] It is characterized by an explosive cutaneous eruption of raised violaceous plaques in association with constitutional symptoms and fever. Sweet's syndrome (SS) is now classified in three groups: classic (or idiopathic), malignancy associated and drug induced. Malignancy is present in approximately 20% of all patients with SS. Both hematologic malignancy and solid tumors occur but acute myelogenous leukemia is most commonly associated. Drug-induced SS is well described following administration of granulocyte-colony stimulating factor, as well as a number of other agents.[35] Uncommon or rare associations include streptococcal, *Salmonella* and nontuberculous mycobacterial infections, vaccination,[36] inflammatory bowel disease and pregnancy.

Clinical presentation is an acute eruption of dull red elevated inflammatory nodules and plaques, which may pustulate in later stages or clear centrally to give an annular appearance. The majority of patients have a persistent fever and neutrophilia with an elevated erythrocyte sedimentation rate. Diagnosis may be confirmed by cutaneous biopsy, which reveals a florid dermal polymorphonuclear cell infiltrate. Cases respond rapidly to systemic corticosteroid therapy, which is usually required for several weeks.

Toxic epidermal necrolysis

Toxic epidermal necrolysis (TEN) is a serious drug-induced skin disorder, which clinically may be difficult to distinguish from staphylococcal scalded skin syndrome (SSSS). Both conditions are characterized by an acute, widespread cutaneous erythema with sloughing of the epidermis in sheets. As described, SSSS occurs mainly in childhood and is caused by staphylococcal toxin-mediated superficial cleavage of the epidermis below the granular layer. It has an excellent prognosis if appropriately treated. In comparison, TEN is usually a drug-induced phenomenon causing full-thickness necrosis of the epidermis, with subsequent impairment of cutaneous barrier function and a high mortality (30%).[37]

Stevens–Johnson syndrome (SJS) and TEN are now recognized as variants of a single entity, with TEN being of greater severity.[23] The precise pathogenesis is poorly understood, although increased circulating levels of fasL, a ligand for the fas keratinocyte death receptor, have been identified in patients with TEN. Perforin and granzyme, proteins contained within lytic granules of cytotoxic T cells, have also been shown to induce keratinocyte apoptosis in TEN.[37]

The most commonly implicated drugs in TEN are antibiotics, particularly sulfonamides, and anticonvulsants, allopurinol and non-steroidal anti-inflammatory drugs. Nevirapine and lamotrigine have also recently been shown to be strongly associated with TEN.[38] TEN may be distinguished from SSSS by histologic examination of biopsied skin, allowing assessment of the level of epidermal splitting and identification of the presence or absence of micro-organisms. Treatment outcome is improved by intensive nursing in a specialist burns unit and by prompt withdrawal of all potential precipitants. No specific treatment has been unequivocally proven to be effective; however, intravenous immunoglobulins and plasmapheresis have been used.

Oral ulceration

Oral ulceration is a frequent clinical funding, with a wide differential diagnosis including infectious disease.

Primary herpes simplex gingivostomatitis usually occurs in early childhood. Fever is followed by development of painful vesicles on the lips, buccal mucosa, tongue and palate, with tender regional lymphadenopathy. Later in life, recurrences are usually less severe and lesions fewer in number.

Aphthous ulceration is common, painful and often occurs in crops. Minor aphthous ulcers are 2–4 mm in diameter with a gray–white surface and red margin. Commonest on the lips, buccal mucosa or floor of the mouth, they rarely occur on the gingiva, palate or dorsal tongue. Major aphthous ulcers may exceed 1 cm in diameter and occur anywhere in the mouth. They are more painful and protracted and often heal with scarring. Herpetiform aphthous ulceration produces painful vesicles and multiple tiny ulcers less than 2 mm in diameter. These coalesce to produce larger lesions and tend to recur frequently. This syndrome resembles herpetic gingivostomatitis, although there is no evidence of viral etiology.

Behçet's syndrome is characterized by the presence of recurrent oral and genital ulcers, and ocular disease. Other features which may be present include central nervous system disease, arthropathy, skin lesions (e.g. pustules, pathergy, erythema nodosum) and vascular disease.[39] Oral lesions in Behçet's syndrome start as small erythematous papules or pustules, which erode to form ulcers. Pain is variable. Recurrent ulceration may predate the syndrome by months or years.

Pemphigus vulgaris affects the oral mucosa in almost all patients and is the presenting feature in 50–70%. Bullae are fragile and therefore rarely seen. Ruptured bullae form painful large irregular erosions on any part of the oral mucosa. Most patients will also go on to develop flaccid cutaneous bullae which rupture easily.[40]

Eosinophilic cellulitis (Wells' syndrome)

Eosinophilic cellulitis is a rare syndrome which may closely mimic bacterial cellulitis or bullous erysipelas.[41] The etiology is unknown. It is characterized by development of indurated areas of erythema, usually on a distal limb, which may be single or multiple. Systemic illness is unusual, although associated fever has been reported and peripheral eosinophilia is common. Early lesions are often pruritic, infiltrated and may blister but then resolve without scarring within a few weeks. Diagnosis is suggested on skin pathology by the demonstration of marked dermal eosinophilia with areas of granulomatous change surrounding aggregates of eosinophilic material, known as 'flame figures'. The condition responds to oral corticosteroid therapy.

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Chapter 13

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Superficial fungal infections

INTRODUCTION

This chapter reviews the different fungal diseases of the skin, nails and hair. The most common of these diseases are dermatophytosis, candidiasis and pityriasis versicolor. Other, less frequent infections of the skin and hair include tinea nigra and piedra. In addition, there are a number of nondermatophytic molds that can cause nail disease (onychomycosis). Superficial fungal infections, such as dermatophytosis and onychomycosis, have become an important problem in persons infected with HIV, transplant recipients and other immunocompromised host groups. Prompt diagnosis of these infections is important as they may lead to more severe complications; however, this can be difficult because of atypical clinical manifestations. Furthermore, in such patients, skin and nail infections can be difficult to treat because the disease is often more extensive and severe.

EPIDEMIOLOGY

The organisms that cause dermatophytosis are molds belonging to the genera *Trichophyton*, *Microsporum* and *Epidermophyton*.[1,2] Many of the 40 or so species that are recognized at present are worldwide in distribution, but others are confined to particular regions.[3] About 10 species are common human pathogens. The dermatophytes can be split into three ecologic groups depending on whether their usual natural habitat is the soil (geophilic species), animals (zoophilic species) or humans (anthropophilic species). Members of all three groups can cause human infections, but their different natural reservoirs have important implications in relation to the acquisition, site and spread of the disease. Infections originating from the soil are the least common. Infections having animal origins are more frequent and particular species are often associated with particular animal hosts. Anthropophilic dermatophytes account for most human infections; these species are contagious and are readily transmitted from person to person.

Tinea capitis (dermatophytosis of the scalp) is a common infection in children. The predominant etiologic agents differ from continent to continent, but the anthropophilic species *Trichophyton tonsurans* has replaced *Microsporum audouinii* as the dominant cause of this disease among urban populations in North, Central and South America.[4] Infections with this organism have also become much more common in the UK, particularly among black African or black Caribbean school children in London. The cause of this rise in anthropophilic infection rates is difficult to ascertain, but it is possible that it is associated with increased migration. In France, and in particular Paris, the main anthropophilic dermatophytes associated with tinea capitis are those that originate from Africa, particularly *Trichophyton soudanense*. Other less common causes of tinea capitis include the animal species *Microsporum canis* and *Trichophyton verrucosum*. Tinea pedis (dermatophytosis of the feet) is a contagious condition and is easily spread from person to person. The predominant agent is the anthropophilic species *Trichophyton rubrum*, but this disease can also be caused by *Trichophyton mentagrophytes* var. *interdigitale*. Transfer within households has been reported, but the main spread occurs in communal baths and showers.[5]

Cutaneous candidiasis is a less common disease than dermatophytosis. *Candida albicans*, the predominant etiologic agent, is a commensal organism found in the mouth and gastrointestinal tract of a significant proportion of the normal population. It is seldom recovered from normal skin, being much less prevalent than *Candida parapsilosis*, but it is a frequent colonizer of moist or damaged skin and nails.

Malassezia spp. are common commensal organisms that colonize the normal skin of the head and trunk during late childhood.[6] In certain circumstances, such as hot humid climatic conditions, these lipophilic organisms produce the disease pityriasis versicolor. In the tropics, up to 50% of the population may be affected. *Malassezia* spp. can be transmitted from person to person, either through direct contact or through contaminated clothing or bedding. In practice, however, infection is endogenous in most cases and spread between individuals is uncommon.

Tinea nigra is a chronic infection of the palms and soles. The disease is rare but has a worldwide distribution, although it is more common in the tropics and subtropics. The etiologic agent, *Phaeoannellomyces werneckii*, is a saprobic mold that is found in the soil and in decomposing vegetation. Human infection is thought to follow traumatic inoculation.

Black piedra is an uncommon hair infection that occurs in humid tropical regions. The natural habitat of the etiologic agent, *Piedraia hortae*, has not been identified. There are some reports of familial infection. White piedra is less common than black piedra. It is found worldwide, but is more prevalent in the tropics and subtropics. The etiologic agents, *Trichosporon* spp., have a widespread natural distribution and are sometimes found on normal skin.

Onychomycosis is a nonspecific term used to describe fungal disease of the nails; tinea unguium is a more specific term used to describe dermatophyte nail infection. At least 80% of fungal nail infections and 90% of toenail infections are due to dermatophytes, in particular *Trichophyton rubrum*.[7] Between 5% and 10% of nail infections are due to *Candida* spp. and the remainder are attributable to nondermatophytic molds. Most prominent among these are *Scopulariopsis brevicaulis*, *Scytalidium dimidiatum* (*Hendersonula toruloidea*), *Aspergillus* spp. and *Fusarium* spp.[7] Unlike the dermatophytes, these molds are not contagious. Onychomycosis is more prevalent in older people and men are more commonly affected than women. Toenails are more frequently involved than fingernails.

PATHOGENESIS AND PATHOLOGY

The dermatophytes are keratinophilic fungi that are normally found growing only in the dead keratinized tissue of the stratum corneum, within and around hair shafts, and in the nail-plate and keratinized nail-bed. The clinical appearances of dermatophyte infections are the result of a combination of direct damage to the tissue by the fungus (mainly in the case of hair and nail infections) and of the immune response of the host. The damage to tissue is due to a combination of mechanical forces and enzymatic activities. Dermatophytes produce a number of keratinolytic proteinases that function best at an acidic pH and these have been recognized as important virulence factors.[1]

The immune response to dermatophytes has been studied in human infections as well as in animal models.[8] The humoral response does not appear to help in the elimination of infection; the highest levels of antibodies are often found in patients with chronic dermatophytosis. Rather, it is the cell-mediated response that is important in ridding the stratum corneum of the infection.[9] Dermatophytes vary in their host interactions. Zoophilic species, such as *Trichophyton verrucosum*, often elicit intense inflammation in humans. This leads to enhanced epidermal proliferation and can result in spontaneous cure.[10] In contrast, anthropophilic species such as *Trichophyton rubrum* often produce chronic or recurrent lesions. Chronic dermatophytosis in otherwise healthy people may be mediated by fungal cell wall components, such as mannan, that diminish the local immune response.[11]

Except for neonatal infections, most cases of superficial candidiasis result from infection of the host from his or her own commensal flora. This shift in the host–fungus relationship results from a number of influences, of which host factors appear to be the most important. Local tissue damage is a critical factor in the pathogenesis of cutaneous candidiasis; most infections occur in moist, occluded sites and follow maceration of the tissue. Chronic mucocutaneous candidiasis is a rare condition that results from inherited defects in the cell-mediated immune response.[12]

Malassezia spp. are present on the normal skin from late childhood. Hot, humid environmental conditions are among the factors that predispose to the development of the cutaneous lesions of pityriasis versicolor.

PREVENTION

Prevention of dermatophytosis must take into account the site of the infection, the etiologic agent and the source of the infection.

Anthropophilic tinea capitis is a common fungal infection in children. It is easily spread from child to child, both in the home and at school. To prevent this, contacts of children infected with *Microsporum audouinii* can be examined for infected fluorescent hairs with Wood's light (a source of ultraviolet light filtered through nickel oxide glass). In the more common nonfluorescent infection with *Trichophyton tonsurans*, detection is more difficult, but the scalp brush sampling method is often helpful in detecting subclinical disease.[13] All those found to be infected must be treated and the importance of good personal hygiene should be stressed. It is seldom practical to exclude infected children from school.

In the case of tinea capitis and tinea corporis caused by zoophilic species, such as *Microsporum canis* and *Trichophyton verrucosum*, it is important to locate the animal source. *Microsporum canis* infection of cats and dogs can often be detected with Wood's light examination. The subsequent course of action will depend upon the value placed on the infected animal. It is more difficult to detect and eliminate *Trichophyton verrucosum* infection in cattle, because infected hairs are not fluorescent and because the fungus can survive for long periods on hairs and scales that have been deposited on the walls of buildings and gates. Fungicidal washes have sometimes been effective in controlling this infection.

Tinea pedis is a contagious condition and is easily spread from person to person. Transfer within households has been reported, but the main spread occurs in communal baths and showers.[5] Educating infected people not to expose others to their infection by not walking barefoot on the floors of communal changing rooms and by avoiding public baths and showers can help to reduce the spread of this disease. Frequent hosing of the floors of public baths and antifungal foot dips near communal baths are helpful preventive measures. Prompt treatment of tinea pedis and the use of separate towels are sensible measures that can help to prevent tinea cruris, tinea manuum and tinea unguium.

Intertriginous candidiasis of the fingernails is often seen in people whose occupation necessitates frequent wetting of the hands. Wearing protective gloves can help to prevent this infection. This condition is also seen in diabetic patients and good glucose control is important in prevention and control.

Good personal hygiene is important in preventing the spread of piedra. Infected people should not share hair brushes or combs with others.

CLINICAL FEATURES

The dermatophytes are the predominant causal organisms of fungal disease of the scalp, toe clefts, soles, palms and nails. In the temperate, developed countries, tinea pedis is the most common form of dermatophytosis. By contrast, in the tropics, tinea capitis and tinea corporis are the most prevalent.

Tinea capitis

The clinical manifestations of tinea capitis are varied and depend on the species of dermatophyte involved and the degree of host response (Table 13.1). The appearance of the lesions can range from mild scaling and hair loss with minimal inflammation to severe inflammation with kerion formation.

In *Microsporum audouinii* infection the lesions consist of well-demarcated patches of partial alopecia. Inflammation is minimal, but fine scaling is characteristic. Most of the hairs in these lesions are broken off near the surface of the scalp. In *Microsporum canis* infection the picture is similar, but there is usually more inflammation. In both these infections the hair surface is coated with small arthrospores (ectothrix infection). The affected hairs show green fluorescence under Wood's light.

In *Trichophyton tonsurans* and *Trichophyton violaceum* infections the lesions are often inconspicuous and inflammation may be minimal. The typical lesions are irregular patches of scaling. The affected hairs often break off at the surface of the scalp, giving a 'black-dot' appearance. The hairs are filled with arthrospores (endothrix infection) and do not fluoresce under Wood's light.

The most florid form of tinea capitis is a kerion. A kerion is a painful inflammatory mass in which the hairs that remain are loose. Thick crusting with matting of adjacent hairs is common. Pus may be discharged from one or more points. A kerion may be limited in extent, but a large confluent lesion may develop (a severe form of kerion) that involves most of the scalp. In most cases this violent reaction results from infection with an animal dermatophyte such as *Trichophyton verrucosum* or *Trichophyton mentagrophytes* var. *mentagrophytes*. However, geophilic or anthropophilic organisms are sometimes involved. In *Trichophyton verrucosum* infections the hairs are covered with chains of large arthrospores but they do not fluoresce under Wood's light.

Favus is now rare, but it is still a distinctive form of fungal scalp infection. The causal organism is *Trichophyton schoenleinii*, an anthropophilic dermatophyte noted for its persistence. Favus presents with hair loss and the formation of cup-shaped crusts known as scutula. These give off a fetid odor and can amalgamate to form dense mats on part or all of the scalp. Longstanding favus can lead to permanent patches of cicatricial alopecia. Infected hairs give off a dull green fluorescence under Wood's light.

Table 13.1 Some characteristics of common dermatophytes causing scalp infection

Organism	Arthrospore size	Arthrospore arrangement	Fluorescence under Wood's light
Microsporum audouinii	Small (2–3 µm)	Ectothrix	Yes
Microsporum canis	Small (2–3 µm)	Ectothrix	Yes
Trichophyton mentagrophytes	Small (3–5 µm)	Ectothrix	No
Trichophyton soudanense	Large (4–8 µm)	Endothrix	No
Trichophyton tonsurans	Large (4–8 µm)	Endothrix	No
Trichophyton verrucosum	Large (5–10 µm)	Ectothrix	No
Trichophyton violaceum	Large (4–8 µm)	Endothrix	No

Tinea capitis must be distinguished from seborrheic dermatitis, psoriasis, bacterial folliculitis and cicatricial alopecia.

Tinea barbae

The animal species *Trichophyton verrucosum* and *Trichophyton mentagrophytes* var. *mentagrophytes* are the principal causes of dermatophyte infection of the beard and mustache areas of the face. *Microsporum canis* is a less common cause. The characteristic appearance is of a highly inflammatory pustular folliculitis (Fig. 13.1). Some infections are less severe and consist of circular, erythematous, scaling lesions.

Tinea faciei

The more common causes of dermatophyte infection of the face are *Trichophyton rubrum* and *Trichophyton mentagrophytes* var. *mentagrophytes*, but many other species may be involved, including *Trichophyton tonsurans* and *Microsporum canis*. The typical annular lesions are erythematous, but scaling is often absent. The lesions are often pruritic and exacerbation after exposure to the sun is common.

Tinea corporis

The clinical manifestations of tinea corporis are varied and often depend on the species of the infective organism. The disease often follows contact with infected animals, but occasional cases result from contact with contaminated soil. *Microsporum canis* is a frequent cause of human infection and *Trichophyton verrucosum* infection is common in rural districts. Infections with anthropophilic species, such as *Trichophyton rubrum*, often follow spread from another site, such as the feet. Infections with *Trichophyton tonsurans* are sometimes seen in children with tinea capitis.

The characteristic lesion is an annular scaling plaque with a raised erythematous border and central clearing. In their most florid form the lesions can become indurated and pustular (Fig. 13.2). This is more common in infections with zoophilic organisms. The differential diagnosis includes discoid eczema, impetigo, psoriasis and discoid lupus erythematosus. Of note, formation of perifollicular pustules (Majocchi's granuloma) is indicative of deep-seated follicular involvement and requires systemic therapy.



Fig. 13.1 Tinea barbae due to *Trichophyton verrucosum*.



Fig. 13.2 Tinea corporis due to *Trichophyton mentagrophytes* var. *mentagrophytes*.

Tinea cruris

Infection of the groin and the perianal and perineal regions is more common in men. The predominant causes are the anthropophilic species *Trichophyton rubrum* and *Epidermophyton floccosum*. The infection often follows spread from another site in the same person (e.g. feet or nails), but person-to-person spread (e.g. through contaminated clothing) is not uncommon.

In color, the lesions are erythematous to brown. They have raised scaling margins and radiate from the groin down the inner border of the thigh. Patients often complain of intense pruritus. The differential diagnosis includes intertriginous *Candida* infection, bacterial intertrigo, psoriasis and seborrheic dermatitis.

Tinea imbricata

This is a chronic infection that is characterized by the development of homogeneous sheets or concentric rings of scaling that can spread to cover large parts of the affected person. Most reports of tinea

imbricata have come from the Pacific Islands and Melanesia but there have been occasional reports from South East Asia and Central and South America. The etiologic agent is the anthropophilic species *Trichophyton concentricum*.

Tinea pedis

Infection of the feet is the most common form of dermatophytosis in the UK and North America. The main organisms involved are the anthropophilic species *Trichophyton rubrum* and, less commonly, *Trichophyton mentagrophytes* var. *interdigitale*.

The most common clinical presentation is interdigital maceration, peeling and fissuring, mostly in the spaces between the fourth and fifth toes. Itching is a common symptom.

Another common presentation associated with *Trichophyton rubrum* is hyperkeratosis of the soles, heels and sides of the feet. The affected sites are pink and covered with fine, white scales. This form of the disease is often chronic and resistant to treatment. If there is extensive involvement of the foot, then the term 'moccasin tinea pedis' is often applied (Fig. 13.3). This presentation is frequently associated with nail infection.

A third form of tinea pedis, associated with *Trichophyton mentagrophytes* var. *interdigitale*, is an acute vesicular infection of the soles. This severe form of the disease may resolve without treatment, but exacerbations tend to occur under hot humid conditions. There is often associated hyperhidrosis.

Tinea pedis can be difficult to distinguish from other infectious causes of toe web infection, such as *Candida* intertrigo and erythrasma. Noninfectious conditions that mimic tinea pedis of the soles include psoriasis and contact dermatitis.

Tinea manuum

Tinea manuum is usually unilateral, the right hand being more commonly affected than the left. Lesions on the dorsum of the hand appear similar to those of tinea corporis, with a distinct border and central clearing. Infection of the palms is more common. This presents as a diffuse scaling hyperkeratosis, with accentuation of the fissuring in the palmar creases. *Trichophyton rubrum* is the most common cause of tinea manuum. The differential diagnosis includes contact dermatitis, eczema and psoriasis.

Tinea unguium

The most common causes of dermatophyte infection of the nails are *Trichophyton rubrum* and *Trichophyton mentagrophytes* var. *interdigitale*, but many other species may be involved. Three clinical forms of tinea unguium are recognized. Distal (or lateral) subungual disease is the most common presentation (Fig. 13.4). This usually begins as a discoloration and thickening of the nail and it can result in destruction of the entire nail-plate and separation of the nail from the nail-bed (Fig. 13.5). In superficial white onychomycosis, crumbling white lesions are evident on the nail surface, particularly the toenails. This condition is most commonly caused by *Trichophyton mentagrophytes* var. *interdigitale*. Proximal subungual disease is the least common presentation of dermatophyte nail infection. In the USA *Trichophyton rubrum* is the principal cause of proximal subungual onychomycosis.

The differential diagnosis includes eczema, lichen planus and onychogryphosis. Unlike dermatophytosis, *Candida* infections of the nails often begin in the proximal nail-plate and are associated with nail-fold infection.



Fig. 13.3 Moccasin tinea pedis due to *Trichophyton rubrum*.

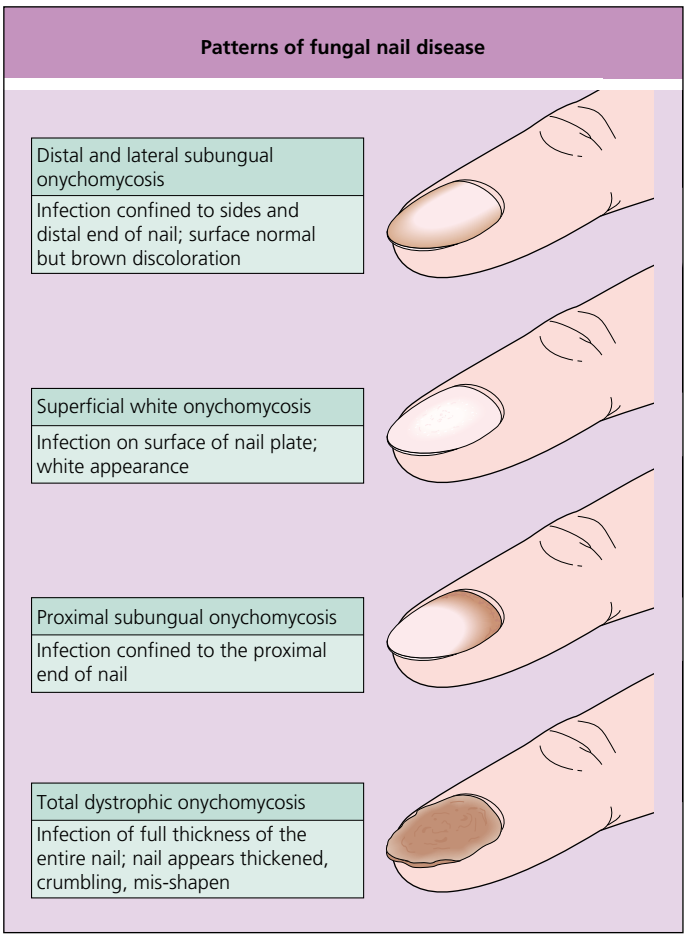


Fig. 13.4 Patterns of fungal nail disease.



Fig. 13.5 Total dystrophic onychomycosis due to *Trichophyton rubrum*.

Candidiasis

The lesions of cutaneous candidiasis (intertrigo) tend to develop in warm, moist sites such as the folds of the skin under the breasts and the groin. The infection is more common in overweight or diabetic people. The initial lesions are papules or vesicopustules that later enlarge and



Fig. 13.6 Interdigital candidiasis.

become confluent. The larger lesions are erythematous and have an irregular margin. Smaller, satellite lesions are often present. Soreness and itching are usual. The differential diagnosis includes dermatophytosis, seborrheic dermatitis, bacterial intertrigo and psoriasis.

Infection of the skin between the fingers or toes can also occur. Infection of the webs of the fingers presents as a macerated, erythematous lesion (Fig. 13.6). It is often uncomfortable and may be painful. This condition is usually seen in people whose occupations necessitate frequent immersion of the hands in water. Infection of the webs of the toes mimics tinea pedis and many cases do occur in conjunction with this form of dermatophytosis.

Chronic mucocutaneous candidiasis is a rare condition that affects people with underlying endocrinologic or immunologic disorders. The disease often develops during the first 3 years of life. The mouth is usually the first site to be affected (Fig. 13.7), but lesions then appear on the scalp, hands, feet and nails (Fig. 13.8). In some patients, disfiguring hyperkeratotic lesions develop on the scalp and face.

Three forms of *Candida* nail infection are recognized: infection of the nail-folds (paronychia), distal nail infection and total dystrophic onychomycosis. The last is a manifestation of chronic mucocutaneous candidiasis. Infection of the nail-folds is more common in women than in men. The periungual skin is raised and painful and a prominent gap develops between the fold and the nail-plate. White pus may be discharged. The infection usually starts in the proximal nail-fold, but the lateral margins are sometimes the first site to be affected. The nail-plate may be invaded.

Distal *Candida* nail infection presents as onycholysis and subungual hyperkeratosis. It is often difficult to distinguish from dermatophytosis, but candidiasis tends to affect the fingernails rather than the toenails. In patients with chronic mucocutaneous candidiasis, the nail-plate is invaded from the outset, causing gross thickening and hyperkeratosis.

Pityriasis versicolor

Pityriasis versicolor is a disfiguring but otherwise harmless condition. The characteristic lesions consist of patches of fine brown scaling that are found particularly on the upper trunk, neck, upper arms and abdomen. In light-skinned people the affected skin may appear darker than



Fig. 13.7 Chronic mucocutaneous candidiasis of the mouth. Courtesy of Jos W M van der Meer, MD, PhD FRCP.



Fig. 13.8 Chronic mucocutaneous candidiasis of (a) the foot and (b) the thumb. Courtesy of Jos W M van der Meer, MD, PhD, FRCP.



Fig. 13.9 Pityriasis versicolor showing depigmented lesions.

normal. The lesions are light pink in color but grow darker, turning a pale brown shade. In dark-skinned or tanned people, the affected skin becomes depigmented (Fig. 13.9).

Hyperpigmented lesions must be distinguished from erythrasma, seborrheic dermatitis, pityriasis rosea and tinea corporis. Hypopigmented lesions can be confused with pityriasis alba and vitiligo.

Malassezia folliculitis

In addition to pityriasis versicolor, *Malassezia* spp. are also associated with two other cutaneous disorders, folliculitis and seborrhoeic dermatitis. There are two main forms of *Malassezia* folliculitis. The first, which is most common in young adults, consists of small, scattered, itching and erythematous follicular papules that develop on the back, chest or upper arms, and slowly enlarge to become pustular. These often appear after sun exposure or antibiotic or immunosuppressive treatment. These patients do not usually have seborrhoeic dermatitis. This condition is often overlooked and may easily be confused with common acne.

In the second form, which is seen in some patients with seborrhoeic dermatitis, there are numerous small follicular papules scattered over the upper and lower chest and back. The rash is more florid and particularly marked on the back.

Tinea nigra

The lesions of tinea nigra, which are found on the palm or sole, consist of one or more flat, dark brown or black, nonscaling patches with a well-defined edge. Inflammation is absent. The lesions, which are small at first, expand and become confluent. The disease is asymptomatic and may remain undiagnosed for long periods. Tinea nigra must be distinguished from malignant melanoma and chemical stains.

Piedra

Black piedra is most often seen on the scalp hair. Small, brown or black, hard nodules, which are difficult to remove, are formed on the distal hair shafts. The appearance of white piedra is similar, but the nodules are softer and pale in color. This condition affects the hairs of the beard and moustache. Less commonly, it involves the scalp or pubic hair.

Onychomycosis

Up to 5% of cases of onychomycosis are due to nondermatophyte molds. With the exception of *Scytalidium dimidiatum*, these molds usually affect nails that have previously been diseased or damaged. This may account for the fact that these infections often affect only one nail. There is nothing specific about the clinical appearance of the lesions (Fig. 13.10). Distal subungual hyperkeratosis with onycholysis of the distal nail-plate is common (see Fig. 13.4). Superficial white lesions are another presentation.

Superficial fungal infections in immunocompromised patients

In general, fungal infections, such as dermatophytosis and onychomycosis, are no more common in immunocompromised persons than in immunocompetent individuals.[14,15] The clinical manifestations of dermatophytosis in the immunocompromised patient are often similar to those seen in normal individuals. However, the clinical presentation can be atypical, particularly in patients with T-cell defects, such as organ transplant recipients and persons who have AIDS. The major features of dermatophytosis in these groups are loss of obvious lesions, minimal scaling and the presence of follicular papules or pustules. In addition, the lesions can be more extensive than normal.

Tinea pedis has been described in both organ transplant recipients and persons who have AIDS. The lesions are often indistinguishable from those seen in normal individuals but can be extensive, with involvement of the dorsum of the foot. In tinea corporis and tinea cruris, the lesions can be extensive but the inflammation is mild and the margin is indistinct. Facial dermatophytosis has been noted in persons who have AIDS, where it can be confused with seborrheic dermatitis. This is because the rash is diffuse and can spread across both cheeks.



Fig. 13.10 Onychomycosis due to *Scytalidium dimidiatum (Hendersonula toruloidea)*.

Although proximal subungual onychomycosis is the most infrequent form of fungal nail disease in the general population, it is common in persons who have AIDS and has been considered a useful clinical marker of HIV infection.[16] Infection of the toenails is much more frequent than fingernail infection. *Trichophyton rubrum* is the usual etiologic agent. In AIDS patients, it can spread rapidly from the proximal margin and superior surface of the nail to produce gross white discoloration of the plate without obvious thickening.

Malassezia infections of the skin can take a number of different clinical forms in immunocompromised persons, including pityriasis versicolor, *Malassezia* folliculitis and seborrheic dermatitis.[14,15] The clinical manifestations of pityriasis versicolor in immunocompromised persons are similar to those seen in normal individuals. However, the lesions are usually more erythematous and may appear raised. *Malassezia* spp. can also cause folliculitis in immunosuppressed individuals. This is characterized by multiple itching follicular papules and pustules on the trunk and face, and is often associated with severe seborrhoeic dermatitis.

Seborrheic dermatitis is chronic relapsing scaling dermatosis of the face, scalp and trunk. It is the commonest cause of dandruff (scalp scaling). The role of *Malassezia* spp. in the pathogenesis of seborrheic dermatitis is controversial and is based on the fact that most cases respond to azole antifungal treatment. Improvement is associated with disappearance of the organisms and relapse with recolonization. Seborrheic dermatitis is a common and troublesome problem, estimated to occur in up to 80% of persons with HIV infection. The lesions take the form of an erythematous scaling rash on the scalp, face, ears, chest and upper back. Scaling of the eyelid margins and around the nasal folds is a common presentation. The rash is often more extensive than in other individuals.

DIAGNOSIS

Superficial fungal infections often present with characteristic lesions but where this is not the case, mycologic investigation can assist in diagnosis. Material should be collected from cutaneous lesions by scraping outward from the margin. Cleansing the site with 70% alcohol before sampling will increase the likelihood of detecting fungus on direct microscopic examination. Nail specimens should be taken from discolored or dystrophic parts of the nail and should include the full thickness of the nail. If distal subungual lesions are present,

debris should be collected from underneath the nail. If there is superficial nail-plate involvement, the scrapings should be taken from the nail surface. Specimens from the scalp should include hair roots and skin scales. Wood's light can sometimes be useful for the selection of sites of active infection, especially if the lesions are inconspicuous or atypical.

Direct microscopic examination of skin and nail material is often sufficient for the diagnosis of a dermatophyte infection, but it gives no indication as to which species is involved. With hair specimens, the size and disposition of the arthrospores can give some indication as to the etiologic agent (see Table 13.1).

Culture is a more reliable method of diagnosis than microscopic examination. It permits the species of dermatophyte to be determined and this can aid the selection of the most appropriate form of treatment. If possible, both microscopic examination and culture should be performed on all specimens. If, however, there is insufficient material for both, microscopic examination should be performed.

Cutaneous candidiasis is often difficult to diagnose if the lesions are other than typical in appearance. Isolation of *Candida albicans* from scrapings is of doubtful significance because the organism is a common colonizer of cutaneous lesions in moist sites. Microscopic demonstration of the organism in scrapings is much more significant. Isolation of *Candida albicans* from nails is seldom significant unless the organism is seen on direct microscopic examination.

Microscopic examination of scrapings from lesions will permit the diagnosis of pityriasis versicolor if there are clusters of round or oval cells together with short broad filaments (which are seldom branched). Because this appearance is pathognomonic for pityriasis versicolor, and because *Malassezia* spp. are part of the normal skin flora, their isolation in culture is not helpful. Direct microscopic examination and culture of scrapings or epilated hairs will permit the diagnosis of tinea nigra and piedra.

It is not unusual to isolate molds other than dermatophytes from abnormal nails cultured on media from which cycloheximide has been omitted. In many cases, these molds are casual, transient contaminants and direct microscopic examination of the material is negative. However, if filaments are seen on microscopic examination but no dermatophyte is isolated, it is possible that the mold is the cause of the infection.

MANAGEMENT

There is now a good selection of topical and systemic agents for the treatment of superficial fungal infections. The choice of treatment and its duration depends on the causative organism, the site of infection and the extent of the disease, as well as on other factors for each individual patient, such as concurrent disease and medication. Topical agents can be used for localized skin infections, but they are seldom successful for sites with a thick keratin layer or when follicular structures are involved. The palms and soles and certainly the nails and hair often require systemic antifungal treatment. Although they respond well to many topical and systemic antifungal agents, persons who have AIDS often suffer from recurrent episodes of superficial fungal infections. If the disease is chronic and extensive, systemic treatment is required.

Tinea capitis

Topical treatment is ineffective on its own in tinea capitis. Terbinafine 250 mg/day and itraconazole 100 mg/day are both effective oral treatments for scalp infection. In adults, either agent should be given for 2–4 weeks. Terbinafine is licensed in some countries for use in children and it appears to be a safe and effective agent in this group.[17] In other countries, the older drug griseofulvin must still be used in children. The recommended dose is 20–25 mg/kg/day for at least 6–8 weeks. A confirmatory culture is often done at 5–6 weeks to determine if longer courses are needed.

Tinea corporis and tinea cruris

The choice of topical or systemic treatment in these conditions depends on the extent of the disease. Localized lesions can be treated with topical antifungal preparations. Numerous imidazoles and allylamines are available in different formulations. These agents should be applied morning and evening for 4–6 weeks. To prevent relapse, treatment should be continued for at least 1 week after the lesions have cleared.

If the disease is widespread or follicular structures are involved (i.e. pustules), or the patient fails to respond to topical preparations, oral treatment is usually indicated. Terbinafine 250 mg/day for 2–4 weeks and itraconazole 100 mg/day for 2 weeks are more effective than griseofulvin 10–20 mg/kg/day for 4–6 weeks. Unlike itraconazole, oral terbinafine has a low potential for drug interactions, making it a useful agent for the treatment of dermatophytosis in persons who have AIDS and other immunocompromised individuals. Terbinafine has been reported to be a safe and effective drug for tinea corporis and cruris in these patient groups.[18,19]

Tinea pedis

Tinea pedis is a chronic infection that seldom clears if left untreated. Infection of the webs of the toes will often respond to topical terbinafine, applied morning and evening for 1–2 weeks. Topical imidazoles can also be used, but they are less effective and must be applied for at least 4 weeks.[20] The recurrence rate following topical treatment is quite high and it is not uncommon for chronic infection to persist despite treatment.

If the disease involves the soles or if there is acute inflammation, oral treatment should be given. Terbinafine 250 mg/day for 2 weeks has been shown to be effective in tinea pedis. Itraconazole 100 mg/day is an alternative, but it must be given for 4 weeks.[21] Chronic tinea pedis is often associated with nail infection. Inadequate treatment of onychomycosis may result in reinfection of the feet. Terbinafine has been reported to be a safe and effective drug for tinea pedis in persons who have AIDS.[18,19]

Tinea manuum

Local treatment with an imidazole or an allylamine will often suffice to clear tinea manuum. In cases that fail to respond to topical preparations, oral treatment is usually indicated. Infections of the palms are difficult to clear with griseofulvin, but oral terbinafine 250 mg/day for 2–6 weeks has been shown to be highly effective.[22] Itraconazole 100 mg/day for 4 weeks is an alternative.

Candidiasis

Most patients with cutaneous candidiasis will respond to topical treatment with terbinafine or an imidazole such as clotrimazole or miconazole, applied for 2–4 weeks. However, relapse is common if any underlying problem is not controlled. If the infection is associated with an underlying skin disease, such as flexural eczema or diaper dermatitis, treatment with a combination preparation containing an azole agent together with hydrocortisone is often helpful.

Long-term treatment with itraconazole or fluconazole has helped many patients with chronic mucocutaneous candidiasis. However, protracted treatment has led to the development of azole-resistant strains of *Candida albicans* in some cases.[23]

Pityriasis versicolor

If left untreated, pityriasis versicolor will persist for long periods. Most patients with this disease respond to topical treatment with terbinafine, azole agents or selenium sulfide shampoo, but more than half relapse within 12 months. Oral treatment with itraconazole 200 mg/

day for 1 week or ketoconazole 400 mg once weekly for 2 weeks is indicated for extensive or recalcitrant lesions. Oral terbinafine and griseofulvin are ineffective.

Malassezia folliculitis

Treatment with a topical imidazole or selenium sulfide is often effective, but oral treatment with ketoconazole 200 mg/day for 1–2 weeks may be required in patients with extensive or recalcitrant lesions. To prevent relapse, maintenance treatment may be given once or twice per week.

Seborrheic dermatitis

This is a difficult condition to treat in persons who have AIDS. Milder forms can often be managed with topical ketoconazole 2% cream with or without a topical corticosteroid. Terbinafine cream may also be effective. Patients with inflamed lesions often benefit from topical application of combined hydrocortisone 1% and antifungal drugs. However, relapse is common once treatment is discontinued.

Tinea nigra

Benzoic acid compound ointment or 10% thiabendazole solution can be applied morning and evening for 3–4 weeks. Topical imidazoles are also effective.

Piedra

Black piedra can be treated with a topical salicylic acid preparation or an imidazole cream. However, relapse is common. Shaving or clipping the affected hairs is often sufficient to clear white piedra. To help prevent recurrence, an imidazole lotion can be applied to the scalp after shampooing.

Onychomycosis

Topical agents should only be used where the infection is confined to the distal ends of the nails. Topical applications of tioconazole, amorolfine or ciclopirox solution should be continued for at least 6 months for fingernails and 12 months or longer for toenails.

Oral terbinafine is the treatment of choice for proven dermatophyte infections of the nail (tinea unguium).[24] However, it is not appropriate for *Candida* infections, nondermatophytic mold infections or mixed infections.[25] Pulsed treatment with terbinafine is at least as effective as continuous treatment while offering potential improvements in patient compliance and cost-effectiveness.[26,27] Pulsed therapy with terbinafine consists of 250 mg twice daily for 1 week each month for 3–4 months. If continuous treatment is given, the dose is 250 mg/day for a period of 6–12 weeks for fingernails and 3–6 months for toenails. Treatment with oral terbinafine will also clear any associated skin infection without the need for additional topical treatment. Terbinafine 250 mg/day for 3 months has been proven to be effective in the treatment of dermatophyte infections of the nail in persons who have AIDS. No drug interactions or significant adverse effects related to the drug have been reported.[28]

Itraconazole is also licensed for the oral treatment of nail infections at a dose of 200 mg/day for 3–6 months. Pulsed treatment with itraconazole has also been advocated because it is as effective as continuous treatment. Itraconazole has a broader spectrum than terbinafine and is more appropriate for patients who present with nondermatophyte or mixed nail infections.[25] However, itraconazole interacts with a number of other drugs and this can limit its usefulness in certain situations. It is not recommended for simultaneous use with protease inhibitors in persons who have AIDS. Fluconazole is not licensed for use in fungal nail disease, but it is sometimes useful in severe *Candida* nail infections.

Oral griseofulvin is only effective in dermatophytosis. It is no longer regarded as a first-line choice for toenail infections, but it works quite well in fingernail infections if given over a 6–9-month period. It must be taken until the affected nail has fully grown out. It should be borne in mind that it has a number of side-effects and can interact with other medications.

Other interventions include chemical dissolution of the nail using 40% urea paste and, in rare instances, surgical removal of the nail. Surgical removal is a painful and disfiguring procedure that should be reserved for cases where there are either contraindications to the use of systemic antifungal agents or a drug-resistant fungus is present.

Oral antifungal treatment of onychomycosis can be followed with topical terbinafine or ciclopirox nail solution in an attempt to prevent recurrence.

REFERENCES



References for this chapter can be found online at http://www.expertconsult.com

Practice point 2

Edmund L Ong

Approach to the acutely febrile patient who has a generalized rash

INTRODUCTION

In assessing patients who have fever and rash, the following five points are essential.

- Is the patient well enough to give a further history?
- Is immediate cardiorespiratory support required?
- From the nature of the rash, does the patient require isolation precautions?
- Is immediate empiric antimicrobial therapy required?
- How rapidly did the clinical picture evolve?

The history obtained should give the following information:

- drugs taken within the past month;
- geographic itinerary of travel;
- immunizations;
- occupational exposure;
- sexually transmitted disease exposure and risk factors for HIV;
- the immunologic status of the patient;
- whether the female patient is pregnant;
- any history of valvular heart disease;
- recent exposure to other ill febrile patients;
- stay in a crowded situation (e.g. soldier dormitory);
- exposure to wild or rural habitats and wild animals;
- exposure to domestic animals (bites);
- prior medical history including allergies; and
- sun exposure.

PATHOGENESIS

Virtually any class of microbe can induce a local skin rash with fever if the microbes are allowed to penetrate the stratum corneum. The systemic effects of micro-organisms on the skin, however, can also produce cutaneous eruptions by:

- multiplying in the skin;
- toxin-mediated effects;
- inflammatory responses; and
- altering the vasculature of skin.

MICROBIOLOGY

The range of organisms causing systemic infections with prominent cutaneous manifestations is described in Table PP2.1(see Chapters 8, 9 and 12). There are other noninfectious causes of fever with a generalized rash and these need to be borne in mind.

CLINICAL FEATURES

Physical examination should include the following:

- vital signs including blood pressure;
- general appearance;
- signs of toxicity;
- evidence of adenopathy;
- presence of mucosal, genital or conjunctival lesions;
- presence of hepatosplenomegaly;
- evidence of arthropathy; and
- signs of meningismus or neurologic dysfunction.

The rash should be assessed with regard to:

- its distribution;
- its pattern of progression;
- the timing of its development relative to the onset of illness and fever (Table PP2.2); and
- its characteristics.

The morphology of skin lesions includes macules, papules, plaques, nodules, vesicles, bullae and pustules. Skin lesions are also characterized by their color and particularly by the presence or absence of hemorrhage. Lesions may also be hyperpigmented or hypopigmented. Blanching erythematous lesions are due to vasodilatation, whereas nonblanching erythemas may be due to extravasation of blood. Purpuric lesions are hemorrhages into the skin and they may be small, petechial or large and ecchymotic. Lesions of erythema multiforme usually begin as round or oval macules and papules that vary in size and have central erythema surrounded by a narrow ring of normal skin, which is also surrounded by another thin ring of erythema to form target lesions. Most cases are idiopathic, but the common infective causes are shown in Table PP2.3. The lesions of erythema nodosum are characterized by tender, erythematous nodules that vary in diameter from 1 cm to several centimeters. Infectious agents are a major cause of this lesion (Table PP2.4).

INVESTIGATIONS

Establishing the microbiologic diagnosis is of great importance in managing the patient. Blood cultures should form part of the essential investigations, along with full blood count and differential white cell count, platelet count, liver function tests, renal function tests and acute phase reactants (e.g. C-reactive protein). Rapid changes (e.g. fall of platelets) are ominous. Skin lesion aspirates or biopsy should be considered, particularly for the identification of meningococcal, staphylococcal and gonococcal infections. A punch biopsy of the

Table PP2.1 Microbiology of cutaneous manifestations associated with systemic infections

Macular or papular rash		**Vesicobullous eruptions**	
Viruses	Adenovirus Measles virus (atypical) Colorado tick fever Coxsackie viruses Cytomegalovirus Dengue virus Echoviruses Epstein–Barr virus Hepatitis B virus HIV-1 Human herpesvirus 6 Lymphocytic choriomeningitis virus Parvovirus B19 (erythema infectiosum) Rubella virus (German measles) Rubeola virus (measles)	Viruses	Coxsackie viruses Echoviruses Herpes simplex virus (disseminated) Vaccinia virus Varicella-zoster virus (varicella and disseminated zoster) Variola virus (smallpox)
Bacteria	*Bartonella bacilliformis* *Bartonella henselae* *Bartonella quintana* *Borrelia burgdorferi* (Lyme disease) *Borrelia* spp. (relapsing fever) *Chlamydia psittaci* *Francisella tularensis* *Leptospira* spp. *Mycobacterium haemophilium* *Mycoplasma pneumoniae* *Pseudomonas aeruginosa* *Rickettsia akari* (rickettsial pox) *Rickettsia prowazekii* (epidemic/louse-borne typhus) *Rickettsia rickettsii* (Rocky Mountain spotted fever) *Rickettsia tsutsugamushi* (scrub typhus) *Rickettsia typhi* (endemic/murine typhus) *Salmonella typhi* *Spirillum minus* (rat-bite fever) *Staphylococcus aureus* *Streptobacillus moniliformis* (rat-bite fever) Streptococci group A (scarlet fever) *Treponema pallidum* (secondary syphilis)	Bacteria	*Listeria monocytogenes* *Mycoplasma pneumoniae* *Rickettsia akari* (rickettsial pox) *Vibrio vulnificus*
		Petechial purpuric eruptions	
		Viruses	Adenovirus Atypical measles Cytomegalovirus (congenital infection) Coxsackie viruses Dengue virus Echoviruses Epstein–Barr virus Rubella virus (German measles) Viruses causing hemorrhagic fevers Yellow fever virus
		Bacteria	*Borrelia* spp. (relapsing fever) *Capnocylophaga canimorsus* *Neisseria gonorrhoeae* *Neisseria meningitidis* *Rickettsia prowazekii* *Rickettsia rickettsii* *Staphylococcus aureus* *Streptobacillus moniliformis*
Fungi (disseminated)	*Blastomyces dermatitidis* *Candida* spp. *Coccidioides immitis* *Cryptococcus neoformans* *Fusarium* spp. *Histoplasma capsulatum*	Protozoa	*Plasmodium falciparum* (malaria)

maculopapular skin lesion of disseminated candidemia is sometimes diagnostic. Occasionally, a Gram stain of a routine buffy coat may reveal the responsible organisms (e.g. staphylococci, meningococci or *Candida* spp.) in a septic patient. Isolation of the causative organisms may be difficult, particularly with viruses and some bacteria. Serologic methods (e.g. serologic test for syphilis, paired viral complement fixation tests), molecular techniques (e.g. polymerase chain reaction for dengue fever virus, cytomegalovirus, HIV and other hemorrhagic fevers) and immunofluorescence microscopy are useful methods for establishing the difficult culturable organisms.

Table PP2.2 Skin lesions and systemic infections

Lesion	Common pathogens	Time of appearance after onset of illness
Toxic erythema	*Staphylococcus aureus* *Streptococcus pyogenes*	At presentation
Rose spots	*Salmonella* spp.	5–10 days
Purpuric lesions (in critically ill patients)	*Neisseria meningitidis* *Rickettsia* spp. *Capnocytophaga canimorsus* Gram-negative bacteria	12–36 hours
Macronuclear lesions	*Candida spp*. *Cryptococcus neoformans* *Histoplasma capsulatum* *Fusarium* spp.	Days
Erythema multiforme, bullous lesions, ecthyma gangrenosum	*Pseudomonas* spp. *Vibrio vulnilicus* Gram-negative bacteria	Days

Table PP2.3 Infective causes of erythema multiforme

- Adenovirus
- *Chlamydia trachomatis*
- *Chlamydia psittaci*
- *Coccidioides imitis*
- Enterovirus
- Epstein–Barr virus
- *Franciscella tularensis*
- Hepatitis B virus
- Herpes simplex virus
- Histoplasma
- HIV
- *Legionnella* spp.
- Mumps virus
- *Mycobacterium tuberculosis*
- *Mycoplasma pneumoniae*
- Orf
- Poliomyelitis virus
- *Pseudomonas aeruginosa*
- *Rickettsia* spp.
- *Salmonella typhi*
- *Streptococcus pyogenes*
- *Treponema pallidum*
- Vaccinia virus
- *Yersinia* spp.

Table PP2.4 Infections associated with erythema nodosum

- Blastomycosis
- Campylobacteriosis
- Cat-scratch disease
- Coccidioidomycosis
- Gonorrhea
- Hepatitis C
- Histoplasmosis
- Inflammatory dermatophyte infections
- Leptospirosis
- Lymphogranuloma venereum
- Psittacosis
- Salmonellosis
- Streptococcal infections
- Tuberculosis
- Tularemia
- Upper respiratory tract viruses
- Yersiniosis

FURTHER READING

Further reading for this chapter can be found online at http://www.expertconsult.com

Practice point | 3 |

Sajeev Handa

Management of foot ulcer

INTRODUCTION

Foot ulceration is a relatively common problem in clinical practice and one that sometimes poses difficult diagnostic and therapeutic dilemmas. Major complications are infection and, in more severe cases, the development of dry and wet gangrene. In certain cases making a determination of superinfection versus colonization can try even the most astute clinician.

PATHOGENESIS

The pathogenesis depends on the type and etiology of foot ulcer. The differential diagnosis includes:

- ischemic arterial ulceration;
- venous ulceration due to increased venous hydrostatic pressure causing local edema with its low exchange of oxygen and metabolites; edematous tissue (particularly skin) is more vulnerable to trauma than healthy tissue and is far less able to combat infection;
- neuropathic ulcers caused by diabetes, tabes dorsalis, leprosy, syringomyelia or hereditary sensory (radicular) neuropathy; the protective pain sensation is ablated, resulting in loss of awareness of trauma, which can cause further deterioration of the ulcer;
- vasculitis;
- infection, including acute pyogenic infections, tuberculous infections, tropical ulcer (chronic phagedenic ulcer secondary to Vincent's organisms), syphilis and yaws;
- patient disabilities, such as reduced vision, limited mobility and previous amputation(s); and
- health-care system failures, such as inadequate patient education, monitoring of glycemic control and foot care.

CLINICAL FEATURES

The ischemic arterial ulcer is typically located on the toes, heel, dorsum of the foot or lower third of the leg. The pain is severe and persistent and worsens at night. The ulcer is generally 'punched out' with a pale or necrotic base.

Venous ulcers are located in the 'gaiter' distribution around the ankle, especially around the medial malleoli. They are less painful, more diffuse and shallow and usually have some evidence of granulation tissue at the base.

Diabetic ulcers are usually located in the plantar or lateral aspect of the foot. They resemble arterial ulcers morphologically but are characteristically painless. Diabetic neuropathy (sensory, motor and autonomic), microvascular and macrovascular lesions, and diminished neutrophil function all conspire to generate diabetic foot ulcers.

Diabetic foot ulcers may be classified under Wagner's grades as follows.

Grade 0	No ulcer in a high-risk foot
Grade 1	Superficial ulcer involving the full skin thickness but not the underlying tissues
Grade 2	Deep ulcer, penetrating down to the ligaments and muscle but not involving the bone and no formation of an abscess
Grade 3	Deep ulcer with cellulitis or abscess formation, often with osteomyelitis
Grade 4	Localized gangrene
Grade 5	Extensive gangrene involving the whole foot

All ulcers may become secondarily infected. Features of infection range from minimal cellulitis with lack of systemic toxicity to extensive cellulitis with associated lymphangitis, purulent drainage, sinus tract formation, osteomyelitis, septic arthritis, abscess formation and sometimes the development of gangrene. Systemic signs and symptoms often occur late and suggest severe infection.

INFECTED ULCER – DIAGNOSIS

A peripheral blood count may demonstrate a leukocytosis (this may be absent in severe cases, especially in diabetes); the erythrocyte sedimentation rate is usually raised. Blood cultures may be positive, especially if the patient is febrile and has not received prior antibiotic treatment.

Obtaining a swab culture of the ulcer itself is an unreliable means of establishing the causative organism(s) in superinfected ulcerations. Ulcers are typically colonized by a multitude of organisms that may or may not be pathogenic. Deep tissue cultures that avoid contact with the ulcer surface or other draining lesions are preferable. In osteomyelitis, bone cultures obtained by percutaneous biopsy or surgical excision are the best specimens for determining the etiology provided the incision site is away from the ulceration itself.

Plain radiographs of the affected area are useful in determining the presence of foreign bodies or air in the soft tissues, which may suggest the presence of gas-forming bacteria. CT scanning and MRI are useful for looking for abscesses as well as early osteomyelitis. The performance of technetium bone scanning for the diagnosis of osteomyelitis in the impaired foot is poor and a 24-hour indium-111 leukocyte scan is more sensitive than a bone scan in diagnosing osteomyelitis associated with a diabetic foot. However, this test is expensive and less specific than MRI and may be difficult to interpret in the presence of local soft tissue inflammation (Chapter 41). High resolution ultrasonography may also be helpful for detecting soft tissue abscesses or sinus tract formation.

MICROBIOLOGY

Mild ulcers may be infected by single organisms. Organisms frequently involved include:

- *Staphylococcus aureus*;
- *Streptococcus pyogenes*; and
- facultative Gram-negative bacilli and anaerobic organisms (which are isolated infrequently).

Severe ulcers, especially the diabetic foot, are usually polymicrobial with aerobic and anaerobic bacterial isolates, including:

- *Staphylococcus aureus*;
- coagulase-negative staphylococci;
- aerobic streptococci and enterococci;
- Enterobacteriaceae (e.g. *Escherichia coli, Klebsiella* spp. and *Proteus* spp.);
- *Pseudomonas* spp.;
- *Corynebacterium* spp.;
- *Bacteroides fragilis* and other Bacteroidaceae; and
- *Clostridium perfringens* and other clostridial spp.

THERAPY

Antibiotics are the mainstay of treatment (Chapter 9) and are recommended in the presence of a surrounding cellulitis, a foul-smelling lesion, fever or deep tissue infection. Empiric antibiotics are necessary until culture results are available (see Table PP3.1). The clinician should assume that *Staphylococcus aureus* is resistant because of the current high prevalence of community-associated methicillin-resistant strains (CA-MRSA). Compared with health-care-associated strains, CA-MRSA strains produce more toxins and appear more virulent. Agents effective against CA-MRSA such as vancomycin, linezolid or daptomycin should therefore be utilized. Step-down treatment with minocycline or trimethoprim–sulfamethoxazole may be feasible based on susceptibility test results and clinical response.

The optimal duration of therapy is unclear; however, for infections that are limited to soft tissue, intravenous therapy may be administered for 7–10 days followed by oral therapy for an additional 14 days. For those in whom osteomyelitis is identified, a minimum of 6–8 weeks' parenteral therapy is recommended if the offending tissue is not removed in its entirety (Chapter 41). Limb-threatening infections require immediate hospitalization, bed rest and a strict nonweight-bearing regimen, even if signs and symptoms of systemic infection are absent. Although medical stabilization, metabolic and glycemic control (in diabetic patients) and antimicrobial therapy are important, debridement should not be delayed. Failure to debride necrotic infected tissue and to drain purulent collections increases the risk of amputation. The initial debridement must be performed independently of the status of the arterial circulation and revascularization should be postponed until sepsis is controlled (Chapter 10).

Surgical consultation for intervention should be considered in infections accompanied by a deep abscess, extensive bone or joint involvement, crepitus, substantial necrosis or necrotizing fasciitis.

Table PP3.1 Selected empiric antimicrobial regimens for infected foot ulcers

Non-limb-threatening infection	
Oral regimens	Trimethoprim–sulfamethoxazole double strength (ds) Minocycline plus penicillin VK Cephalexin, cefprozil, cefuroxime, cefdinir, cefpodoxime or a fluoroquinolone Amoxicillin–clavulanate extended release plus trimethoprim–sulfamethoxazole ds or a fluoroquinolone Alternative: clindamycin plus a fluoroquinolone
Limb-threatening infection	
Parenteral regimens	Vancomycin plus ampicillin–sulbactam, piperacillin–tazobactam or ticarcillin–clavulanate Vancomycin plus ertapenem, imipenum or meropenem Daptomycin, linezolid or tigecycline may be substituted for vancomycin and ciprofloxacin, levofloxacin, moxifloxacin plus metronidazole for the β-lactam/β-lactamase inhibitor combination If methicillin-sensitive *Staphylococcus aureus* is identified then vancomycin may be switched to oxacillin, nafcillin or cefazolin The activity of clindamycin against CA-MRSA is geographically variable and is not recommended for use as a sole agent for severe infections Rifampin (rifampicin) is highly active against CA-MRSA and is commonly used in combination with trimethoprim–sulfamethoxazole or doxycycline but should never be used alone. It is particularly useful in patients with ulceration complicated by abscess formation

It should be noted that definitive management of the ulcer will require treatment of the underlying cause. For example, up to 60% of diabetic patients with nonhealing ulcers have associated arterial insufficiency. Therefore, the arterial circulation must be critically evaluated in all diabetics presenting with a foot ulcer. Once the ulcer has healed, a lifelong program of proper footwear, education and close follow-up for routine callus and nail care must be maintained. In addition, tetanus vaccination status must be ascertained in all patients presenting with ulceration or infection.

FURTHER READING

Further reading for this chapter can be found online at http://www.expertconsult.com

Practice point | 4 |

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Managing the patient with recurring skin infections

BACKGROUND

Skin infections come in many forms. Most commonly, troublesome skin infection is synonymous with cellulitis, an entity that perfectly illustrates the cardinal signs of inflammation. Cellulitis is therefore an acute, usually noncontagious inflammation of the connective tissue of the skin, resulting from bacterial infection and characterized by localized warmth, erythema, pain and tenderness, swelling and reluctance to mobilize the affected area (Fig. PP4.1). When such a problem is recurrent, this can become extremely tiresome and even disabling for the afflicted individual.

Cellulitis is usually consequent upon a break developing in the skin surface or its appendages, such as a laceration, cut, fissure, puncture wound, insect bite, animal or human bite, scratch, abrasion, blisters or friction burn, such as might occur with shoes that are too tight. Organisms normally confined to the skin surface are admitted to the dermis where they proliferate and lead to cellulitis.

Erysipelas is a specific form of cellulitis usually caused by group A streptococci. The skin is red and very tender with a raised, well-demarcated area of inflammation often on the lower extremities or face. It is thought to be toxin mediated with intense superficial inflammation and lymphatic involvement. This can result in lymphatic obstruction and make the patient susceptible to recurrent episodes. Direct microbiologic cultures of skin and aspirate are often negative.



Fig. PP4.1 Severe recurrent cellulitis associated with obesity.

RECURRENT CELLULITIS

Recurrent cellulitis implies that factors facilitate the recurrent entry of organisms into the dermis. Effective management of recurrent cellulitis involves identifying these factors and, if possible, remedying them. Recurrent cellulitis may cause local persistent lymphedema, resulting in permanent hypertrophic fibrosis.

With respect to location, most often it is the lower limbs that are involved in recurrent cellulitis. The site may be the arm if, for example, the patient has received radiotherapy to the axillary area as part of breast cancer treatment. Other sites, such as the vulva and perianal region (sometimes in association with *Enterobius vermicularis*) can also be problematic.

To make matters more complex, cellulitis of the lower extremities is more likely to be complicated by thrombophlebitis in elderly patients, which in turn can encourage recurrence of cellulitis.

A number of clinical scenarios and risk activities render patients particularly vulnerable to recurrent episodes of cellulitis. These include:

- tinea pedis or onychomycosis;
- fissures between toes;
- diabetes mellitus – there may be a family history;
- peripheral vascular (arterial) disease – there may be a history of smoking, angina pectoris or hypertension;
- ischemic or venous ulceration of the skin (including sickle cell disease);
- post-deep venous thrombosis;
- eczema and dermatitis;
- immunodeficiency states, for example patients with HIV infection (who may be more prone to recurrent staphylococcal skin sepsis), neutropenia (granulocytopenia), Job's syndrome (hyper IgE syndrome with recurrent staphylococcal cellulitis) or use of immunosuppressive or corticosteroid drugs – always establish the medication history;*

*Unusual organisms such as *Campylobacter jejunii* may cause an erysipelas-like cellulitis in patients with hypogammaglobulinaemia, probably as a result of a lack of serum bactericidal activity. Reference should be made to patients with agammaglobulinemia who develop *Campylobacter jejunii* cellulitis because of a lack of serum bactericidal effect (see Kerstens *et al*. in Further reading). *C. fetus* gives a similar picture in patients with deficient cell-mediated immunity.



Fig. PP4.2 Severe recurrent cellulitis in a lymphedematous leg following radical surgery for rhabdomyosarcoma.

- lymphatic obstruction, for example post-radiotherapy (e.g. post-mastectomy), post-block dissection of lymph nodes for cancer (Fig. PP4.2), elephantiasis (e.g. due to infections by *Wuchereria bancroftii*, *Brugia malayi*, *Onchocerca volvulus*) or Milroy's disease;
- scar cellulitis (e.g. previous burn or skin graft sites and in areas from which veins were harvested for coronary artery bypass grafting);
- trauma related, for example cosmetic piercings (studs, rings), intravenous drug users, recurrent localized trauma, self-harm or Munchausen's syndrome;
- nasal carriage of staphylococci;
- lepromatous leprosy;
- underlying occult osteomyelitis;
- very poor personal hygiene (e.g. associated with alcoholism); and
- morbid obesity – largely associated with recurrent lower limb cellulitis (see Fig. PP4.1).

MICROBIOLOGY

In immunocompetent individuals, cellulitis is usually the result of Gram-positive aerobic cocci, particularly *Staphylococcus aureus* and *Streptococcus pyogenes*, or sometimes a combination of both. It can be clinically difficult to decide which of them is causing the problem.

A minority of *Staph. aureus* strains may produce the Panton–Valentine leukocidin toxin (PVL), a cytotoxin that causes leukocyte destruction and tissue necrosis. Outbreaks of recurrent cellulitis, boils and abscesses have been reported within families by this strain. Awareness of the high transmissibility and virulence of the PVL-producing strain is crucial in avoiding recurrence, eradicating reservoirs and preventing severe complications such as necrotizing pneumonia.



Fig. PP4.3 Mixed *Aspergillus* and *Prototheca* cellulitis in a neutropenic patient.

Non-group A streptococci, particularly groups B, C and G, are sometimes implicated in cellulitis, occurring in patients with lymphatic obstruction or post-vein harvesting for coronary artery bypass grafting.

Recurrent cellulitis due to streptococci may be seen in association with chronic lymphedema (e.g. from lymph node dissection, post-irradiation, Milroy's disease, elephantiasis).

Neutropenic patients may develop cellulitis due to other organisms, such as Gram-negative bacilli (e.g. *Proteus, Serratia, Enterobacter* spp.) and fungi. Rarely, the infection can be mixed with fungal and algal species (e.g. *Aspergillus* and *Prototheca* spp., Fig. PP4.3). *Campylobacter* species may cause both septicemia and cellulitis in hypogammaglobulinemic patients. The organism is often isolated from tissue biopsies and blood cultures.

Other organisms may be involved as part of a mixed picture, depending upon the source of the organisms. Incontinent patients may contaminate their lower limbs with urine and feces while intravenous drug users can inoculate their own tissues (Fig. PP4.4) with a variety of organisms from contaminated needles. Patients whose cellulitis is the result of deliberate self-harm may also yield multiple organisms on culture. This is an extremely difficult diagnosis to make and requires the highest levels of clinical acumen. For example, self-inoculation with milk has been reported as the cause of recurrent cellulitis.

IS IT REALLY CELLULITIS?

Sometimes the apparent recurrent cellulitis problem may not in fact be cellulitis, and the following should be considered:

- acute gout can resemble recurrent cellulitis and certain diuretics may predispose to gout;
- recurrent deep venous thrombosis;
- migratory necrolytic erythema associated with underlying neoplasia, particularly glucagonoma of the pancreas;
- inflammatory carcinoma of the breast, which produces a picture of localized cellulitis unresponsive to antibiotics;
- herpes zoster, which can cause recurrent rash that may be complicated by superinfection;
- erythema nodosum, especially if it recurs;



Fig. PP4.4 Intravenous drug user with severe recurrent cellulitis of the left arm.

- palmoplantar pustulosis and pyoderma gangrenosum, such as that associated with inflammatory bowel disease, can be mistaken for cellulitis;
- scurvy and pellagra; and
- fixed drug eruptions.

ASSESSMENT AND DIAGNOSIS

Unless pus has formed or an open wound is present, it is often difficult to isolate the responsible organism from a case of cellulitis. Aspiration of material from the advancing edge of the lesion, skin biopsy and blood cultures yield potential pathogens in only about 25% of cases. The etiology of most cases of cellulitis will usually be *Staph. aureus* and/or *Strep. pyogenes*.

In unusual circumstances, such as patients who are immunocompromised or those not responding to empiric therapy, or indeed whenever the clinical history points toward other infective or noninfective diagnostic options, further investigations may be warranted. This may become particularly important where the patient is suffering recurrent attacks. For example, among those with peripheral vascular disease or diabetes mellitus, minor injuries or cracked skin in the feet or toes can serve as an entry point for recurrent infection.

Attention should accordingly be directed toward establishing the presence or absence of factors that might be supporting the development of recurrent cellulitis and might be amenable to correction. The following range of tests can be applied selectively according to circumstances:

- microbiologic – samples for microscopy, Gram staining, culture and sensitivity, swabs from areas of abscess or bullae formation, needle aspiration of the advancing edge of cellulitis, full skin biopsy, interdigital skin and/or nail scrapings (especially where tinea pedis is present), blood culture (positive in only a few patients), nasal swabs (especially for *Staph. aureus* carriage, including methicillin-resistant *Staph. aureus*, MRSA), perianal cellophane tape (for *Enterobius* ova), throat swab (for *Strep. pyogenes* in those with erythema nodosum) and bullous fluid (for immunofluorescence antibody test for varicella-zoster);
- imaging – tissue scanning (plain radiographs, ultrasound, CT, MRI and indium leukocyte scanning) may identify collections of pus meriting drainage, foreign bodies or underlying osteomyelitis (if gas is seen in the tissues, the differential diagnosis then includes gangrene and fasciitis, which are generally considered to be surgical emergencies), Doppler scans (which may assist in identifying deep venous thrombosis or peripheral arterial disease);
- hematologic and immunologic – blood films (macrocytosis associated with alcohol excess and microfilaria in suspected filariasis), differential white cell count (to identify neutropenia, eosinophilia, e.g. in filariasis), hemoglobin electrophoresis in sickle-cell disease, immunoglobulin levels and subsets, complement levels, T-cell subsets;
- serology – HIV-1 and HIV-2, antistreptolysin titer (may point toward erythema nodosum as the diagnosis), hepatitis C, hepatitis B (may point toward occult intravenous drug abuse), filariasis, onchocerciasis if the patient is at risk;
- biochemistry – blood glucose, urate levels, liver function tests; and
- skin biopsy – may help with rarer causes of cellulitis.

MANAGEMENT

Managing the acute phase of recurrences

Tissue penetration sufficient to achieve adequate local antibiotic concentrations can be problematic. For acute exacerbations, intravenous therapy may therefore be necessary. Useful combinations include (flucl)oxacillin–benzylpenicillin, (flucl)oxacillin–amoxicillin and clindamycin–ciprofloxacin.

Other antibiotics may be indicated, depending upon the clinical scenario:

- where allergy to β-lactam drugs is an issue – macrolides, levofloxacin or moxifloxacin;
- where MRSA is an issue – doxycycline and rifampin (rifampicin) can be given orally; vancomycin remains the first choice for parental therapy although linezolid, quinupristin–dalfopristin and more recently daptomycin and tigecycline are alternative options;
- where anaerobes are an issue – metronidazole, clindamycin or quinupristin–dalfopristin; and
- where *Campylobacter* spp. are an issue – macrolides, quinolones or carbapenems, with treatment according to sensitivities; regarding *Campylobacter*, macrolides, quinolones and imipenem are dependent on susceptibility with plasma treatment.

Surgical care includes debridement of devitalized tissue. Incision and drainage may be indicated if suppuration occurs. Treat local effects of cellulitis by elevating the affected limb.

Prevent recurrences

Adequate patient education and training are essential.

Skin and foot care for tinea pedis and onychomycosis includes:

- patient training regarding proper skin hygiene and suitable footwear;
- treating affected toe webs or feet with topical antifungals;
- consideration of oral antifungals such as itraconazole or terbinafine for severe chronic tinea pedis or onychomycoses; and
- expert podiatry – cuts and fissures should be washed and kept clean while healing.

For cases caused by edema, treat any underlying cause (e.g. cardiac failure) and relieve edema using support stockings, specialized bandaging and nocturnal elevation of the affected area. Diuretics may have a role.

Immunocompromised patients will remain vulnerable to recurrent infections and therefore may need prolonged antibiotics until their immune status improves.

There is no convincing evidence for the value of antibiotic prophylaxis and there is a risk of antibiotic resistance. Penicillin, erythromycin and clindamycin have all been advocated. Early institution of antibiotics may help in cellulitis of the lower extremities. The patient must be trained to spot the early signs of a recurrence, and given a supply of antibiotics (such as amoxicillin or flucloxacillin) to take. They should be advised to seek medical advice as soon as possible.

Nasal carriage of *Staph. aureus* can be treated with mupirocin if it is thought to be associated with recurrent disease.

CONCLUSION

Recurrent cellulitis is responsible for much morbidity. Diagnosis is not always straightforward and it presents a significant management challenge.

FURTHER READING


Further reading for this chapter can be found online at http://www.expertconsult.com



Chapter 14

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Lymphadenopathy

INTRODUCTION

The body has approximately 600 lymph nodes, but only those in the cervical, submandibular, axillary or inguinal regions may normally be palpable in healthy people. Lymphadenopathy is a change in the size and/or consistency of a lymph node or lymph node group (regional lymphadenopathy), or may be generalized involving multiple sites and multiple lymph node groups. The lymph node system is the major component of the body's surveillance system against foreign invaders and functions as a filter to trap micro-organisms, tumor cells, immune complexes and foreign material.

The lymphoid system grows rapidly during childhood and achieves twice the adult size in early adolescence. Thereafter it starts regressing, reaching adult maturity at about the age of 20–25 years. Lymphadenopathy, particularly peripheral, is thus a common finding in childhood, adolescence and young adulthood.[1] Lymphadenopathy may be divided into acute and chronic lymphadenitis, i.e. inflammatory lymphadenopathy, lymphadenopathy that accompanies lymphoproliferative diseases, infiltrative lymphadenopathy secondary to malignant disease and reactive lymphadenopathy that may be infectious or noninfectious. Table 14.1 summarizes the differential diagnosis of lymphadenopathy.

PATHOGENESIS AND PATHOLOGY

The normal lymph node

Lymph nodes are widely distributed throughout the human body, particularly at potential portals (Fig. 14.1). The normal lymph node is an oval, encapsulated, soft structure, 1–2 cm in diameter with an average weight of approximately 1 g.

Histologically the lymph node can be divided into three regions (Fig. 14.2):[2]

- the cortex, the outmost layer, which is composed mainly of B lymphocytes and macrophages arranged in primary follicles;
- the paracortical region, underneath the cortex, which is composed mainly of T lymphocytes and dendritic cells; and
- the medulla, the innermost region, which has fewer lymphocytes than the other two regions but more plasma cells that secrete immunoglobulins.

Afferent lymphatic vessels empty the lymph drained from the tissues into the subcapsular sinus; from there the lymph flows through the cortex, paracortex and medulla, allowing phagocytic and dendritic cells to trap any foreign material. The efferent lymphatic vessels carry lymph rich in lymphocytes and antibodies into the circulatory system.

The lymph node has two functions:

- it acts as a defensive barrier; and
- it serves as a factory for lymphocyte maturation and differentiation and as an antibody production site during antigenic challenge.

Lymphadenitis

Lymphadenitis is an inflammation of the lymph node. The initial phase of an acute inflammation consists of swelling and hyperplasia of the sinusoidal lining cells and infiltration by leukocytes and edema. This leads to distention of the node's capsule which causes local pain. The process may progress to abscess formation causing the node to become fluctuant depending on the causative micro-organism and the host response. The node may break into the skin and produce a draining sinus. Following the infection the node resumes its normal architecture or, if severely damaged, may obliterate completely. Acutely inflamed lymph nodes are most commonly caused by entrapped microbes.

Chronic lymphadenitis is typically a proliferative process with either follicular hyperplasia or paracortical lymphoid hyperplasia depending on the cause of the inflammation; such nodes are nontender.

EPIDEMIOLOGY

In children the cause of lymphadenopathy is apparent in most cases. In approximately 80% of the cases it is benign, mainly reactive–infectious in origin. In contrast, lymphadenopathy in adults more often reflects serious disease. One study revealed a 0.6% annual incidence of unexplained lymphadenopathy in the general population. Of 2556 patients with unexplained lymphadenopathy, 3.2% required a biopsy but only 1.1% had a malignancy. The probability of a neoplasm affecting enlarged peripheral lymph nodes increases steadily with age; in those older than 50 years who are referred for biopsy because of longstanding enlarged lymph nodes, more than 60% of cases of lymphadenopathy are due to a malignancy.[2,3] In contrast, in primary care settings, patients 40 years of age and older with unexplained lymphadenopathy have about a 4% risk of cancer versus a 0.4% risk in patients younger than age 40.[4] In tropical and subtropical regions leading causes may include parasitic diseases as well as infections.

Lymphadenopathy is defined as generalized whenever three or more anatomically discrete groups of lymph nodes are involved. The different infectious etiologies of generalized lymphadenopathy are shown in Table 14.1. Occasionally lymph nodes that are not palpable may be involved as is the case with lymph node involvement in anthrax or typhoid (abdominal), sarcoidosis and tuberculosis (mediastinal).

Table 14.1 Differential diagnosis of lymphadenopathy

Etiology	Regional	Generalized	Suppurative/caseating
Infectious lymphadenopathy			
Bacterial (acute)			
Streptococcal	+		+
Scarlet fever	+	+	
Staphylococcal	+		+
Diphtheria	+		
Ludwig's angina	+		
Tuberculosis	+	+	+
Syphilis	+	+	
Chancroid	+		
Plague	+		+
Tularemia	+		+
Rat-bite fever	+*		
Anthrax	+		
Melioidosis		+	
Glanders	+		+
Cat-scratch disease	+	+	
Typhoid fever		+	
Rickettsial			
Boutonneuse fever	+*		
Scrub typhus	+		
Rickettsialpox	+		
Chlamydial			
Lymphogranuloma venereum	+		+
Viral			
Measles		+	
Rubella		+	
Infectious mononucleosis		+	
HIV/AIDS		+	
Cytomegalovirus infection		+	
Dengue		+	
West Nile fever		+	
Lassa fever		+	
Genital herpes	+	+	
Epidemic keratoconjunctivitis (adenovirus)	+		+
Pharyngoconjunctival fever (adenovirus)	+		
Mycotic			
Histoplasmosis		+	+
Coccidioidomycosis	+		

(Continued)

Table 14.1 Differential diagnosis of lymphadenopathy—cont'd

Etiology	Regional	Generalized	Suppurative/caseating
Paracoccidioidomycosis	+		
Cryptococcosis	+	+	
Protozoan			
Kala-azar		+	
Leishmaniasis	+		+
African trypanosomiasis	+	+	
Chagas disease		+	
Toxoplasmosis	+	+	
Helmintic			
Loa loa	+		+ (Bubo)
Onchocerciasis	+		+ (Bubo)
Filariasis		+	
Noninfectious lymphadenopathy			
Sarcoidosis		+	
Connective tissue disorders		+	
Kawasaki disease	+	+	
Rosai–Dorfman disease	+	+	
Kikuchi's disease	+	+	
Castleman's disease	+	+	
Drug hypersensitivity		+	
Silicone breast implant	+		
Infiltrative malignant			
Metastatic carcinoma	+	+	
Metastatic melanoma	+		
Leukemia		+	
Infiltrative nonmalignant			
Lipid storage disease		+	
Amyloid		+	
Primary lymphoproliferative			
Lymphoma		+	
Angioimmunoblastic lymphadenopathy		+	
Lymphomatoid granulomatosis		+	
Malignant histiocytosis		+	
Drug induced			
Allopurinol, atenolol, captopril, carbamazepine, cephalosporins, gold, hydralazine, penicillin, phenytoin, primidone, pyrimethamine, quinidine, sulfonamides, sulindac		+	

*Ulceroglandular.

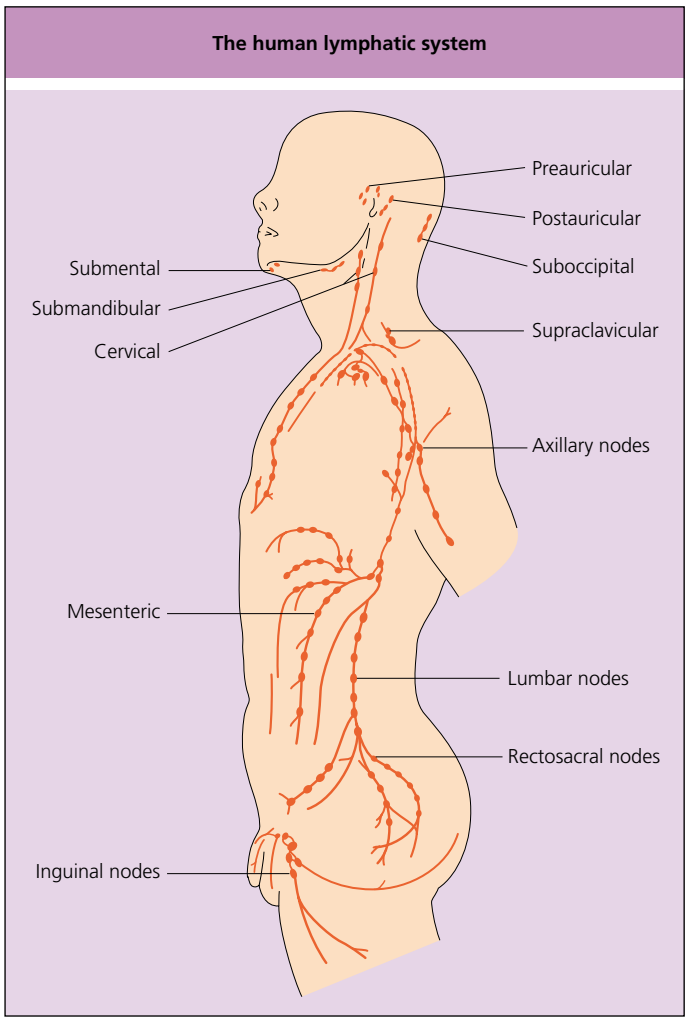


Fig. 14.1 The human lymphatic system.

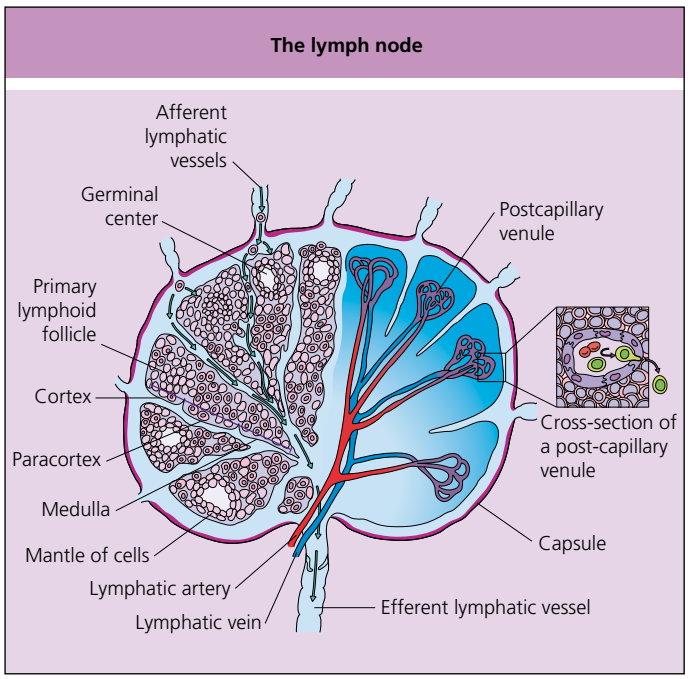


Fig. 14.2 The lymph node.

Viral diseases are the major cause of generalized lymphadenopathy. The bacterial diseases that may cause generalized lymphadenopathy include tuberculosis, typhoid fever, brucellosis and leptospirosis. In the differential diagnosis of generalized lymphadenopathy important parameters are the age of the patient, epidemiologic factors, relevant traveling, contact with sick individuals, accompanying signs and symptoms (rash, splenomegaly), and laboratory findings.

CLINICAL FEATURES

Regional lymphadenopathy and lymphadenitis

Acute suppurative lymphadenitis

Acute suppurative lymphadenitis is commonly caused by pyogenic infections, arising from draining of the organisms causing the initial focus of infection (especially *Staphylococcus aureus* or group A streptococci). The most commonly involved sites are the submandibular, cervical, inguinal and axillary lymph node groups.

The affected lymph node is extremely tender and firm, although it may be fluctuant, and the overlying skin may be red and warm. There are usually systemic manifestations. Acute cervical lymphadenitis due to a pyogenic infection is more common in children than adults. In both children and adults it is commonly due to staphylococcal infections of the face or neck and, uncommonly, it may be a complication of streptococcal pharyngitis.[5] In adults, anaerobic bacteria, of which the predominant species are *Prevotella* spp., *Peptostreptococcus* spp., *Propionibacterium acnes* and *Fusobacterium* spp., are recovered in 30% of cervical lymphadenitis, 13% are anaerobes alone and 17% are mixed anaerobic–aerobic bacteria.[6]

Acute pyogenic cervical lymphadenitis is unilateral. In contrast, acute bilateral cervical lymphadenitis is commonly due to viral upper respiratory infection, infectious mononucleosis, streptococcal pharyngitis or localized periodontal infections. Acute suppurative axillary lymphadenitis is a severe infection with prominent systemic manifestations and axillary pain that radiates to the shoulder and down to the arm. The axilla, arm, shoulder and supraclavicular and pectoral areas are markedly edematous, but there are no signs of skin infection or lymphangitis. The portal of entry of the infecting bacteria (group A streptococci or *S. aureus*) is often a traumatic lesion of the arm.[7] Rapidly enlarging lymph nodes may be accompanied by systemic manifestations, including toxic shock syndrome, without obvious genital or skin lesions.[8,9]

Patients who have chronic granulomatous disease experience recurrent pyogenic infections, of which the most common manifestations are lower respiratory tract infections, suppurative lymphadenitis, subcutaneous abscesses and hepatic abscesses.[10] The infecting pathogens are catalase-positive organisms such as *S. aureus*, *Serratia marcescens*, *Burkholderia* (*Pseudomonas*) *cepacia* and *Aspergillus* spp. The histologic appearance of the lymph node is one of inflammation with granuloma formation and necrosis.[10,11]

Cat-scratch disease

Cat-scratch disease typically manifests after a cat scratch or bite as regional lymphadenopathy distal to the involved lymph node. The mode of transmission is presumably direct contact with the causative agent, primarily *Bartonella henselae*. The disease occurs worldwide, with healthy children and adolescents being most frequently affected.[12] A history of a trivial cat scratch or a bite by a kitten can be elicited in most cases.[13] Rarely, a dog or monkey is implicated. Occasionally, typical cat-scratch disease cases can be caused by other pathogenic *Bartonella* sp. (i.e. *Bartonella clarridgeiae*).[14]

Tender lymphadenopathy develops within 1–3 weeks after inoculation. Commonly, an erythematous papule at the site of inoculation precedes the development of lymphadenopathy and may last for

Table 14.2 Oculoglandular syndromes

Disease	Infecting organism	Features
Cat-scratch disease	*Bartonella henselae*	Parinaud's sign in 3%, conjunctivitis in 6%
Tularemia	*Francisella tularensis*	Parinaud's sign in 5%
Lymphogranuloma venereum	*Chlamydia trachomatis*	Parinaud's sign in <1%
Pharyngoconjunctival fever	Adenovirus 3, 7	Common in children
Epidemic keratoconjunctivitis	Adenovirus 8, 19, 37	Occasionally seen in adults
Chagas disease	*Trypanosoma cruzi*	Romaña's sign

several weeks. Regional lymph node enlargement is the sole manifestation in one-half of the patients. Most commonly the cervical, axillary or epitrochlear lymph nodes are involved, but any peripheral nodes at multiple sites may be enlarged. In one-third of the patients, low-grade fever is present, and about 15% have systemic manifestations such as malaise, headache, splenomegaly and sore throat. Unusual clinical manifestations occur in 10% of patients; the most frequent of these is the oculoglandular syndrome of Parinaud,[13,15] which is conjunctivitis with ipsilateral preauricular lymphadenitis (Table 14.2). The adenopathy subsides spontaneously within several months. Occasionally, aspiration of a suppurative lymph node is needed to relieve pain.

The diagnosis is based on epidemiologic exposure and can be confirmed by detection of serum antibody to *B. henselae*.[13] Occasional cat-scratch disease cases caused by other pathogenic *Bartonella* may, however, be serologically negative.[14]

In atypical presentations or whenever a neoplastic or mycobacterial process is suspected, a lymph node biopsy or aspirate may be needed.

Mycobacterial lymphadenitis

This is the most common form of extrapulmonary tuberculosis in the Western world.[16] Tuberculous cervical lymphadenitis is caused by spread of *Mycobacterium tuberculosis* from a lung infection. Scrofula often presents as a one-sided red, painless mass, located along the upper border of the sternocleidomastoid muscle or in the supraclavicular area or axilla. In areas in which both tuberculosis and HIV are prevalent, tuberculous lymphadenitis may be the presenting sign in 50% of young children and is often associated with intrathoracic disease[17] (Fig. 14.3). Currently most tuberculous lymphadenitis is caused by *M. tuberculosis* and atypical mycobacteria, particularly *M. scrofulosum* and *M. avium* complex (MAC).[18] The process is indolent and slowly progressive and is not accompanied by systemic symptoms.



Fig. 14.3 Tuberculous lymphadenitis of the axilla.

If systemic signs appear, or the process is outside the cervical lesion, miliary tuberculosis should be suspected. With *M. bovis* infection the primary focus is usually the tonsil or the pharynx.

The diagnosis of mycobacterial lymphadenitis is confirmed microbiologically and with histology of the afflicted lymph node. Fine needle aspiration frequently reveals the presence of granuloma but only rarely yields smears that are positive or material that is culture positive.[18] Microbiologic and histologic examinations are complementary as *M. tuberculosis* has been isolated from lymph nodes not showing granuloma, and granulomata have occasionally grown atypical mycobacteria. Acid-fast bacilli are only rarely seen in smears except in HIV co-infected patients. New diagnostic techniques such as DNA hybridization, PCR and blotting techniques hold promise for more accurate and rapid diagnosis[19] (see Chapter 174).

Plague and tularemia

Both these infections are zoonoses that produce rather similar manifestations, mostly fever and regional lymphadenitis.[20] Plague and tularemia may be used as bioterrorism agents as they are highly infectious when dispersed by the airborne route.[21,22]

Plague is caused by *Yersinia pestis* (see Chapter 120). Most human cases are in the bubonic form transmitted by the bite of infected fleas. After an incubation period of 2–8 days patients develop sudden fever, chills, malaise and headache. Usually by the same time or within a few hours, a painfully swollen regional lymph node appears in the draining region of the inoculation site, commonly in the axilla, neck or groin. The primary lesion is occasionally found at the bite site and may develop into an area of cellulitis or an abscess. Over the next few days, the discrete lymph nodes become matted together to form the characteristic bubo with extensive surrounding edema (Fig. 14.4). The matted lymph nodes are exquisitely tender. Isolation of *Y. pestis* from an aspirated bubo, blood, bone marrow and multiple organs is possible but bacterial growth is slow; rapid identification can be accomplished by a characteristic Gram or Wayson stain. Such patients should be immediately isolated, reported and treated.[22]

Tularemia (see Chapter 121) occurs only in the northern hemisphere. Over 80% of infections are acquired by handling infected animals or by tick or deer fly bites. In the Western world most cases are sporadic, whereas in other parts of the world large water-borne, arthropod-borne and airborne outbreaks have been reported. Infection commonly manifests as an ulceroglandular or oculoglandular syndrome.[21] The most common portals of entry are the skin and the conjunctiva, with an ulcer or a pustule, or in the case of the conjunctiva or eyelid, conjunctivitis, a papule or an ulcer, developing 1–10 days after exposure. Regional lymphadenopathy follows and the enlarged lymph nodes may suppurate. Systemic manifestations are common



Fig. 14.4 Bubonic plague, axillary bubo and accompanying edema.

Table 14.3 Differential diagnosis of infectious inguinal lymphadenopathy

Sexually transmitted diseases	Other diseases
Syphilis	Pyogenic infections
Lymphogranuloma venereum	Cellulitis
Chancroid	Plague
Genital herpes	Filariasis
Granuloma inguinale	Onchocerciasis

but systemic toxicity and prostration is absent. Presumptive diagnosis is made on epidemiologic grounds and confirmed by specific serologic tests.[23]

Inguinal lymphadenopathy

Sexually transmitted diseases (STDs) and metastatic genital neoplastic disease are the most common causes of inguinal lymphadenopathy. The differential diagnosis of infectious inguinal lymphadenopathy is shown in Table 14.3.

Chancroid

Chancroid is caused by *Haemophilus ducreyi* and in some parts of the world (e.g. Thailand) it is one of the most common causes of genital ulcer with inguinal lymphadenopathy (see Chapter 60). The chancroid ulcer is a painful, nonindurated lesion that appears 1 day to several weeks after inoculation. Inguinal lymphadenitis occurs in between one-third and one-half of untreated cases. The lymph nodes are enlarged, painful and tender. The process is most commonly unilateral and, without treatment, can progress to suppuration with periadenitis and involvement of the overlying skin (bubo).

Culture provides the definitive diagnosis; however, *H. ducreyi* is a fastidious organism and immediate direct inoculation into specific culture media is required for bacterial growth and isolation. Chemotherapy is sufficient in most uncomplicated cases but abscesses that are more than 5 cm in diameter may need surgical drainage[24] (see also Chapter 60).

Lymphogranuloma venereum

Lymphogranuloma venereum is a rare STD caused by *C. trachomatis* serovars L1, L2 and L3. The typical vesicular lesions appear 1–2 weeks after inoculation but the incubation period varies between 5 and 20 days. The lesions often go unnoticed by the patient. Inguinal lymphadenopathy appears 1–6 weeks after the vesicles disappear. The lymphadenopathy is most commonly unilateral but is bilateral in 30–40% of patients. The nodes are painful and the groove sign (cleavage of the enlarged nodes by the inguinal ligament) is seen in 25% of patients (Fig. 14.5). The involved lymph nodes frequently coalesce to form a bubo. If untreated, the nodes, which are filled with bacteria, rupture and a nonhealing fistula is formed.

The anorectal syndrome, which occurs mainly in women and homosexual men, results from involvement of the pelvic lymph nodes. In the male, abscess formation may occur in the dorsal lymphatic of the penis and cause tissue destruction and elephantiasis of the penis. *Chlamydia trachomatis* can be isolated from both blood and aspirates of lymph nodes in approximately 30% of cases. Incision of the bubo is not warranted for diagnosis and positive serologic tests (complement fixation antibody test or microimmunofluorescence test) in the appropriate clinical setting are highly sensitive and specific for the diagnosis[25] (see Chapter 60).



Fig. 14.5 Groove sign of lymphogranuloma venereum. There is cleavage of extensive lymphadenopathy by the inguinal ligament.

Syphilis

The lymphadenopathy of primary syphilis is easily differentiated from chancroid and lymphogranuloma venereum because nodes involved in syphilis are firm, only moderately enlarged, nonsuppurative and painless. The classic primary chancre appears 14–30 days after inoculation and is a nonexudative, painless ulcer. In the immunocompetent patient only one chancre appears but in the immunocompromised patient, especially in patients who have AIDS, multiple chancres may be seen. In women and homosexual men, the chancre may be located in the perianal region or in the anal canal. Regional painless lymphadenopathy is characteristic at this stage of disease. The chancre usually heals and disappears after 3–6 weeks, but the lymphadenopathy may persist for longer. The symptoms of secondary syphilis appear 2–8 weeks after the chancre has healed, with generalized lymphadenopathy and various skin lesions in the majority of patients. Epitrochlear lymphadenopathy suggests the diagnosis.[26]

Genital herpes

The typical vesicles associated with inguinal lymphadenopathy usually suggest the correct diagnosis. Rarely, lymph node enlargement may appear before the rash develops.[27]

The lymphadenopathy associated with primary herpes is either unilateral or bilateral; in severe cases generalized lymphadenopathy may also occur. Genital lesions and lymphadenopathy are common in genital herpes in the immunocompetent host; however, massive lymphadenitis is frequently seen in the immunocompromised patient. The diagnosis is based on isolation of herpes simplex virus from a skin lesion, preferably from a vesicle[28] (see also Chapter 58).

Granuloma inguinale

Granuloma inguinale (donovanosis) is caused by *Calymmatobacterium granulomatis*. The disease is rare in the Western world but is a major cause of genital ulcer in south-east India, Brazil and some parts of Africa. The penile papules of granuloma inguinale appear within days of inoculation and rapidly ulcerate to form a red, granulomatous, painless ulcer with a characteristic surface that bleeds easily on contact. Subcutaneous spread into the inguinal region results in swellings (pseudobuboes) that are not a true adenitis. Lymphedema and elephantiasis occasionally result from scarring and blockage of the lymphatics. Granuloma inguinale should be differentiated from other genital ulcerative lesions with inguinal lymphadenopathy. The diagnosis is established through the demonstration of the typical intracellular Donovan bodies in stained smears obtained from the lesions[29] (see Chapter 60).

Parasitic lymphadenopathy

Toxoplasmosis

Lymphadenopathy is an important clinical sign of acquired primary toxoplasmosis in the immunocompetent and occurs in up to 84% (mean 64%) of cases in different studies. Lymphadenopathy is typically found in the neck, most commonly in the posterior and anterior cervical regions, followed by the suboccipital region, the axillae and then the groins. It is usually found at single sites in adults (in 90%), but multiple sites are more common in children. Retroperitoneal or mesenteric lymphadenopathy may occur occasionally and cause abdominal pains. Toxoplasmic lymphadenopathy has been described in unusual sites such as the lung hilus, the mammary gland, parotid gland and chest wall.[30] Lymphadenopathy may be the only symptom of toxoplasmosis but generally there are additional symptoms – often fever and rarely splenomegaly and/or hepatomegaly. In approximately 15% *Toxoplasma* lymphadenopathy is associated with fever, headache, myalgia and sore throat that may mimic infectious mononucleosis.

On palpation, the lymph nodes are usually discrete, of varying firmness, and may or may not be tender; they rarely suppurate or ulcerate. A chronic lymphadenopathy fluctuating in size over several months has also been described.[30] The histologic appearance should be differentiated from lymphoma, cat-scratch disease and Kikuchi's lymphadenitis.

Enlarged glands will usually resolve within 1–2 months in 60% of patients. However, 25% of patients take 2–4 months to return to normal, 8% take 4–6 months and in 6% the enlarged lymph nodes do not return to normal until much later.

The diagnosis of toxoplasmosis cannot be made solely on clinical grounds. Histologic features in lymph node biopsies are suggestive of toxoplasmosis but are not diagnostic.[30]

Antibodies to *Toxoplasma gondii* are usually detectable within 2 weeks of infection and reach a peak within 2 months. *Toxoplasma* lymphadenopathy in immunocompetent patients normally resolves without treatment.

Acute infection by *Toxoplasma gondii* is common worldwide. The prevalence of seropositivity by the fourth decade of life in North America is 30–50% and higher than 90% in certain European countries.[31]

Toxoplasmosis has been estimated to cause between 3% and 7% of clinically significant lymphadenopathy. Of major importance is the diagnosis in pregnant women, because of the consequences to the fetus and in infected HIV patients, because of the accompanying central nervous system (CNS) disease. In other immunocompromised patients (e.g. transplant recipients) disseminated toxoplasmosis may occur. In addition, differential diagnosis of *Toxoplasma* from lymphoma may, on occasion, save unnecessary invasive diagnostic workup.

Leishmaniasis (see Chapter 117)

Enlarged lymph nodes occur in the majority (77%) of people with confirmed cutaneous leishmaniasis in South America and in patients who have *Leishmania major* acquired in equatorial Africa.[32,33] Lymphadenopathy may precede the cutaneous lesion by some 2 weeks in two-thirds of the cases. Cultures of the lymph nodes obtained by aspiration or biopsy are more frequently positive (86%) than cultures obtained from the skin lesions. In Brazil a lymphadenopathy (whether localized or generalized) may often be the first, and sometimes the only, symptom of cutaneous leishmaniasis.[34,35] Lymph node histology may show necrotizing or suppurative granulomas, sometimes with discharging sinuses. Patients who have leishmanial lymphadenopathy often have fever, hepatomegaly, splenomegaly, intense skin reaction and lymphocyte proliferation when exposed to suitable antigenic stimulation, but fewer previous infections. In an endemic area, therefore, an unexplained lymphadenopathy should prompt an investigation for leishmaniasis.

African trypanosomiasis (see Chapter 105)

Human African trypanosomiasis, or sleeping sickness, is an illness endemic to sub-Saharan Africa, caused by the flagellate protozoan, *Trypanosoma brucei*, which exists in two morphologically identical subspecies: *Trypanosoma brucei rhodesiense* (East African or Rhodesian African trypanosomiasis) and *Trypanosoma brucei gambiense* (West African or Gambian African trypanosomiasis). Both of these parasites are transmitted to human hosts by bites of infected tsetse flies (*Glossina* species), which are found only in Africa. Humans are infected following a fly bite, which occasionally causes a skin chancre at the site. These injected trypomastigotes further mature and divide in the blood and lymphatic system, causing symptoms of malaise, intermittent fever, rash and wasting. Eventually, the parasitic invasion reaches the CNS, causing behavioral and neurologic changes, such as encephalitis and coma.

In the first stage of the disease the major findings are painless skin chancre that appears about 5–15 days after the bite, intermittent fever (refractory to antimalarials), general malaise, myalgia, and headache usually 3 weeks after the tsetse fly bite, accompanied by generalized lymphadenopathy, pruritus, urticaria and facial edema (minority of patients). Axillary and inguinal lymphadenopathy is noted more often in patients with the East African form; cervical lymphadenopathy is more commonly observed in patients with the West African form. The classic Winterbottom sign is clearly visible (i.e. enlarged, nontender, mobile posterior cervical lymph node) (Fig. 14.6). Fevers, tachycardia, irregular rash, edema and weight loss ensue. Lymphadenopathy has to be differentiated from tuberculosis lymphadenitis, HIV and cancer, all of which are common in this part of the world. A definitive diagnosis of infection requires detection of trypanosomes in blood, lymph nodes, cerebrospinal fluid, skin chancre aspirates or bone marrow.

The standard serologic assay to diagnose West African trypanosomiasis is the card agglutination test for trypanosomiasis (CATT), results of which are available in only 10 minutes. It is highly sensitive (96%) but less specific because of cross-reactivity with animal trypanosomes. Commercial antibody tests for East African trypanosomiasis are not available. For early- and late-stage disease, treatment usually results in resolution of symptoms and rapid clearance of parasitemia on repeat blood smears. Most patients experience full recovery following treatment.[36,37]

American trypanosomiasis (Chagas disease)[38] (see Chapter 118)

Lymphadenopathy appears in around two out of three patients with the acute disease; additional signs and symptoms also occur. An enlarged



Fig. 14.6 Winterbottom's sign: lymph node enlargement in the posterior neck, a sign of African trypanosomiasis.

liver and spleen are mainly observed in children, whereas generalized lymphadenopathy is observed in 60% of all patients. Subcutaneous edema, either generalized or localized to the face and/or lower extremities, is observed in 30–50% of cases.

Filariasis[39] (see Chapter 115)

The most common symptoms of filarial lymphatic disease include fever, inguinal or axillary lymphadenopathy, distinctive lymphangitis spreading retrogradely from the lymph node where the filaria reside to the periphery, testicular and/or inguinal pain, skin exfoliation, and limb or genital swelling. Such attacks last for 3–7 days and recur between 6 and 12 times per year. Attacks subside spontaneously without specific therapy. Affected lymph nodes are enlarged and painful, and the surrounding lymph vessels also appear inflamed and indurated. With the continuation of lymphangitis and lymphadenitis, pitting edema of the skin appears; this is transformed into brawny edema of the involved area causing thickening of the subcutaneous tissue and hyperkeratosis. Coarsening of the skin ensues with deep fissuring and nodular and papillomatous hyperplastic skin changes. Occasionally local lymphedema appears causing penile, testicular, labial or limb enlargement. In some patients passage of cloudy milk-like urine may denote chyluria. Occasionally local thrombophlebitis may complicate the clinical picture. With an infection by *Brugia malayi* an abscess of the local lymphatic apparatus may develop leaving characteristic scars.

Onchocerciasis[40]

This infection is also known as hanging groins, leopard skin, river blindness or sowda. The major manifestations of the disease are dermatitis, onchocercoma, lymphadenitis and visual impairment or blindness. Mild to moderate lymphadenopathy is common, particularly in the inguinal and femoral areas. Involved nodes are characteristically firm and nontender; at times they may reach gigantic proportion and be associated with lymphedema causing elephantiasis (hanging groins). Itching skin and chronic papular onchodermatitis with depigmentation are bothersome, sometimes accompanied by lymphadenopathy in conjunction with secondarily infected skin lesions.

Wuchereria bancrofti infection[41]

Lymphadenopathy is the striking feature of infection with *Wuchereria bancrofti*. Filaria are frequently found in the enlarged lymph node. While the peripheral blood smear and involved organs are characterized by intense eosinophilia, the histology of the lymph node may not reveal in parallel eosinophilic infiltration. At times, the genitalia may be involved with funiculitis, epididymitis, scrotal pain and skin changes and disfiguration of the penis. Scrotal lymphedema, hydrocele and elephantiasis may be present. In addition, characteristic gigantic swelling of the leg below the knee and of the arm below the elbow described as elephantiasis may develop as a result of lymphatic involvement. Occasionally obstruction of the retroperitoneal lymphatics occurs, leading to rupture of lymphatic vessels into the kidney and appearance of chyluria.

Viral lymphadenopathy

HIV

Lymphadenopathy is very common in HIV-infected individuals and may occur at any stage of HIV infection. Generalized lymphadenopathy was one of the characteristic manifestations of the AIDS epidemic, even before its etiology was recognized.[42] In the San Francisco Men's Health Cohort Study marked lymphadenopathy was present in 29% of seropositive men[43] and 29.2% among 1616 HIV-positive persons in India.[44] Acute retroviral syndrome is often associated with generalized lymph node enlargement (see Chapter 89). In the majority of cases it usually appears during the second week of the illness. The lymphadenopathy commonly involves the cervical, axillary and inguinal regions.[45] In the early stage of the infection the lymph nodes are characterized histopathologically by pronounced follicular hyperplasia that, with progression of the disease, evolves into a pattern of follicular involution.[46]

Lymph node enlargement in patients with HIV, especially with immunosuppression, may indicate a serious local or systemic condition, and should be evaluated carefully. Rapid enlargement of a previously stable lymph node or a group of nodes requires evaluation to identify the cause and to determine whether treatment is needed. Similarly, nodes that are abnormal in consistency, tender to palpation, fluctuant, asymmetrical, adherent to surrounding tissues or accompanied by other symptoms should be evaluated promptly.

A multitude of opportunistic infections and malignancies can cause lymphadenopathy in patients with HIV (see Chapter 91). The likely causes of lymphadenopathy, and thus the diagnostic workup, will depend in part on the patient's degree of immunosuppression (Table 14.4). Sudden enlargement, pain or tenderness of a lymph node warrants further diagnostic procedures.

In the evaluation of patients with HIV with lymph node enlargement, the history should include accompanying symptoms, particularly constitutional symptoms such as fever, sweats, fatigue and unintentional weight loss. In addition, the patient should be asked about travel history, country of origin, disease exposures (e.g. tuberculosis) and risk behaviors (e.g. sexual activity). A comprehensive physical examination should be done with emphasis on lymph node distribution, size, mobility and consistency. The size and consistency of the spleen and liver should also be examined. In patients with a CD4 cell count >200 cells/μl a tuberculin skin test should be done. Laboratory studies should include complete blood count, including a careful evaluation of the peripheral blood smear. CD4 cell count should be done as early as possible since the whole differential diagnosis will depend on this number. Evaluation of hepatic biochemistry, renal function and urine analysis are useful to identify underlying systemic disorders that may be associated with lymphadenopathy. Additional studies, such as lactate dehydrogenase (LDH), uric acid, calcium and phosphate, may be indicated if malignancy is suspected. Tests for specific organisms (e.g. *Bartonella henselae*) should be done according to clinical assessment.

Imaging studies should include chest X-ray and, when appropriate, tomography. Mediastinal or hilar lymphadenopathy is not a part of the persistent generalized lymphadenopathy (PGL) syndrome and whenever isolated intrathoracic lymphadenopathy is detected, a thorough investigation for mycobacterial disease is recommended. In such cases tuberculosis is found in more than half of the patients.[47]

Table 14.4 Causes of lymphadenopathy in HIV-positive patients

Generalized lymphadenopathy	Localized lymphadenopathy
HIV (acute or persistent generalized lymphadenopathy)	Local infection
Viral diseases (Epstein–Barr virus, cytomegalovirus, hepatitis B virus, hepatitis C virus)	Tuberculosis
Tuberculosis	Fungal disease (histoplasmosis, coccidioidomycosis, etc.)
Syphilis (secondary)	Lymphogranuloma venereum, chancroid
Bartonella henselae	Lymphoma or other malignancy
IRIS (immune reconstitution syndrome)	Castleman's disease
Drugs (e.g. abacavir)	Kaposi's sarcoma
Multicentric Castleman's disease	

Fine needle aspiration biopsy (FNA-B) is a worthwhile procedure and may allow a rapid diagnosis, obviating the need for surgery and enabling swift treatment to be undertaken in both adults and children.[48,49] If FNA-B is nondiagnostic (false-negative results are relatively common), an open biopsy for definitive evaluation should be done. Biopsy specimens should be sent for (myco)bacterial and fungal cultures, acid-fast staining for mycobacteria and cytologic examination.

Most patients with PGL will not require any treatment. In most patients highly active antiretroviral therapy (HAART) will reduce viral load and lymphadenopathy will subside. When a specific diagnosis is made, specific therapy should be instituted.

Human T-lymphocyte leukemia virus 1 (HTLV 1)[50]

Generalized lymph node enlargement is the most common manifestation of HTLV-1 infection. Additional characteristic findings include skin lesions, hepatosplenomegaly, hypocalcemia, lymphocytosis with abnormal circulating lymphocytes and hyperimmunoglobulinemia. Geographic-dependent findings are tropical spastic paraparesis and myelopathy in some tropical areas. Untreated, the disease undergoes rapid clinical deterioration.

Infectious mononucleosis (see Chapter 155)

Infectious mononucleosis is a disease of teenagers and young adults. It is classically characterized by fever, tonsillopharyngitis, lymphadenopathy, splenomegaly and atypical lymphocytes on peripheral blood smear. Epstein–Barr virus (EBV) is the cause in 80–90% of cases, followed by cytomegalovirus (CMV; 8–16%) and toxoplasmosis (1–2%).[51] It is usually a benign and self-limiting process. Patients exhibit generalized lymphadenopathy and localized lymph node enlargement with or without systemic manifestations. Lymphadenopathy is observed in the vast majority of children and young adults with infectious mononucleosis but only in approximately 45% of patients older than 40 years.[52] Lymph nodes are usually moderately enlarged and not very tender; they can be found at the posterior cervical, axillary, epitrochlear, submandibular, submental and groin regions. Lymph node histology demonstrates paracortical immunoblastic proliferation as seen in many viral infections.[53]

Serious complications of infectious mononucleosis include meningoencephalitis with seizures, myelitis, peripheral neuropathy, splenic rupture, upper airway obstruction, interstitial pneumonitis and severe hepatitis with liver failure. Death is rather rare.

Rarely, a progressive fatal disease develops in patients who have X-linked lymphoproliferative disorder and in other immunocompromised patients.[54] Another infrequent outcome is severe chronic active Epstein–Barr virus infection (SCAEBV).[55] Most often, complete recovery occurs within 1–26 weeks after onset of the disease although in some cases chronic fatigue syndrome may follow and remain for a prolonged period, even years (see Chapter 70).

MANAGEMENT

Lymphadenopathy may be the presenting sign in many diseases. The physical examination of a patient needs to include a description of the most important lymph node groups (cervical, clavicular, axillary, inguinal) and a search for lymph node enlargement of unconventional sites (suboccipital, scalenal, epitrochlear, popliteal, etc.) when indicated.

In adults, small lymph nodes, the size of a small olive, can be normally palpated in the inguinal region and in children in the suboccipital and submental regions. Enlarged and certainly persistent supraclavicular, scalenal, axillary and epitrochlear lymph nodes will usually require investigation, including aspiration or a biopsy of the node.

Mode of presentation

In acutely ill patients who have a tender enlarged lymph node, bacterial etiology is most likely, frequently but not always caused by Gram-positive cocci. A thorough ear, nose and throat examination is mandatory in lymphadenitis or lymphadenopathy of the cervical, submental and head regions. In endemic regions, plague, anthrax and tularemia should be suspected. An acutely ill patient found to have generalized lymphadenopathy (more than three sites) should be evaluated for systemic infections including infectious mononucleosis, typhoid fever, rickettsiosis, leptospirosis, miliary tuberculosis and tularemia, as well as disseminated streptococcal and staphylococcal infections. In mildly symptomatic younger individuals and in symptomatic transplant patients with generalized lymphadenopathy the most likely etiologies will be EBV, CMV and HIV; all of these require special laboratory investigations.[56]

Disease progression

Lymphadenopathy of long duration (>1 month) mandates a thorough investigation. While lymphadenopathy caused by the agent of cat-scratch disease, *Bartonella henselae*, and by *Toxoplasma gondii* can persist for several months, the presumed etiology may be in doubt if the enlarged lymph nodes persist for more than 6 months; in such cases a biopsy of the lymph node is indicated. Fine needle aspiration has replaced the previously often used open surgical biopsy, and is suitable for most diagnostic purposes (except for the diagnosis of lymphoma). The average rate of diagnosis of a biopsied lymph node is 50–60%. Among patients in whom a diagnosis cannot be established through a lymph node biopsy, 25% will develop a lymphoma within 1 year. Therefore patients who undergo a nondiagnostic lymph node biopsy need to be followed up.

The role of age

As mentioned above, lymphadenopathy is exceedingly common in the pediatric age group and represents a benign process in approximately 80% of cases. As such, a trial of antibiotics is justified prior to an extensive workup. In adults, particularly if over 50 years of age, lymphadenopathy (regional or generalized) that persists for several weeks requires a thorough evaluation and consideration for biopsy.

Physical characteristics of the enlarged lymph node

The size, consistency and relation to surrounding and underlying tissues are important clues to the diagnosis of enlarged lymph nodes. Lymph nodes involved by an infective process tend to be large, soft and tender. Signs of local inflammation may be present and draining sinuses may be seen in tuberculous cervical lymphadenitis; inguinal draining sinuses are occasionally seen in lymphogranuloma venereum and chancroid. Nodes involved by lymphoma are characterized as rubbery, matted together and usually nontender. Metastatic lymph nodes due to carcinoma are usually firm, nontender and fixed to the surrounding tissues.

Location

Specific locations of enlarged lymph nodes are frequently associated with specific etiologies (Table 14.5). As intrathoracic and intra-abdominal lymph nodes are usually not palpable, appropriate imaging studies are necessary (ultrasound, CT).

Head and neck lymphadenopathy

The oropharyngeal cavity (including the teeth) is the most common cause for head and neck lymphadenopathy, followed by the nasal cavity and the skin covering the head and neck. In children and young adults enlarged lymph nodes must be differentiated from epidermoid

Table 14.5 Location of enlarged lymph node and associated disease

Site of enlarged lymph node	Associated disease or condition
Occipital	Scalp infections, insect bites, head lice, allergy to hair shampoo
Posterior auricular	Rubella, infected ear piercing, otitis externa, HIV infection
Anterior auricular	Eye and conjunctival infection, tularemia
Posterior cervical	Toxoplasmosis
Submental	Dental and oral cavity infections
Anterior cervical and submandibular	Oral cavity infections, infectious mononucleosis, cytomegalovirus, HIV, tuberculosis
Supraclavicular	Neoplasia
Mediastinal	Sarcoidosis, tuberculosis, histoplasmosis, blastomycosis, anthrax, neoplasia (lymphoma, metastasis)
Axillary	Cat-scratch disease, pyogenic infections of the arm, neoplasia
Epitrochlear	Viral diseases, cat-scratch disease, tularemia, hand infections, secondary syphilis
Abdominal/retroperitoneal	Tuberculosis, yersiniosis, neoplasia
Inguinal	Genital herpes, syphilis, lymphogranuloma venereum, granuloma inguinale, filariasis, pediculosis pubis, neoplasia

cysts, thyroglossal cysts, branchial cysts and parotid and submental enlarged glands. Symmetric lymph node enlargement is usually benign and of viral etiology in most instances; unilateral lymph node enlargement raises a wider differential diagnosis list including viral etiology. The most common causes of unilateral enlargement include inflammation of a draining lymph node of a local (bacterial) infection, cat-scratch disease, toxoplasmosis and neoplasia. If asymmetrical cervical lymph node enlargement persists beyond a few weeks and serologic tests for toxoplasmosis and cat-scratch disease are negative, a biopsy, usually with a fine needle, is indicated. An abnormal chest radiograph with associated unilateral cervical lymph node enlargement is highly suspicious (in 80% of patients) of neoplastic etiology or granulomatous disease, and in such instances a lymph node surgical biopsy may be more accurate than fine needle aspiration. Supraclavicular adenopathy, particularly in adults, is most likely to be neoplastic, frequently secondary to gastric cancer. Rare situations that may cause cervical lymphadenopathy include Kimura disease (eosinophilic hyperplastic lymphogranuloma) and Gianotti–Crosti syndrome (hepatitis B-associated lymphadenopathy in children).

Axillary lymphadenopathy

Infections causing unilateral axillary lymphadenopathy include local infectious processes of the arm and hand, hidradenitis suppurativa, cat-scratch disease, HIV, toxoplasmosis and tularemia, streptococcal and staphylococcal lymphadenitis, sleeping sickness and African trypanosomiasis. Postvaccinational lymphadenopathy was regularly seen following smallpox vaccination and occurs occasionally following anthrax vaccination. It is also seen post measles vaccination where characteristic Warthin–Finkeldey multinucleated giant cells are seen on biopsy. Bacille Calmette–Guérin (BCG) vaccination is also occasionally accompanied by an enlarged local lymph node from which *M. bovis* may be isolated. Asymptomatic unilateral axillary lymph node enlargement is suspicious of being of neoplastic etiology. Bilateral axillary lymph node enlargement can be practically caused by all etiologies: viral, bacterial, protozoal, neoplastic, allergic and noninfectious inflammatory diseases.

Thoracic lymphadenopathy

Major etiologies causing thoracic lymphadenopathy include neoplasia, tuberculosis, sarcoidosis, endemic mycosis and anthrax. In children, mediastinal lymphadenopathy, uni- or bilateral with or without visible lung X-ray findings, is characteristic for primary tuberculosis; malignant lymphoma, however, may also present in this manner. The tuberculin test is usually positive in tuberculosis; however, if negative, it should be repeated in 14 days. If still negative a lymph node biopsy is indicated. Occasionally disseminated atypical mycobacterial infection (e.g. *M. cheloni*, *M. avium-intracellulare*) may also manifest as thoracic or generalized lymphadenopathy.

In adults, unilateral mediastinal and hilar lymphadenopathy without any other symptoms or signs suggests a neoplastic etiology and a biopsy to confirm the diagnosis is required. An exception may be the patient with HIV, or a person who arrives from an area endemic for tuberculosis or endemic fungal infection (such as cryptococcosis, histoplasmosis and coccidioidomycosis). Bilateral hilar lymphadenopathy in the asymptomatic young adult is commonly caused by sarcoidosis. In patients who have parenchymal involvement in addition to the hilar lymphadenopathy, a search for tuberculosis needs to be undertaken with sputum investigations (smear and culture) and, if necessary, bronchoscopy. Bronchoscopy increases the yield for *M. tuberculosis* in sputum-negative patients by 50–75%. The presence of an ulcerating granuloma on bronchoscopy will augment the positive rate even further.

Abdominal lymphadenopathy

Infectious etiologies of abdominal lymphadenopathy are few and include mesenteric and intestinal tuberculosis, *Yersinia enterocolitica* infection, Whipple's disease and intestinal anthrax; occasionally Crohn's disease will be accompanied by enlarged mesenteric lymph nodes. More commonly enlarged abdominal lymph nodes are of neoplastic etiology. Occasionally abdominal lymph node enlargement can be seen during attacks of familial Mediterranean fever (FMF) and similar disorders, such as Muckle–Wells syndrome, chronic infantile neurologic cutaneous articular syndrome (CINCA/NOMID), tumor necrosis factor (TNF) receptor-associated periodic syndrome (TRAPS) and hyperimmunoglobulinemia D with periodic fever syndrome (HIDS).[57]

Inguinal lymphadenopathy

Inguinal lymph node enlargement is very common in a variety of sexually transmitted diseases, namely syphilis, chancroid, lymphogranuloma venereum, granuloma inguinale, genital herpes, pediculosis pubis and HIV. In women there may be inflammation of the Bartholin or Skene glands of the labia, but also cat-scratch disease, purulent infections of the upper and lower leg (streptococcal and staphylococcal), Kikuchi's disease and toxoplasmosis. In endemic areas or in travelers or immigrants returning from endemic areas the differential diagnosis should include filariasis, Bancroftian filariasis, onchocerciasis and human plague.

REFERENCES

References for this chapter can be found online at http://www.expertconsult.com

Practice point | 5 |

Yoav Keynan
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Evaluation and management of the solitary enlarged lymph node

INTRODUCTION

The conditions resulting in enlargement of lymph nodes have been reviewed in Chapter 14. Lymph node enlargement should first be differentiated from other masses that originate from nonlymphoid tissue. The differential diagnosis of solitary lymph node enlargement is similar to that of generalized lymphadenopathy although the frequency of viral and protozoal infections is decreased in the former. The proportions of the different causes depend on the patient's age and the anatomic location of the lymph node; in histologic studies reported in the literature malignant causes are over-represented but this does not seem to be the 'real world'. Overall, staphylococcal and streptococcal infections are the most common causes of localized lymphadenopathy, especially when the enlarged nodes are cervical or axillary. In 17% to over 40% the etiology of solitary lymph node enlargement is not established; this is probably an underestimate since these figures are based on cases in which biopsy was deemed necessary.

In general, a lymph node will increase in size in response to several processes, the most frequent being:

- infiltration by malignant cells;
- infiltration by inflammatory cells in response to an infectious agent being filtered from afferent lymphatics or blood; and
- proliferation of lymphocytes in response to antigenic stimuli.

In most infections the histologic changes in an enlarged lymph node are nonspecific, while a few show a distinctive histologic appearance. Caseating necrosis suggests mycobacterial or fungal etiology, and granulomatous inflammation without necrosis is typical for several infectious and noninfectious processes, among which cat-scratch disease, Kikuchi's disease, sarcoidosis, tularemia and lymphogranuloma venereum are the leading causes. In most cases, however, the histologic features are insufficient to determine the exact infectious etiology.

Kikuchi's disease affects younger women and may be accompanied by constitutional symptoms suggesting lymphoma or a systemic infection. Lymph node biopsy reveals infiltration by plasmocytoid cells and histiocytes and coagulative necrosis (Fig. PP5.1). These findings, as well as the other symptoms, make it difficult to distinguish Kikuchi's disease from lymphoma and systemic lupus erythematosus. The course of Kikuchi's disease is usually self-limited with a favorable prognosis.

Noninfectious causes of an enlarged lymph node can be divided to two broad categories, inflammatory and infiltrative, with further subdivision into immunologic and lymphomyeloproliferative in the former and metastatic and storage in the latter (see Table PP5.1).

The infectious causes of solitary lymph node enlargement include viruses, bacteria, rickettsia, fungi and protozoa. Many of these agents are capable of causing generalized as well as localized lymphadenopathy. Table PP5.2 summarizes the infectious etiologies of solitary lymph node enlargement.

CLINICAL MANIFESTATIONS

Enlarged lymph node(s) may lead the patient to seek medical care or may be an incidental finding. Not every palpable lymph node has an underlying defined pathologic process – inguinal lymph nodes of up to 2 cm in diameter are considered normal for adults and axillary lymph nodes are commonly palpable in laborers. Enlarged cervical and submandibular lymph nodes are commonly found in infants and children and may indicate subclinical dental and oropharyngeal infections or are remnants of past infections.

Evaluation of the enlarged lymph node includes a detailed history that includes the duration of the lymphadenopathy; presence of pain, drainage, skin or mucosal lesions; and any associated local infections. Any accompanying systemic symptoms, history of travel, sexual behavior, occupation, exposure to animals, previous vaccinations and tattooing may provide useful clues and guide the diagnostic approach.

The physical examination should assess the size, texture, adherence to surrounding tissues, tenderness, discharge and fluctuance. Careful examination of the skin, mucous membranes and genitalia is of extreme importance in patients with regional adenopathy. All major lymph node groups should be palpated and examination of the abdomen should include assessment of the size of the liver and spleen.

INVESTIGATIONS

The history and the physical examination guide the diagnostic and therapeutic approach. A history of skin breakdown and a fluctuant lymph node make staphylococcal infection the most likely diagnosis; in other cases, however, the etiology may not be apparent. Basic tests include complete blood count and blood film, and swabs from skin lesions, ulcers and pharynx in the case of cervical adenopathy. A fluctuant node should be aspirated and stained with Gram, Ziehl–Neelsen, silver stain (when indicated) and potassium hydroxide (KOH). Specimens should be cultured for bacteria, mycobacteria and fungi, with additional specific tests according to clinical need. Blood cultures should be obtained from febrile patients. Serologic tests assist the diagnosis of some bacterial pathogens (antistreptolysin O, VDRL, treponemal tests, etc.); a tuberculin skin test may provide helpful information. Images of the chest, abdomen and pelvis are indicated when a systemic cause of infectious or noninfectious lymphadenopathy is suspected. Histology with specific staining for mycobacteria, fungi and cat-scratch disease is indicated when culture does not establish a diagnosis or when there is no response to empiric therapy.

Excisional biopsy is usually preferred to needle aspirate and is the mainstay of diagnosis for entities such as nontuberculous lymphadenopathy. The application of 16S DNA gene amplification as well as

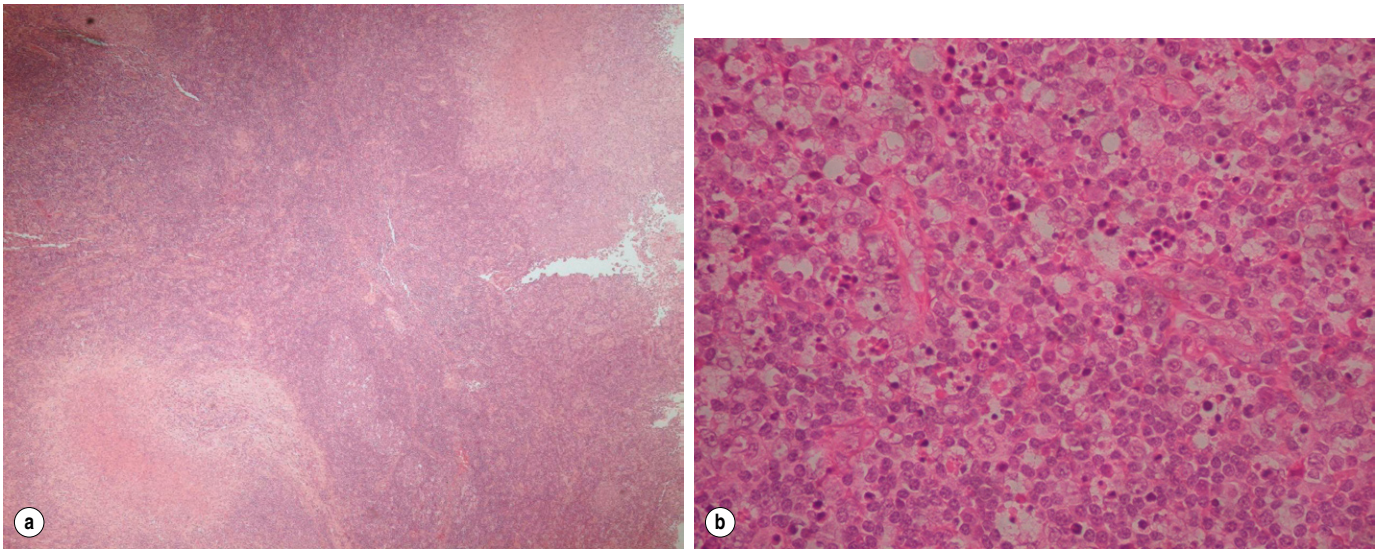


Fig. PP5.1 Kikuchi's disease. (a) Section of a lymph node with extensive paracortical area of coagulative necrosis (H & E). (b) Lesion with infiltrating of histiocytes intermingled with small lymphocytes, immunoblasts, apoptotic bodies (arrowhead) and foamy macrophage (arrow) (H & E). Courtesy of Doron Rimar and Yaaov Schendler.

Table PP5.1 Noninfectious causes of lymphadenopathy

Inflammatory	Immunologic	Rheumatoid arthritis Systemic lupus erythematosus At the site of vaccination Sarcoidosis Drugs (anticonvulsants) Local allergic reactions
	Lymphomyeloproliferative	Leukemia Lymphoma Castleman's disease Sinus histiocytosis with massive lymphadenopathy (Rosai–Dorfman disease) Kikuchi's disease
Infiltrative	Metastatic malignancy	Skin, melanoma, head and neck, breast, sarcoma
	Storage	Gaucher's disease Niemann–Pick disease

specific target gene amplification are promising diagnostic methods for *Bartonella*, *Coxiella burnetii* and *Tropheryma whipplei*. However, further studies are needed in order to establish the role of these tests in the diagnosis of lymphadenopathy.

MANAGEMENT

Ideally, management should be guided by the findings of clinical and laboratory investigations. Identifying the causative organism leads to targeted therapy. Cervical adenopathy associated with pharyngitis or odontogenic infection can be treated with penicillin. When suppurative lymphadenopathy is accompanied by lymphangitis, or when a skin infection is thought to be the portal of entry, staphylococcal and streptococcal infections are the most likely diagnoses and penicillinase-resistant penicillin is an appropriate choice. The increasing incidence of community-acquired methicillin-resistant *Staphylococcus aureus* (MRSA) in some geographic areas should be kept in mind if patients fail to respond to β-lactam-based antistaphylococcal therapy. Cat-scratch disease is self-limited and no antimicrobial therapy is required. Aspiration may relieve pain and aid diagnosis, obviating the need for excisional biopsy.

If no specific diagnosis is evident after a detailed history, physical examination and laboratory tests, the course of action is determined by clinical suspicion. A node of less than 1 cm in a person under 40 years of age with no features suggesting malignancy (hard, fixed node) can be observed for a few weeks and in many cases spontaneous resolution will occur. If the disease process does not become apparent, the node is larger than 2 cm, or enlarges during the observation period or fails to respond to empiric therapy, a biopsy is indicated.

Table PP5.2 Infectious etiologies of solitary lymph node enlargement

Infectious agent	Example	Site	Generalized adenopathy	Comments
Bacteria Pyogenic	Group A streptococci *Staphylococcus aureus* *Corynebacterium diphtheriae*	Cervical Regional Regional		

Table PP5.2 Infectious etiologies of solitary lymph node enlargement—cont'd

Infectious agent	Example	Site	Generalized adenopathy	Comments
Mycobacteria	*Mycobacterium tuberculosis* *Mycobacterium scrofulaceum* Nontuberculous mycobacteria	Regional Cervical Regional	+	
Treponema	Syphilis Endemic treponema, yaws	Regional Regional	+	
Other	*Francisella tularensis* *Bacillus anthracis* *Yersinia pestis* *Burkholderia pseudomallei, mallei* *Bartonella* *Streptobacillus moniliformis, Spirillum minus* *Haemophilus ducreyi*	Regional Regional Regional Regional Regional Regional Regional	+ 	
Rickettsia/Chlamydophila	*Rickettsia tsutsugamushi* *Rickettsia akari* *Rickettsia africae, sibirica, australis, honei* *Rickettsia slovaca* *Rickettsia mongolotimonae* *Chlamydia trachomatis* (lymphogranuloma venereum)	Regional Regional Regional Cervical Regional Regional	+ +	Lymphangitis-associated rickettsiosis
Fungi	*Histoplasma capsulatum* *Coccidioides immitis* *Paracoccidioides brasiliensis*	Regional Regional Regional		
Protozoa	*Toxoplasma gondii* *Trypanosoma brucei* *Leishmania* spp. (cutaneous)	Cervical Cervical	+	 *L. braziliensis* regional lymphadenopathy may precede skin lesions
Helminths	Filariasis *Loa loa* *Onchocerca volvulus*		+	
Viruses	Herpes simplex virus type 2 Human herpesvirus 8 Adenoviruses HIV	Inguinal Cervical, preauricular 	 +	Mucocutaneous lesion may be absent Kaposi's sarcoma Especially types 3 and 7 pharyngoconjunctival fever Usually generalized; localized lymphadenopathy may be the presenting symptom of immune reconstitution

FURTHER READING

Further reading for this chapter can be found online at http://www.expertconsult.com

Chapter 15

Michel Drancourt
Luke Herbert

Conjunctivitis, keratitis and infections of periorbital structures

Conjunctivitis

Conjunctivitis is the inflammation of the conjunctiva, with bacterial conjunctivitis being the eye disease most commonly seen by general practitioners. The incidence of infectious conjunctivitis presented to general practitioners in the Netherlands was measured to be 13.9 per 1000 person-years.[1] Recent reports have confirmed that bacterial conjunctivitis has an excellent prognosis with a high frequency of spontaneous remission. Infectious conjunctivitis must be differentiated from noninfectious conjunctivitis, due to dry eye and acute and chronic inflammatory conditions of the conjunctiva such as Stevens–Johnson syndrome or mucous membrane pemphigoid. Exogenous causes include pollution and medication (conjunctivitis medicamentosa). Rarely conjunctival or eyelid tumors can cause conjunctivitis (masquerade syndrome). Molluscum contagiosum lesions near the eye can shed viral particles into the eye and cause a reactive follicular conjunctivitis, without evidence of conjunctival infection. Large crops of periocular molluscum lesions can be seen in patients with AIDS.

The distinction between noninfective and infectious causes is usually clear from the history. Except for chlamydial conjunctivitis, infectious conjunctivitis rarely lasts longer than 3 weeks, beginning to resolve after 10 days. Conjunctivitis medicamentosa can be a difficult condition to diagnose and may follow an infectious conjunctivitis. Clues include a history of initial improvement on starting a new type of eye drop, followed by worsening and complaints that the eye drops sting. Thorough history taking is essential because patients may not recall all the different eye drops that they have been using during an episode of conjunctivitis. Withdrawal of all topical medication and reassessment 2 or 3 days later is helpful.

COMMUNITY-ACQUIRED INFECTIOUS CONJUNCTIVITIS

Conjunctivitis in neonates

In neonates, infectious conjunctivitis is named ophthalmia neonatorum in the first 3 weeks of life. It occurs worldwide, but is uncommon where there is adequate infant eye prophylaxis. The most serious cause is *Neisseria gonorrhoeae* (gonococcal conjunctivitis), while more common causes are *Chlamydia trachomatis*,[2] chemical irritation and herpes simplex virus. Globally gonococcal ophthalmia neonatorum is an important cause of blindness. The risk of ophthalmia neonatorum for a child born to an infected mother is 30–50% for gonorrhea[3] and 15–35% for chlamydial infection.[4] Transmission usually occurs in the birth canal.

Gonococcal conjunctivitis presents as a hyperacute conjunctivitis 24–48 hours after birth and earlier after premature rupture of membranes. Lid edema, chemosis and profuse purulent secretion rapidly progress to corneal opacities and ulceration, and endophthalmitis may occur. Chlamydial conjunctivitis presents 5–12 days after birth with a watery discharge, which becomes purulent more slowly. Follicles are absent because the conjunctival lymphoid tissue in infants is immature. Untreated the condition usually resolves in 3–4 weeks, but can take up to a year. Rarely membranes and micropannus form, resulting in significant stromal scarring in later life. Pneumonitis, rhinitis, vaginitis and otitis can follow the conjunctivitis.

Conjunctivitis in infants

Brazilian purpuric fever is caused by a rare invasive clone of *Haemophilus influenzae* biogroup *aegyptius*. Systemic disease occurs in children 1–3 weeks after an episode of conjunctivitis. It is seen over a widespread area in Brazil and there is a case mortality of 70%. The clinical picture is similar to that of meningococcal sepsis.

The Brazilian purpuric fever clone of *H. influenzae* biogroup *aegyptius* is resistant to trimethoprim but is sensitive to chloramphenicol and ampicillin. During epidemics, systemic rifampin (rifampicin) (20 mg/kg/day for 2 days) may be used as prophylaxis against Brazilian purpuric fever for children who have conjunctivitis.

Conjunctivitis in adults

Bacterial conjunctivitis is ubiquitous and is more common in warmer months and regions. It is transmitted by contact with ocular or upper respiratory tract discharges of people who have the infection, fomites, medical equipment or shared cosmetics. In some areas insect vectors are involved. Tearing, irritation and sticky discharge without preauricular lymphadenopathy are early clinical features. The conjunctiva is pink (not red). The lids may be stuck together by a mucopurulent exudate on awakening.

Chlamydial infection results in three distinct clinical pictures:

Neonatal chlamydial conjunctivitis is described above.

Trachoma is a scarring condition of the conjunctiva and cornea in adults caused by recurrent childhood infection with *C. trachomatis*.[2] Trachoma occurs worldwide and is endemic in poorer rural areas of developing countries. Blindness due to trachoma is still common in parts of the Middle East, northern and sub-Saharan Africa, parts of the Indian subcontinent, South East Asia and China. There are pockets of infection in South America, among Australian Aborigines, in the Pacific islands and in native American reservations in southwest USA.

The acute chlamydial conjunctivitis is characterized by a diffuse follicular reaction in the conjunctiva of the superior tarsal plate and at the limbus with soft follicles. Papillary hypertrophy is a nonspecific sign.

With resolution of the follicles there is subconjunctival scarring and a loss of conjunctival mucin-producing goblet cells. Blinding complications result from chronic re-infection,[5] severe dry eyes and entropion leading to corneal scarring, bacterial superinfection and ulceration.

Adult inclusion conjunctivitis is an acute condition associated with sexually transmitted chlamydial infection; it presents with a mucopurulent discharge after a 4- to 12-day incubation period. It is often monocular and develops into a chronic follicular conjunctivitis, often with epithelial keratitis and limbal follicles. Tender preauricular lymphadenopathy is common. Untreated the disease has a chronic course and may progress to keratitis and possibly iritis.

VIRAL CONJUNCTIVITIS

Viral conjunctivitis is common and occurs worldwide. Epidemics frequently occur and may be propagated by eye clinics. Transmission is by direct or indirect contact with ocular or upper respiratory tract secretions. Airborne transmission occurs. Viral particles can remain infectious on surfaces for more than 1 month. The incubation period is from 2 days to 2 weeks, and infected people remain contagious for up to 2 weeks after the symptoms begin.

The main patterns of viral conjunctivitis are:

- epidemic keratoconjunctivitis (EKC);
- pharyngoconjunctival fever (PCF);
- acute hemorrhagic conjunctivitis (AHC).

The symptoms and signs of these patterns of infection are similar, but vary in degree. All of these patterns present as an acute follicular conjunctivitis, with watering, grittiness, redness, ecchymosis and lid edema, often with flu-like symptoms. Preauricular lymphadenopathy and bilateral involvement are common.

In EKC most patients have some focal epitheliopathy and photophobia by 2 weeks. Subepithelial opacities appear after 2 weeks in 50% of patients and occasionally impair vision. These lesions cause occasional recurrences of grittiness. The natural history is of slow resolution, which can take more than 1 year. In PCF there is a similar ophthalmic picture, but the systemic features are more pronounced.

In AHC ecchymosis is more prominent and the onset of symptoms more rapid. Rarely a polio-like radiculopathy follows AHC.

Diagnosis of infectious conjunctivitis

Microbiologic investigation is necessary only in neonatal, hyperacute, severe, unusual or chronic cases. Microscopy of conjunctival smears can be useful, and conjunctival swabs are cultured on blood agar and (especially in children) chocolate agar. *Haemophilus influenzae* biogroup *aegyptius* and *Streptococcus pneumoniae* are common causes. *Haemophilus influenzae* type b, *Moraxella* spp., *Branhamella* spp., *Neisseria meningitidis* and *Corynebacterium diphtheriae* are also involved.[6,7] The diagnosis of trachoma is made on clinical grounds in endemic areas.

Expressed follicles have a characteristic microscopic appearance involving macrophages (Leber's cells), plasma cells and lymphoblasts. *Chlamydia trachomatis* types A–C are involved. Secondary bacterial superinfection is common and causes severe disease. Antibodies to *C. trachomatis* can be demonstrated in the tears and serum. Enzyme-linked immunoassay or immunofluorescent monoclonal antibody stains of conjunctival scrapings are rapid and convenient ways to make the diagnosis of chlamydial conjunctivitis.[8] Most methods available for typing the viruses that cause acute conjunctivitis are polymerase chain reaction (PCR)-based.

Most epidemics of viral conjunctivitis are caused by adenoviruses, commonly serotypes 8, 19 and 37 for EKC, and types 3 and 8 for PCF. Picornaviruses such as enterovirus type 70 and Coxsackie virus type A24 cause AHC. Conjunctivitis can be a feature of many other viral diseases, including influenza, rubella, rubeola, chickenpox and glandular fever.

Prevention of infectious conjunctivitis

Topical prophylaxis was described in 1881 by Credé. His silver nitrate eye drops substantially reduced the incidence of gonococcal conjunctivitis; however, they are inactive against chlamydia, and toxicity is common. Recently, 2.5% aqueous povidone-iodine has been shown to be safer, cheaper and more effective.[9] Maternal treatment before birth is the best prevention.

Control of epidemics associated with eye clinics involves setting up a triage system for those suspected of viral conjunctivitis. These patients are seen in a separate room with dedicated personnel. The use of gloves and noncontact examination techniques are strongly recommended. Staff infected with viral conjunctivitis should be furloughed until 2 weeks after the start of symptoms, by which time they are considered to be no longer contagious.

Trachoma is transmitted by contact with ocular or nasopharyngeal secretions, either directly through fomites or insect vectors (the flies *Musca sorbens* in Africa and the Middle East and *Hippelates* spp. in the Americas). Untreated active lesions can be infectious for years. Prevention of transmission is the most important public health measure.[10] Education about personal hygiene and regular washing of the face is very important, and these hygiene measures require only a tiny amount of water. There has been mass treatment of the population with topical tetracycline or erythromycin in hyperendemic areas.

Management of acute conjunctivitis

Topical broad-spectrum antibiotic drops are used 2-hourly until the symptoms subside, which should occur rapidly. In Europe, chloramphenicol is used; in the USA, neomycin, polymyxin and bacitracin are used. Gentamicin and tobramycin are useful against Gram-negative organisms, but cause a higher rate of local toxic reactions. Erythromycin or the quinolones may also be used.

Treatment of chlamydial ophthalmia neonatorum comprises 2 weeks of erythromycin 10 mg/kg every 12 hours for the first week and every 8 hours for the second week of life. The Brazilian purpuric fever clone of *H. influenzae* biogroup *aegyptius* is resistant to trimethoprim but is sensitive to chloramphenicol and ampicillin. During epidemics systemic rifampin (rifampicin) (20 mg/kg/day for 2 days) may be used as prophylaxis against Brazilian purpuric fever for children who have conjunctivitis.

The drug of choice for treating *N. gonorrhoeae* and *N. meningitidis* is an extended-spectrum cephalosporin such as ceftriaxone 1 g (25–40 mg/kg) every 12 hours for 3 days. Repeated irrigation of the conjunctival sac is recommended to reduce inflammatory microbial mediators to the cornea. Treatment of chlamydial conjunctivitis is oxycycline 100 mg every 12 hours or erythromycin stearate 500 mg every 6 hours. Topical treatment is relatively ineffective.

The management of viral conjunctivitis is supportive, with warm or cold compresses reducing symptoms of itch, plus lubrication and cycloplegics if there is an element of keratitis (see below). Antibacterial prophylaxis is probably unnecessary and can cause an allergic or toxic conjunctivitis.

Neonates who have a purulent discharge are presumed to have gonococcal infection and are treated with ceftriaxone 25–50 mg/kg intramuscularly or intravenously to a maximum of 125 mg. Frequent irrigation removes bacteria and microbial products. A single intramuscular dose of 125 mg of ceftriaxone is effective therapy for gonococcal ophthalmia neonatorum.[11] Parents should be screened for sexually transmitted diseases.

Keratitis

Infectious keratitis can be caused by bacteria, viruses, fungi or parasites[12] and can be suspected in various clinical situations, herein classified as community-acquired keratitis, contact lens-related keratitis and health care-associated keratitis. Infectious keratitis is a medical

emergency and improper management can lead to loss of vision. Noninfectious keratitis is important in that it can be confused with infectious keratitis.

COMMUNITY-ACQUIRED KERATITIS

Typical acute microbial keratitis is the major syndrome. Patients present with a corneal epithelial defect and a stromal infiltrate (Fig. 15.1). The other main clinical problems are herpesvirus infections, with recurrent inflammation leading to scarring, and infection in a 'compromised' cornea (i.e. after local or systemic immunosuppression, injury or chronic disease). Algorithms for the initial division of keratitis into the main clinical situations are shown in Figure 15.2. Microbial keratitis is responsible for 30% of cases of blindness in some developing countries. In hot climates fungal infection is more frequent, although bacterial keratitis still accounts for 60% of cases.[13]

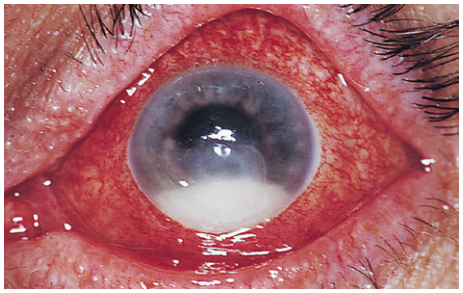


Fig. 15.1 Typical microbial keratitis. Note accumulation of inflammatory cells at the dependent part of the anterior chamber of the eye (hypopyon) and mid-corneal defect. Courtesy of Myron Yanoff.

Surgery, particularly corneal grafts and corneal sutures, and concomitant use of topical corticosteroids increase the risk of infection. Other than vitamin A deficiency, systemic predisposing conditions are relatively unimportant clinically. Vitamin A deficiency is particularly important in children who are malnourished and who have a concomitant infection, especially measles, in which keratomalacia (corneal melting) can occur suddenly. *Staphylococcus* and *Micrococcus* spp., *Streptococcus* and *Pseudomonas* spp. and the Enterobacteriaceae (*Citrobacter, Klebsiella, Enterobacter, Serratia* and *Proteus* spp.) accounted for 87% of cases of bacterial keratitis in one series.[14]

Pseudomonas infection is recognized for its swift suppurative course to perforation, secondary to proteolytic enzyme release. It is particularly common in contact lens wearers (hard and soft). *Serratia marcescens* corneal ulcers have been associated with the wearing of contact lenses and with contaminated eye drops. *Neisseria gonorrhoeae, Corynebacterium diphtheriae, Haemophilus* spp. and *Listeria* spp. are capable of penetrating an intact corneal epithelium. Other bacteria and fungi produce disease only after a loss of corneal epithelial integrity. *Mycobacterium tuberculosis* is a rare cause of keratitis in the course of tuberculous scleritis.[15] Also, *Nocardia* spp. can cause keratitis with amikacin being the drug of choice for treatment.[16]

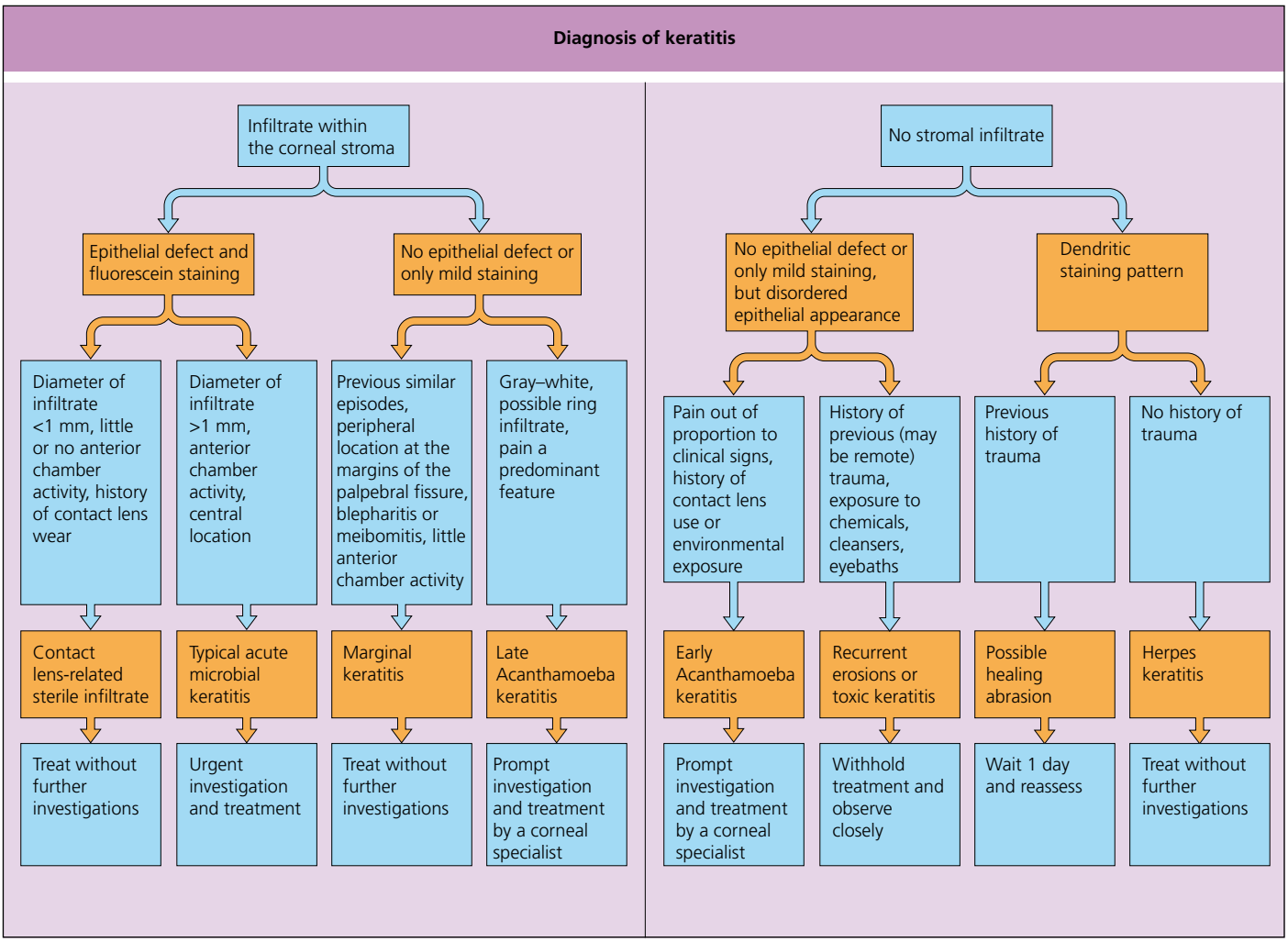


Fig. 15.2 Diagnosis of keratitis. Courtesy of Myron Yanoff.

If the clinical picture progresses after a 2-day treatment, the patient should be admitted to hospital for supervised treatment. If after the first week there is no response to therapy, consider stopping therapy and reculturing or switching to a different antibiotic guided by antibiotic sensitivity testing.

Viral keratitis: herpes simplex virus type 1 or 2

Epidemiology

Herpes simplex keratitis has an annual incidence of new cases of 8/10 000. The incidence peaks at ages 5–10 years and 35–40 years. Males are affected twice as often as females. Approximately 50% of patients have a history of herpes labialis. Primary ocular infection occurs in 5%.[17] Corneal infection is usually with herpes simplex virus type 1 (in 98% of cases), except in neonatal herpes infection, when 80% of cases are due to herpes simplex virus type 2.

Pathogenesis and pathology

The pattern of dendritic ulcers is caused by direct infection of corneal epithelium. It is thought that delayed-type hypersensitivity to viral antigens causes the inflammation that leads to disciform keratitis.[18] Recurrent herpes simplex virus keratitis has been described in the course of Alport's syndrome.[19]

Clinical features

Primary ocular infection is usually asymptomatic, although a vesicular reaction may be seen. Occasionally there is a follicular conjunctivitis. Corneal damage is caused by recurrent disease.

Recurrence occurs in around 10% of cases at 1 year and 50% by 20 years. Triggers include fever, trauma and ultraviolet light. Recurrence is a consequence of latency.[20] Latency develops as virus spreads to a sensory ganglion and remains dormant there. It is thought that infection can spread across a ganglion, for instance from the mandibular to the ophthalmic division of the trigeminal nerve, and hence from lip to eye. Spread from eye to eye is uncommon except in atopic patients in whom delayed-type hypersensitivity is impaired. These patients are at risk of bilateral disease, larger geographic ulcers and disseminated infection. With each recurrence corneal scarring increases, and it is thought that viral particles remaining within the cornea cause the continuing inflammation and edema seen in disciform keratitis.

Management

Either gentamicin 1.5% with cefuroxime 5% or the fluoroquinolones ofloxacin 0.3% or ciprofloxacin 0.3% are effective against 90% of expected isolates in most clinical settings.[21,22]

Aminoglycosides are toxic to the epithelium in moderate doses and quinolones are preferred as monotherapy. In cases in which streptococci are likely (e.g. chronic ocular surface disease, children) there may be quinolone resistance, and combination with cefuroxime 5% is recommended. There are increasing reports of quinolone resistance in some settings.[23]

Initial treatment is with drops every hour day and night for 2 days, followed by review.[24] If the clinical picture has improved or not worsened, treatment is then reduced to drops every hour by day for 3 days. After this time the ulcer should be sterile. Treatment is reduced to prophylactic levels every 6 hours until the epithelium has healed. One trap for the inexperienced is that intensive treatment with ciprofloxacin 0.3% can cause a white ciprofloxacin precipitate to form in the corneal stroma adjacent to an epithelial defect. This may be confused with progression of the infection.

Untreated herpes simplex keratitis usually resolves within 1–2 weeks, but it can progress, resulting in the development of geographic ulcers. Resolution is more rapid if the dendritic ulcer is debrided. Antiviral treatment also speeds up resolution, and no benefit has been shown for a combination of debridement and antiviral agents when compared with the use of antiviral agents alone. The choice of antiviral agent depends on availability. In Europe aciclovir 3% ophthalmic ointment five times a day is almost universally preferred because it is effective and has a low incidence of toxicity.[25] In the USA, trifluridine 1% 2-hourly is used.

Oral aciclovir or famciclovir is effective but comparatively expensive. It may be useful when there is a desire to reduce corneal exposure to topical agents. Systemic antiviral treatment is recommended as prophylaxis against encephalitis for infants who have primary infection.

Prophylactic treatment with oral aciclovir 400 mg every 12 hours reduces the recurrence rate of stromal (severe) disease from 28% to 14%.[26]

The management of the chronic sequelae of herpes simplex keratitis may require topical corticosteroid therapy under close ophthalmic supervision. Topical antiviral treatment reduces recurrence rates in these patients.[27]

Viral keratitis: herpes zoster ophthalmicus

Epidemiology

The varicella-zoster virus is a herpesvirus that causes chickenpox as a primary infection and shingles on recurrence. Around 90% of adults are seropositive for varicella-zoster virus. The rate of recurrence increases with age, and second recurrences can occur in those who have impaired immunity.

Pathogenesis and pathology

Reactivated virus replicates in the ganglion, producing local inflammation and premonitory pain before it travels down peripheral nerves to the skin or eye. A perineuritis and vasculitis occurs, and in the eye a disciform keratitis, iritis and cyclitis are common manifestations.

Clinical features

The clinical appearance is typical. Symptoms of eye involvement include photophobia and decreased vision. Hutchinson's sign, a rash on the side of the nose, is associated with ocular inflammation (Fig. 15.3).

Management

Oral aciclovir 800 mg five times a day for 7 days accelerates skin healing and reduces the incidence of episcleritis, keratitis, iritis, postherpetic neuralgia and probably acute pain.[28] The newer antivirals famciclovir and valaciclovir have improved bioavailability and are increasingly used instead of aciclovir. There is little role for topical antivirals. Systemic corticosteroids used with antiviral therapy have some benefit in shortening the clinical syndrome, but they do not reduce the incidence of postherpetic neuralgia.[29] There is no clinical consensus on whether the benefits of treatment outweigh the risks.



Fig. 15.3 Herpes zoster ophthalmicus. Inflamed right periorbital skin, with conjunctivitis and a lesion on the nose. Courtesy of Myron Yanoff.

Disciform keratitis, iritis and cyclitis respond to conventional topical corticosteroid treatment, but prolonged therapy, sometimes for years, is required.

AIDS: varicella-zoster epithelial keratitis

A chronic, painful epithelial keratitis has been described in patients with AIDS and severe immunodeficiency. Clinically characterized by a variable dendrite-like keratitis, the associated severe, burning pain is the most dramatic feature. Response to treatment is variable; some patients have responded to oral or topical aciclovir, but such patients tend to relapse when treatment stops.[30]

Fungal keratitis

Fungal infections are seen in two distinct clinical settings. In hotter climates the cornea is inoculated with infected vegetable matter as a result of agricultural or other trauma. Infection can then occur in otherwise healthy eyes. In temperate climates infection most often occurs in association with chronic ocular surface disease or prolonged corticosteroid use.

The main cause of fungal keratitis worldwide is trauma. In the developed world risk factors include protracted epithelial ulceration, therapeutic wearing of soft contact lenses, corneal transplant and topical corticosteroid therapy. Patients who have exposure keratitis and a history of previous herpes simplex or herpes zoster keratitis are at risk.

The septate filamentous fungal species, especially *Fusarium* and *Aspergillus* spp., are the most common cause of fungal keratitis. With increasing distance from the equator the relative incidence of candidal infection increases. Dematiaceous filamentous fungi such as *Curvularia* spp. are of low virulence and cause indolent infections.

Clinical features

Fungal keratitis usually presents as a typical microbial keratitis. It is not possible to diagnose the type of infection on clinical grounds. Clinical context is the most important guide to appropriate investigation and therapy. In filamentary fungal keratitis the corneal surface is gray or dull. Satellite lesions are common in the surrounding stroma and may be seen with an intact epithelium (Fig. 15.4). Candidal lesions appear in corneas that are already abnormal. They are often quite localized at first with a collar button configuration.

CONTACT LENS-RELATED KERATITIS

The wide use of contact lenses has led to increased prevalence of specific opportunistic pathogens including *Pseudomonas* spp., nontuberculous mycobacteria such as *Mycobacterium chelonae*,[31] fungi such as *Fusarium*[32] and free-living *Acanthamoeba* amebae.[33] The wearing of contact lenses increases the risk of developing bacterial keratitis.[34]

The annual incidence is 5/10 000 for daily wear of soft lenses and 20/10 000 for overnight wear of soft lenses, although estimates 10-fold higher than this have been made. The largest risk factor is the overnight wearing of soft lenses. This increases the risk fivefold compared with the daytime wearing of lenses and 20-fold compared with the wearing of rigid lenses.[35] One particular situation is microbial keratitis associated with orthokeratology, with *Pseudomonas aeruginosa* and *Acanthamoeba* spp. being the leading causes; cases have been mostly reported in East Asian patients.[36] The incidence of fungal keratitis associated with contact lenses steadily increased up to >50% in Florida.[37] Higher incidence has been noted in the 2004–2006 multicountry *Fusarium* keratitis epidemic[38] which has been associated with the use of particular soft contact lenses and contact lens solutions. *Fusarium* keratitis evolves towards corneal ulceration.[39]

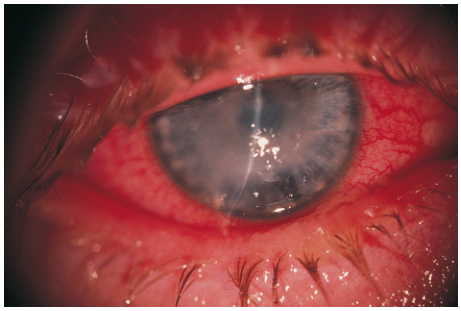


Fig. 15.4 Fungal keratitis. The corneal surface looks rough, and there are several satellite lesions best seen here at the periphery on the left side of the cornea. Courtesy of Myron Yanoff.

Acanthamoeba keratitis is a painful, vision-threatening infection, which can lead to ulceration of the cornea, loss of visual acuity and eventually blindness and enucleation. *Acanthamoeba* keratitis is associated with trauma to the cornea and contact lens wear; the use of contaminated saline solution is the major risk factor for *Acanthamoeba* keratitis. Approximately 10–15% of cases are associated with agricultural and other trauma, but most are associated with contact lens use. Poor lens hygiene by soft contact lens wearers and the use of homemade saline- or chlorine-based disinfection systems are risk factors.

A 360° or paracentral stromal ring infiltration is a characteristic of *Acanthamoeba* keratitis. A nonhealing corneal ulcer is often the first clue to *Acanthamoeba* keratitis. In early epithelial disease there may be punctate keratopathy, pseudodendrites, epithelial wrinkling, diffuse or focal subepithelial infiltrates and radial perineural infiltrates. Ring infiltrates and corneal ulceration may present later.[40,41] Pain out of proportion to the clinical signs is common, but not constant. The clinical picture may resemble herpetic or fungal keratitis, and a high index of suspicion is warranted in contact lens wearers who have apparent herpetic disease. Bacterial co-infection or superinfection in late disease occurs in around 10% of cases and causes an atypical presentation.

The clinical course of *A. histolytica* keratitis is prolonged. Early diagnosis is important, but treatment started before proper diagnostic tests are carried out only confuses the clinical picture. Unlike bacterial keratitis, there is no justification for starting therapy without investigation. A delay of 1–2 days while a patient is referred for expert opinion and investigation is less harmful than early inappropriate therapy. The grave prognosis of *A. histolytica* keratitis in the past was due to the slow inexorable progression of disease rather than fulminant corneal destruction.

Diagnosis

In some parts of the world broad-spectrum therapy is used without initial investigation and it has been suggested that this practice could be extended.[42] The laboratory diagnosis of infectious keratitis relies on culture-based and PCR-based detection of the pathogen in a suitable ocular specimen. A 'keratitis kit' has been developed in order to standardize both the clinical specimens and laboratory tests. Moreover, corneal scraping also debrides the ulcer, allows antibiotics to enter the base of the ulcer and provides microbiologic data (provided that antimicrobial therapy has not been given within the previous 24 hours). The cornea is anesthetized with nonpreserved amethocaine 1%, five or six drops at 2-minute intervals. Using a slit-lamp, necrotic material is removed from the center of the ulcer. A 23 gauge needle tip is scraped parallel to the cornea to remove infected tissue. A fresh needle is used for each specimen. If a slit-lamp is not available, scraping with a scalpel blade is safer than using a needle. The diagnostic yield is greater from the sides of the ulcer than from necrotic areas.

Slides should be made for Gram stain and Giemsa stain, and appropriate bacterial cultures (and fungal cultures if suspected) should be prepared directly from the needle. Bending the tip of the needle before use facilitates this. The sharp side is used to scrape the cornea and the smooth surface is used to plate out the specimen without penetrating the surface of the agar. For amebal analysis, specimens can be plated on non-nutrient agar, which is later overlain with *Escherichia coli* in the laboratory.[43] Tracks of bacterial clearing show

where the amebae have been grazing. Bacterial and fungal cultures should also be obtained. In contact lens wearers the lenses and the lens case should be cultured.

Microscopy techniques vary. Fluorescent microscopy can be carried out using calcofluor white (which stains the walls of cysts), with acridine orange or with an immunofluorescent antibody. Confocal microscopy of a wet preparation can be used to identify motile trophozoites, which have a large karyosome and a contractile vacuole. Biopsy specimens should also be stained with hematoxylin and eosin, periodic acid-Schiff and methenamine silver to demonstrate *A. histolytica* cysts.

Definitive diagnosis of *Acanthamoeba* keratitis is made by microscopic observation of amebal trophozoites and cysts in corneal scrapings or biopsy, or on their cultivation from infected tissues. Confocal microscopy has been used as an aid in the diagnosis.[44] Also, PCR-based molecular tests applied to corneal specimens and tear fluid allow us to identify and genotype *Acanthamoeba* by 18S rDNA sequencing.[45] MALDI-TOF mass spectrometry is a promising rapid method, not only for the identification of isolates, but also the detection/identification of amebae in corneal specimens.[46] A real-time PCR assay[47] and a multiplex real-time PCR assay[48] have also been developed for the rapid detection of most, but not all, the *Acanthamoeba* spp. genotypes involved in keratitis. As for bacterial keratitis, microscopic examination of Gram-stained corneal smears or biopsy may reveal bacteria.[49] Acid-fast bacteria may be observed in cases of *Mycobacterium* spp. and *Nocardia* spp. keratitis.[49]

Viral culture or immunofluorescence[50] can be used to confirm infection, but in practice the diagnosis is clinical. In epithelial disease there is a characteristic dendritic ulcer (Fig. 15.5). Corneal sensation is often impaired, and there is usually a history of similar attacks in the past.

Management

The diamidines, propamidine isethionate 0.1% and hexamidine 0.1%, and the cationic antiseptics, polyhexamethyl biguanide (PHMB) 0.02% and chlorhexidine 0.02%, are probably the most effective medications,[41,51] although availability can be a problem. Local manufacture of chlorhexidine or PHMB may be possible from 20% disinfectant preparations. The azoles (clotrimazole, fluconazole, ketoconazole and miconazole) have also been suggested for use as a third agent, as for amebal keratitis.

Initial treatment starts immediately after the epithelium is debrided using a cationic antiseptic and a diamidine, each hour day and night for 48 hours, then hourly by day for 3 days. Dosage is then reduced to 3-hourly to reduce local toxicity. If toxicity is suspected, the frequency of the diamidine should be reduced. It may take 2 weeks to achieve a response to treatment. Pain relief with systemic nonsteroidal anti-inflammatory agents in cycloplegia is very important. The use of corticosteroids in controlling inflammation is controversial. Some authorities say that they are contraindicated at any stage. Others advocate the use of a weak topical corticosteroid to control inflammation after at least 2 weeks of antiamebic therapy, emphasizing the importance of continued antiamebic therapy until several weeks after the corticosteroid is stopped.

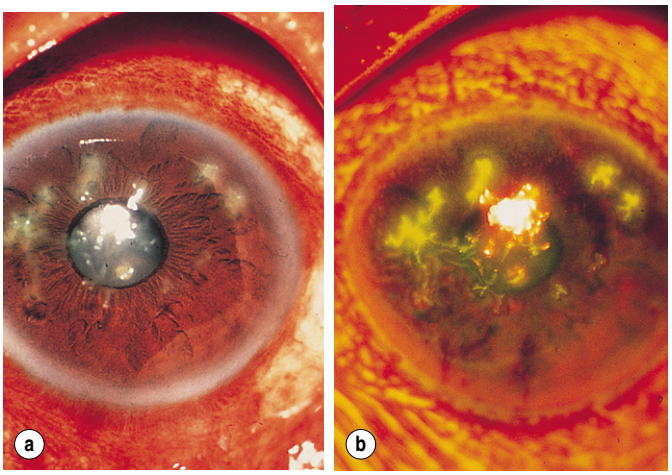


Fig. 15.5 Herpes simplex virus dendritic keratitis, showing branching epithelial lesions seen (a) without staining and (b) with rose bengal staining. Rose bengal stains the devitalized cells at the edges of the dendritic lesions. Courtesy of Myron Yanoff.

Historic data are the best guide to selecting antifungal therapy.[52] The greatest experience in the use of antifungals for keratitis comes from India. However, this experience may not apply to situations where there is a local or systemic disorder of immunity. No currently available antifungal agent has a favorable profile of activity and toxicity. Response to antifungal treatment is poor compared with that to antibacterial treatment. Surgical management (corneal transplant) may be necessary. Combined topical and systemic therapy is often used and is usually prolonged. Corneal drug penetration is helped by daily scraping of the epithelium and necrotic stroma. The choice of antifungal agent is often dictated by availability. Most topical antifungal agents must be made locally from tablets or intravenous preparations.

Among the polyenes, amphotericin B is fungicidal, but is toxic to the eye in high concentrations. It is used diluted in sterile water at 0.1–0.15% two to four times every hour for the first 1–2 days. Natamycin 5% has poor corneal penetration, but is useful for superficial filamentary fungal infections.

Among the pyrimidines, flucytosine 1% can be prepared from a commercially available intravenous or tablet form and is used in combination only (early resistance occurs) with amphotericin B for candidal keratitis. Concomitant systemic use may be helpful.

Among the imidazoles, miconazole 10 mg/ml in polyethoxylated castor oil (intravenous preparation) was effective in more than 60% of cases in a prospective trial in India[53] when used topically every 2 hours. Ketoconazole 2% prepared as an aqueous solution from tablets is probably effective against *Aspergillus flavus*, but not *Aspergillus fumigatus* or *Fusarium* spp. Systemic and topical administration is often combined. Econazole 1% is used topically in combination with systemic itraconazole, and clotrimazole 1% vaginal cream has also been used. Fluconazole is given systemically or as a 0.2–0.5% topical aqueous solution. Itraconazole used orally has some activity against filamentous fungi (see Chapter 149). It has poor tissue penetration, but a good clinical response when used against candidal infections. *Fusarium* spp. respond less well. Silver sulfadiazine 1% is used for antibiotic prophylaxis in patients who have burns. It has moderate antifungal activity *in vitro*, but has been widely used in developing countries as a topical antifungal with good rates of success, particularly against *Fusarium* spp.

Chlorhexidine gluconate 0.2% solution is at least as effective as natamycin in treating fungal infections and is suggested as an inexpensive agent for use in the developing world.[54]

Initial treatment for fungal keratitis depends upon local experience. In London, UK we use topical econazole 1% (or amphotericin B 0.15 or 0.3%) hourly for 48 hours, then 2 hourly for 72 hours and then reduce the dosage depending upon the initial response. Long-term treatment is required. Systemic treatment is used as an adjunct: itraconazole 200 mg daily initially, reduced to 100 mg for longer term use, or fluconazole 200 mg daily for *Candida* infections. If surgical debridement is performed, a 2 mm clear margin is advised. The inflammatory reaction results from live organisms and fungal debris. Because most antifungal agents are fungistatic, reduction of inflammation also depresses local immunity so that the organisms are not killed. Concomitant corticosteroid use is therefore not recommended for fungal keratitis.

Prevention

Good lens hygiene, the use of hydrogen peroxide 3% for at least 3 hours[55] and avoidance of tap water in contact lens care are important preventive measures.[36] The role of mechanical rubbing is still debated.[56]

KERATITIS FOLLOWING OCULAR SURGERY

Penetrating keratoplasty has been complicated by *Gemella haemolysans* keratopathy[57] diagnosed by corneal biopsy. In children, penetrating keratoplasty may be complicated by bacterial keratitis in up to 1–3% of patients due to Gram-positive bacteria, chiefly *Streptococcus pneumoniae*.[58]

Laser *in situ* keratomileusis (LASIK) may be complicated by infectious keratitis. A three-patient cluster of *Nocardia asteroides* keratitis was traced to inappropriate use of the same blade and microkeratome in all patients.[49] *Propionibacterium acnes* has been recovered in one case.[59] Notably, several nontuberculous, rapidly growing mycobacteria caused post-LASIK keratitis including *Mycobacterium chelonae*.

Methicillin-resistant *Staphylococcus aureus* keratitis has been described following refractive surgery.[60] *Staphylococcus aureus* and *Streptococcus pneumoniae* in developing countries, and *Staphylococcus epidermidis* in developed countries, are responsible for infectious keratitis following keratoplasty.[61] Also, outbreaks of viral keratoconjunctivitis have been reported in ophthalmology departments.[62]

Infections of Periorbital Structures

PERIORBITAL CELLULITIS

The orbit is the bony structure surrounding the eye. Infection of the contents of the bony orbit is called orbital cellulitis. Orbital infection commonly occurs as a result of contiguous spread from adjacent structures. Periorbital cellulitis is divided into preseptal or postseptal (orbital) depending on the site of infection. Orbital cellulitis is sight-threatening and occasionally life-threatening.

Preseptal cellulitis is 5–10 times more common than orbital cellulitis in infants and toddlers and is not sight-threatening or life-threatening, but it can, rarely, spread to become orbital cellulitis. It is commonly caused by *H. influenzae* or *Streptococcus* spp. and follows an upper respiratory tract infection. In adults it is most often seen after minor trauma such as an infected bite or scratch. One case of *Tropheryma whipplei*, the Whipple's disease bacterium, orbital manifestation has been reported.[63]

The source and agents of infection causing orbital cellulitis vary with age. The paranasal sinuses (the ethmoid, maxillary and frontal sinuses) are the main sources. Orbital cellulitis is uncommon in neonates, but when it occurs it is usually secondary to conjunctivitis or a developmental abnormality such as a ruptured dacryocele. In infants respiratory tract infections may cause preseptal cellulitis; in older children and adults dental abscesses and trauma become important causes. Endogenous orbital cellulitis is rare.

Clinical features

In adults there is frequently a history of sinusitis, headache or recent tooth extraction or abscess. Children often have an antecedent upper respiratory tract infection. Preseptal cellulitis is characterized by eyelid swelling and edema, which is usually unilateral and mainly involves the upper lids. Lower lid swelling occurs when the cellulitis is secondary to dacryocystitis. Edematous conjunctiva can prolapse between the lids.

In orbital cellulitis features of preseptal cellulitis are variably present, but there is also proptosis, decreased ocular mobility and even decreased vision or a relative afferent pupil defect. These signs are caused by increased intraorbital pressure due to edema or abscess. If a subperiosteal abscess forms there may be nonaxial displacement of the globe and a palpable fluctuant mass in the orbit. Headache, fever and leukocytosis are common.

Diagnosis

Blood and local cultures are mandatory. A CT scan can distinguish between preseptal and orbital cellulitis and show the site of an orbital or subperiosteal abscess:[64]

- preseptal cellulitis produces edema of the lids and tissues anterior to the orbital septum;
- orbital cellulitis produces edema of the orbital tissues and proptosis.

Orbital cellulitis is often associated with signs of primary or secondary sinus disease.

Staphylococcus and *Streptococcus* spp. and, in those under 4 years of age, *H. influenzae* are the main pathogens that cause preseptal and orbital cellulitis in children. In subperiosteal abscesses in children over 9 years of age and adults there is often a mixed infection of aerobes and anaerobes[65] from extending sinus or dental infections.

Management

Preseptal cellulitis responds well to systemic antibiotics. One or more broad-spectrum agents that cover *Staphylococcus* and *Streptococcus* spp. and *H. influenzae* should be used. A response is usual within 24 hours.

Orbital cellulitis requires prompt diagnosis and inpatient treatment with intravenous antibiotics. Monitoring of vision, pupillary reaction, extraocular movements and central nervous system function should be carried out during the first 1–2 days until the infection begins to resolve. If a subperiosteal abscess or sinusitis is identified and the clinical picture is not improving after 24 hours, surgical management is required. Orbital or brain abscesses are less common and should be drained immediately.

LACRIMAL SYSTEM INFECTIONS

Dacryoadenitis

Dacryoadenitis is usually due to a viral infection of the lacrimal gland. Patients present with adenopathy, fever, malaise and leukocytosis. The causes include infectious mononucleosis, herpes zoster, mumps, trachoma, syphilis, tuberculosis and sarcoidosis. Dacryoadenitis occasionally occurs in dehydrated patients as an ascending staphylococcal infection associated with a purulent discharge. On CT scanning there is diffuse lacrimal gland swelling without bony defects.

Diagnosis

The condition is usually self-limiting. Investigation other than CT scanning is reserved for chronic dacryoadenitis, and such patients should be referred for specialist investigation to exclude neoplasia.

Management

Treatment is generally symptomatic. Corticosteroids can help speed up resolution. It can be difficult to distinguish dacryoadenitis from idiopathic lacrimal gland inflammation (pseudotumor), although the presence of enlarged preauricular lymph nodes makes a viral diagnosis more likely.

Canaliculitis

There are chronic and acute forms. Acute dacryoadenitis may be caused by herpes simplex or herpes zoster and is often unrecognized except as a conjunctivitis and by its sequelae: scarred closed canaliculi and a punctum.

Chronic canaliculitis is usually unilateral and is characterized by pain or tenderness at the inner canthus. A chronic conjunctivitis may mask the more specific signs. The lacrimal punctum may pout and 'sulfur granules' may be expressed. These sulfur granules are

pathognomonic of infection with *Actinomyces israelii*, an anaerobic Gram-positive branching filamentous bacterium. Less common causes are *Aspergillus* and *Candida* spp.

Treatment is by incision of the infected canaliculus and wash-out of all 'sulfur' material, usually with a penicillin-containing irrigation fluid.

Dacryocystitis

Pathogenesis and pathology

This condition occurs in chronic and acute forms. In infants it is usually an indolent condition resulting from incomplete development of the lacrimal drainage system. There is a mucopurulent discharge and recurrent conjunctivitis. Colonization is usual with *H. influenzae*, *Streptococcus pneumoniae*, staphylococci and *Klebsiella* and *Pseudomonas* spp. In adults an acquired blockage of the lacrimal drainage system can cause an acute or chronic infection. An acute infection can be precipitated by instrumentation for investigation of a suspected blocked lacrimal duct. For this reason, mucoceles or chronic dacryocystitis should not be probed or syringed. Acute dacryocystitis presents with a painful swelling over the lacrimal sac.

Treatment is with warm compresses and systemic antibiotics. A large abscess should be drained by a stab through the skin or inferior fornix conjunctiva. A dacryocystorhinostomy will prevent recurrence.

REFERENCES

References for this chapter can be found online at http://www.expertconsult.com

Chapter 16

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Endophthalmitis

Endophthalmitis is fortunately an uncommon condition; however, it may result in severe visual impairment or loss of an eye.

EPIDEMIOLOGY

Definition and nomenclature

Endophthalmitis is an infection within the vitreous and may involve the cornea and, in severe cases, the sclera (panophthalmitis). A number of classifications of this condition have been published but from a practical point of view, categorization by the clinical setting, taking into account such factors as the events preceding infection and the time to diagnosis, is most appropriate. Categories include postoperative endophthalmitis (acute within 2 weeks of operation), delayed onset (more than 2 weeks after operation), conjunctival filtering bleb associated, post-traumatic endophthalmitis and endogenous endophthalmitis. Each of these subtypes may have characteristic clinical features and a spectrum of common causative pathogens (Table 16.1). This classification fits with the clinical classification of uveitis.[1]

Incidence and prevalence of endophthalmitis

Although recent eye surgery is the most common cause of endophthalmitis, accounting for more than 70% of cases, the incidence of infection after cataract extraction, the most commonly performed eye surgery, is very low, at 0.07–0.13%.[2,3] Endophthalmitis may occur after any other form of ocular surgery, but appears to be more common after glaucoma filtering procedures. Nontuberculous mycobacteria are emerging agents in this situation.[4] It has been estimated at 1:5233 consecutive bevacizumab intravitreal injections.[5]

Endophthalmitis after penetrating ocular trauma is common, representing 7–30% of all endophthalmitis cases; 3–26% of penetrating eye injuries develop infection. It is more common when trauma is associated with a retained intraocular foreign body or when the injury is contaminated with vegetable matter.[6] The leading organisms in this setting are staphylococci, especially *Staphylococcus aureus*, and *Bacillus* spp. Endogenous bacterial and fungal endophthalmitis are the least common forms, accounting for less than 2–8% of cases; they usually follow bloodstream spread of organisms and are commonly associated with a number of chronic medical conditions, such as diabetes mellitus, chronic renal failure, chronic immunosuppression, invasive medical procedures (including urinary catheterization and intravascular central lines) and intravenous drug abuse.

Table 16.1 Microbial etiology of endophthalmitis

Category of endophthalmitis		Common causative organisms
Postoperative	Acute	Coagulase-negative staphylococcus *Staphylococcus aureus* *Streptococcus* spp. Gram-negative bacilli *Pseudomonas* spp.
	Delayed	*Staphylococcus epidermidis* *Propionibacterium acnes* *Candida* spp.
	Filtering bleb	*Streptococcus* spp. *Haemophilus influenzae* *Staphylococcus aureus*
Post-traumatic	Bacterial	*Staphylococcus aureus*; other staph. spp. *Bacillus* spp. *Pseudomonas* spp. Other Gram-negative bacilli; anaerobes; corynebacteria; streptococci
	Fungal	*Penicillium* spp.; *Fusarium* spp.; *Acremonium* spp; other filamentous fungi
Endogenous	Bacterial	Enteric Gram-negative bacilli Fungi (including *Candida albicans, Aspergillus* spp.) *Streptococcus* spp.
	Fungal	Yeasts (*Candida albicans, Cryptococcus* spp.) Filamentous fungi (*Aspergillus* spp., *Acremonium* spp., *Fusarium* spp., *Paecilomyces* spp.)

PATHOGENESIS AND PATHOLOGY

Although a broad range of organisms can cause endophthalmitis, the most common causative infectious agents are bacteria. Virtually any bacterium, including those usually accepted as saprophytes, can cause infection, although members of the normal ocular microflora are the most commonly implicated.

Acute and delayed-onset postoperative endophthalmitis

Infecting organisms are usually introduced into the eye via incisions at the time of surgery. Nosocomial outbreaks of endophthalmitis caused by contaminated irrigation fluids, intraocular lenses and donor corneas have been recognized. Contaminated phacoemulsification probe was demonstrated as a source of nosocomial endophthalmitis following phacoemulsification cataract extraction surgery.[7] Infiltration of pathogens in the immediate postoperative period may be associated with inadequately buried sutures, suture removal or the presence of vitreous wicks.

In over 70% of cases, the pathogenic organism is a Gram-positive bacterium. *Staphylococcus epidermidis* and other coagulase-negative staphylococci are now the most frequently isolated bacteria from post-surgical endophthalmitis, representing 50–55% of all culture-positive cases.[8] *Staphylococcus aureus* and *Streptococcus* spp. are cultured from 10–30% of postoperative infections, whereas Gram-negative organisms, including *Pseudomonas* spp., *Proteus* spp. and *Citrobacter* spp., are implicated in only 7–20%. The change in prevalence of *Staph. epidermidis* probably represents, at least in part, a past failure to recognize coagulase-negative staphylococci as potential ocular pathogens.

Delayed-onset endophthalmitis is often caused by *Staph. epidermidis, Corynebacterium* spp. and *Candida* spp. A specific syndrome of chronic localized infection may occur with *Propionibacterium acnes*. Filtering bleb endophthalmitis is frequently caused by streptococci (60%) and *Haemophilus influenzae* (20%), although *Staph. aureus* remains a prominent pathogen.[9] Fungal endophthalmitis is usually observed as a cluster of cases caused by the use of contaminated irrigating solution,[10] intraocular lens,[11] ventilation system[12] and hospital construction activity[13] but sporadic cases have also been observed.[14] Postoperative fungal infection is very rare; a 27-case series has been observed over a 5-year period of time.[14] In this series, endophthalmitis was caused mainly by *Aspergillus* spp. with a median 10.5-day latent period.[14]

Post-traumatic endophthalmitis

Post-traumatic infection may be polymicrobial (10–40%), caused by anaerobic organisms, especially *Clostridium* spp. *Staph. aureus* remains a common agent, although saprophytes such as *Bacillus* spp., especially associated with intraocular foreign bodies, may induce fulminating endophthalmitis.[15] Spread of organisms through corneal abrasions and penetrating corneal ulcers, particularly those involving *Staph. aureus* or *Pseudomonas aeruginosa*, may lead to endophthalmitis.

Endogenous endophthalmitis

Endogenous infection may be associated with a recognizable infective focus elsewhere in the body and this may provide an indication as to the likely causative organism. Ocular involvement, however, may also be the first and only manifestation of systemic infection, particularly in the course of endocarditis. The diagnosis of endogenous endophthalmitis should prompt investigation (three blood cultures, serology for fastidious bacteria and echocardiography) to eliminate endocarditis.[16,17] More recently, *Streptococcus* spp. other than *Strep. pneumoniae, Staph. aureus* and Enterobacteriaceae from gastrointestinal sources have become more prominent.[18] Intravenous drug use may be associated with infection with *Candida* spp., *Aspergillus* spp. and *Bacillus cereus*, although more common pathogens, including *Staph. aureus*, may be involved. Exceptionally, late-onset endophthalmitis may develop after bacille Calmette–Guérin (BCG) immunotherapy for bladder carcinoma.[19] Other mycobacterial organisms are also exceptional, including *Mycobacterium haemophilium*.[20]

Fungal endophthalmitis

Fungal endophthalmitis may occur as exogenous or endogenous infection. After trauma, fungal endophthalmitis may represent up to 10% of cases, particularly if penetration with vegetable matter has occurred.[21] Extension of a fungal corneal ulcer may also lead to endophthalmitis. Fungi most commonly identified in this situation are usually saprophytic and may include *Aspergillus* spp., *Fusarium* spp., *Acremonium* spp. and *Paecilomyces* spp.

Endogenous fungal endophthalmitis has been seen with increasing frequency over the past two decades, concurrent with an increased recognition of systemic fungal infections. *Candida albicans* is the most frequently reported causative agent after hematogenous dissemination from other infected body sites, particularly infected central venous catheters, and often in immunocompromised patients.[22] Direct intravenous inoculation as a result of narcotic abuse or contaminated infusion solutions has also been reported. Other fungi less commonly implicated in endogenous fungal endophthalmitis include *Cryptococcus neoformans, Aspergillus* spp. and *Paecilomyces* spp.

PREVENTION

The prevention of endophthalmitis is based on identification and pre-treatment of high-risk patients, and reduction in the conjunctival commensal flora.

Preoperative precautions

High-risk patients

Host factors that lower resistance to infection, such as chronic immunosuppression or diabetes mellitus, have been reported as significant risk factors for postoperative endophthalmitis. Reduction in immunosuppressive medications, when possible, and optimal control of blood glucose are essential in such groups. Pre-existing infection of external ocular tissue, for example chronic blepharitis, conjunctivitis and lacrimal outflow obstruction, should be identified and treated with appropriate topical antibiotics.

Antimicrobial prophylaxis

The aim of preventive treatment is to reduce eyelid and ocular surface microflora; this may be achieved by using topical antibiotics, topical antiseptic agents or subconjunctival antibiotics at the time of surgery.

Topical antibiotics

Although there is no consensus as to the optimal use of preoperative topical antibiotics in intraocular surgery, several studies have demonstrated significant falls in bacterial colonization of the conjunctiva with the application of topical antibiotics and have thus suggested a reduction in the incidence of postoperative endophthalmitis with the use of such antibiotics preoperatively. Topical antibiotics have been reported to be most effective in decreasing conjunctival bacterial colony counts when administered 2 hours before surgery.[23]

Until the early 1980s, gentamicin (3 mg/ml) was consistently found to be the most effective antibiotic in this situation compared with other agents such as chloramphenicol (5 mg/ml), bacitracin (10 mg/ml), neomycin (5 mg/ml) and polymixin (2.5 mg/ml).[24] However, the increase in gentamicin resistance among *Staph. epidermidis*, now the most common cause of postoperative endophthalmitis, suggests that it may no longer be the optimal agent for prophylaxis.[25] Vancomycin, when used prophylactically, has been shown to be active against staphylococci, but the potential risk of emerging resistance in enterococci and to a lesser extent in methicillin-resistant *Staph. aureus* has led some authorities to discourage this practice. Although no specific recommendations have been developed for ophthalmology, it seems appropriate to restrict the use of vancomycin to the treatment of, for example, serious keratitis, endophthalmitis or orbital cellulitis caused by β-lactam resistant Gram-positive organisms or alternatively to the treatment of enterococci and *Staph. aureus* in patients unable to tolerate β-lactam antibiotics. More recently, fluoroquinolones

(all compounded at a concentration of 3 mg/ml) have been shown to be very effective in reducing conjunctival and eyelid bacterial flora when used preoperatively.[26]

Subconjunctival antimicrobials

Subconjunctival antibiotics can be administered after intraocular surgery based on the rationale that, at the completion of the ocular procedure, it is appropriate to inhibit growth of any bacteria that may have gained entry into the eye during surgery. During routine cataract surgery, aqueous fluid samples have been demonstrated to be culture positive in up to 43% of cases.[27] Conflicting results as to the value of this modality have been reported and penetration into the vitreous is relatively poor.

Topical antiseptics

Application of 5% aqueous povidone–iodine solution alone has been shown to be nontoxic and to decrease perioperative conjunctival bacterial colony counts and reduce the incidence of postoperative endophthalmitis significantly. In combination with topical antibiotics, povidone–iodine has been found to sterilize the conjunctiva in more than 80% of treated patients.[28]

Intraoperative precautions

Although the judicious use of preoperative antibiotics can reduce infection rates considerably, they do not replace meticulous aseptic technique in intraocular procedures. An appropriate operating room environment, with efficient ventilation to reduce bacterial contamination, is essential; surgical techniques should be modified to minimize entry of ocular surface microbes into the eye during the surgical procedure, and adhesive-backed plastic drapes to isolate the eyelids and lashes from the operative field are recommended.

Implantation of intraocular lenses with prolene haptics appears to increase the risk of endophthalmitis, probably because coagulase-negative staphylococci bind well to this particular plastic. Binding to polymethylmethacrylate material is less, and therefore its use may reduce risk.[29] Care must be taken to minimize contact with the external eye during insertion of an intraocular lens to prevent contamination with conjunctival flora. A foldable lens insertion device has been developed to facilitate this. There is always the threat of infection from personnel and equipment in the operating room, and from contaminated irrigation solutions.

CLINICAL FEATURES

Clinical signs of endophthalmitis vary greatly depending on the preceding events, the nature of the infecting organism, the degree of tissue inflammation and the duration of disease. Early diagnosis requires the maintenance of a high index of suspicion as classic features of infection may be absent.

Acute postoperative endophthalmitis

Acute postoperative endophthalmitis usually occurs within 2 weeks of surgery, whereas the presentation of infection after penetrating trauma will often be more rapid. As a general principle, the more rapid the onset of symptoms, the more virulent the organism, with *Staph. aureus, Streptococcus pyogenes, Bacillus* spp. and Gram-negative bacilli being implicated in very rapid onset of infection within 24–72 hours of surgery. This presentation is characterized by marked anterior chamber inflammation, by the rapid development of a fibrinous anterior chamber exudate with hypopyon (which produces severe pain, more prominent than general postoperative discomfort) and by a progressive decrease in visual acuity. A marked vitreous inflammatory reaction, often obscuring visualization of the retina, frequently follows (Figs 16.1–16.3). Associated features may include marked conjunctival, lid and corneal edema, but systemic features are virtually never seen.

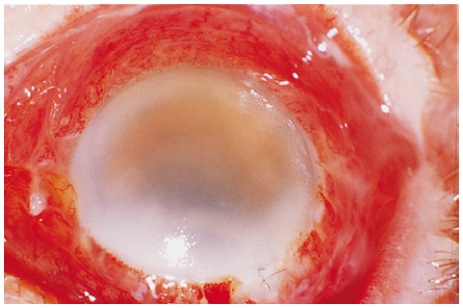


Fig. 16.1 Corneal edema and fibrinous anterior chamber exudate in a traumatic foreign body-induced endophthalmitis. Any posterior vitreal or retinal view is obscured by the anterior corneal and aqueous haze.

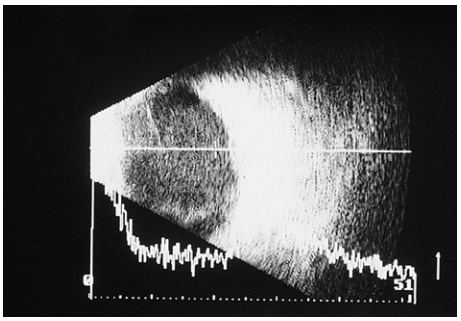


Fig. 16.2 B-scan ultrasound of the eye showing total vitreous opacity of a severe endophthalmitis in patient seen in Figure 16.1. This horizontal 'cut' through the eye shows the normally 'transparent' vitreous cavity to be filled with inflammatory debris, but there is no obvious retinal detachment.

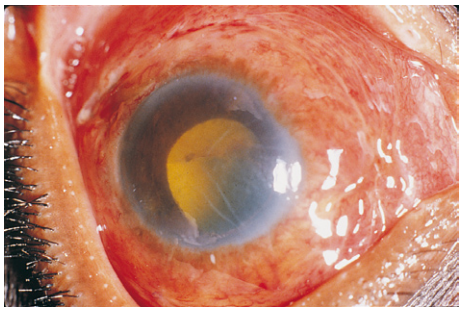


Fig. 16.3 A dense vitreous abscess in advanced endophthalmitis. This partially treated postoperative endophthalmitis has vitreous cellular and protein deposits obscuring the retinal view.

Delayed-onset postoperative endophthalmitis

Delayed-onset endophthalmitis has a more insidious course and frequently overlaps with acute postoperative endophthalmitis if less virulent organisms are implicated. Symptoms may not manifest until weeks or even months after surgery, although early mild clinical features with progressive worsening over time are not uncommon (Fig. 16.4). When delayed-onset endophthalmitis is caused by *P. acnes*, it usually develops months after cataract extraction; patients will often have a history of corticosteroid-responsive postoperative inflammation with a fluctuating course over many months. The most common clinical signs are posterior capsular deposits and chronic iridocyclitis.[30]

Fungal infection may also have a delayed clinical onset. Anterior chamber reaction is seen with progressive white infiltrates, often adherent to the iris and posterior corneal surface (Figs 16.5, 16.6). Fluffy white fungal ball infiltrates ('string of pearls') occurring in the vitreous are characteristic (Fig. 16.7). Patients who have chronic postoperative endophthalmitis caused by coagulase-negative staphylococci may present with a hypopyon and diffuse vitritis, which occasionally obscures the view of the fundus. Visual loss is usually more severe than that in endophthalmitis caused by *P. acnes* or fungi.

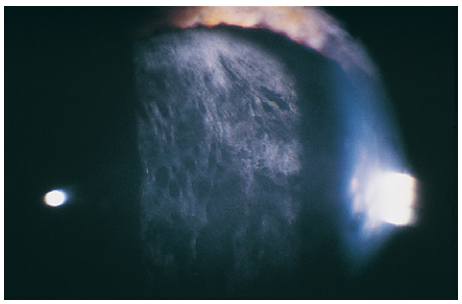


Fig. 16.4 The typical posterior capsular opacities seen in a late-onset *Staphylococcus epidermidis* endophthalmitis. These deposits are actual coccal colonies, which are frequently removed at subsequent vitrectomy surgery to open up the capsular bag to intraocular antibiotics.

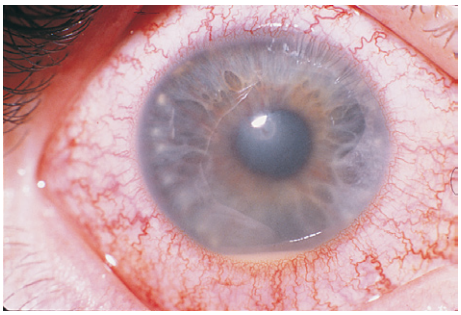


Fig. 16.5 A 'quiet' endogenous fungal endophthalmitis with small hypopyon. This eye is relatively quiet with little chemosis, injection or pain, but has a small hypopyon and some small fungal 'balls' on the temporal iris.

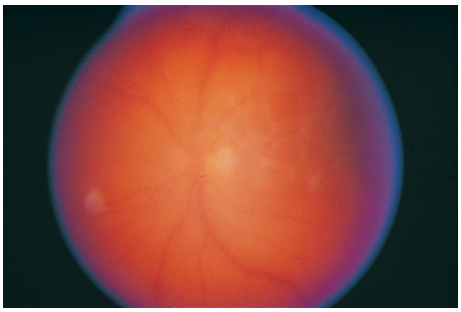


Fig. 16.6 The degraded ophthalmoscopic retinal view obtained in the patient seen in Figure 16.5. The corneal edema and anterior chamber activity make vitreous and retinal observation difficult.

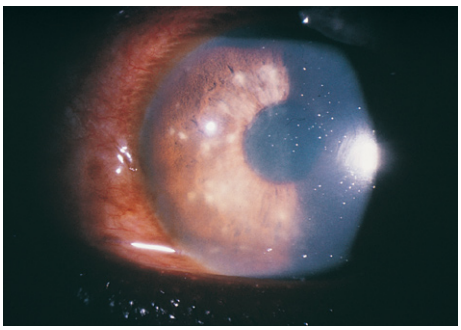


Fig. 16.7 Fungal 'fluff balls' on the iris seen in a fungal endophthalmitis. Although these are not pathognomonic, their appearance raises the real possibility that the infection is of fungal origin.

Filtering bleb endophthalmitis

A conjunctival filtering bleb is a collection of fluid under the conjunctiva resulting from the formation of a fistula at operation through the sclera from the anterior chamber in an endeavor to reduce pressure in the anterior chamber. Endophthalmitis associated with a conjunctival filtering bleb is actually an acute presentation of endophthalmitis, but may occur months to years after the operation with rapid development of symptoms. Intraocular spread occurs from an initial bacterial

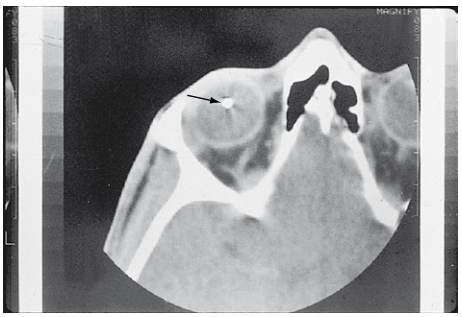


Fig. 16.8 Horizontal CT scan section of eye seen in Figures 16.1 and 16.2 revealing a metallic intraocular foreign body in the vitreous cavity (arrow). This CT scan demonstrates that the vitreous opacity seen by ultrasound is 'invisible' to this investigation.

penetration through the mucosa of the bleb, often in association with bacterial conjunctivitis. Infection is often associated with late bleb leakage and is facilitated by antimetabolites (e.g. 5-FU or mitomycin) used to ensure bleb survival.[6] A purulent discharge and an injected bleb are commonly seen, ultimately in association with typical signs of endophthalmitis. Viridans streptococci, *H. influenzae*, *Staph. aureus* and *Moraxella* spp.[31] are commonly implicated.

Post-traumatic endophthalmitis

Presentation will vary depending on the nature and severity of the ocular trauma or the type of retained foreign body, for example steel or vegetable matter, and the virulence and concentration of the organism initially deposited into the intraocular tissues (Fig. 16.8). The more virulent this organism, including *Staph. aureus* and *B. cereus*, the more rapid the onset of pain and associated ophthalmic features of infection. *B. cereus*, fungi and to a lesser extent *Nocardia* spp. and atypical mycobacteria should be considered when injury to the eye is related to plant or vegetable matter.[32]

Endogenous endophthalmitis

Endogenous endophthalmitis usually has an insidious onset with a slow decrease in visual acuity caused by vitritis and localized areas of chorioretinitis. It may be suspected when other systemic symptoms of infection are present or in certain groups of patients, including those in whom bacteremia or fungemia is common, such as those with infective endocarditis and those with intravascular or urinary catheters, or patients who abuse intravenous drugs. Rarely, a fungal endophthalmitis, with a predilection for *Paecilomyces* infection, may occur in otherwise healthy individuals with no antecedent trauma.[33]

Differential diagnosis

The differential diagnosis of postoperative inflammation includes sterile uveitis related to retained lens cortex; operative complications such as vitreous loss, hemorrhage and iris trauma; pre-existing uveitis; and toxicity of foreign material such as irrigation solutions introduced during surgery. These presentations are often difficult to distinguish from similar symptoms caused by infective endophthalmitis and careful sequential monitoring of such eyes or intraocular sampling for culture is appropriate to facilitate early diagnosis and treatment.

DIAGNOSIS

Microbiologic investigations in endophthalmitis

Confirmation of the diagnosis of infective endophthalmitis is essential to rational management. Because most postoperative infections are caused by normal ocular flora, there is some correlation between the

results of external swabs of the eyelid margins and conjunctivae with intraocular isolates.[34] These should not be used to determine causative pathogens; routine preoperative cultures have a low predictive value and are not recommended. The optimal specimen for laboratory processing in endophthalmitis is an intraocular aspirate; aqueous and vitreous specimens should be obtained, although the latter appears more reliable, as some 30–55% of concomitant aqueous specimens are negative in the presence of positive vitreous isolates.[35] In the case of late endophthalmitis, aqueous vitreous and some capsule material should also be cultured. Foreign bodies should be processed in traumatic endophthalmitis, whereas a swab of the bleb may assist in bleb-associated infections.

Because specimen volumes are often very limited, the traditional approach of direct inoculation onto culture media for aerobic and anaerobic bacteria and fungi remains important. Rapid results by microscopy of Gram stains are useful, with positive results occurring in some 50–70% of well-collected vitreous aspirates.[36] An appropriate clinical history should accompany specimens to the laboratory so that culture can be prolonged for fastidious organisms and so that skin commensals such as *P. acnes* are not routinely discarded as contaminants.

Polymerase chain reaction (PCR)-based detection of the 16S rRNA gene for detection of bacteria, and of the 18S rRNA gene for the detection of fungi, are of great help in the diagnosis of endophthalmitis.[37–40] These techniques are particularly helpful when the patient received intraocular antibiotic/antifungal treatment prior to intraocular sampling.[41] Previous methods based on the analysis of restriction profile[42] are now being replaced by direct sequencing of the PCR product.[40,41] Because of the high risk of contamination, nested PCR[37] should be replaced by real-time PCR, targeting specific pathogens such as *Neisseria meningitidis*.[43]

MANAGEMENT

Antibiotic chemotherapy

Early institution of antimicrobial therapy is essential to optimal outcome in the management of infective endophthalmitis. Controversy continues as to the best therapeutic approach.

Intravitreal antibiotics

Intravitreal injection of antibiotics is the most effective way of rapidly achieving high intraocular antibiotic levels. Direct intravitreal injections of nonpreserved (i.e. intravenous) formulations of antibiotics are now the recommended route of administration in endophthalmitis treatment. The rationale of such therapy is that, although antibiotics when administered topically, subconjunctivally or systemically may attain therapeutic concentrations in the anterior chamber, concentrations in the vitreous are much lower. Intravitreal injections may produce significant retinal toxicity, although single injections have been shown to be safe and effective.

The optimal combination of antibiotics in empiric therapy normally covers Gram-positive and Gram-negative organisms. Over recent years, vancomycin has replaced first-generation cephalosporins for Gram-positive activity because of the increasing incidence of *Staph. epidermidis* infection resistant to methicillin and other β-lactam drugs. Reports of retinal toxicity caused by gentamicin led to the use of amikacin to cover Gram-negative organisms, as this latter antibiotic was believed to be less toxic. Amikacin has now been used widely in controlled and uncontrolled clinical situations. However, more recently, gentamicin and amikacin have been associated with macular infarction[44] and this has led to increased use of other broad-spectrum Gram-negative antimicrobial agents, particularly ceftazidime. An initial single injection of 0.2 mg vancomycin maintains therapeutic concentration for at least 3 days and a repeat injection at that time for at least 4 days.[45] Repeat injections of aminoglycosides should be avoided, except in severe cases. Intravitreal amphotericin B injections (1–5 μg) can be utilized in fungal endophthalmitis, with a single repeat inoculation after 72–96 hours in progressive infection; again, however, toxicity limits longer duration of therapy.

Subconjunctival antibiotics

The data regarding subconjunctival antibiotic penetration are conflicting, possibly because of varying degrees of inflammation in the eye or poor and variable sampling techniques. Penetration is affected by the transscleral and transcorneal permeability of the agent, but high aqueous levels can be achieved with vancomycin, gentamicin and β-lactam antibiotics for up to 4 hours; however, vitreous concentrations are generally poor. Subconjunctival injections are an irritant and are painful, thus limiting the duration of this form of therapy to a few days.

Topical antibiotic therapy

The efficacy of topical antibacterial applications in endophthalmitis is not well studied, although significant concentrations of antibiotics in the anterior segment can be obtained with frequent administration of highly concentrated (fortified) solutions. A combination of vancomycin and fortified gentamicin (e.g. 1.5%) provides broad-spectrum cover, but solutions must be prepared by a qualified pharmacist as they are not commercially available. Third-generation cephalosporins (such as cefotaxime and ceftazidime) and fluoroquinolones (such as ciprofloxacin) may be used to replace gentamicin to provide appropriate Gram-negative cover. The use of collagen shields to produce frequent topical application of antibiotic solutions has been explored, but is limited by potential corneal toxicity. Iontophoresis also increases anterior chamber concentrations, but its efficacy remains unproved.

Systemic antibiotics

In humans, intraocular penetration of systemically administered antibiotics is generally poor. However, intravenous antibiotics are a common adjunctive therapy in endophthalmitis, justified in that they may enhance concentrations of antibiotics achieved with intravitreal agents and extend the duration of therapeutic activity. Intraocular inflammation and/or performance of a vitrectomy may alter the blood–retina barrier to allow improved intraocular penetration.

Posterior chamber concentrations of newer and broad-spectrum agents, including ceftazidime, imipenem and ciprofloxacin, appear improved, particularly in terms of efficacy against Gram-negative bacilli such as *Pseudomonas* spp. However, none is reliably active against *Staph. epidermidis*, the most common cause of postoperative endophthalmitis, and resistance may develop rapidly.

A multicenter randomized trial, the Endophthalmitis Vitrectomy Study (EVS), sponsored by the National Eye Institute of the National Institutes of Health, followed a cohort of 420 patients who had clinical evidence of endophthalmitis within 6 weeks after cataract surgery or secondary intraocular lens implantation.[24] Patients were randomly assigned to therapy, with or without pars plana vitrectomy and with or without systemic antibiotics (ceftazidime and amikacin), but each patient underwent intravitreal injection of vancomycin and amikacin. When outcome was assessed 9–12 months after the operation, no difference in final visual acuity or media clouding between groups with and without systemic antibiotics could be determined. Well conducted as this study was, a number of questions remain unanswered, particularly in relation to generalization of the results. Do the results, for example, extrapolate to Gram-negative infection or to other categories of ocular surgery? Moreover, a significant percentage of coagulase-negative staphylococci and *Streptococcus* spp., the most common Gram-positive pathogens, are resistant to ceftazidime and amikacin, although vancomycin was active against all.

Duration of therapy is very variable. In spite of the presence of an ocular foreign body such as an intraocular lens, endophthalmitis caused by *Staph. epidermidis* will settle rapidly with appropriate management.

Length of therapy can be assessed by a reduction in cellular activity in both the anterior and posterior chambers and by improvement in visual acuity.

Guidelines have been published for the treatment of fungal endophthalmitis.[46] Itraconazole, a lipophilic antifungal imidazole, has been shown to be efficacious in *Aspergillus* endophthalmitis. Fluconazole, a hydrophilic imidazole, achieves useful concentrations in intraocular tissue and has also proved successful in *Candida* endophthalmitis. Voriconazole, a newer broad-spectrum antifungal with intraocular penetration, has been used successfully.[46,47] Amphotericin B, often the only available therapy in filamentous fungal infections other than those caused by *Aspergillus* spp., does not achieve significant concentrations in intraocular tissue. Nevertheless, it is widely used in both conventional and liposomal formulations in fungal endophthalmitis with some evidence of success.

Specific recommendations

Endophthalmitis may cause irreparable visual loss within 24–48 hours. Initial therapy must frequently be empiric, because of the low sensitivity of Gram stain film and the 24–48 hour delay until culture results become available. Antibiotic therapy should be chosen to cover the spectrum of pathogens likely to be implicated. In this situation classification of endophthalmitis by clinical setting provides useful information. Suggested initial antibiotic therapy for acute postoperative, filtering bleb and post-traumatic endophthalmitis is illustrated in Table 16.2.

Localized bleb infection without endophthalmitis can usually be managed initially with topical therapy. Because of the increased prevalence of *H. influenzae* in this infection, a combination of antibiotics that covers this pathogen and provides broad-spectrum activity against Gram-positive and Gram-negative organisms should be chosen. New combinations have been recently reviewed.[48]

Adjunctive therapy

Vitrectomy

The place of pars plana vitrectomy remains a controversial issue in the management of endophthalmitis. The theoretic rationale for such a procedure is that it offers a reduction in organism load, a reduction in traction effect on the retina with less potential for detachment, collection of adequate culture material and possibly improved distribution of intravitreal antibiotics. Evidence for its efficacy has been conflicting, often as a result of poorly controlled studies. The EVS was designed to determine definitively the value of vitrectomy in the presence of intravitreal antibiotics. The final conclusions of this study were that visual acuity was improved significantly in patients treated with vitrectomy only if the initial vision was light perception, but not if initial vision was hand movements or better; that is, the most severe cases on presentation benefited most from vitrectomy.[49]

Corticosteroids

The use of corticosteroids to reduce the inflammatory response to infection and thus preserve ocular tissue is widely practiced with administration by several routes, including intraocular, periocular, topical and systemic, but its place in therapy remains contentious. Experimental animal studies demonstrate superior outcomes utilizing corticosteroids with antibiotics even in endophthalmitis caused by pseudomonads and fungi. In general, clinical studies do not report deterioration in outcomes if corticosteroids are used in combination with antibiotics, at least at the ocular level.[50]

Management of intraocular lens

In most cases of acute postoperative pseudophakic endophthalmitis, removal of the intraocular lens is not necessary and does not influence outcome. In fact, it may be hazardous and predispose to anterior

Table 16.2 Initial empiric recommendations for antimicrobial therapy of endophthalmitis*

Category of endophthalmitis		ANTIMICROBIAL THERAPY		
		Topical	**Intravitreal**	**Systemic**
Postoperative	Acute	Cefazolin (5 mg/ml) + gentamicin (8–15 mg/ml) or amikacin (25–50 mg/ml) Vancomycin (50 mg/ml) + gentamicin or amikacin or ceftazidime (5 mg/ml) or ciprofloxacin (3 mg/ml)	Cefazolin (2.25 mg) + amikacin (400 µg) Vancomycin (1 mg) + amikacin or ceftazidime (2.25 mg)	Cefazolin (2 g q8h) + gentamicin (4–5 mg/kg q24h)† or amikacin (15 mg/kg q24h)† Vancomycin + gentamicin or amikacin or ceftazidime (2 g q8h) or ciprofloxacin (400 mg q12h)
	Delayed	Vancomycin + gentamicin or amikacin or ceftazidime or ciprofloxacin	Vancomycin + amikacin or ceftazidime	Vancomycin + gentamicin or amikacin or cefotaxime (2 g q6h) or ceftazidime or ciprofloxacin
	Filtering bleb	Vancomycin + ceftazidime or ciprofloxacin	Vancomycin + ceftazidime	Vancomycin +/or cefotaxime, ceftazidime, ciprofloxacin
Post-traumatic		Vancomycin + gentamicin or amikacin or ceftazidime or ciprofloxacin	Vancomycin + amikacin or ceftazidime	Vancomycin + gentamicin or amikacin or ceftazidime or ciprofloxacin

*Dosages given in brackets apply to all citations of each specific drug within the relevant column.
†Dosages must be adjusted to reflect the patient's age, body weight and renal function.

segment hemorrhage and retinal detachment. Exceptions may occur when the pathogen is a fungus and in cases of late-onset endophthalmitis caused by *P. acnes* when conservative treatment with intravitreal vancomycin and corticosteroids is unsuccessful. In such cases, complete capsulectomy and lensectomy may be necessary to provide cure.

Outcome

Up to 50% of patients who have endophthalmitis suffer major visual loss within 24–48 hours of onset, emphasizing the essential need for early diagnosis and prompt treatment.[50] Approximately 30% of patients in recent studies of endophthalmitis achieved a final visual acuity of 20/60 or better after treatment.[51,52]

Certain factors are highly correlated with poor visual outcome; these include severity of infection, delay in time to diagnosis and institution of treatment, virulence of infecting organisms and intraocular complications such as vitreous hemorrhage and retinal detachment. Poor vision at the time of diagnosis correlates with either a virulent organism, such as *Bacillus* spp., *Streptococcus* spp. or Gram-negative bacilli or fungi, or a delay in diagnosis even with a low-virulence organism. Normally, however, *Staph. epidermidis* endophthalmitis has an excellent outcome, although even in this situation some 10% of patients develop blindness.[53] Culture-negative cases of endophthalmitis generally have a better visual outcome than do culture-positive groups, which may relate to the lower virulence of more fastidious organisms or to the veracity of diagnosis.

The outcome of post-traumatic endophthalmitis and endophthalmitis related to a conjunctival filtering bleb is poor, probably because of the intrinsic virulence of organisms implicated; however, for converse reasons, prognosis in delayed ophthalmitis, including that caused by *P. acnes*, is usually more favorable.

The primary complication of endophthalmitis is retinal detachment that may occur at any time before, during or after treatment. Prognosis in this situation is poor, although surgical repair can salvage useful vision in a substantial number of patients.[54]

REFERENCES


References for this chapter can be found online at http://www.expertconsult.com

Chapter 17

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Infectious retinitis and uveitis

INTRODUCTION

Strictly speaking, uveitis is inflammation of any etiology in one or more parts of the uveal tract of the eye which includes the choroid, the iris and the ciliary body. In practice, however, uveitis has come to mean any inflammation of the intraocular structures. Inflammation localized to certain structures can be denoted by more specific terms. For example, inflammation of the iris (iritis) or ciliary body (cyclitis) is called anterior uveitis. Inflammation of the vitreous (vitritis), retina (retinitis) or choroid (choroiditis) is called posterior uveitis. Inflammation of the entire globe is called panuveitis. Inflammation localized to the outer coats of the eye or optic nerve without accompanying intraocular inflammation (e.g. scleritis, keratitis, optic neuritis) is not considered a uveitis.

The anterior segment of the eye consists of the cornea, anterior chamber, lens, iris, posterior chamber and ciliary body; the posterior segment refers to the vitreous cavity and posterior structures including the retina and optic nerve (Fig. 17.1).

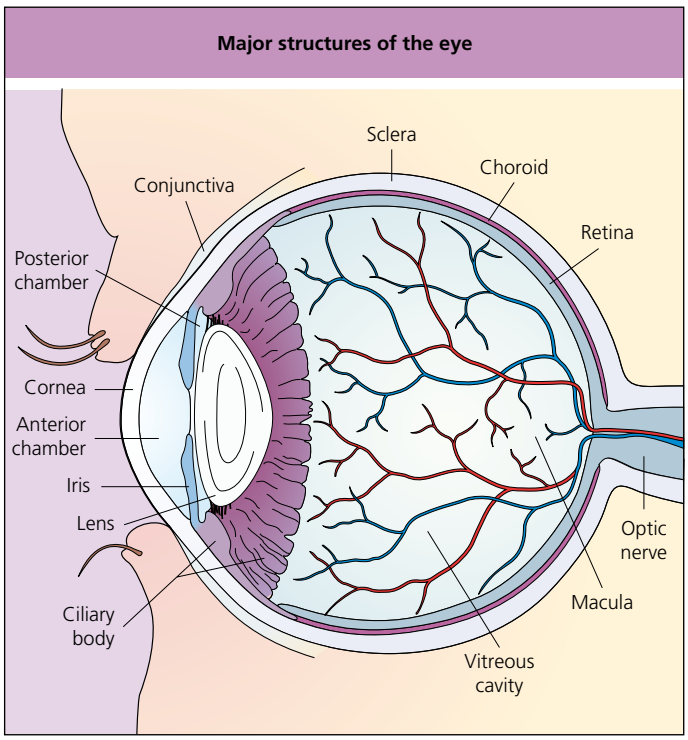


Fig. 17.1 Major structures of the eye.

The location, distribution and ophthalmoscopic appearance of inflammatory lesions is useful to the ophthalmologist in suggesting likely causes of uveitis (Table 17.1).[1] Intermediate uveitis centers about the equator of the eye between the anterior and posterior parts of the uveal tract, whereas anterior uveitis involves the anterior chamber and posterior uveitis involves the posterior segment of the eye.

A diagnosis of retinal vasculitis is made by finding inflammatory sheathing of the retinal vessels on ophthalmoscopic examination and leakage of dye from the involved vessels on intravenous fluorescein angiography (Fig. 17.2).[1] Retinal vasculitis is sometimes isolated, but more commonly occurs in conjunction with posterior uveitis. It is a nonspecific finding and can occur in ischemic conditions as well as infection. Endophthalmitis, although overlapping to some degree with uveitis and retinitis, is considered separately (see Chapter 16).

There are many known causes of uveitis, including infections, autoimmune disorders, various other systemic diseases and trauma. Many cases of uveitis remain of uncertain origin despite extensive investigation. In trying to determine the cause of any particular case of uveitis, the ophthalmologist considers a number of features, including the distribution and morphologic characteristics of the lesions, the chronicity of the disorder and the presence of underlying systemic diseases. In select cases, invasive diagnostic testing in the form of anterior chamber paracentesis, vitreous biopsy or even retinal biopsy may be necessary to rule out an infectious etiology.

This chapter describes the major manifestations of uveitis and retinitis, with an emphasis on the infectious causes of these syndromes. Uveitis and retinitis in immunosuppressed patients and neonates are considered separately because the causative agents differ from those in immunocompetent and adult patients. Only the infectious aspects pertinent to the eye will be discussed.

EPIDEMIOLOGY

Uveitis and retinitis are uncommon in clinical practice. In Minnesota, USA, the annual incidence of new cases of uveitis has been found to be 17/100 000 population,[2] though it has been estimated that in the Western world uveitis accounts for approximately 10% of the visual handicap.[3] Typically, a general ophthalmologist is likely to see only a dozen patients with uveitis each year. The majority are anatomically 'anterior uveitis', accounting for 50–60% of all uveitis cases in a tertiary care center and 90% in primary care settings.[4] A specific etiology can be identified in only about half of the patients with uveitis. In some instances, uveitis provides the first evidence of an underlying systemic disease. HLA-B27 and its associated seronegative spondyloarthropathies are the most commonly identifiable causes of anterior uveitis in the Western world.[5] Uveitis and retinitis can occur at any age and the incidence is about equally divided between the sexes. In one study, the mean age of patients with uveitis was 45 years.[1]

Table 17.1 Anterior, intermediate and posterior uveitis. **Definitions and common symptoms.**

	Anterior uveitis	Intermediate uveitis	Posterior uveitis
Ophthalmoscopic signs that define the type of uveitis	Inflammatory cells in the anterior chamber with or without keratic precipitates or iris lesions	Inflammatory cells more highly concentrated in anterior vitreous than in anterior chamber	Inflammation of retina, choroid, retinal vessels, posterior vitreous humor, or a combination of these
Additional clinical signs	Ciliary flush (perilimbal injection of the sclera) Posterior synechiae	Macular edema Inflammatory exudate on pars plana	Retinal vasculitis Optic disk edema Macular edema
Symptoms	Pain Redness Photophobia	Floaters	'Floaters' Blurred vision



Fig. 17.2 Retinal inflammatory vascular sheathing (vasculitis). This case occurred secondary to sarcoidosis.

Infectious uveitis or retinitis in neonates is almost always the result of congenital infection – toxoplasmosis, rubella, cytomegalovirus (CMV), herpesvirus (i.e. the TORCH syndrome, see Chapter 52). Each of the TORCH agents can involve the uvea or retina. Among immunosuppressed patients, uveitis or retinitis is found most commonly in patients who have AIDS. However, hematogenous fungal endophthalmitis occurs primarily in patients with other forms of immunosuppression.

Although uveitis does not appear to be more prevalent in any particular part of the world, there is geographic variation in the underlying causes. For example:

- acquired ocular toxoplasmosis is quite common in Brazil, but rare in the rest of the world;
- Behçet's disease is prevalent in Turkey and the Middle East, but unusual elsewhere;
- leprosy has been eradicated in most developed countries, but can still be found in less developed regions;
- human T-cell lymphotropic virus type-1 (HTLV-1)-associated uveitis is commonly found in Japan;
- CMV retinitis associated with AIDS and a CMV-associated vitritis secondary to highly active antiretroviral therapy (HAART) occurs in patients from developed countries but is rarely seen in developing areas of the world; and
- onchocerciasis (river blindness), which primarily affects the cornea, but can produce retinitis and choroiditis, is seen only in equatorial Africa and Central America.

CLINICAL FEATURES

Common symptoms of intraocular inflammation, irrespective of the cause, are ocular pain, photophobia, 'floaters' (specks that appear to float in the visual field) and impaired vision (see Table 17.1). Both eyes may be affected simultaneously, but unilateral involvement does not rule out a systemic cause. Anyone with these symptoms should have an ophthalmologic evaluation with pharmacologic dilatation of the pupil with examination by slit lamp and indirect ophthalmoscopy. The findings allow the process to be characterized as anterior, intermediate or posterior uveitis, or panuveitis (see Table 17.1).

Additional clinical signs include conjunctival injection, anterior segment cells and 'flare' (protein floating in aqueous fluid, seen on slit lamp examination), keratic precipitates (white cells adherent to the posterior cornea), iris nodules (granulomas), posterior synechiae, posterior segment cells (Fig. 17.3), optic disk edema, retinal vasculitis, retinitis and choroiditis. A hypopyon refers to layered inflammatory

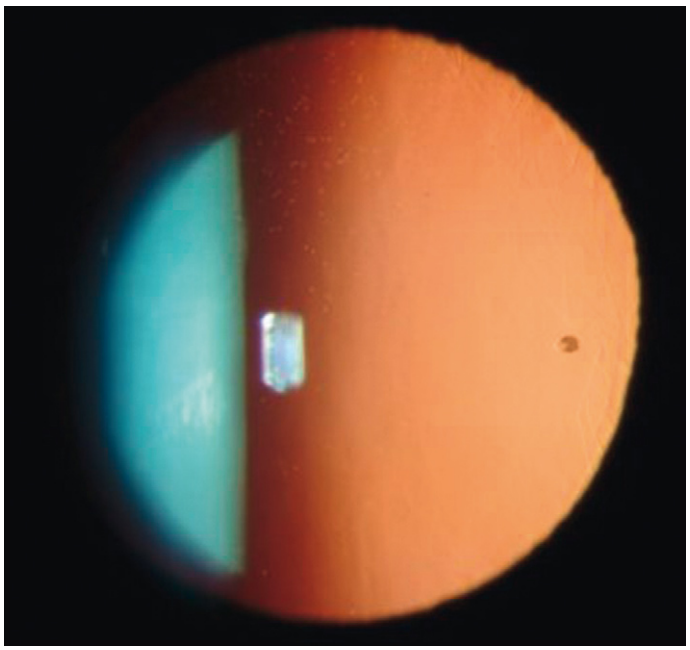


Fig. 17.3 A retroillumination view through the dilated pupil highlights the inflammatory cells suspended in the vitreous.

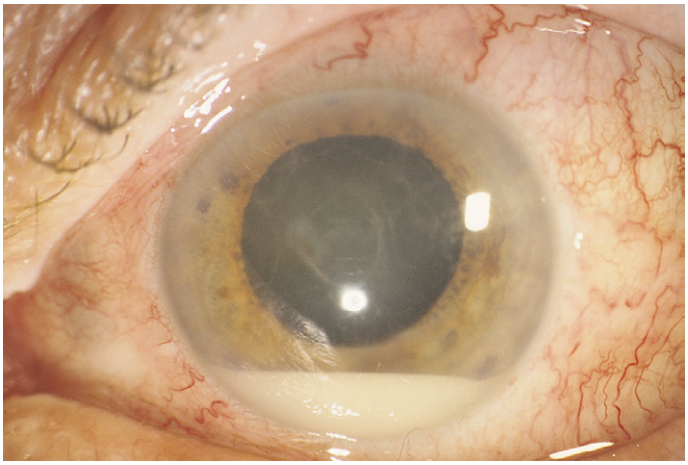


Fig. 17.4 Hypopyon. The finding of a hypopyon (layered inflammatory cells in the anterior chamber of the eye) usually denotes a severe anterior uveitis.

cells that settle gravitationally in the inferior aspect of the anterior chamber (Fig. 17.4). In cases of severe acute uveitis, there may be so much intraocular inflammation that a cloudy media results. Ocular ultrasonography should be performed in such cases.

The characteristic causes and clinical presentations of uveitis seen in a general community-based ophthalmologic practice and in a tertiary referral center have been found to differ (Table 17.2).[1] In the community ophthalmic practice, about 90% of uveitis was anterior and most cases of anterior uveitis were acute. By contrast, in the tertiary referral center only 50–60% of uveitis was anterior, with posterior uveitis and panuveitis significantly more prevalent.[4] In both settings, a specific diagnosis could be made in only about half of patients from data gathered at the initial visit. An infectious cause was documented in 21% of the referred patients and 14% of the community-based patients. The uveitis was attributed to a systemic disease, usually rheumatologic, in a smaller number of patients. The most common causes of uveitis in both settings were:

- HLA-B27-associated anterior uveitis;
- CMV retinitis;
- herpesvirus-associated uveitis, caused either by herpes simplex virus (HSV) or varicella-zoster virus (VZV); and
- *Toxoplasma* retinochoroiditis (see Table 17.2).

Thus, three of the four most common identified causes of uveitis were infectious, but infectious causes accounted for well under half of all cases of uveitis in either setting. Anterior uveitis is much more common than posterior uveitis, and the specific cause is more likely to be identifiable.

PATHOGENESIS AND PATHOLOGY

Most of the infectious agents discussed in this chapter gain access to the ocular structures via hematogenous spread of micro-organisms from other sites. The uveal tract is highly vascular, offering a ready target for seeding by blood-borne microbes. Some organisms (e.g. herpesviruses, *T. gondii*) have a propensity for the retina itself. Furthermore, circulating inflammatory cells and mediators of inflammation have a potent impact on the uveal tract and adjacent structures. In some types of infectious uveitis (e.g. tuberculous and possibly spirochetal uveitis), the inflammation may be produced by a combination of microbial

Table 17.2 Uveitis in community-based and tertiary ophthalmology centers*

	Patients in community-based ophthalmology practice	Patients in tertiary referral center
Mean age of patients (y)	46	45
Males (%)	49	52
Percentage with:		
Anterior uveitis	91	60
Intermediate uveitis	1	12
Posterior uveitis	5	15
Panuveitis and other	3	13
Percentage of cases of anterior uveitis that are chronic	9	63
Percentage in which specific diagnosis was made	47	58
Percentage attributable to infection	14	21
Percentage associated with systemic disease (including infections)	13	9
Most common causes of uveitis	HLA-B27-associated anterior uveitis (15%) CMV retinitis (7%) Traumatic uveitis (5%) VZV uveitis (4%) *Toxoplasma* retinochoroiditis (4%)	CMV retinitis (33%) HLA-B27-associated anterior uveitis (8%) Pars planitis syndrome (6%) HSV-associated uveitis (5%) *Toxoplasma* retinochoroiditis (4%)
Most common causes of posterior uveitis	CMV retinitis *Toxoplasma* retinochoroiditis	CMV retinitis *Toxoplasma* retinochoroiditis

*The characteristics of uveitis seen in a community-based ophthalmology practice and in a tertiary referral center. The figures for the proportion of cases in which a specific diagnosis was made are based on data gathered at, or requested at, the first visit; cases of CMV retinitis and 'masquerade' syndrome are omitted. The figures for the proportion of cases associated with systemic disease include cases due to varicella, candidiasis, coccidioidomycosis and syphilis. Data from McCannel *et al.*[1]

invasion and immunologic mechanisms. Occasionally, uveitis occurs as a 'sympathetic' reaction to an adjacent infection (e.g. anterior uveitis in patients with HSV keratitis). It is hypothesized that other infections gain access to the eye by spreading along nerves, such as the acute retinal necrosis (ARN) syndrome associated with VZV.

The eye is unique among organs in showing a neovascular response to certain stimuli, especially prolonged inflammation. New vessels may form in the cornea, iris, retina, optic nerve and choroid, presumably as a result of the production of various protein growth factors such as vascular endothelial growth factor (VEGF). These newly formed, acquired vessels themselves can cause severe loss of vision. Together with specific treatment of the underlying cause, laser photocoagulation, corticosteroids or intravitreal injection with anti-VEGF agents may be needed to treat ocular neovascularization.[6]

PREVENTION

Patients who have systemic infections associated with an appreciable risk of intraocular infection should be screened by an ophthalmologist for early detection and treatment. An example is the periodic examination of patients who have HIV infection and a low $CD4^+$ lymphocyte count to detect CMV retinitis. Likewise, patients who have HIV infection who develop ophthalmic zoster are at risk of developing necrotizing herpetic retinitis and are candidates for ophthalmologic screening examination. Patients who have *Candida* fungemia merit ocular examination to detect metastatic retinitis. Neonates who have congenital HSV infection must be screened carefully for ocular lesions, which may first appear up to several months after birth.

MOLECULAR DIAGNOSTICS

Molecular diagnostic techniques are playing an increasing role in the care of infectious uveitis and retinitis. The polymerase chain reaction (PCR) was first used to identify *T. gondii* DNA in ocular tissue in 1990.[7] Since then PCR of intraocular fluids has been utilized in the diagnosis of HSV, VZV, CMV, fungi (including *Fusarium*, *Candida* and *Aspergillus*) mycobacteria, protozoal eye disease and infectious bacterial endophthalmitis.[8] PCR is considered a helpful diagnostic adjunct, particularly when sample sizes are small and inadequate for culture, complementing other diagnostic processes including enzyme-linked immunosorbent assay (ELISA), cytology and culture. Protocol refinement, including the use of a nested PCR protocol followed by restriction enzyme digestion, has led to the amplification and characterization of diverse mycobacterial species. It has also been used to identify immunoglobulin H rearrangements in lymphomatous uveitis. More recently 16s ribosomal DNA typing has been employed to identify bacteria in vitreous samples.[9] Internal transcribed spacer 1 (ITS1) and ITS2 5.8S ribosomal DNA typing has also been used to diagnose fungal endophthalmitis.[10] Most microbiologic techniques have difficulty identifying active disease. Reverse transcription PCR (RT-PCR) techniques hold promised for identifying active versus latent infections.

MANAGEMENT

There are two major principles of therapy for intraocular infections. The first is to treat the infection. Drugs for the treatment of ocular diseases may be given by topical administration, periocular injection, systemic administration (orally or intravenously, or both) and intravitreal injection.

The second principle is to suppress intraocular inflammation, lest there be persisting damage to the retina and other crucial structures. This is usually accomplished by using corticosteroids, which may be given by similar topical, oral, periocular or intravitreal routes. Rarely systemic immunosuppression is employed.

Uveitis in immunocompetent adults and children

Viral causes of uveitis

Uveitis, especially anterior uveitis, may occur in the course of many viral infections, most commonly those caused by rubeola virus and the herpesvirus family (Table 17.3). The uveitis is generally self-limited and does not require treatment.

Measles often causes conjunctivitis and keratitis; the keratitis rarely leads to bacterial ulceration and perforation of the cornea. Other rare complications are chorioretinitis or neuroretinitis; these may occur, together with measles encephalitis, 1–2 weeks after the onset of the rash.

The ocular lesions of rubeola are generally self-limited and no specific treatment is available. Involvement of the optic disk (optic neuritis)

Table 17.3 Viral causes of retinitis and uveitis*

Viral infection	Systemic infection	Features of retinitis or uveitis	Other ocular disease
Measles	Uveitis occurs 1–2 weeks after onset of rash	Common: anterior uveitis Rare: chorioretinitis or neuroretinitis	Common: conjunctivitis, keratitis Rare: optic neuritis
SSPE (see Fig. 17.6)	Uveitis occurs some years after infection	Chorioretinitis involves macula	Papillitis; motility disturbances
Herpes simplex virus	Primary infection; lids and conjunctiva	Common: anterior uveitis Rare: posterior uveitis, retinitis, ARN	Dendritic corneal ulcer common
Varicella-zoster virus	Convalescence from varicella or trigeminal zoster (often with nasociliary branch involved)	Common: mild, anterior uveitis, self-limited; rarely, chorioretinitis or ARN	Granulomatous keratic precipitates; synechiae; glaucoma, cataract
Epstein–Barr virus	Infectious mononucleosis	Rare: mild anterior uveitis or chorioretinitis	Common: follicular conjunctivitis
Influenza, adenovirus, mumps	Systemic infection usually evident	Rare: bilateral, mild, self-limited, anterior uveitis Rare: neuroretinitis or optic neuritis	None

*These ocular problems are seen in immunocompetent adults and children.



Fig. 17.5 Salt-and-pepper fundus. The pigment alterations in the macula give a 'dirty' appearance to the retina. The lesion occurred following congenital rubella infection. The vertical black line across the fovea is a focusing stick.

may cause severe visual loss, but this may improve spontaneously over subsequent months. There may be residual pigmentary retinopathy with the salt-and-pepper appearance (Fig. 17.5).

Subacute sclerosing panencephalitis (SSPE), which results from measles infection in very early life, commonly causes chorioretinitis, usually about the time that the neurologic signs of the disease become evident. The chorioretinitis is often focal, involving the macula, and there is mild vitritis (Fig. 17.6). Cortical blindness may occur. The prognosis of the ocular lesions is poor. There is no specific treatment.

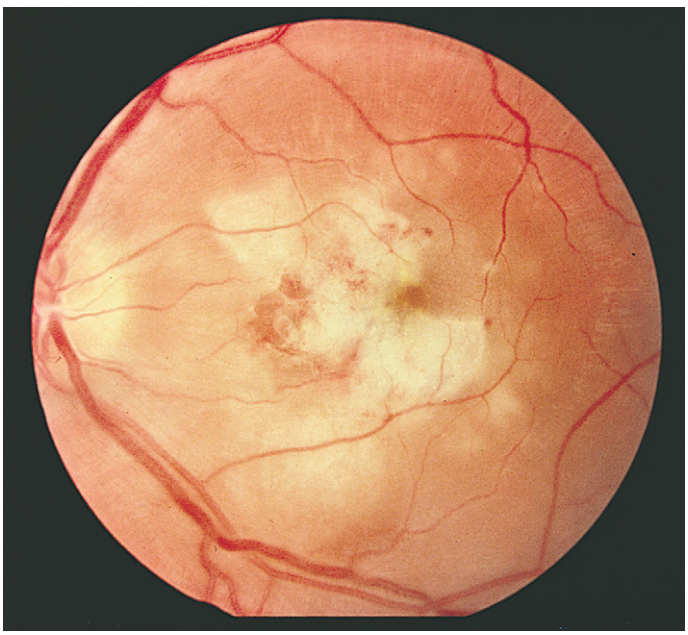


Fig. 17.6 Macular retinitis. This patient has subacute sclerosing panencephalitis.

Herpes simplex virus infection of the eye in children and adults usually presents as recurrent keratitis with characteristic dendrites (see Chapter 155). There may be an associated or isolated anterior uveitis and it has been estimated that 9% of anterior uveitis is attributable to HSV.[11] Rarely, HSV keratitis may spread along the axons to produce retinitis and posterior uveitis (see below).

Varicella, especially in the convalescent stage, may cause a mild, self-limited anterior uveitis. A more serious lesion, keratitis, may develop in patients who have trigeminal zoster, especially during convalescence from zoster that has involved the nasociliary branch of the trigeminal nerve. It may be accompanied by anterior uveitis which may be treated with 1% trifluridine ophthalmic drops or oral aciclovir or valaciclovir. Rarely, trigeminal zoster may lead to chorioretinitis and a form of necrotizing herpetic retinitis referred to as the ARN syndrome (see below). Cases have been reported with primary varicella infection of childhood (chickenpox).[12]

During infectious mononucleosis caused by Epstein–Barr virus (EBV) infection, a bilateral, mild, follicular conjunctivitis may be seen. Rarely, a mild anterior uveitis can occur as well. Recently, a choroiditis resembling that seen with histoplasmosis, but with vitritis, has been ascribed to acute or chronic EBV infection.[13]

Other viral infections may produce uveitis on occasion. Influenza virus infection may cause a mild, transient, bilateral anterior uveitis. Mumps virus infection may cause a mild, evanescent, bilateral anterior uveitis, which may appear up to 4 weeks after the onset of clinical infection. Optic neuritis, sometimes with neuroretinitis, may occur after mumps infection, but nearly always resolves spontaneously.

HLA-B27-associated uveitis

An autoimmune form of recurrent, bilateral anterior uveitis is common in patients who harbor the HLA-B27 antigen. It has been estimated that 55% of Caucasian patients with acute anterior uveitis are HLA-B27 positive versus 8–10% of the general population.[14] HLA-B27-associated uveitis is the most common type of nonidiopathic anterior uveitis.[5] Although usually considered immune-mediated rather than infectious, it may be triggered by systemic infection with Gram-negative bacteria, *Mycoplasma pneumoniae*, *Bartonella henselae* or *Chlamydia trachomatis*, as seen in Reiter's syndrome. Reiter's syndrome consists of the triad of conjunctivitis, urethritis and uveitis.[15] Several serologic studies from Scandinavian countries and Australia suggest that *Yersinia* infections may be important contributors.[16] Other data recently implicated *Helicobacter pylori* as a possible inciting agent.[17]

A recent retrospective cohort study found the average age of onset for HLA-B27 uveitis patients to be 36 years and was slightly more common in males. Bilaterality occurred in 21% and was more common in females. At the time of uveitis diagnosis, 33% of males had a systemically associated disease (most commonly ankylosing spondylitis) versus 17% of females. However, after the onset of uveitis the risk of developing systemic disease was statistically similar in males and females. At 10-year follow-up, the long-term visual prognosis of HLA-B27 uveitis was good.[18]

Bacterial causes of uveitis

Many bacterial species are associated with ocular infections (Table 17.4). Ocular lesions are common in the course of syphilis (*Treponema pallidum* infection). In the primary stage it may manifest as eyelid or conjunctival chancres. In the secondary stage, 1–3 months later, patients may develop a bilateral (though occasionally asymmetric) anterior uveitis, sometimes with iris nodules (granulomas). Less commonly is a choroiditis with large, white, 'geographic' lesions. There may also be retinal vasculitis, vitritis and papillitis.[19] Conjunctivitis, scleritis and episcleritis have also been reported.[20]

Uveitis remains the most common ocular finding in tertiary syphilis, affecting 2.5–5% of patients.[21] In addition to the Argyll Robertson pupil that may be present, other ophthalmic findings including papillitis, periphlebitis, vitritis and serous retinal detachment. One recent

Table 17.4 Bacterial causes of retinitis and uveitis*

Bacterial infection	Systemic infection	Features of retinitis or uveitis	Other ocular disease
Yersinia spp.	Infection occult, not proven	Suggested important cause of anterior uveitis in patients with HLA-B27 antigen	None
Borrelia burgdorferi	Features of extraocular Lyme disease	Nonspecific anterior or posterior uveitis	Conjunctivitis
Treponema pallidum	Uveitis usually during secondary syphilis; interstitial keratitis is delayed manifestation of congenital syphilis	Common: bilateral anterior uveitis Rare: choroiditis (large while lesions), retinal vasculitis, papillitis	Acute bilateral interstitial keratitis (age 5–10 years, after congenital infection)
Bartonella spp. (see Fig. 17.7)	Nonspecific systemic symptoms antedate ocular symptoms	Bilateral papillitis: optic disk edema, white retinal lesions, vitritis	None
Metastatic endophthalmitis	Often extraocular source is evident	Often bilateral: focal or diffuse uveitis or retinitis	None

*These ocular problems are seen in immunocompetent adults and children.

evaluation of the clinical findings in tertiary syphilis described a group of patients with an acute syphilitic posterior placoid chorioretinits.[22] In the setting of HIV, early syphilis tends to be associated with a florid uveitis and VDRL-positive syphilitic meningitis. Late syphilis produces a chronic posterior uveitis with associated subclinical neurosyphilis;[23] this transition to the tertiary stage is often accelerated in those with HIV. Syphilis should be considered in the differential diagnosis of any posterior segment inflammation or any bilateral anterior segment inflammation. The diagnosis is made serologically and treatment is according to the stage of the syphilis (see Chapter 57).

Bartonella henselae in immunocompetent patients has been reported as a cause of stellate neuroretinitis, also known as macular star (Fig. 17.7). Cats are thought to be an important reservoir of *B. henselae*, and most infections have occurred in patients with a history of exposure to cats.[24] *Bartonella* infection should be suspected in any patient with optic disk edema and intraocular inflammation, especially if retinal lesions are seen.

Borrelia burgdorferi infection may cause a wide variety of ocular problems. Conjunctivitis and keratitis occur commonly in the early stages of infection. In the later stages of Lyme disease there may be iridocyclitis, retinal vasculitis, exudative retinal detachment, vitritis and optic disk edema. Orbital pseudotumor and orbital myositis have also been described.[25] The treatment is with systemic antibiotics appropriate for the stage of the disease (see Chapter 43).

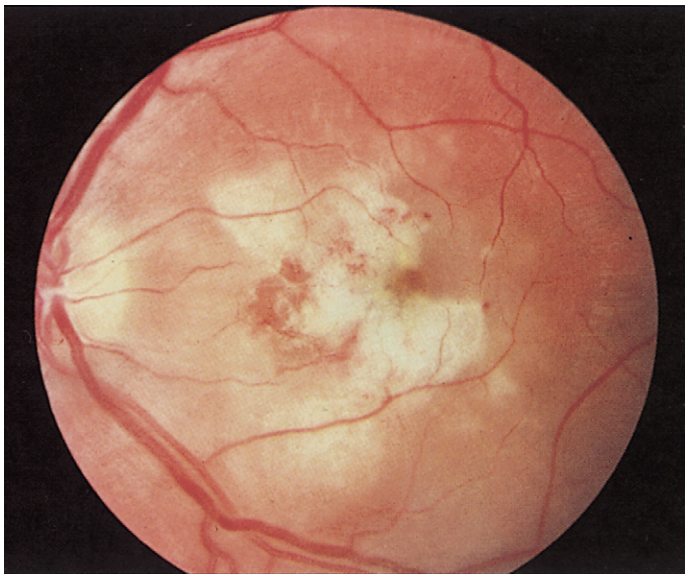


Fig. 17.7 Neuroretinitis with a macular star associated with *Bartonella* infection. Note the swelling of the optic disk with hard exudate in the macula in the so-called 'stellate' pattern.

The infectious agent of Whipple's disease, *Tropheryma whipplei*, is associated with a multisystem disorder, which can include panuveitis, retinitis and choroiditis. The diagnosis can be made by biopsy of affected tissue.[26]

Rarely, other bacterial infections have been associated with uveitis distinct from endogenous endophthalmitis. The list includes *Nocardia* infection (see Fig. 17.10), leptospirosis, brucellosis and tularemia.

Mycobacterium tuberculosis infection can cause anterior uveitis, posterior uveitis or isolated choroiditis. This infection should be a consideration in every case of nonspecific uveitis of unknown cause, though data from the USA suggest a tubercular etiology in less than 0.5% of patients managed in a tertiary care uveitis service.[27] If eye-related pathology is present, there is nearly always active extraocular disease. The anterior uveitis may be acute or chronic, unilateral or bilateral, and there may be granulomatous keratic precipitates. A periphlebitis resembling that seen in sarcoidosis may occur, along with choroidal infiltrates; these infiltrates represent miliary tubercles. More recently PCR analysis of the aqueous has become a popular aid to diagnosis. A recent analysis suggested that one-third of those with suspected TB-related anterior uveitis and two-thirds with posterior disease had a PCR-positive aqueous aspirate.[28] TB-associated uveitis usually improves within 2 weeks of the start of specific antituberculous treatment.

Bacterial endophthalmitis is considered in detail in Chapter 16.

Fungal causes of uveitis

The most common fungal species that cause metastatic endophthalmitis are *Candida* spp. (Table 17.5), especially *C. albicans*. The hallmark lesion is a yellow-white, fluffy patch of retinitis with indistinct borders (Fig. 17.8), almost always associated with vitritis. Some cases of *Candida* endophthalmitis have an indolent course and some even improve spontaneously. Treatment of *Candida* infections is discussed in detail in Chapter 149.

Whereas metastatic *Candida* infection usually occurs in a setting of active fungemia, *Histoplasma* ocular infection usually occurs without evident extraocular infection (see Table 17.5). The lesions arise from previous hematogenous spread to the choroid, producing a granuloma that usually becomes an inactive scar. Disk edema with optic neuritis can occur acutely. As a late complication, choroidal neovascular membranes can emanate from old choroidal scars, leading to retinal edema, hemorrhage and permanently decreased central vision.

Table 17.5 Fungal causes of retinitis and uveitis*

Fungal infection	Systemic infection	Features of retinitis or uveitis	Other ocular disease
Candida spp. (see Fig. 17.8)	Risk factors for candidemia	Whitish fluffy patch of retinitis and some vitritis	Anterior segment cells with hypopyon; corneal abscess
Histoplasma capsulatum (see Fig. 17.9)	History of residence in an endemic area; no extraocular infection evident	Choroidal granulomas, scars; optic neuritis; no vitritis	No anterior segment inflammation

*These ocular problems are seen in immunocompetent adults and children.

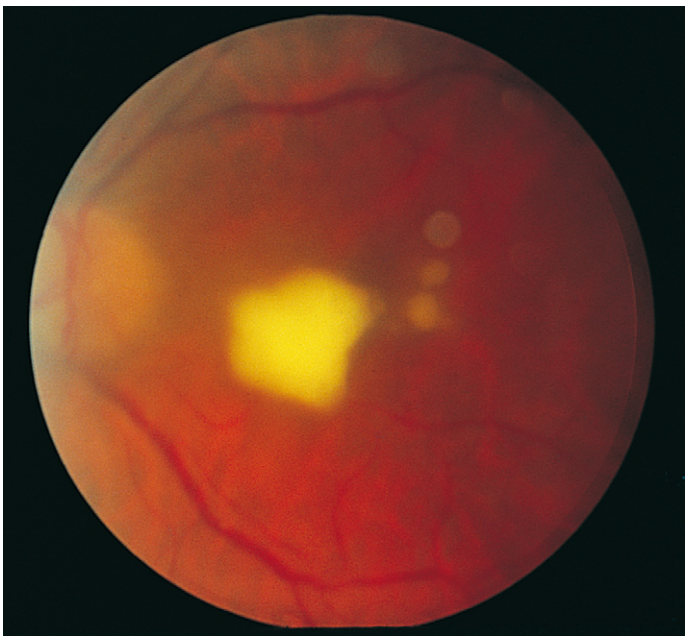


Fig. 17.8 A focal area of superficial retinitis and vitritis. This occurred secondary to *Candida albicans*.



Fig. 17.9 The classic ocular findings of previous histoplasmosis. Note the peripapillary atrophy and punched-out yellowish chorioretinal scars. An old choroidal neovascular membrane is present in the center of the histoplasmosis scar temporal to the macula.

A diagnosis of ocular histoplasmosis is suspected by finding the characteristic choroidal lesions (Fig. 17.9) in a patient who has resided in an area endemic for the fungal infection. Serologic or skin tests for histoplasmosis are usually positive but do not prove the diagnosis. The primary visual risks are associated with late neovascular vessel growth from the chorioretinal scars. Laser photocoagulation and intravitreal administration of anti-VEGF agents are the primary treatment modalities. There is no indication for antifungal treatment.

Rarely, other fungi such as *Coccidioides*, *Blastomyces* and *Aspergillus* spp. and *Cryptococcus neoformans* may cause chorioretinitis by hematogenous spread. *Nocardia* spp. (Fig. 17.10) also occasionally cause chorioretinitis.

Parasitic causes of uveitis

Toxoplasma gondii infection is a common cause of posterior segment infection in children and adults (Table 17.6). It has been estimated to afflict 1.26 million individuals in the USA alone.[29] In the immunocompetent, it represents the most common cause of infectious posterior uveitis worldwide. In many countries, most cases of ocular toxoplasmosis are thought to represent reactivations following primary intrauterine infection (see Chapter 52). However, in some countries, such



Fig. 17.10 A *Nocardia* chorioretinal abscess. This abscess is in the macula of a patient on systemic immunosuppression following heart transplant.

Table 17.6 Parasitic causes of retinitis and uveitis*

Parasitic infection	Systemic infection	Features of retinitis or uveitis	Other ocular disease
Toxocara canis (see Fig. 17.11)	Children 6 months to 4 years of age; no extraocular infection	Usually unilateral; pale granulomatous mass or focal retinitis; traction bands; vitritis	No anterior segment inflammation
Toxoplasma gondii (see Figs 17.12, 17.13)	Usually acquired in utero but not evident systemically	Recurrent self-limiting attacks of chorioretinitis and vitritis ('headlight in the fog')	May be keratic precipitates (granulomatous reaction) in anterior chamber

*These ocular problems are seen in immunocompetent adults and children.

as Brazil, postnatally acquired infection is the more common antecedent of ocular toxoplasmosis. Postnatally acquired toxoplasmosis is followed by ocular toxoplasmosis in fewer than 5% of cases.

The lesions of ocular toxoplasmosis are found preferentially in the nerve fiber layer of the retina, but can affect any layer including the choroid.[30] Healed lesions of congenital toxoplasmosis are flat, atrophic, variably pigmented chorioretinal scars that have a propensity for the macular area of the fundus (Fig. 17.11). There may be recurrent bouts of uveitis, caused by the rupture of cysts, releasing trophozoites. Most patients have their first reactivation before 30 years of age and it has been estimated that recurrence occurs in 79% of patients.[31] Ophthalmoscopic examination shows vitritis and one or more white retinal lesions that are usually round or oval and up to 5 mm in diameter. There may be many such lesions surrounding an old scar (Fig. 17.12). Aggregates of vitreous cells over the active lesions are the rule. The appearance of the vitreous haze and the white granuloma has been likened to a 'headlight in the fog'. There may be retinal hemorrhages, sheathing of arterioles and venules, and papillitis. In the anterior segment, there may be a granulomatous reaction in the anterior chamber with keratic precipitates and conjunctival injection.

A diagnosis of ocular toxoplasmosis is based on the characteristic appearance of the lesions together with a positive serum antibody test for *T. gondii* (see Chapter 183). PCR of vitreous aspirates has also been utilized (see above). Other possible causes of localized necrotizing retinitis with vitritis, such as syphilis or tuberculosis, should be considered (Table 17.7).

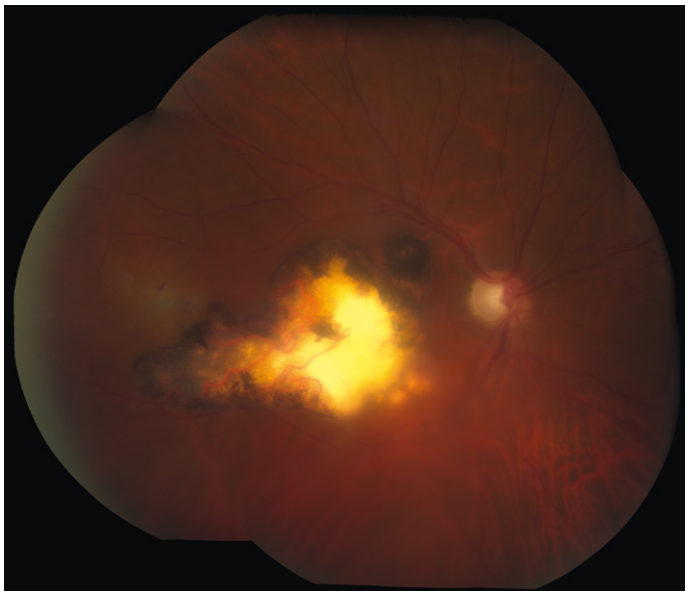


Fig. 17.12 A reactivated area of retinal toxoplasmosis. The lesion is the area of whitening in the macula of the right eye. Overlying vitritis creates the classic appearance of a 'headlight in the fog'.

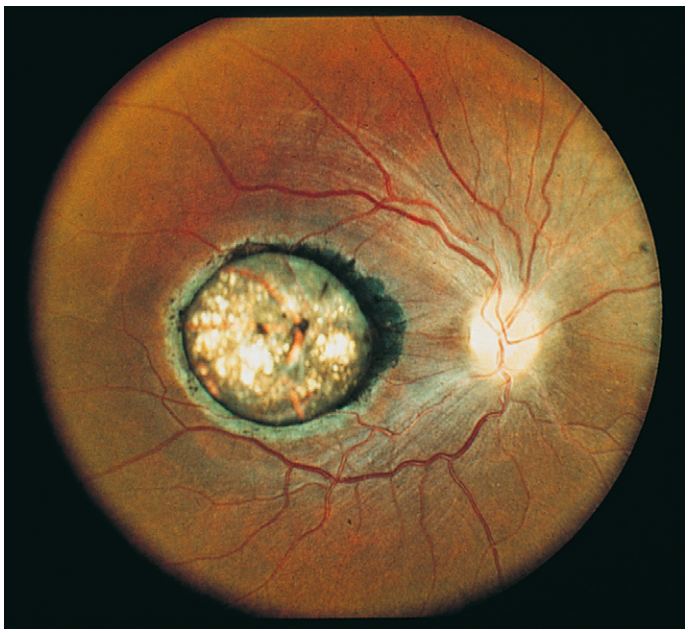


Fig. 17.11 An old, inactive congenital macular toxoplasmosis scar. The patient's vision was 20/400.

In otherwise healthy persons, flare-ups of ocular toxoplasmosis tend to be self-limited over a period of weeks to months. Not all active lesions need to be treated. The highest priority for treatment is for lesions that threaten the fovea, the optic nerve or large areas of the nerve fiber layer, as well as those that produce enough vitritis to impair vision. Once the fovea is directly involved, visual acuity is usually permanently compromised. Severe vision loss (visual acuity ≤20/200) in at least one eye has been described in up to 24% of ocular toxoplasmosis patients.[31]

There is no consensus among uveitis specialists on treatment because evidence is sparse. Typically, treatment is the same as for other systemic forms of toxoplasmosis – pyrimethamine, sulfadiazine and clindamycin (see Chapter 150), though the use of antiparasitics does not affect visual outcome.[31] Many ophthalmologists avoid pyrimethamine initially because of its marrow-suppressive effects, preferring to rely on the other two agents. Others have advocated the use of trimethoprim–sulfamethoxazole alone and in combination with clindamycin and suggested similar efficacy.[32] Corticosteroids are often used concomitantly, but not without the anti-infective drugs. Laser photocoagulation may be used for active lesions unresponsive to medication and pars plana vitrectomy may be used to clear vitreous opacities.

Ocular infection by *Toxocara canis*, the dog roundworm, occurs in young children, especially boys, aged from 6 months to 4 years (see Table 17.6). *Toxocara cati*, the cat roundworm, has also been implicated, but the organisms have never been positively identified

Table 17.7 Characteristics of some infections causing posterior uveitis/retinitis without extraocular manifestations

	Usual number of lesions	Appearance of lesions	Distribution of lesions	Accompanying vitritis
Toxoplasma gondii (see Figs 17.12, 17.13)	One to a few	'Headlight in fog'	Random, but heavier in macular area	Almost always
Toxocara canis (see Fig. 17.11)	One	Granuloma	Macula, optic disk or periphery	Always
Histoplasma capsulatum (see Fig. 17.9)	Multiple	Choroidal granulomas	Various	Almost never
Treponema pallidum	Diffuse retinochoroiditis	Bilateral anterior uveitis; large white geographic choroidal lesions	Random	Always
Borrelia burgdorferi	Focal or diffuse	Anterior uveitis, retinal vasculitis, exudative retinal detachment	Random	Usually
Mycobacterium tuberculosis	One or a few	Retinitis or chorioretinitis	Random	Usually

in the human eye. The typical presenting complaint is decreased vision, strabismus or leukocoria (a whitish lesion in the pupil). *Toxocara* infection is acquired by the ingestion of soil contaminated with embryonated eggs (see Chapter 184). The larvae are believed to reach the eye by hematogenous spread. The ocular findings are generally unilateral.

There are several ophthalmoscopic presentations of ocular toxocariasis (Fig. 17.13). The features they have in common are a whitish or yellowish retinal mass, representing a granuloma surrounding the larva, and the eventual formation of traction bands between the vitreous and the granuloma, which may result in retinal folds and exudative retinal detachment. There may be diffuse posterior uveitis, sometimes called nematode endophthalmitis. Unlike bacterial endophthalmitis, there is little pain and no anterior segment inflammation. Other presentations include localized retinitis affecting the macula or the peripheral retina, and acute optic neuritis, in which the granuloma overlies the optic disk. A macular star (neuroretinitis) may be seen as a result of leakage from vessels in the optic disk.

The major differential diagnoses of ocular toxocariasis are noninfectious diseases such as retinoblastoma and Coats' disease, a congenital vascular disorder. Retinoblastoma should always be considered and ruled out. Peripheral blood eosinophilia is rare in ocular toxocariasis. A serum ELISA antibody titer to the parasite of 1 to 8 or more supports the diagnosis. A positive ELISA titer of the intraocular fluid, which may be found even if the serum titer is negative, is highly indicative of the infection. Treatment is directed toward stemming the intraocular inflammation, clearing any media opacity and preventing permanent distortion in the retinal architecture. Corticosteroids, usually applied locally in the eye, and cataract and/or vitrectomy surgery are the mainstays of treatment.

Diffuse unilateral subacute neuroretinitis has been attributed to two nematodes which invade the subretinal space and induce inflammation, *Ancylostoma caninum* (definitive host is dog, found in the southeast USA) and *Baylisascaris procyonis* (definitive host is raccoon, found in the northwest USA). Early in the clinical course, vitritis, grayish outer retinal lesions and optic disk edema are noted. Later in the course of the disease the retina takes on a 'retinitis pigmentosa'-like appearance with pigment epithelial changes, arteriolar attenuation and optic nerve atrophy. In 25% of cases, a worm is visualized on clinical examination. The condition is treated by applying laser photocoagulation directly to the visualized nematode, which if achieved early may arrest the severe vision loss that results.

Rarely, uveitis occurs in other parasitic infections such as cysticercosis, myiasis and onchocerciasis.

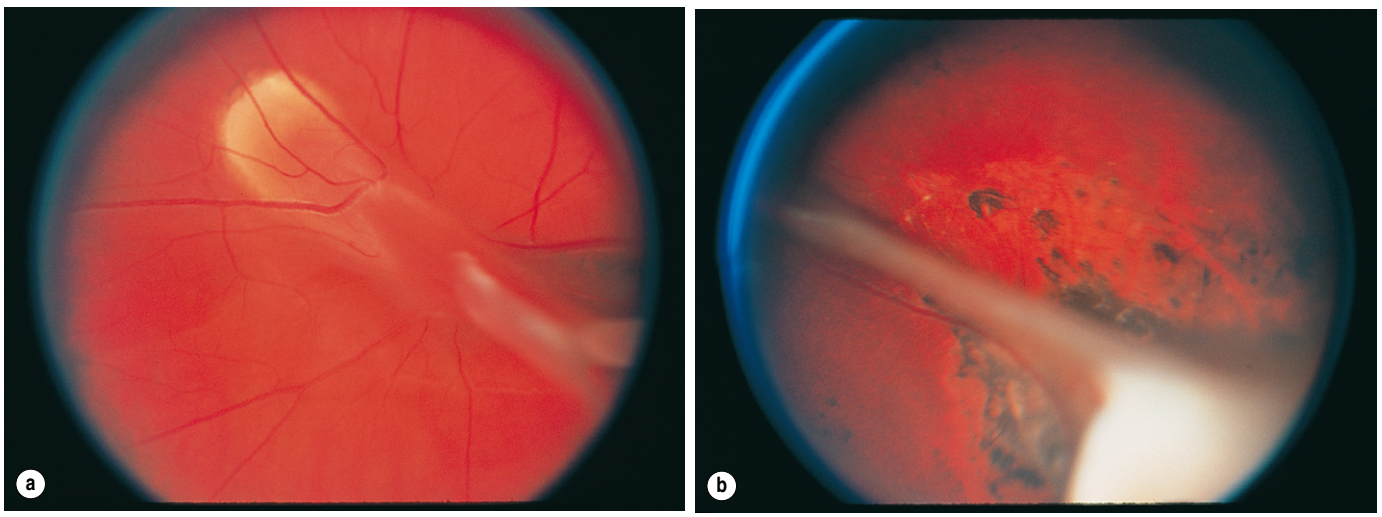


Fig. 17.13 Ocular toxocariasis. (a) The posterior pole of a left eye affected by toxocariasis. There is severe macular distortion and dragging of the retina toward a granuloma in the inferotemporal retinal periphery. (b) The periphery of the inferotemporal retina in the same eye showing the granuloma.

Table 17.8 Infectious retinitis and uveitis in neonates

	Systemic infection	Features of uveitis/retinitis	Other ocular disease
Viral infections			
Herpes simplex virus	Disseminated infection usually evident	Anterior uveitis early; optic neuritis, retinal hemorrhages and necrosis later; sparing of choroid	Conjunctivitis, keratitis common
Varicella-zoster virus	Features of congenital varicella-zoster (rare)	Chorioretinitis	Microphthalmia; cataracts
Cytomegalovirus (CMV)	Features of congenital CMV infection	Retinitis in 20–25% of infants with symptomatic CMV infection; resembles that seen in adults with AIDS	None
Rubella	Congenital rubella syndrome	Mild, self-limiting chorioretinitis & vitritis in 25–50% with congenital rubella syndrome; unilateral or bilateral; eventual salt-and-pepper fundus	None
Bacterial infections			
Treponema pallidum	Stigmata of congenital syphilis	Salt-and-pepper fundus; occasionally optic atrophy, retinal vascular sheathing	Delayed manifestation: acute interstitial keratitis (5–10 yrs)
Fungal infections			
Candida spp. (see Fig. 17.8)	Candida fungemia; risk factors	Whitish fluffy patch of retinitis, some vitritis	None
Parasitic infections			
Toxoplasma gondii (see Figs 17.12, 17.13)	Rarely, encephalitis, hydrocephalus at birth	Rarely, chorioretinitis at birth	None

Uveitis in neonates

There are several causes of uveitis in neonates (Table 17.8).

Herpes simplex virus

Congenital infection by HSV is usually acquired directly from the infected birth canal. Ocular findings may first appear from 1 week to several months after birth About 80% of isolates are HSV-2 and this strain is associated with more severe ocular infections.

Congenital HSV infection may cause a wide variety of ocular lesions. Conjunctivitis and keratitis are most common, and are usually seen in the acute phase of the infection. Posterior segment changes tend to occur later in the infection. Retinal involvement ranges from scattered hemorrhages to widespread retinal necrosis similar to that seen in the ARN syndrome. Residual changes include pigment migration and clumping (salt-and-pepper appearance), macular chorioretinal scars, optic atrophy and preretinal neovascularization. Systemic treatment is required.

Cytomegalovirus

Congenital infections by CMV are usually asymptomatic (see Chapter 52). With symptomatic infections, retinitis occurs in 20–25% of cases. If retinitis is not evident at birth, it is unlikely to occur later. The retinitis resembles that seen in adult patients with HIV infection (see below).

Rubella

In the congenital rubella syndrome, ocular manifestations occur in 25–50% of affected children. The classic finding is a mild, self-limited chorioretinitis that may be unilateral or bilateral. Vision is usually not impaired. A common sequela is a salt-and-pepper fundus caused by changes in the retinal pigment epithelium (see Fig. 17.5).

Syphilis

The most common ocular manifestation of congenital syphilis (see Chapter 57) is a salt-and-pepper fundus. The lesions are almost always bilateral, but may be sectoral or exclusively peripheral. They are the result of chorioretinitis, which may be evident at birth or appear in the first few years of life. Visual acuity is rarely affected. In more severe cases, there may be diffuse sheathing of the retinal vessels, optic atrophy and migration of the retinal pigment epithelium in a manner resembling retinitis pigmentosa. Acute interstitial keratitis is a delayed manifestation of congenital syphilis. It may be accompanied by anterior uveitis and secondary glaucoma may develop. The lesions of keratitis are self-limited, but treatment of the syphilitic infection is indicated. Hutchinson's triad in congenital syphilis consists of peg-shaped teeth, deafness and interstitial keratitis.

Other causes

Candida spp. may cause retinitis and vitritis in neonates. Risk factors are prematurity, low birth weight, sepsis, malnutrition and treatment with broad-spectrum antibiotics. The clinical presentation and treatment are the same as for adults (see Chapter 149).

Toxoplasma chorioretinitis is usually acquired in utero. Although the ocular complications usually present long after the neonatal period, they are sometimes evident at birth in the TORCH syndrome (see above, and Chapter 52). Manifestations include encephalitis, hydrocephalus and bilateral chorioretinitis. Treatment is described in Chapter 150.

Endogenous spiroplasma infection has also been described in neonates, typically manifesting as anterior uveitis and unilateral cataract.

Acute retinal necrosis syndrome

The ARN (acute necrotizing herpetic retinitis) syndrome is a recently described, rare syndrome of vaso-obliterative retinal and choroidal vasculitis, diffuse retinal necrosis and vitritis. It typically affects healthy immunocompetent people and has no predilection for race or sex. ARN is bilateral in one-third of patients (BARN), although the two eyes need not be affected simultaneously and involvement in the fellow eye may occur years later. Although reported in children as young as 8 years of age, it occurs most often in adults with a bimodal age distribution with peaks at 20 and 50 years of age. Conclusive evidence now exists that VZV is the primary cause; HSV is less common.[33] Patients infected with HIV who develop herpes zoster of the first division of the trigeminal nerve appear to have a high risk of subsequent ARN, often bilateral, over the next few weeks to months.[34]

Recent immunologic research suggests that specific human leukocyte antigens (HLAs) may predispose to ARN. Some 55% of Caucasian ARN patients were found to have the HLA-DQw7 antigen versus 19% of controls. More aggressive and fulminant disease has been associated with HLA-DR9.[35]

Acute retinal necrosis causes diffuse arteritis and phlebitis with sheathing of the retinal vessels and striking white areas of full thickness retinal necrosis (Fig. 17.14). Broad areas of the peripheral retina are involved early and the macula is usually spared initially. There is often mild inflammation of the anterior segment. As the disease progresses there is increasing vitritis. Optic neuropathy with disk edema may appear. Diagnosis is typically based on the characteristic clinical presentation though PCR of intraocular aspirates may be helpful.

Retinal detachment follows in up to 75% of patients. The detachments are notoriously difficult to repair. Previous prophylactic laser treatment of healthy retina posterior to the areas of necrosis reduces the incidence of retinal detachment.

The mainstay of treatment for ARN is aciclovir, given in high dosage intravenously (e.g. 12–15 mg/kg q8h for 7–14 days). Oral aciclovir or one of its prodrugs should be continued for at least 2–3 months to lessen the risk of fellow eye involvement. Systemic corticosteroids are employed to decrease inflammation, but never prior to the institution of antiviral therapy. Retinal detachments require complex vitreoretinal surgery for successful repair, often with silicone oil placement. It has been suggested that patients who have AIDS and who develop ophthalmic zoster should be given long-term oral prophylaxis with aciclovir, but there is no proof of benefit from this approach.[34]

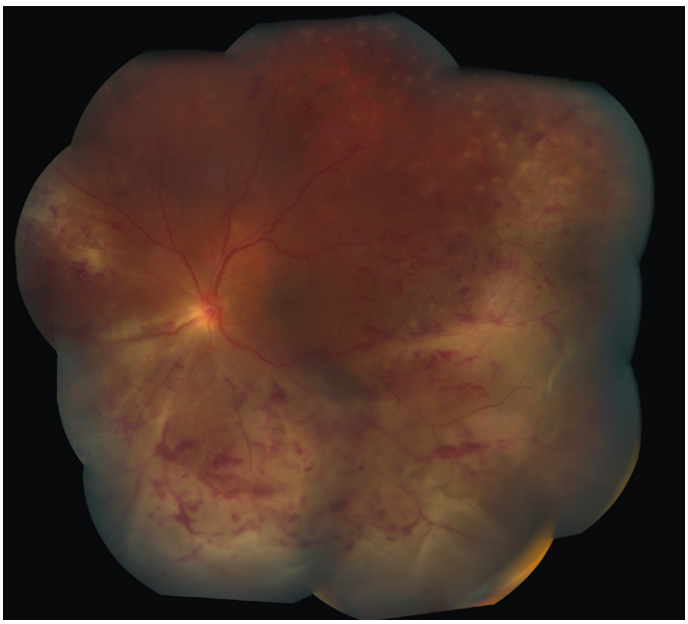


Fig. 17.14 Typical appearance of the retina in acute retinal necrosis syndrome. There is dense peripheral retinal whitening with a geographic border. Satellite lesions are common. The view is hazy owing to vitritis.

Uveitis and retinitis in immunosuppressed patients

Because of the AIDS pandemic, ophthalmologists encounter a variety of intraocular infections that were previously either unknown or extraordinarily rare.

Cytomegalovirus retinitis

The most common ocular infection in HIV-positive adult patients is CMV retinitis. In developed countries prior to 1996, CMV retinitis occurred in about 35% of adult patients who had AIDS. Since HAART has become widely available in developed countries, the incidence of CMV retinitis has dropped dramatically. CMV retinitis rarely occurs in patients who have $CD4^+$ lymphocyte counts over 50 cells/mm^3.[36] Pediatric patients who have AIDS have a much lower incidence of CMV retinitis than adults.

'Floaters' and blurred vision are the most common complaints, if any. Often, an active infection is noted incidentally on routine ophthalmologic screening examination. In 40% of patients with CMV retinitis, the lesions are bilateral at presentation. Nearly 75% of patients present to the ophthalmologist with disease that is considered immediately sight-threatening. Pain, redness or more than a mild anterior uveitis are unusual with CMV retinitis.

The hallmark lesion of CMV retinitis is a necrotizing, full-thickness retinitis resulting in retinal cell death (Fig. 17.15). Retinal tissue adjacent to major retinal blood vessels or the optic disk is often affected initially. The areas of active retinitis have a granular appearance and are dirty-white in color. Hemorrhage is common, owing to damage to vascular endothelial cells. The appearance has been likened to a 'brush fire' and to 'ketchup and cottage cheese'. The retinitis spreads contiguously as well as by producing satellite lesions. It is common to see areas of healed retinitis alongside areas of active necrosis. Areas of burned-out necrosis show an absence of retinal tissue while the underlying retinal pigment epithelium assumes a salt-and-pepper appearance.



Fig. 17.15 Cytomegalovirus infection with granular retinal whitening along the major blood vessels with mild hemorrhage. The view is clear because there is only mild vitritis.

Loss of vision occurs because of both the death of retinal cells and retinal detachment. About 10–20% of eyes with CMV retinitis can be expected to suffer a detached retina. The risk is time dependent; after 1 year the risk approaches 50%. Although vitreous surgery to repair the detached retinas is anatomically successful in more than 90% of instances, visual results are often limited by the underlying disease process.

Treatment of HIV-associated CMV retinitis is discussed in detail in Chapter 91. Immunologic improvement with antiretroviral therapy may result in an immune recovery uveitis in patients with prior or undiagnosed CMV disease (see Chapter 92). It has been hypothesized to be secondary to a reaction to CMV antigens typically occurring 2–16 weeks (median 4 weeks) following an increased CD4 count. Treatment with corticosteroids has not been shown to reactivate CMV retinitis.[37,38]

Other causes of uveitis and retinitis

About 75% of patients with HIV-1 infection have a non-sight-threatening retinopathy that may be caused by the HIV-1 itself.[39] The lesions consist of multiple, bilateral, cottonwool spots and scattered retinal hemorrhages (Fig. 17.16). If there is concern that such lesions may be due to CMV, close observation over a period of days to weeks with documentation by photographs is recommended.

Opportunistic infections with *Pneumocystis jirovecii*, *Histoplasma capsulatum*, *Cryptococcus neoformans* and atypical mycobacteria may produce multifocal choroiditis in patients who have AIDS. These infections are rarely sight threatening, but they should alert the clinician to the presence of a systemic infection. Unfortunately, it is generally not possible for the ophthalmologist to distinguish between these possible opportunistic infections on the basis of the eye examination alone.[39]



Fig. 17.16 HIV retinopathy. There are multiple superficial white patches in the retina (cottonwool spots). These do not affect vision and typically wax and wane over time.

Other important posterior segment infections that can occur in HIV-positive patients include toxoplasmosis, syphilis and infection with HSV and VZV. Whereas toxoplasmosis in immunocompetent patients produces a slowly progressive, focal, relapsing chorioretinitis, in immunocompromised patients it can produce a severe diffuse retinitis. Serologic testing may be helpful. Nearly one-third of patients who have AIDS and *Toxoplasma* retinitis have concurrent toxoplasmosis of the central nervous system. Ocular syphilis can also mimic CMV retinitis, but the lesions of secondary syphilis are usually a choroiditis, rather than a retinitis.

Acute retinal necrosis syndrome

The ARN syndrome (see above) occurs in both healthy and immunosuppressed people, including those with AIDS. Acute retinal necrosis differs from CMV retinitis in that the lesions of ARN are typically peripheral and the course is much more rapid. In addition, significant vitreous cells are a prominent feature of ARN, but not of CMV retinitis. Patients who have HIV infection and who develop zoster of the first division of the trigeminal nerve appear to have a high risk of subsequent ARN, which is often bilateral, over the next few weeks to months.[34] Therefore, when a rash of ophthalmic zoster appears, patients who have AIDS should be followed carefully for signs of ARN.

A specific type of rapidly progressive ARN is progressive outer retinal necrosis (PORN). PORN is secondary to VZV infection and has been described in patients who have AIDS and a low $CD4^+$ lymphocyte count.[40] Without aggressive therapy, PORN results in bilateral loss of vision within days to weeks. The recommended treatment is a combination of foscarnet and either ganciclovir or aciclovir, given intravenously or via intravitreal injection.

Perhaps the most common cause of noninfectious anterior uveitis in HIV-positive patients is a dose-related reaction to rifabutin. Patients receiving rifabutin for prophylaxis against *Mycobacterium avium-intracellulare* in conjunction with another agent that interferes with the metabolism of rifabutin (such as clarithromycin or ritonavir) and patients weighing less than 65 kg who are receiving 600 mg of rifabutin are at particular risk. The incidence of rifabutin-induced uveitis is 20% among AIDS patients taking the medication, but it has been reported in HIV-negative patients as well. Treatment with corticosteroids and discontinuation of the medication usually results in prompt reversal of the inflammation. Systemic cidofovir has also been reported to cause uveitis in patients who have AIDS.

REFERENCES

References for this chapter can be found online at http://www.expertconsult.com

Practice point 6

Michel Drancourt

Management of red eye

Red eye is a common sign of ocular infections (etiologies are presented in Chapters 15–17) and noninfectious conditions, some of which are vision-threatening etiologies requiring same-day referral to an ophthalmologist.

WHEN TO REFER THE PATIENT TO THE OPHTHALMOLOGIST?

Clinical interview and examination are required to identify the affected eye segment and select situations requiring same-day referral to an ophthalmologist (Table PP6.1). Such situations include a history of trauma and contact lens wear, severe ocular pain, impaired vision, foreign body sensation, corneal opacity or infiltrate at penlight examination and hypopyon (layer of white cells in the anterior chamber). In addition, unilateral red eye associated with nausea and vomiting, suggestive of acute angle-closure glaucoma, and masquerade syndromes due to malignant etiologies including B cell lymphoma and metastases of cancer such as breast cancer[1] (see Table PP6.1) should be referred.

WHICH OCULAR SEGMENT IS AFFECTED?

Conjunctivitis

Conjunctivitis is usually a benign, self-limited condition and probably the most common cause of red eye in the community setting. Conjunctivitis is responsible for diffuse or localized redness (episcleritis) in one or both eyes. Patients complain of morning crusting and daytime redness and discharge. Morning watery discharge of viral conjunctivitis contrasts with day-long purulent discharge in the course of bacterial conjunctivitis. Viral conjunctivitis, a self-limited infection, most often bilateral, typically caused by adenovirus, may be part of a viral prodrome followed by adenopathy, fever, pharyngitis and upper respiratory tract infection, or the eye infection may be the only manifestation of the disease.

Neisseria gonorrhoeae causes a hyperacute bacterial conjunctivitis that is severe and sight threatening, requiring immediate ophthalmologic referral. The eye infection is characterized by a profuse purulent discharge with redness, irritation and tenderness with chemosis, lid swelling, and tender preauricular adenopathy.

Conjunctivitis is the major clinical manifestation of active trachoma, a disorder that is largely limited to endemic areas in developing countries. Adult inclusion conjunctivitis is a chronic (weeks to months), indolent follicular conjunctivitis caused by certain serotypes of sexually transmitted *Chlamydia trachomatis*. Concurrent asymptomatic urogenital infection is typically present.

Table PP6.1 Clinical signs and symptoms indicating same-day referral to an ophthalmologist in patient with red eye

Medical history	Contact lens wearing Ocular surgery Ocular trauma
Symptoms	Nausea, vomiting Severe ocular pain Impaired vision Foreign body sensation
Signs	Corneal opacity Corneal infiltrate Hypopyon (white cells in the anterior chamber)

Keratitis

Keratitis is responsible for red eye along with photophobia and foreign body sensation. In addition, herpes simplex keratitis produces watery discharge. There may be a faint branching gray opacity on penlight examination, best visualized with application of fluorescein. Epidemic keratoconjunctivitis is a particularly fulminant cause of keratitis due to adenovirus types 8, 19 and 37. Multiple corneal infiltrates are barely visible with a penlight to the skilled observer but fluorescein staining reveals multiple punctate staining lesions.

Overnight wear of contact lenses is associated with a higher incidence of bacterial keratitis characterized by a corneal opacity or infiltrate (typically a round white spot) seen with a penlight in association with red eye, photophobia and foreign body sensation. Mucopurulent discharge is typically present. Fulminant cases may present with an associated hypopyon (layer of white cells in the anterior chamber). Amebal keratitis is also associated with contact lens wearing.[2]

Anterior uveitis

Anterior uveitis (or iritis) may be responsible for red eye (Fig. PP6.1; Table PP6.2). Patients with iritis may present in a similar fashion to those with an active corneal process but there is no foreign body sensation per se. The cardinal sign of iritis is ciliary flush: injection that gives the appearance of a red ring around the iris. Typically, there is no discharge and only minimal tearing. Patients with iritis should be referred to an ophthalmologist within a matter of days.

Toxoplasmosis is a surprisingly common cause of uveitis in the normal host, suspected on the basis of a very typical chorioretinal

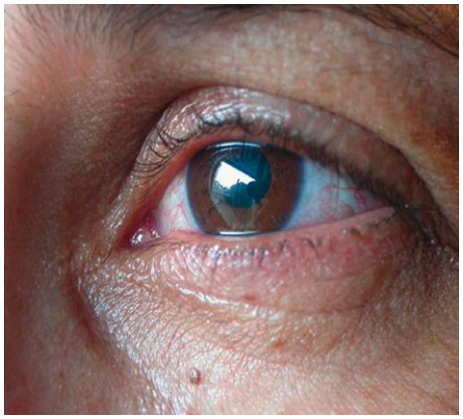


Fig. PP6.1 Red eye in a patient diagnosed with Whipple's disease uveitis.

Table PP6.2 Major groups of conditions responsible for the differential diagnosis of red eye

Aetiology	Associated clinical signs/symptoms
Acute angle-closure glaucoma	Nausea, vomiting
Conjunctivitis	
Viral	Morning – watery discharge
Bacterial	Fever, pharyngitis
Allergic	Day-long purulent discharge
	Watery discharge
Keratitis	
Viral	Photophobia
Bacterial	Impaired vision
Amebal	Foreign body sensation
	Corneal infiltrate
	Corneal opacity
	Hypopion
Anterior uveitis	
Viral	
Bacterial	
Parasite	
Masquerade syndromes	
Mycoses	

lesion. Most chorioretinal scarring from toxoplasmosis is due to infection during gestation, but scarring is increasingly being recognized as a result of primary infections as well. Other etiologies recognized after extensive investigations include viruses and fastidious bacteria of spirochetes, *Borrelia* and *Bartonella* spp.[3]

WHEN TO PERFORM MICROBIOLOGICAL SAMPLING?

The question then arises of when, how and which specimens to sample? Cultures are not necessary for the initial diagnosis and therapy of conjunctivitis except for patients with symptoms of hyperacute conjunctivitis in whom Giemsa and Gram stains may be helpful to identify Gram-negative diplococci suggestive of *Neisseria gonorrhoeae*. A point-of-care rapid test for adenoviral conjunctivitis has been favorably evaluated by modeled cost-effectiveness analysis.[4] The diagnosis of adult inclusion conjunctivitis can be confirmed with Giemsa or direct fluorescent antibody staining of conjunctival smears, by culture or by polymerase chain reaction (PCR) of swabbed specimens.

Keratitis lesions are sampled for microbiologic diagnosis based on culture and PCR-based detection of major pathogens including herpesvirus, bacteria and amebae.

In patients with uveitis of unknown etiology which is bilateral, recurrent, sight threatening or nonresponsive to therapy will require extensive evaluation for etiology. Vitreous humor should be sampled to exclude ocular lymphoma by cytology and interleukin 10 measurements and to search for selected pathogens (see below).

WHEN TO PROVIDE ANTI-INFECTIOUS TREATMENT?

Viral conjunctivitis is a highly contagious, self-limited process for which there is no specific antiviral agent.

Bacterial conjunctivitis is also likely to be self-limited in most patients, although treatment probably shortens the clinical course and reduces person-to-person spread of this highly contagious condition. Appropriate choices for bacterial conjunctivitis include erythromycin ophthalmic ointment, sulfa ophthalmic drops or polymyxin/trimethoprim drops. These agents cover the most common pathogens responsible for bacterial conjunctivitis, and patients should respond to this treatment within 1–2 days. Patients who do not respond should be referred to an ophthalmologist. The fluoroquinolones are effective and well tolerated; they are the treatment of choice for corneal ulcers and are extremely effective against *Pseudomonas*. However, fluoroquinolones are not first-line therapy for routine cases of bacterial conjunctivitis because of concerns regarding emerging resistance and cost. The exception is conjunctivitis in a contact lens wearer; once keratitis has been ruled out, it is reasonable to treat these individuals with a fluoroquinolone due to the high incidence of *Pseudomonas* infection.[5] A contact lens wearer with a red eye should discontinue contact lens wear. If the diagnosis is conjunctivitis, contact lens wear can resume when the eye is white and has no discharge for 24 hours after the completion of antibiotic therapy. The lens case should be discarded and the lenses subjected to overnight disinfection or replaced if disposable. Systemic therapy with doxycycline, tetracycline, erythromycin or azithromycin is required to eradicate adult inclusion conjunctivitis.

The duration of symptoms in patients with herpes simplex keratitis is reduced with treatment with topical or oral antiviral agents. Immunocompromised patients may require topical and systemic treatment, and longer duration of therapy. A small percentage of patients develop chronic or recurrent inflammation, or recurrent viral keratitis, both of which are treated with prophylactic oral antiviral agents. Some patients also benefit from treatment with topical corticosteroid agents, used in conjunction with antiviral prophylaxis. Fortified topical antibiotics and fluoroquinolones are still the mainstay of bacterial keratitis therapy, and new antimycotic agents such as voriconazole could be used for treating amebal keratitis.

Specific anti-infectious treatment will be prescribed in addition to steroids in cases of uveitis, in collaboration between the ophthalmologist and the infectious disease/clinical microbiology practitioner.

REFERENCES

References for this chapter can be found online at http://www.expertconsult.com

Chapter 18

Diederik van de Beek

Acute and chronic meningitis

INTRODUCTION

Meningitis is an infection of the meninges and subarachnoid space, but can also involve the cortex and brain parenchyma (meningoencephalitis) because of the close anatomic relation between the cerebrospinal fluid (CSF) and the brain. Inflammatory involvement of the subarachnoid space with meningeal irritation leads to the classic triad of headache, fever and meningism, and to a pleocytosis in the CSF. Involvement of the brain cortex and parenchyma may result in behavioral changes, focal neurologic abnormalities and impairment of consciousness. Although these latter signs are classically considered to be encephalitis, the meningitis and encephalitis should rather be seen as a continuum. Thus, although somewhat artificial, this chapter will be limited to those infections in which the meninges are inflamed. For a treatise of encephalitis the reader is referred to Chapter 19.

Acute Bacterial Meningitis

EPIDEMIOLOGY

The incidence of acute bacterial meningitis is 5–10/100 000 persons per year in high-income countries, resulting in 15 000–25 000 cases in the USA annually.[1,2] Vaccination strategies have substantially changed the epidemiology of community-acquired bacterial meningitis during the past two decades. The routine vaccination of children against *Haemophilus influenzae* type b has virtually eradicated *H. influenzae* meningitis in the developed world.[1] As a consequence, *Streptococcus pneumoniae* has become the most common pathogen beyond the neonatal period and bacterial meningitis has become a disease predominantly of adults. The introduction of conjugate vaccines against seven serotypes of *S. pneumoniae* that are among the most prevalent in children aged 6 months to 2 years has reduced the rate of invasive pneumococcal infections in young children and in older persons.[1] The integration of the meningococcal protein–polysaccharide conjugate vaccines into vaccination programs in several countries further reduced the disease burden of bacterial meningitis in high- and medium-income countries.[3]

Streptococcus pneumoniae affects all ages and causes the most severe disease in the very young and the very old.[4] Of the more than 90 pneumococcal serotypes, a few dominate as the causes of meningitis. The increase of drug-resistant strains of *S. pneumoniae* is an emerging problem worldwide. The prevalence of antibiotic-resistant strains in some parts of the USA is as high as 50–70% with important consequences for treatment.[5]

Neisseria meningitidis is mainly responsible for bacterial meningitis in young adults; it causes sporadic cases and epidemics.[6] Its incidence shows a peak in winter and early spring and varies greatly around the world. Small outbreaks typically occur in young adults living in close quarters, such as dormitories of military camps or schools. Major epidemics have occurred periodically in sub-Saharan Africa (the so-called 'meningitis belt'), Europe, Asia and South America. During these epidemics, attack rates can reach several hundred per 100 000, with devastating consequences.

The group B streptococcus (*S. agalactiae*) is a pathogen of neonates and often causes a devastating sepsis and meningitis.[7] It colonizes the maternal birth canal and is transmitted to the child. The colonized newborn can develop group B streptococcal disease of early onset (developing at less than 7 days of age; median 1 day) or late onset (developing later than 7 days of age).

Listeria monocytogenes causes meningitis preferentially in neonates, in adults with alcoholism, immunosuppression or iron overload, and in pregnant women.[8] There is often an encephalitic component to presentation, with early mental status alterations, neurologic deficits and seizures.

Haemophilus influenzae causes meningitis in young children. In countries with routine vaccination against *H. influenzae* type b it has become a rare disease.[9]

Bacterial meningitis also occurs in hospitalized patients ('physician associated meningitis' or 'nosocomial meningitis'). In a large city hospital, almost 40% of cases may be nosocomial.[10] Most cases occur in patients undergoing neurosurgical procedures, including implanting of neurosurgical devices, and in patients with focal infections of the head. The organisms causing nosocomial meningitis differ markedly from those causing community-acquired meningitis and include Gram-negative rods (*Escherichia coli*, *Klebsiella* spp., *Pseudomonas aeruginosa*, *Acinetobacter* spp., *Enterobacter* spp. and others), staphylococci and streptococci other than *S. pneumoniae*.[11,12]

PATHOPHYSIOLOGY AND PATHOLOGY

Specific bacterial virulence factors for meningeal pathogens include specialized surface components that are crucial for adherence to the nasopharyngeal epithelium, the evasion of local host defense mechanisms and subsequent invasion of the bloodstream (Fig. 18.1).[13] In pneumococcal disease, presence of the polymeric immunoglobulin A receptor on human mucosa, which binds to a major pneumococcal adhesin, CbpA, correlates with the ability of pneumococci to invade the mucosal barrier. Viral infection of the respiratory tract may also promote invasive disease.[4] From the nasopharyngeal surface, encapsulated organisms cross the epithelial cell layer and invade the small subepithelial blood vessels. Binding of bacteria to upregulated receptors (e.g. platelet activating-factor receptors) promotes migration through the respiratory epithelium and vascular endothelium, resulting in invasive disease.

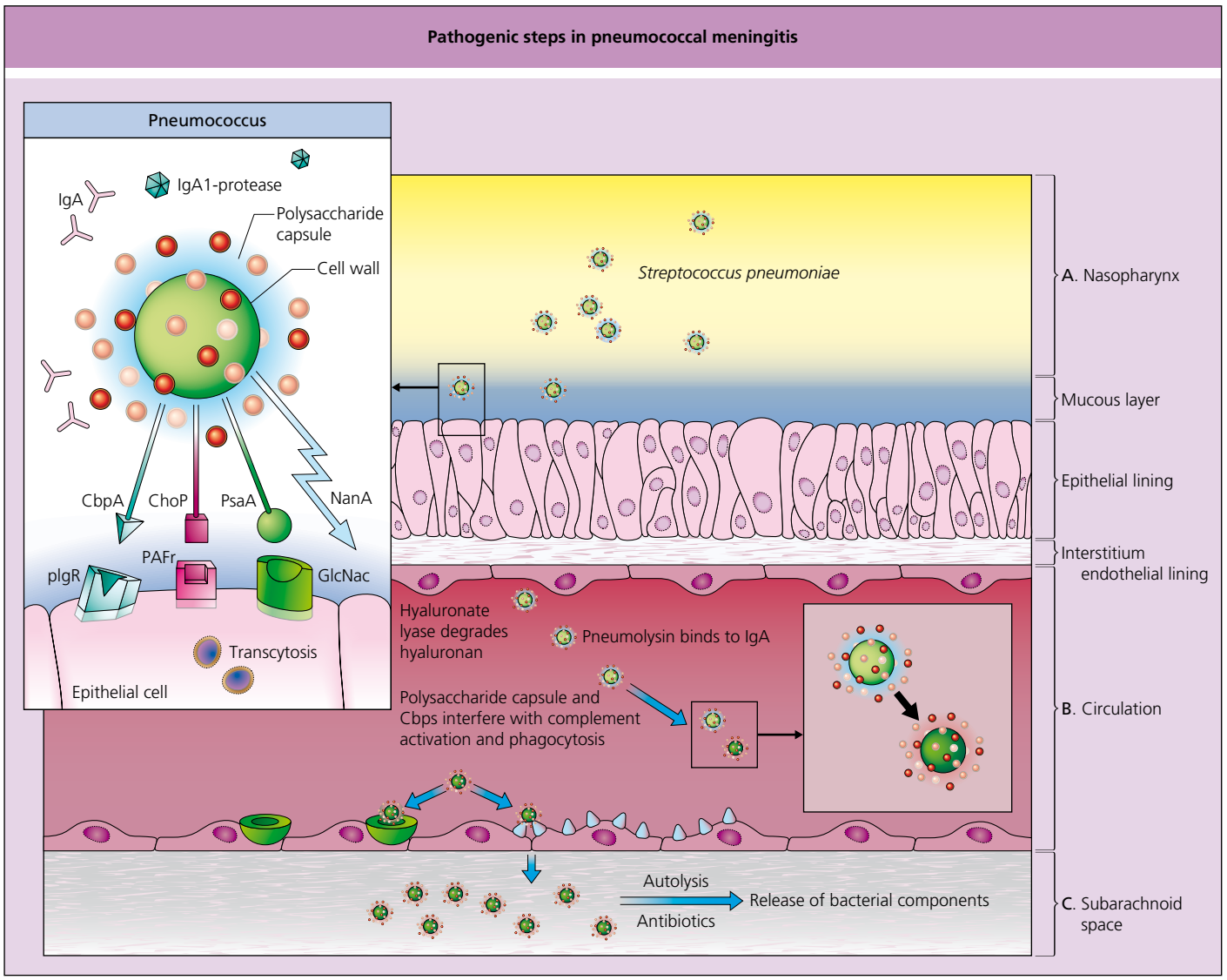


Fig. 18.1 Pathogenic steps in pneumococcal meningitis. Neuraminidase (NanA) decreases viscosity of the mucus and exposes *N*-acetyl-glucosamine (GlcNAc)-receptors on epithelial cells, which interact with pneumococcal surface-associated proteins (such as PsaA). IgA1 proteases cleave opsonizing host IgA. Cytokines stimulate cell-surface expression of platelet-activating-factor receptors (PAFr). Binding to PAFr via cell-wall phosphocholine (ChoP) induces internalization and migration (transcytosis) of pneumococci through the epithelial barrier, which is facilitated by binding of choline-binding protein A (CbpA) to the polymeric Ig-receptor (pIgR). After penetrating the mucosal epithelium, hyaluronate lyase facilitates bacterial spreading by degrading hyaluronan, an important component of the interstitial matrix. Once in the bloodstream the polysaccharide capsule and Cbps enhance intravascular survival by interfering with activation of the complement cascades and phagocytosis. Pneumolysin reduces serum opsonic activity by binding to IgG. By changing the composition of surface components (phase variation) pneumococci adapt to the host environmental conditions and migration through the blood–brain barrier. Attachment to endothelial cells occurs to several glycoconjugates. Activated endothelial cells upregulate PAFr and thereby enable pneumococci (mainly transparent phase variants) to invade the subarachnoid space via transcytosis. The poor local host defenses in the subarachnoid space facilitate multiplication of bacteria, which undergo autolysis (mediated by autolysins or antibiotics) and release bacterial compounds. Reproduced with permission from Weisfelt *et al.*[4]

In the bloodstream, bacteria must survive host defenses, including circulating antibodies, complement-mediated bactericidal mechanisms and neutrophil phagocytosis. Encapsulation is a shared feature of the principal hematogenous meningeal pathogens. To survive the various host conditions they encounter during infection, pneumococci undergo spontaneous and reversible phase variation, which involves changes in the amount of important surface components.[4,14] The capsule is instrumental in inhibiting neutrophil phagocytosis and complement-mediated bactericidal activity. Several defense mechanisms counteract the antiphagocytic activity of the bacterial capsule. Activation of the alternative complement pathway results in cleavage of C3 with subsequent deposition of C3b on the bacterial surface, thereby facilitating opsonization, phagocytosis and intravascular clearance of the organism.[15] Impairment of the alternative complement pathway occurs in patients with sickle-cell disease and those who have undergone splenectomy, and these groups of patients are predisposed to the development of pneumococcal meningitis. Functional deficiencies of several components involved in the activation and function of complement-mediated defenses have been identified (i.e. mannose-binding lectin, properdin, lack of terminal complement components), which increase the susceptibility for invasive meningococcal infections.[16]

The blood–brain barrier is formed by cerebromicrovascular endothelial cells, which restrict blood-borne pathogen invasion. Cerebral capillaries, as opposed to other systemic capillaries, have adjacent endothelial cells fused together by tight junctions that prevent intercellular transport.[17] Bacteria are thought to invade the subarachnoid space via transcytosis. Nonhematogenous invasion of the CSF by

bacteria occurs in situations of compromised integrity of the barriers surrounding the brain. Direct communication between the subarachnoid space and the skin or mucosal surfaces as a result of malformation or trauma gives rise to meningeal infection. Bacteria can also reach the CSF as a complication of neurosurgery, spinal anesthesia or ventriculostomy placement.

Physiologically, concentrations of leukocytes, antibodies and complement components in the subarachnoid space are low, which facilitates multiplication of bacteria. Pneumococcal cell-wall products, pneumolysin and bacterial DNA induce a severe inflammatory response via binding to Toll-like receptor (TLR)-2.[18] Once engaged, this signaling receptor transmits the activating signal into the cell, which initiates the induction of inflammatory cytokines.[19] Endotoxin is a major component of the outer membrane of the meningococcus and is crucial in the pathogenesis of sepsis and meningitis.[6] The host responds to bacterial endotoxin with proinflammatory gene expression and activation of coagulation pathways.

The subarachnoid inflammatory response is accompanied by production of multiple mediators in the central nervous system (CNS). Tumor necrosis factor (TNF), interleukin 1β and interleukin 6 are regarded as the major early response cytokines that trigger the inflammatory cascade, which induces various pathophysiologic alterations implicated in pneumococcal meningitis (Fig. 18.2).[13] TNF and interleukin 1β stimulate the expression of chemokines and adhesion molecules, which play an important part in the influx of leukocytes from the circulation to the CSF. Upon stimulation with bacterial components, macrophages and granulocytes release a broad range of potentially tissue-destructive agents, which contribute to vasospasm and vasculitis, including oxidants (e.g. peroxynitrite) and proteolytic enzymes such as matrix metalloproteinases.[20] Matrix metalloproteinases, zinc-dependent enzymes produced as part of the immune response to bacteria that degrade extracellular matrix proteins, also contribute to the increased permeability of the blood–brain barrier.

The major element leading to increased intracranial pressure in bacterial meningitis is the development of cerebral edema, which may be vasogenic, cytotoxic or interstitial in origin. Vasogenic cerebral edema is a consequence of increased blood–brain barrier permeability.[21] Cytotoxic edema results from an increase in intracellular water following alterations of the cell membrane and loss of cellular homeostasis. Cytotoxic mechanisms include ischemia and the effect of excitatory amino acids. Secretion of antidiuretic hormone also contributes to cytotoxic edema by making the extracellular fluid hypotonic and increasing the permeability of the brain to water. Interstitial edema occurs by an increase in CSF volume, either through increased CSF production via increased blood flow in the choroid plexus, or decreased resorption secondary to increased CSF outflow resistance.

The exact mechanisms that lead to permanent brain injury are incompletely understood. Cerebral ischemic necrosis probably contributes to damage to the cerebral cortex (see Fig. 18.2). Cerebrovascular complications occur in 15–20% of patients with bacterial meningitis.[22] Other abnormalities include subdural effusion or empyema, septic sinus thrombosis, subarachnoid hematomas, compression of intracranial structures due to intracranial hypertension, and herniation of the temporal lobes or cerebellum. Gross changes, such as pressure coning, are rare.[23]

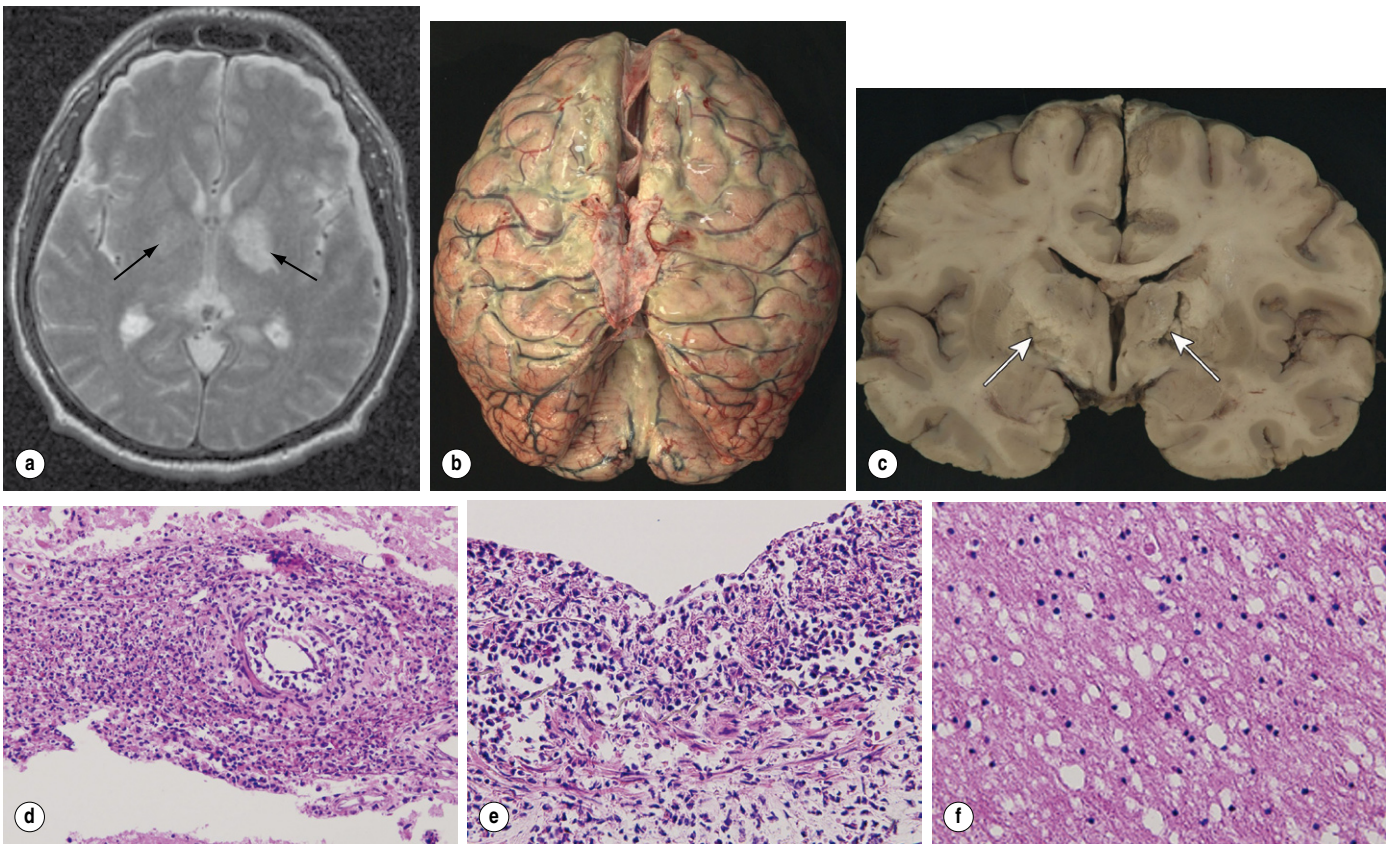


Fig. 18.2 Multiple complications in a patient with pneumococcal meningitis. (a) T2-proton-density-weighted MRI of the brain shows a transverse view of a hyperintense signal (arrows) in the basal ganglia that indicates bilateral oedema. (b) A post-mortem view of the brain of the same patient shows yellowish-coloured meninges as a result of extensive inflammation. (c) Confirmation of the bilateral infarction of the basal ganglia (arrows). The microscopic substrate in the same patient shows a meningeal artery with (d) lymphocytic infiltration in and around the vessel wall, (e) extensive subpial necrotizing cortical inflammation, and (f) edema in the white matter. Reproduced with permission from van de Beek *et al.*[21]

There is diffuse acute inflammation of the pia-arachnoid, with migration of neutrophil leukocytes and exudation of fibrin into the CSF. Pus accumulates over the surface of the brain, especially around its base and the emerging cranial nerves, and around the spinal cord. The meningeal vessels are dilated and congested and may be surrounded by pus (Fig. 18.3). Pus and fibrin are found in the ventricles and there is ventriculitis, with loss of ependymal lining and subependymal gliosis. Infection may block CSF circulation, causing obstructive hydrocephalus or spinal block. In many cases death may be attributable to related septicemia, although bilateral adrenal hemorrhage (Waterhouse–Friederichsen syndrome) may well be a terminal phenomenon rather than a cause of fatal adrenal insufficiency as was once imagined. Patients with meningococcal septicemia may develop acute pulmonary edema.

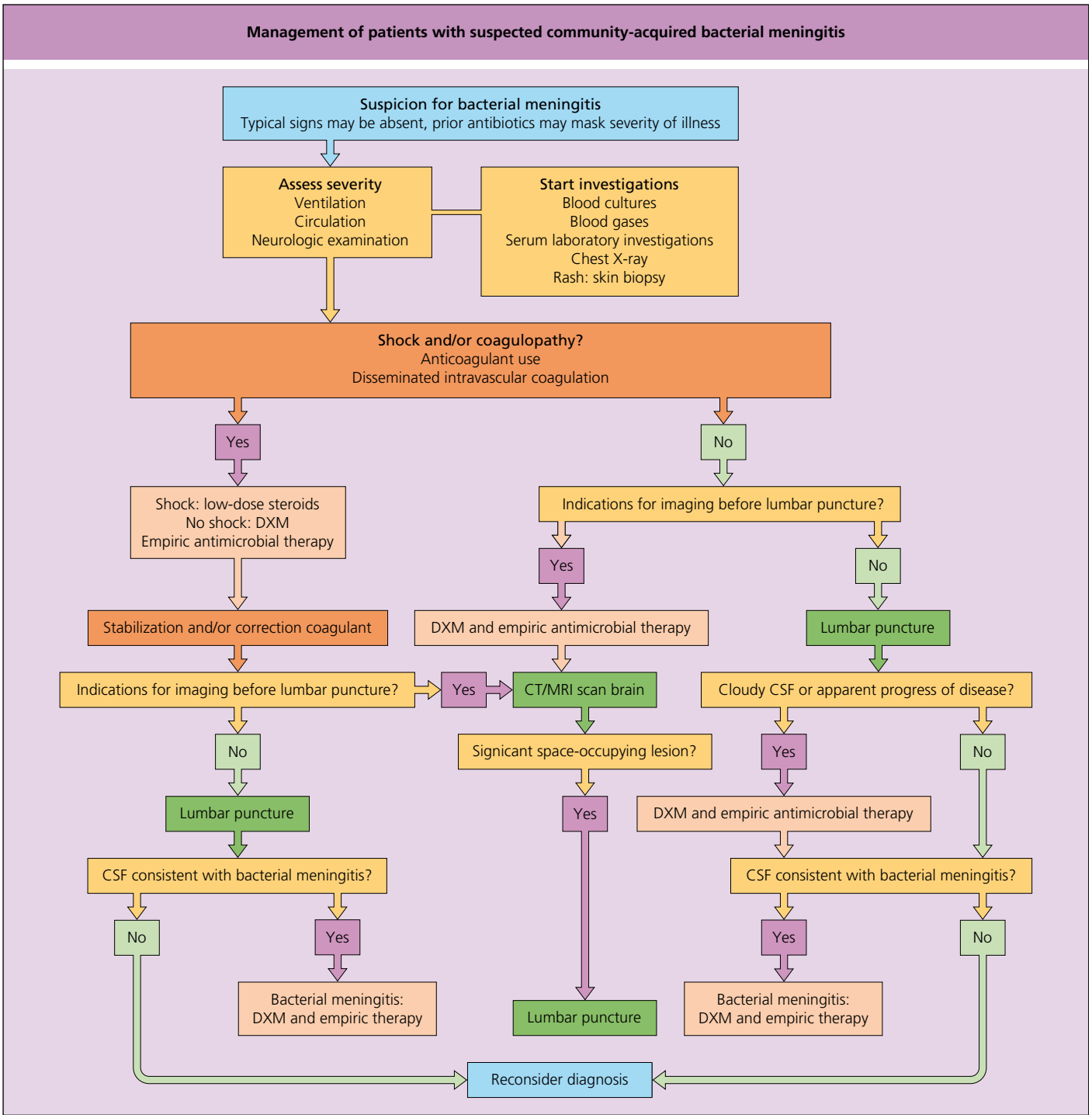


Fig. 18.3 Algorithm for the management of patients with suspected community-acquired bacterial meningitis. CSF, cerebrospinal fluid; DXM, dexamethasone. This material was previously published by van de Beek et al as part of an online supplementary appendix of reference 22. Copyright 2006 Massachusetts Medical Society. All rights reserved.

CLINICAL FEATURES

The clinical presentation of a patient with bacterial meningitis may vary depending on age, underlying conditions and severity of illness.[7,22] Clinical findings of meningitis in young children are often minimal, and in childhood bacterial meningitis and in elderly patients classic symptoms such as headache, fever, nuchal rigidity and altered mental status may be less common than in younger and middle-aged adults. Infants may become irritable or lethargic, stop feeding, and are found to have a bulging fontanel, separation of the cranial sutures, meningism and opisthotonos, and they may develop convulsions.

A meta-analysis of 845 patients aged over 30 years showed poor sensitivity and specificity for symptoms predicting community-acquired bacterial meningitis such as headache, nausea and vomiting.[24] A prospective study of 696 adults with community-acquired bacterial meningitis found an incidence of 44% for the classic triad of fever, neck stiffness and change in mental status (Glasgow Coma Scale ≤14).[2] However, 95% of patients with culture-proven bacterial meningitis presented with at least two signs or symptoms of headache, fever, neck stiffness and alterations in mental status. Purpuric rash is one of the hallmarks of meningococcal disease, but can be subtle.[6]

At physical examination, Kernig's sign, Brudzinski's sign and meningismus lack adequate sensitivity to be used in isolation to exclude bacterial meningitis. In a prospective study of 297 adults, Kernig's sign and Brudzinski's sign had poor sensitivity (5%) with high specificity (95%) in predicting meningitis.[25] Naturally, physicians do not rely on a single test for diagnosis and combine a number of historical and physical examination findings to form a clinical impression.

DIAGNOSIS AND MANAGEMENT

Given the high mortality of acute bacterial meningitis, starting treatment and completing the diagnostic process should be carried out simultaneously in most cases. The first step is to evaluate vital functions, obtain two sets of blood cultures, and start antimicrobial therapy and adjunctive dexamethasone when indicated (Fig. 18.3). At the same time, the severity of the patient's condition and the level of suspicion for the presence of bacterial meningitis should be determined. Performance and contraindications of lumbar puncture are covered in Practice Point 6.

Retrospective studies suggest a link between delay in the administration of antimicrobial therapy and adverse outcome.[23] In a prospective study involving 156 patients with pneumococcal meningitis who were admitted to the intensive care unit, a delay of more than 3 hours after presentation to the hospital before the initiation of antimicrobial therapy was associated with an increased 3-month mortality.[26]

In patients with suspected bacterial meningitis in whom lumbar puncture is postponed because of coagulation disorders (e.g. disseminated intravascular coagulation) or severe septic shock, or in whom cranial imaging is indicated before lumbar puncture, blood cultures should be drawn and antimicrobial therapy should be initiated without delay (see Fig. 18.3). In patients who have not undergone prior imaging and in whom disease progression is apparent, therapy should be started directly after lumbar puncture, as well as in all patients with cloudy CSF (suggestive of bacterial meningitis). In patients without apparent progress of disease and no cloudy CSF on lumbar puncture, antimicrobial therapy should be initiated after early CSF analysis (e.g. Gram stain, leukocyte count) confirms the diagnosis.

CSF analysis

When CSF analysis shows increased white blood cell counts, confirming a diagnosis of meningitis, many clinicians would like to determine who has life-threatening bacterial meningitis and who the less concerning viral meningitis. The CSF abnormalities of bacterial meningitis include raised opening pressure in almost all patients, polymorphonuclear leukocytosis, decreased glucose concentration and increased protein concentration. In bacterial meningitis, the white blood cell count is typically >1000 cells/μl, while in viral meningitis it is <300 cells/μl, although there is considerable overlap.[23] The neutrophil count is higher in bacterial than in viral meningitis. More than 90% of cases present with CSF white cell counts of >100/μl. In immunocompromised patients, CSF white blood cell counts may be lower, although acellular CSF is probably rare, except in patients with tuberculous meningitis.[27] The normal CSF glucose concentration is between 2.5 and 4.4 mmol/l which is approximately 65% of the serum glucose. In bacterial meningitis the glucose concentration is usually <2.5 mmol/l or <40% of the serum glucose. The CSF protein in bacterial meningitis is usually >50 mg/dl.[2,28]

Gram stain is positive in identifying the organism in 50–90% of cases and CSF culture is positive in 80% of untreated patients.[2,28] Gram staining of CSF permits the rapid identification of the causative organism (sensitivity 60–90%; specificity >97%).[22] Latex particle agglutination tests that detect antigens of *N. meningitidis*, *S. pneumoniae*, *H. influenzae* and *S. agalactiae* can provide diagnostic confirmation but are not routinely available. Increasingly, laboratories are offering broad-range polymerase chain reaction (PCR) that can detect small numbers of viable and nonviable organisms in CSF. When the broad-range PCR is positive, a PCR that uses specific bacterial primers to detect the relevant pathogens can be done. However, although these tools are promising, further refinements are needed before PCR can be routinely recommended.

Skin biopsy

Microbiologic examination of skin lesions is routine diagnostic workup in patients with suspected meningococcal infection. It differentiates well between meningitis with and without hemodynamic complications and the result is not affected by previous antibiotic treatment.[22,29,30]

Antibiotic treatment

Empiric antibiotic treatment should be based on the most common bacterial species that cause the disease according to the patient's age or clinical setting and on antibiotic susceptibility patterns of the pathogens. Neonatal meningitis is largely caused by group B streptococci, *E. coli*, and *L. monocytogenes*.[7] Initial treatment, therefore, should consist of penicillin or ampicillin plus a third-generation cephalosporin (cefotaxime or ceftriaxone), or penicillin or ampicillin and an aminoglycoside (Table 18.1).[31,32]

Due to the emergence of multidrug-resistant strains of *S. pneumoniae*, vancomycin is often added to the initial empiric antimicrobial regimen in adult patients. Although intermediate penicillin resistance is common in some countries, the clinical importance of penicillin resistance in the meningococcus has yet to be established. In countries with low rates of pneumococcal penicillin resistance (such as The Netherlands),[33] penicillin is still recommended as a first-line agent. In the UK, the addition of vancomycin is also not considered necessary and is not recommended unless the patient presents from one of the geographic regions associated with high-level ceftriaxone resistance, such as Spain, southern Africa and the USA. In addition, in patients aged >50 years, treatment with ampicillin should be added to the above antibiotic regimen for additional coverage of *L. monocytogenes*, which is more prevalent among this age group.[22]

Recommendations directed at common specific organisms in adults are described below and general recommendations based on the isolated micro-organism are listed in Table 18.2.

Antibiotic prophylaxis

Prophylaxis is indicated for intimate contact of patients with meningococcal meningitis, which covers those eating and sleeping in the

Table 18.1 Recommendations for empiric antimicrobial therapy in suspected community-acquired bacterial meningitis

Predisposing factor	Common bacterial pathogens	Initial intravenous antibiotic therapy
Age		
<1 month	*Streptococcus agalactiae, Escherichia coli, Listeria monocytogenes*	Ampicillin plus cefotaxime or an aminoglycoside
1–3 months	*Streptococcus pneumoniae, Neisseria meningitidis, S. agalactiae, Haemophilus influenzae, E. coli, L. monocytogenes*	Ampicillin plus vancomycin plus ceftriaxone or cefotaxime*
3–23 months	*S. pneumoniae, N. meningitidis, S. agalactiae, H. influenzae, E. coli*	Vancomycin plus ceftriaxone or cefotaxime*
2–50 years	*N. meningitidis, S. pneumoniae*	Vancomycin plus ceftriaxone or cefotaxime*
>50 years	*N. meningitidis, S. pneumoniae, L. monocytogenes,* aerobic Gram-negative bacilli	Vancomycin plus ceftriaxone or cefotaxime plus ampicillin†
With risk factor present‡	*S. pneumoniae, L. monocytogenes, H. influenzae*	Vancomycin plus ceftriaxone or cefotaxime plus ampicillin†

*In areas with very low penicillin-resistance rates monotherapy penicillin may be considered.
†In areas with very low penicillin-resistance and cephalosporin-resistance rates, combination therapy of amoxicillin and third-generation cephalosporin may be considered.
‡Alcoholism, altered immune status.
This material was published previously as part of an online supplementary appendix to reference 22. Copyright 2006 Massachusetts Medical Society. All rights reserved.

same dwelling as well as those having close social and kissing contacts, or health-care workers who perform mouth-to-mouth resuscitation, endotracheal intubation or endotracheal tube management.[22] Patients with meningococcal meningitis treated with monotherapy of penicillin or ampicillin should also receive chemoprophylaxis, since carriage is not reliably eradicated by these drugs. Recommendations for prophylactic therapy are listed is Table 18.2.

Adjunctive dexamethasone treatment

Animal models of bacterial meningitis showed that bacterial lysis, induced by antibiotics, leads to subarachnoid inflammation (Fig. 18.4). The severity can be attenuated by treatment with steroids. Several trials were done to assess adjunctive steroids in bacterial meningitis. Dexamethasone is a glucocorticosteroid with excellent penetration in the CSF. In a meta-analysis of randomized trials dexamethasone reduced meningitis-associated hearing loss in children with influenzal meningitis.[34]

Most available studies on dexamethasone therapy in adults with bacterial meningitis suffered from methodologic flaws. In 2002, a European randomized controlled trial showed that dexamethasone, given before or with the first dose of antibiotics, met with a 0.59 relative risk of unfavorable outcome in adults with bacterial meningitis and 0.48 relative risk of mortality. This effect was most apparent in pneumococcal meningitis, in which mortality was decreased from 34% to 14%.[35] The benefits were not undermined by an increase of severe neurologic disability in survivors or by corticosteroid-induced complications. In a post-hoc analysis of pneumococcal meningitis patients who died less than 4 days after admission, the mortality benefit was due to reduced mortality from septic shock, pneumonia or acute respiratory distress syndrome; there was no significant reduction in mortality due to neurologic causes.[36]

A subsequent review in adults, which included five clinical trials, confirmed the corticosteroid-associated reduction in mortality and neurologic sequelae.[37] The reduction in case fatality in patients with pneumococcal meningitis was 21%. In meningococcal meningitis, mortality and neurologic sequelae were not significantly altered. Adverse events were equally divided between the treatment and placebo groups. Treatment with dexamethasone did not worsen long-term cognitive outcome.[38] Based on these results, dexamethasone has become routine therapy in adults with suspected bacterial meningitis.

An updated Cochrane analysis showed that corticosteroids were associated with lower case fatality (RR 0.83) and less severe hearing loss (RR 0.65) and long-term neurologic sequelae (RR 0.67). In children the beneficial effect was less convincing than in adults.[34] Subsequently, randomized studies in adults with pyogenic meningitis showed no significant benefit in Malawi,[39] and significant benefit in mortality (RR 0.43) in Vietnamese patients with pyogenic meningitis.[40]

Use of dexamethasone in bacterial meningitis remains controversial in certain patients. First, there seems to be no clear benefit in patients in the Malawi setting. Secondly, patients with septic shock and adrenal insufficiency benefit from corticosteroid therapy in physiologic doses and for more than 4 days; however, without adrenal insufficiency, corticosteroids may be detrimental. A review of nine studies in sepsis or septic shock showed a trend towards increased mortality with corticosteroid administration.[41] Animal studies of bacterial meningitis suggest that the initiation of steroid therapy before or with the first dose of antibiotics is more effective than after the first dose of antimicrobial therapy, but the time point at which dexamethasone loses effects is unknown. Thirdly, by reducing permeability of the blood–brain barrier, steroids can impede penetration of antibiotics into the CSF, as was shown for vancomycin in animal studies, and lead to treatment failures, especially in drug-resistant pneumococcal meningitis. However, an observational study in suspected pneumococcal meningitis showed appropriate concentrations of vancomycin in CSF when steroids were used.[42]

Intensive care management

Monitoring in an ICU is recommended to recognize changes in consciousness and the development of new neurologic signs, monitor for subtle seizures and treat severe agitation. Bacterial meningitis may be associated with septic shock, which is an important predictor of outcome. Patients with bacterial meningitis are at risk of hyponatremia, although often mild.[43] Hyponatremia may be a result of cerebral salt wasting, the syndrome of inappropriate antidiuretic hormone secretion, or aggressive fluid resuscitation. It is a dilemma whether intravenous fluids should be restricted in hyponatremia. Hypernatremia is predictive of unfavorable outcome and mortality.[44] Severe brain injury in meningitis rarely leads to decreased antidiuretic hormone secretion resulting in diabetes insipidus. Whether adequate fluid management improves prognosis is still uncertain.

Decline in consciousness

In patients with a decline in consciousness or failure to improve upon antimicrobial therapy, brain imaging is indicated. A decline in

Table 18.2 Specific antimicrobial therapy in community-acquired bacterial meningitis based on CSF culture results and *in-vitro* susceptibility testing

Micro-organism, susceptibility	Standard therapy	Alternative therapies
Streptococcus pneumoniae		
Penicillin MIC		
<0.1 mg/l	Penicillin G or ampicillin	Cefotaxime or ceftriaxone, chloramphenicol
0.1–1.0 mg/l	Cefotaxime or ceftriaxone	Cefepime, meropenem
>2.0 mg/l	Vancomycin plus cefotaxime or ceftriaxone*	Fluoroquinolone†
Cefotaxime or ceftriaxone MIC		
>1.0 mg/l	Vancomycin plus cefotaxime or ceftriaxone‡	Fluoroquinolone†
Neisseria meningitidis		
Penicillin MIC		
<0.1 mg/l	Penicillin G or ampicillin	Cefotaxime or ceftriaxone, chloramphenicol
0.1–1.0 mg/l	Cefotaxime or ceftriaxone	Chloramphenicol, fluoroquinolone, meropenem
Listeria monocytogenes	Penicillin G or ampicillin¶	Trimethoprim–sulfamethoxazole, meropenem
Group B streptococcus	Penicillin G or ampicillin¶	Cefotaxime or ceftriaxone
Escherichia coli and other Enterobacteriaceae	Cefotaxime or ceftriaxone¶	Aztreonam¶, fluoroquinolone, meropenem¶, trimethoprim–sulfamethoxazole, ampicillin¶
Pseudomonas aeruginosa	Ceftazidime¶ or cefepime¶	Aztreonam¶, ciprofloxacin¶, meropenem¶
Haemophilus influenzae		
β-Lactamase negative	Ampicillin	Cefotaxime or ceftriaxone, cefepime, chloramphenicol, fluoroquinolone
β-Lactamase positive	Cefotaxime or ceftriaxone	Cefepime, chloramphenicol, fluoroquinolone
Chemoprophylaxis§ *Neisseria meningitidis*	Rifampin (rifampicin), ceftriaxone, ciprofloxacin, azithromycin	

CSF, cerebrospinal fluid; MIC, minimum inhibitory concentration.
*Consider addition of rifampin (rifampicin) if dexamethasone is given.
†Gatifloxacin or moxifloxacin; no clinical data on use in patients with bacterial meningitis.
‡Consider addition of rifampin (rifampicin) if the MIC of ceftriaxone is >2 mg/l.
¶Consider addition of an aminoglycoside.
§Prophylaxis is indicated for close contacts who are defined as those with intimate contact, which covers those eating and sleeping in the same dwelling as well as those having close social and kissing contacts; or health-care workers who perform mouth-to-mouth resuscitation, endotracheal intubation or endotracheal tube management. General recommendations for intravenous empiric antibiotic treatment have included penicillin, 2 MU q4h; amoxicillin or ampicillin, 2 g q4h; vancomycin, 15 mg/kg q8h; third-generation cephalosporin: ceftriaxone, 2 g q12h, or cefotaxime, 2 g q4–6h; cefepime, 2 g q8h; ceftazidime, 2 g q8h; meropenem, 2 g q8h; chloramphenicol, 1–1.5 g q6h; fluoroquinolone: gatifloxacin, 400 mg q24h, or moxifloxacin, 400 mg q24h, although no data on optimal dose needed in patients with bacterial meningitis; trimethoprim–sulfamethoxazole, 5 mg/kg q6–12h; aztreonam, 2 g q6–8h; ciprofloxacin, 400 mg q8–12h; rifampicin (rifampin) 600 mg q12–24h; aminoglycoside: gentamicin, 1.7 mg/kg q8h. The preferred dose for chemoprophylaxis: rifampin (rifampicin), 600 mg po q12h for 2 days; ceftriaxone, 250 mg im; ciprofloxacin, 500 mg po; azithromycin, 500 mg po.
This material was published previously as part of an online supplementary appendix to reference 22. Copyright 2006 Massachusetts Medical Society. All rights reserved.

consciousness in bacterial meningitis is compatible with meningoencephalitis (see Fig. 18.4). Proinflammatory mediators in the subarachnoid space contribute to an increased permeability of the blood–brain barrier, cerebral edema and increased intracranial pressure. On neuroimaging, early signs of brain edema are the disappearance of Sylvian fissures and a narrowing of ventricular size. In an advanced stage of brain edema and raised intracranial pressure, basal cisterns and sulci may become obliterated.

Seizures occur in about 20% of patients with bacterial meningitis.[45] These patients tend to be older, are more likely to have focal abnormalities on brain CT and to have *S. pneumoniae* as the causative microorganism, and they have a higher mortality. If a patient with a falling conscious level has a normal brain CT and normal serum electrolytes, then an electroencephalogram (EEG) should be performed to look for seizure activity. The high mortality warrants a low threshold for starting antiepileptic therapy in those with clinical suspicion of seizures.

In bacterial meningitis a purulent exudate forms over the cerebral hemispheres where it interferes with CSF absorption by the arachnoid villi, resulting in communicating hydrocephalus.[21] When the inflammatory exudate involves the basal cisterns and surrounds the cranial nerves at the base of the brain (basilar meningitis), it may block CSF flow at the foramina of Luschka and Magendie, resulting in obstructive hydrocephalus. Obstructive hydrocephalus may also complicate infratentorial subdural empyema.[46] In patients with communicating hydrocephalus, lumbar punctures can be performed (with measurement of CSF pressure) or the temporary insertion of a lumbar drain. In patients with mild enlargement of the ventricular system without clinical deterioration, spontaneous resolution may occur and 'watchful waiting' may be justified. Acute obstructive hydrocephalus requires ventricular drainage.

Focal neurologic abnormalities

In meningitis, focal cerebral abnormalities (hemiparesis, monoparesis or aphasia) are most commonly caused by stroke or seizures, or a combination of the two. Activation of inflammation and coagulation are closely related and interdependent. In a patient with rapid deterioration, subdural empyema should be considered. Clues to the diagnosis are the presence of sinusitis and mastoiditis (and recent surgery for either of these disorders).[46] Abnormalities of the cranial nerves are caused by the meningeal inflammatory process or by an increase

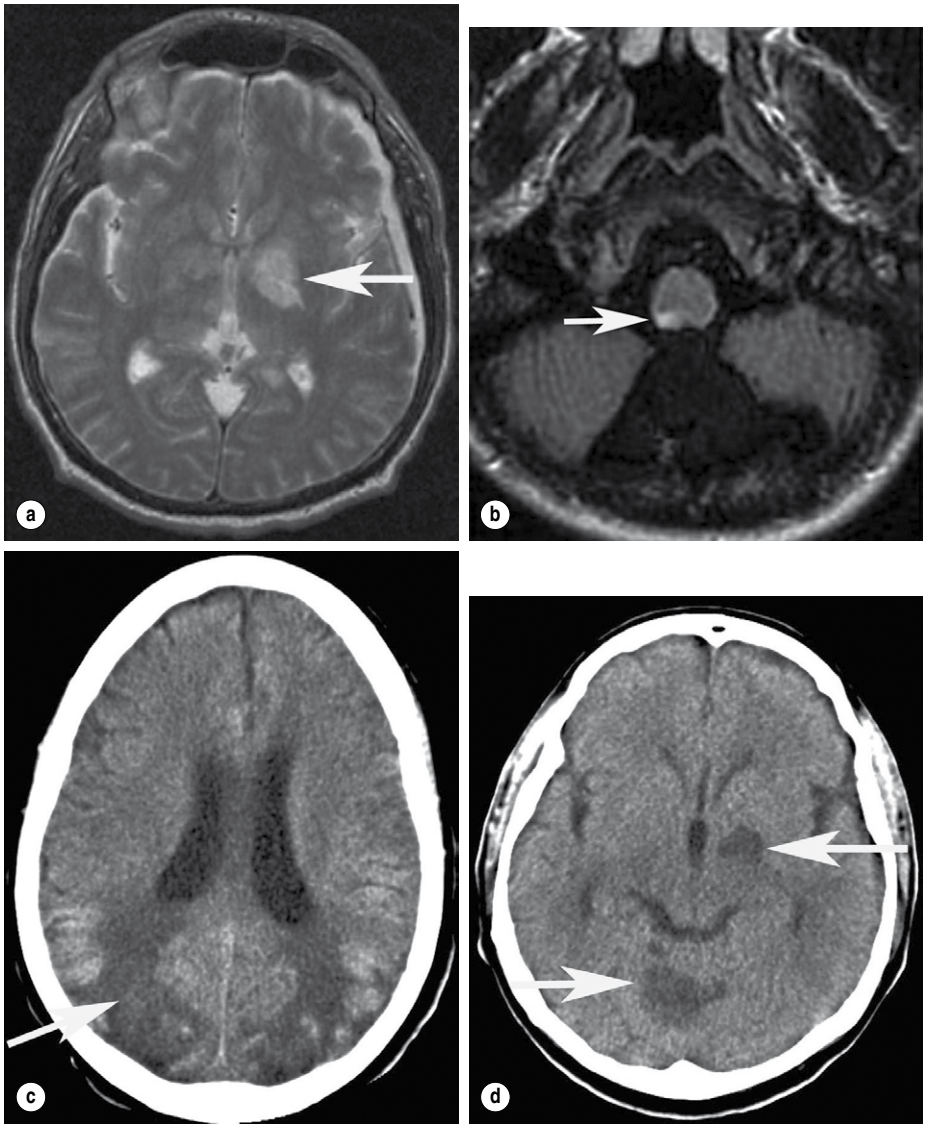


Fig. 18.4 Neuroimaging of cerebrovascular complications of bacterial meningitis. (a) MRI showing bilateral infarction in the basal ganglia causing decline in consciousness. (b) MRI showing a focal zone of signal abnormality in the right dorsal aspect of the medulla oblongata, which had restricted diffusion, suggestive of cerebral infarction. (c) CT showing bilateral occipital brain oedema indicating infarction. (d) CT showing hypodense areas in left basal ganglia and cerebellum consistent with infarction.

in CSF pressure. The most frequent cranial nerve abnormality is the involvement of the eighth cranial nerve, which is reflected in a hearing loss in 14% of patients.

Arthritis

The coexistence of bacterial meningitis and arthritis has been described in several studies; it occurs in 7% of patients overall, more in meningococcal meningitis (12%).[47] It is caused either by hematogenous bacterial seeding of joints (septic arthritis) or by immune-complex deposition in joints (see Chapter 40).

Recurrent bacterial meningitis

Recurrent bacterial meningitis occurs in 5% of community-acquired bacterial meningitis cases; predisposing conditions are head injury and CSF leak, and occasionally humoral immundeficiency.[48,49] The high prevalence of remote head injury and CSF leakage justifies an active search for anatomic defects and CSF leakage. Detection of β-2 transferrin in nasal discharge is a sensitive and specific method to confirm a CSF leak and thin-slice CT of the skull base is best to detect small bone defects. The detection of a small bone defect does not prove CSF leakage. Surgical repair has a high success rate.

When to repeat a lumbar puncture

A repeat CSF analysis should only be carried out in patients whose condition has not responded clinically after 48 hours of appropriate antimicrobial and adjunctive dexamethasone treatment. It is essential when pneumococcal meningitis caused by penicillin-resistant or cephalosporin-resistant strains is suspected. Gram staining and culture of the CSF should be negative after 24 hours of appropriate antimicrobial therapy.

OUTCOME

Community-acquired bacterial meningitis in adults is a severe disease with high fatality and morbidity rates. Meningitis caused by *S. pneumoniae* has the highest case fatality rates, reported from 19% to 37%.[7,22] Whereas neurologic complications are the leading cause of death in

younger patients, elderly patients die predominantly from systemic complications.[50] Of those who survive, up to 50% develop neurologic sequelae, including cognitive impairment.[51] For meningococcal meningitis mortality is around 5%.[52] The strongest risk factors for an unfavorable outcome in bacterial meningitis are impaired consciousness, low CSF white cell count and infection with *S. pneumoniae*.[2,53]

POST-TRAUMATIC BACTERIAL MENINGITIS

This is often indistinguishable clinically from spontaneous meningitis. However, in obtunded or unconscious patients with a recent or previous head injury, few clinical signs may be present. Fever and deterioration in the level of consciousness or loss of vital functions may be the only signs of meningitis. The rare finding of a CSF leak supports the possibility of meningitis. The range of bacteria causing meningitis in these patients is broad and consideration should be given to broad-spectrum antibiotics including metronidazole for anaerobic pathogens.[31]

Viral Meningitis

EPIDEMIOLOGY

Acute viral meningitis and meningoencephalitis represent the majority of viral CNS infections and frequently occur in epidemics with a seasonal distribution.[54] Enteroviruses cause an estimated 90% of cases in countries that immunize against mumps, while arboviruses constitute the majority of the remaining reported cases in the USA.[55] Most cases occur from late spring to autumn, reflecting the increased incidence of enteroviral and arboviral infections during these seasons.

PATHOPHYSIOLOGY AND PATHOLOGY

The pathogenesis of meningitis and meningoencephalitis are similar and requires that viruses reach the CNS by hematogenous or neuronal spread. Viruses most frequently access the CNS after a high-titer secondary viremia and cell-free or cell-associated CNS entry. Other than direct entry via cerebral vessels, virus can initially infect the meninges and then enter the parenchyma across either ependymal cells or the pial linings. Viruses exhibit differences in neurotropism and neurovirulence.

CLINICAL FEATURES

Clinical manifestations of patients presenting with viral meningitis vary with age, immune status and viral etiology. The clinical examination of a patient with suspected meningitis has been described previously in this chapter.

Epidemiologic features such as the season of the year, prevalent diseases within the community, travel, recreational activities (e.g. caving or hiking), occupational exposures and animal contacts (e.g. insect or animal bite) may provide helpful clues to the diagnosis. Late summer and early fall are seasons when enteroviral infections are encountered in temperate climates. Similarly, during warm summer months, mosquito propagation may enhance the likelihood of transmission of arthropod-borne viruses.

Patients with enteroviral meningitis often present with nonspecific symptoms such as fever of 3–5 days' duration, malaise and headache.[56] Nuchal rigidity and photophobia are the hallmark sign and symptom for meningitis, but 33% of patients with viral meningitis have no evidence of meningismus.[57] Children may present with seizures secondary to fever, electrolyte disturbances or the infection itself. In the immunocompromised host, enteroviral infection is both a diagnostic quandary and a potentially life-threatening disease. While physical examination of the patient usually does not suggest an etiologic diagnosis, a few considerations are essential.

Patients with herpes simplex virus (HSV) meningitis are infected with HSV type 2 and present with a rather benign disease. This is in contrast with herpes simplex encephalitis (HSE) caused by HSV type 1 which is a serious illness with significant risks of morbidity and death (Chapter 155). Patients with herpes meningitis typically have recurrent episodes.[58]

LABORATORY FINDINGS

Initial CSF samples, while frequently suggestive of the diagnosis, are neither sensitive nor specific enough to differentiate viral from bacterial meningitis.[23] The CSF in patients with viral meningitis typically exhibits pleocytosis with 10–500 leukocytes and a slightly elevated protein level (>100 mg/dl). The glucose level in the CSF is typically greater than 40% of a simultaneously drawn serum sample.

Cultures of specimens of body fluids other than CSF may be useful in establishing the etiologic diagnosis in selected patients with meningoencephalitis.[31] Specific clinical findings should also direct other sites for culture (e.g. stool, skin, sputum).

Molecular techniques have advanced identification of viral agents. PCR provides a rapid and reliable test for verifying the etiology of certain types of meningitis. These techniques provide results within 24–36 hours and therefore may limit the duration of hospitalization, antibiotic use and excessive diagnostic procedures. For other infections, molecular techniques are the standard for diagnosing viral meningitis.

Diagnostic serologic assays have simplified the diagnosis of viral infections of the CNS. The ELISA assay that detects IgM antibodies in the CSF from patients with presumed Japanese encephalitis (JE) is both sensitive and specific, as most patients have antibodies at the time of hospitalization and virtually all acquire them by the third day of illness.[59] Unlike herpesviruses, which are ubiquitous agents with generally high basal seroprevalence levels in the general population, seroprevalence levels for individual arboviruses are generally low.

ANTIVIRAL TREATMENT

Antiviral therapy exists for HSV-1, HSV-2, varicella-zoster virus (VZV), cytomegalovirus (CMV) and human immunodeficiency virus (HIV).[60–62] The introduction of aciclovir has resulted in a sharp decline in mortality and morbidity from HSE.[62] Most authors recommend the use of intravenous aciclovir for HSV meningitis, although no definitive clinical trials have been conducted. There are no data on benefit of antiviral treatment or on suppressive therapy for recurrent HSV meningitis.

In the normal host, viral meningitis is a relatively benign self-limited disease. A prospective study in children less than 2 years of age, for example, found that even in the 9% of children who develop evidence of acute neurologic disease (complex seizures, increased intracerebral pressure or coma) long-term prognosis is excellent. During follow-up (42 months), children with acute CNS complications performed neurodevelopmental tasks and achieved developmental milestones as well as did children with an uncomplicated course.[63,64]

In case reports, immunoglobulin preparations, given systemically or intrathecally, retarded mortality and morbidity in agammaglobulinemic patients with enteroviral meningitis. Enteroviral infections in neonates frequently produce overwhelming viremia and CNS disease. A blind, randomized controlled trial did not demonstrate clinical benefit for enterovirus-infected neonates with severe life-threatening disease who received intravenous immunoglobulin.[65,66]

Pleconaril, an inhibitor of enterovirus replication, was tested in two placebo-controlled clinical trials. Of 607 randomized patients in a multicenter, double-blind, placebo-controlled study of pleconaril, 240 patients were confirmed to have enterovirus infection.[67,68] Resolution of headache in patients with concomitant moderate to severe nausea at baseline occurred at a median of 9.5 days in the absence of therapy and was reduced to 7.0 days for pleconaril recipients ($p = 0.009$). Over 50% of untreated patients had a persistent headache that was greater than 1 week in duration. Pleconaril shortened the course of illness compared to placebo recipients, especially in the early disease course. However, the benefit was achieved only modestly in a subgroup analysis of patients with more severe disease after adjusting for confounding variables.

SUPPORTIVE THERAPY

After establishing a presumptive diagnosis and instituting therapy, the clinician must also vigilantly anticipate and treat complications associated with the viral CNS disease or the therapeutic interventions,[55] as described in this chapter for patients with bacterial meningitis. Seizures secondary to direct viral CNS damage, inflammatory vasculitis and electrolyte changes require anticonvulsant therapy.

Chronic Meningitis

CLINICAL FEATURES

Chronic meningitis is defined by symptoms of meningeal inflammation with CSF pleocytosis that persist for more than 4 weeks. Symptoms and signs of chronic meningitis evolve over several days to weeks. Patients complain of headaches, often associated with signs of infection (fever, anorexia). Nuchal rigidity may be subtle or absent. Many forms of chronic meningitis involve the base of the brain and lead to cranial nerve palsies, often affecting eye movements and facial musculature. As the syndrome progresses, signs of brain involvement with seizures, mental status changes, confusion or hallucinations, and focal neurologic deficits develop. Hydrocephalus and increased intracranial pressure may accompany the syndrome.

TUBERCULOUS MENINGITIS

One of the most common causes of chronic meningitis is *Mycobacterium tuberculosis* (Table 18.3). Tuberculous meningitis (TBM) results from the rupture of a tubercle into the subarachnoid space. It is the most

Table 18.3 Microbial causes of chronic meningitis

Pathogen		Predominant type of CSF pleocytosis	Predisposition and risk factors	Associated clinical manifestations
Bacteria	*Actinomyces* spp.	Neutrophils	Mouth and ear lesions	CNS lesions, endophthalmitis
	Borrelia burgdorferi (Lyme disease)	Lymphocytes	Tick bite	Cranial nerve palsy (VII nerve)
	Brucella spp.	Lymphocytes, neutrophils	Unpasteurized dairy products	Undulant fever, hepatomegaly
	Leptospira spp.	Neutrophils	Exposure to urine of infected animals	Hepatomegaly, hepatitis, thrombocytopenia
	Mycobacterium tuberculosis	Neutrophils, monocytes, lymphocytes	Immunodeficiency, high endemic prevalence	Cranial nerve palsy (VI nerve)
	Nocardia spp.	Neutrophils	Immunodeficiency	Abscesses
	Treponema pallidum	Eosinophils, lymphocytes	Sexually transmitted diseases	Cranial nerve palsy (VII and VIII nerves)
	Tropheryma whipplei	Neutrophil	Gastrointestinal Whipple disease	Cognitive decline, gait ataxia, supranuclear gaze palsy
Viruses	Cytomegalovirus	Lymphocytes; neutrophils (in HIV)	Immunodeficiency	Fever, retinitis
	Echovirus	Lymphocytes	Agammaglobulinemia	Dermatomyositis
	Lymphocytic choriomeningitis virus	Lymphocytes	Exposure to rodents	Orchitis, leukocytopenia, thrombocytopenia
	Mumps virus	Neutrophils	No vaccination	Parotitis, orchitis, oophoritis
	HIV	Lymphocytes	HIV risk factors	Mononucleosis-like illness
Fungi	*Aspergillus* spp.	Lymphocytes or neutrophils	Immunodeficiency, surgery	Lung involvement
	Candida spp.	Neutrophils	Antibiotics, surgery, immunodeficiency	Disseminated disease
	Coccidioides spp.	Lymphocytes	Endemic areas	Lung involvement
	Cryptococcus spp.	Lymphocytes	Immunodeficiency	Encephalitis, headache
	Histoplasma spp.	Lymphocytes	Endemic areas, immunodeficiency	Fever, oral lesions, hepatosplenomegaly
	Pseudallescheria spp.	Neutrophils	Immunodeficiency	Skin lesions, endophthalmitis
	Sporothrix spp.	Neutrophils	Immunodeficiency	Skin lesions, endophthalmitis
Parasites	*Taenia solium*	Eosinophils	Endemic areas	Elevated intracranial pressure, calcified lesions on head imaging
	Angiostrongylus spp.	Eosinophils	Raw seafood	Fever

important cause of chronic meningitis. In the less developed world TBM remains a common cause of bacterial meningitis, particularly in populations with a high prevalence of HIV infection.[27]

Most patients with TBM have progressive headache and signs of meningeal irritation, followed by cranial nerve involvement, other neurologic deficits and progressive mental status changes over a period of weeks.[27,69] These prodromal symptoms can last from 2 to 8 weeks until the classic features of meningitis become more apparent. Patients commonly present to hospital, when the infection is well established. They will usually complain of headache and vomiting; many will present confused or comatose. Examination reveals neck stiffness in most, although rarely as marked as in pyogenic meningitis. Cranial nerve palsies (especially third, sixth and seventh nerves) are found in 25% of patients. Ten percent of patients will present with a mono- or hemiparesis. Rarely, TBM presents as an acute meningoencephalitis that can be difficult to distinguish from pyogenic bacterial or viral meningitis.[69] Seizures are rare in adults with TBM, but more common in children. HIV infection does not appear to alter the clinical presentation of TBM, although evidence of other extrapulmonary disease is more likely in HIV-infected patients.

Tuberculous meningitis may be a consequence of either primary infection or reactivation of disease. The diagnosis can be confirmed by a positive CSF culture; however, *M. tuberculosis* is recovered from the CSF in only 38–88% of cases.[27] A moderate lymphocytic pleocytosis is most common. The glucose can be very low; the protein is often very high. CSF smears for acid-fast bacilli are positive in only a minority of cases (10–20%). Tuberculin skin tests are frequently negative in TBM.

The treatment of TBM follows the model of short course chemotherapy of pulmonary tuberculosis (Table 18.4): an 'intensive phase' of treatment with four drugs, followed by a prolonged 'continuation phase' with two drugs. When there is no suspicion of multidrug resistance, the first 2 months of treatment should be with isoniazid, rifampicin, pyrazinamide and either streptomycin, ethambutol or ethionamide.[27] The British Thoracic Society (BTS) and the Infectious Diseases Society of America (IDSA) favor ethambutol as the fourth drug, although they acknowledge the lack of evidence from controlled trials. British and American guidelines suggest between 9 and 12 months total antituberculosis treatment for TBM, although a recent systematic review concluded that 6 months might be sufficient provided the likelihood of drug resistance is low.

The use of steroids in the treatment of TBM was first reported in the early 1950s.[70] Continued research supports the use of steroids while adequately treating with multiple antimycobacterial drugs in TBM. A meta-analysis of 595 patients found that the use of adjunctive steroids led to fewer deaths and reduced incidences of death and disability.[70,71] A randomized, double-blind, placebo-controlled trial of 545 patients found that patients receiving dexamethasone had a reduced risk of death (RR 0.69) that was consistent across subgroups of differing clinical severity.[72] Although there were significantly fewer serious adverse events in the patients receiving dexamethasone, this study did not report an associated reduction in the proportion of patients who developed severe disabilities, and the lack of neurosurgical intervention might limit the generalizability of the results.

The largest randomized study recommend a stratified dexamethasone treatment regimen in patients with TBM.[72] Under this protocol, patients with a Glasgow Coma Scale score of less than 15 or who have a focal neurologic deficit are treated with intravenous dexamethasone for 4 weeks (0.4 mg/kg per day in week 1, 0.3 mg/kg per day in week 2, 0.2 mg/kg per day in week 3, and 0.1 mg/kg per day in week 4), followed by a taper of oral dexamethasone (4 mg/day, 3 mg/day, 2 mg/day and 1 mg/day, each for a period of 1 week). Patients with a normal mental status and no neurologic findings receive intravenous dexamethasone for 2 weeks (0.2 mg/kg per day in week 1, then 0.1 mg/kg per day in week 2), followed by the same oral taper as described above. It is recommended that the steroid treatment should start as soon as possible after initiation of first-line antituberculous drugs.

Tuberculous meningitis leads to severe CNS inflammatory processes with secondary damage; mortality of up to 50% has been reported.[27]

OTHER INFECTIOUS CAUSES OF CHRONIC MENINGITIS

Secondary syphilis may cause chronic meningitis.[73] The disease is slowly progressive, and generally symptoms have been present for more than 1 month before presentation. Cranial nerve palsies are common; the facial and acoustic nerves are the most frequently affected. Diagnosis is based on a positive VDRL test in CSF. Treatment consists of high-dose penicillin (see Table 18.4).

The diagnosis of meningitis associated with Lyme disease, caused by *Borrelia burgdorferi*, should be considered in patients who live or have traveled through endemic regions, particularly those with a history of a tick bite or erythema chronicum migrans.[74,75] Meningitis may persist for weeks and may be associated with cranial nerve palsies and peripheral neuropathies. Syphilis, other spirochetal diseases and collagen vascular diseases

Table 18.4 Treatment recommendations for common treatable causes of chronic infectious meningitis

Agent	Therapy	Dose	Route
Herpesviruses	Aciclovir	10 mg/kg q8h	iv
Mycobacterium tuberculosis	Isoniazid Rifampin (rifampicin) Ethambutol Pyrazinamide	10 mg/kg/q24h (up to 300 mg/q24h) 10 mg/kg/q24h (up to 600 mg/q24h) 25 mg/kg/q24h 25 mg/kg/q24h (up to 2.5 g/q24h)	po or iv po or iv po or iv po or iv
Brucella spp. (>8 years)	Doxycycline plus gentamicin	100 mg q12h 1.7–2 mg/kg q8h	po iv
Brucella spp. (<8 years)	Trimethoprim– sulfamethoxazole plus gentamicin	5 mg/kg trimethoprim q12h 25 mg/kg sulfamethoxazole q12h 2 mg/kg q8h	po po iv
Treponema pallidum	Penicillin G	2 g q4h	iv
Borrelia spp.	Ceftriaxone	2–3 g q24h	iv
Cryptococcus spp.	Amphotericin B plus flucytosine	0.5–0.8 mg/kg/q24h 37.5 mg/kg q6h	iv po
Coccidioides spp.	Fluconazole	400–600 mg/q24h	po

may result in a false-positive Lyme serology. Treatment consists of high-dose ceftriaxone, although a clinical trial showed oral doxycycline to be equally effective in European adults with mild neuroborreliosis.[74–76]

Whipple's disease is a rare, systemic infectious disease caused by the bacterium *Tropheryma whipplei*.[77] Neurologic manifestations occur in three situations: neurologic relapse of previously treated Whipple's disease, neurologic involvement in classic Whipple's disease, and isolated neurologic symptoms due to *T. whipplei* without evidence of intestinal involvement. Many patients have systemic symptoms, such as fever, weight loss, peripheral lymphadenopathy and arthralgia. The predominant symptoms include cognitive impairment, ophthalmoplegia, ataxia and upper motor neuron disorder. A review summarized outcomes for 30 patients described in the literature: 18 (60%) had improvement and 10 died (33%); in 1 patient, the disease stabilized. Treatment consisted of high-dose ceftriaxone, followed by maintenance therapy with co-trimoxazole or hydroxychloroquine in combination with doxycycline.[78]

Presentations of chronic meningitis caused by *Cryptococcus neoformans* range from a subacute meningoencephalitis to fever of unknown origin.[79] People at highest risk are those with defects in cellular immunity such as occurs in AIDS, hematologic malignancies and prolonged use of high-dose corticosteroids. CSF shows moderate lymphocytic pleocytosis; however, in patients who have AIDS, inflammation may be virtually absent. Cryptococcal antigen latex agglutination is positive in more than 90% of cases, whereas microscopy of India ink preparations to visualize the yeast in CSF is less sensitive (but may be more readily available). Treatment recommendations are provided in Table 18.4 (see also Chapter 91).

Coccidioides immitis grows in the dry sandy soils of the south-west USA, and Central and South America.[80] Acute infection is acquired by inhalation of the spores, and meningitis develops within a few months. There are few distinguishing features of the disease; some patients who have generalized disease have erythema nodosum; hydrocephalus is a common complication. CSF eosinophilia in patients who have lived or traveled through endemic regions should alert the clinician to the possibility of *Coccidioides* meningitis. Complement-fixing antibodies are present in the CSF in 75–95% of cases and CSF cultures are positive in more than 50%. Treatment is usually with an azole or amphotericin B, depending on the clinical manifestations and the immune status of the host.

Histoplasma meningitis is a rare complication of histoplasmosis.[81] The diagnosis should be considered in patients who live or have traveled through endemic regions – the Ohio River Valley of the USA, the Caribbean and South America. CSF cultures are positive in 27–65% of cases. Blood should be cultured and a buffy coat of the blood should be examined for the presence of the fungus. *Histoplasma* polysaccharide antigen is found in the urine, blood or CSF in 61% of patients and in an even higher proportion of patients who have AIDS. Adequate and prolonged antifungal therapy is indicated in all cases of CNS histoplasmosis.

Neurocysticercosis is endemic in Mexico, South America and Asia. Infection is acquired by eating food contaminated with eggs of *Taenia solium*.[82] Seizures are the most common manifestation. Intraventricular and basilar cysts (racemose cysticercosis) may present with signs of obstructive hydrocephalus. The CSF shows a lymphocytic pleocytosis with eosinophils. CT scans of the head show multiple calcified lesions. Serology of blood and CSF may provide support for the diagnosis. Treatment should be tailored according to the type of neurocysticercosis and may consist of albendazole, steroids, analgesics, antiepileptic drugs, surgical resection of lesions and placement of ventricular shunts.

Angiostrongylus cantonensis, the rat lung worm, is most prevalent in Asia and the Pacific Islands and is acquired by the ingestion of raw or inadequately cooked shellfish or snails.[83] Symptoms are typical of chronic meningitis, and rash with pruritus is also common. Infection results in peripheral eosinophilia and chronic eosinophilic meningitis, which resolves spontaneously within 2 months. There is no effective therapy.

Other even less common causes of infectious chronic meningitis are organisms that usually cause abscesses, which may leak into the subarachnoid space to cause chronic meningitis. These conditions include blastomycosis, paracoccidioidomycosis, phaeohyphomycosis, mucormycosis, actinomycosis, nocardiosis and toxoplasmosis. Less common fungi causing this syndrome include *Sporothrix schenckii*, chromoblastomycoses and *Aspergillus* spp.

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Chapter 19

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Encephalitis and myelitis

INTRODUCTION

Encephalitis is defined by the presence of an inflammatory process in the brain, associated with clinical evidence of neurologic dysfunction. It is one of the most challenging syndromes for clinicians to diagnose and manage, particularly because a specific pathogen is identified in fewer than 50% of cases.[1] Diagnostic difficulties in establishing an etiology include the vast number of infectious causes of encephalitis, the many noninfectious etiologies that present with syndromes suggestive of encephalitis, and the confusion regarding the role of molecular versus serologic testing. Guidelines for the diagnosis and management of encephalitis have recently been published and address many of these issues.[2]

Myelitis refers to inflammation of the spinal cord, as a result of a direct infectious process or through postinfectious or other mechanisms;[3] in association with encephalitis, this syndrome is often referred to as encephalomyelitis. Encephalitis and myelitis are often considered together because there is overlap in terms of epidemiology, microbiology and approach to management. The current chapter will focus on the syndromes of encephalitis and myelitis in immunocompetent hosts, with a more detailed discussion of pathogen-specific infections in the chapters dedicated to these microbes.

EPIDEMIOLOGY

Encephalitis causes significant morbidity and mortality, and represents a significant burden on the health-care system. The average duration of hospitalization for patients with encephalitis is 12 days, with a cumulative cost for hospitalization exceeding $650 million per year in the USA alone.[4] The case-fatality rate among patients with encephalitis varies from 3.8% to 7.4% and is significantly higher in patients also infected with human immunodeficiency virus (HIV).[5,6] These aggregate data mask the fact that in some infections, such as those caused by rabies virus or *Naegleria fowleri*, the mortality rate is almost 100%. Additionally, these estimates of disease burden do not take into account the significant morbidity among survivors of encephalitis, with the resultant loss of productivity and need for prolonged rehabilitation or skilled nursing care.

Limited data exist on the incidence and epidemiology of encephalitis. A county-wide, population-based study of encephalitis diagnosed between 1950 and 1981 in Minnesota reported an incidence of 7.4 cases per 100 000 person-years;[5] similar rates have been reported in population-based studies in Finnish children.[7,8] A more recent study evaluating US hospital discharge survey data between 1988 and 1997 identified approximately 19 000 encephalitis hospitalizations annually, for a rate of 7.3 encephalitis hospitalizations per 100 000 US population.[4] While these studies of disparate populations have shown strikingly similar rates, these data predate the introduction of West Nile virus (WNV) in the USA, which in recent years accounted for 1000–2000 cases annually during summer and early fall.[9] Outside of the USA, there are many important viruses, such as measles, rabies and Japanese encephalitis virus (JEV), that contribute significantly to the disease burden in these areas (Fig. 19.1). For instance, JEV causes 20 000 illnesses each year worldwide; 20–30% of these infections result in death, and over 50% of survivors have neurologic sequelae.[10]

Several factors appear to increase the risk of encephalitis regardless of etiology. Rates of encephalitis are higher in males than females, and are highest at the lower and upper age ranges.[4,5] One study reported a rate of encephalitis in children <1 year of age of 18.4/100 000 child-years, almost double that seen in older children.[8] HIV co-infection is present in 15% of cases in population-based studies of encephalitis.[4] Furthermore, HIV-infected and other immunocompromised hosts are at risk for a broader array of pathogens than those found in the general population. While encephalitis does not appear to follow a seasonal pattern, specific pathogens, particularly those transmitted by arthropods, have well-defined temporal patterns. Other exposures or risk factors associated with specific infectious causes of encephalitis, and myelitis, are listed in Table 19.1.

MICROBIOLOGY

Infectious causes of encephalitis and myelitis are diverse and include viruses, bacteria, fungi and parasites. The spectrum of agents has shifted in recent years, primarily as a result of a decrease in vaccine-preventable conditions such as measles, mumps, rubella and varicella.[8,11] While the specific etiologies have changed, the overall incidence has remained stable due to increasing rates in other previously under-recognized or emerging pathogens.[8,12] Examples of emerging causes of encephalitis and myelitis worldwide in the last decade include WNV,[9] Nipah virus,[13,14] enterovirus 71,[15] *Balamuthia mandrillaris*[16,17] and Chandipura virus.[18]

A useful paradigm for organizing the vast number of causative agents is to categorize them based on the strength of association with encephalitis and myelitis. The most commonly identified etiologies in the USA are herpes simplex virus 1 (HSV-1), WNV and the enteroviruses, followed by other herpesviruses (Table 19.2). Other agents may be highly endemic regionally (e.g. La Crosse virus) or internationally (e.g. rabies virus, JEV). Bacterial agents, including *Ehrlichia* spp. and *Rickettsia rickettsii* are potentially treatable causes of encephalitis and myelitis, and prompt administration of antimicrobial therapy may be lifesaving.[19,20]

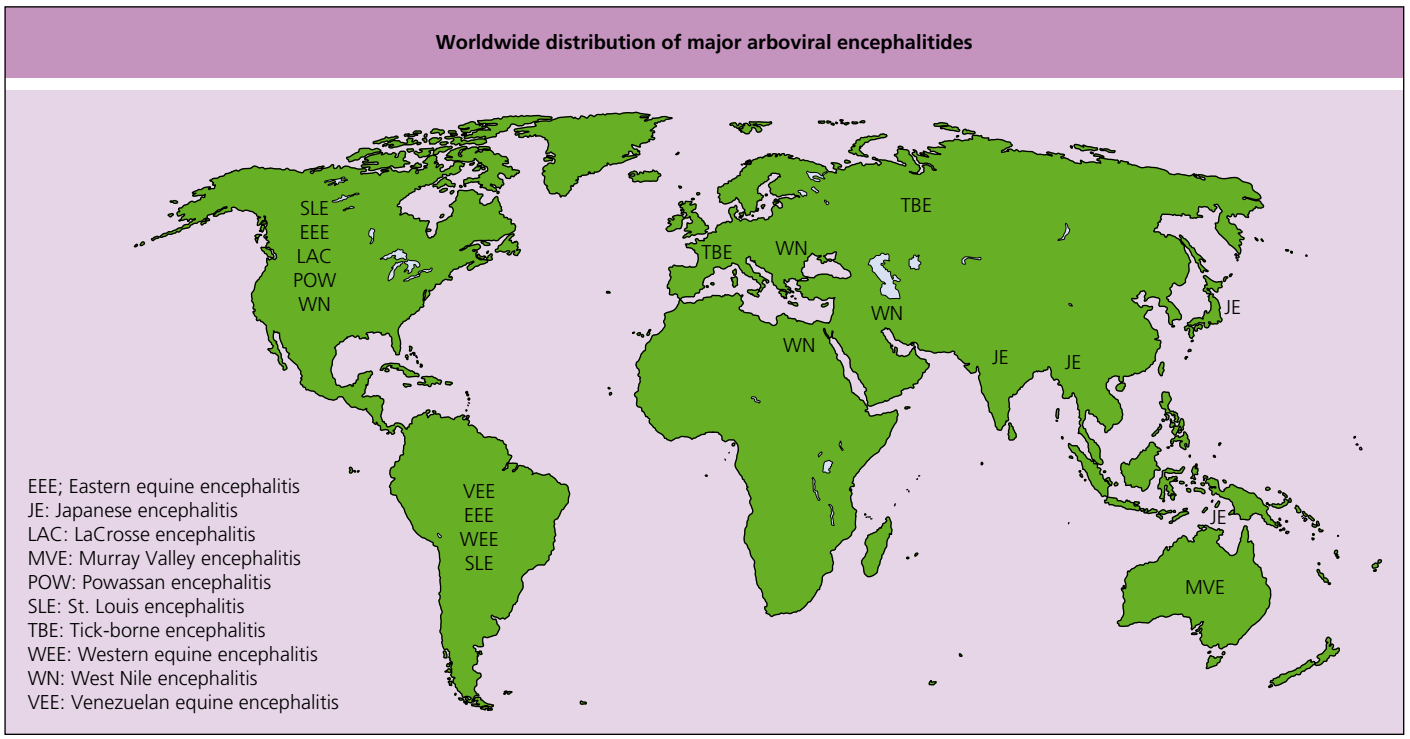


Fig. 19.1 Worldwide distribution of major arboviral encephalitides. Courtesy of the Centers for Disease Control and Prevention.

A second category includes agents less commonly identified, but which are neurotropic and are well-associated causes of sporadic encephalitis and myelitis (Table 19.3). These agents fall into two categories:

- uncommon pathogens with prominent central nervous system (CNS) symptoms (e.g. Eastern equine encephalitis and Venezuelan equine encephalitis viruses); and
- relatively common infections in which meningoencephalitis occurs in a minority of cases (e.g. *Borrelia burgdorferi*, *Coxiella burnetii*).

A number of these have well-defined geographic distributions (e.g. Chandipura virus in India, Nipah virus in Malaysia) and consideration of these agents is indicated if there is travel to or residence in an endemic area.

Most problematic is the third category of agents, those anecdotally associated with encephalitis and myelitis but with minimal neurotropism and limited laboratory data confirming direct CNS invasion (Table 19.4). For instance, *Mycoplasma pneumoniae* is the most common agent identified, usually by serology, in several large case series of encephalitis and myelitis.[21–23] However, the significance of serologic diagnosis of acute *M. pneumoniae* infections is unknown since *Mycoplasma* infections are relatively common and most serologic testing lacks specificity;[22] detection of bacteria in cerebrospinal fluid (CSF) by culture or by nucleic acid amplification tests, such as polymerase chain reaction (PCR), is diagnostic of CNS infection, but is rarely found. Other organisms associated with encephalitis, but with similar difficulties in establishing causality, include influenza virus,[24] rotavirus,[25] human herpes virus-6,[26] parvovirus[27] and others listed in Table 19.4.

Perhaps the most challenging aspect of encephalitis for patients, families and clinicians is that, despite the multitude of causal organisms, no pathogen is identified in the majority of cases. Studies conducted in geographically distant sites on disparate populations consistently report that between 50% and 70% of cases remain unexplained.[4,7,28,29] One of the most recent and ambitious studies of encephalitis performed prospective testing on 1570 well-defined cases over a 7-year period using a standardized diagnostic algorithm including extensive molecular testing. Despite this rigorous approach, no etiologic cause was identified in 63% of patients.[30] Furthermore, approximately 10% of patients initially thought to have an infectious cause of encephalitis were ultimately diagnosed with a noninfectious condition.

CLINICAL FEATURES

Encephalitis is infrequently confirmed by pathologic means. Therefore, signs and symptoms of neurologic dysfunction are used as surrogate markers; these are often nonspecific. Encephalopathy, defined by a disruption of brain function in the absence of direct brain inflammation, may mimic encephalitis, although fever is less common and there is usually minimal CSF pleocytosis. Causes of encephalopathy include metabolic disturbances, hypoxia, ischemia, intoxications, organ dysfunction, paraneoplastic syndromes or systemic infections.

Encephalitis can present with purely parenchymal findings, but more commonly has associated meningeal inflammation, representing an overlap syndrome termed meningoencephalitis; for the purposes of this chapter, these terms are used interchangeably. The clinical signs and symptoms of encephalitis are determined by the specific area of the brain involved and by the severity of the infection. Some organisms show neurotropism for particular anatomic sites; for example, HSV-1 infection almost universally involves the temporal lobe and the presentation typically includes temporal lobe seizures.[31] Diffuse brain involvement, as is frequently seen with arboviral infections, is associated with global impairment in neurologic function and coma.

Fever and headache frequently precede the onset of altered mental status, which can range from mild confusion to obtundation. Rarely, patients with inflammation localized to extracerebral portions of the CNS may have intact cognition. For instance, primary varicella infection is associated with cerebellar inflammation, with findings of ataxia and nystagmus but no cognitive deficits.[32] Other neurologic manifestations may include behavioral changes (such as psychosis), focal paresis or paralysis, cranial nerve palsies, or movement disorders such as chorea.[30] The frequency of seizures varies based on the pathogen; generalized seizures are common with *Bartonella* (cat scratch) encephalopathy[33] but are noted in less than 10% of cases of WNV encephalitis.[34]

Table 19.1 Exposures associated with agents causing encephalitis and myelitis

Risk factor/exposure	Agent
Arthropods	
Mosquito bite*	Alphaviruses (Western equine encephalitis virus, Eastern equine encephalitis virus, Venezuelan equine encephalitis virus) Flaviviruses (West Nile virus, Japanese encephalitis virus, St Louis encephalitis virus) Bunyaviruses (California/La Crosse virus)
Tick bite	Powassan virus, *Anaplasma phagocytophilum*, *Ehrlichia* spp., *Rickettsia rickettsii*, tick-borne encephalitis virus, *Borrelia burgdorferi*
Sandflies	*Bartonella bacilliformis*, Chandipura virus (India)
Tsetse flies	*Trypanosoma brucei gambiense*, *Trypanosoma brucei rhodesiense* (Africa)
Wild/domestic animals	
Bats	Rabies virus, Nipah virus, *Histoplasma capsulatum*
Dogs	Rabies virus
Cats	*Bartonella hensalae*, *Toxoplasma gondii*, rabies virus
Rodents*	Lymphocytic choriomeningitis virus, *Leptospira* spp.
Raccoon	*Baylisascaris procyonis*, rabies virus
Sheep/goats	*Coxiella burnetii*
Birds*	*Cryptococcus neoformans*, *Chlamydia psittaci*
Old world monkeys	B virus
Horses*	Hendra virus (Australia)
Swine	Nipah virus (South East Asia)
Skunks	Rabies virus
Parturient animals (especially farm animals)	*Coxiella burnetii*
Fresh water	*Naegleria fowleri*, *Leptospira* spp.
Soil (aerosolized or ingestion)	*Balamuthia mandrillaris*, *Baylisascaris* procyonis, endemic fungi
Undercooked freshwater fish, chicken or pork	*Gnasthostoma* spp. (South East Asia, Mexico)
Raw or undercooked fresh water prawns, crabs or frogs, or unwashed produce (with snails/slugs)	*Angiostrongylus cantonensis* (South East Asia, Pacific Islands, Caribbean)
Unpasteurized milk	*Coxiella burnetii*, tick-borne encephalitis virus, *Listeria monocytogenes*
Season	
Fall	Enteroviruses, arthropod-borne pathogens (see above)
Winter	Influenza virus
Spring	Enteroviruses, arthropod-borne pathogens (see above)
Summer	Enteroviruses, arthropod-borne pathogens (see above)
Recreation	
Camping/hunting	Arthropod-borne pathogens (see above)
Spelunking	Rabies virus, *Histoplasma capsulatum*
Sexual activity	HIV, syphilis, herpes simplex virus 2

*Rodents, birds and horses may serve as reservoir hosts for arboviruses, but transmission to humans is generally from an arthropod vector.

Rabies infections deserve special mention because of their severity and worldwide importance. Rabies virus infections, with few exceptions, are fatal illnesses, and the time to progression from onset of symptoms to death is typically less than 2 weeks.[35] Symptoms initially are nonspecific, but may include fever, headache, pharyngitis and weakness. The diagnosis should be considered in any patient with a rapid progression of illness, particularly if hydrophobia, autonomic instability, extreme agitation and seizures are present. Paresthesia at the inoculation site is a unique feature of rabies.

Table 19.2 Relatively common causes of encephalitis and myelitis in immunocompetent patients

Etiology	Epidemiology	Clinical features	Diagnosis
Viruses			
Enteroviruses (includes coxsackieviruses, echoviruses and enterovirus 71)	Peak incidence in late summer and early fall; enterovirus 71 a cause of large outbreaks in Asia, with children primarily affected	Range from aseptic meningitis (most common) to encephalitis; enterovirus 71 causes rhombencephalitis	CSF PCR or culture. Stool or throat swab PCR or culture suggestive, but not diagnostic, of CNS involvement
Epstein–Barr virus	Either during acute infection or reactivation, primary CNS lymphoma	Infectious mononucleosis symptoms during acute infection, cerebellar ataxia, sensory distortion ('Alice-in-Wonderland' syndrome)	CSF PCR (may be falsely positive through detection of latently infected macrophages), serology (IgM positive in acute infection)
Herpes simplex virus (HSV) 1 and 2	HSV-1 accounts for 5–10% of encephalitis, typically reactivation disease; HSV-2 seen in neonates	Temporal lobe seizures (apraxia, lip smacking), behavioral abnormalities	CSF PCR, CSF serologies if >1 week of symptoms
Japanese encephalitis virus (JEV)	Mosquito-borne, most common worldwide cause of encephalitis, endemic throughout Asia; vaccine preventable	Seizures, Parkinsonian features, acute flaccid paralysis variably seen. MRI classically with thalamic and basal ganglia involvement	Serology, CSF IgM, CSF antigen (cross-reacts with other flaviviruses)
La Crosse virus	Mosquito-borne, endemic in midwestern and eastern USA; peak incidence in school-aged children	Varies from subclinical illness to seizures and coma	Serology
Rabies virus	Vaccine preventable; most common vector is bat, and bites often unrecognized; dogs important in developing countries; worldwide distribution	Often with numbness or neuropathic pain at site of bite, progressing to hydrophobia (with drooling), agitation, delirium, autonomic instability, coma; paralytic form with ascending paralysis in 30%	Antibodies (serum, CSF), PCR of saliva or CSF, IFA of nuchal biopsy or CNS tissue; coordinate testing with local health department
St Louis encephalitis virus (SLE)	Mosquito-borne, endemic to western USA, with periodic outbreaks in central/eastern USA; peak incidence in adults >50 years	Tremors, seizures, paresis, urinary symptoms, SIADH variably present	Serology (cross-reacts with other flaviviruses)
Tick-borne encephalitis virus	Transmitted via tick or ingestion of unpasteurized milk; endemic to Eastern and Central Europe, Far East	Weakness ranging from mild paresis to acute flaccid paralysis	Serology
Varicella-zoster virus	Acute infection (chickenpox) or reactivation (shingles)	Vesicular rash (disseminated or dermatomal), cerebellar ataxia, large vessel vasculitis	DFA or PCR of skin lesions, CSF PCR, serum IgM (acute infection)
West Nile virus (WNV)	Mosquito-borne, emerging cause of epidemic encephalitis throughout USA, Europe; endemic in Middle East; peak incidence adults >50 years	Weakness and acute flaccid paralysis, tremors, myoclonus, Parkinsonian features, MRI with basal ganglia and thalamic lesions	CSF IgM, paired serology (cross-reactivity with WNV and SLE)
Bacteria			
Bartonella henselae (and other *Bartonella* spp.)	Typically follows scratch or bite from cat or kitten; highest incidence in children	Encephalopathy with seizures (often status epilepticus), with rapid recovery; often peripheral lymphadenopathy noted; CSF usually paucicellular	Serology (acute usually diagnostic), PCR of lymph node, CSF PCR rarely positive
Mycobacterium tuberculosis	Most common in developing countries; disease in extremes of age or immunocompromised	Subacute basilar meningitis, lacunar infarcts, hydrocephalus; CSF formula often with low glucose, high protein; often have associated pulmonary findings	CSF AFB smear, culture, PCR; respiratory cultures highly suggestive
Ehrlichia/Anaplasma	Tick-borne bacteria causing human monocytotrophic and human granulocytotrophic ehrlichiosis, respectively; former endemic to southern and central USA, latter to northeast USA and Midwest	Acute onset of fever and headache; rash seen in <30% of cases of *Ehrlichia* in adults; leukopenia, thrombocytopenia and elevated LFTs frequent manifestations	Morulae in white blood cells, PCR of whole blood, serology (seroconversion may occur several weeks after symptoms)
Rickettsia rickettsii	Tick-borne infection found throughout North America, with peak incidence in southeast and south central USA	Acute onset of fever and headache; petechial rash in 85% of cases beginning 3 days after onset of symptoms	Serology (seroconversion may occur several weeks after symptoms), PCR or IHC staining of skin biopsy of rash

AFB, acid-fast bacilli; CSF, cerebrospinal fluid; DFA, direct fluorescent antibody; IFA, indirect fluorescent antibody; IHC, immunohistochemical; LFTs, liver function tests; PCR, polymerase chain reaction; SIADH, syndrome of inappropriate antidiuretic hormone secretion.

Table 19.3 Less common causes of encephalitis and myelitis in immunocompetent patients

Etiology	Epidemiology	Clinical features	Diagnosis
Viruses			
Eastern equine encephalitis virus	Coastal states (Atlantic and Gulf); children and elderly disproportionately affected	Ranges from subclinical to fulminant; mortality 50–70%	Serology
Hendra virus	Endemic in Australia; associated with horse exposure	Nonspecific	Contact local health department or Special Pathogens Branch at CDC
B virus	Transmitted by bite of old-world macaque	Vesicular eruption at site of bite followed by neurologic symptoms, including transverse myelitis	Culture and PCR of vesicles and CSF
Lymphocytic choriomeningitis virus	Peak incidence in fall and winter	Orchitis, parotitis	Serology
Measles virus	Vaccine-preventable illness; measles inclusion body encephalitis onset 1–6 months after infection; subacute sclerosing panencephalitis (SSPE) a late manifestation (>5 years after infection)	Measles encephalitis nonspecific; SSPE has a subacute onset with progressive dementia, myoclonus, seizures and ultimately death	CSF antibodies, brain tissue PCR; EEG changes often diagnostic
Mumps virus	Vaccine-preventable illness	Parotitis, orchitis; hearing loss frequent	Serology, throat swab PCR, CSF culture or PCR
Murray Valley encephalitis virus	Peak incidence in aboriginal children; indigenous to Australia and New Guinea	Nonspecific presentation; case fatality 15–30%	Serology (may cross-react with other flaviviruses)
Nipah virus	Epidemics in South East Asia	Myoclonus, dystonia, pneumonitis	Serology (Special Pathogens Branch, CDC)
Powassan virus	Tick-borne; endemic in New England states and Canada	Nonspecific	Serology
Rubella virus	Vaccine-preventable illness	Neurologic findings typically occur at same time as rash and fever	Serology, CSF antibodies
Vaccinia	Infection or vaccination as precipitating event; thought to be autoimmune phenomenon	Vaccinia rash (localized or disseminated)	CSF antibodies, serum IgM (natural infection)
Venezuelan equine encephalitis virus	Central/South America; rarely in border states of USA (Texas, Arizona)	Myalgias, pharyngitis, upper respiratory symptoms variably present	Serology, viral cultures (blood, oropharynx), CSF antibody
Western equine encephalitis virus	Summer and early fall onset; Western USA and Canada, Central and South America	Nonspecific	Serology
Bacteria			
Borrelia burgdorferi	Tick-borne infection, with encephalitis in early disseminated Lyme disease, encephalopathy in late disease	Facial nerve palsy (often bilateral), meningitis, radiculitis; may be associated with or follow erythema migrans rash	Serology (serial EIA and Western blot), CSF antibody index, CSF PCR
Coxiella burnetii	Animal exposures (particularly placenta and amniotic fluid)	Nonspecific	Serology
Treponema pallidum	Sexually transmitted disease, with meningoencephalitis seen in early disseminated disease and progressive dementia in late disease	Protean manifestations including temporal lobe focality (mimics herpes simplex virus), general paresis, psychosis, dementia	CSF VDRL (specific but not sensitive), serum RPR with confirmatory FTA-ABS
Tropheryma whipplei	–	Progressive subacute encephalopathy; oculomasticatory myorhythmia pathognomonic; variably enteropathy, uveitis	CSF PCR, PAS-positive cells in CSF, small bowel biopsy

Table 19.3 Less common causes of encephalitis and myelitis in immunocompetent patients—cont'd

Etiology	Epidemiology	Clinical features	Diagnosis
Protozoa			
Balamuthia mandrillaris	Central America (natives and immigrants)	Subacute progressive disease characterized by space-enhancing lesions, often with cranial nerve palsies and hydrocephalus (similar to tuberculosis)	Serology (research laboratories), brain histopathology
Naegleria fowleri	Summer months; children and adolescent boys at highest risk; swimming in fresh water, and particularly water sports, a risk factor	Anosmia, progressive obtundation; typically a neutrophilic pleocytosis	Motile trophozoites on wet mount of warm CSF, brain histopathology
Helminths			
Baylisascaris procyonis	Pica, particularly near raccoon latrine	Obtundation, coma; typically with significant CSF and peripheral eosinophilia	CSF and serum antibodies (Perdue Department of Veterinary Pathology)

CSF, cerebrospinal fluid; EIA, enzyme immunoassay; FTA-ABS, fluorescent treponemal antibody absorption; PAS, periodic acid-Schiff; PCR, polymerase chain reaction; RPR, rapid plasma reagin; SSPE, subacute sclerosing panencephalitis.

Table 19.4 Selected pathogens of unknown neurotropic potential that are anecdotally associated with encephalitis and myelitis

Etiology	Epidemiologic and clinical features	Diagnosis
Viruses		
Adenovirus	Sporadic cases; children and immunocompromised at greatest risk; variably associated respiratory symptoms	Viral culture or PCR from respiratory site, CSF, or brain tissue
Human herpes virus 6	Most commonly reported in immunocompromised, particularly bone marrow transplant recipients; latent infection of neural tissues making significance of detection in brain tissue difficult to determine	CSF PCR
Hepatitis C	Hepatitis C seropositive patient	CSF PCR
Human metapneumovirus	Newly described pathogen almost exclusively in children	Respiratory tract PCR
Influenza virus	Sporadic disease in children, with most reports from Japan and South East Asia; upper respiratory symptoms; acellular CSF, 10% with bilateral thalamic necrosis; high mortality	Respiratory tract culture, PCR or rapid antigen; CSF and brain PCR infrequently positive
Parvovirus B19	Sporadic cases, variably associated with skin rash	IgM antibody, CSF PCR
Rotavirus	Typically children, winter months, usually with diarrhea	Stool antigen, CSF PCR (CDC)
Bacteria		
Chlamydia spp.	Anecdotal reports with *C. psittaci* and *C. pneumoniae*	Nasopharyngeal swab; respiratory or CSF PCR
Mycoplasma pneumoniae	In some pediatric series, the most common cause of encephalitis; respiratory symptoms variably present, but pneumonia rare; often with white matter involvement consistent with ADEM	PCR of nasopharyngeal swab or respiratory culture; serum IgM, CSF PCR rarely positive

ADEM, acute disseminated encephalomyelitis; CSF, cerebrospinal fluid; PCR, polymerase chain reaction.

Myelitis may occur in patients with or without concomitant encephalitis. Myelitis is specifically characterized by motor weakness, rising sensory deficit, and early bowel and bladder involvement.[3,36] The motor weakness may be of the upper motor neuron type (manifested as spasticity, hyperreflexia, and extensor plantar reflexes) or the lower motor neuron type (manifested as flaccid weakness and decreased or absent deep tendon reflexes). Acute flaccid paralysis is seen in a number of viral causes of myelitis, including infection caused by enteroviruses (e.g. poliovirus and enterovirus 71) and flaviviruses (e.g. WNV, JEV and tick-borne encephalitis virus).[2,36,37] However, in most cases a specific viral cause is never determined. Chronic myelitis is often caused by retroviruses;[3,36] human T-cell leukemia virus 1 (HTLV-1) causes disease primarily in the thoracic spinal cord with resultant progressive spastic paraparesis, and HIV may cause spastic paraparesis and sensory ataxia.

When both halves of the spinal cord are affected, the entity is referred to as transverse myelitis.[3,36] Patients with transverse myelitis

present with a syndrome mimicking transection of spinal cord with involvement of the motor, sensory and autonomic pathways; virtually all patients have paresthesias, numbness or radicular dysesthesias with an associated sensory level. Autonomic symptoms can include constipation, bowel or bladder incontinence, and urinary retention. Transverse myelitis may be produced by direct invasion of the spinal cord with organisms such as viruses or *Borrelia burgdorferi*; vasculitis of the anterior spinal artery caused by varicella-zoster virus (VZV), tuberculosis and syphilis may also lead to transverse myelitis. In most cases, there is no identifiable cause.

Evidence of inflammation or infection at sites distant from the CNS may be useful in making a microbiologic diagnosis in patients with encephalitis and myelitis. For instance, rickettsial diseases, VZV and WNV often have associated skin manifestations. Stomatitis and ulcerative lesions in the mouth or an exanthem in a peripheral distribution might suggest enterovirus infection. Patients with tuberculous and fungal meningoencephalitis may have suggestive pulmonary findings. Regional lymphadenopathy is typically present in patients with *Bartonella* encephalopathy. Diarrhea may be seen with enterovirus, adenovirus or rotavirus infections.

A syndrome frequently misclassified as encephalomyelitis, based on the similar clinical presentation, is postinflammatory encephalomyelitis, occurring days to weeks after an infectious illness or immunization. The most widely cited example is acute disseminated encephalomyelitis (ADEM), seen primarily in children and adolescents, and characterized by poorly defined white matter lesions on MRI that enhance following gadolinium administration (Fig. 19.2).[38,39] Postinflammatory encephalomyelitis is presumed to be mediated by an immunologic response to an antecedent antigenic stimulus, and is thought to account for 5–15% of cases.[4,40] As many as 90% of patients report an infectious illness, or receipt of an immunization, in the weeks before onset of ADEM.[39] Viral infections associated with ADEM include measles, mumps, rubella, varicella-zoster, Epstein–Barr, cytomegalovirus, herpes simplex, hepatitis A and Coxsackie virus. Immunizations temporally associated with ADEM include vaccines for JEV, yellow fever, measles, influenza, smallpox, anthrax and rabies virus; however, a direct causal association with these vaccines is difficult to establish. Onset of ADEM generally begins between 2 days and 4 weeks following the antigenic stimulus, with rapid onset of encephalopathy, with or without meningeal signs;[39] neurologic features depend upon the location of the lesions.

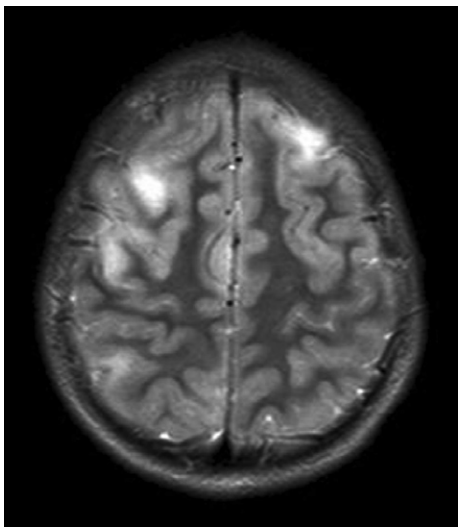


Fig. 19.2 Brain MRI of a 3 year old with new onset seizures showing increased T2 signal affecting the white matter in diffuse regions of the cerebral cortex consistent with acute disseminated encephalomyelitis.

PATHOLOGY AND PATHOGENESIS

Most viruses and other infectious agents that cause encephalitis and myelitis gain access to the CNS via the hematogenous route, causing diffuse neurologic dysfunction. The initial site of entry and replication varies depending on the organism and may include the respiratory tract (e.g. measles virus), gastrointestinal tract (e.g. enteroviruses) or skin (e.g. arboviruses). Following local infection, a viremia follows, leading to invasion and replication with the CNS.[41] Why certain viruses are more neurotropic than others is unknown, although it has been postulated that the small size of the arboviruses allows them to escape clearance by the reticuloendothelial system; arboviruses likely enter the CNS via cerebral capillaries, with vascular endothelial cell infection preceding infection of the neural parenchyma.[42] Recent studies have identified hypertension as a risk factor for the development of WNV neuroinvasive disease, suggesting that defects in the blood–brain barrier may be a risk factor for the development of WNV encephalitis.[43]

Another mechanism that organisms such as rabies virus and polioviruses use to gain access to the CNS is retrograde spread through neuronal networks. After introduction through the skin, usually via a bite, rabies virus replicates in the skeletal muscle and travels to the CNS via the peripheral nerves. In cases of prolonged incubation periods, the rabies virus likely remains close to the site of viral entry.[44] After spread of the virus to the brain via peripheral nerves, rabies virus disseminates rapidly throughout the CNS. Despite the high fatality rate, rabies CNS infection causes relatively benign neuropathologic changes without evidence of neuronal death.

A third pathogenic mechanism that organisms use to gain entry into the CNS is exemplified by free-living amebae such as *Naegleria fowleri*. These organisms enter transnasally and pass through the cribriform plate with invasion into the frontal lobes of the brain.[45]

The pathogenesis of herpes simplex encephalitis (HSE) is incompletely understood.[46–48] Serologic studies suggest that one-third of cases are the result of primary infection and two-thirds are a result of reactivation.[49] For primary HSE, theorized routes of entry into the CNS include hematogenous dissemination, migration from the nasopharyngeal mucosa through the cribriform plate, or retrograde spread from viral-infected ganglion to the brain tissue.[50] In cases of HSV reactivation, one theory is that the virus replicates in the trigeminal ganglion and spreads to the frontal and temporal lobes via the tentorial nerves.[46]

In acute viral encephalitis, the classic histopathologic findings include perivascular mononuclear cell inflammation, neuronal destruction, neuronophagia and microglial nodules.[40] Most of the pathology occurs in the gray matter. In contrast, the white matter is primarily affected in postinfectious encephalitis where there is perivenular mononuclear inflammation, edema and demyelination of brain tissue with a relative sparing of the axons.[9]

Infectious agents can also cause a clinical syndrome of encephalitis without CNS parenchymal involvement. For example, tuberculous and fungal meningitis can lead to hydrocephalus and cranial nerve palsies, causing symptoms similar to encephalitis. Alternatively, agents causing vasculitis lead to infarcts of the brain with resultant focal neurologic deficits mimicking encephalitis.

In patients with encephalomyelitis following infection or vaccination (i.e. as in ADEM), perivascular inflammation of mononuclear cells (T cells and macrophages) and perivascular demyelination are observed.[39] Other histopathologic features that have been described include varying degrees of cerebral edema, perivascular lymphocytic infiltrates with fibrin deposition and perivascular hemorrhagic necrosis.

DIAGNOSIS

Although many cases of encephalitis and myelitis remain undiagnosed, and many of the causes are not treatable, a thorough diagnostic evaluation is important. Identification of a specific agent may allow for the

discontinuation of potentially toxic antimicrobial therapy and may be useful for prognosis, potential prophylaxis for contacts, counseling of patients and families, and initiating public health interventions.[2]

The initial laboratory testing of an individual should include a complete blood count, tests of renal and hepatic function, and coagulation studies. Results of these studies are often nonspecific but may suggest a particular etiology. For example, a low white blood cell count, low platelet count and elevated liver transaminases might suggest *Ehrlichia* or *Anaplasma* infection. A baseline chest radiograph should be obtained, as a focal infiltrate would be suggestive of particular pathogens (e.g. fungal or mycobacterial infections), and might prompt further diagnostic studies such as bronchoscopy.

Neuroimaging studies are important to perform in all patients with encephalitis; MRI is more sensitive at detecting abnormalities than CT, and is the preferred study.[2] Diffusion-weighted MRI is superior to conventional MRI for the detection of early signal abnormalities in viral encephalitis caused by HSV, enterovirus 71 and WNV. In addition, some neuroimaging patterns may suggest infection caused by certain organisms (Table 19.5). In patients with herpes simplex encephalitis, there may be significant edema and hemorrhage in the temporal lobes. Patients with flavivirus encephalitis may display characteristic patterns of mixed intensity or hypodense lesions on T1-weighted images in the thalamus, basal ganglia and midbrain. In patients with ADEM, MRI generally reveals multiple focal or confluent areas of signal abnormality in the subcortical white matter. MRI of the spine is abnormal in about 90% of patients with transverse myelitis,[3] commonly manifested as an area of increased T2-weighted hyperintensity in the central region of the spinal cord occupying two-thirds or more of the cross-sectional area; swelling of the spinal cord is seen in about 50% of patients.

Electroencephalography (EEG) should also be performed in patients with encephalitis. Although findings of the EEG are rarely specific for a given pathogen, results can be helpful in identifying the degree of cerebral dysfunction, detecting subclinical seizure activity, and may provide information about the specific area of the brain involved. For example, many patients with herpes simplex encephalitis demonstrate a temporal lobe focus with periodic lateralizing epileptiform discharges (PLEDs).

Lumbar puncture with CSF analysis (cell count and differential, glucose and protein) and a measurement of the opening pressure should universally be performed unless there is a specific contraindication.[51] Mild to moderate CSF abnormalities are characteristic of most patients with viral encephalitis. Most patients have a mononuclear cell pleocytosis with cell counts ranging from 10 to 1000/mm^3.[40] Early in the disease process, CSF pleocytosis may be absent or there may be an elevation in neutrophils. The CSF protein concentration is typically elevated, but usually less than 100–200 mg/dl, while the CSF glucose concentration is typically normal. Neutrophilic pleocytosis (particularly when the total WBC count is >1000/mm^3, CSF protein >200 mg/dl or CSF glucose less than two-thirds of serum concentrations) suggests a bacterial, mycobacterial, fungal, parasitic or noninfectious etiology, and is distinctly unusual in patients with viral infections. CSF viral cultures are of limited value in patients with viral encephalitis because of low sensitivity and are generally not recommended.[52] Although of low yield, CSF cultures for bacteria, fungi and mycobacteria should be performed as identification of a pathogen has significant implications for treatment.

Brain biopsy, previously considered instrumental in the etiologic diagnosis of encephalitis, has largely been replaced by CSF molecular tests.[2] Histopathology of brain tissue, however, may help confirm whether a patient has encephalitis as opposed to another CNS process. For certain types of infection, brain biopsy may be diagnostic. In rabies infections, for example, Negri bodies are a distinctive histopathologic feature. Intranuclear eosinophilic amorphous bodies surrounded by a halo may be seen in such diseases as herpes simplex encephalitis. Pathologic examination of the brain may also be very helpful in the diagnosis of *Balamuthia mandrillaris* and other free-living amebic infections; amebic trophozoites and cysts can be detected by hematoxylin-eosin stained sections of necrotic regions of brain tissue.

In addition to the above studies, there are often epidemiologic clues that can help guide specific diagnostic testing, such as season of the year, geographic locale, prevalence of disease in a community, travel history, hobbies and recreational activities, occupation, insect and animal exposure, and vaccination history (see Table 19.1). Specific clinical findings should also direct other sites for culture or other diagnostic tests. For example, respiratory viral cultures should

Table 19.5 Possible etiologic agents of encephalitis based on neuroimaging findings

Neuroimaging finding	Possible infectious agents
Arteritis and infarctions	Varicella-zoster virus, Nipah virus, *Rickettsia rickettsii, Treponema pallidum*
Calcifications	Cytomegalovirus (cortical), *Toxoplasma gondii* (periventricular), *Taenia solium*
Cerebellar lesions	Varicella zoster virus, Epstein-Barr virus, *Mycoplasma pneumoniae*
Hydrocephalus	*Mycobacterium tuberculosis*, *Cryptococcus neoformans*, *Coccidioides immitis*, *Histoplasma capsulatum*, *Balamuthia mandrillaris*
Focal lesions in basal ganglia, thalamus and/or brain stem	Epstein–Barr virus, Eastern equine encephalitis virus, Murray Valley encephalitis virus, St Louis encephalitis virus, Japanese encephalitis virus, West Nile virus, enterovirus 71, influenzae virus (acute necrotizing encephalopathy), human transmissible spongiform encephalopathies
Space-occupying lesions	*Toxoplasma gondii*, *Balamuthia mandrillaris*, *Taenia solium*
Subependymal enhancement	Cytomegalovirus
Temporal and/or frontal lobe involvement	Herpes simplex virus, varicella-zoster virus, human herpesvirus 6, West Nile virus, enteroviruses, *Treponema pallidum* (medial lobes)
White matter abnormalities	Varicella zoster virus, cytomegalovirus, Epstein–Barr virus, human herpesvirus 6, HIV, Nipah virus, JC virus, measles virus (subacute sclerosing panencephalitis), *Baylisascaris procyonis*, acute disseminated encephalomyelitis

This is not meant to be a comprehensive list to detail all etiologic agents based on neuroimaging finding, but to suggest that certain etiologies have been associated with findings on neuroimaging studies.

be performed if patients have respiratory symptoms. If the patient has a rash, skin lesions should be cultured or biopsied.

Testing for specific agents includes various laboratory methods, such as antigen detection, culture, serology and molecular diagnostics.[2] The optimal method varies, depending both on the particular agent and the duration of symptoms. For instance, for many of the herpesviruses, molecular testing of CSF is the assay of choice, while serology is the preferred methodology for many of the arboviruses. However, very early in the course of disease, PCR may be positive for arboviruses, while after several weeks, there is evidence of HSV intrathecal antibody production.

More in-depth discussion of diagnostic testing is provided in the agent-specific chapters; however, there are some pathogens that should be routinely considered in all patients with encephalitis. Herpes simplex encephalitis is a treatable and relatively common cause of encephalitis, and an HSV PCR should be performed on the CSF of all patients with a clinical diagnosis of encephalitis. False-negative PCR tests can occur within the first 72 hours of onset, and if herpes simplex encephalitis is strongly suspected (e.g. in a patient with temporal lobe involvement), a repeat HSV PCR on a second sample of CSF from later in the disease course is recommended.[53,54] Enterovirus and varicella PCR should be done on CSF since these are also common causes of encephalitis.[30] Testing for *M. pneumoniae* deserves special mention since it is one of the leading agents identified serologically among pediatric patients with encephalitis;[21,22] diagnostic testing should include IgM on acute serum and IgG on paired samples, with attempts to amplify the bacteria from CSF and nasopharyngeal swabs in suggestive cases.

Testing for other agents should be individualized for each patient, with consideration of the patient's exposures, travel, season of the year and clinical and laboratory characteristics (see Tables 19.1–19.4). Many infections require acute and convalescent (i.e. paired) serum samples to determine a diagnosis.[2] Although results of paired serologies are not available during the acute presentation, analysis may be useful for the retrospective diagnosis of an infectious agent; therefore, a serum specimen collected during the acute phase of the illness should be stored and tested in parallel when the convalescent serum sample is drawn. Since arboviruses are a leading cause of encephalitis, testing for geographically relevant viruses should be considered during the appropriate season. Recently, IgM and IgG capture ELISAs have become useful and widely available for the diagnosis of arboviral encephalitis.[55,56] For WNV, detection of intrathecal IgM antibody is both a specific and a sensitive method for diagnosis.[57] It is important to realize that there is substantial cross-reactivity among the flaviviruses (e.g. WNV, SLE and JEV); plaque-reduction neutralization assays may be helpful in distinguishing which flavivirus is involved in the event of elevated titers.

Serologic testing for *Rickettsia*, *Ehrlichia* and *Anaplasma* spp. should be performed in all encephalitis patients during the appropriate season and with travel to, or residence in, endemic areas, especially since these are treatable etiologies. Empiric therapy should not be withheld in patients with a compatible clinical presentation, however, since antibodies are not always present early in the course of illness.[20] Detection of antibodies against free-living amebae (e.g. *Balamuthia*) in patients with compatible clinical and laboratory findings is suggestive of amebic meningoencephalitis.[16,17]

MANAGEMENT

One of the most important first steps in management of patients with encephalitis is to consider treatable causes of encephalitis. Specific antiviral therapy is generally limited to infections causes by herpesviruses (especially HSV-1 and VZV) and HIV.[2] Aciclovir (10 mg/kg intravenously q8h in children and adults with normal renal function; 20 mg/kg intravenously q8h in neonates) should be initiated in all patients with suspected viral encephalitis and continued until HSV-1 has been excluded by PCR. Empiric therapy for acute bacterial meningitis should be initiated when clinical and laboratory testing is compatible with bacterial infection.[51] If rickettsial or ehrlichial infections are suspected, empiric doxycycline should also be used. Clinicians should consider conditions that mimic infectious encephalitis, particularly if no etiology is identified in the first week of illness.

In patients suspected to have postinfectious encephalomyelitis (i.e. ADEM), steroids or other immunotherapies are often recommended.[2] High-dose intravenous corticosteroids (methylprednisolone 1 g intravenously daily for at least 3–5 days, followed by an oral taper for 3–6 weeks) are generally recommended for ADEM; plasma exchange should be considered in patients who respond poorly to corticosteroids. Although there is no clear evidence of effective therapy for transverse myelitis, intravenous methylprednisolone (followed by oral prednisone) may shorten the duration of illness and improve outcome;[3] one study, however, did not confirm this benefit.[58] Some subsets of patients may also receive clinical benefit from plasma exchange or cyclophosphamide,[59] although more studies are needed.

In addition to directed therapy for patients with encephalitis, aggressive supportive care is critical, and minimizing secondary brain injury should be made a high priority. Complications of encephalitis include elevated intracranial pressure, hydrocephalus, stroke and seizures; these should be managed expectantly, often in an intensive care setting.[60]

REFERENCES

References for this chapter can be found online at http://www.expertconsult.com

Chapter **20**

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Gregory C Townsend

Brain abscess and other focal pyogenic infections of the central nervous system

Focal pyogenic infections of the central nervous system (CNS) include brain abscess, spinal cord abscess, subdural empyema, epidural abscess and suppurative intracranial phlebitis. These conditions are characterized by the presence of one or more localized and well-defined collections of purulent material within the cranial vault or the paraspinal space. They exert their effects largely by direct involvement and destruction or encroachment of the brain or spinal cord parenchyma, by elevation of intracranial pressure or by interference with flow of blood or cerebrospinal fluid (CSF).

Infections in contiguous structures generally lead to infections relating to their anatomy (Fig. 20.1). The frontal and ethmoidal paranasal sinuses underlie the anterior cranial fossa and often lead to infections in or near the frontal lobe. The sphenoid sinuses adjoin the sella turcica, temporal lobes and cavernous sinuses; sphenoid sinusitis may lead to infections in the frontal or temporal lobes or pituitary gland or to cavernous sinus thrombosis. Middle ear infections and mastoids may spread to the temporal lobe, cerebellum or brain stem. The focal nature of these infections often manifests by focal neurologic deficits, rather than global CNS dysfunction.

Brain Abscess

Brain abscess is a focal suppurative process of the brain parenchyma. The diagnosis and management of brain abscess have undergone considerable changes during the past several years resulting from the availability of noninvasive radiographic diagnostic techniques, the use of antimicrobials that penetrate across the blood–brain barrier and into abscesses, and the refinement of minimally invasive surgical procedures.

EPIDEMIOLOGY

Brain abscess is uncommon The lifetime incidence is approximately 1.3 per 100 000 person-years, although the overall incidence decreased from 2.7 per 100 000 person-years in 1935–44 to 0.9 per 100 000 person-years in 1965–81. The highest rates were observed in children 5–9 years old and adults over 60 (approximately 2.5 per 100 000 person-years each).[1] Brain abscesses account for approximately 1 in 10 000 hospital admissions in the USA.[2]

The patients' predominant age varies with the predisposing factors; brain abscesses following otitis media are most common among young children and older adults, while those due to paranasal sinusitis are commonly among older children and young adults.[2–4] Brain abscesses are approximately two to three times as common among males as among females.[2]

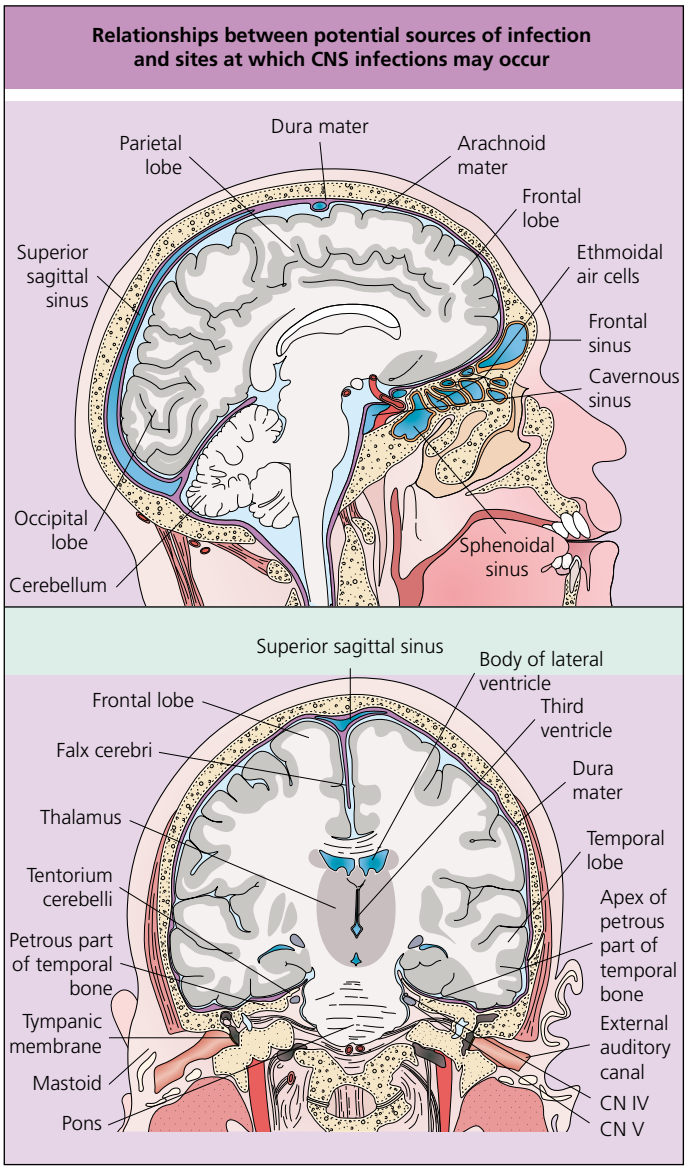


Fig. 20.1 Anatomic relationships between potential contiguous sources of infection and sites at which focal pyogenic central nervous system infections may occur.

The major predisposing factors are:

- an associated contiguous focus of infection (e.g. sinusitis, subacute or chronic otitis media and mastoiditis);
- trauma (e.g. penetrating head injury, post-neurosurgery);
- hematogenous spread from a distant focus (e.g. in association with pulmonary, skin, abdominal and pelvic infections, endocarditis, cyanotic heart disease and esophageal dilation or sclerosis of varices); and
- cryptogenic (no recognized focus).

Brain abscesses associated with a contiguous focus account for approximately 40–50% of the total; the percentage of cases without an identified predisposing factor has been reduced to approximately 15% with the use of newer, more sensitive imaging procedures. In addition, abscesses have been reported in immunocompromised individuals or after a cerebrovascular accident.

The commonest underlying conditions in developed countries are otitis media and mastoiditis.[4] However, the role of these conditions has declined in association with early antimicrobial therapy for ear infections. The current risk of brain abscess in cases of otitis media is less than 0.5%.[5] Brain abscess is also associated with cyanotic heart disease and trauma.[6] Other associated head and neck infections include paranasal and dental infections. A report from Turkey observed bacterial meningitis to be the most common predisposing factor in children.[7]

Brain abscesses associated with otitis media and mastoiditis are most common in the temporal lobe and cerebellum (Table 20.1). Brain abscesses associated with sinusitis occur primarily in the frontal or the temporal lobes. The frontal lobe is also most commonly affected following dental infections. Abscess can occur following complicated neurosurgical procedures. Post-traumatic abscesses usually occur with a penetrating wound, but also occur in closed head injuries such as facial trauma. Bullet and shrapnel wounds to the brain can result in necrotic tissue leaving metal fragments that can become a nidus for infection. Injuries include penetrating pencil tip and lawn dart injuries in children. Abscess presentation can occur months to years after the precipitating event. In one study, the median time to development of brain abscess was 113 days[8] and can occur up to 52 years after war-related penetrating head injuries.[9]

Abscesses occurring in a distant primary site following hematogenous spread are often multiple; approximately 10–15% of patients with brain abscesses have multiple abscesses. They tend to occur in the distribution of the middle cerebral artery at the junction of the gray and white matter, where microcirculatory flow is poorest. Cyanotic congenital heart disease and chronic pyogenic lung diseases (e.g. lung abscess, bronchiectasis) are common predisposing factors. Hereditary hemorrhagic telangiectasia (Osler–Weber–Rendu disease) is also associated with brain abscess; it is thought that in these cases pulmonary arteriovenous malformations allow septic microemboli to bypass the normal pulmonary filter and gain access to the cerebral circulation. Abscesses can follow dental extractions and other manipulations, dilatation of esophageal strictures and endoscopic sclerosis of esophageal varices.

Table 20.1 Site of brain abscess based on predisposing condition

Predisposing condition	Site
Otitis media or mastoiditis	Temporal lobe Cerebellum
Paranasal sinusitis	Frontal lobe Temporal lobe
Dental infection/manipulation	Frontal lobe
Trauma/neurosurgery	Related to wound
Meningitis	Cerebellum Frontal lobe
Cyanotic heart disease	Middle cerebral artery distribution
Pyogenic lung disease	
Bacterial endocarditis	
Gastrointestinal source	
T-cell deficiency	
Neutropenia	

PATHOGENESIS AND PATHOPHYSIOLOGY

The main pathogenetic factors are a source of virulent organisms and the presence of ischemic or devitalized brain tissue. The vulnerability of compromised tissue to brain abscess is evidenced by their occurrence after trauma or cerebrovascular accident, in association with cyanotic heart or lung disease and in areas of poor local perfusion such as the junction of gray and white matter.

Experimental models demonstrated differences in the abilities of various organisms to induce brain abscesses. There are two main postulated mechanisms by which brain abscess may occur in association with a contiguous focus of infection:

- direct extension through infected bone; and
- hematogenous spread through emissary or diploic veins or spread through local lymphatics.

Infections associated with otogenic infections may also spread through the internal auditory canal, between suture lines or through cochlear aqueducts. Abscesses developing after trauma or neurosurgical procedures may follow deep wound injury with direct inoculation into the brain parenchyma or may be a result of extension of a superficial infection through compromised tissue.[10,11]

The sites most commonly involved by solitary brain abscess are the frontal and temporal lobes, followed by the frontoparietal region, the parietal, cerebellar and occipital lobes.[12] These areas are those most likely to be associated with a contiguous focus or hematogenous seeding. Although rare, abscesses may occur in other areas, such as the pituitary gland, thalamus, basal ganglia and brain stem, and may be associated with specific predisposing conditions. For example, abscesses of the pituitary are often associated with pre-existing pituitary adenomas and with sphenoidal sinusitis.

Experimental animal data, surgery and autopsy findings and radiographic examinations indicate that brain abscesses develop in a four-stage process:[13] an early and late cerebritis (days 1–3 and 4–9, respectively) and an early and late capsule formation (days 10–13 and day 14 and later, respectively). These represent a continuum rather than discrete steps. The evolution of this process is dependent upon the causative organism(s), local factors, host immunologic status and antimicrobial therapy.

The microbiology depends upon the site of the initiating infection, the patient's underlying condition and the geographic locale (Table 20.2).[14–18] The organisms most commonly isolated are anaerobes, streptococci, the Enterobacteriaceae, *Staphylococcus aureus* and fungi; approximately 30–60% are polymicrobial. The predominate bacteria are anaerobes that generally are members of the oropharyngeal flora and are associated with otorhinolaryngeal infections. However, anaerobes of gastrointestinal and female genital origin can also spread hematogenously and cause an abscess. The anaerobes most commonly isolated include anaerobic streptococci, *Bacteroides* (including *B. fragilis*), *Prevotella*, *Porphyromonas*, *Clostridium*, *Propionibacterium*, *Fusobacterium*, *Eubacterium*, *Veillonella* and *Actinomyces* spp. The aerobic pathogens include aerobic and microaerophilic streptococci which include alpha-hemolytic streptococci, *Streptococcus milleri* and *Streptococcus pneumoniae*. Aerobic Gram-negative rods are common following neurosurgery or head trauma and the common ones are *Klebsiella pneumoniae*, *Escherichia coli*, *Pseudomonas* spp. and *Proteus* spp. *Staph. aureus* is common after trauma.

High frequency of brain abscess due to *K. pneumoniae* is mainly seen in South East Asia.[19] It was found with or without meningitis and as

Table 20.2 Likely pathogens and suggested empiric therapy for brain abscess based on predisposing condition

Predisposing condition	Likely pathogens	Empiric therapy
Otitis media or mastoiditis	Aerobic, anaerobic and microaerophilic streptococci Anaerobic Gram-negative bacilli (i.e. *Prevotella* spp., *Bacteroides* spp.) *Staphylococcus aureus* Enterobacteriaceae *Pseudomonas aeruginosa*	Third-generation cephalosporin + metronidazole ± antistaphylococcal penicillin or a penicillin
Sinusitis	Aerobic, anaerobic and microaerophilic streptococci Anaerobic Gram-negative bacilli (i.e. *Prevotella* spp., *Bacteroides* spp.) Enterobacteriaceae *Haemophilus* spp. *Fusobacterium* spp.	Penicillin or third-generation cephalosporin + metronidazole
Trauma or post-neurosurgery	*Staphylococcus aureus* Coagulase-negative staphylococci Enterobacteriaceae *Streptococcus* spp. *Pseudomonas aeruginosa* *Clostridium* spp.	Vancomycin + third-generation cephalosporin ± metronidazole
Congenital heart disease	Aerobic and microaerophilic streptococci *Staph. aureus* *Haemophilus* spp.	Third-generation cephalosporin + vancomycin
Pyogenic lung disease	Streptococci *Nocardia asteroides* *Actinomyces* spp. *Fusobacterium* spp. Anaerobic Gram-negative bacilli (i.e. *Prevotella* spp., *Bacteroides* spp.) *Nocardia* spp. Alpha-hemolytic streptococci *Enterococcus* spp. *Haemophilus* spp.	Penicillin or third-generation cephalosporin plus metronidazole Trimethoprim-sulfamethoxazole Vancomycin + ampicillin and gentamicin + antistaphylococcal penicillin
Gastrointestinal source	Enterobacteriaceae *Bacteroides fragilis*	Third-generation cephalosporin + metronidazole
T-cell deficiency	*Toxoplasma gondii* *Nocardia* spp. *Mycobacterium* spp. *Listeria monocytogenes* *Cryptococcus neoformans*	Variable
Neutropenia	Enterobacteriaceae *Pseudomonas aeruginosa* Fungi, especially *Aspergillus*, *Mucor* and *Candida*	Third- or fourth-generation cephalosporin, meropenem Amphotericin B

a metastatic infection that is associated with community-acquired primary liver abscess. Fungi were the organisms most commonly isolated in a report from Saudi Arabia[20] and are particularly common causes of brain abscesses in immunocompromised patients; *Aspergillus* spp. are especially common in patients who have bone marrow and solid organ transplants.[21] Patients with T-cell immunity defects (including AIDS patients) are predisposed to infections with intracellular organisms such as *Toxoplasma gondii*, *Nocardia* spp., *Cryptococcus neoformans*, *Mycobacterium* spp. and fungi.[15]

Parasites that can cause brain abscess or infection include *Taenia solium* (cysticercosis), *Entamoeba histolytica*, *Schistosoma japonicum* and *Paragonimus* spp.[22]

PREVENTION

The appropriate use of antibiotics in patients with predisposing infections such as otitis media and mastoiditis is the primary means of prevention. Other measures include surgical correction of cyanotic congenital heart disease, maintenance of dental hygiene, management of pyogenic lung infections and attention to proper sterile techniques during neurosurgical procedures. In patients who have underlying T-cell defects, measures to prevent exposure to *T. gondii* are recommended.[17]

CLINICAL FEATURES

The clinical manifestations are due largely to the presence of a space-occupying lesion.[12,23] The most common symptoms are headache (75% of patients), which is usually hemicranial, altered mental status, focal neurologic findings (especially hemiparesis) (>60%), fever (50%), nausea and vomiting (25–50%) and seizures (usually generalized) (30%). Nuchal rigidity may occur if the abscess is near the meninges. Vomiting often develops in association with increased intracranial pressure.

Other signs and symptoms vary depending on the abscess stage, size and anatomic location:

- abscesses of the frontal lobe are characterized by headache, drowsiness, global mental status changes, inattention, hemiparesis with unilateral motor signs and expressive speech disturbances;
- temporal lobe abscesses may present with ipsilateral headache and aphasia (if the abscess involves the dominant hemisphere) and a visual field defect;
- cerebellar abscesses are associated with vomiting, ataxia, nystagmus and dysmetria; and
- brain stem abscesses generally present with headache, facial weakness, hemiparesis, vomiting and dysphagia.

Papilledema is present in the older child and adults, and bulging fontanels may be present in the younger infant. Rapid deterioration with nuchal rigidity suggests the possibility of abscess rupture into the intraventricular or subarachnoid space. A ruptured abscess may produce purulent meningitis associated with signs of neurologic damage.

Laboratory findings may include a peripheral leukocytosis and a left shift, but approximately 40% of patients have normal leukocyte concentrations. Serum sodium levels may be lowered resulting from inappropriate antidiuretic hormone production. Platelet counts may be high or low. The erythrocyte sedimentation rate is often elevated. An elevated C-reactive protein is both sensitive (77–90%) and specific (77–100%) when used to distinguish brain abscess from cerebral neoplasms.

The differential diagnosis of brain abscess includes subdural empyema, epidural abscess, bacterial meningitis, cerebral neoplasm, cranial osteomyelitis, mycotic aneurysm, suppurative thrombophlebitis, venous sinus thrombosis, cerebrovascular infarct or hemorrhage, headace and migraine, and encephalitis.

DIAGNOSIS

Radiographic imaging with contrast-enhanced CT or MRI has contributed greatly to diagnosis and management of brain abscess.[24,25] Plain skull radiographs are insensitive but the presence of air indicates the need for further evaluation. Technetium-99 (^{99m}Tc) brain scanning is very sensitive and is the procedure of choice where CT or MRI is unavailable; there is some evidence that ^{99m}Tc scanning may be more sensitive than CT in the early cerebritis stage. ^{99m}Tc-HMPAO labeled leukocyte single photon emission CT (SPECT) has been examined as a potential means of distinguishing brain abscess from other focal cerebral parenchymal lesions, such as neoplasms. Ultrasonography may also be used if other techniques are unavailable.

CT scanning, preferably with contrast, provides a rapid means of detecting the size, the number and the location of abscesses, and has become the mainstay of diagnosis and follow-up care. It is used to confirm the diagnosis, to localize the lesion, and to monitor the progression after treatment. However, CT scan results can lag behind clinical findings. The characteristic appearance of brain abscess on CT scan varies with the stage of the disease.[26] During the cerebritis stage, cerebral edema is prominent; no abnormalities may be seen in the early cerebritis stage. As capsule formation progresses, the abscess appears as a lesion with a hypodense center composed of necrotic debris surrounded by ring enhancement, which may in turn be surrounded by hypodense cerebral edema (Fig. 20.2). Although highly sensitive, CT scanning is not specific. These findings may also be seen in patients with cerebral neoplasms, cerebrovascular accidents or granulomas.

Many authorities consider MRI to be the first method for diagnosis. It permits accurate diagnosis and excellent follow-up because of its superior sensitivity and specificity. Compared with CT scanning, it offers better ability to detect cerebritis, greater contrast between cerebral edema and the brain, and early detection of satellite lesions and the spread of inflammation into the ventricles and subarachnoid space. MRI is more sensitive than CT in the early cerebritis stage of brain abscess, where it appears as slightly low intensity on T1-weighted images and very low intensity on T2-weighted images, and may be more sensitive in diagnosing lesions in the posterior fossa due to the absence of bone artifact[24,25] (Fig. 20.3). It may allow distinction of abscess fluid from cerebrospinal fluid, which may be important if intraventricular rupture is suspected. Enhancement with gadolinium-DTPA allows evaluation of disruption of the blood–brain barrier and permits greater distinction of the radiographic appearance of the central abscess, capsule and surrounding edema. Examination by ^{1}H magnetic resonance spectroscopic imaging has been proposed as a means of distinguishing brain abscess from other focal cerebral parenchymal lesions.

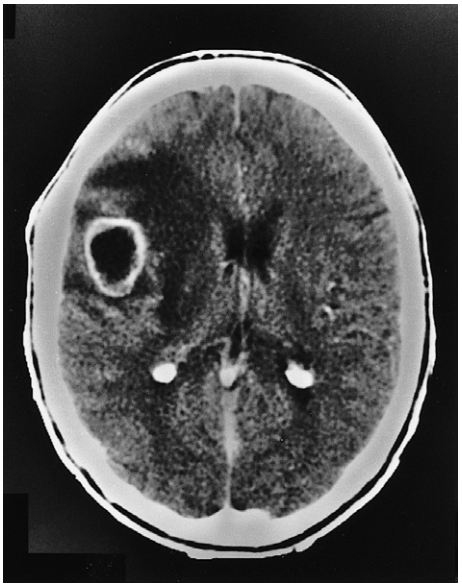


Fig. 20.2 Contrast-enhanced CT scan of the head in the coronal projection of a 43-year-old man with an atrial septal defect that persisted after attempts at surgical repair. The patient presented with seizures after undergoing dental work for which he did not receive antimicrobial prophylaxis. Note the ring-enhancing lesion in the right frontoparietal region with edema and mass effect.

It must be noted that the appearance of edema and contrast enhancement on CT and MRI may be diminished or absent in immunocompromised patients, possibly due to poor host inflammatory response.

Lumbar puncture should be avoided in patients who have known or suspected brain abscess. The yield of CSF culture is low (less than 10% positive) and the risk of herniation is considerable (approximately 15–30%). The number of white blood cells is generally elevated. It reaches 100 000/µl or higher when a rupture of the abscess into the CSF occurs. Many red blood cells are generally observed at that time, and the CSF lactic acid level is then elevated above 500 mg/l. In patients in whom both diagnoses are considered, blood cultures should be obtained and appropriate empiric therapy should be initiated, after which an imaging procedure should be performed. Lumbar puncture may be performed if there is no evidence of a mass lesion or signs of raised intracranial pressure.

Serologic testing for anti-*Toxoplasma* IgG antibody in blood and anticysticercal antibodies on CSF specimens can aid in the diagnosis of toxoplasmosis or neurocysticercosis. These may be of value in the immunocompromised patient.

Specimens obtained during surgery or stereotactic CT-guided aspiration should be sent for aerobic, anaerobic, mycobacterial and fungal culture and, when indicated, for protozoa. In one study, the Gram stain revealed organisms in 82% of cases and the culture was positive in 88%.[24] Special stains should be performed which include fungal stains (e.g. methenamine silver, mucicarmine), an acid-fast stain for mycobacteria and a modified acid-fast stain for *Nocardia* spp. In patients with HIV infection, polymerase chain reaction (PCR) examination of CSF may be useful in diagnosing tuberculous abscesses.

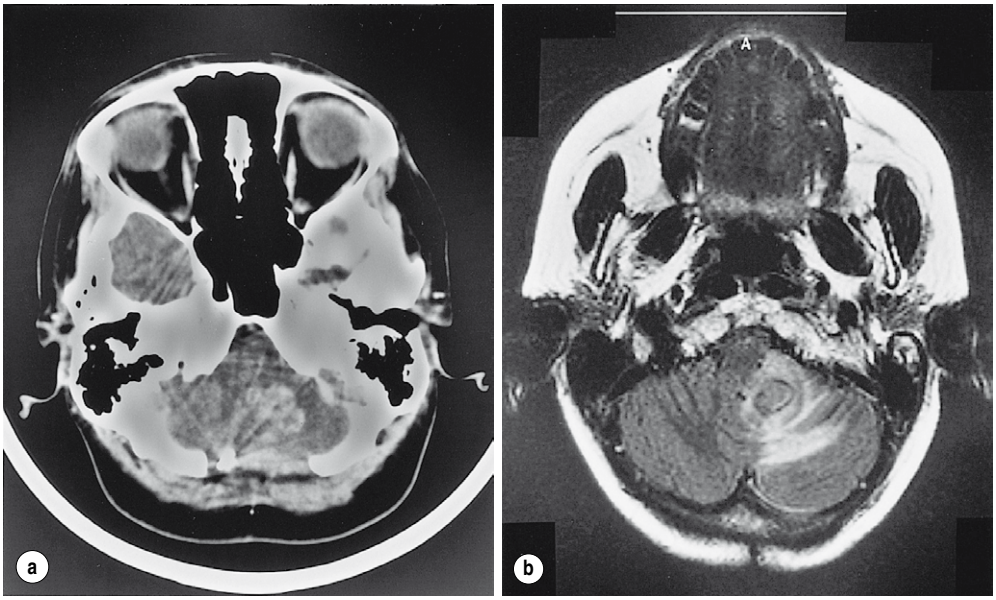


Fig. 20.3 Contrast-enhanced CT and MRI scans of the head in the coronal projection of a 43-year-old woman with headaches after a recent fall on her head. (a) CT scan image reveals a cystic ring-enhancing lesion in the left cerebellum. Note the prominent bone artifact. (b) T1-weighted MRI scan image reveals an enhancing cystic lesion in the left cerebellum with significant surrounding edema. Bone artifact is absent. Both CT and MRI scans were felt to be most consistent with a primary or metastatic neoplasm, but culture of material obtained at stereotactically guided aspiration grew *Staphylococcus aureus*.

MANAGEMENT

Before abscess encapsulation and localization, antimicrobial therapy, accompanied by measures to control increasing intracranial pressure, are essential.[27] Once an abscess has formed, surgical excision or drainage combined with prolonged antibiotics (usually 4–8 weeks) remains the treatment of choice. Some neurosurgeons advocate complete evacuation of the abscess, while others advocate repeated aspirations as indicated. The procedures used are aspiration through a burr hole and complete excision after craniotomy. In addition to its therapeutic effect, drainage permits microbiologic evaluation of abscess material, which guides antimicrobial therapy. Since aspiration is as effective as excision in most cases and is less invasive, it has become the procedure of choice. Stereotactic CT-guided aspiration permits accurate access even to areas that had been difficult to reach by aspiration, such as the brain stem, cerebellum and thalamus,[28] and multiple abscesses may be drained. Neuroendoscopic aspiration has also been used with success in a small number of patients.

Emergency surgery should be performed if a single abscess is present. Abscesses larger than 2.5 cm are excised or aspirated, while those smaller than 2.5 cm or are at the cerebritis stage are aspirated for diagnostic purposes only. In cases of multiple abscesses or in abscesses in essential brain areas, repeated aspirations are preferred to complete excision. High-dose antibiotics for an extended period may be an alternative approach in this group of patients.

A number of factors should be considered when trying to decide the appropriate approach to therapy.

Knowledge of the etiologic agent(s) by recovery from blood, CSF, abscess or other normally sterile sites is essential because it allows for the most appropriate selection of antimicrobials.

The duration of the symptoms before diagnosis is an important factor. Antibiotic therapy during the early stage, before expanding mass lesion exists, may prevent progress from cerebritis to abscess. Patients who have symptoms for less than 1 week have a more favorable response to medical therapy than patients with symptoms persisting longer than 1 week.

Growing evidence suggests that under certain circumstances brain abscess may be treated without surgical drainage. Small abscesses (less than 2.5 cm diameter) and abscesses in the cerebritis stage may respond to antimicrobial therapy alone. Medical therapy alone may also be indicated if the patient is a poor surgical candidate. In these cases, prolonged courses of antibiotics (at least 8 weeks of parenteral therapy) and close monitoring with sequential CT or MRI scans are necessary; MRI may be especially useful here because of its lack of ionizing radiation. Patients treated with medical therapy alone usually demonstrate clinical improvement before significant changes in the CT scan are observed. CT scanning and MRI should eventually show a decrease in the size of the lesion, a decrease in accompanying edema, and a lessening of the enhancement ring. Improvement on CT scans is generally observed within 1–4 weeks (average, 2.5 weeks) and complete resolution in 1–11 months (average, 3.5 months). Radiographic abnormalities may persist for months after successful therapy (Fig. 20.4).

The antimicrobial treatment of the brain abscess is generally long (6–8 weeks) because of the prolonged time needed for brain tissue to repair and close abscess space. The initial course is through an intravenous route, often followed by an additional 2–6 months of appropriate oral therapy. A shorter course (3–4 weeks) may be adequate in patients who had surgical drainage. Because of the difficulty involved in the penetration of various antimicrobial agents through the blood–brain barrier, the choice of antibiotics is restricted, and maximal doses are often necessary.

Initial empiric antimicrobial therapy should be based on the expected etiologic agents according to the likely predisposing conditions, the primary infection source and the presumed pathogenesis of abscess formation. Antibiotics should be parenteral, have activity against the likely pathogens, penetrate into abscess fluid (and the site of any underlying infection) in adequate concentrations and be bactericidal. The combination of penicillin or a third-generation cephalosporin (cefotaxime or ceftriaxone) plus metronidazole is effective as empiric therapy in most cases (see Table 20.2). An antistaphylococcal penicillin (such as flucloxacillin, nafcillin or oxacillin) should be used if staphylococci are suspected. Vancomycin should be used instead if methicillin-resistant *Staph. aureus* (MRSA) is suspected or identified, or if the patient is allergic to β-lactam antimicrobials. Cefepime or ceftazidime are administered to treat *Pseudomonas aeruginosa*. Patients with HIV infection may require therapy for toxoplasmosis.

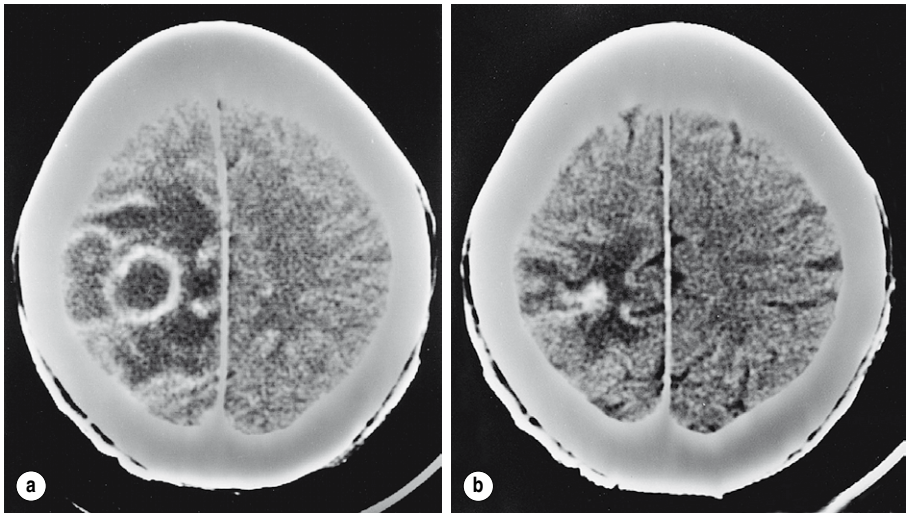


Fig. 20.4 Contrast-enhanced CT scans of the head in the coronal projection of a 66-year-old woman with a group B streptococcal brain abscess demonstrating evolution of the abscess during and after surgical and antimicrobial therapy. (a) The original scan demonstrates a hypodense necrotic center surrounded by an enhancing capsule and hypodense edema. (b) Seven weeks later, after stereotactically guided aspiration and a full course of antimicrobial therapy, the central cavity can no longer be seen, although the enhancement and surrounding edema persist to a small degree.

Open craniotomy with debridement, intraventricular lavage and intraventricular as well as intravenous antibiotics are recommended after intraventricular rupture of brain abscess. Changes in therapy should be guided by results of microbiologic examination and by clinical and radiographic progress.

Adjunctive therapy with corticosteroids, mannitol and hyperventilation may be indicated where there is evidence of increased intracranial pressure. Corticosteroid use is controversial. Steroids can retard encapsulation, increase necrosis, reduce antibiotic penetration into the abscess, alter CT scans and can produce a rebound effect when discontinued. If used to reduce cerebral edema, therapy should be of short duration. The appropriate dosage, timing and any effect of steroid therapy on the course of the disease are unknown. The routine use of corticosteroids in the absence of increased intracranial pressure cannot be recommended.

Prognosis has improved considerably, particularly since the introduction of CT scanning. Mortality is now less than 30%. Long-term sequelae may occur in about one-third of patients and include mental retardation, seizures and focal neurologic deficits. Poor prognostic factors include delayed or missed diagnosis, multiple lesions, deep-seated lesions, intraventricular rupture, severe impairment of mental or neurologic status (including coma), fungal etiology and extremes of age.

Subdural Empyema and Intracranial Epidural Abscess

Subdural empyema and epidural abscess are focal collections of purulent material between the dura mater and arachnoid mater, and outside the dura mater, respectively. Subdural empyema accounts for approximately 15–20% of all focal intracranial infections.

EPIDEMIOLOGY

Subdural empyema in adults is most often a complication of acute or chronic bacterial paranasal sinusitis, otitis media or mastoiditis.[29] It is the most common intracranial complication of sinusitis, accounting for approximately 60% of such cases.[30] The frontal and ethmoidal sinuses are the foci in over half of the cases. Hematogenous spread from a distant source may also occur. In children the most common predisposing condition is bacterial meningitis. Other predisposing conditions include trauma, neurosurgical procedures, infection of a pre-existing subdural hematoma, ethmoidectomy, polypectomy and nasal surgery. Hematogenous spread from a distant location (mainly from the lung) is uncommon. As with brain abscess, there is a male predominance.[31]

Most cases of intracranial epidural abscess in the past were due to paranasal sinusitis (particularly frontal), otitis media, mastoiditis or cranial trauma. Currently many cases occur after neurosurgical procedures. Males are again more commonly afflicted.[32]

PATHOGENESIS AND PATHOPHYSIOLOGY

As with brain abscesses, extension of infection into the epidural or subdural space from a contiguous focus may occur by extension through infected bone or by hematogenous seeding through emissary veins. Intracranial epidural abscess is generally associated with subdural empyema and overlying osteomyelitis, and usually consists of a localized lesion with a central collection of pus surrounded by a wall of inflammatory reaction that may calcify. These abscesses rarely spread downwards into the spinal canal because the dura is very tightly attached around the foramen magnum. In patients with subdural empyema, infection can spread rapidly through the subdural space until limited by its natural boundaries. These include the falx cerebri, tentorium cerebelli, base of the brain, foramen magnum posteriorly and the anterior spinal canal. Within the compartments defined by these boundaries, the progressing infection behaves as an expanding mass lesion.

As the lesion expands, intracranial pressure increases and the cerebral parenchyma is compromised. Interference with flow of blood or of CSF may cause cerebral edema and hydrocephalus. Septic thrombosis of veins within the affected subdural or epidural space may lead to thrombosis of cavernous sinuses or cortical veins, leading to infarction of brain tissue.

Organisms commonly isolated from adult patients with subdural empyema and intracranial epidural abscess include anaerobes, aerobic streptococci, staphylococci, *Strep. pneumoniae*, *Haemophilus influenzae* and other Gram-negative bacilli. Polymicrobial infections are common; in one study, more than 50% of infections were polymicrobial.[31,33] In children, the most common agents are those that are responsible for the underlying meningitis. In the past, this has been *H. influenzae* in children outside the neonatal age; however, as the

relative frequency of *H. influenzae* meningitis declines, its role in subdural empyema may diminish as well. Anaerobes were identified in children in cases associated with sinusitis.[34]

Organisms commonly isolated from epidural abscesses associated with paranasal sinuses or ears are microaerophilic or anaerobic streptococci, *Propionibacterium*, *Peptostreptococcus* and *Prevotella* spp. When isolated associated with neurosurgery, the most likely organisms are staphylococci, especially *Staph. aureus*, and aerobic Gram-negative bacteria. The infection can also spread inward from osteomyelitis of the skull or be introduced by fetal monitoring probes during delivery.[35]

CLINICAL FEATURES

The signs and symptoms result from the infection and the slowly expanding intracranial mass. The most prominent early symptoms associated with subdural empyema and intracranial epidural abscess are fever and headache. The headache is often focal at onset but may become generalized. These symptoms are usually followed by papilledema and focal neurologic defects. Abscesses near the petrous portion of the temporal bone may be associated with palsies of cranial nerves V and VI, causing unilateral facial pain and lateral rectus muscle weakness. Periorbital edema and subgaleal abscess (Pott's puffy tumor) may be found in up to about one-third of patients. Signs of increased intracranial pressure (such as vomiting, gait disturbances and mental status changes) and meningeal irritation may follow and may be accompanied by seizures, hemiparesis and hemisensory defects.[31] Epidural abscess complicating sinus infections can cause purulent nose or ear drainage.[36]

DIAGNOSIS

CT and MRI scanning are the diagnostic procedures of choice. Imaging reveals a hypodense lesion with displacement of the arachnoid mater in both entities, with accompanying displacement of the dura mater noted in patients who have subdural empyema. Mass effect is more common with subdural empyema than with epidural abscess (Fig. 20.5). Capsule formation with contrast enhancement may be seen in either condition, but is more common with epidural abscess (Fig. 20.6). Cranial osteomyelitis may also be noted in patients who have underlying contiguous foci of infection. Gadolinium-enhanced MRI may detect lesions not noted on CT (because the lesions may be isodense with the cerebral tissue on CT). As with brain abscess, lumbar puncture is contraindicated in patients with known or suspected subdural empyema or epidural abscess (see Practice Point 6). Clinical deterioration was reported in 33 of 280 patients (11.3%) who underwent lumbar puncture with subdural empyema[31] and in 1 of 12 patients (8.3%) with epidural abscess.[32]

The differential diagnosis of epidural abscess includes metastatic tumors, disc and bony disease (including vertebral discitis and osteomyelitis), meningitis and early stage of herpes zoster.

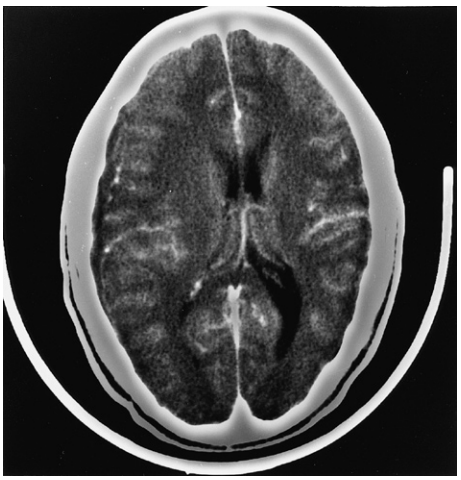


Fig. 20.5 Contrast-enhanced CT scan of the head in the coronal projection of a 23-year-old man with fever and headache. There is a small isodense extra-axial fluid collection in the subdural space on the right, with significant mass effect shown by right-to-left midline shift and effacement of the right lateral ventricle. There was also opacification of the frontal and ethmoid sinuses, suggesting sinusitis as the source of this subdural empyema.

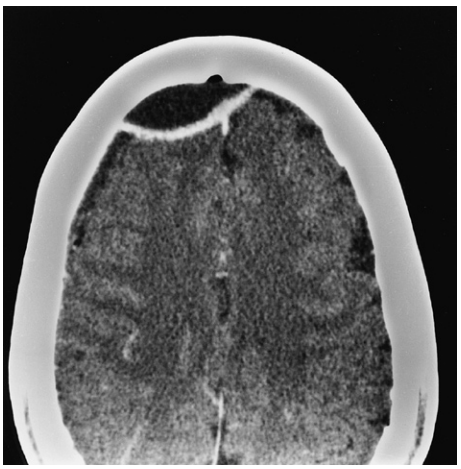


Fig. 20.6 Contrast-enhanced CT scan of the head in the coronal projection of a 19-year-old man with otitis media who presented with sinus congestion 1 week earlier. Plain films of the sinuses revealed opacification of the right maxillary and ethmoidal sinuses and an intracranial air–fluid level. Note the intracranial gas in the right frontal region abutting a hypodense region in the epidural space with ring enhancement and surrounding edema, representing an intracranial epidural abscess.

MANAGEMENT

Treatment requires a combination of drainage and antibiotic therapy. Surgical evacuation is necessary for management of most patients. It can provide immediate decompression and specimens for microbiologic studies and should be accomplished by craniotomy or by the use of burr holes. Although the optimal surgical procedure has not been established, in one study of 699 patients with subdural empyema, the use of limited procedures such as burr holes was associated with a worse prognosis than more extensive procedures such as craniotomies.[37] It may also be necessary to debride the primary infection. Samples should be submitted for Gram stain and aerobic and anaerobic cultures. Antimicrobial therapy alone may be used for a limited number of patients with very small fluid collections.[31,32]

The choice of empiric antimicrobial therapy depends upon the patient's age, predisposing conditions and the primary infection site (Table 20.3). Antimicrobial choice should be guided and eventually be specific after the results of Gram stain of aspirated material and cultures are available. In adults, the wide variety of possible pathogens and the potential for polymicrobial infection dictate the use of broad-spectrum therapy. In children, therapy should be directed against the likely causes of meningitis. Parenteral antimicrobial therapy should be continued for 3–6 weeks, with close monitoring of clinical status and radiographic appearance.

Table 20.3 Pathogens and suggested empiric antibiotic regimens for subdural empyema and intracranial epidural abscess based on underlying condition

Predisposing condition	Likely pathogens	Empiric therapy
Sinusitis	Aerobic, anaerobic and microaerophilic streptococci Anaerobic Gram-negative bacilli (i.e. *Prevotella* spp., *Bacteroides* spp.) *Staphylococcus aureus* Enterobacteriaceae *Haemophilus influenzae* *Streptococcus pneumoniae*	Third-generation cephalosporin + metronidazole ± antistaphylococcal penicillin or a penicillin
Otitis media or mastoiditis	Aerobic, anaerobic and microaerophilic streptococci Anaerobic Gram-negative bacilli (i.e. *Prevotella* spp., *Bacteroides* spp.) *Staph. aureus* Enterobacteriaceae *Haemophilus influenzae*	Third-generation cephalosporin + metronidazole ± antistaphylococcal penicillin or a penicillin
Trauma	*Staph. aureus* Coagulase-negative staphylococci Enterobacteriaceae *Pseudomonas aeruginosa*	Vancomycin + third-generation cephalosporin
Dental infection	Aerobic, anaerobic and microaerophilic streptococci *Fusobacterium* spp. Anaerobic Gram-negative bacilli (i.e. *Prevotella* spp., *Bacteroides* spp.)	Penicillin or third-generation cephalosporin + metronidazole
Neonate	Enterobacteriaceae Group B streptococci *Listeria monocytogenes*	Third-generation cephalosporin + ampicillin
Infant or child	*Streptococcus pneumoniae* *Haemophilus influenzae* *Neisseria meningitidis*	Third-generation cephalosporin ± vancomycin

Suppurative Intracranial Phlebitis

Suppurative intracranial phlebitis is inflammation of the blood vessels within the cranium resulting from infection.[38]

EPIDEMIOLOGY, PATHOGENESIS AND PATHOPHYSIOLOGY

These usually follow infections of the paranasal sinuses, middle ear, mastoids, face, oropharynx and, in particular, the nasal furuncle. They may be associated with subdural empyema, epidural abscess or bacterial meningitis. Conditions associated with increased blood viscosity or hypercoagulability increase the risk of nonsuppurative thrombosis of the intracranial venous sinuses as well as of suppurative intracranial phlebitis

Spread generally occurs along emissary veins. The venous sinuses most commonly involved are the cavernous sinus, lateral sinus and superior sagittal sinus. If there is sufficient involvement of the vasculature, then cerebral edema and hemorrhagic infarction may result. The infarcts tend to occur in venous watershed regions. Involvement of the superior sagittal sinus or of the lateral sinuses may block CSF reabsorption and lead to hydrocephalus and increased intracranial pressure. Involvement of contiguous structures can lead to brain abscess, subdural empyema, epidural abscess or meningitis, or distant seeding and infection of the lungs and other organs.

The microbiology of suppurative intracranial phlebitis is similar to that of subdural empyema and intracranial epidural abscess, with *Staph. aureus*, streptococci and anaerobes being most commonly identified.

CLINICAL FEATURES

Clinical manifestations vary with the location of the involved venous sinuses or cortical veins.

Cavernous sinus thrombosis is associated with palsies of cranial nerves III, IV, V and VI, producing loss of corneal reflexes, ophthalmoplegia and hypesthesia over the upper part of the face. Papilledema and visual loss may result from obstruction of retinal venous return.

Lateral sinus thrombosis involves cranial nerves V and VI, resulting in altered facial sensation and lateral rectus muscle weakness. Obstruction of venous CSF resorption may cause communicating hydrocephalus and increased intracranial pressure. Cranial nerves IX, X and XI may also be affected.

Involvement of the superior sagittal sinus may also diminish CSF resorption. In addition, obstruction of venous drainage from the motor cortex region of the cerebral hemispheres may lead to weakness of the legs.

Cortical vein thrombosis may be neurologically silent or produce only transient defects if collateral venous drainage can compensate for thrombosis. If collateral flow is inadequate, the lesion will manifest as progressive neurologic defects. The precise nature of the defects depends on the location of the veins involved. Unilateral or bilateral extremity weakness, hemiparesis, aphasia, seizures and mental status changes may be seen.

DIAGNOSIS

MRI is more sensitive than CT and is the diagnostic procedure of choice. The appearance on MRI is that of increased signal within the

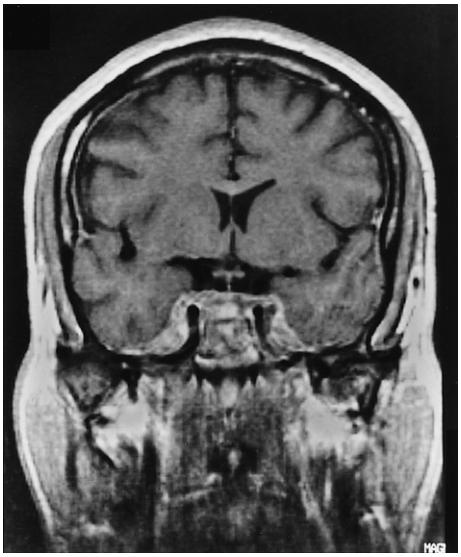


Fig. 20.7 Contrast-enhanced MRI scan of the head in the sagittal projection of a 29-year-old man with sinus congestion and headache. There is non-uniform signal intensity of the cavernous venous sinuses, indicating cavernous sinus thrombosis. The sphenoid, ethmoidal and maxillary paranasal sinuses also demonstrated abnormal signal intensity.

involved vessel (Fig. 20.7). Sensitivity of MRI can be enhanced by the use of magnetic resonance imaging angiography. CT may be used when MRI is unavailable. If CT or MRI is unremarkable and suppurative intracranial phlebitis is still suspected, angiography should be performed.

MANAGEMENT

Empiric antimicrobial therapy is similar to that employed for subdural empyema and intracranial epidural abscess (see Table 20.2). Control of increased intracranial pressure may be necessary with adjunctive measures such as corticosteroids, hyperosmolar agents and hyperventilation. Anticoagulant therapy has been used with some success, but carries the risk of hemorrhagic infarction. Surgery may be required for drainage of associated abscesses.

Spinal Epidural Abscess

Spinal epidural abscess is a focal infection of the paraspinal epidural space. It requires prompt recognition and appropriate management to avoid potentially serious complications.

EPIDEMIOLOGY

Spinal epidural abscess is rare and occurs in 2–25 patients per 100 000 admitted to hospital.[39] The increased sensitivity and accuracy of diagnosis, aided by the use of MRI, has increased the rate of diagnosis of this infection over the last 30 years.[40] The incidence is similar in males and females and the median age of onset is approximately 50 years.

PATHOGENESIS AND PATHOPHYSIOLOGY

Spinal epidural abscess may be secondary to epidural catheter placement (risk of 0.5–3%)[41] and contiguous source of infection, such as vertebral osteomyelitis, pyogenic infectious discitis, penetrating trauma or decubitus ulcers, or may arise by hematogenous spread from a distant source.[29,40–43] The use of face masks when epidural catheters are placed has been recommended as a means to reduce spinal abscesses after epidural catheter placement.

A history of back trauma, spinal and paraspinal procedures, diabetes mellitus, alcoholism, intravenous drug use, HIV infection, tattooing, acupuncture, local spinal injections, and contiguous bony or soft tissue infection are common risk factors.[42] In addition to pulmonary infections, endocarditis and bacteremia as seen in patients who have brain abscess and subdural empyema, possible sources for hematogenous seeding of the spinal epidural space include cutaneous, intra-abdominal, pelvic and genitourinary infections. Spread from these sources occurs via the paravertebral venous plexus. In about a third of patients no identifiable source for the infection can be found.

The abscess can extend longitudinally in the epidural space, damaging the spinal cord by direct compression, interruption of the arterial blood supply and local vasculitis, thrombosis and thrombophlebitis of nearby veins, and by bacterial toxins and mediators of inflammation. Longitudinal extension is common in infection arising in the epidural space. The average extent is three to five spinal cord segments, but the whole length of the spinal column may be affected.

In the acute stage the abscess may have pus, but after about 2 weeks it may contain only granulation tissue.

In most cases in adults, the thoracic spine is involved, while cervical and lumbar involvement are more common in children. There is a male predominance.[43]

The organism responsible for the majority of cases is *Staph. aureus*, accounting for almost three-quarters of all cases in a meta-analysis of 915 cases reported.[43] The rate of proportion of infections due to MRSA is steadily rising, reaching 40% of all *Staph. aureus* in one report.[42] Other staphylococci, aerobic and anaerobic streptococci, *Escherichia coli* and *P. aeruginosa* are also common.[43] Polymicrobial infections may occur, but are uncommon. *Mycobacterium tuberculosis* can cause up to 25% of the cases.[44]

CLINICAL FEATURES

There are four clinical stages:

- fever and focal back pain;
- nerve root compression with nerve root pain;
- spinal cord compression with accompanying deficits in motor, sensory, and bowel and bladder sphincter function; and
- paralysis.

Respiratory compromise may also be present if the cervical cord is involved.[45]

Progression tends to be rapid with infection due to direct hematogenous spread, and may be accompanied by severe pain. Progression to the second stage tends to occur slowly in those with abscesses secondary to vertebral osteomyelitis, but may accelerate after that. Headache, meningismus and focal tenderness are common signs and symptoms.

DIAGNOSIS

Clinical suspicion is essential, as the presentation may not be typical. A recent study showed that the mean duration of symptoms between onset of symptoms and the first emergency room visit or admission was 5 and 9 days, respectively, and the median number of visits to the emergency room before admission was two (1–8).[46] Sedimentation rate is usually elevated and the leukocyte count may be elevated or normal.[46]



Fig. 20.8 Contrast-enhanced MRI scan of the spine in the coronal projection of a 28-year-old man with a 1-week history of headache, fever and sweats. Physical examination demonstrated meningismus but no focal neurologic deficits. Scans in the sagittal section demonstrated a substance nearly isointense with the spinal cord and running nearly the length of the cord. This scan clearly demonstrates impingement and anterior displacement of the cord by the spinal epidural abscess.

Plain radiographs may reveal changes of osteomyelitis or discitis. Gadolinium-enhanced MRI has supplanted CT and myelography as the diagnostic procedure of choice for spinal epidural abscess[47,48] (Fig. 20.8). MRI is highly sensitive (91%) and can identify osteomyelitis and intramedullary spinal cord lesions. It also enables early detection of an abscess while it is still small prior to spinal cord compression. If MRI is not available, myelography should be performed.

Recovery of etiologic organism(s) from blood, abscess contents or CSF is important. CSF may show high protein and pleocytosis. Gram stain is usually negative and cultures are positive in less than a 25% of the specimens.[42]

The differential diagnosis includes many conditions that cause back pain and neurologic deficits, including disc and bony disease, discitis, osteomyelitis, meningitis, malignancy and early stage herpes zoster.

MANAGEMENT

Urgent decompression and surgical drainage or aspiration plus parenteral antimicrobial therapy are indicated for the management of most patients.[49] Decompression and drainage are ordinarily achieved by laminectomy, but CT-guided aspiration may be performed in selected patients. Two studies reported the outcome in patients who received only medical therapy to be similar to those who had surgery.[50,51] The use of a nonsurgical conservative approach is rarely used but may be attempted in those individuals with significant surgical risk who have an identified pathogen (through blood culture or aspiration). Close neurologic monitoring and follow-up MRI that demonstrates reduction and resolution of the abscess are needed in these cases. If there is neurologic deterioration, surgery should be performed without delay.

An antistaphylococcal penicillin should be instituted as empiric therapy in all patients; vancomycin may be used in patients who are allergic to penicillin and those with MRSA. Antibiotics effective against Gram-negative organisms (i.e. third-generation cephalosporins) and anaerobes (i.e. metronidazole) should be added in patients whose underlying source might have been an intra-abdominal, pelvic or genitourinary infection. The usual duration of parenteral therapy should be at least 3–4 weeks; if osteomyelitis is present, it should be continued for 8 weeks. Therapy length can be adjusted according to resolution of the abscess on MRI. Follow-up MRI is generally performed at about 4 weeks if the patient is improving, or earlier if clinical deterioration occurs.

Spinal Cord Abscess

This is a rare condition defined as a focal suppurative processes of the spinal cord parenchyma. It usually involves the thoracic segment of the spinal cord and is generally hematogenous in origin. The lungs are usually the source of infection and it can occur in intravenous drug users and secondary to congenital dermal sinuses. The predominant pathogens are *Staph. aureus*, streptococci, *Listeria monocytogenes* and *Burkholderia cepacia*.[52] Symptoms mimic those of spinal epidural abscess. Diagnosis may be made by CT, MRI or myelography. Treatment consists of surgical debridement and prolonged antimicrobial therapy. Antibiotics should be directed against *Staph. aureus* and possibly Gram-negative bacilli.

REFERENCES

References for this chapter can be found online at http://www.expertconsult.com

Chapter 21

Thomas P Bleck

Tetanus and botulism

Tetanus and botulism are completely preventable diseases that nevertheless continue to cause serious morbidity and mortality, especially in the developing world. Both are caused by members of the genus *Clostridium*; in both cases, disease is caused by exotoxins that conform to an A–B model, each being composed of an enzymatic (A) portion and a binding (B) portion. The biologic activity resides in the A portion, whereas the B subunits may bind to target cells but are biologically inactive. Common to both toxins described, A and B are domains of a single protein that is cleaved by proteolytic activity of the bacterium (Fig. 21.1). Tetanus is caused by tetanospasmin (TS), a neural toxin that predominantly interferes with the inhibition of spinal cord reflexes, and is produced by the obligate anaerobic bacterium *Clostridium tetani*. Botulism is caused by one of several neurotoxins (types A, B, E and F cause human disease) produced by *C. botulinum* (Table 21.1). There are three forms of the latter illness: food-borne botulism from ingestion of preformed toxin; wound botulism; and botulism from intestinal colonization, usually seen in infants.[1,2]

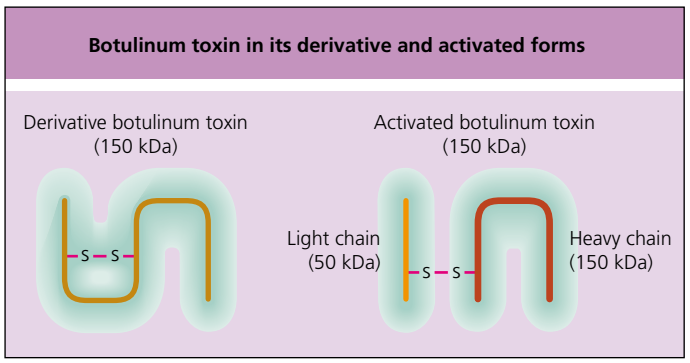


Fig. 21.1 Clostridial toxins as produced by the bacteria (left) and after nicking to produce light and heavy chains linked by a disulfide bond.

Table 21.1 Characteristics of groups of *C. botulinum* and the toxins that produce human disease

Group	Toxins produced	Proteolysis	Heat resistance of spores	Disease severity
Group I	A, B, F	Yes	High	Severe
Group II	B, E, F	No	Low	Less severe

EPIDEMIOLOGY

Tetanus is still common in developing tropical countries, where it is an important cause of death, particularly in neonates. The World Health Organization estimated in 2002 that about 180 000 newborns died worldwide from tetanus. In the developed world, however, active immunization and better hygiene, wound care and management of childbirth have meant that the disease is now rare. The annual incidence of tetanus has fallen from nearly 4 to 0.2 per million population in the USA since 1947 (Fig. 21.2); mortality has dropped even more, with the death:case ratio falling from about 50% to below 30%.[3] In England and Wales there were 175 cases of tetanus (and 19 deaths) in the period from 1984 to 2000.[4]

Neonatal tetanus usually occurs within 3–14 days of birth in infants delivered under nonsterile conditions to nonimmunized women. The umbilical cord stump is the usual portal of entry, particularly if cultural practices dictate the application of animal dung to the stump. At other ages acute wounds are the portal of entry for *C. tetani* in about 80% of cases, with the remainder associated with chronic decubitus ulcers, gangrene, abscesses or parenteral drug abuse. Tetanus is more likely to occur in the summer months when gardening and other pastimes bring people into contact with soil.

All three forms of botulism occur throughout the world, but for food-borne botulism, the causative strains, the responsible foods and the resulting illness vary in different geographic areas. Accurate data about the incidence of botulism are difficult to obtain but it is estimated that, in France, there were about 300 food-borne outbreaks between 1979 and 1988, and in the USA there were 474 outbreaks (a large proportion from Alaska) involving nearly 1050 persons between 1950 and 1990. Outbreaks were most frequent in the summer or autumn. In the UK the incidence is much lower than in Europe or the USA, with only nine outbreaks between 1922 and 1988. In 1989 the largest ever outbreak of food-borne botulism in the UK affected 27 people who had eaten hazelnut yoghurt. The illness was caused by type B toxin formed by bacteria growing in canned hazelnut conserve used to flavor the yogurt and that had been inadequately heat treated.[5]

Nearly 1000 cases of infant botulism (roughly equally divided between type A and type B *C. botulinum*) were reported in the USA between 1976 and 1990. Almost half the cases were reported from California, with an incidence of 7 per 100 000 live births. Cases occur most frequently in the second month of life and 95% of cases are in infants less than 6 months old.[1]

Most cases of food-borne botulism are associated with home-preserved meats, fish and vegetables but the common vehicles are often idiosyncratic to a country or culture.[6] In the USA (apart from Alaska), Spain, Italy and China most cases follow consumption of home-preserved vegetables (home-canned asparagus, beans and peppers in the USA, home-fermented bean curd in China) contaminated

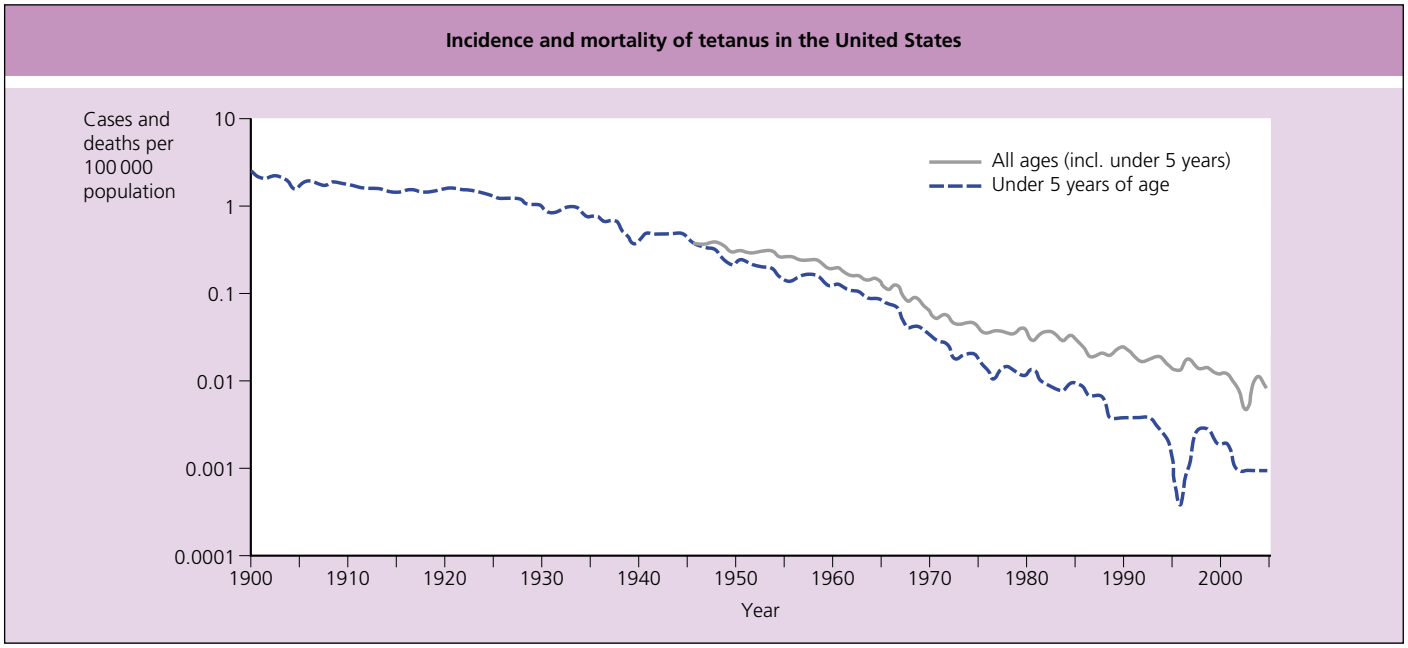


Fig. 21.2 Incidence and mortality of tetanus in the United States. From the US Centers for Disease Control and Prevention.

with type A *C. botulinum*. In Alaska, Japan, Canada and Scandinavia cases are usually due to type E toxin and follow eating fermented foods or preserved fish products; in central continental Europe cases typically arise from home-cured meats and are caused by nonproteolytic strains of type B *C. botulinum*. Commercially prepared foods are only rarely implicated. There is a significant association between infant botulism and ingestion of honey.

Occasionally, botulism follows wound infections with *C. botulinum*. The wounds are often compound fractures or penetrating wounds; cases have also been reported in intravenous drug users[7] and complicating sinusitis in chronic cocaine sniffers.

PATHOGENESIS AND PATHOLOGY

Tetanus

The clostridia are Gram-positive, anaerobic, spore-forming bacilli. In tetanus, spores of *C. tetani* contaminate a wound and germinate under anaerobic conditions. The proliferating organisms elaborate the tetanus toxin TS, one of the most potent of the known poisons, with an estimated lethal dose of 2.5 ng/kg. It is produced by a plasmid-encoded gene and is synthesized as a 151 kDa polypeptide. As with botulinum and diphtheria toxins, this polypeptide is then split by clostridial proteolytic cleavage into the light (L) chain (approximately 50 kDa and containing the enzymatic (A) domain) and the heavy (H) chain (approximately 100 kDa and containing the binding (B) domain).

From the standpoint of pathogenesis, the important difference between tetanus and botulinum toxin is that botulinum toxin remains outside of the central nervous system, while tetanus toxin enters the central nervous system and exerts most of its activity in the brain stem and spinal cord. The activity of each toxin at the cellular level is very similar, although the subunit of the transmitter release mechanism targeted by each toxin type varies.

Most of the tetanus toxin gets into the bloodstream, and then enters the central nervous system (CNS) via neurons to exert its toxicity (Fig. 21.3). The effect of each toxin is via a three-stage process: binding, internalization and induction of paralysis.[8] The H chain interacts with the ganglioside GT1 of neurons at neuromuscular junctions, both locally and distally (via the bloodstream), and enables the L chain to enter the cytoplasm of the neuron. The tetanospasmin is then transported intra-axonally at 75–250 mm/day in a retrograde manner to the cell body in the ventral horns of the spinal cord and the motor nuclei of cranial nerves and then, via trans-synaptic spread, to other neurons within the CNS. Within the neurons tetanospasmin acts as a zinc-dependent protease that cleaves synaptobrevin, a protein component of synaptic vesicles,[9] and prevents release of neurotransmitters at the presynaptic membrane. Inhibitory interneurons in the spinal cord (using glycine as a transmitter) and descending inhibitory projection neurons from the brain stem (using γ-amino butyric acid; GABA) are most severely affected, but cells of the autonomic nervous system are also involved.

There are no gross or histologic abnormalities in tetanus. Any changes described in fatal cases reflect terminal hypoxia and autonomic dysfunction.

Botulism

Food-borne botulism follows ingestion of preformed toxin, but in other forms of the disease toxin is produced *in vivo* and released when vegetative cells lyse. The toxin is not released from spores. Botulinum toxins are the most poisonous substances known, and are synthesized as a polypeptide of molecular weight 150–165 kDa, which is then broken into a heavy chain of about 100 kDa and a light chain joined by a disulfide bond. The mechanism of action is similar or identical to that of tetanospasmin, resulting in cleavage of synaptobrevin and inhibition of release of acetylcholine at peripheral cholinergic synapses. The heavy chain binds rapidly to the membrane of the presynaptic α motor neuron and then some (or all) of the toxin molecule translocates through the membrane. Finally, there is a slow paralytic step that may partially depend upon temperature and activity of the neuron. The process is slowly reversible and the synapse is inactivated for a long time. Initial recovery of function depends upon the budding and growth of new presynaptic end-plates, but the original synapse eventually can regain activity.

Infant botulism results from colonization of the infant's gastrointestinal tract with as many as 10^8 proteolytic *C. botulinum* organisms

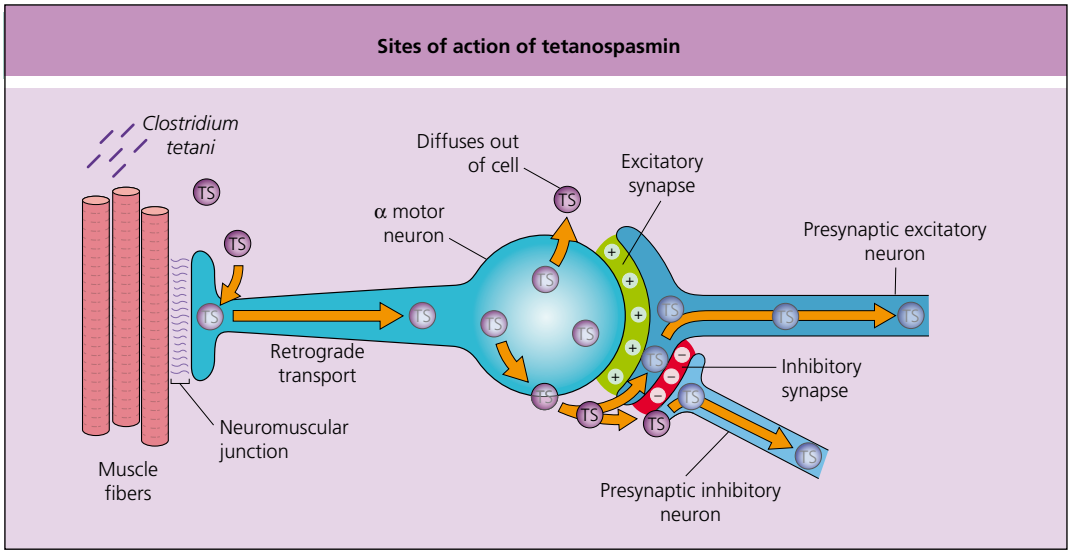


Fig. 21.3 Sites of action of tetanospasmin. Tetanospasmin (TS) is produced by *Clostridium tetani* at the site of the wound and binds and internalizes at the neuromuscular junction into the α motor neuron. It then travels by retrograde axonal flow to the cell body and diffuses out into the synapses and extracellular space of the CNS. It enters other neurons and travels further into the CNS. Its major effect is to inhibit transmitter release from the glycinergic presynaptic inhibitory neuron but it can also inhibit release of transmitters at the excitatory synapses and of acetylcholine at the neuromuscular junction.

per gram of feces.[10] The mechanisms that relate to colonization and toxin absorption from the infant gut are unclear, and both the organism and toxin may sometimes continue to be excreted in the feces for several months after the illness has resolved. Wound botulism typically results from soil contamination of severe head wounds, as occurs in warfare, or following the subcutaneous injection of contaminated material.

Death results from respiratory paralysis; there are no specific pathologic findings on gross or histologic examination in any of the forms of botulism.

PREVENTION

Tetanus

Tetanus is a completely preventable disease. Generally, concentrations of antibody to tetanospasmin as low as 0.01 IU/ml are regarded as protective against clinical tetanus, although cases have occurred in patients who have antibody concentrations at least 10-fold higher than this. Active immunization with tetanus toxoid is extremely effective. A primary series of three doses of tetanus toxoid given in infancy, either with diphtheria toxoid and acellular or whole cell pertussis (DTaP or DTP) or diphtheria toxoid alone (DT), with a booster at school entry, is virtually 100% effective for 5–10 years. Another dose should be given to school leavers. Older children and adults should receive the appropriate combined vaccine (TDaP) every 10 years.[11] Immunity wanes in the elderly, who should be specially targeted for booster immunization.[12] Neonatal tetanus can be prevented by ensuring that all pregnant women are immune.

The need for both active and passive immunization against tetanus, with toxoid and specific human tetanus immunoglobulin (HTIG), should be reviewed after any injury that brings an individual to medical attention (Table 21.2).[13]

Clean, minor wounds do not need any special treatment. Previously nonimmunized persons over 7 years of age should receive a three-dose series of TDaP, the first two doses 4–8 weeks apart and the third after 6–12 months. All other wounds, including frostbite, burns and others, should be considered to render the patient prone to tetanus. Foreign bodies and ischemic tissue should be removed. If the patient has not received a primary series then the age-appropriate tetanus vaccine and HTIG (250 IU intramuscularly), or equine tetanus antitoxin if HTIG is not available within 24 hours, should be given.

Botulism

The key to preventing botulism is adequate processing and storage of food to destroy spores and prevent their germination and toxin production. Spores are not killed by boiling at 212 °F (100 °C) but are

Table 21.2 Recommendations for use of tetanus prophylaxis in wound management

History of tetanus toxoid administration	CLEAN, MINOR WOUNDS		ALL OTHER WOUNDS	
	Tetanus toxoid*	Immunoglobulin	Tetanus toxoid*	Immunoglobulin
Unknown or less than three doses	Yes and proceed with basic immunization	No	Yes and proceed with basic immunization	Yes (250 IU HTIG or 3000 IU equine tetanus antitoxin)
Three or more doses	No, unless >10 years since last dose	No	No, unless >5 years since last dose	No

*Administered as Td (i.e. low dose of diphtheria toxoid) in adults.

destroyed by heating at 250°F (121°C) for 2.5 minutes (the type of temperature achieved under pressure processing of low-acid foods). Once toxin is formed, it can be inactivated by boiling or heating at 176°F (80°C) for 30 minutes.

Toxin production by strains of *C. botulinum* is inhibited at a pH below 4.6, in saline, and at low temperatures (below 38°F (3.3°C)); the respective values differ somewhat for different strains. Commercial canneries pay particular attention to less acid (pH >4.6) fruit and vegetables; the canning and curing of meats relies on a reduced heat treatment to kill vegetative bacteria and sodium chloride and nitrite to inhibit spore growth. Vacuum packaging of food may encourage the growth of anaerobes and there are concerns that botulinum toxin may be produced before spoilage is obvious. Toxin has been detected in mushrooms and coleslaw kept in modified atmosphere packaging. This should be preventable by piercing the packaging of fresh vegetables with air holes to allow sufficient oxygen to be present.

Honey has been associated with infant botulism and honey is not recommended as a food for infants less than 1 year old. However, other dietary sources of spores are involved, as the incidence of infant botulism has only declined by about half after the association with honey was discovered and publicized.

CLINICAL FEATURES

Tetanus

Although tetanus can occur at any age, those over 60 are at greatest risk in the developed world since they have lowest immunization levels; elderly women are at greatest risk since they are less likely to have been immunized during earlier military service.

The incubation period to the first symptom ranges from 1 day to several months but most cases start between 3 and 21 days after an acute injury. There is a correlation between the distance of the injury from the CNS and the duration of the incubation period. The time between the first symptom and the first reflex spasm is termed the period of onset. There are four clinical forms of tetanus – neonatal, localized, cephalic and generalized – depending on the predominant site of toxin action.[14]

Localized tetanus consists of fixed muscle rigidity and painful spasms, sometimes lasting weeks or months, confined to an area close to the site of the injury. It is rare and generally mild but may herald generalized tetanus. Cephalic tetanus is a particular form of localized tetanus associated with wounds to the head or face or with chronic otitis media, and manifested by atonic palsies involving the motor cranial nerves. The incubation period is often only 1–2 days and generalized tetanus may follow.

Generalized tetanus (which is by far the most common form) typically starts with rigidity and spasm of the masseter muscles, causing trismus or lockjaw and the characteristic risus sardonicus – a grimace through clenched teeth and closed mouth with wrinkled forehead and raised eyebrows (Fig. 21.4). Other muscles, first in the neck, then the thorax, back and extremities, become rigid and go into spasms, producing opisthotonos, abdominal rigidity and apnea. Tetanic spasms are intermittent, irregular and unpredictable, although they are often triggered by external stimuli, sometimes very trivial such as a sudden noise or puff of cold air, or even the internal stimulus of a distended bladder or bowel. Each spasm is sudden, painful and generalized, resulting in opisthotonos, leg extension and arm flexion; pharyngeal spasm causes dysphagia and spasm of the glottis may cause immediate asphyxiation and death. Cognitive functions are not affected. Severe tetanus is accompanied by abnormalities of the autonomic nervous system, including hypo- or hypertension, arrhythmias and flushing.

Neonatal tetanus typically starts with poor sucking and irritability, followed by trismus and tetanospasms. It has a higher death:case ratio than tetanus at other ages.

With intensive care the death rate from tetanus (which is due to respiratory dysfunction or autonomic cardiovascular instability) may be as low as 10–20%, with higher rates in infants and in the elderly. A rating scale for the severity and prognosis of tetanus may be used (Table 21.3). In general, the more rapid the evolution of symptoms and signs, the worse the prognosis but the belief that a short incubation period leads to a worse prognosis has been challenged.[14]

Complications related to spasms include vertebral and long bone fractures, glottic obstruction and asphyxia, and intramuscular hematomas. Rhabdomyolysis is common in generalized tetanus. Other complications are those related to general debility and prolonged intensive care.



Fig. 21.4 Facial spasm and risus sardonicus in a Filipino patient who has tetanus.

Botulism

Food-borne botulism usually develops 12–36 hours after ingestion of the toxin, although the interval may be as short as 6 hours or as long as 10 days. Patients who have type E toxin-mediated disease tend to have shorter, and those with type B tend to have longer incubation periods. Wound botulism occurs at a mean of 7.5 days (range 4–18 days) after the injury.[15]

Table 21.3 Rating scale for severity and prognosis of tetanus

Score 1 point for each of the following

- Incubation period <7 days
- Period of onset <48 hours
- Acquired from burns, surgical wound, compound fracture, septic abortion
- Narcotic addiction
- Generalized tetanus
- Pyrexia >104°F (40°C)
- Tachycardia >120 beats/min (>150 beats/min in neonates)

Total score provides indication of severity and prognosis

Score	Severity	Mortality
0–1	Mild	<10%
2–3	Moderate	10–20%
4	Severe	20–40%
5–6	Very severe	>50%

Note: Cephalic tetanus is always scored as severe or very severe, and neonatal tetanus as very severe.

Table 21.4 Frequency of symptoms in types A, B and E food-borne botulism[16]

Symptoms	Type A disease (% of cases)	Type B disease (%)	Type E disease (%)
Dysphagia	25–96	77–100	63–90
Dry mouth	26–83	96–100	55–88
Diplopia	50–90	57–100	85
Dysarthria	25–100	69–100	50
Fatigue	8–92	69–100	Not known
Weakness of arm	16–86	64–86	Not known
Constipation	73	17–100	25–38
Weakness of leg	16–76	64–86	Not known
Dyspnea	35–91	34	88
Vomiting	70	50–100	88–100
Dizziness	8–86	30–100	63
Diarrhea	35	8–14	10
Paresthesiae	20	12–14	Not known

Typically, botulism first affects the muscles supplied by the cranial nerves with disturbances of vision and difficulties in swallowing and speech, followed by descending weakness of muscles of the trunk and extremities that is bilateral but not necessarily symmetric. Cardiovascular, gastrointestinal and urinary autonomic dysfunction may follow. The presentation may be related to the type of toxin: autonomic symptoms occur earlier and are more prominent in intoxication with type B and E toxins.

Common presenting symptoms are diplopia, dysphagia, dysarthria, dry mouth and fatigue (Table 21.4).[16] Ptosis and ophthalmoplegia are common physical signs, together with facial weakness and a decreased gag reflex. The pupils are dilated or fixed in less than 50% of cases. Frequently, there is weakness of the extremities, although deep tendon reflexes are usually normal. Patients are usually afebrile and have no sensory deficits. Patients who have wound botulism have a similar presentation but acute gastrointestinal symptoms are lacking.

Constipation is the first sign of infant botulism, with neurologic signs developing either concurrently or up to several weeks later. The neurologic signs progress in a similar fashion to those in other forms of botulism but they may be overlooked by the parents, who merely note the infant is irritable, lethargic or unable to suck. There is a wide range of clinical illness associated with infant botulism; 50% of cases develop ventilatory failure.

DIAGNOSIS

Tetanus

The diagnosis of tetanus depends upon clinical features, and epidemiologic history and laboratory tests are usually unhelpful. There is often a moderate leukocytosis, but the CSF is normal, except for increased intracranial pressure associated with increased muscle tone (raising intrathoracic pressure, which is then transmitted). Neither electroencephalography nor electromyography is helpful. Occasionally, characteristic Gram-positive bacilli with terminal or subterminal spores may be visualized in aspirates from a wound but anaerobic cultures are rarely positive and the organism may be grown from wounds in the absence of disease. However, neither stains nor cultures of wounds are diagnostically useful.

Botulism

Routine laboratory tests are not helpful in the diagnosis of botulism. The diagnosis is best confirmed by assay of botulism toxin in the patient's blood, gastric washings or feces by means of toxin neutralization tests in mice. Toxin may also be demonstrated in the incriminated food. This test takes anything from 6 to 96 hours to perform and the initial diagnosis must therefore be based on clinical findings. *Clostridium botulinum* may be cultured or the toxin detected by an enzyme-linked immunosorbent assay in the patient's feces, particularly in infant botulism and other cases resulting from intestinal colonization.

Electrophysiologic studies show normal nerve conduction velocities, but the electromyogram is often abnormal with facilitation (an incremental increase) of the amplitude evoked in the muscle when high-frequency (20–50 per second) repetitive stimuli are applied to the relevant nerve. Testing at this frequency is uncomfortable and is usually not performed unless the electromyographer is specifically asked to examine for botulism. These abnormalities may persist for several months after the onset of illness.

DIFFERENTIAL DIAGNOSIS

Tetanus

Strychnine poisoning is the only true mimic of tetanus, although there are several other diseases that may overlap to some extent. Strychnine poisoning develops more rapidly than tetanus and there is usually no muscle rigidity between spasms; serum analysis for strychnine should be performed in suspect cases. Other causes of trismus include dystonic reactions to phenothiazines (which may be ruled out by administration of benztropine 1–2 mg intravenously or diphenhydramine 50 mg intravenously) and dental abscesses. Tetany from hypocalcemia or alkalosis tends to affect the extremities rather than the axial muscles and there is no trismus.

Botulism

The diseases most often confused with botulism are Guillain–Barré syndrome (particularly the Miller–Fisher variant, predominantly affecting the cranial nerves), myasthenia gravis and the Eaton–Lambert myasthenic syndrome, other forms of food poisoning and tick paralysis. Guillain–Barré syndrome frequently has sensory complaints and the Miller–Fisher syndrome includes prominent ataxia. Myasthenias lack autonomic dysfunction and are less fulminant than botulism. In tick paralysis a careful search will reveal the Dermacentor tick still attached.

MANAGEMENT

Tetanus

A detailed guide to the general management of the patient with tetanus is available.[14] Human tetanus immune globulin (HTIG), 500 IU as a single intramuscular injection should be given at the time of diagnosis in order to prevent further circulating toxin from reaching the CNS.[13,17] The use of intrathecal HTIG to neutralize toxin that has entered but is not yet fixed to nervous tissue[18] has not been consistently beneficial and is not routinely recommended; injections are potent stimuli for tetanic spasms.

The source of toxin should be removed by wound debridement and removal of foreign bodies. Only vegetative forms of *C. tetani* will be sus-

ceptible to antibiotics. Therapy with metronidazole (15 mg/kg intravenously followed by 20–30 mg/kg/day intravenously for 7–14 days) should be used to eradicate *C. tetani*, even though antibiotics are not likely to penetrate into the anaerobic conditions that support growth of the organism.[19] Penicillin is less suitable (it is a central GABA antagonist).

A benzodiazepine (midazolam administered intravenously at 5–15 mg/h is suitable) should be used to produce sedation, decrease rigidity and control spasms. Airway protection during spasms is paramount. If ventilation is compromised, the patient should be sedated, intubated, provided with a soft nasal feeding tube and transferred to a quiet and darkened area. A tracheostomy may be needed, and is often advisable as the patient may require much higher doses of sedation to tolerate an endotracheal tube than a tracheostomy tube. If benzodiazepines do not adequately control the spasms then the patient will need long-term neuromuscular blockade.

The management over the next few weeks is that of any ventilated patient plus specific therapy for autonomic nervous system complications and control of spasms.[20] Sympathetic hyperactivity is treated with combined α- and β-blockade or morphine. Intrathecal baclofen has been shown to be effective in controlling muscle rigidity.[18] Epidural blockade with local anesthetics may be needed. Hypotension requires fluid replacement and noradrenaline (norepinephrine) administration. Parasympathetic overactivity is rare, but if bradycardia is sustained then a pacemaker may be needed.

Clinical tetanus does not induce immunity against further attacks of the disease and all patients should be fully immunized with tetanus toxoid (in the form appropriate for their age) during convalescence.

Botulism

Elimination of any unabsorbed toxin from the gastrointestinal tract should be encouraged in patients who have suspected botulism. Administration of an emetic or gastric lavage is recommended if ingestion of the suspect food has occurred within the preceding few hours and (unless there is a paralytic ileus) purgation or high enemas should be administered even several days after food ingestion.

The mainstay of therapy for botulism is meticulous supportive care. Patients should be admitted to an intensive care unit and their respiratory function monitored by repeat vital capacity measurements. Intubation should be performed if vital capacity falls below 12 ml/kg.

Equine antitoxin, containing antibodies to types A, B and E toxin, is available through public health services in many countries. There are few data concerning its use in humans but it is clearly effective in experimental animals. It has to be given as early as possible in the course of the illness but its use needs careful consideration in view of the risk of serious anaphylaxis or serum sickness. A test dose is administered into the skin or the conjunctiva. If there is no hypersensitivity, then one vial is given intravenously and one vial intramuscularly for an average adult; further doses may be given 2–4 hours later if the symptoms persist. Human botulinum immune globulin is available for infants through the California Department of Public Health (http://www.infantbotulism.org).

Antibiotics do not help except as part of meticulous debridement of the wound in wound botulism.

The relevant public health authorities should be notified promptly of a suspected case of botulism so that the necessary investigation may be conducted.

The severity and duration of food-borne botulism are related to the amount of toxin ingested. Respiratory failure occurs in 20–35% of patients; the mean duration of respiratory support is 7 weeks for those requiring mechanical ventilation. Recovery from botulism is usually complete but persistent dysphagia, diplopia and prolonged weakness are rare complications of severe cases.[21]

There has been a steady decline in mortality associated with botulism over the past century; the rate was about 70% in the period 1910–19 and about 9% during 1980–89. Mortality is higher in type A disease than in type B.[6] The prognosis for infants hospitalized with botulism and given meticulous supportive care is very good, with less than 1.3% case fatality and full recovery.

REFERENCES

References for this chapter can be found online at http://www.expertconsult.com



Chapter **22**

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Sarah J Tabrizi
John Collinge

Prion diseases of humans and animals

The prion diseases or transmissible spongiform encephalopathies are a group of closely related transmissible neurodegenerative conditions of humans and animals. In recent years prion diseases have captured the public attention with the evolving epidemic of bovine spongiform encephalopathy (BSE) in Europe, and the subsequent appearance of a novel phenotype of Creutzfeldt–Jakob disease (CJD), variant CJD (vCJD) in humans, which is experimentally linked to dietary exposure to BSE. Recently, the transmission of vCJD by blood transfusion has generated concern about secondary transmission.

The nature of the transmissible agent in these diseases has been a subject of intense controversy. The understandable initial assumption that the agent must be some form of virus was challenged by the failure to directly demonstrate such a virus (or an immunologic response to it), and by the remarkable resistance of the transmissible agent to treatment expected to inactivate nucleic acids (such as ultraviolet radiation or treatment with nucleases). Such findings had led to suggestions by Alper and others that the transmissible agent may be devoid of nucleic acid,[1] and led Griffith to suggest in 1967 that the transmissible agent might be a protein.[2] Progressive enrichment of brain homogenates for infectivity resulted in the isolation of a protease-resistant sialoglycoprotein, designated the prion protein (PrP), by Prusiner and co-workers in 1982.[3] This protein was the major constituent of infective fractions and was found to accumulate in affected brains and sometimes to form amyloid deposits. The term prion (from *pro*teinaceous *in*fectious particle) was proposed by Prusiner in 1982 to distinguish the infectious pathogen from viruses or viroids.[3] Prions were defined as 'small proteinaceous infectious particles that resist inactivation by procedures which modify nucleic acids'.

The unifying hallmark of the prion diseases is the aberrant metabolism of the prion protein (PrP), which exists in at least two conformational states with different physicochemical properties. The normal form of the protein, referred to as PrP^{C}, is a highly conserved cell surface protein attached via a glycosylphosphatidylinositol (GPI) anchor (Fig. 22.1). It is expressed in a wide range of cell types, and particularly in neuronal cells. PrP^{C} is a sialoglycoprotein of molecular weight 33–35 kDa with a high content of α-helical secondary structure that is sensitive to protease treatment and soluble in detergents (Fig. 22.1). The disease-associated isoform, referred to as PrP^{Sc}, is found only in infected brains as aggregated material, is partially resistant to protease treatment and insoluble in detergents, and has a high content of β-sheet secondary structure.

Due to its physicochemical properties, the precise atomic structure of the infectious particle or prion is still undetermined but considerable evidence argues that prions are composed largely, if not entirely, of an abnormal isoform of PrP. According to the protein-only hypothesis of prion replication, PrP^{Sc} recruits PrP^{C} into the infectivity-associated isoform, an event that is central to prion propagation (Fig. 22.2).

EPIDEMIOLOGY

Animal prion diseases

Scrapie is the prototypic prion disease (Table 22.1). It has been recognized as an enzootic disease of sheep and goats for more than 250 years. Present in many countries, its prevalence in the UK has been

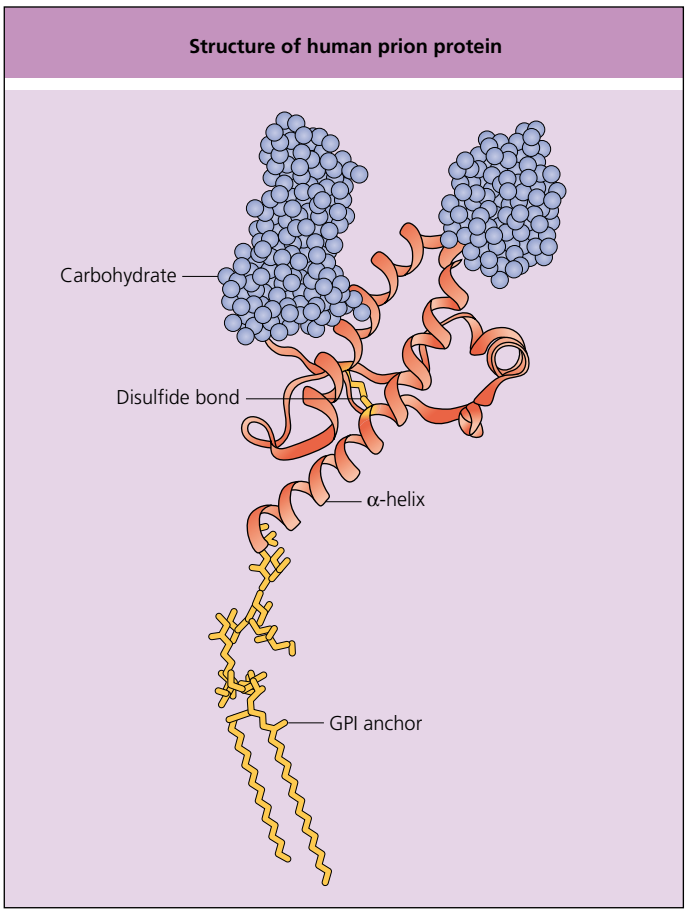


Fig. 22.1 Structure of the human prion protein. Model of glycosylated human prion protein indicating positions of *N*-linked glycans (blue), the single disulfide bond linking helixes 2 and 3, and the glycosylphosphatidylinositol (GPI) anchor to the outer surface of the cell membrane. Courtesy of Dr Richard Sessions and Mr Ray Young.

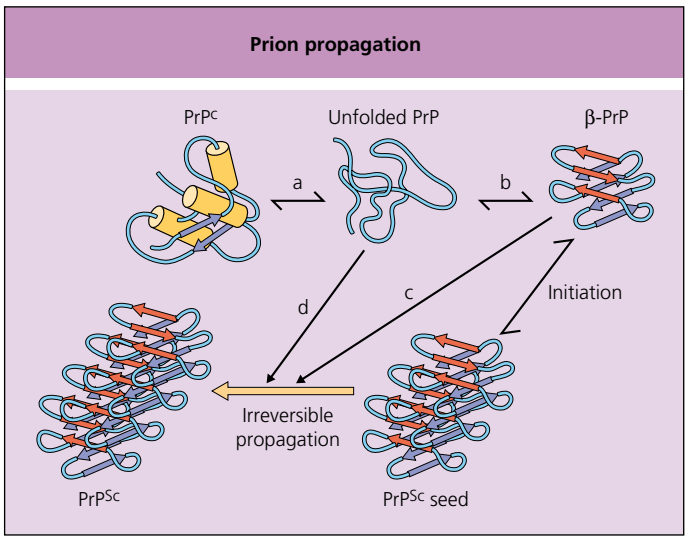


Fig. 22.2 Prion propagation. Schematic representation of a possible mechanism for prion propagation. The predominantly α-helical form of the normal prion protein (PrP^C) proceeds via an unfolded state (a) to refold into a predominantly β-sheet form (β-PrP) (b). Prion replication may require a critical 'seed' size. Further recruitment of β-PrP (c) or unfolded PrP (d) occurs as an essentially irreversible process. Courtesy of Prof Tony Clarke and Mr Ray Young.

Table 22.1 Animal prion diseases

Disease	Host	Etiology
Scrapie	Sheep and goats	Thought to involve both horizontal and vertical transmission
Transmissible mink encephalopathy	Captive mink	Probably food-borne, although the origin of infectious prions is uncertain
Chronic wasting disease	Captive and free-ranging mule deer and Rocky Mountain elk	Origin unknown. There is evidence for horizontal transmission
Bovine spongiform encephalopathy (BSE)	Cattle	Food-borne in the form of contaminated meat and bone meal
Feline spongiform encephalopathy	Domestic and zoo cats	Feed contaminated with bovine spongiform encephalopathy prions
Exotic ungulate encephalopathy	Captive bovidae	Feed contaminated with bovine spongiform encephalopathy prions

estimated as 0.5–1% of the sheep population. The etiology of natural scrapie has been the subject of intense debate for many years, but it is now clear that it is an infectious disease,[4] for which susceptibility is genetically modulated by the host.

Following its discovery in 1985, BSE reached epidemic proportions, with over 180 000 confirmed cases in UK cattle, and much smaller numbers in many other European countries. It has been estimated that over 2 million cattle were infected with BSE in the UK.[5] Smaller epidemics have also been described in Switzerland, Ireland, Portugal and France, and cases have been reported in Japan, Canada and the USA. Epidemiologic studies point to contaminated offal used in the manufacture of meat and bone meal and fed to cattle as the source of prions responsible for BSE.[6] Because the UK has a relatively large sheep population in which scrapie is endemic, it was hypothesized that scrapie-contaminated sheep offal was the initial source of BSE. An alternative view is that BSE prions originated spontaneously in cattle and that infection was subsequently amplified by recycling of infected cattle that had subclinical disease. The host range of BSE appears to be unusually wide, affecting many other animal species (Table 22.1). Rare atypical forms of BSE were first identified in 2004 in Italy and France on the basis of an apparently higher (BSE-H) or lower (BSE-L) molecular mass of partially protease-digested PrP^{Sc} compared with typical BSE.[7,8] The pathogenicity of atypical BSE in humans is predicted by transgenic studies.[9]

Prion disease of wild and captive cervids, known as chronic wasting disease (CWD), has been increasingly documented in the USA and Canada, principally Colorado and Wyoming.[10] Deposition of PrP^{Sc} is widespread in peripheral cervid tissues, offering a mechanism for horizontal transmission through environmental contamination by carcasses.[11] Several polymorphisms of the cervid PrP gene are known to influence susceptibility to CWD.[10] Although CWD will have been consumed by humans, no atypical prion strains have been detected in hunters or local populations,[12] and transgenic studies with 'humanized' mice support the existence of a strong barrier to transmission between cervid and human.[13]

Human prion diseases

The human prion diseases are unique in biology in that they manifest as sporadic, genetic and infectious diseases (Table 22.2). The majority of cases of human prion disease occur sporadically as Creutzfeldt–Jakob disease (sporadic (s) CJD) at a rate of roughly 1 per 10^6 population across the world, with an equal incidence of disease in men and women. The etiology of sCJD is unknown, although hypotheses include somatic mutation of the PrP gene (referred to as *PRNP*), and the spontaneous conversion of PrP^C into PrP^{Sc} as a rare stochastic event. There is a common coding polymorphism at codon 129 of *PRNP* encoding either methionine or valine (Fig. 22.3). Homozygosity at this position (denoted 129MM or 129VV) predisposes people to the development of sporadic and iatrogenic CJD.[14,15] Additionally, susceptibility single nucleotide polymorphisms have been identified near to *PRNP*, indicating additional genetic effects at the locus which presumably act through altered expression of the gene.

Approximately 15% of human prion diseases are inherited with an autosomal dominant mode of inheritance. Inherited human prion diseases have been shown to segregate with more than 30 different missense and insertion mutations in the coding sequence of *PRNP* (see Fig. 22.3).[16] Although the human prion diseases are experimentally transmissible, the acquired forms have, until recently, been confined to rare and unusual situations. For example, kuru was caused by cannibalism among the Fore linguistic group of the Okapa district of the Eastern Highlands in Papua New Guinea.[17] The disease had its origins at the beginning of the 20th century and was the leading cause of death in this population by the middle of the century, killing over 3000 people in the total population of 30 000. Mainly adult women as well as children of both sexes were affected, to give an annual disease-specific mortality of approximately 3%. It is thought that the roughly sevenfold higher incidence of disease in adult women than adult men was the result of higher exposure of women to infectious brain material. Since the cessation of cannibalistic practices around 1956, the disease has all but died out, with only a handful of cases currently occurring in older people who were presumably exposed to kuru as young children, indicating an incubation time in these cases of greater than 50 years.[18] In kuru, all codon 129 genotypes were affected as the epidemic evolved, with codon heterozygotes (129MV) having

Table 22.2 Human prion diseases

Disease	Incidence	Etiology	Age of onset or incubation period and duration of illness
Sporadic Creutzfeldt–Jakob disease (CJD)	1 case per 1 million population	Unknown but hypotheses include somatic mutation or spontaneous conversions of PrP^{C} into PrP^{Sc}	Age of onset is usually 45–75 years; age of peak onset is 60–65 years; 70% of cases die in under 6 months
Inherited prion diseases (CJD, FFI, GSS)	10–20% of cases of human prion disease	Autosomal dominant *PRNP* mutation	Onset tends to be earlier in familial CJD compared to sporadic CJD. Can be wide phenotypic variability between and within families
Kuru	>2500 cases among the Fore people in Papua New Guinea	Infection through ritualistic cannibalism	Incubation period 5–>40 years; duration of illness 12 months
Iatrogenic Creutzfeldt–Jakob disease	About 90 cases to date	Infections from contaminated human growth hormone, human gonadotropin, depth electrodes, corneal transplants, dura mater grafts, neurosurgical procedures	Incubation periods of cases from human growth hormone 4–30 years; duration of illness 6–18 months
Variant Creutzfeldt–Jakob disease	Over 200 predominantly young adults in the UK and France*	Infection by bovine spongiform encephalopathy-like prions	Mean age of onset 26 years; mean duration of illness 14 months

*To October 2000.
FFI, fatal familial insomnia; GSS, Gerstmann–Straussler–Scheinker syndrome; *PRNP*, prion protein gene; PrP^{C}, normal form of prion protein; PrP^{Sc}, disease-associated isoform of prion protein.

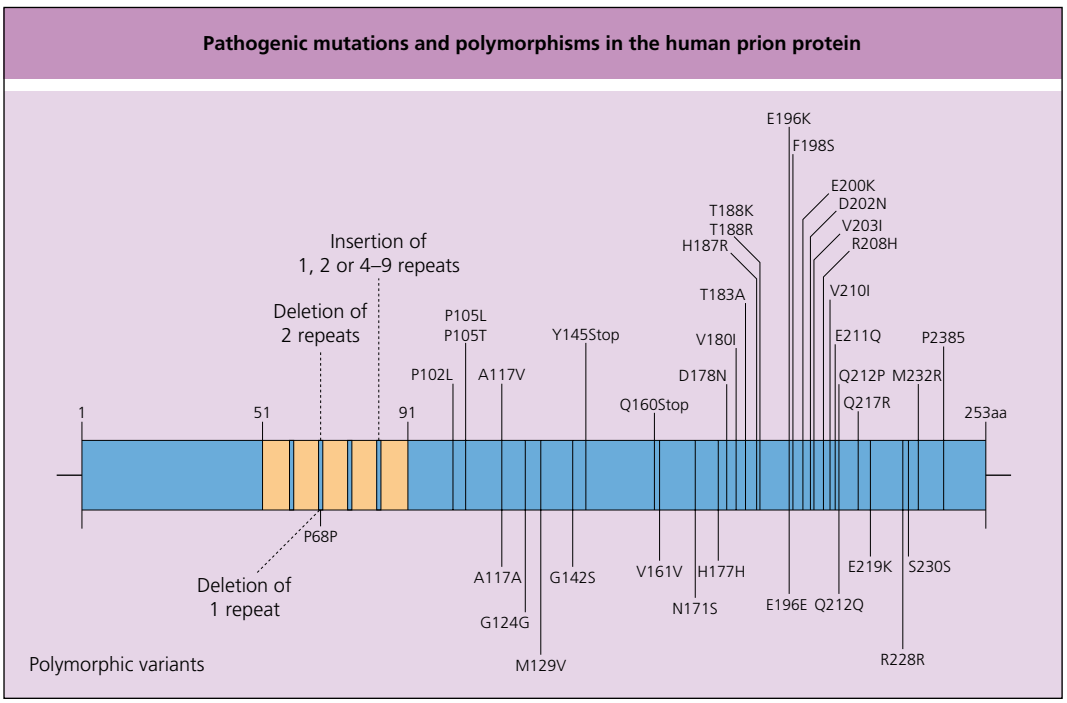


Fig. 22.3 Pathogenic mutations and polymorphisms in the human prion protein. The pathogenic mutations associated with human prion disease are shown above the PrP coding sequence. These consist of 4–9 octapeptide repeat insertions within the octarepeat region between codons 51 and 91, a deletion of two octapeptide repeats; and various point mutations causing missense amino acid substitutions. Point mutations are designated by the wild-type amino acid preceding the codon number, followed by the mutant residue, using single-letter amino acid conventions. Polymorphic variants are shown below the PrP coding sequence. Deletion of one octapeptide repeat is not associated with disease.

the longest mean incubation time.[19] Elderly women survivors of the epidemic of kuru are overwhelmingly codon 129MV, supporting the concept that kuru imposed strong balancing selection on the Fore.

Other examples of acquired human prion diseases have resulted from iatrogenic transmission of CJD during corneal transplantation, contaminated electroencephalographic (EEG) electrode implantation and surgical operations using contaminated instruments or apparatus. In addition, iatrogenic CJD has occurred as a result of implantation of dura mater grafts and treatment with growth hormone or gonadotropin derived from the pituitary glands of human cadavers.[20]

The appearance of CJD cases in teenagers and young adults in the UK during the mid-1990s prompted considerable concern that they might have acquired the illness as a result of exposure to BSE. By March 1996, it became clear that the unusual clinical presentation and neuropathology was remarkably consistent in these new cases.[21] Up until February 2008 there have been 163 deaths from probable or pathologically confirmed vCJD in the UK, predominantly teenagers and young adults, and over 200 deaths worldwide. Molecular strain typing, which focuses on the biochemical properties of PrP^{Sc} from the brains of BSE-infected cattle and patients who have CJD, has demonstrated that vCJD is different from sporadic CJD but similar to BSE.[22,23] Moreover, the incubation times and profile of neuropathologic lesions of vCJD and BSE prions are indistinguishable in inbred lines of mice.[24] These data argue that BSE and vCJD are the same strain. All reported cases of vCJD have been 129MM, a genotype shared by ~40% of the British Caucasian population.

Polymorphisms in the human *PRNP* gene are not the sole genetic influence on disease susceptibility and incubation time. Studies with inbred lines of mice show that large differences occur even with the same amino acid sequence of the prion protein, suggesting that other genes may contribute to the observed variation. Studies of quantitative trait loci (QTL) linked to prion disease incubation periods in mice have identified susceptibility loci on chromosomes 2, 4, 8, 11, 12 and 15.[25] These QTL studies provide strong evidence that genetic loci other than the coding region of *PRNP* have a major influence on scrapie incubation time in experimental prion disease.

Iatrogenic secondary transmission of vCJD has now occurred by blood transfusion from preclinical vCJD.[26–28] Three patients have been identified from a cohort of 23 who have survived more that 5 years after receiving vCJD-implicated blood. In 2004 a 62-year-old patient was diagnosed with vCJD post-mortem, 6.5 years after transfusion of a single unit of red cells.[27] Later in 2004 an elderly patient who died of an unrelated cause 5 years after transfusion of a single unit of red cells was found to have PrP^{Sc} deposition in lymphoreticular tissues consistent with vCJD infection.[26] In 2006 a patient was confirmed to have vCJD at autopsy, having received a unit of vCJD-implicated red cells 6 years earlier.[28] This patient was diagnosed whilst alive and had PrP^{Sc} deposition in tonsillar tissue. At an early stage of his disease, MRI was negative for the pulvinar sign. Both clinically affected patients were genotype 129MM, but the subclinically infected patient was 129MV, indicating for the first time the potential of individuals with this genotype to replicate vCJD prions. The fact than no PrP^{Sc} was detectable in the tonsil of this patient may reflect the selection of a novel strain in 129MV or alternatively relate to the amount and distribution of PrP^{Sc} seen at an early stage of infection. With an average of around 6 years, the shortest incubation times of secondary vCJD are, as expected, considerably shorter than the shortest incubation times of primary vCJD of around 12 years. Six thousand patients have been notified of their exposure to vCJD-implicated blood products.

As there is no blood test for pre- or subclinical carriers of vCJD, estimates of the prevalence of the carrier state rely on screening of surgical lymphoreticular tissue.[29,30] The larger study, conducted on 12 674 anonymized surgical appendectomy specimens, identified three positive samples, resulting in an estimated prevalence of vCJD infection of 237/million (95% CI 49–692). Subsequent genetic analysis of two of these positive samples identified 129VV genotypes, found in around 10% of the UK population.[31] A large prospective longitudinal study of surgical tonsil samples is currently being undertaken in the UK to clarify prevalence and obtain fresh tissue for strain type analysis.

CLINICAL FEATURES

The human prion diseases can be divided etiologically into inherited, sporadic and acquired forms with CJD, Gerstmann–Straussler–Scheinker syndrome (GSS) and kuru now seen as clinicopathologic *syndromes* rather than individual disease entities. The identification of one of the pathogenic *PRNP* mutations in a patient with neurodegenerative disease allows the diagnosis of an inherited prion disease and subclassification according to mutation. Over 30 pathogenic mutations have been described in two groups:

- point mutations resulting in amino acid substitutions in PrP or production of a stop codon resulting in expression of a truncated PrP;
- alteration of integral copies of an octapeptide repeat present in a tandem array of five copies in the normal protein (see Fig. 22.3).

They are all autosomal dominantly inherited conditions. Kindreds with inherited prion disease have been described with phenotypes of classic CJD and GSS, together with a range of other neurodegenerative disease phenotypes. Some families show remarkable phenotypic variability which can encompass both CJD- and GSS-like cases, as well as other cases which do not conform to either CJD or GSS phenotypes. Such atypical prion diseases may lack the classic histologic features of a spongiform encephalopathy entirely, although PrP immunohistochemistry is usually positive. Progressive dementia, cerebellar ataxia, pyramidal signs, chorea, myoclonus, extrapyramidal features, pseudobulbar signs, seizures and amyotrophic features are seen in variable combinations. *PRNP* analysis is also used for presymptomatic genetic testing in affected families.

Classic CJD is a rapidly progressive dementia accompanied by myoclonus. Decline to akinetic mutism and death is rapid and often occurs within 3–4 months. Cerebellar ataxia, extrapyramidal and pyramidal features and cortical blindness are also frequently seen. The electroencephalogram (EEG) may show characteristic pseudo-periodic sharp wave activity which is helpful in diagnosis but present only in around 70% of cases. To some extent, demonstration of a typical EEG is dependent on the number of EEGs performed and serial EEG is indicated to try and demonstrate this appearance. Cerebrospinal fluid (CSF) immunoassay for the neuron-specific 14-3-3 protein may be helpful. A combination of both 14-3-3 CSF analysis and EEG is recommended in the investigation of suspected classic CJD cases to increase the sensitivity of pre-mortem case definition. A raised 14-3-3 protein is not, however, specific for classic CJD and is raised in viral encephalitis or recent stroke; rather it is a marker of rapid neuronal injury and loss. More concerning with respect to the differential diagnosis is that it may also be raised in rapidly progressive forms of Alzheimer's disease, which may be confused with CJD. MRI scanning, particularly the use of diffusion weighted sequences, is highly sensitive in classic CJD. Caudate and putamen hyperintensity is well known, but cortical ribbon hyperintensity and thalamic high signal in 129MV patients is increasingly recognized. Atypical cases of classic CJD are well-recognized however, and can still present diagnostic difficulties.

The clinical features of kuru consist of a progressive cerebellar ataxia accompanied by dementia in the later stages and death, which usually occurs within 12 months. Iatrogenic prion disease arising from intracerebral or optic inoculation usually manifests clinically as classic CJD, whilst that arising from a peripheral route of inoculation, such as pituitary growth hormone, commonly presents like kuru with a progressive ataxia. GSS commonly presents as a chronic cerebellar ataxia with pyramidal features; dementia occurs much later in the clinical course which is longer than that seen in classic CJD. Fatal familial insomnia (FFI) is characterized by progressive untreatable insomnia, dysautonomia and dementia and selective thalamic degeneration, and is most commonly associated with a missense mutation at codon 178 of *PRNP*. The FFI phenotype has also been described occurring sporadically with no causative mutation in *PRNP* identified.

The early clinical presentation of vCJD resembles kuru more than classic CJD and consists of behavioral and psychiatric disturbances, peripheral sensory disturbance and cerebellar ataxia. Common early psychiatric features include dysphoria, withdrawal, anxiety, insomnia and apathy. Neurologic symptoms preceded psychiatric symptoms in 15% of cases studied, and were present in combination with psychiatric symptoms in 22% of cases from the onset of disease. No common early neurologic features were noted, but paresthesiae and/or pain in the limbs is seen in around half of the cases. However, a significant proportion of patients exhibited neurologic symptoms within 4 months of clinical onset, and these included poor memory, pain, sensory

symptoms, unsteadiness of gait and dysarthria. Disorientation, hallucinations, paranoid ideation, confabulation, impaired self-care and the commonest neurologic features (cerebellar signs, chorea, dystonia, myoclonus, upper motor neuron signs and visual symptoms) developed late in the course of the illness. The duration of disease is longer in vCJD, with mean patient survival times of about 13 months, compared with about 4 months for classic CJD. Moreover, whereas classic CJD is predominantly a late-onset disease, with a peak onset between 60 and 65 years, the median age of onset of vCJD is 26 years.

The EEG is not helpful in the diagnosis of vCJD; whilst generalized slowing is usually present, the characteristic periodic changes associated with classic CJD are not. The CSF 14–3–3 protein is less helpful, and may often be negative. MRI, however, is useful in the diagnosis of vCJD; in the majority of cases high signal is noted in the posterior thalamus (pulvinar) bilaterally on dual echo (T_2- or proton density-weighted) MRI (Fig. 22.4). Other common MRI features of vCJD are medial thalamic and periaqueductal gray matter high signal, and the notable absence of cerebral atrophy. All cases to date are *PRNP* 129MM (unpublished data).

vCJD can be diagnosed by detection of characteristic PrP^{Sc} immunostaining on tonsil biopsy. Importantly, PrP^{Sc} is only detectable in tonsil and other lymphoreticular tissues in vCJD, and not other forms of human prion disease, indicating that it has a distinctive pathogenesis.[32,33] The PrP^{Sc} type detected on Western blot in vCJD tonsil has a characteristic pattern designated type 4, by the London classification. Tonsil is the tissue of choice for diagnostic biopsy in the investigation of possible vCJD. Tonsil biopsy is well tolerated with minor discomfort, and has, so far, shown 100% sensitivity and specificity.[32] If the tonsil biopsy is positive with the specific vCJD banding pattern (Fig. 22.5), then a more invasive brain biopsy becomes unnecessary.

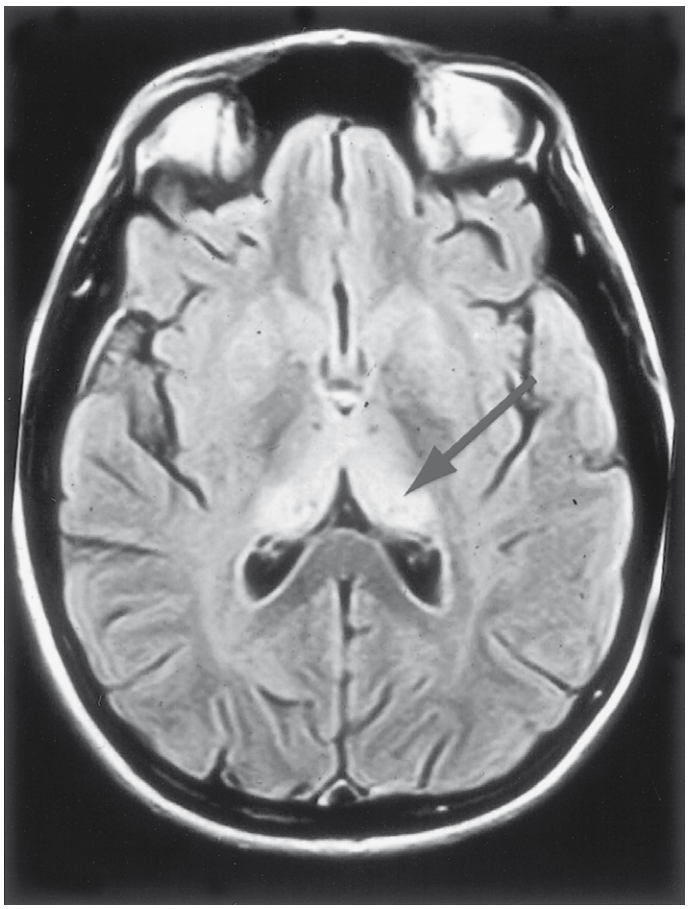


Fig. 22.4 Axial T_2-weighted MRI brain demonstrating high signal bilaterally in the posterior thalamus (arrowed) – the 'pulvinar sign' in a patient with vCJD.

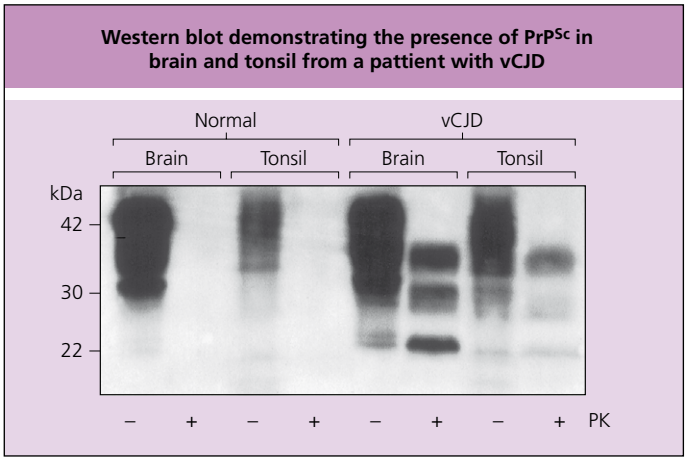


Fig. 22.5 Western blot demonstrating the presence of PrP^{Sc} in brain and tonsil from a patient with vCJD. The presence of PrP^{Sc} is revealed after proteinase K (PK) treatment, which digests the normal form of PrP (PrP^{C}) but not the pathologic form (PrP^{Sc}). Courtesy of Ms Susan Joiner and Dr Andy Hill.

PATHOGENESIS AND PATHOLOGY

Molecular strain typing

The marked clinical heterogeneity observed in sporadic CJD has yet to be explained. However, it has been clear for many years that distinct isolates, or strains, of prions can be propagated in the same host and these are biologically recognized by distinctive clinical and pathologic features in experimental animals (for review, see[34]). It is therefore likely that a proportion of the clinicopathologic heterogeneity in CJD, and other human prion diseases, relates to the propagation of distinct human prion strains. The identification of these prion strains would allow an etiology-based classification of CJD by typing of the infectious agent itself.

The existence of prion strains has been difficult to accommodate within the protein-only model of prion propagation. As they can be serially propagated in inbred mice with the same *PRNP* genotype, they cannot be encoded by differences in PrP primary structure. Furthermore, strains can be re-isolated in mice after passage in intermediate species with different PrP primary structures. Conventionally, distinct strains of conventional pathogens are explained by differences in their nucleic acid genome. However, in the absence of such a scrapie genome, alternative possibilities must be considered. A wealth of experimental evidence now suggests that PrP^{Sc} itself may encode strain-specific phenotypic properties. Different subtypes of PrP^{Sc} were associated initially with two strains of transmissible mink encephalopathy in hamsters.

Several human PrP^{Sc} types have been identified which are associated with different phenotypes of CJD.[22,35,36] The different fragment sizes seen on Western blots, following treatment with proteinase K, suggest that there are several different human PrP^{Sc} conformations, referred to as 'molecular strain types'. These types can be further classified by the ratio of the three PrP bands seen after protease digestion, representing di-, mono- and unglycosylated fragments of PrP^{Sc}. By the London classification system, sporadic CJD is associated with PrP^{Sc} types 1–3, while type 4 human PrP^{Sc} is uniquely associated with vCJD and characterized by glycoform ratios which are distinct from those observed in classic CJD.[22,35]

Importantly, these biochemical changes in PrP^{Sc} are transmissible to the PrP in a host. This has been demonstrated in studies with CJD isolates, with both PrP^{Sc} fragment sizes and the ratios of the three PrP glycoforms maintained on passage in transgenic mice expressing human PrP.[22] Furthermore, transmission of human prions and bovine

prions to wild-type mice results in murine PrP^{Sc} with fragment sizes and glycoform ratios which correspond to the original inoculum.[22] Variant CJD is associated with PrP^{Sc} glycoform ratios which are distinct from those seen in classic CJD. Similar ratios are seen in BSE, and BSE when transmitted to several other species.[22]

BSE and vCJD have now been propagated in *PRNP*-null transgenic mice expressing the human prion protein modeling three different codon 129 genotypes. Transgenic mice for human 129MM propagate either type 2 or type 4 PrP^{Sc} with respective neuropathologies consistent with human sporadic CJD or vCJD, whereas transgenic mice homozygous for human 129VV either propagate novel type 5 PrP^{Sc} and a distinct pattern of neuropathology, or develop clinical prion disease in the absence of detectable PrP^{Sc}. Transmissions to human 129MV mice were complex, with four distinct phenotypes, including the propagation of type 4 PrP^{Sc} in the absence of florid plaques.

These findings argue that primary BSE prion infection, as well as secondary infection by iatrogenic routes, may not be restricted to a single disease phenotype, including extensive transmission as a subclinical carrier state. Further, these studies raise the possibility that some humans infected with BSE prions may develop a clinical disease indistinguishable from classic CJD associated with type 2 PrP^{Sc}. All these data strongly support the 'protein only' hypothesis of infectivity and suggest that strain variation is encoded by a combination of PrP conformation and glycosylation pattern.

Pathology

The animal and human prion diseases share a number of characteristic features, the most consistent being the neuropathologic changes that accompany disease in the central nervous system. Indeed, it was the neuropathologic similarities between scrapie and kuru which strongly suggested that the two diseases might be closely related, and that kuru, like scrapie, might also be transmissible by inoculation. Subsequently, brain extracts from patients who have kuru produced a progressive neurodegenerative condition in inoculated chimpanzees after a prolonged incubation period of 18–21 months. The neuropathologic similarities between kuru and CJD prompted similar transmission experiments from CJD patients.

Although the brains of patients or animals who have prion disease frequently show no recognizable abnormalities on gross examination, microscopic examination of the central nervous system typically reveals characteristic histopathologic changes, consisting of neuronal vacuolation and degeneration, which gives the cerebral gray matter a microvacuolated or 'spongiform' appearance (Fig. 22.6b) and a reactive proliferation of astroglial cells (Fig. 22.6b), which is often out of all proportion to the degree of nerve cell loss. Although spongiform degeneration is frequently detected, it is not an obligatory neuropathologic feature of prion disease; astrocytic gliosis, although not specific to the prion diseases, is more constantly seen.

The lack of an inflammatory response is also an important characteristic. Although it is by no means a constant feature, some examples of prion disease are characterized by deposition of amyloid plaques composed of insoluble aggregates of PrP. Amyloid plaques are a notable feature of kuru and GSS but they are infrequently found in the brains of patients who have classic CJD.

Although there is wide variation in the neuropathologic profiles of different forms of human prion disease, the histopathologic features of vCJD are remarkably consistent and distinguish it from other human prion diseases. Large numbers of PrP-positive amyloid plaques are a consistent feature of vCJD but they differ in morphology from the plaques seen in kuru and GSS in that the surrounding tissue takes on a microvacuolated appearance, giving the plaques a florid appearance (Fig. 22.6a).[21]

vCJD is clearly very different in its pathogenesis from other human prion diseases, and this is reflected in the tissue distribution of PrP^{Sc} in vCJD. As mentioned, it is readily detectable in lymphoreticular tissue, and using highly sensitive immunoassays, PrP^{Sc} has been found in retina, optic nerve, rectum, adrenal gland and thymus in vCJD postmortem tissue (Fig. 22.7).[33]

Pathogenesis

Detection of PrP^{Sc} in brain material by immunohistochemical or immunoblotting techniques is considered to be diagnostic of prion disease (Fig. 22.6c). However, certain examples of natural and experimental prion disease occur without accumulation of detectable protease-resistant PrP^{Sc}, and the time course of neurodegeneration is not equivalent to the time course of PrP^{Sc} accumulation in mice expressing lower than normal levels of PrP^{C}.[37] Moreover, PrP^{Sc} is not toxic to cells that do not express PrP^{C}, and although mice expressing PrP without a glycosylphosphatidylinositol anchor to the cell membrane may be infected with prions, they do not develop neurodegeneration. Additional evidence that PrP^{Sc} may not be the neurotoxic species has been demonstrated in mice inoculated with $^{Sc}237$ hamster prions. These mice replicate prions to high levels in their brains but do not develop any signs of clinical disease during their normal lifespan.

Recently, PrP^{Sc} fractionation experiments suggest that the most infectious entity of prion disease is an oligomer (about 14–28-mer) of misfolded PrP, analogous to the oligomers implicated as the prime cause

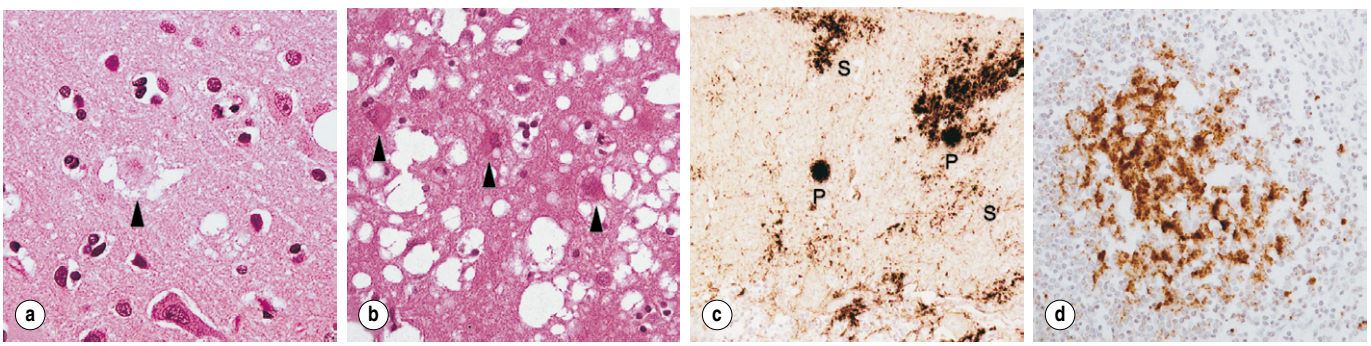


Fig. 22.6 Neuropathology of prion disease – histopathologic findings in vCJD. (a) Florid plaques, a characteristic feature of vCJD pathology. They consist of a round amyloid core (arrowed) surrounded by a ring of vacuoles (hematoxylin and eosin stain). (b) Spongiform degeneration in prion disease. This area shows severe vacuolization (spongiosis); there is severe neuronal loss and many strongly reactive astrocytes (arrowed) (hematoxylin and eosin stain). (c) Immunostaining of the pathologic prion protein. The specimen is pretreated to denature the normal PrP and staining with a prion protein antibody reveals the presence of plaques (P) and synapses (S) staining positively for PrP^{Sc}. (d) Detection of pathologic prion protein in the follicular dendritic cells in a tonsil. Accumulation of prion protein in lymphoreticular organs, such as spleen, tonsils or appendix, is a specific finding in vCJD and is not present in other forms of CJD. Therefore tonsillar biopsies can be used to specifically diagnose vCJD when clinical symptoms are only emerging. Courtesy of Dr Sebastian Brandner.

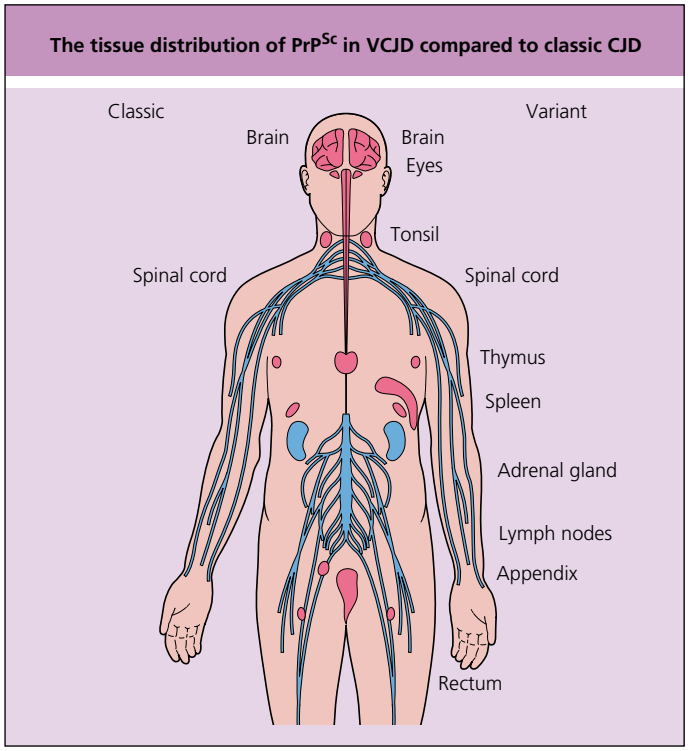


Fig. 22.7 The tissue distribution of PrP^{Sc} in vCJD compared to classic CJD. In vCJD PrP^{Sc} is found in lymphoreticular tissue as well as brain and spinal cord. Using highly sensitive immunodetection methods, PrP^{Sc} has also been found in the optic nerve, retina, adrenal gland and rectum. Courtesy of Dr Jonathon Wadsworth and Mr Ray Young.

of disease in other neurodegenerative conditions such as Alzheimer's and Parkinson's disease. However, more precise characterization of the infectious and toxic entity of prion disease will require the synthesis of prion from recombinant substrates. Although there has been some success, synthetic prions have only been generated in such low concentrations that analysis of molecular structure is impossible.[34]

The essential role of host PrP^{C} for prion propagation and pathogenesis is demonstrated by the fact that mice in which the PrP gene has been disrupted (referred to as $Prnp^{0/0}$) are resistant to scrapie infection,[37] and that re-introduction of the murine PrP^{C} transgene restores susceptibility to infection.

Gene-targeted $Prnp^{0/0}$ mice have also been studied to probe the normal function of PrP^{C}. Two independently generated lines of gene-targeted $Prnp^{0/0}$ mice developed normally and appeared to suffer no gross phenotypic abnormalities. The relative normality of these PrP null mice was thought to result from effective adaptive changes during development. However data from *Prnp* conditional knockout mice suggest this is not the case; these mice undergo ablation of neuronal PrP expression at 9 weeks of age. The mice remain healthy without evidence of neurodegeneration or an overt clinical phenotype, demonstrating that acute loss of neuronal PrP in adulthood is tolerated, and that the pathophysiology of prion diseases is not due to loss of normal PrP function. The normal function of PrP is not known but evidence from PrP knockout mice reveals defects in neurophysiologic and biochemical function. Electrophysiologic studies have demonstrated that fast inhibition and long-term potentiation mediated by δ-aminobutyric acid receptors were impaired in hippocampal slices from $Prnp^{0/0}$ mice,[38,39] and that calcium-activated potassium currents were disrupted.[40]

These abnormalities of synaptic inhibition are reminiscent of the neurophysiologic defects seen in patients who have CJD and in scrapie-infected mice,[38] and suggest a direct role for PrP in the modulation of neuronal excitability. Normal PrP has also been shown to bind copper ions,[41,42] with femtomolar affinity,[43] and a role for PrP in copper metabolism or transport has also been suggested. Other suggested functions include as a nerve growth factor[44] or self-renewal of hemopoietic stem cells.[45]

Thus, it appears that neither accumulation of PrP^{Sc}, nor loss of normal PrP function, is the cause of the neurodegeneration in prion diseases. It is possible that a toxic intermediate species is produced in the conversion of PrP^{C} to PrP^{Sc}, and that the steady state level of such an intermediate could then determine the rate of neurodegeneration.[34]

Although the pathologic consequences of prion infection occur in the central nervous system, and experimental transmission of these diseases is most efficiently accomplished by intracerebral inoculation, most natural infections do not occur by these means. Indeed, administration to sites other than the central nervous system is known to be associated with much longer incubation periods, which may extend to 20 years or more. Experimental evidence suggests that this latent period is associated with clinically silent prion replication in the lymphoreticular tissue, whereas neuroinvasion takes place later.

The *M* cells in the intestinal epithelium mediate prion entry from the gastrointestinal lumen into the body, and follicular dendritic cells (FDCs) are thought to be essential for prion replication and for accumulation of disease-associated PrP^{Sc} within secondary lymphoid organs. Inhibition of the lymphotoxin (LTβ) signaling pathway with a soluble receptor that depletes FDCs abolishes prion replication in spleens and prolongs the latency of scrapie after intraperitoneal challenge. B-cell deficient mice are resistant to intraperitoneal inoculation with prions, possibly because of impaired FDC maturation. Opsonization by complement system components may also be important in peripheral neuroinvasion, as mice that are genetically engineered to lack complement factors, or mice deleted of the C3 complement component by the administration of cobra venom, are resistant to peripheral prion inoculation. Recent evidence suggests that infection of organs may play an important role in shedding of prions in milk (mastitis) or urine (nephritis).

PREVENTION

Because there are currently no treatments for these invariably fatal diseases, prevention is particularly important. Perhaps the most effective example of prevention was the cessation of cannibalistic practices among the Fore people of Papua New Guinea in the 1950s, which resulted in the disappearance of kuru. The replacement of growth hormone derived from the pituitary glands of human cadavers with recombinant growth hormone was implemented to avoid the continued iatrogenic transmission of CJD to young children who have growth hormone deficiency. Similarly, because CJD has resulted from the use of prion-contaminated surgical instruments or apparatus after neurosurgical or ophthalmic procedures, it is advised that surgical instruments be incinerated in cases where CJD is confirmed so as to avoid future iatrogenic transmission of prion disease. Current policy in the UK is to quarantine surgical instruments until a suspected diagnosis is confirmed (see http://www.advisorybodies.doh.gov.uk/acdp/tseguidance/index.htm). Experimental studies have confirmed that prions adhere readily to metal following a contact time with infected brain of as little as 5 minutes.[46]

When it was realized that BSE was caused by feeding prion-contaminated foodstuffs to cattle, a number of preventive measures were introduced in the UK. In July 1988 a ban on feeding ruminant-derived protein to other ruminants was introduced to break the cycle of infection via feed. A ban on specified bovine offals was introduced in the UK in 1989 to prevent inclusion in the human food chain of bovine tissues thought to contain the highest titer of prions; these included tissues from the lymphoreticular system and the central nervous system. The European Union imposed a worldwide ban on the export of British cattle, products derived from them (with the exception of products for technical uses) and mammalian meat and bone meal in March 1996 after the announcement that BSE and vCJD might be linked. Since then, more than 1.35 million cattle over 30 months

old have been culled in the UK in a further attempt to limit human exposure to BSE. The 'over thirty month' (OTM) rule is one of the UK BSE controls to prevent further BSE-infected cattle from entering the human food chain because cattle over 30 months are more likely to develop BSE than younger animals. Therefore, since 1996 there has been a ban on selling meat in the UK from slaughtered cattle over 30 months old. The cost of tackling BSE to the British and European taxpayer has been estimated at over £7000 million. These measures appear to have been effective in reducing the incidence of BSE in the UK and the number of newly identified BSE cases has declined sharply. The EU-imposed worldwide ban on British beef exports was lifted in late 1999 after the EU were satisfied that appropriate measures had been taken to counteract the likelihood of BSE-infected animals getting into the human food chain.

Prior to the realization of blood transmission of vCJD, the UK government decided in 1998 that all blood donations should be leukodepleted. Since then the majority of European countries have followed this strategy. The UK National Blood Transfusion Service now imports all plasma and plasma derivatives from BSE-free countries, and blood donors are screened to exclude anyone with a blood relative with classic CJD or vCJD. Several countries have instituted policies of deferral of blood donors who have resided in the UK for a cumulative period of 6 months or more from 1980 until the end of 1996. In view of the potential exposure to the vCJD agent in other European countries in addition to the UK, the US Food and Drug Administration has a blood donation deferral policy of a cumulative 10-year residence in France, Portugal and Ireland. The American Red Cross Blood Banks have adopted a deferral policy of 3 months residence in the UK and 6 months residence in any other European country. The efficacy of these risk reduction procedures is not known, and a screening test for blood infectivity is urgently needed.

Transmission of classic CJD has occurred on surgical instruments as prion infectivity is resistant to conventional sterilization.[47] Surgery may be an epidemiologic risk factor for classic CJD.[48,49] The presence of vCJD in peripheral tissues commonly involved in routine surgery has raised concerns about this mechanism of secondary transmission. A three-stage detergent/enzymic procedure has now been demonstrated to be effective at decontamination of prions and is used by some NHS Trusts.[50]

An effective assay for low concentrations of prion may lead to a useful blood test. The new methodology implements a cyclical amplification and sonication of PrP^{Sc} derived from brain homogenates in a dilution of normal brain homogenate. This promising technique is known as protein misfolding cyclic amplification (PMCA).[51] It is likely that refinements will continue to take place over the ensuing years.[52]

DEVELOPMENT OF THERAPIES

Prion diseases are invariably fatal, and whilst curative therapies for prion infection are conceivable, such therapies, if developed, will not be available for some years.[53] Such approaches are likely to involve targeting PrP itself.[53] However, the development of neuroprotective agents, and pre- and post-exposure prophylaxis are also important. In addition, early firm diagnosis will be crucial to allow such treatments to be initiated before extensive brain damage occurs.

A number of compounds have been shown to be effective at clearing PrP^{Sc} in cell culture systems. These include the acridine and phenothiazine derivatives quinacrine and chlorpromazine, Congo-red, sulphated polyanions and anti-PrP antibodies (reviewed in[54]). A few compounds have been shown to prolong survival in animal models after intraperitoneal inoculation with prions. These include pentosan polysulphate, cyclic tetrapyrroles and CpG oligodeoxynucleotides. Treatment of mice with anti-prion monoclonal antibodies results in delay of clinical onset beyond 300 days (control animals were affected after about 190 days) after intraperitoneal administration of mouse prions.[55] However, clinical duration was not prolonged when treatment was started after neurologic onset, and there was no effect if prions were administered intracerebrally.

The PRION-1 trial of quinacrine in the UK has been completed.[56] Pentosan polysulphate has been used in a small number of patients with various human prion diseases, in many cases with direct intracerebral delivery. However, given the very small sample size, several adverse events and lack of placebo group, it is very difficult to conclude whether this treatment has any benefit (see http://www.cjd.ed.ac.uk/bone.pdf for details).

Currently, an international research effort is underway to develop therapies aimed at both pre- and post-exposure prophylaxis, in addition to neuroprotective agents that may slow down disease progression.

REFERENCES


References for this chapter can be found online at http://www.expertconsult.com

Chapter 23

Roger Bayston

Infections in hydrocephalus shunts

Hydrocephalus shunts drain excess cerebrospinal fluid (CSF) from the cerebral ventricles, usually to the peritoneal cavity (ventriculoperitoneal, VP) or to the right cardiac atrium (ventriculoatrial, VA) (Fig. 23.1).

EPIDEMIOLOGY

The incidence of infection varies according to the age at which the shunt is inserted. Up to 25% of operations in premature infants with hydrocephalus after periventricular hemorrhage result in infection, whereas in older children the incidence is 3–8%.[1,2] Although the incidence of shunt infections has generally fallen, it is still unacceptably high. Some centers have reported rates of infection near to zero[3,4] but this is rare.

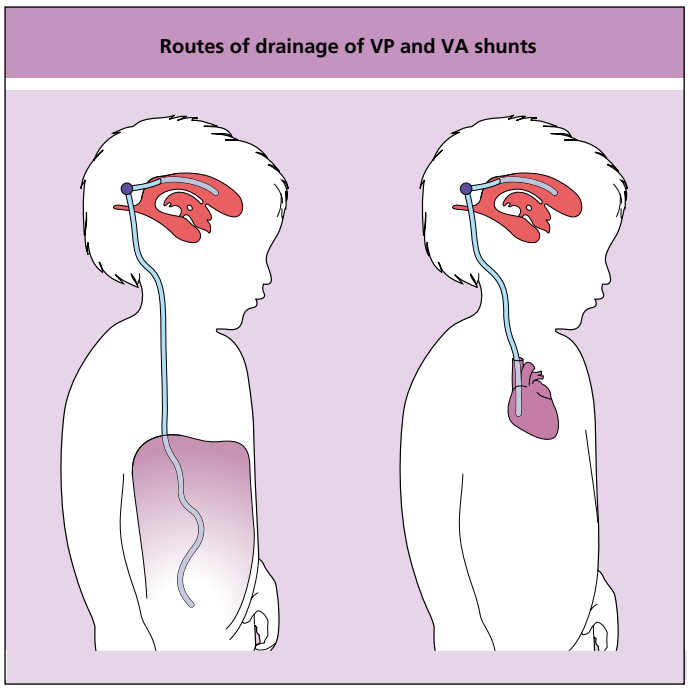


Fig. 23.1 Routes of drainage of ventriculoperitoneal and ventriculoatrial shunts. Ventriculoperitoneal shunts drain CSF from the cerebral ventricles to the peritoneal cavity via catheter tubing implanted superficially over the rib cage. The lower end of the peritoneal catheter lies free in the abdomen. Ventriculoatrial shunts drain CSF via a convenient neck vein such as the jugular and the superior vena cava to the right atrium.

PATHOGENESIS AND PATHOLOGY

The source of the organisms is almost invariably the patient's skin, from which they gain access to the device during its insertion.[5,6] Where there is a serious breakdown in surgical asepsis or where the operating room environment or air is grossly contaminated, these may be alternative sources. Although the bacterial population on the surface of the patient's skin can be reduced to almost zero by thorough skin preparation, resident bacteria in follicles remain and recolonization by resident bacteria occurs rapidly. It is therefore not unusual to find coagulase-negative staphylococci (CoNS) in the incision during the procedure. In nonimplant surgery these are irrelevant, but where a shunt is inserted they are highly likely to adhere to and colonize its inner surfaces. *Staphylococcus epidermidis* predominate in shunt infections. After adhering to the shunt material, they multiply and produce copious amounts of exopolysaccharide ('slime'), enabling the formation of a biofilm. Because of nutrient depletion, growth is very slow and this accounts for the often long periods between surgery and clinical presentation of infection. *Staphylococcus aureus* causes a different clinical picture and is more frequently involved in external shunt infections (Fig. 23.2). Unlike *Staph. epidermidis*, it produces toxins which evoke a very active inflammatory response, leading to erythema and suppuration.



Fig. 23.2 External shunt infection in a premature infant with poor nutritional status. The infection can be caused by organisms introduced at surgery or they may gain access through minor skin abrasions and pressure necrosis. Differing from the more common internal shunt infections, they are usually caused by *Staphylococcus aureus* and constitute a wound infection enhanced by a foreign material.

Table 23.1 Clinical features of ventriculoatrial and ventriculoperitoneal shunt infections of surgical origin

	VA shunts	VP shunts
Time from surgery to presentation	Weeks, months, several years	<9 months
Intermittent fever	75%	<50%
Anorexia, lassitude, poor sleep pattern	>80%	>50%
Shunt obstruction	<1%	≥75%
Other features	Chills, rigors: 20% Arthralgia: 50% [late onset cases (1–15 years)] Rash: 70% [late onset cases (1–15 years)] Nephritis: 30% [late onset cases (1–15 years)]	Abdominal pain, bloating: ~75% Swelling, erythema over shunt tubing: ~60% Headache, vomiting, etc. (i.e. recurrence of hydrocephalus): ≥75%

Percentages indicate the approximate proportion of cases in which features are present. It is important to realize that each case is different and that many of these features may be absent or modified.

The clinical presentation of infection in VA shunts differs from that in VP shunts (Table 23.1). In the former, bacteria from the shunt enter the bloodstream directly to cause intermittent fever which, in infections caused by *Staph. epidermidis*, propionibacteria or coryneforms, may continue for months or years with little other evidence of infection. However, antibody to bacterial components is produced in large quantities and immune complex disease may ensue, with deposits of C3, C4, IgG and IgM on the synovial and glomerular basement membranes. Hypertension, renal failure (shunt nephritis) and arthropathy may result.[7] In contrast, in VP and lumboperitoneal (LP) shunt infections, the bacteria are discharged into the peritoneal cavity, provoking the greater omentum to seal off the distal catheter. This and associated adhesions give rise to shunt obstruction and raised CSF pressure (Fig. 23.3). Occasionally, peritoneal abscesses are seen, and the absorptive capacity of the peritoneum may be lost. In both VP and VA shunts ventriculitis is seen in most cases, although the inflammatory response is usually feeble.

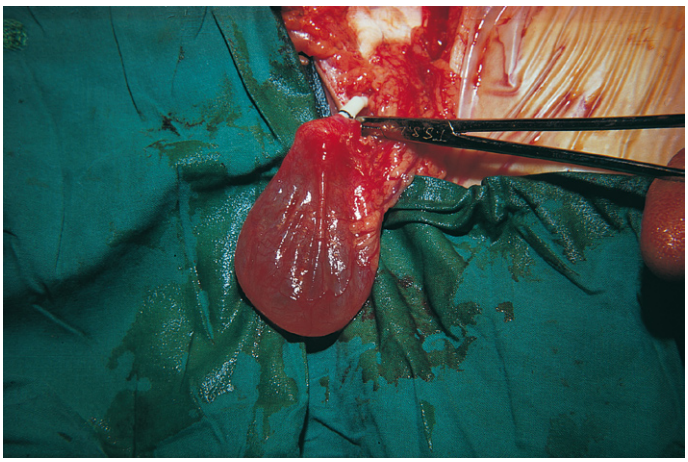


Fig. 23.3 Cystic obstruction of a ventriculoperitoneal shunt caused by shunt infection with *Staphylococcus epidermidis*. Bacteria and bacterial products entering the peritoneal cavity via the shunt catheter evoke an inflammatory response involving the greater omentum, which seals off the catheter outlet. The resulting cyst fills with CSF, giving rise to recurrence of the hydrocephalus. Cystic obstruction can occur from noninfective causes but unlike those cases caused by infection, which present within 6–9 months of surgery, they can arise at any time.

Only a few shunt infections are due to causes other than surgery. In babies or adults whose nutritional status is poor, erosion of the skin over the shunt can take place, leading to secondary infection with *Staph. aureus* or Gram-negative bacteria. An unusual but well-documented cause of VP shunt infection is visceral perforation by the distal catheter, which results in polymicrobial infection of the cerebral ventricles.[8] However, peritonitis from this cause is rare. Following the increasing use of VP shunts in the elderly with normal pressure hydrocephalus, mixed enteric infections arising from diverticulitis may be seen.

Cerebrospinal fluid shunts, including VA, appear to be unusually free of risk from hematogenous spread, and no cases have been documented from such sources as dental surgery. However, VP shunts can become infected during abdominal surgery or continuous ambulatory peritoneal dialysis.

PREVENTION

Studies have shown that understanding of the causes of shunt infections and surgical experience are very important and shunt surgery should be carried out only by experienced personnel or by fully supervised trainees. In view of the source of infection, most attempts at prevention have been targeted at the surgical procedure. Povidone-iodine or chlorhexidine, both alcohol based, should be used for skin preparation, the latter having the greater activity. Assiduous surgical aseptic technique is extremely important, with attention to 'no touch' techniques and glove changing. The use of prophylactic antibiotics might appear to be reasonable, but they have not been found to have a statistically significant beneficial effect in properly designed trials unless the infection rate exceeds 15%.[9] Those who feel obliged to use them despite this are advised to administer 10 mg vancomycin hydrochloride and 3 mg gentamicin in 1–2 ml sterile water for injection intraventricularly[10] as soon as the ventricular catheter is inserted (this should be inserted first), and 1.5 g cefuroxime (25 mg/kg for children) intravenously at induction of anesthesia. Intravenous antibiotics alone are not recommended.

Because of the general lack of success in further reducing the infection rate beyond acceptable minima, an innovative process (Bactiseal, Codman & Shurtleff Inc.) has been developed for treatment of shunts in order to reduce bacterial colonization and infection. In preclinical tests high-level protection of all shunt surfaces has been demonstrated for approximately 2 months. Antibacterial activity is undiminished in the presence of high CSF protein levels such as those found after hemorrhage or meningitis.[11] While the six clinical trials carried out so far vary considerably in design and quality, all except one (where there was no randomization and no case definition for infection) show a reduction in infection rate.[12]

CLINICAL FEATURES

The clinical features of VA shunt infection differ considerably from those of VP and LP shunts (see Table 23.1). Although the shunt lumen becomes colonized at implantation, in VA shunts symptoms considered serious enough to warrant specialist medical attention may not appear for months or years.[7] Many patients have intermittent low-grade fever, but some do not. Some report chills and occasionally rigors. Transient rashes are common. Sore throat and muscular and joint pains are common complaints. Anemia is almost universally found and there is increasing lassitude, anorexia, irritability and poor sleep. Dyspepsia may also be a problem. In later stages, arthralgia becomes more common. Unfortunately, these features are nonspecific and are often mistaken for those of other conditions. As the disease progresses, nephritis and vasculitis may appear. The vasculitic

rash is usually confined to the lower extremities and can be frankly hemorrhagic and can ulcerate. Nephritis is indicated by hypertension, hematuria and proteinuria, edema and often loin pain. Endocarditis has been reported only rarely.

In contrast, infections in VP and LP shunts almost always present within 6 months of surgery.[13,14] Fever is present in fewer than 50% of cases and is usually intermittent and mild. Chills and rigors are rare. There may be abdominal discomfort, bloating, pain or tenderness and occasionally persistent flatulence. In cases presenting within 1 or 2 weeks of surgery, there may be failure of the abdominal wound to heal, with CSF leak and sometimes catheter protrusion. In LP shunts there is often spinal pain. However, the most constant symptoms are those of hydrocephalus caused by obstruction at the distal end and the differential diagnosis is between infective and noninfective shunt obstruction. In the former there is often erythema and tenderness over the lower shunt track, whereas these features are absent in noninfective obstruction. In addition, distal VP shunt obstruction occurring more than 9 months after shunt surgery is very unlikely to have an infective cause.[14,15] A very small number of cases present as acute abdomen, with fever, abdominal pain and tenderness suggesting appendicitis or peritonitis.[16] These may present at any time and may lead to unnecessary laparotomy. The tenderness is not necessarily associated with the location of the shunt tip.

Ventriculoperitoneal shunt infections can also present at any time after perforation of the bowel, vagina or other organ.[17] The distal catheter often protrudes from the anus or vagina and CSF leaks from these sites. Presentation is usually as meningitis rather than shunt obstruction or peritonitis, with few abdominal features. Considering the often large numbers of bacteria seen in the CSF in these cases, the patients are not usually severely ill and recovery is often uneventful after shunt removal, without need for laparotomy.

DIAGNOSIS

Blood should be drawn for culture in all cases of suspected shunt infection. However, in infected VP and LP shunts the positive culture rate is less than 5%, except where *Staph. aureus* or Gram-negative bacilli are involved. In VA infections the positivity rate is much higher and blood should be drawn for culture on several occasions. However, in longstanding infections blood cultures may remain negative, possibly because of high antibody and opsonin titers. All isolates, however doubtful, should be saved until a definitive diagnosis is made. Attempts should be made to compare consecutive isolates, by antibiograms and by proprietary kits such as API Staph, or by molecular typing techniques if available. Differentiation between contaminants and pathogens, however, remains a problem. Critical evaluation of tests for 'slime' production shows that these tests, as currently formulated, are of no value in the diagnostic laboratory.[18]

Aspiration of CSF from the shunt reservoir carries little risk of introducing infection. The CSF sample should be examined promptly and a portion should be centrifuged for Gram film and culture, whatever the cell count, because bacteria are not infrequently found in the absence of a significant cellular response. As with blood cultures, isolates should be kept and identified, although the isolation of an organism from a shunt aspirate, particularly if it is also seen on Gram film, is diagnostic of shunt infection. It is important to realize that no isolate should be disregarded, whatever its identity. Gram film is essential to detect *Propionibacterium acnes*, which will require anaerobic incubation for up to 2 weeks.

It should be noted that the shunt might be infected only distal to the reservoir. In such cases reservoir aspiration may yield normal CSF that is culture negative.

In the presence of symptoms and a suggestive history, negative blood and CSF cultures cannot rule out shunt infection completely and a high index of suspicion should be retained. If symptoms persist, consideration should be given to removing the shunt empirically and it is then imperative that it is sent immediately to the laboratory in a sterile container for Gram stain and culture. Cultures should be incubated for at least 5 days. The Gram stain is very important; where organisms are isolated on culture without being seen on microscopy they are almost invariably contaminants, except where antimicrobials have been given immediately before shunt removal.

In view of the difficulties both of laboratory and clinical diagnosis, serologic tests have been developed. For example, a whole-cell agglutination test using *Staph. epidermidis* has proved useful in diagnosing VA shunt infections.[7] The agglutinin titer in individuals without shunts rises with age. In patients who have VA shunt infection caused by coagulase-negative staphylococci, the titer rises before symptoms appear and, over several months, it can rise to 15–30 times the normal level for age. It can therefore be used as a screening test. When used in this way, shunt nephritis is not seen because a diagnosis is invariably made sufficiently early for it to be avoided.[7] Ventriculoperitoneal and LP shunts do not discharge directly into the bloodstream, which may explain why the agglutination test is not useful in these cases. The plasma C-reactive protein (CRP) can be helpful in distinguishing between infective and noninfective distal VP shunt obstruction. For VP shunts, if symptoms of obstruction appear within 6–9 months of surgery, and the plasma CRP is raised with no other cause, shunt infection is likely and should be confirmed by CSF aspiration.

MANAGEMENT

Three factors are important in the antimicrobial chemotherapy of shunt infections. The first is the mode of growth of the organisms in the shunt lumen. The concentration of antimicrobials required to kill biofilm organisms is often several logs higher than the conventional minimum inhibitory concentration.[19]

The second is the inherent multiresistance of many strains of coagulase-negative staphylococci. Almost all are resistant to penicillin and at least 50% are resistant to methicillin and therefore to cephalosporins. Resistance to aminoglycosides is also common. However, only rare clinical isolates of *Staph. epidermidis* are resistant to vancomycin, although some strains of *Staph. haemolyticus* and a few of *Staph. epidermidis* are resistant to teicoplanin. Similarly, the incidence of resistance to rifampin (rifampicin) and lincosamines is low in most centers.

The third factor is the lack of a vigorous inflammatory response in the CNS to most shunt infections, so that most systemically administered antimicrobials fail to penetrate the CSF, this being particularly true of aminoglycosides, β-lactams, glycopeptides and streptogramins. Of the few drugs that give acceptable CSF concentrations in such circumstances, chloramphenicol is bacteriostatic and ineffective in treating shunt infections; rifampin is highly active against most organisms causing shunt infections but cannot be given alone because of rapid development of resistance; and trimethoprim is active against fewer Gram-positive bacteria than rifampin. However, linezolid is active against bacteria commonly found in shunt infection, including those that are methicillin resistant, and it has good CSF penetration even in the absence of meningeal inflammation.[20]

These factors explain the generally disappointing results achieved whenever attempts are made to treat shunt infections without shunt removal. The shunt should therefore be removed early and an external ventricular drain (EVD) inserted to control CSF pressure (Table 23.2). This course of action is supported by many reports, clearly indicating that shunt retention is associated with a greater chance of relapse, a longer hospital stay and a greater risk of death. However, a recent report has claimed success in *Staph. epidermidis* shunt infections using intraventricular vancomycin and intravenous or oral rifampin without shunt removal.[21] The success of the regimen appears to depend on early diagnosis, the elective placement of a contralateral reservoir for antibiotic therapy, and *Staph. epidermidis* as the cause.

Table 23.2 Treatment of shunt infections caused by *Staphylococcus epidermidis*, other coagulase negative staphylococci and other susceptible Gram-positive bacteria

- Shunt removal, insertion of external drain with ≥5 cm tunnel
- Intraventricular vancomycin 20 mg q24h plus intravenous/oral rifampin (rifampicin) 15 mg/kg q24h (pediatric) or 600 mg q24h (adults), both in two divided doses
- After 7–10 days of treatment, if clinical response and if CSF cultures negative, re-shunt if necessary. Stop both antibiotics on day of re-shunting

Antimicrobials should be begun as soon as the diagnosis is confirmed. Vancomycin is recommended for CoNS, *Staph. aureus*, coryneforms and for those enterococci that are susceptible. As the drug does not give adequate CSF concentrations when given intravenously,[22] it should be given intraventricularly via a reservoir or through the clamped EVD tube. A Rickham reservoir should be incorporated in the system if the chosen EVD does not have a reservoir or injection port. Alternatively, an Ommaya reservoir can be inserted contralaterally.[21] The standard dose of intraventricular vancomycin is 20 mg daily, although this should be reduced to 10 mg daily for those with small ventricles. It should be noted that the dose depends on ventricular volume rather than on age or body weight. The vancomycin should be diluted in 1–2 ml sterile water for injection. In addition to vancomycin, rifampin should be given intravenously in a total dose of 15 mg/kg per day (two divided doses) for children or 300 mg every 12 hours for adults along with intravenous or intramuscular flucloxacillin (250–500 mg every 6 hours or 60–125 mg every 6 hours for children) or oxacillin (500 mg every 6 hours; for children >40 kg body weight, use adult dose, <40 kg 25–50 mg/kg every 4 hours). The intravenous drugs can be given orally in most cases after a few days. Alternatively, trimethoprim can be given intravenously, 3 mg/kg every 8 hours for children and 250 mg every 12 hours for adults. Teicoplanin offers no obvious advantage over vancomycin.

Intraventricular vancomycin given in the doses recommended above leads to CSF concentrations that commonly reach 5–10 times the expected plasma concentrations, but no toxicity has been encountered. Attempts should not be made to titrate the dose to keep the CSF concentrations below the toxic plasma levels. There is no indication for the use of intravenous vancomycin in addition to that given by the intraventricular route except in the case of methicillin-resistant *Staph. aureus* shunt infections, for which vancomycin might be the only available agent. The drug may be given by the intravenous route alone if the CSF cell count and protein concentration are sufficiently raised to indicate a vigorous inflammatory response, but this is the case only with *Staph. aureus* infections.

Using this regimen, CoNS should no longer be detectable in the CSF on microscopy or culture by day 4 and any fever should have resolved. A new shunt, if needed, can be inserted by day 7–10 of the regimen, the last dose of antibiotics being given on that day. It is unwise to wait for a few days after stopping treatment, as this is the period of greatest risk for secondary infection from the EVD. Using this regimen for CoNS, *Staph. aureus*, coryneforms and propionibacteria, successful eradication and re-shunting within 10 days without relapse can be expected in almost all cases.[23]

For shunt infections caused by Gram-negative bacilli, the shunt should again be removed and the treatment for Gram-negative meningitis (Chapter 18) instituted.

A notable exception to the rule of shunt removal is community-acquired bacterial meningitis in shunted persons. Such patients should be treated in the same way as those without shunts and can be expected to respond at least as well. On no account should these patients be subjected to shunt removal.[24]

REFERENCES


References for this chapter can be found online at http://www.expertconsult.com

Practice point | 7 |

Allan R Tunkel

When to do a lumbar puncture for the evaluation of meningoencephalitis

Lumbar puncture, with removal of cerebrospinal fluid (CSF), is an essential procedure in the evaluation and proper management of patients with meningitis and encephalitis. In patients with suspected bacterial meningitis, for example, CSF analysis allows assessment of whether the cell count and biochemical profile are consistent with the diagnosis (i.e. neutrophilic pleocytosis, elevated protein and decreased glucose) and permits performance of tests, such as Gram stain and culture, to establish the bacterial etiology.[1] Furthermore, the CSF profile may suggest other etiologies, and allow discontinuation of antibacterial therapy or management of other treatable infections or conditions. In patients with suspected encephalitis, performance of specialized tests on CSF (e.g. virus-specific IgM and nucleic acid amplification tests, such as polymerase chain reaction) can help determine the etiology of encephalitis and verify continuation of appropriate empirical therapy or allow discontinuation of therapy based on negative results; identification of a specific etiology may also be important for prognosis, potential prophylaxis, counseling of patients and family members, and public health interventions.[2]

Despite the benefits of CSF analysis in the management of patients with meningitis and encephalitis, lumbar puncture is sometimes not performed in an expeditious manner, or not performed at all, because of concerns of potential adverse events. Complications associated with lumbar puncture have ranged from mild alterations in comfort to life-threatening brain herniation.[1] The most common complication of lumbar puncture is headache, generally occurring in 10–25% of patients. Headache results from continued leakage of CSF at the site of the procedure, and is usually absent when the patient is supine, appearing rapidly when the patient stands. The risk of headache can be reduced with use of small-gauge, atraumatic needles and reinsertion of the stylet before needle removal.[3] Other complications of lumbar puncture include painful paresthesias, infection, local bleeding, and spinal subdural or epidural hematoma.

However, the most important potential complication following lumbar puncture is brain herniation (Fig. PP7.1).[4] In patients who undergo lumbar puncture, there is normally a transient lowering of lumbar CSF pressure as a result of removal of CSF and continued leakage of CSF from the opening made in the arachnoid membrane; this lowering of CSF pressure is rapidly communicated throughout the subarachnoid space.[5] In patients who happen to have an intracranial mass lesion, there is already a relative pressure gradient with downward displacement of the cerebrum and brain stem that can be further increased by lumbar puncture. This can then precipitate brain herniation. Furthermore, brain herniation can occur in patients with acute bacterial meningitis as a result of the development of cerebral edema and increased intracranial pressure. The exact incidence of brain herniation following lumbar puncture is unknown, but the possibility is such that patients should be carefully evaluated, always with an appropriate clinical examination and sometimes with a neuroimaging study such as CT scan of the head, to reduce the likelihood of precipitating this devastating complication.

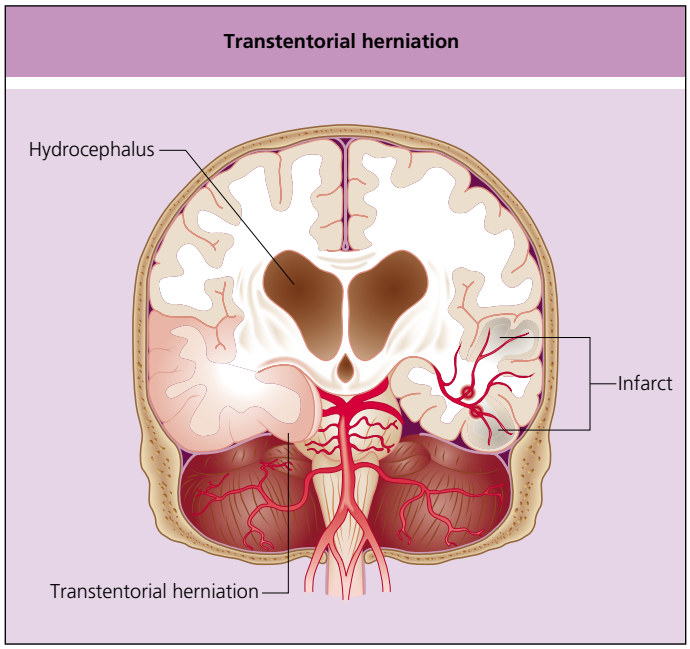


Fig. PP7.1 Transtentorial herniation is caused by diffuse swelling of the brain, or hydrocephalus; the herniation may be asymmetrical when lateral focal lesions are present. From van de Beek *et al.*[4]

In one study of 301 adults with bacterial meningitis,[6] an attempt was made to determine the clinical characteristics at baseline prior to CT that could be used to identify patients who would have abnormalities on neuroimaging. These were:

- age ≥60 years;
- a history of central nervous system (CNS) disease (e.g. mass lesion, stroke, focal infection);
- an immunocompromised state (e.g. HIV infection, immunosuppressive therapy, transplantation);
- a history of seizure ≤1 week before presentation; and
- specific neurologic findings (i.e. an abnormal level of consciousness, an inability to answer two consecutive questions correctly or to follow two consecutive commands, gaze palsy, abnormal visual fields, facial palsy, arm drift, leg drift or abnormal language).

None of these features was present at baseline in 96 of the 235 patients who underwent CT scan of the head. Of these 96 patients, the CT findings were normal in 93, yielding a negative predictive value of 97%. Of the three patients who did not have normal findings, only one had mass effect on CT and all underwent lumbar puncture with no evidence of brain herniation. On the basis of these findings, and review of other literature and expert opinion, a panel of the Infectious Diseases Society of America developed guidelines for adult patients who should undergo CT of the head prior to lumbar puncture, which were as follows:[1]

- immunocompromised state;
- history of CNS disease;
- new onset seizure;
- papilledema;
- abnormal level of consciousness; or
- focal neurologic findings.

The decision of when to perform a lumbar puncture in patients with suspected meningitis or encephalitis is further complicated by the fact that a normal CT scan of the head does not necessarily mean that lumbar puncture is safe, and it has been suggested that in patients with clinical signs of impending herniation, it is best to delay lumbar puncture as the procedure may precipitate brain herniation.[7] These clinical signs include a deteriorating level of consciousness (particularly a Glasgow Coma Scale of ≤11), brain-stem signs (e.g. papillary changes, posturing, or irregular respirations) and a very recent seizure. These findings would argue against the use of lumbar puncture or, if performed, use of a 22- or 25-gauge needle in order to remove the smallest amount of CSF for essential studies to minimize leakage after the procedure.[5] This is followed by careful monitoring for any clinical signs of impending herniation.

Despite concerns regarding the potential risk of brain herniation, CSF analysis should continue to be performed in patients who are appropriately evaluated prior to lumbar puncture.

REFERENCES


References for this chapter can be found online at http://www.expertconsult.com

Practice point | 8 |

Iain Stephenson
Martin J Wiselka

Approach to the patient who has fever and headache

INTRODUCTION

Fever and headache are common presenting symptoms with a wide differential diagnosis. The physician needs to be able to recognize the early features of meningitis and initiate appropriate treatment without delay as the consequences of a missed diagnosis or inadequate treatment can be fatal.

PATHOGENESIS AND MICROBIOLOGY

Inflammation of the meninges may result from a number of pathologic processes including infection, acute vasculitis, malignant infiltration and subarachnoid hemorrhage. Organisms can enter the meninges via local or blood-borne spread.

The organisms causing bacterial meningitis vary with age and geographic region (Table PP8.1). Meningococcal meningitis is most common in infants, children and young adults. Outbreaks of infection occur more frequently during the winter months and may affect nurseries, schools, universities and residential accommodation. The incidence of *Haemophilus influenzae* b and meningococcal types a and c meningitis has decreased dramatically since the introduction of conjugate vaccines; however, the current meningococcal vaccine does not protect against group b disease and there continue to be outbreaks of meningococcal infection in sub-Saharan Africa (type a) and the Hajj pilgrimage in Saudi Arabia (type W135). Pneumococcal meningitis may follow an initial otitis media, sinus infection or pneumonia. Most cases occur in infants with a second rise in incidence in the middle-aged and elderly.

Listeria monocytogenes is associated with pregnancy, advanced age and underlying immunosuppression or malignancy. If meningitis follows surgery or trauma the causative organisms may include Gram-negative and anaerobic bacteria. Tuberculous and fungal meningitis occur most frequently in patients who have resided in endemic areas or have had contact with infected cases and may be associated with immunosuppression, including underlying HIV infection.

Viral meningitis is predominantly caused by the enteroviruses (70%), but can also be caused by mumps, herpes simplex virus, varicella-zoster virus, measles virus, adenoviruses and Epstein–Barr virus.

CLINICAL FEATURES

The cardinal features of meningeal inflammation are headache, neck stiffness and photophobia. The speed of progression of the illness and any prodromal symptoms are often helpful in making the diagnosis. The presence of intracranial shunts, previous head trauma or surgery, travel history, age of the patient and underlying immunosuppression will influence the range of potential pathogens. Symptoms and signs of meningitis may be nonspecific in very young or elderly patients. Fulminant meningococcal sepsis, when purpuric lesions and hypotension may be prominent, is often present without meningitis or meningeal signs.

The vital signs should be documented and careful examination may reveal the presence of a purpuric rash (meningococcus; Fig. PP8.1), viral exanthem or lymphadenopathy. Evidence of meningism should be sought, including presence of neck stiffness, photophobia and any focal neurology or papilledema. Kernig's and Brudzinski's signs are of limited diagnostic value.[1] Examination of the tympanic membrane may reveal an underlying otitis media. Urinary dipstick examination should be performed as a urinary tract infection may occasionally present with meningism.

Table PP8.1 Likely organisms causing bacterial meningitis

Group of patients	Likely organisms
Neonates	Group B streptococci *Escherichia coli* *Listeria monocytogenes*
Children	*Neisseria meningitidis* *Streptococcus pneumoniae* *Haemophilus influenzae*
Adults	*Neisseria meningitidis* *Streptococcus pneumoniae* *Haemophilus influenzae* *Listeria monocytogenes*
Elderly persons/underlying malignancy	*Neisseria meningitidis* *Streptococcus pneumoniae* *Haemophilus influenzae* *Listeria monocytogenes* Gram-negative organisms
Post-traumatic surgery	*Streptococcus pneumoniae* *Haemophilus influenzae* *Escherichia coli* *Klebsiella pneumoniae* *Enterobacter* spp. *Pseudomonas* spp. *Staphylococcus aureus* Anaerobic organisms



Fig. PP8.1 Purpuric rash associated with meningococcal infection.

INVESTIGATIONS

Routine investigations include blood count, biochemistry and clotting profile. Antigen detection and culture of body fluids – blood, sputum, urine, cerebrospinal fluid (CSF), throat swab and skin scrapings (particularly meningococcal-related purpura) – may reveal the causative pathogen. Meningococcal, tuberculosis, herpes simplex and enterovirus genome detection by the polymerase chain reaction (PCR) is available in many centers. Comparison of meningococcal antibody titers in acute and convalescent serum samples can yield a retrospective diagnosis. Chest radiograph may reveal underlying infection.

Examination of the CSF remains important in many cases. Acute bacterial meningitis is usually associated with polymorphonuclear leukocytosis in the CSF with raised protein and low glucose levels. Viral meningitis characteristically gives a lymphocytosis with normal protein and glucose levels. However, there is a wide differential diagnosis of a lymphocytic CSF (Table PP8.2). A low CSF glucose (<2/3 serum glucose) usually indicates a bacterial, fungal, tuberculous or carcinomatous cause.

Table PP8.2 Some causes of lymphocytic cerebrospinal fluid

Viruses	Enteroviruses Mumps virus Herpes simplex virus
Bacteria	Partially treated bacterial meningitis Early bacterial meningitis Cerebral abscess Tuberculosis Brucellosis Spirochaetes (treponemes, leptospirosis, *Borrelia* spp.)
Fungi	Cryptococcosis Histoplasmosis
Protozoa	Toxoplasmosis Amebiasis
Inflammatory conditions	Seropositive conditions (e.g. lupus) Seronegative conditions (e.g. Behçet's syndrome, Kawasaki's disease)
Chemicals	Irritants and drugs
Carcinomatous conditions	Usually secondary deposits Cerebral lymphoma

MANAGEMENT

Immediate management

If bacterial meningitis is suspected, immediate hospital admission should be arranged and blood cultures and intravenous antibiotic therapy instituted without delay. Resuscitation may be required and all patients who have suspected or confirmed bacterial meningitis should be closely monitored, preferably in a high-dependency or intensive care unit. There is compelling evidence that immediate empiric antibiotic therapy can improve the outcome of meningococcal sepsis and meningitis. Following admission and any immediate resuscitation the two major questions for the clinician managing the patient are whether to do a head scan (CT or MRI) or a lumbar puncture.

Role of head scanning

The indications for performing an urgent scan before lumbar puncture include focal neurologic signs, altered level of consciousness, papilledema or symptoms suggestive of raised intracranial pressure, convulsions and suspected subarachnoid hemorrhage. A CT or MRI head scan can identify the presence of cerebral edema, hydrocephalus or a mass effect, and exclude alternative diagnoses of intracranial hemorrhage, subdural collection or abscess formation (Fig. PP8.2).

Role and safety of lumbar puncture

Immediate lumbar puncture should be avoided in patients with fulminant meningococcal disease and a widespread purpuric rash as there is a significant risk of cerebral edema and coning. In other cases lumbar puncture remains a very important diagnostic investigation which is recommended in all patients with suspected meningitis, except where there is a clear contraindication.[2] It is safe to perform a lumbar puncture in uncomplicated suspected viral meningitis without prior scanning.[3] If the CSF is clear, further investigations should be performed to establish the cause of fever and headache.

Choice of empiric therapy

Empiric antimicrobial therapy should cover all likely pathogens; treatment can then be modified if any organisms are identified. The role of corticosteroids remains controversial. Early dexamethasone treatment has been shown to be associated with an improved outcome in

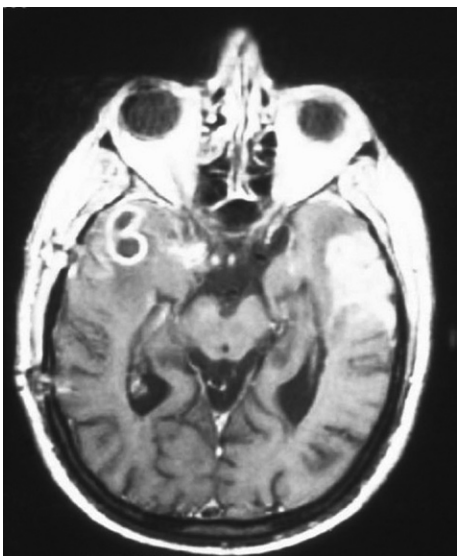


Fig. PP8.2 Post-contrast MRI scan showing cerebral abscesses.

meningitis caused by *Haemophilus influenzae* and *Streptococcus* pneumoniae,[4] but the effect of corticosteroids is less clear in other forms of bacterial meningitis. This is discussed in Chapter 18.

Management of the complications of meningitis

Bacterial meningitis may be accompanied by all the features of sepsis syndrome, with multiorgan failure requiring intensive care support. Early hemofiltration may be beneficial in seriously ill patients, but there are no controlled trials; there are anecdotal reports of the use of extracorporeal membrane oxygenation (ECMO).

Complications of meningitis can include cerebral abscesses and subdural empyema, secondary hydrocephalus and venous sinus thrombosis. Sensorineural deafness occurs in about 10% of patients who have pneumococcal meningitis and a smaller proportion of patients who have meningococcal meningitis. Audiologic assessment is recommended for all patients who have recovered from bacterial meningitis.

PUBLIC HEALTH ISSUES

Public health physicians should be notified immediately of any patients with known or suspected meningitis so that appropriate action can be taken, including provision of antibiotic prophylaxis to close contacts of patients with meningococcal or *Haemophilus* meningitis and more extensive prophylaxis and vaccination campaigns if these are indicated.[5]

REFERENCES

References for this chapter can be found online at http://www.expertconsult.com

Practice point | 9 |

Marc A Tack

Empiric antimicrobial therapy for suspected infection of the central nervous system

INTRODUCTION

The selection of an appropriate initial antimicrobial regimen for the management of infections of the central nervous system (CNS) is frequently empiric. The key clinical determinants that will guide this therapy include the patient's age, clinical presentation, immune status and medical history. The common utilization of vaccines against the most frequent bacterial pathogens in meningitis has made knowledge of a patient's vaccination record a key component of the history. Previous trauma or instrumentation of the CNS will also significantly impact the choice of antimicrobial therapy while cultures are pending. The clinical presentation of bacterial infections of the CNS can vary from rapidly life-threatening illnesses such as pneumococcal meningitis to more insidious infections such as neuroborreliosis. A detailed history including the use of immunosuppressive agents such as corticosteroids or disease-modifying agents utilized in the treatment of autoimmune illnesses is necessary to adequately assess a patient's risk of immune dysfunction. Use of these agents will result in an expanded list of potential pathogens and thus alter the selection of empiric therapy. A previously healthy adult who has acute bacterial meningitis poses different etiologic considerations from those of a patient with arthritis treated with a tumor necrosis factor-blocking drug. Similarly, surgical manipulation compromising the integrity of the blood–brain barrier, such as placement of a ventriculostomy or epidural pump, dictates empiric antimicrobial therapy targeted toward pathogens that gain entry by these routes.

PATHOGENESIS

The mechanisms of infection that account for most CNS infectious processes are well established. Bacteremia is the frequent cause of hematogenous seeding and acute meningitis. Direct extension of local infections, such as sinusitis and otitis, have been implicated as frequent causes of brain abscesses and surgical manipulation (such as the placement of a ventriculoperitoneal shunt) or trauma may allow direct seeding of the CNS.

MICROBIOLOGY

The most frequent pathogens associated with bacterial meningitis in a previously healthy host are *Streptococcus pneumoniae* and *Neisseria meningitidis*. Routine vaccination against *Strep. pneumoniae* in children as well as the use of licensed meningococcal vaccine in high-risk groups such as military recruits and college students is likely to result in a change in the frequency of these pathogens in the near future. *Haemophilus influenzae*, formerly a frequently encountered organism, is now rarely implicated owing to routine pediatric immunization. The emergence of strains of *Strep. pneumoniae* highly resistant to penicillin and ceftriaxone has major implications in the selection of empiric treatment of meningitis. In neonates, *Strep. agalactiae* is frequently isolated. In elderly individuals, immunocompromised patients and patients with iron overload, *Listeria monocytogenes* must be included in the differential diagnosis of acute bacterial meningitis (Table PP9.1).

Patients with foreign bodies or who have undergone recent neurosurgical procedures are at increased risk for infections caused by Gram-positive pathogens such as *Staphylococcus aureus* and coagulase-negative staphylococci, although a host of other organisms including fungi have been reported. Common skin flora such as diphtheroids and coagulase-negative staphylococci may cause infection in patients undergoing procedures penetrating the CNS, including epidural injections. Aerobic Gram-negative bacilli have also been implicated in trauma and postoperative patients. A detailed review of bacterial meningitis is found in Chapter 18.

CLINICAL FEATURES

The most common presenting clinical features of acute bacterial meningitis are fever, headache and meningismus. This triad of symptoms is seen in over 85% of cases and should be easily recognizable to the experienced clinician. Confusion and altered sensorium are also frequently noted. Fever and a change in baseline mental status should always raise the potential diagnosis of meningitis and requires rapid evaluation.

Physical examination frequently reveals nuchal rigidity, a positive Kernig's or Brudzinki's sign (although see comment in Practice Point 8) and the presence of photophobia.

Table PP9.1 Common bacterial pathogens associated with predisposing factors for meningitis

Age of patient	Most common bacterial pathogens
0–4 weeks	*Streptococcus agalactiae*, *Escherichia coli*, *Listeria monocytogenes*, *Klebsiella pneumoniae*
4 weeks to 2 years	*Streptococcus agalactiae, Streptococcus pneumoniae, Haemophilus influenzae*
2–18 years	*Haemophilus influenzae* (decreasing), *Neisseria meningitidis*, *Streptococcus pneumoniae*
18–60 years	*Streptococcus pneumoniae, Neisseria meningitidis*
Over 60 years	*Streptococcus pneumoniae*, *Listeria monocytogenes*, others

Presumptive evidence of meningococcal meningitis may be apparent on physical examination. Of patients who have meningococcemia, with or without meningitis, 50% manifest a macular erythematous papular rash early in the course of the disease. This rash rapidly appears petechial and then becomes purpuric.

The presentation of acute bacterial meningitis in neonates may be subtle as meningismus is uncommon and irritability and temperature instability may be the only indications of meningitis. Refusal to feed, lethargy, a high-pitched cry, vomiting, diarrhea and respiratory distress should alert the clinician to this diagnosis.

An insidious onset is frequently noted in elderly patients and in patients with co-morbid disease, as well as immunosuppressed hosts. Fever is frequently absent and headache, confusion or altered level of consciousness may be the only clinical finding.

Other less common causes of meningitis may frequently present with varying clinical syndromes. The presentation of cryptococcal meningitis in an AIDS patient manifests as an insidious illness; persistent fever and headache over several days or even weeks is often the only clinical complaint. A CD4 count below 50 is typically noted with this syndrome. Lyme meningitis, more common in highly endemic areas, often presents as a subacute basilar meningitis with one or more cranial nerve palsies and radiculopathy. Tuberculous meningitis may also present in an insidious manner, necessitating a high index of suspicion for early recognition and successful treatment.

The clinical manifestations of a brain abscess are usually more attributable to the anatomic location and space-occupying effect of the lesion than to the actual infection or pathogen. The classic triad (fever, headache and a focal neurologic deficit) is seen in only 50% of patients. Headache, which frequently is moderate to severe, is the most common manifestation; the next most common is fever. Eventually, altered mental state, ranging from confusion to coma, ensues. Neurologic findings may be focal, but this depends on the size and location of the abscess. Seizures as well as papilledema are more common in brain abscess than in meningitis.

The clinical presentation of infections of the CNS in patients with recent neurosurgical manipulation, with foreign implanted devices or recent epidural injection will vary depending upon the etiologic agent. Patients with a foreign body infection caused by *Staph. aureus* will likely appear toxic with fever and other common signs of meningeal inflammation, while patients with infection from coagulase-negative staphylococci will likely present with a complaint of a mild headache over several days. Clinical obstruction of a previously functioning ventricular drainage shunt should raise suspicion for infection and warrants evaluation of the cerebrospinal fluid (CSF).

INVESTIGATIONS

Rapid diagnosis and initiation of appropriate antimicrobial therapy are essential in patients who present with clinical manifestations of acute CNS infection. A lumbar puncture should be performed in all patients unless there are specific contraindications to this procedure. Patients who present with papilledema or focal neurologic findings should undergo a CT scan before lumbar puncture to determine whether there is a space-occupying lesion under increased intracranial pressure; it will determine the presence of a brain abscess in most cases as well. Initiation of antibiotic therapy should not be withheld awaiting imaging studies when bacterial meningitis is suspected. In patients with a CSF shunt or reservoir, samples should be obtained from these sites.

CSF should be examined using a cytospin technique for the presence of bacterial and fungal pathogens utilizing appropriate stains. Glucose and protein levels in the CSF should be obtained, together with a simultaneous serum glucose level for comparison. Cultures of CSF for bacterial, fungal and mycobacterial organisms should be obtained. If cryptococcal meningitis is suspected, a cryptococcal antigen assay should be performed on CSF as well an India ink stain. Bacterial latex agglutination tests are frequently used but are of limited benefit. Blood cultures should be sent in all cases because of the high rate of concomitant bacteremia. Other specimens, such as sputum, urine and sinus aspirates, should be obtained and evaluated when clinically appropriate.

MANAGEMENT

The rapid initiation of appropriate antimicrobial therapy in acute bacterial meningitis is imperative. As noted above, initiation of antibiotics should not be delayed while awaiting an imaging study before lumbar puncture. In general, the patient should receive the first dose of antibiotics within 30 minutes of arrival at the hospital if bacterial meningitis is suspected. Appropriate therapy should be based on the age, clinical presentation and predisposing factors, and therapy should be modified when identification of the pathogen and sensitivity testing become available.

In the past several years the incidence of resistance of community isolates of *Strep. pneumoniae* to penicillin has risen to the point where the author believes that vancomycin should be included with a third-generation cephalosporin (ceftriaxone or cefotaxime) as the initial empiric treatment of choice for acute bacterial meningitis in adults. In patients aged under 3 months or over 60 years, ampicillin should be included in the empiric regimen for the treatment of potential listeriosis. Therapy should be adjusted when antimicrobial sensitivities of the cultured isolate become available.

Patients who develop a CNS infection after trauma or postoperatively are at higher risk of Gram-negative bacillary infections. An antipseudomonal cephalosporin (ceftazidime or cefepime) or meropenem in combination with vancomycin is an appropriate empiric regimen. The frequent β-lactam resistance seen with coagulase-negative staphylococci, a common pathogen in this setting, dictates the use of vancomycin. This is also the empiric regimen the author suggests for patients who have foreign bodies in place. In patients with an indwelling reservoir, direct administration of vancomycin may be utilized (5–10 mg initially, diluted in preservative-free saline). In patients with organisms difficult to eradicate, intrathecal antimicrobial treatment should be considered.

Brain abscesses are typically caused by streptococci, Gram-negative bacilli and anaerobes and empiric antimicrobial therapy must target these pathogens and penetrate purulent collections. A third-generation cephalosporin (ceftriaxone or cefotaxime) in combination with metronidazole is appropriate in this clinical setting. Meropenem is another appropriate treatment option. The diagnosis and treatment of brain abscess is presented in detail in Chapter 20.

Although there are no controlled clinical trials, most clinicians treat acute bacterial meningitis for 7–14 days. The duration of therapy for brain abscesses is prolonged and determined by clinical and radiographic response. AIDS patients who are infected with cryptococcal meningitis require prolonged therapy followed by chronic suppression (see Chapter 178 for further discussion).

FURTHER READING

Further reading for this chapter can be found online at http://www.expertconsult.com



Chapter **24**

Rafik Bourayou
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Laryngitis, epiglottitis and pharyngitis

Laryngitis

Croup is a common respiratory tract infection of infants and children younger than 6 years of age, with a peak incidence between 7 and 36 months.[1,2] It is characterized by varying degrees of inspiratory stridor (noisy breathing on inspiration), barking cough, and hoarseness as a result of laryngeal and/or tracheal obstruction. Before the advent of treatment with corticosteroids and racemic adrenaline (epinephrine) for severe croup, intubation, tracheotomy and death were typical outcomes. Nowadays, mortality from croup has become a rarity in developed countries. Most children can be managed in the primary care setting.

EPIDEMIOLOGY

Croup is the most common cause of stridor in children and accounts for up to 15% of emergency department and primary care visits for respiratory infections in the USA.[3] The disease mainly affects those aged between 6 months and 3 years, with a peak annual incidence in the second year of life of nearly 5%. However, croup does occur in babies as young as 3 months old and in adolescents.[1] There is a slight male preponderance (male:female ratio, 3:2). Although croup is observed throughout the year, the majority of cases occur with parainfluenza viral infections, typically in the autumn. Those with the respiratory syncytial virus present mainly in the winter. There is often a smaller spring peak. The generalization of treatment with oral corticosteroids and nebulized adrenaline during the 1990s reduced the rate of hospitalization. Nowadays, most cases of croup are managed in the primary care or emergency room setting, with 1.5–31% of patients requiring admission[4] and less than 5% requiring endotracheal intubation.[5]

PATHOGENESIS AND PATHOLOGY

Parainfluenza is the etiologic agent in 50–70% of patients who are hospitalized for croup.[6] Other pathogens causing croup include influenza virus, respiratory syncytial virus, metapneumovirus, adenovirus, rhinovirus, enterovirus and, rarely, measles virus and herpes simplex virus. When croup is caused by influenza virus, the clinical picture is usually more severe than that caused by a parainfluenza virus.[7]

A strong association has been described between both human metapneumovirus and coronavirus HCoV-NL63 infection and croup in children.[8,9] However, a likely possibility is that the increasing number of viruses seen in association with croup is merely a reflection of improvements in methods of detection.

Laryngeal diphtheria is now very rare in immunized populations. However, outbreaks have been reported in case series from Russia and India. Measles remains an important cause of croup in nonimmunized children. Treatment with vitamin A has been reported to be effective for prevention of secondary infections, especially croup, in children with severe measles.

In acute laryngotracheitis, there is erythema and swelling of the lateral walls of the trachea, just below the vocal cords. Histologically, the involved area is edematous, with cellular infiltration in the lamina propria, submucosa and adventitia. The infiltrate contains histiocytes, lymphocytes, plasma cells and neutrophils. In bacterial croup – laryngotracheobronchitis and laryngotracheobronchopneumonitis – the tracheal wall is infiltrated with inflammatory cells; in addition, ulceration, pseudomembranes and microabscesses are present. There is thick pus within the lumen of the trachea and the lower air passages. In spasmodic croup, there is non-inflammatory edema in the subglottic region.[3]

PREVENTION

Prevention of disease depends mainly on good hand washing and preventing the spread of oral secretions. Masks and handkerchiefs inoculated with antiviral drugs have been used in experimental trials; however, after several minutes of breathing, when the mask becomes wet, the protection seems to diminish.

Vaccines are available to prevent some of these diseases. Effective measles vaccines have been used for approximately 30 years, so the disease has decreased dramatically in most countries. Certain adenoviral vaccines have been used with some degree of success, mostly in military personnel. Vaccines against parainfluenza viruses would have the most impact in preventing laryngitis and croup; however, these are still experimental. The ability of influenza vaccine to prevent laryngitis has not been studied.

CLINICAL FEATURES

Croup usually begins with nonspecific respiratory symptoms, including rhinorrhea, sore throat and cough. Fever is generally low grade (38–39°C) but can exceed 40°C. Within 1–2 days, the characteristic signs of hoarseness, barking cough and inspiratory stridor develop, often suddenly, along with a variable degree of respiratory distress. Symptoms may fluctuate depending on whether the child is calm or agitated. They are perceived as worsening at night and most emergency department visits occur at night between 10 pm and 4 am. Croup symptoms are generally short lived, with about 60% of children showing resolution of their barky cough within 48 hours. However, a few

children continue to have symptoms for up to 1 week. Spasmodic croup typically presents at night with the sudden onset of 'croupy' cough and stridor. The child may have mild upper respiratory complaints but more often appears completely well prior to the onset of symptoms.

The diagnosis of croup should be made clinically. The child's symptoms may range from minimal inspiratory stridor to severe respiratory failure secondary to airway obstruction. In mild cases, respiratory sounds at rest are normal; however, mild expiratory wheezing may be heard. Children with more severe infection have inspiratory and expiratory stridor at rest with suprasternal, intercostal and subcostal retractions. Air entry may be poor. Lethargy and agitation may be a result of hypoxemia. Warning signs of severe respiratory disease include tachypnea, tachycardia out of proportion to fever and hypotonia. Children may be unable to maintain adequate oral intake, which results in dehydration. Cyanosis is often a late ominous sign.

Determination of disease severity relies on clinical assessment. The most commonly used scoring system has been that of Westley *et al.*[10] (Table 24.1), which evaluates the severity of croup by assessing five factors: level of consciousness, cyanosis, stridor, air entry and retractions. This system has been extremely valuable in treatment trials but has little use in the routine clinical setting. However, the Alberta Clinical Practice Guideline Working Group has developed another clinically useful severity assessment table. Using this classification scheme, 85% of children in 21 general emergency departments in Alberta, Canada,[11] were determined to have mild croup and less than 1% had severe croup. The assessment is as follows:

- mild croup: occasional barking cough, no audible rest stridor, and either mild or no suprasternal or intercostal retractions;
- moderate croup: frequent barking cough, easily audible rest stridor, and suprasternal and sternal retractions at rest, with little or no agitation;
- severe croup: frequent barking cough, prominent inspiratory and, occasionally, expiratory stridor, marked sternal retractions, agitation and distress; and
- impending respiratory failure: barking cough (often not prominent), audible rest stridor, sternal retractions may not be marked, lethargy or decreased vigilance, and often dusky appearance with no supplemental oxygen.

Table 24.1 Westley croup score[10]

Symptom	Descriptor	Score
Stridor	None When agitated At rest	0 1 2
Retractions	None Mild Moderate Severe	0 1 2 3
Air entry	Normal Decreased Markedly decreased	0 1 2
Cyanosis in room air	None With agitation At rest	0 4 5
Level of consciousness	Normal Disoriented	0 5
Total score		0–17

Mild croup: scores 1–2; moderate croup: scores 3–8; severe croup: scores >8.

DIAGNOSIS

Radiologic studies are not recommended in a child who has a typical history of croup and who responds appropriately to treatment. Plain films of the airway and a chest radiograph may be obtained to rule out findings suggestive of another etiology. Anteroposterior films may demonstrate symmetric subglottic narrowing ('steeple sign') (Fig. 24.1),[12] although this may be absent in up to 50% of cases and may be present in the absence of croup. If radiographs are justified by an atypical clinical picture, the child must be closely monitored during imaging by skilled personnel with appropriate airway management equipment, as airway obstruction can worsen rapidly.

Cardiorespiratory monitoring, including continuous pulse oximetry, is indicated in children with severe croup but it is not necessary in mild cases. Oxygen saturation may be near normal in severe croup and yet significantly lowered in some children with mild to moderate disease.[10] This apparent discrepancy may relate to the degree of lower airway disease present.

MANAGEMENT

Over the past 50 years, there has been considerable controversy regarding many therapies for croup, including the role of humidified air and the optimal type (warm versus cold) and the roles of corticosteroids and racemic adrenaline (epinephrine).[3] However, the marked success of corticosteroids in the outpatient management of croup and the effectiveness of nebulized adrenaline (epinephrine) in more severe cases have led to the resolution of many of the controversies. The general consensus is that children with croup should be made as comfortable as possible, and clinicians should take special care during assessment and treatment not to frighten or upset them because agitation causes substantial worsening of symptoms. Sitting the child comfortably in the lap of a parent or caregiver is usually the best way to lessen agitation.

Oxygen is the immediate treatment of choice for children with severe viral croup who have considerable upper airway obstruction with significant oxygen desaturation (SaO_2 <90%). This therapy has not been subjected to a randomized controlled trial and is unlikely ever to be. It is the initial treatment before the administration of pharmacologic treatment in the hospital setting.

During much of the 20th century, treatment with humidified air (mist therapy) was the cornerstone of the management of croup.[3]

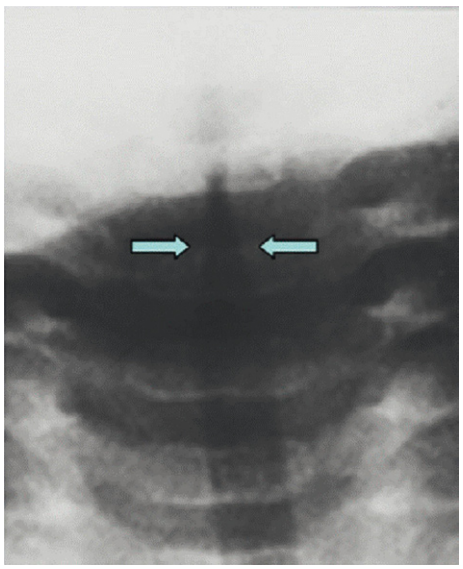


Fig. 24.1 Anteroposterior neck film demonstrating steeple sign (arrows) in a case of croup.

More recently, however, the effectiveness of mist therapy has been questioned. In a recent trial[13] comparing the effects of high humidity (100%), low humidity (40%), and blow-by humidity (in which a plastic hose is held near the child's nose and mouth) in children with mild croup, there were no significant differences in the croup score responses among the three groups; each group had significant improvement (about 33%) over baseline in the croup score 60 minutes after administration. In two other small trials, control subjects who received nebulized saline also had improvement in their croup scores over baseline values.[10] Since none of these studies included an untreated control group, it is not possible to determine whether or not the improvements were due to the moist air. A recent Cochrane Review of data from three other studies concluded that there was no evidence that inhalation of humidified air in children with mild-to-moderate croup resulted in a substantial improvement in the croup score.[14]

Corticosteroid therapy is now routinely recommended by all experts. In a cotton-rat laryngotracheitis model,[15] corticosteroids reduced the degree of inflammation and cell damage; although the viral load was increased, the duration of shedding was not prolonged. Meta-analyses of randomized trials[16] have consistently demonstrated significant improvement in patients treated with corticosteroids as compared with controls. For example, in a meta-analysis of 37 trials, patients who were given corticosteroids had significantly lower croup scores at 6 hours, a decrease in return visits and a decrease in time spent in emergency rooms or hospitals.[16]

Trials of corticosteroids in croup have involved a variety of drugs, dosages and routes of administration. The regimens studied most frequently have been single-dose dexamethasone (0.6 mg/kg of body weight given orally or intramuscularly) and nebulized budesonide (2 mg in 4 ml of water); some studies have involved additional doses (up to four doses of dexamethasone or nebulized budesonide given over a period of 2 days). No studies have directly compared the outcomes of single-dose therapy with the outcomes of 2-day treatment schedules. The 1992 recommendation by the Canadian Paediatric Society to use dexamethasone for treatment was followed by a marked decrease in hospitalizations for croup in Ontario, providing further support for the use of corticosteroids.[2] Similar findings were noted in Perth, Australia.

A potential concern with corticosteroids, however, is their immunosuppressive effects, which might predispose the patient to infectious complications. Trials have not been powered to assess these risks, but such complications would be expected to be rare with standard (single-dose) therapy.

Nebulized adrenaline (epinephrine) has been extensively studied for the treatment of croup. Early controlled trials demonstrated that the administration of 2.25% racemic adrenaline (epinephrine) (0.5 ml in 2.5 ml of saline) by intermittent positive-pressure breathing resulted in a significant reduction in the croup severity score,[10,17] but this benefit lasted for less than 2 hours. Later trials also showed that nebulized L-epinephrine diluted in 5 ml of saline at a ratio of 1:1000 was as effective as racemic adrenaline (epinephrine) in the treatment of croup.[18] In severe croup, repeated treatments with adrenaline (epinephrine) have been used and have often decreased the need for intubation.

GUIDELINES

The American Academy of Pediatrics has no guidelines for the management of croup. The Infectious Diseases and Immunization Committee of the Canadian Paediatric Society published a brief statement in 1992, recommending corticosteroid therapy for children admitted to the hospital with croup. The Alberta Medical Association published a guideline for the diagnosis and management of croup in 2004, which was updated in 2007.[11] An algorithm for the management of croup in the outpatient setting is shown in Figure 24.2.

Epiglottitis

Epiglottitis is an acute inflammation of the epiglottis or supraglottis that may lead to the rapid onset of life-threatening airway obstruction and is considered an otolaryngologic emergency. Since the widespread implementation of a conjugate vaccine for *Haemophilus influenzae* type b (Hib) nearly 2 decades ago, the incidence of epiglottitis has significantly declined in children. Securing the airway should be accomplished immediately in a controlled setting. Coordinated communication between the otolaryngologist, anesthesiologist, and intensive care physician is vital to the care provided to these critically ill patients.

PATHOGENESIS AND PATHOLOGY

Haemophilus influenzae type b (Hib) can colonize the pharynges of otherwise healthy children through respiratory transmission from intimate contact. These bacteria may penetrate the mucosal barrier, invading the bloodstream and causing bacteremia and seeding of the epiglottis and surrounding tissues. Bacteremia may also lead to infection of the meninges, skin, lungs, ears and joints.

Hib infection of the epiglottis leads to acute onset of inflammatory edema, beginning on the lingual surface of the epiglottis where the submucosa is loosely attached. Swelling significantly reduces the airway aperture. Edema rapidly progresses to involve the aryepiglottic folds, the arytenoids and the entire supraglottic larynx. The tightly bound epithelium on the vocal cords halts edema spread at this level. Aspiration of oropharyngeal secretions or mucous plugging can cause respiratory arrest.

Despite the dramatic decrease of Hib-related infections after the introduction of the vaccine, recent reports have shown that Hib may still cause epiglottitis despite adequate vaccination.[19,20] It should be noted, however, that vaccination failure may have prevailed with use of the older, purified polysaccharide vaccine.[19] Infectious agents in the postvaccination era associated with epiglottitis include group A *Streptococcus pneumoniae*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Haemophilus parainfluenzae* and β-hemolytic streptococci.

EPIDEMIOLOGY

Traditionally, epiglottitis was most commonly caused by Hib and primarily reported in children aged 2–7 years. The introduction of the Hib conjugate vaccine in 1988 dramatically changed the epidemiology of acute epiglottis. At the Children's Hospital of Buffalo, a rate of 3.5 cases of epiglottis per 10 000 admissions in 1969–1977 decreased to 0.3 cases per 10 000 admissions in 1995–2003. Hib was the causative organism identified in 84% of the cases in the earlier years, but was completely absent in the later segment of the study.[21] A 5-year retrospective review of the incidence of epiglottitis at the Children's Hospital of Philadelphia indicated a frequency of 10.9 per 10 000 admissions before 1990. Only 1.8 episodes per 10 000 admissions were noted 5 years after introduction of the vaccine.[22] A Finnish study also demonstrated a decreased prevaccination era incidence of 50 and 60 cases annually in 1985 and 1986, respectively, to only two cases in 1992 after widespread administration of the Hib vaccine.[23] In a Swedish study, the incidence of epiglottitis also decreased substantially from 20.9 in 1987 to 0.9 in 1996 for children younger than 5 years.[24]

There is a male preponderance of acute epiglottitis, with male-to-female ratios ranging from 1.2:1 to 4:1. Most studies have not demonstrated a seasonal variation in the incidence of acute epiglottitis.

Mortality rates have decreased considerably since the introduction of the Hib vaccine and the consequent shift in disease from young children to adults. Death rates are now less than 1% for children but approach 7% for adults. When deaths have occurred, a large percentage transpired due to delay in diagnosis or shortly after arrival at a medical facility for appropriate care.[25]

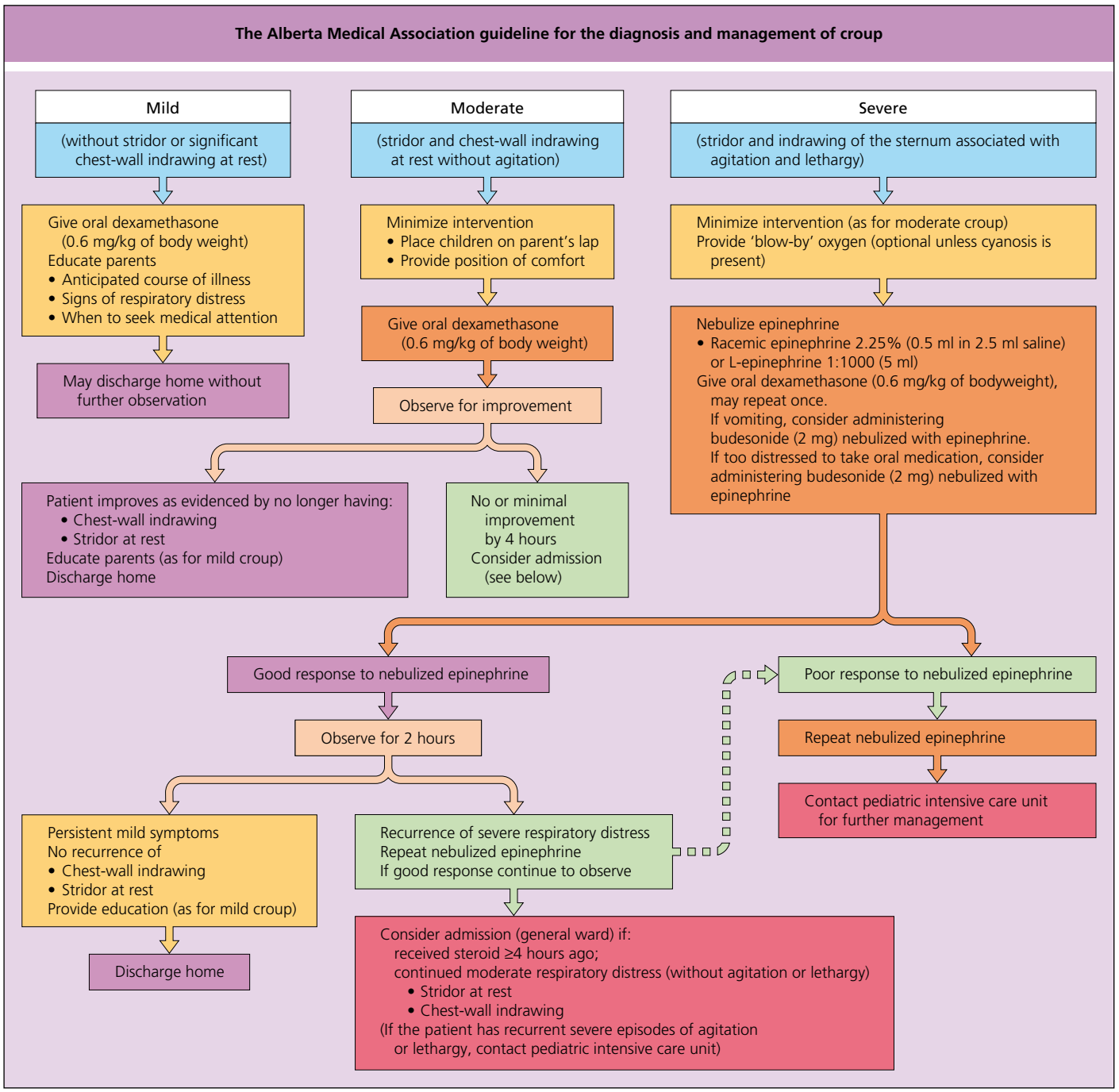


Fig. 24.2 The Alberta Medical Association guideline for the diagnosis and management of croup.[11]

PREVENTION

In the only study to date seeking specific risk factors for epiglottitis, day-care attendance was the strongest predictor for disease but the association was modified by whether the subject had had an upper respiratory illness in the previous 4 weeks. There was also the suggestion that northern European ancestry was a risk factor as well. Fortunately, the incidence of epiglottitis (and meningitis) has decreased markedly since the advent of the Hib vaccination. Whether the incidence of Hib disease in adults may change in the future is unknown, because long-term immunity from vaccination may prove to be either more or less effective than that due to natural infection.

CLINICAL FEATURES

Patients with epiglottitis often have an underlying illness, presumed to be viral. They then have sudden onset of fever, with the neck extended forward, drooling and air hunger. Affected children are anxious and lean forward to open their airway. The diagnosis is easily made by viewing the epiglottitis, which is swollen and red (Fig. 24.3). Intubation is often required. Culturing swabs from the epiglottis in children almost always obtains *Haemophilus influenzae* type b. Some children have been discharged without intubation after receiving only one dose of ceftriaxone when the epiglottis did not appear reddened, but subsequent epiglottic and blood cultures have been positive for Hib. They were

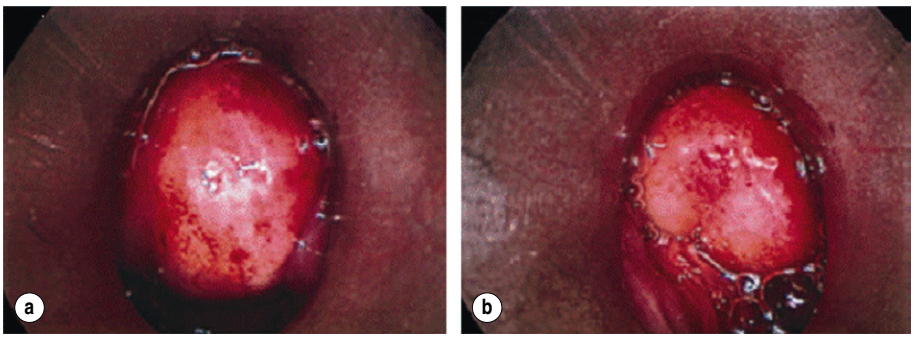


Fig. 24.3 Acute epiglottitis with views of the cherry red epiglottis on direct laryngoscopy.

cured completely. The duration of hospital treatment averages 3 days. Intubation is needed for less than 24 hours in most cases.[26]

In adults other pathogens may be obtained.[27] In most adults the disease is less severe and of slower onset. The airway obstruction occurs because of a progressive cellulitis of the supraglottic area. Thus at presentation, antibiotic treatment and intubation at the first sign of increasing respiratory compromise may avert the need for tracheotomy. The use of steroids to reduce inflammation and decrease the need for tracheotomy is appealing but unproven.

DIAGNOSIS

Visualization of the posterior pharynx is the best way to confirm the diagnosis of epiglottitis. Because airway obstruction is the most feared complication of this disease, this examination should be done in a manner and place where immediate intubation can be performed if necessary.

Lateral neck radiographs may demonstrate the classic thumb sign (Fig. 24.4). It is actually a rounded mass shadow of the normal leaf-like epiglottis resulting from the thickening and edema of the inflamed epiglottic tissue. Another radiologic feature of acute epiglottitis is the 'vallecula sign', which is the result of partial or complete obliteration of a well-defined air pocket bounding the base of the tongue and the epiglottis. The poor sensitivity (38%) and specificity (78%) of plain films limits the utility of this radiographic modality in the current age of technologic advances, whereby the larynx can be safely and accurately visualized with flexible laryngoscopy.[28]

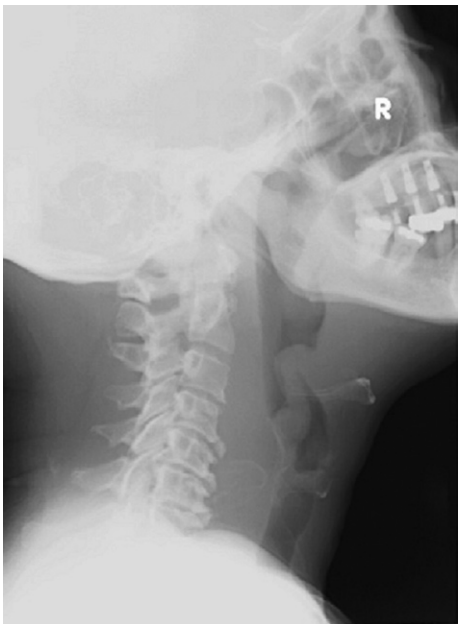


Fig. 24.4 Lateral neck film demonstrating thumb sign with edema of the epiglottis.

A complete blood cell count with differential, blood cultures and epiglottic cultures (when an artificial airway has been placed) are obtained after the airway is secure and the patient is stable. Elevated white blood cell counts are frequently present, but positive blood culture results are extremely variable (6–15%).

MANAGEMENT

Securing the airway is the initial step in the management of epiglottitis. Various studies have been performed to identify predictive factors for the need for airway intervention. A combination of features such as stridor, drooling, acute onset or rapid progression, hoarseness, respiratory distress, dyspnea, chest wall retractions and upright position have been associated with the need for airway intervention.[29]

Appropriate antibiotics include ceftriaxone, cefotaxime and cefuroxime (for non-meningitic infections). Ampicillin should not be used due to the high frequency of ampicillin-resistant strains of Hib. Steroids are commonly employed to decrease mucosal edema of the epiglottis, but no data exist in the literature to prove any benefit from their use.

Most children can be successfully extubated after 24 hours of antibiotic therapy and some extubate themselves before that time has expired. Family members and day-care contacts should receive rifampin prophylaxis (300 mg q12h for 2 days) to avoid secondary infection.

Pharyngitis

Pharyngitis is a very common inflammatory condition of the pharynx accompanied by a sore throat and occasionally difficulty in swallowing. It is usually viral but may be caused by bacterial or fungal infection. Gastroesophageal reflux disease (GERD) or particularly extraesophageal reflux (EER) can also cause an acid pharyngitis in adults and children. Serious complications of pharyngitis may include peritonsillar abscess or retropharyngeal abscess.

EPIDEMIOLOGY

Pharyngitis can be separated into one group of illnesses with associated nasal symptoms (which are most commonly viral in origin) and another that causes only pharyngitis. It is important to distinguish between these infections because rheumatic fever and acute glomerulonephritis may complicate untreated group A β-hemolytic streptococcal infections, but they can usually be prevented by appropriate antibiotic treatment.

Adenoviruses, rhinoviruses, coronaviruses, enteroviruses and parainfluenza viruses most frequently cause self-limiting viral infections. Other viral infections, such as respiratory syncytial virus (RSV) and Epstein–Barr virus (EBV), are less common but still frequently

occur. Bacterial causes of upper respiratory infections are led by group A β-hemolytic streptococci (GAS) but can also be caused by *Haemophilus influenzae*, *Bordetella pertussis*, *Chlamydia pneumoniae*, *Corynebacterium haemolyticum*, *Mycoplasma pneumoniae* and *Yersinia enterocolitica* among others.

These upper respiratory infections are often difficult to differentiate and hence difficult to diagnose, frequently leading to futile overtreatment in many cases. Further complicating the issue is the fact that primary viral infections are often succeeded by secondary 'opportunistic' bacterial infections, making undertreatment a problem in a significant minority of infections. Additionally, individuals with allergies are sometimes more prone to secondary bacterial infections. The clinician is therefore challenged to weigh multiple factors involved in deciding whether an infection is viral or bacterial in origin, and whether antibiotic treatment is warranted.

Viral upper respiratory infections frequently occur in mini-epidemics (RSV, parainfluenza, influenza, varicella, measles). They are more common in the winter except for those caused by enteroviruses, which are more common in the summer.[30] Some viral infections occur year round, with no seasonal pattern (adenoviruses). Group A β-hemolytic streptococcal infections are more common in the winter, but many other nonviral respiratory infections do not appear to be seasonally linked (*Chlamydia* and *Mycoplasma* spp.). Some bacterial infections appear to be linked to preceding viral infections and hence occur more commonly in the winter. Pharyngeal colonization may occur throughout the year.

Influenza infections vary significantly from year to year. In the USA one subtype was predominant each year until about 1990, since which time both H3N2 and H1N1 strains have been circulating simultaneously. When there is a major shift in antigen type, significant excess morbidity occurs as the new strain infects the community. Increasing air travel has accelerated the rate at which the influenza viruses travel around the world and has perhaps been responsible for the increasing frequency with which the viruses are detected. The shifted strain outbreaks have most affected the elderly and children with congenital heart and lung disease. Minor influenza drifts have occurred also, but cause less disease. There has also been a recent decrease in the average age at which children acquire upper respiratory diseases because of the increasing use of day care for children. Although the long-term effect of this is unknown, in the short term it appears that it has been responsible for a significant increase in the number of ear infections in children less than 2 years old.[31]

Rheumatic fever, a complication of group A β-hemolytic streptococcal pharyngitis, has waxed and waned in importance.[32] After a century of prominence, the disease was in considerable decline in developed countries for 40 years. Recently, clusters of rheumatic fever cases have occurred, for example in Salt Lake City, Utah, USA, where it is hypothesized that the re-emergence of certain M types has been responsible.

PATHOGENESIS AND PATHOLOGY

The pathogenesis of the sore throat due to pharyngitis is poorly understood. Volunteers given rhinoviral infections produce bradykinin and lysylbradykinin, which are known inflammatory mediators that can excite nerve endings in the pharynx to cause pain.[33] There is also suggestive evidence from laboratory animals that adenovirus, RSV and other viral infections directly invade the pharyngeal cells and produce an inflammatory response. This leads to the well-described 'red, sore throat'. Additionally, adenovirus and EBV often produce lymphoid hyperplasia and tonsillar exudation. Herpes simplex virus (HSV) and coxsackievirus infections frequently lead to ulcerations of the oral mucosa. Herpes simplex virus ulcers are more common in the anterior part of the mouth and coxsackievirus ulcers occur more frequently in the posterior part of the pharynx, but this is only a guide and both viruses can cause ulcers in any part of the oropharynx. Herpes simplex often produces a significant gingivitis as well.

Streptococcal pharyngitis often involves the posterior pharynx, with petechiae on the uvula and soft palate.[34] When one sees this clinical sign, GAS is often isolated by throat culture. A confusing factor is that up to 10% of patients who have EBV infections will have a secondary group A β-hemolytic streptococcal pharyngitis during their illness. *Corynebacterium diphtheriae* can also cause pharyngitis, producing a characteristic gray membrane across the structures of the posterior pharynx. This is seldom seen today except in a few geographic areas where diphtheria outbreaks have occurred recently, such as Russia. There are also noninfectious causes of pharyngitis, such as Behçet's syndrome, Kawasaki disease, Marshall's syndrome and Stevens–Johnson syndrome.

PREVENTION

Preventing pharyngitis is desirable but difficult to achieve. Mostly aerosolized oral secretions, hand-to-mouth contact with multiple individuals and the use of common utensils, glassware, etc. spread viral pharyngitis. Certain viruses are known to be particularly resilient; RSV has been cultured from tabletops hours after being inoculated.[35] Measles has been known to be contracted from the air in a physician's waiting room, as long as 1 hour after the child with measles had left the room. Other viruses may be less durable and less contagious, but close contact is obviously not necessary to transmit many of these agents. Prevention of disease depends mainly on good hand washing and preventing the spread of oral secretions. Masks and handkerchiefs inoculated with antiviral drugs have been used in experimental trials, but after several minutes of breathing, when the mask becomes wet, the benefit seems to diminish.

There are vaccines available to prevent some of these diseases. Effective measles vaccines have been used for approximately 30 years, so the disease has decreased dramatically in most countries. Certain adenoviral vaccines have been used with some degree of success, mostly in military personnel. Vaccines for RSV and parainfluenza viruses are currently under development. These vaccines could have a significant effect on the population's health, particularly on that of the youngest children.

Transmission of streptococcal pharyngitis seems to require closer contact than for most viruses. Studies performed in the military during the Second World War showed that soldiers in barracks sleeping on either side of the index case were more likely to have disease than those further away.

To date, there are no immunizations available to prevent streptococcal disease, although trials evaluating group B and group A vaccines are under way. For patients who have had prior group A disease and subsequent rheumatic fever, penicillin prophylaxis is recommended. Most patients receive intramuscular benzathine penicillin, 1.2 million units, once per month; although oral regimens are acceptable, they have poorer compliance rates.

CLINICAL FEATURES

Pharyngitis is a ubiquitous infection. A 'sore throat' affects most people at least once every year. Most cases of viral pharyngitis are associated with an upper respiratory infection (nasopharyngitis). Generally nasopharyngitis has a prodrome that may include malaise, diaphoresis, fever, headache and general aches and/or pains. Coryza and sore throat then begin. Many infections will progress to produce a cough and/or laryngitis. Some viral infections produce predominantly coryza, others more pharyngitis, and others more cough or laryngitis. Coxsackieviruses often cause ulcers in the posterior pharynx along with a sore throat. Measles can cause a severe pharyngitis, but the associated symptoms of conjunctivitis, rash and Koplik's spots make the disease easily diagnosable. Parainfluenza and influenza viruses can give a particularly painful pharyngitis, with frequently associated symptoms of cough and laryngitis.

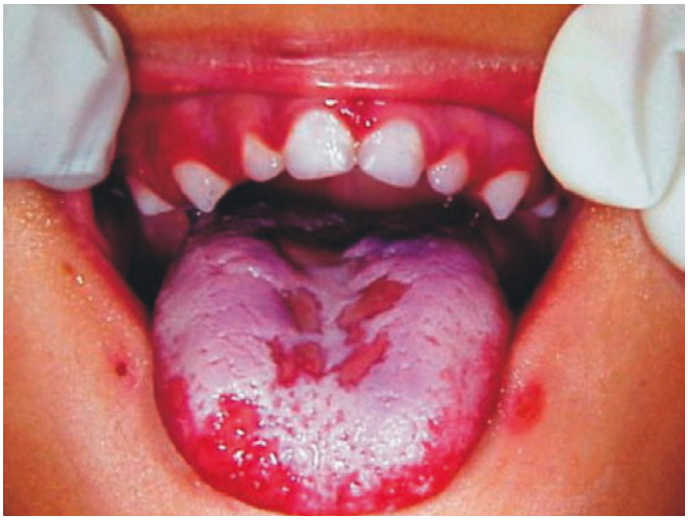


Fig. 24.5 Primary infection of HSV-1

The DNA viruses EBV, adenovirus, cytomegalovirus and HSV can produce significant pharyngitis. They also tend to last longer than the other viral causes of pharyngitis. These viruses produce other upper respiratory symptoms such as nontender cervical adenopathy of, in the case of HSV, tongue and mouth ulcers. Herpes simplex virus pharyngitis has been described as a disease in which 'the gums swell up and swallow the teeth' (Fig. 24.5). Rhinoviruses and RSV infections give upper respiratory symptoms as well as pharyngitis in infants.

The syndrome of acute HIV infection ('seroconversion illness') is well described and may cause symptoms in up to 50% of patients. It is a mononucleosis-like illness in which pharyngitis is a prominent feature. Patients will also have fever, lymphadenopathy, rash and myalgias. The symptoms are nonspecific.

It is important to diagnose bacterial causes of pharyngitis because, unlike viral causes, many can be treated specifically with antibiotics. Proper treatment can avoid significant morbidity and/or mortality. Pharyngitis caused by GAS is the most common infection causing significant pharyngeal edema, frequently with petechiae on the soft palate and uvula (Fig. 24.6). Tender cervical nodes are common. Small children may complain of abdominal pain, which may be due to mesenteric adenitis. Headache and raised temperature are also common. Some patients who have a streptococcal sore throat have a characteristic red 'scarlet fever' rash that begins in the groin and axillary areas and spreads over the body (Fig. 24.7). The rash is sandpaper-like and may itch. A strawberry tongue is also often present. Other patients have a characteristic rash on the face. Without treatment the illness usually resolves over 3 or 4 days, but rheumatic fever may ensue.



Fig. 24.6 GAS tonsillitis.



Fig. 24.7 Scarlatine rash.

The recommended treatment is penicillin, or clindamycin, erythromycin or azithromycin for those allergic to penicillin. There is concern about the recurrence rate of streptococcal pharyngitis in adequately treated patients but there is no evidence for microbiologic resistance to therapy. It is more likely that the organism is reacquired or sequestered in a sanctuary site and simply re-emerges after therapy is discontinued. Some have used rifampin at the end of therapy in an attempt to alleviate this possibility. While all therapies have similar response profiles, azithromycin has a higher culture-positive recurrence rate.

The risk of rheumatic fever without antibiotic treatment is difficult to assess. Wannamaker and colleagues documented rates as high as 3% in 1952;[36] however, more typical rates of 0.4% were found in a pediatric population in 1961.[37] Certainly M protein type, genetic susceptibility and previous infection play a part in this diversity of recurrence rates. Other β-hemolytic streptococcal infections (groups C, G and B) can cause pharyngitis but not rheumatic fever. For these, antibiotic treatment may provide symptomatic relief.

Occasionally, pharyngitis can be secondary to an abscess in the peritonsillar area. This is usually easily diagnosed by an asymmetry of the tonsillar pillars. The affected side is asymmetrically enlarged and protrudes anteriorly into the mouth. *Haemophilus influenzae* (nontypeable and types a–f) can cause pharyngitis and type b can also cause epiglottitis or meningitis. Many individuals are carriers but are not ill.

Corynebacterium diphtheriae causes diphtheria, which is easily diagnosed because of the gray pseudomembrane in the posterior pharynx along with pharyngitis (Fig. 24.8). The disease has recently become endemic in parts of the former Soviet Union. *Arcanobacterium* (previously *Corynebacterium*) *haemolyticum* is a common cause of pharyngitis and can also cause a scarlatiniform rash. It is the cause of many non-GAS throat infections.[38] *Neisseria gonorrhoeae* can also cause pharyngitis. The appearance of the pharyngitis is nondiagnostic, so a heightened awareness is required to make this diagnosis.[39] *Chlamydia*



Fig. 24.8 Diphtheria pharyngitis with gray pseudomembranes in the posterior pharynx.

pneumoniae and *Mycoplasma pneumoniae* can cause pharyngitis, but generally will go on to cause cough also, often with wheezing and pneumonia.[40,41] *Candida albicans* can cause pharyngitis but normally only in the immunocompromised host. The pharyngitis is hyperemic, with white plaques on the buccal mucosa.

Aphthous stomatitis is a common feature of mouth ulcers. The etiology is unclear. Small painful ulcers appear on the buccal mucosa, but can also appear in the posterior pharynx. The ulcers are usually stress related and last approximately 1 week. Very extensive aphthous ulceration can also be seen as a complication of HIV infection. Behçet's syndrome may cause aphthous stomatitis. Kawasaki disease, most common in young children, can cause significant redness of the oral mucosa. Most children with Kawasaki disease also have fever, a strawberry tongue and, importantly, conjunctivitis. This condition is frequently confused with streptococcal disease. Stevens–Johnson syndrome can result in pharyngitis, stomatitis and perioral swelling and ulcerations. Marshall's syndrome or PFAPA (periodic fever, aphthous stomatitis, pharyngitis, cervical adenitis) syndrome is a pediatric periodic disease characterized by recurrent febrile episodes associated with head and neck symptoms.[42]

DIAGNOSIS

Prevalence of group A β-hemolytic streptococcus (GABHS) during school outbreaks of pharyngitis is between 15% and 50% in healthy school-age children. Since toddlers with GABHS respiratory tract infection may not present with classic symptoms, the diagnosis of GABHS pharyngitis among children should be based on laboratory tests in conjunction with the clinical findings. Culture isolation of GABHS from the pharynx is the gold standard, but 2 days are needed for it to be informative. On the other hand, the rapid antigen detection test (RADT) detects the presence of GABHS within a few minutes and has high sensitivity and specificity.

Rapid testing has many additional benefits: early treatment within 48 hours provides symptomatic relief for the child and limits spreading of the organism. In addition, it allows the practitioner to treat only those cases with GABHS, thus avoiding prescribing antibiotics for viral infections. Group B, C and G streptococci can also cause significant morbidity, but only group A leads to rheumatic fever, so the reason for treatment is not only to eliminate the pharyngitis but also to prevent the subsequent rheumatic disease. Viral causes of pharyngitis do not normally require specific diagnosis, but serologic tests are available for mononucleosis (EBV) and cytomegalovirus. Adenoviruses, RSV and parainfluenza viruses can be diagnosed using rapid antigen tests, which are available but rarely used in uncomplicated community-acquired infections.

MANAGEMENT

For group A streptococcal pharyngitis the recommended therapy is 6–10 days of oral penicillin or amoxicillin. Erythromycin and clindamycin are acceptable alternatives. There have been studies showing that one dose of ceftriaxone intramuscularly or oral azithromycin or cefaclor for 5 days is equally effective at eliminating carriage of GAS, but recurrent pharyngeal colonization occurs with all treatment regimens. Viral causes of pharyngitis can be most suitably treated with supportive measures: gargles, lozenges, etc. *Mycoplasma* and *Chlamydia* spp. infections can be treated with erythromycin or tetracycline (depending on age). Diphtheria and *Arcanobacterium* spp. infections should be treated with erythromycin or penicillin. *Legionella* spp. infections should be treated with tetracycline. *Haemophilus influenzae* type b and *Yersinia enterocolitica* infections should be treated with a third-generation cephalosporin.

REFERENCES

References for this chapter can be found online at http://www.expertconsult.com

Chapter 25

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Otitis, sinusitis and related conditions

Otitis

Otitis media (OM) is a frequent disease in early childhood and is the first reason for prescription of antibiotics in pediatric practice. Viruses are the leading cause and in 60–80% of cases OM resolves spontaneously within 48 hours. Overuse of antibiotics is due to current misunderstanding of the microbial ecology and is also explained by the fear of secondary complications. However, it is well known that unnecessary antibiotic treatment may favor the development of multiresistant pathogens. Appropriate prescription of antibiotics for OM is based on clinical findings, the patient's age and the bacterial ecology.

EPIDEMIOLOGY

Acute otitis media (AOM) is common in young children and is associated with upper respiratory tract infection.[1] The immaturity of the immune system plays a major role in its development but other risk factors – such as male sex, lower age (peak age of incidence is between 6 and 18 months),[2] family history and above all the early entry into day care[3] – are well known. The frequency of AOM increases with allergy and passive smoking that modify the ciliated respiratory membrane. Esophageal reflux can also favor AOM by way of throat inflammation.[4]

PHYSIOPATHOLOGY

The middle ear and rhinopharynx are covered by a ciliated respiratory mucous membrane. The middle ear communicates with the pharynx by the Eustachian tube that allows physiologic drainage of the mucus secreted by the middle ear. Viral aggression against the respiratory epithelium, so frequent in early childhood, decreases ciliary movement, favoring bacterial adhesion. At the same time, the inflammation provokes Eustachian tube obstruction. Together, these mechanisms facilitate bacterial proliferation in the middle ear, with the formation of pus.[1]

Acute otitis media develops into two phases:

- first, the congestive phase (red eardrums, not inflated with normal reliefs); and
- second, the purulent phase (tympanic inflammation and effusion behind the eardrum).

The principal causative pathogens are *Haemophilus influenzae* (HI), *Moraxella catarrhalis* (MC) and *Streptococcus pneumoniae* (SP), the last being associated with a more frequent risk of complications.

PREVENTION

Vaccination

Vaccination by the octavalent vaccine or 14-valent anti-SP vaccine in the 1980s reduced the frequency of AOM but only in children aged more than 6 months. Moreover, efficacy was limited to the first 6 months following vaccination.[5] The new anti-SP conjugated heptavalent vaccine (7-valent SP conjugate vaccine or PCV7) is effective against the invasive forms of diseases caused by SP in children who were immunized at 2, 4 and 6 months of age.[6] Immunized children had fewer episodes of AOM caused by the seven serotypes (4, 6B, 9V, 14, 18C, 19F, 23F) contained in the vaccine but had more episodes of AOM caused by the other serotypes. The use of PCV7 has a strong impact on penicillin nonsusceptible SP carriage in children with AOM.[7] Unfortunately, since the introduction of PCV7, a multiresistant serotype, 19A SP, not included in PCV7, has emerged in the USA.[8]

Prevention of respiratory viral infections

Viruses can be co-factors of AOM. AOM and sinusitis are the most common bacterial complications of upper respiratory tract infections in children.[9] The use of the vaccine against influenza reduced the frequency of AOM caused by influenza by 36%. However, the benefit of vaccination is low because influenza is responsible for only 5% of cases of AOM.[10] Respiratory syncytial virus (RSV) is closely associated with AOM. The use of anti-RSV immunoglobulins decreases the frequency of bronchiolitis but does not modify the frequency of AOM.

Antibiotic prophylaxis

Prophylaxis against AOM with antibiotics is not currently recommended as this favors the selection of multiresistant pathogens. However, in patients who had multiple episodes of AOM within 6 months and/or have proven immunodeficiency, amoxicillin or trimethoprim treatment may be considered.[11]

Tympanostomy tubes and adenoidectomy

The efficacy of these procedures in the prevention of AOM has not been demonstrated but they do avoid hearing disorders due to repeated episodes of AOM. They are beneficial in chronic otitis media with effusion.[11]

DIAGNOSIS AND CLASSIFICATION

The diagnosis of AOM is difficult and should be based on rigorous criteria. Indeed, according to the severity of symptoms and associated systemic signs, the child may (or may not) receive antibiotic treatment initially.[12] Otoscopic examination allows the diagnosis of AOM. However, the technique can be problematic due to issues that may arise during examination (e.g. small auditory canals, cerumen obstruction, child restlessness).[13] Rhinopharyngitis and/or child screams during examination induce congestion and hypervascularization of the eardrum.

Congestive otitis and purulent otitis are separate conditions:

- Congestive otitis (CO) is an acute inflammation of the middle ear. Eardrums are congested but reliefs are normal and there is no tympanic convexity. More often CO is caused by viral infection. Secondary bacterial infection is possible and justifies medical follow-up.
- Purulent otitis (PO) (Fig. 25.1) is an inflammation of the tympanic membrane associated with a retrotympanic effusion, which can sometimes be exteriorized by otorrhea. Common symptoms are severe ear pain (expressed in a young child by irritability, tears and sleeplessness), fever, asthenia and anorexia. Ear pain arising at night and fever are frequent but not mandatory. Associated nonspecific signs include gastrointestinal disorders, cough and rhinorrhea in the context of associated viral infection.

Some clinical associations indicate a diagnosis of bacterial otitis media – for example, otitis with conjunctivitis indicates HI; high fever with ear pain indicates SP. Acute otitis media without systemic signs is called seromucous otitis. External otitis must be distinguished from AOM with otorrhea, in which there is no perforation of the tympanic membrane and pain persists in spite of drainage of secretions.

BACTERIOLOGY

Bacteria are retrieved in 50–90% of cases of AOM.[14] The principal pathogens identified by aspiration and culture of middle ear fluid are SP (the most frequent, 20–35%), HI (nontypeable), MC and *Strep. pyogenes* (group A streptococcus). *Staphylococcus aureus* (less than 5%) and *Pseudomonas aeruginosa*, retrieved in few cases, may also cause chronic OM.[14] Since the use of PCV7, the cost of non-invasive pneumococcal diseases such as AOM has increased in the US population, as well as the frequency of HI AOM.[15] Moreover, PCV7 has reduced the rate of vaccine-type-associated AOM by 57%.[16] *Strep. pyogenes* is more frequent in Europe than in the USA, especially in children more than 2 years old. Respiratory viruses (RSV and influenza mostly, but also enterovirus and rhinovirus) are also found alone or in association with bacteria in the auricular liquid.

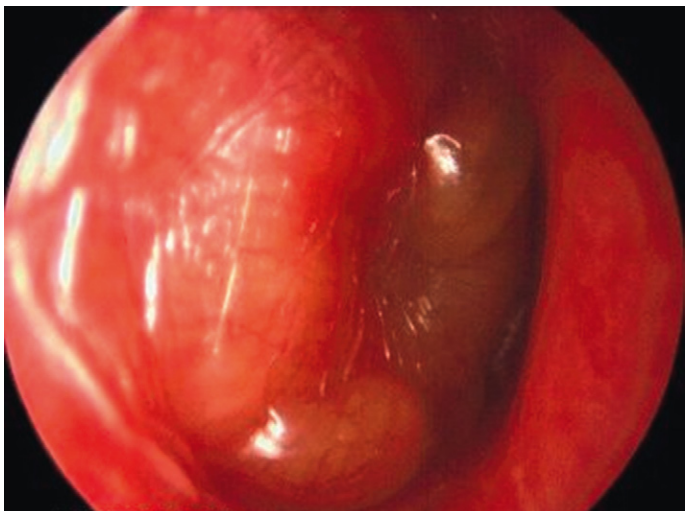


Fig. 25.1 Acute otitis media compared with normal tympanic membrane.

In light of this information, the treatment of AOM remains empiric because the responsible pathogen is not identifiable in the majority of cases. Modification of the bacterial ecology over the past few years, as well as increasing resistance, may influence antibiotic treatment.

SP strains isolated in AOM can be resistant to β-lactams by modification of protein-linking penicillins (PLPs). Sensitivity decrease concerns G penicillin, amoxicillin and oral cephalosporins. The rate of penicillin-resistant SP is 12% in the USA and more than 30% in Asia.[14] More than 80% of AOM SP strains are resistant to macrolides. The major serotypes involved in AOM are 19A, 19F, 23F, 6B and 14; all are contained in the heptavalent-conjugated vaccine except 19A. Since the generalization of SP vaccination in USA, there has been an emergence of nonvaccinal serotypes with decreased sensitivity to β-lactams.[8] Nontypeable HI causes most cases of AOM. HI secretes β-lactamases, which inactivate amoxicillin and sometimes C1G. Occasionally there is modification in the target of β-lactams, the PLPs. These strains are called 'BLNAR' (β-lactamase-negative, ampicillin-resistant). They are fortunately sensitive to high-dose amoxicillin and to C3G. Macrolides are naturally only slightly active or inactive against HI. *M. catarrhalis* is resistant to amoxicillin due to secretion of β-lactamases. Amoxicillin–clavulanic acid, C2G and C3G are active against these strains.

Acute otitis media in children less than 3 months of age is caused by pathogens such as Enterobacteriae and *Staph. aureus* that may disseminate rapidly into systemic life-threatening infection. Treatment requires parenteral antibiotic therapy after thorough research of the pathogen by tympanocentesis.

TREATMENT

In March 2004 the American Academy of Pediatrics (AAP) proposed guidelines for treatment of AOM.[17] The goal of treatment is sterilization of the middle ear and reduction of pain. Prescription of antibiotics depends essentially on two factors: the age of the child (younger or older than 2 years) and the severity of systemic signs. For children younger than 2 years, initial antibiotic treatment is recommended. For children older than 2 years with few or no symptoms, symptomatic treatment only is recommended, with patient re-evaluation after 48–72 hours. This attitude is called 'watchful waiting' and is justified because immediate antibiotic treatment has only a modest effect on the course of AOM. For example, a study compared the safety, efficacy, acceptability and cost of non-antibiotic intervention for children with mild AOM versus amoxicillin treatment. Results showed the same parent satisfaction in both groups and no serious adverse events in patients who did not receive antibiotics.[18]

Antibiotics are not recommended for treatment of congestive otitis and seromucous otitis, unless symptoms last more than 3 days in cases of congestive otitis and more than 3 months in cases of seromucous otitis. Clinical studies comparing antibiotics versus placebo have shown that the majority of cases of AOM recover spontaneously. The spontaneous cure is more frequent when the child is more than 6 months old and is infected by HI. Conversely, most serious complications are more frequent before 2 years of age.

The goal of antibiotics is to treat the source of infection and to sterilize the middle ear to avoid systemic dissemination. Antimicrobial agents must be active against both HI and SP and must reach adequate levels in serum and the middle ear. Successful sterilization occurs when drug concentrations exceed minimum inhibitory concentration (MIC) by 50%. For this reason, the first choice is amoxicillin because of its efficacy against SP and nontypeable HI, as well as its safety and low cost. The dose of amoxicillin to administer raises controversies. Some recommend 40–45 mg/kg/day in two or three

divided doses in children who have received more than three doses of PCV7, and 80–90 mg/kg/day in two doses in other children, including those recently treated with antibiotics. This higher dose is safe and appropriate for SP with reduced sensitivity to penicillin.[19] Other recommended antibiotics are the β-lactams: amoxicillin–clavulanic acid, cefpodoxime proxetil or cefuroxime axetil. If β-lactams are contraindicated because of the risk of failure due to current resistance to HI and SP, erythromycin–sulfafurazole or pristinamycin are suitable alternatives.

If antibiotic treatment is instituted, dosage and timing of administration must be strictly followed. Re-evaluation of patients within 48–72 hours must be undertaken if symptoms persist and to check the eardrum at the end of treatment in case of recurrent AOM or a past history of treatment failure. The duration of treatment should be as short as possible to reduce the cost and the risk for both development of resistant pathogens and drug-related adverse events.[20] In *in-vitro* and animal studies the middle ear is sterilized after 5 days on antibiotic treatment. However, there appears to exist a higher risk of recurrence following short-term treatment, with the effusion persisting for a longer period.[21] Thus, a short course of treatment (5 days) is recommended for children without the risk of SP multidrug resistance and for children more than 2 years of age. In children younger than 2 years a 5-day course of antibiotics is less efficient, especially for those in day-care centers, and an 8-day course is more effective.

Treatment is considered to have failed if symptoms persist after 48 hours on medication or if AOM recurs within 4 days following the end of treatment. Obtaining a bacterial sample is then necessary to adapt the treatment. Pathogen-resistant β-lactamases producing nontypeable HI or SP with reduced susceptibility to penicillin are likely to be identified.

Amoxicillin (100 mg/kg/day for 10 days) or ceftriaxone (50 mg/kg/day for 3 days) are treatments recommended in cases of SP with decreased sensitivity to amoxicillin. In cases of resistant HI, both oral and intravenous C3G have the same effect as amoxicillin–clavulanic acid.

Penicillin-resistant SP is emergent in South Africa, France and the USA, with an MIC to penicillin of more 2 mg/ml in children with AOM or sinusitis. Associated risk factors include recent antimicrobial treatment, children younger than 1 year and children in day care. High-dose amoxicillin (80/150 mg/kg/day), amoxicillin (40 mg/kg/day) and amoxicillin–clavulanic acid (45 mg/kg/day), ceftriaxone (50 mg/kg/day), amoxicillin–clavulanic acid (90 mg/kg/day) or clindamycin (50 mg/kg/day) can be used. In penicillin allergy there is significant cross-reactivity with other antimicrobial agents such as cephalosporins. Macrolides can be used against SP but are less effective against nontypeable HI and resistant SP. Trimethoprim–sulfamethoxazole (TMP–SMX) can be effective but resistance rates reach 20–50% against SP. The best choice is to combine TMP–SMX and macrolides. When cultures are negative (30–45% of cases), careful patient monitoring is recommended.

Therapeutic abstention is possible for children who will be examined by a doctor in case of persistent signs, for children more than 2 years old and if there are no systemic signs. Benefits of short-term antibiotic treatment versus longer treatment are equivalent in efficacy but there is better compliance, fewer adverse effects, improved parental satisfaction, less impact on emergence of resistant flora and lower costs. The adjuvant treatments include analgesics and antipyretics. Ibuprofen did not show better efficacy than paracetamol for treatment of pain in AOM and has more side-effects. Codeine can be used for severe pain. Auricular drops have no indication in AOM.

COMPLICATIONS

Some 60–80% of cases of uncomplicated AOM resolve within 24–48 hours without antibiotic treatment. Following treatment of AOM with antibiotics, the occurrence of mastoiditis, labyrinthitis, brain abscesses, facial nerve palsy and septic thrombophlebitis has become exceptional. However, some retrospective reviews have shown that the use of antibiotics to treat purulent AOM in children did not influence the development of acute mastoiditis.[22,23]



Fig. 25.2 Mastoiditis: local inflammatory signs in front of the mastoid.

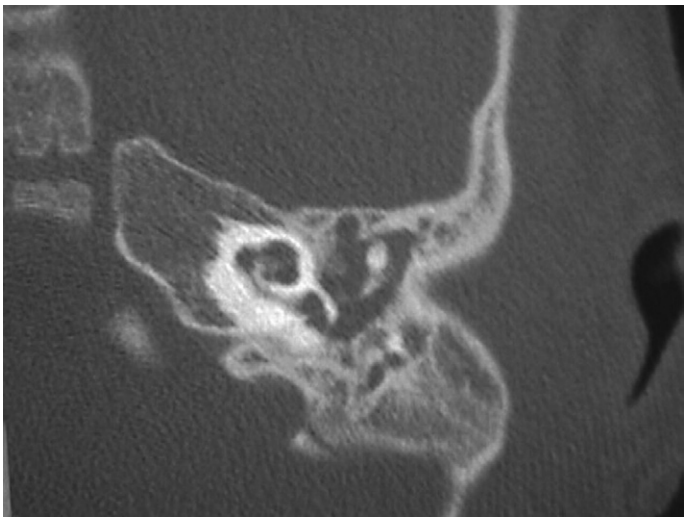


Fig. 25.3 CT scan of mastoiditis.

Mastoiditis particularly affects children younger than 2 years. Systemic signs are associated with local inflammatory signs in front of the mastoid (Figs 25.2 and 25.3). Virulent pathogens such as group A streptococcus, SP and type b HI in non-immunized subjects are commonly found. Mastoiditis may progress to cerebral abscess, central venous thrombosis, osteomyelitis and hydrocephaly. *Staph. aureus* and Enterobacteriae cause subacute mastoiditis. Parenteral antibiotic treatment is mandatory.

Another AOM complication is labyrinthitis caused by dissemination of infection in the inner ear. Vestibular syndrome is the key feature. Benign facial nerve palsy may also occur; this has a favorable outcome with antibiotic and corticosteroid treatment.

Sinusitis

Sinusitis refers to inflammation of the lining of the paranasal sinuses (infectious or noninfectious; bacterial or viral). Symptoms of acute sinusitis last less than 4 weeks, symptoms of subacute sinusitis last

less than 4–8 weeks and symptoms of chronic sinusitis last more than 12 weeks. Sinusitis can be further classified according to the anatomic site (maxillary, ethmoidal, frontal and sphenoidal).

CLINICAL FEATURES

Sinusitis can cause different symptoms in children at varying ages. Younger children often present with persistent rhinorrhea (often purulent), cough and foul-smelling breath; fever is generally low grade. Facial pain and headaches are rare. The differential diagnosis with a viral upper respiratory tract infection (URTI) is difficult to establish and no single sign or symptom has strong diagnostic value. However, sinusitis should be considered in patients who present with symptoms of a viral URTI of more than 10 days' duration.[24]

During a viral throat infection, there is inflammation of the mucous membrane and a continuum between the ordinary cold, acute viral congestive rhinosinusitis and acute bacterial sinusitis characterized by the presence of pus in the paranasal sinuses.

In older children and adults, signs are more localized and the most frequent symptoms are facial pain, headaches, fever, nasal congestion or obstruction and daytime cough. Clinical examination may reveal facial tenderness or swelling over the maxillary or frontal sinuses.

In chronic sinusitis (signs present for at least 12 weeks), cough is prominent. This cough is usually present throughout the day and occasionally precipitates emesis, especially after wakening. Chronic headache may also be present: the pain is often dull in nature and radiates to the top of the head or in temporal regions. Purulent or mucopurulent nasal discharge often completes this cluster of signs.

The localization of sinusitis varies according to age because of the chronology of development of the sinuses: patients between 6 months and 5 years develop ethmoidal sinusitis; those older than 3 years develop maxillary sinusitis and those older than 10 years develop frontal sinusitis. Sphenoid sinusitis is exceptional among children. Maxillary sinusitis is common in children older than 3 years and resolves spontaneously in most cases.

Two clinical pictures can be distinguished:

- acute purulent maxillary sinusitis with fever >39°C lasting more than 3 days, headaches, purulent rhinorrhea and sometimes periorbital edema; and
- subacute maxillary sinusitis with cough, purulent rhinorrhea and nose obstruction, which may last more than 10 days without amelioration.

Headaches, prolonged fever associated with morning cough and purulent sputum are clear signs of bacterial sinusitis. Frontal sinusitis is rare. It particularly affects children older than 10 years and teenagers. The pain is located above the eye socket, is unilateral and throbbing in nature, and is increased by anteflexion of the head. Complications can be serious (eyes, osteomyelitis of the cranial bones). Most cases of sinusitis are caused by bacterial or viral infections but may also be related to allergy.[25] Between 97.8% and 99.5% of sinusitis is viral initially.[26] Bacterial sinusitis starts with a viral disease or an allergic rhinitis with secondary bacterial infection. Persistent obstruction of the sinus ostium by inflammatory edema causes secondary bacterial colonization. In addition, there is a diminution of mucociliary transport which may impair the normal secretion flow, creating an optimal environment for bacterial multiplication.[27] The American Academy of Pediatrics (AAP) defines acute bacterial sinusitis as a bacterial infection of the paranasal sinuses lasting less than 30 days in which symptoms resolve completely.[28]

Microbiologic diagnosis of sinusitis is difficult and requires a sample of sinus secretions not contaminated by the resident bacterial flora of the nose. This sample may be obtained by a needle puncture of the sinus. This is an invasive procedure, which may explain the lack of data concerning bacterial pathogenic agents of sinusitis in children. The most common infectious agents are SP (30–66%), HI (20–30%) and MC (12–28%). Conjugate vaccines have been developed to target SP and HI. Since the introduction of these vaccines, a significant decrease in the frequency of SP has been observed and the bacterial data have changed, with predominance of non-b HI (41%), SP (25%), MC (14%), *Strep. pyogenes* (12%) and *Staph. aureus* (8%). There is also a decrease of SP resistant to penicillin from 44% in 1997 to 27% in 2005 and an increase of HI with β-lactamase (37% versus 44%), although these differences are not statistically significant.[29]

Rare cases of sinusitis of dental original must be considered in patients with pain following recent dental procedures. In most cases, anaerobic bacteria of the oropharynx colonize necrotic dental tissue and then colonize the sinuses. Treatment with antibiotic therapy is essential.[30] Finally, some studies explore the link between gastro-esophageal reflux and chronic sinusitis.[4]

DIAGNOSIS

The diagnosis of sinusitis is clinical. Plain film radiographs may help when diagnosis is difficult (the radiograph shows an opaqueness of the sinus, which may be unilateral or the sinus shape is asymmetrical, and thickening of the mucous membrane is greater than 4 mm; liquid levels are exceptional). Radiography must be interpreted according to age. In fact, in young children only maxillary and ethmoidal sinuses are aerated. However, opacification of the sinus or a mucous thickening may be present even if the child is asymptomatic. CT scan (computed tomography) is used in cases of sphenoidal sinusitis (not seen on standard radiography), ethmoiditis and complicated maxillary sinusitis (Fig. 25.4).[31]

COMPLICATIONS

Sinusitis may be severe in children. The AAP published recommendations about sinusitis in the child and advocated the use of antibiotics.[32] Complications of sinusitis are caused by bacterial spread to nearby structures such as the orbit, bone or central nervous system via the venous system. Although extremely rare, bacterial spread by hematogenous dissemination can cause distant site infections.[33] Orbital cellulitis is the most common complication, especially in ethmoidal sinusitis.

Purulent acute ethmoiditis (exteriorization of ethmoiditis in the orbit wall which is very tenuous) represents a real pediatric emergency, which can occur in children aged more than 1 year. This diagnosis must be considered in a child presenting with edema of the superior eyelid associated with swelling of the internal corner of the eye and nose root (Fig. 25.5). It is a severe complication of an apparently common viral rhinopharyngitis, with a high fever in the presence of general signs. The etiologic agents are *Staph. aureus* and Hib (before generalization of vaccination). *Staph. aureus* is suspected if there is a

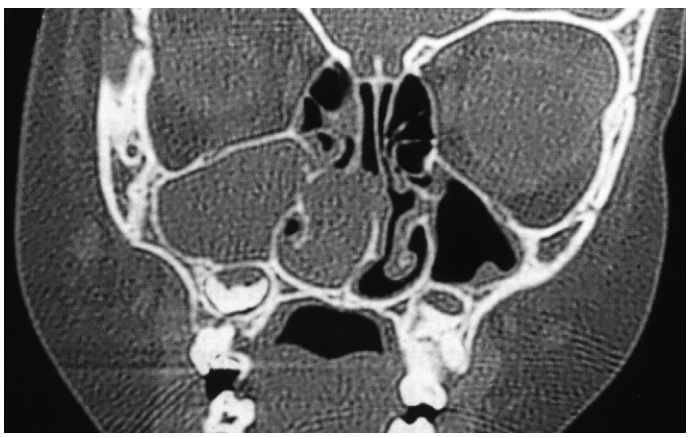


Fig. 25.4 CT scan of acute maxillary sinusitis. Note the opaqueness of the right maxillary sinus.

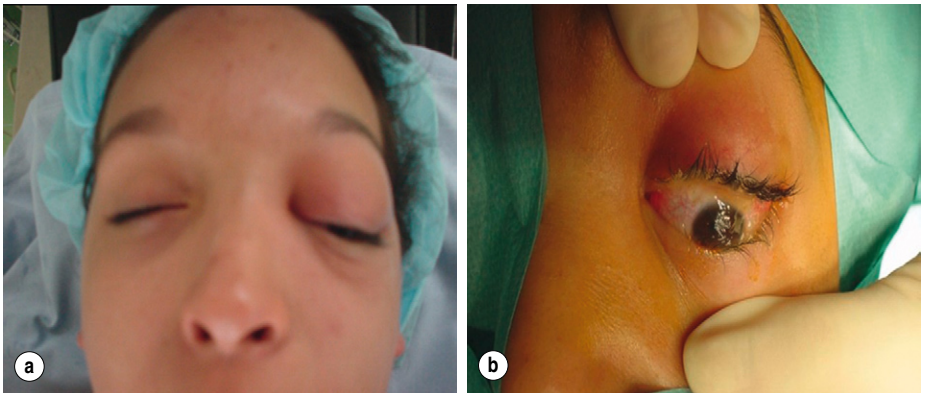


Fig. 25.5 Acute ethmoiditis. (a) Swelling of the internal corner of the eye and nose root. (b) Edema of superior eyelid.

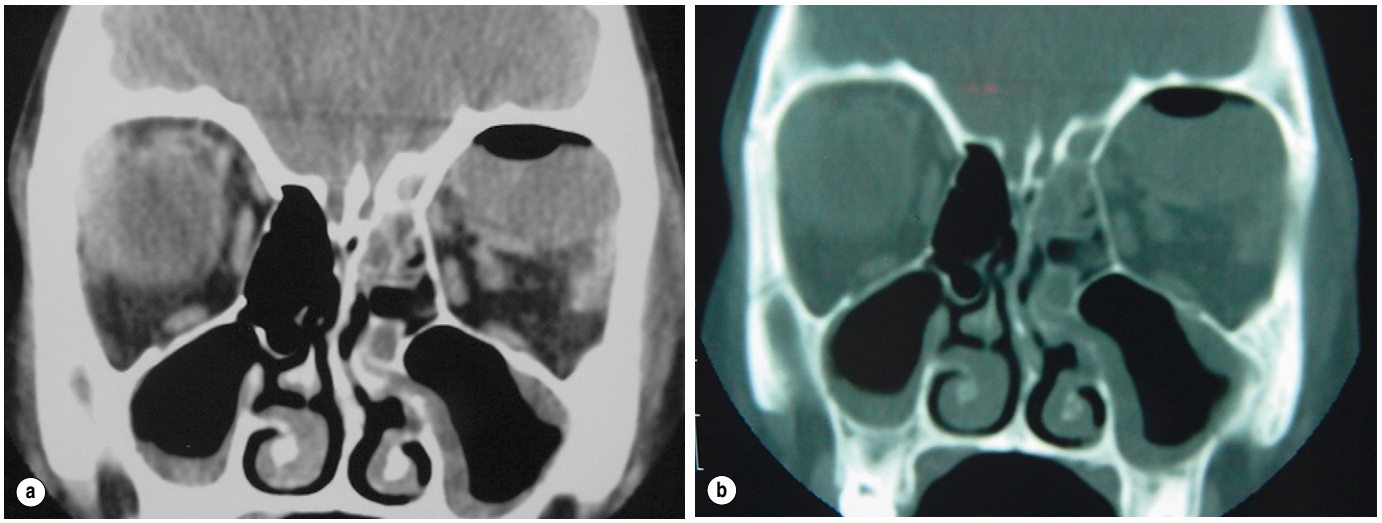


Fig. 25.6 (a, b) CT scans of left acute ethmoiditis.

suggestion of facial trauma or cellulitis of the face. Complications are serious because of the nearness of the brain and its venous channels, i.e. vascular complications (thrombosis of the cavernous sinus) and neuromeningeal complications (purulent meningitis, brain abscess and cerebral empyema). Cerebral computed tomography must be ordered if any neurologic sign is present (Fig. 25.6). Visual involvement may include loss of vision or loss of eye-globe mobility.

Ethmoiditis requires parenteral empiric antibiotic therapy in a hospital setting. Nose pus swab and hemocultures may guide the antibiotic choice. The antibiotic must target HI and *Staph. aureus*. An intravenous third-generation cephalosporin, such as cefotaxime or ceftriaxone associated with an aminoglycoside, is active against *Haemophilus* and methicillin-sensitive staphylococci. Penetration and meningeal distribution are adequate. To cover methicillin-resistant *Staph. aureus*, a third-generation cephalosporin (e.g. fosfomycin) is advised because of the extensive meningeal and osseous distribution. Duration of treatment is 10 days in a hospital setting. In anaerobic infection, an antibiotic of the imidazole family will be added in first intention (e.g. metronidazole, ornidazole). Surgical intervention can be considered according to the efficacy of antibiotics, ophthalmologic involvement and CT data.

Intracranial complications are more common in older children and adults (e.g. cerebral abscess of the frontal lobe or subdural abscess). These patients present with fever, neck rigidity and neurologic abnormalities. Osteomyelitis of the frontal bone may be observed in maxillary sinusitis.

MANAGEMENT

Antibiotic treatment is indicated in cases of purulent sinusitis and hospitalization may be required in severe forms of ethmoiditis. Most cases of mild maxillary sinusitis will resolve spontaneously without antibiotics. A meta-analysis of randomized controlled trials in 2008 assessed the efficacy of antibiotics in acute sinusitis compared with placebo. This study showed a higher success rate with antibiotics and faster symptom resolution; side-effects were higher with antibiotics, but complications and recurrence were comparable in both groups.[33]

A randomized, placebo-controlled trial of antimicrobial treatment for children with clinically diagnosed acute sinusitis showed that neither amoxicillin nor amoxicillin–clavulanate offered any clinical benefit compared with placebo.[34] However, for severe maxillary or frontal sinusitis, and for subacute forms in children with risk factors (asthma, heart disorder, etc.), antibiotics are indicated. For children without risk factors, antibiotics may be discussed – for example, symptomatic

treatment with a follow-up visit after 3–4 days or antibiotics immediately. Treatment is the same as for AOM since the pathogenic agents are the same.

Amoxicillin is used as first-line therapy for uncomplicated acute bacterial sinusitis. Second-line antibiotics should be used in case of failure with amoxicillin: amoxicillin–clavulanic acid (80 mg/kg q8h), cefpodoxime proxetil (10 mg/kg/12h) or cefdinir (14 mg/kg/day). For penicillin-allergic patients, macrolides must be used (azithromycin, erythromycin–sulfisoxazole) or trimethoprim–sulfamethoxazole. Antibiotics may be continued for 7 days after symptom improvement.[35] Besides antimicrobial therapy, symptomatic treatment is necessary – analgesics, antipyretics and nasal obstruction management (nasal solution, iso- or hypertonic, and vasomotor treatment in children aged more than 30 months for less than 5 days).[36]

The benefits of anti-inflammatory drugs (steroids and NSAIDs) were not demonstrated. This treatment may reduce swelling of mucous membranes and sinus openings, allowing better air circulation and reducing bacterial multiplication that have been facilitated by oxygen decline.

Fungal Sinusitis

Fungi contribute to sinusitis in two ways. Invasive fungal infections affecting immunocompromised hosts, such as patients with neutropenia, diabetes mellitus and AIDS are discussed in Chapter 178. In contrast, allergic fungal sinusitis is believed to be an allergic reaction to aerosolized environmental fungi, including Aspergillus and dematiaceous species, in an immunocompetent host. Although not associated with invasive disease, systemic antifungals such as itraconazole may have a limited therapeutic benefit in some patients.

REFERENCES


References for this chapter can be found online at http://www.expertconsult.com

Chapter 26

James R Yankaskas

Bronchitis, bronchiectasis and cystic fibrosis

INTRODUCTION

Genetic, environmental and infectious factors can contribute to acute and chronic inflammation of the airways of the lung. The nature, severity and duration of these insults may produce acute or chronic inflammation with associated cough, dyspnea, sputum and obstructive lung disease. Clinically, these are classified as *bronchitis* (acute or chronic) when the main symptoms are cough and sputum production or *bronchiectasis* when the airways are structurally damaged and dilated, and abundant (>60 ml/day) sputum is expectorated. The diagnosis and clinical management of these various airways diseases are related to the underlying pathogenic processes and to differences among patients.

This chapter describes the pathophysiology, clinical features and management of bronchitis and bronchiectasis. Guidelines for diagnosis and therapy are provided. Cystic fibrosis (CF) is a common genetic disease that leads to progressive bronchitis and bronchiectasis. The molecular, cellular and organ-level pathogenesis of CF has been elucidated and new treatments are being developed. Some of these advances are applicable to bronchiectasis caused by other diseases. It must be emphasized that clinical outcomes are highly variable and treatment decisions must be based on the presentation and responses of the individual patient.

Bronchitis

EPIDEMIOLOGY

Bronchitis is defined as inflammation of the bronchial mucous membranes. Acute bronchitis is manifest by the development of a cough, with or without sputum, that typically occurs during the course of an acute viral illness. Such cough commonly develops in the first week of upper respiratory tract infections (URIs) induced by rhinoviruses in 30% of patients.[1] Acute bronchitis develops in 60% of patients during influenza A infections.[2] In the USA, over 34 million annual office visits are for acute sinusitis, bronchitis or URIs. A majority of these patients are treated with antibiotics and such prescriptions comprise 31% of the total antibiotic prescriptions written.[3]

Chronic bronchitis is defined by the clinical criteria of productive cough for more than 3 months per year for at least 2 years.[4] More than 12 000 000 Americans (about 5% of the population) have chronic bronchitis. The male-to-female distribution is about 2 to 1, but the prevalence is increasing in females. Chronic bronchitis is a major category of chronic obstructive pulmonary disease (COPD) and accounts for significant morbidity and mortality, especially in individuals over age 55. COPD accounted for nearly 83 000 deaths in 1989 and was the fifth leading cause of death in the USA.[5] COPD was also a contributing factor in death due to heart disease and other illnesses.

PATHOGENESIS AND PATHOLOGY

Acute bronchitis is most commonly due to infection of the respiratory epithelium with viruses, such as rhinoviruses, adenoviruses and influenza. Acute bronchitis may also be caused by infections with *Mycoplasma pneumoniae*, *Chlamydia pneumoniae* or *Bordetella pertussis*. The pathogenic effects of these organisms are incompletely understood, but they infect and directly damage airway epithelia, cause release of proinflammatory cytokines, increase production of secretions and decrease mucociliary clearance. Airways damaged by such infections may be more susceptible to irritation by inhaled toxins or bacteria. The role of secondary bacterial infections in the development of symptoms is not clear.

Chronic bronchitis develops as the result of a recurring or persistent injury and the resultant inflammatory responses. Cigarette smoking is the principal etiologic factor. Air pollutants such as sulfur dioxide or occupational exposures may also contribute. The pathologic effects are an increase in the proportion of goblet cells in the surface epithelium and an increase in the size of submucosal glands (Fig. 26.1). The distribution of these pathologic changes along the airway tree depends in part on the composition of the inhaled toxins and may involve peripheral bronchioles as well as central bronchi. There is an influx of polymorphonuclear leukocytes (PMNs), surface epithelial cell hyperplasia and metaplasia, and inflammatory mucosal edema. Genetic diseases that impair airway defenses may amplify these effects. Primary ciliary dyskinesia[6] decreases mucociliary transport secondary to altered ciliary structure and function. α_1-Antitrypsin deficiency produces an imbalance in the defenses against neutrophil elastase and leads to panacinar emphysema and bronchitis, particularly in smokers.[7]

PREVENTION

Chronic bronchitis primarily occurs in cigarette smokers. Avoidance of inhaled toxins, particularly cigarette smoke, is of paramount importance in reducing the incidence and progression of chronic bronchitis. The loss of lung function, as measured by spirometry, is more rapid in active cigarette smokers. Such individuals can gain significant benefits from stopping or significantly decreasing their cigarette consumption. Sputum production usually decreases within weeks. The accelerated decline of lung function seen in smokers slows to that of nonsmokers of the same age.[8] Thus, the importance of avoiding primary and secondhand cigarette smoke cannot be overemphasized.

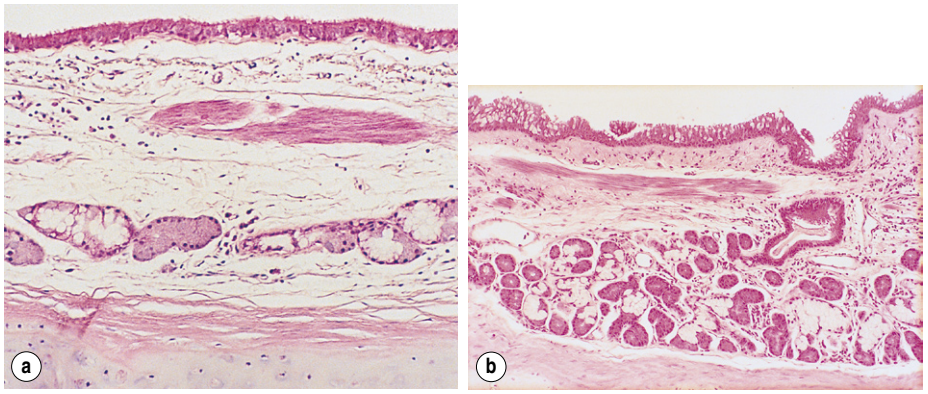


Fig. 26.1 (a) Bronchial wall from normal patient. Normal pseudostratified columnar epithelium with few goblet cells overlies smooth muscle and a submucosal gland. Cartilage is at the bottom of the figure. H&E stain. (b) Bronchial wall from a patient with chronic bronchitis. Hyperplastic epithelium with mucous cell metaplasia overlies a hypertrophied submucosal gland. H&E stain.

CLINICAL FEATURES

Acute bronchitis typically develops during the course of an acute URI. Pharyngitis, coryza, low-grade fever and malaise precede the development of a cough with scanty sputum. Dyspnea is rare. In the absence of other lung disease, most symptoms subside over several days, although the cough may persist for weeks to several months. The quantity of sputum and frequency of cough decrease with time and no long-term sequelae occur.

The key symptoms of individuals with chronic bronchitis are chronic cough, production of sputum, wheezing and exertional dyspnea. These symptoms develop insidiously, often over many years. Presentation for medical care typically occurs during an acute exacerbation. Upon direct inquiry, patients often recall persistent dyspnea and sputum production following URIs for several years prior to presentation. The sputum is purulent, yellow or green, and may be blood-streaked. The daily volume ranges from scanty up to about 60 ml. The cough and sputum are usually most severe soon after awakening. Exertional dyspnea and fatigue are first noticed during exacerbations and later become persistent.

Acute or subacute exacerbations are characterized by increases in cough, sputum production, dyspnea and wheezing.[9] These symptoms often follow acute URIs and tend to be more frequent during winter months. The sputum often changes in color to darker yellow or green. Sputum cultures may identify one of the bacterial pathogens listed in Table 26.1 but the pathogenic role of these microbes is not certain.[10,11] Pulmonary function decreases during such exacerbations and may lead to further complications. The progressive disease often leads to increasingly frequent exacerbations over time, further loss of lung function and worse symptoms.

Patients with severe obstructive airways disease may develop the serious complications of hypoxemic and/or hypercapnic respiratory failure. Hypoxemia develops initially during exercise or sleep and may cause morning headaches. Prolonged hypoxemia may cause cyanosis, polycythemia, pulmonary hypertension and cor pulmonale. Secondary signs of right heart failure, such as jugular venous distention, hepatomegaly and peripheral edema develop in late disease and may become chronic. The respiratory acidosis that signals hypercapnic respiratory failure may be of gradual onset and be compensated by a metabolic alkalosis through renal retention of bicarbonate. Individuals with scanty sputum production and greater degrees of emphysema often achieve adequate oxygenation and ventilation until late in the course of the disease. Exacerbations in the setting of severe disease often produce superimposed acute respiratory failure that is life threatening and requires intensive care.

Table 26.1 Viral and bacterial pathogens in bronchitis

Common	Uncommon
Viruses: – rhinoviruses – adenoviruses – influenza A and B – parainfluenza *Haemophilus influenzae* *Haemophilus parainfluenzae* *Moraxella catarrhalis* *Neisseria* spp. *Streptococcus pneumoniae*	*Chlamydia pneumoniae* *Klebsiella pneumoniae* *Mycoplasma pneumoniae* *Pseudomonas aeruginosa*

DIAGNOSIS

The history of persistent cough and daily sputum is essential to the diagnosis of chronic bronchitis. The extent and duration of cigarette smoking quantify the major risk factor. The presence of antecedent reactive airways disease (e.g. childhood asthma) may increase the risk for developing chronic bronchitis and COPD. Physical examination reveals tachypnea and late expiratory wheezes on auscultation. Patients with advanced disease develop hyperinflation of the lungs with increased anterior–posterior diameter of the thorax and a depressed diaphragm. In such patients, breath sounds and heart sounds are muted. Patients with severe disease may use accessory muscles of respiration and/or pursed-lip breathing. Medium or coarse inspiratory crackles appear in patients with bronchitis and excess airway secretions. Patients with severe disease may develop central and peripheral cyanosis or neck vein distention, hepatomegaly and peripheral edema as signs of hypoxemia and right heart failure.

Pulmonary function tests are essential to establish the diagnosis of obstructive lung disease, to measure the severity of airway obstruction and to follow the course of illness. Spirometry performed before and after inhaled β-adrenergic agonists often reveals decreased FEV_1 and decreased FEV_1:FVC ratio. Improved flows following inhaled β-adrenergic agonists indicate the presence of reversible bronchoconstriction. Forced vital capacity (FVC) is also decreased in patients with very severe obstruction. In such individuals, lung volumes should be measured to distinguish restrictive and obstructive respiratory impairments and to quantify lung hyperinflation and gas trapping. The diffusing capacity for carbon monoxide (DLCO) may be reduced in patients with severe obstruction or emphysema. The chest X-ray shows increased lung volumes. In patients with emphysema the heart may appear small and the bronchovascular markings decreased. Patients with chronic bronchitis often have enlarged hearts, engorged apical vessels, bronchial cuffing and other signs of fluid overload. Arterial blood gases may reveal mild to severe hypoxemia. Patients with severe disease may develop respiratory acidosis and a compensatory metabolic alkalosis.

The complete blood count may reveal polycythemia and the serum electrolytes a metabolic alkalosis. Sputum Gram stain and culture are important to assess the abundance of PMNs and to help identify bacterial pathogens that may be associated with acute exacerbations.

MANAGEMENT

The symptoms of acute bronchitis are best managed with symptomatic treatment. Nonsteroidal anti-inflammatory drugs and decongestants are useful for pharyngitis, sinusitis and coryza. Antibiotics are indicated for clinically significant bacterial bronchitis, but such a complication is difficult to distinguish from viral bronchitis. Antibiotics are prescribed for 53–66% of patients with acute sinusitis, bronchitis or URIs,[3] indicating excess usage. This practice promotes the development of antibiotic-resistant bacteria, which may lead to greater morbidity in the community. Therefore, antibiotics should be reserved for patients with acute bronchitis who have increased numbers of PMNs and numerous bacteria in Gram-stained sputum samples or who do not respond to symptomatic therapy.

The most important feature of managing chronic bronchitis is the avoidance of exposure to irritants, particularly cigarette smoke. Thus, smoking cessation is of primary importance for each individual. Support for smoking cessation can be provided by individual counseling, provision of smoking cessation literature or through smoking cessation groups. Nicotine gum or transdermal nicotine may be useful to reduce withdrawal symptoms.

No specific therapy is available to treat chronic bronchitis. Symptomatic therapy is directed at reducing mucosal edema, mucus hypersecretion, bronchial smooth muscle constriction and airway inflammation (Table 26.2). Inhaled β-adrenergic agonists and anticholinergic drugs may be of benefit to patients with reactive airways disease, as demonstrated on pulmonary function tests.[12] Theophylline may be useful in patients with nocturnal symptoms or severe hyperinflation and respiratory muscle fatigue. In addition to relaxing bronchial smooth muscle, β-adrenergic agonists enhance mucociliary clearance. Inhaled drugs with intermediate (4–6 hours) and long (8–12 hours) duration of action are available. These are generally preferred to systemic treatments. Metered dose inhalers (MDIs) appear to have comparable efficacy to nebulizers, but effective treatment requires coordination of actuation and the breathing cycle. The beneficial effects can be enhanced by the use of a spacer to improve deposition of drugs in the lungs. Dry powder inhalers (DPIs) can be easier to use and more effective. Anticholinergic drugs relax bronchial smooth muscle and have intermediate and long durations of action. Ipratropium bromide is available as an MDI and tiotropium as a DPI. The effects of anticholinergics and β-agonists appear to be roughly equivalent.[13] The responses of individual patients to such drugs must be assessed to determine the optimum treatment.

Systemic and inhaled corticosteroids provide a beneficial effect by reducing severity of airway inflammation. This typically results in decreased airway obstruction and decreased mucus secretion. Long-term use of systemic corticosteroids may be complicated by osteoporosis, central obesity and/or glucose intolerance. Once a beneficial steroid effect has been demonstrated with systemic therapy, these side-effects can be minimized by use of moderate to high doses of inhaled steroids. Typical drugs include beclometasone 800–1600 µg per day and fluticasone 88–1760 µg per day. Other forms of therapy such as cromolyn sodium, expectorants and chest physiotherapy have not been demonstrated to have significant effects in chronic bronchitis or COPD.

The role of bacterial infection in causing acute exacerbations of chronic bronchitis is controversial. It is likely that less than half the cases of acute exacerbation are due to bacterial infection. A number of controlled studies have failed to show significant benefit of antibiotic therapy.[14,15] Nevertheless, antibiotics have a role in patients who demonstrate a significant increase in the number of PMNs in expectorated sputum and dominant bacteria on Gram stain or in culture. Previous positive responses to antibiotic therapy may also support repeated use in individual patients. Antibiotics effective against the common bacterial species are listed in Table 26.3. Patients with exacerbations of sufficient severity to require hospitalization should be treated with

Table 26.2 Therapeutic options for acute exacerbations of chronic bronchitis

Class/agent	Examples	Notes
Bronchodilators		
β-Adrenergic agonists	Albuterol, salmeterol	Inhaled administration preferred
Anticholinergics	Ipratropium bromide	Inhaled administration mandatory
Theophylline		Second-line agent
Anti-inflammatory agents		
Corticosteroids	Prednisone, fluticasone	Administration by inhalation may reduce side-effects
Expectorants		
rhDNase	Dornase alpha	Efficacy in COPD not established
Iodinated compounds	Iodinated glycerol	Limited efficacy
Reducing agents	*N*-acetyl cysteine	Limited efficacy
Airway clearance measures		
Controlled coughing		Efficacy not established
Physical therapy		Efficacy not established
Supplemental oxygen		Corrects significant hypoxemia
Antibiotics		Indications and efficacy controversial

Table 26.3 Oral antibiotics for acute exacerbations of chronic bronchitis

Agent	Dose
Amoxicillin	250–500 mg q8h
Amoxicillin–clavulanic acid	875/125 mg q8h
Ampicillin	500 mg q6h
Azithromycin	500 mg day 1, then 250 mg q24h
Cefaclor	500 mg q8h
Cephalexin	500 mg q6h
Ciprofloxacin	500–750 mg q12h
Clarithromycin	500 mg q12h
Doxycycline	100 mg q12h
Erythromycin	500 mg q6h
Ofloxacin	400 mg q12h
Tetracycline	500 mg q6h
Trimethoprim–sulfamethoxazole	160/800 mg q12h

parenteral antibiotics with comparable antibacterial spectra, and the treatment modified based on sputum culture results.

Hypoxemia may be diagnosed by ambulatory, exercise or nocturnal pulse oximetry, as well as by arterial blood gases. Patients with significant hemoglobin desaturation (SaO_2 <90%) should receive supplemental oxygen. Nocturnal oxygen has been shown to improve survival[16] and continuous treatment has greater effects than nocturnal treatment alone.[17] Some patients with COPD also have obstructive sleep apnea and detailed sleep studies may be required to establish the effectiveness of oxygen and/or continuous positive airway pressure (CPAP) by nasal mask to prevent nocturnal hypoxemia.[18] Noninvasive assisted ventilation by nasal mask has been used for some patients with severe hypercapnic respiratory failure.[19] Patients with acute respiratory failure and significant respiratory acidosis or hypoxemia often require hospitalization, parenteral antibiotics, ICU care and mechanical ventilation.[20]

Bronchiectasis

EPIDEMIOLOGY

Bronchiectasis is defined as abnormal dilatation of the bronchi.[21,22] It typically involves medium-sized bronchi and results from destruction of the muscular and elastic components of the walls. Bronchiectasis is classified as cystic, cylindrical or varicose, based on the morphologic structure of the airways. Chronic airway inflammation is the essential pathologic feature, resulting from genetic abnormalities that impair airway defense mechanisms or from chronic respiratory infections.[23] Such infections have become relatively rare in the USA; the current prevalence of bronchiectasis is less than 1 in 10 000.

PATHOGENESIS

The principal diseases that cause bronchiectasis are listed in Table 26.4. The genetic diseases cause deficits in airway defense or immunologic mechanisms that permit the development of chronic bacterial infections in the airways. Bacterial and inflammatory cell-derived proteolytic and oxidative molecules cause progressive airway wall damage that eventually produces bronchiectasis. Immune reactions to fungi can produce the central bronchiectasis that is associated with allergic bronchopulmonary aspergillosis (ABPA). Chronic infections with *Mycobacterium tuberculosis*[23] or nontuberculous mycobacteria (particularly *Mycobacterium avium* complex)[24] and *Bordetella pertussis* infections are recognized infectious causes. Bacteria that secondarily infect damaged airways following other injuries probably propagate airway damage, but the time course and relative contributions of the different organisms have not been established.

Table 26.4 Principal causes of bronchiectasis

Genetic
Cystic fibrosis
Immunoglobulin deficiency
Primary ciliary dyskinesia
Infectious
Bordetella pertussis
Tuberculosis
Nontuberculous mycobacteria (especially *M. avium* complex)
Inflammatory
Allergic bronchopulmonary aspergillosis (ABPA)
α_1-Antitrypsin deficiency
Bronchial obstruction
Other
Bronchopulmonary sequestration
Congenital cartilage abnormalities
Yellow nail syndrome

CLINICAL FEATURES

Daily cough and production of purulent sputum are the most typical symptoms of bronchiectasis. Sputum production can range from less than 10 ml to greater than 150 ml daily and tends to correlate with disease extent and severity. Bronchiectasis associated with cystic fibrosis, which becomes generalized and progresses relentlessly, is described in greater detail below. Occasional individuals have no discernible sputum production ('dry bronchiectasis'). The clinical course is usually of progressive symptoms and respiratory impairment. Airway obstruction progresses and leads to increasing exertional and resting dyspnea. Acute exacerbations may be precipitated by viral or newly acquired bacterial pathogens, as with chronic bronchitis. The main clinical differentiating feature is the quantity of sputum production. In addition to progressive respiratory failure, patients with bronchiectasis are prone to hemoptysis due to hypertrophied bronchial arteries that are closely apposed to the inflamed airways.[25] Hemoptysis from this source can be massive, even fatal.

DIAGNOSIS

Diagnosis is suggested by clinical symptoms and a physical examination with hyperinflated chest and medium- to low-pitched inspiratory crackles and sometimes expiratory wheezes. Chest X-ray may demonstrate hyperinflation and bronchiectatic cysts or dilated bronchi with thickened walls forming tram track patterns radiating from the lung hila. High-resolution chest CT scans readily demonstrate mild and severe forms of bronchiectasis. CT scans have largely replaced bronchography as a diagnostic examination.

Sputum culture may identify characteristic pathogens, including *H. influenzae, Strep. pneumoniae* and/or *Pseudomonas aeruginosa*. Sputum acid-fast bacilli (AFB) smears and cultures should be performed to evaluate mycobacterial disease. Spirometry is essential to determine the severity of airway obstruction and to evaluate the course of disease.

MANAGEMENT

Specific etiologies such as tuberculosis should be identified and treated whenever possible. Standard therapy includes measures to clear excess secretions from the airways. Chest physiotherapy based on chest percussion and postural drainage is accepted as the most effective technique. Alternatives such as pneumatic vests and aerobic exercise or flow interrupter valves may be effective, but their use must be individualized. Bronchodilators have a role in patients with objective spirometric or subjective clinical responses. Acute exacerbations are managed with intensification of airway clearance measures and the use of antibiotics directed at pathogens identified in recent sputum cultures. The use of prophylactic oral or inhaled antibiotics has been advocated to control the major symptoms of bronchiectasis and such treatment must be tailored to the individual responses. Surgical resection of localized bronchiectatic lung is occasionally indicated.[26]

Cystic Fibrosis

EPIDEMIOLOGY

Cystic fibrosis is the most common lethal genetic disease in Caucasians.[27] This autosomal recessive disease is caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene located on chromosome 7. The incidence is 1 in every 3300 live Caucasian births, with a gene carrier rate of 1 in 29. Other ethnic groups have lower carrier rates, with the Hispanic birth incidence being 1 in 9500, the Native American 1 in 11 200, the African-American one in 15 300 and the Asian 1 in 32 100 live births.

When cystic fibrosis was first described in 1938, survival past infancy was rare. Improved treatments for pancreatic insufficiency, lung infections and other complications have increased the median survival from less than 1 year to more than 36 years (Fig. 26.2). Over 24 500 CF patients have been identified in the United States.[28] The median survival in 2006 was 36.9 years. Adults (≥18 years old) now account for 44.5% of CF patients and survival can extend to 78 years.[28]

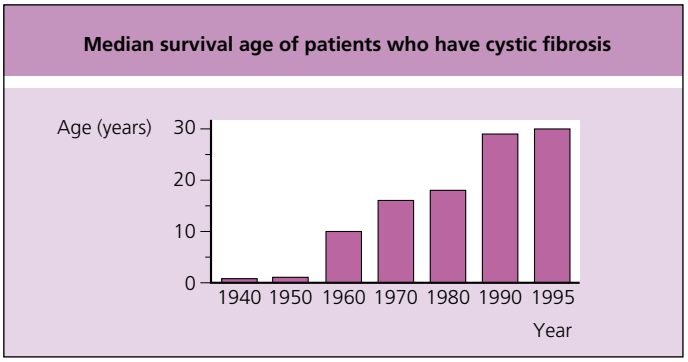


Fig. 26.2 Median survival age of patients who have cystic fibrosis. The median survival age has increased dramatically. Data from Cystic Fibrosis Foundation, Bethesda, MD.

PATHOGENESIS

The CFTR gene encodes a 1480 amino acid protein, with 12 membrane-spanning regions, two nucleotide-binding folds and a regulatory ('R') domain (Fig. 26.3). This protein is localized to the apical membranes of epithelia lining the organs affected by the disease, particularly the airways, pancreatic duct, sweat gland duct, intestines and reproductive tract. CFTR protein acts as a Cl^- channel[29] and as a regulator of epithelial Na^+ channels[30] and other Cl^- channels.[31] Over 1500 different mutations in the CF gene have been identified, encompassing several functional abnormalities (Fig. 26.4):

- Class I mutations prevent protein production.
- Class II mutations produce proteins that fail to traffic to the apical cell membrane. The most common CF mutation, ΔF508, is in this class; it accounts for 68% of US mutations.
- Class III mutations traffic properly but have defective regulation.
- Class IV mutations traffic properly but have defective Cl^- conductance.
- Some exon splice-site mutations have been labeled class V mutations and may permit transcription of some normal CFTR mRNA, conferring a less severe clinical phenotype.[32]

All classes of mutation alter Cl^- permeability and regulation of ion transport in the affected epithelial cells.

The pathogenesis of airways disease in cystic fibrosis is partially understood. CFTR mutations produce decreased Cl^- permeability and increased net Na^+ absorption by bronchial epithelial cells. The effects on bronchiolar epithelial cells and on submucosal gland secretion are not fully defined. It has been suggested that CF mutations lead to relative dehydration of the airway surface liquid.[33] This impairs mucociliary clearance and leads to secondary bacterial infection and airways inflammation. This chronic infection and inflammation forms a vicious cycle that produces progressive airway obstruction, bronchiectasis and eventually respiratory failure. This scheme of pathogenesis is illustrated in Table 26.5. Some existing and potential treatments, directed at the specific pathogenic processes, are indicated.

CLINICAL FEATURES

Cystic fibrosis is classically recognized from the triad of bronchiectatic airways disease, exocrine pancreatic insufficiency and elevated sweat chloride. Most patients have onset of cough and chronic respiratory tract

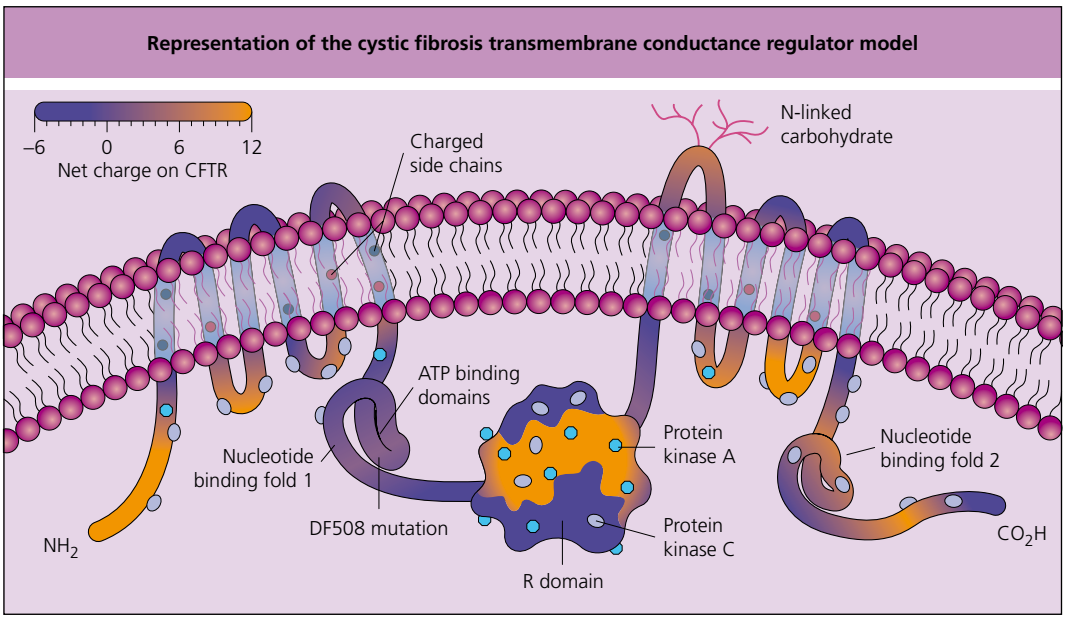


Fig. 26.3 Representation of the CFTR model, based on structural, hydropathy and expression studies. The membrane-spanning domains are arranged in groups of six, each associated with a nucleotide-binding fold. These features are similar to those of the multidrug resistance 'P' glycoprotein. The 'R' domain is unique to CFTR.

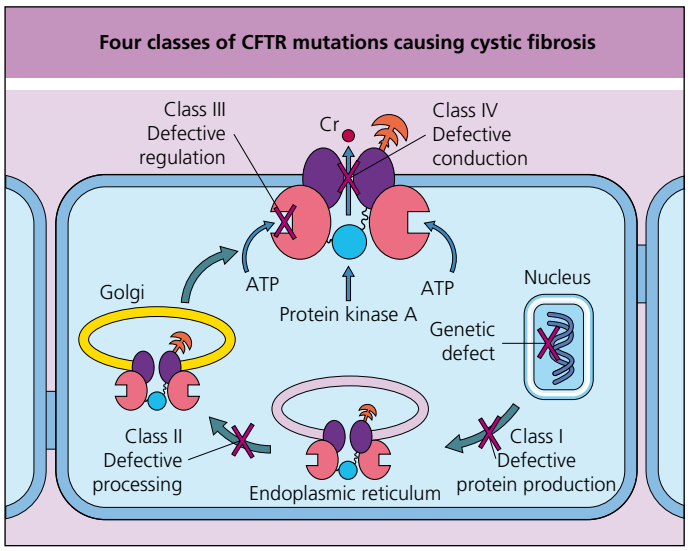


Fig. 26.4 CFTR mutations are divided into classes based on the mechanisms of dysfunction. See text for detailed descriptions. Figure provided by Cystic Fibrosis Foundation, Bethesda, MD.

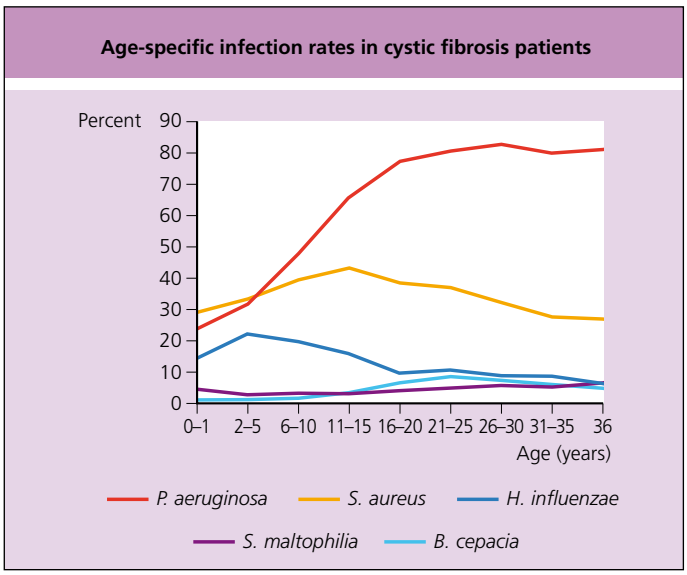


Fig. 26.5 Age-specific infection rates in cystic fibrosis patients. Bacteria isolated from CF sputum samples vary with age and demonstrate the trend toward *Pseudomonas aeruginosa* as the dominant pathogen. Figure provided by Cystic Fibrosis Foundation, Bethesda, MD.

infections during infancy or childhood. Early respiratory tract pathogens include *Staphylococcus aureus* and *Haemophilus influenzae*. *Pseudomonas aeruginosa*, particularly mucoid variants, appears in greater prevalence with increasing age and becomes the dominant pathogen by the teenage years (Fig. 26.5). It is common to isolate several different bacteria from the sputum of adolescent and adult CF patients. *Burkholderia cepacia* has become a significant problem in some centers, and recently non-tuberculous mycobacteria has emerged as a new issue (see Practice Point 15). Pathologically, there is inflammation and obstruction of both bronchioles (Fig. 26.6) and bronchi, with submucosal gland hypertrophy (Fig. 26.7). Bronchiectasis tends to start in the upper lobes and becomes generalized. Chest X-rays show hyperinflated lungs, cystic bronchiectasis and occasionally upper lobe atelectasis (Fig. 26.8).

The clinical course of chronic cough, mucus hypersecretion and airway obstruction is progressive and is punctuated by acute exacerbations, characterized by the features listed in Table 26.6. When such exacerba-

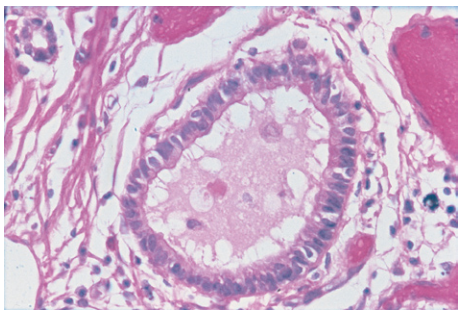


Fig. 26.6 A CF bronchiole is completely occluded by mucoid secretions and surrounded by fibrotic tissue. H&E stain.

Table 26.5 Treatment approaches to cystic fibrosis airways disease

		APPROACH	
Abnormality	**Solution**	**Available**	**Investigational**
Abnormal CF gene	Provide normal gene		Gene therapy
Abnormal CFTR protein	Provide normal protein. Activate mutant form		Protein therapy ?Phosphodiesterase inhibitors ?Phosphatase inhibitors ?Others
Abnormal salt transport	Block Na^+ uptake. Increase Cl^- efflux		Amiloride UTP
?Abnormal mucus	Decrease viscosity	Dornase alpha (rhDNase) (*in vitro*)	Gelsolin
Impaired clearance	Augment ciliary action	Airway clearance techniques	
?*Pseudomonas* infection	Reduce bacterial count	Antibiotics	
Inflammatory response	Decrease host reaction	Anti-inflammatory drugs (corticosteroids, ibuprofen)	Antiproteases. Pentoxifylline. Intravenous immunoglobulin
Bronchiectasis	Replace irreversibly damaged areas	Lung transplantation	

The pathogenesis of cystic fibrosis lung disease is based on a vicious cycle of airway infection, inflammation and obstruction (first column). Treatment can be directed at different pathogenic mechanisms (second column). Currently available and proposed treatment options are shown in final columns. Table provided by Cystic Fibrosis Foundation, Bethesda, MD.

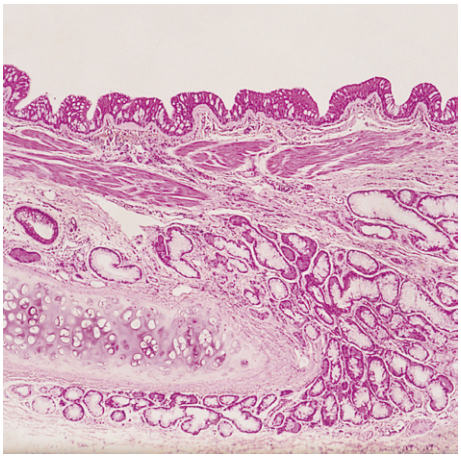


Fig. 26.7 A CF submucosal gland demonstrates marked hypertrophy and dilated gland ducts with mucoid secretions. The surface epithelium has marked goblet cell metaplasia. H&E stain.

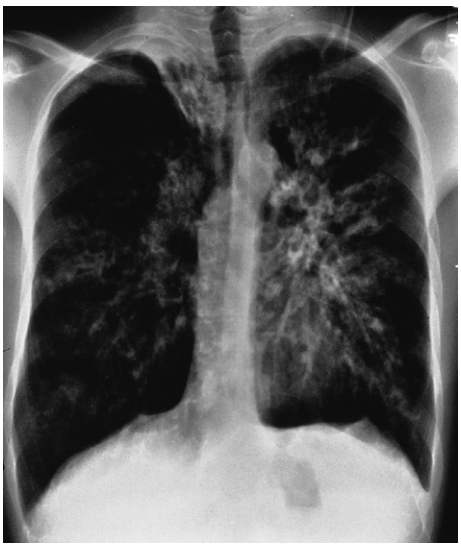


Fig. 26.8 A typical postero-anterior chest X-ray of a 24-year-old man with CF. The lungs are hyperinflated due to airway obstruction and bronchiectasis. The right upper lobe atelectasis is chronic.

Table 26.6 Signs and symptoms of a cystic fibrosis pulmonary exacerbation

- Increased cough
- Increased sputum
- Increased dyspnea
- School or work absenteeism
- Reduced exercise tolerance
- Weight loss >1 kg or >5% of body weight
- Decreased FEV_1 (10% from baseline)
- New chest findings
 - Rales
 - Wheezes
- New radiographic findings

Table provided by Cystic Fibrosis Foundation, Bethesda, MD.

tions are effectively treated, pulmonary function may return to baseline levels. With more severe disease, exacerbations become more frequent and less reversible, culminating in fatal respiratory failure (Fig. 26.9).

DIAGNOSIS

The standard diagnostic criteria for cystic fibrosis are the combination of characteristic lung disease with airway obstruction, bronchiectasis

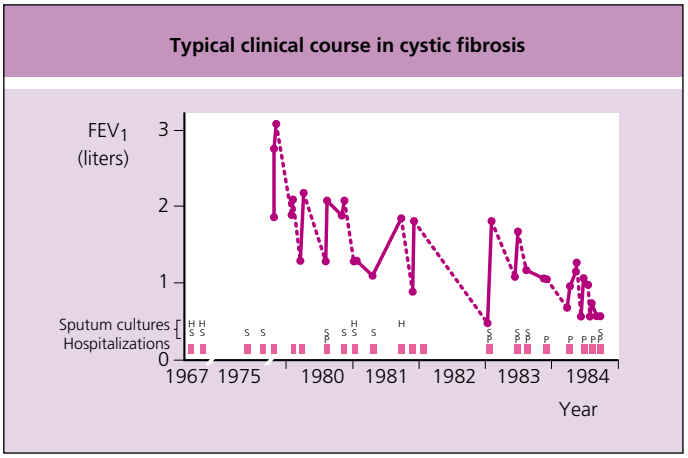


Fig. 26.9 Typical clinical course in CF. Serial pulmonary function measurements (FEV_1) demonstrate a typical clinical course. Measurements are connected by solid lines during therapy for acute exacerbations. The lower bars indicate periods of hospital treatment. Pulmonary function decreases and the exacerbations are more frequent and less responsive to treatment in advanced disease. H, Haemophilus; P, Pseudomonas; S, Staphylococci.

and infection with typical bacterial pathogens; exocrine pancreatic insufficiency which occurs in more than 85% of CF patients; and elevated sweat chloride which occurs in more than 98% of patients. The diagnosis initially may be suggested by a family history of CF or by the presence of meconium ileus at birth, noted in 17% of cases. Of patients reported to the Cystic Fibrosis Foundation (CFF) Patient Registry, 51% presented with acute or persistent respiratory symptoms, 43% with failure to thrive or malnutrition, 35% with steatorrhea and 21% with meconium ileus or intestinal obstruction.

Developments in the understanding of the molecular and physiologic pathogenesis of cystic fibrosis have led to additional diagnostic criteria. CFTR mutational analysis is offered by several companies that test for up to 70 common mutations and can detect mutations in about 95% of CF cases. Abnormal CFTR function in airway epithelia can be assessed by *in-vivo* measurements of nasal electrical potential difference and its response to selected modulators of ion transport.[34] The clinical application of these tests has been summarized by a CFF-sponsored Consensus Committee.[35]

MANAGEMENT (see also Practice Point 15)

Exocrine pancreatic insufficiency and malnutrition are managed with oral pancreatic enzymes and dietary supplements. Pulmonary disease causes the major morbidity in CF and eventually death in 95% of patients. Daily clearance of airway secretions is essential (Table 26.7).[36] This can be accomplished by chest physiotherapy, which enhances sputum production and increases pulmonary function.[37] Physical exercise augments airway clearance and improves cardiovascular function. Special breathing techniques, including forced expiratory technique, autogenic drainage and active cycle of breathing,[38] have been useful in some individuals. Mechanical devices, including the flutter valve[39] and external thoracic compression devices, may improve patient independence, but their efficacy is less well established.

Antibiotics are used extensively. Acute exacerbations are treated with intravenous antibiotics directed at the major pulmonary pathogens, especially *Pseudomonas* species and *Staph. aureus*. Because of the high bacterial burden, two antibiotics with different mechanisms of action and with *in vitro* efficacy against each major bacterium are selected. Pharmacokinetic studies of β-lactams, aminoglycosides and

Table 26.7 Standard therapy for cystic fibrosis lung disease

Airway clearance
Chest physical therapy
Physical exercise
Special breathing techniques
Mechanical devices
Antibiotics
Intravenous
Oral
Inhaled
Others
Bronchodilators
Supplemental oxygen
Anti-inflammatory agents (ibuprofen, corticosteroids)
Recombinant human deoxyribonuclease (rhDNase)

sulfa drugs demonstrate increased clearance in CF patients, necessitating the use of higher doses. Typical antibiotic choices are listed in Table 26.8. Home intravenous antibiotic therapy has cost and convenience advantages,[40] but its clinical efficacy in this setting has not been rigorously established. The benefits of chronic oral antibiotics are controversial, but some aerosolized antibiotics have demonstrated efficacy.[41]

CF patients are particularly susceptible to the complications of massive hemoptysis and pneumothorax. Episodes of massive hemoptysis, defined as more than 240 ml blood per 24 hours, are managed with antibiotics, transient cough suppression and reduction in chest physiotherapy, and bronchial artery embolization.[42] Such therapy is usually effective and does not compromise candidacy for eventual lung transplantation. Large pneumothoraces are managed by chest tube drainage. Recurrent pneumothoraces may require repeated chest tubes or abrasion pleurectomy.[43] Hypoxemia is best treated with supplemental oxygen plus standard pulmonary therapy. Ventilatory assistance can be effectively provided by mask ventilation.[44,45]

Lung transplantation has become an effective form of therapy.[46] From the first heart–lung transplant for CF in 1983 to 2007, more than 3200 heart–lung or sequential double lung transplants (the preferred operation in the USA) for CF have been performed worldwide. Transplant evaluation is indicated when FEV_1 is less than 30% predicted or increasing functional impairment leads to frequent hospitalizations and less response to standard treatments. In May 2005 the United States changed from a waiting time list to the lung allocation score (LAS) that considers urgency and benefit.[47] The 1- and 5-year survivals after lung transplantation for CF are 81% and 55%, respectively. These are comparable to the survival of patients who received lung transplants for other diseases.[48] Deaths in the first year are primarily due to operative complications and infections. After 1 year most deaths are caused by obliterative bronchiolitis, the pathologic marker of chronic rejection.

CONCLUSION

Inflammatory airways diseases are highly prevalent, causing significant morbidity and mortality. Different pathogenic factors cause distinct patterns of disease, including bronchitis and bronchiectasis.

Table 26.8 Inhaled and parenteral antibiotics commonly used for cystic fibrosis

Parenteral (normally two effective agents against each bacterial isolate)	
Class/drug	**Pertinent efficacy**
Aminoglycosides*	
Gentamicin	*Staph. aureus*, *H. influenzae*, *Pseudomonas*
Tobramycin	*Staph. aureus*, *H. influenzae*, *Pseudomonas*
β-Lactams*	
Ceftazidime	*Pseudomonas*
Piperacillin	*H. influenzae, Pseudomonas*
Ticarcillin–clavulanate	*Staph. aureus, H. influenzae, Pseudomonas*
Monobactam	
Aztreonam	*Pseudomonas*
Carbapenem	
Imipenem–cilastatin	*Staph. aureus*, *H. influenzae*, *Pseudomonas*
Fluoroquinolones	
Ciprofloxacin	*Staph. aureus*, *H. influenzae*, *Pseudomonas*
Sulfa drugs*	
Trimethoprim–sulfamethoxazole	*Staph. aureus*, *B. cepacia*
Glycopeptides	
Vancomycin	Oxacillin-resistant *Staph. aureus*
Aerosolized	
Drug	**Common doses**
Tobramycin	80–300 mg q8–12h
Colistimethate sodium	75–150 mg q8–12h
Aztreonam	75 mg q8–12h

*Higher doses required because of increased clearance in cystic fibrosis.

The ability to stop the progression of these diseases is often limited by chronic inflammation and by structural alterations in the airways. Nevertheless, antibiotics, anti-inflammatory drugs and other forms of therapy can modulate acute exacerbations and, potentially, the progression of these diseases. Elucidation of the pathogenic mechanisms, as is being done in cystic fibrosis, may uncover new and more effective means of treatment. The diagnostic tests, continued monitoring and choice of therapeutic options must be tailored to the individual clinical presentation and responses of each patient.

REFERENCES



References for this chapter can be found online at http://www.expertconsult.com



Chapter 27

Michael S Niederman

Community-acquired pneumonia

INTRODUCTION

Pneumonia is a respiratory infection of the alveolar space, which can vary from a mild outpatient illness to a severe illness necessitating hospitalization and intensive care. In 2004, pneumonia, along with influenza, was the eighth leading cause of death in the USA, the sixth leading cause of death in those over age 65, and the principal cause of death from infectious diseases.[1] When the infection occurs in patients who are living in the community it is termed community acquired pneumonia (CAP), while it is called nosocomial pneumonia if it arises in patients who are already in hospital.[2,3] However, the distinction between these two forms of infection is becoming increasing blurred because of the complexity of patients who are now living outwith hospital, including those in nursing homes, those receiving chronic hemodialysis and those recently admitted to hospital, all of whom have contact with the health-care environment and may import multidrug-resistant (MDR) organisms when they come to the hospital with 'healthcare-associated pneumonia' (HCAP). Thus, the relationship between bacteriology and the site of origin of infection is a reflection of several factors, including the co-morbid illnesses present in the patient who develops pneumonia, their host-defense status and their environmental exposure to specific pathogens.[4] This discussion focuses on patients who develop pneumonia out of the hospital (including CAP and HCAP), who are not HIV infected and who do not have traditional immune suppression (cancer chemotherapy, immune suppressive medications).

The complexity of CAP management has increased in recent years, not only because of the presence of more co-morbid illness in at-risk individuals, but also because the etiologic pathogens are changing. Historically, CAP was regarded as a bacterial illness caused by one pathogen, *Streptococcus pneumoniae*, but now the number of identified pathogens has expanded to include not only bacteria, but also viruses (influenza), fungi and a number of other recently identified organisms (e.g. *Legionella* spp. and *Chlamydophila pneumoniae*). In addition to an expanding number of etiologies, the ability to treat CAP is being challenged by the rising frequency of antimicrobial resistance among many bacteria, including pneumococcus.

EPIDEMIOLOGY

In 1994, over 5.6 million people were diagnosed with CAP in the USA, but the majority, 4.6 million, were treated out of the hospital.[5] Data from 2005 showed that there were 1.3 million hospitalizations for pneumonia in the USA, more in females than males, and approximately 60% in those over the age of 65.[1] Community-acquired pneumonia has a seasonal variability, with a rise in frequency during the winter months, paralleling the times of influenza and viral infection, illnesses which can interfere with host defense and predispose to secondary bacterial pneumonia. Certain pathogens, such as *Legionella* spp., are more common in the late summer and early fall, reflecting the water-borne sources of this organism.

The cost of care for patients with CAP in the USA was estimated to be over $40 billion in 2005, including both direct and indirect costs.[1] The elderly account for a disproportionate amount of this cost, largely because they often require inpatient treatment, reflecting a high frequency of co-morbid illness. Although those over age 65 account for only about one-third of all cases of CAP, they are responsible for 60% of those hospitalized with CAP.

The elderly have both an increased incidence of pneumonia and an increased mortality, compared to younger populations. The high frequency and enhanced mortality of pneumonia in older patients are well known, but controversy still continues about whether this is a consequence of aging itself or the result of the co-morbid illnesses that become increasingly common in the aging population.

PATHOLOGY AND PATHOGENESIS

Pneumonia is an infection of the gas exchanging units of the lung, most commonly caused by bacteria, but occasionally by viruses, fungi, parasites and other infectious agents. In the immunocompetent individual, it is characterized by a brisk filling of the alveolar space with inflammatory cells and fluid. If the alveolar infection involves an entire anatomic lobe of the lung, it is termed 'lobar pneumonia'. Multilobar illness can be present in some instances and may lead to more severe clinical manifestations. When the alveolar process occurs in a distribution that is patchy, and adjacent to bronchi, without filling an entire lobe, it is termed a 'bronchopneumonia'.

Pneumonia occurs when a patient's host defenses are overwhelmed by an infectious pathogen. This can happen because the patient has an inadequate immune response, often as the result of underlying chronic medical diseases (congestive heart failure, diabetes, renal failure, chronic obstructive lung disease, malnutrition), because of anatomic abnormalities (endobronchial obstruction, bronchiectasis), as a result of acute illness-associated immune dysfunction (as can occur with sepsis or acute lung injury) or because of therapy-induced dysfunction of the immune system (corticosteroids, endotracheal intubation). Some commonly used therapies may actually reduce the mortality risk of pneumonia, including angiotensin-converting enzyme (ACE) inhibitors and statins.[6] Admission hyperglycemia may increase mortality risk in CAP, but it is unclear if therapy can mitigate this risk.[7]

Pneumonia can even occur in patients who have an adequate immune system if the host defense system is overwhelmed by a large inoculum of micro-organisms, which can occur in a patient with massive aspiration of gastric contents. Patients with impaired gastrointestinal or neurologic function may also aspirate, and this process

involves failure to protect the lower respiratory tract from the entry of oropharyngeal secretions, which are often overgrown with potentially pathogenic Gram-negative bacteria.[8] In patients outside the hospital, a normal immune system can also be overcome by a particularly virulent organism to which the patient has no pre-existing immunity (such as certain bacteria or viruses) or to which the patient has an inability to form an adequate acute immune response. The epidemic spread of severe acute respiratory syndrome (SARS), due to a virulent virus, is one example of this phenomenon.

Bacteria can enter the lung via several routes, but aspiration from a previously colonized oropharynx is the most common mechanism for pneumonia.[9] Although most pneumonias result from microaspiration, some patients can also aspirate large volumes of bacteria if they have impaired neurologic protection of the upper airway (stroke, seizure) or if they have intestinal illnesses that predispose to vomiting. Other routes of entry include inhalation, which applies primarily to viruses, *Legionella pneumophila* and *Mycobacterium tuberculosis*; hematogenous dissemination from extrapulmonary sites of infection (right-sided endocarditis); and direct extension from contiguous sites of infection (such as liver abscess).

Based on these mechanisms, previously healthy individuals often develop infection with virulent pathogens such as viruses, *Legionella pneumophila*, *Mycoplasma pneumoniae*, *Chlamydophila pneumoniae* and *Strep. pneumoniae* (pneumococcus). On the other hand, chronically ill patients can be infected by these organisms, as well as by organisms that commonly colonize the oropharynx (primarily enteric Gram-negatives) but only cause infection when immune responses are inadequate. These organisms include enteric Gram-negative bacteria (*Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Acinetobacter* spp.), as well as fungi.

Severe forms of pneumonia develop when the infection is not contained (inadequate immune response) or, alternatively, if the inflammatory response to infection is unable to be localized to the site of infection (excessive immune response) and it 'spills over' into the systemic circulation (sepsis) or to the rest of the lung (acute respiratory distress syndrome). The normal lung immune response to infection is generally 'compartmentalized' and thus most patients with unilateral pneumonia have an inflammatory response that is limited to the site of infection. In patients with localized pneumonia, tumor necrosis factor (TNF), interleukin (IL)-6 and IL-8 levels are increased in the pneumonic lung and generally not increased in the uninvolved lung or in the serum.[10] Patients with severe pneumonia have increased serum levels of TNF and IL-6. It remains uncertain why localization does not occur in all individuals, but it is likely that genetic polymorphisms in the immune response may explain some of these differences, with patients who have certain inherited patterns of response being more prone than others to severe forms of pneumonia, and even mortality from this illness.[11,12] For example, CAP severity is increased with genetic changes in the IL-10 1082 locus, which are often present along with changes in the TNF 308 locus.[11,12] Currently, there are a large number of genes that have been identified as being able to affect the severity and outcome of CAP, but the ability to use this information to impact patient management does not currently exist.

ETIOLOGY

Etiologic pathogens (overview)

An etiologic pathogen is identified in only about half of all CAP patients, reflecting the limited value of even extensive diagnostic testing and the likelihood that we do not know all the organisms that can cause this illness. For example, in the past three decades, a variety of new CAP pathogens have been identified, including *Legionella pneumophila*, *Chlamydophila pneumoniae*, hantavirus, metapneumoviruses and coronaviruses (including the SARS virus). In addition, antibiotic-resistant variants of common pathogens such as drug-resistant *Streptococcus pneumoniae* (DRSP) and methicillin-resistant *Staphylococcus aureus* (MRSA) have become more prominent.

The number one pathogen for all patient populations with CAP is *Strep. pneumoniae*, or pneumococcus (including DRSP), and some studies have suggested that it may be responsible for many of the patients with no established etiologic diagnosis using standard diagnostic methodology.[13] In addition, atypical pathogens such as *M. pneumoniae*, *C. pneumoniae* and *Legionella pneumophila* are also common in patients with CAP, but may exist as co-pathogens, along with bacterial organisms.[14] Viruses may be present in up to 20% of all patients, particularly influenza, parainfluenza, adenovirus and respiratory syncytial virus.[15] Because a diagnosis of viral pneumonia requires specialized testing, usually acute and convalescent titers, this diagnosis is often not established. *Haemophilus influenzae* is a common organism in patients who smoke cigarettes and in those with chronic obstructive lung disease. Enteric Gram-negatives are not common causes of CAP, being found in only a few patients, and most of those with these organisms actually have HCAP which is treated similarly to nosocomial pneumonia.[3,16] Seasonal variations of pathogens may also be seen – pneumococcus and respiratory viruses are more common in winter in temperate countries.

In approaching management, it is important to stratify patients into different populations that are at risk for infection with specific pathogens (Table 27.1). The classification is based on the severity

Table 27.1 Common pathogens causing CAP and HCAP in specific patient populations (in order of decreasing frequency)

Outpatient, no cardiopulmonary disease or modifying factors	*Streptococcus pneumoniae*, *Mycoplasma pneumoniae*, *Chlamydophila pneumoniae* (alone or as mixed infection), *Haemophilus influenzae*, respiratory viruses, others (*Legionella* spp., *Mycobacterium tuberculosis*, endemic fungi)
Outpatient, with cardiopulmonary disease and/or modifying factors, or HCAP with no resistance risk factors	All of the above plus DRSP, enteric Gram-negatives and possibly anaerobes (with aspiration)
Inpatient, with cardiopulmonary disease and/or modifying factors, or HCAP with no resistance risk factors	*Strep. pneumoniae* (including DRSP), *H. influenzae*, *Mycoplasma pneumoniae*, *C. pneumoniae*, mixed infection (bacteria plus atypical pathogen), enteric Gram-negatives, anaerobes (aspiration), viruses, *Legionella* spp., others (*Mycobacterium tuberculosis*, endemic fungi, *Pneumocystis jirovecii*)
Inpatient, with no cardiopulmonary disease or modifying factors	All of the above, but DRSP and enteric Gram-negatives are unlikely
Severe CAP, with no risks for *Pseudomonas aeruginosa*	*Strep. pneumoniae* (including DRSP), *Legionella* spp., *H. influenzae*, enteric Gram-negative bacilli, *Staphylococcus aureus*, *Mycoplasma pneumoniae*, respiratory viruses, others (*C. pneumoniae*, *Mycobacterium tuberculosis*, endemic fungi)
Severe CAP, with risks for *P. aeruginosa*, or HCAP with resistance risk factors	All of the above pathogens, plus *P. aeruginosa*

DRSP, drug-resistant *Streptococcus pneumoniae*; HCAP, health-care-associated pneumonia.

of illness and the presence of clinical risk factors for specific pathogens, referred to as 'modifying factors'. Patients with severe CAP may have a slightly different spectrum of organisms, being commonly infected with pneumococcus, atypical pathogens (especially *Legionella* spp.), enteric Gram-negatives (including *P. aeruginosa*), *Staph. aureus* and *H. influenzae*. As mentioned, HCAP patients are often at risk for infection with MDR Gram-negatives and Gram-positives, but not all HCAP patients are at the same risk (Table 27.1). In fact, these organisms are only a consideration for the HCAP patient with at least two of three risk factors, which include severe illness, poor functional status and prior antibiotic therapy.[16] Table 27.2 shows that certain clinical conditions are associated with specific pathogens and these associations should be considered in all patients when obtaining a history. One common, and important, association is infection with MRSA in patients with recent influenza infection.[17]

Table 27.2 Clinical associations with specific pathogens

Condition	Commonly encountered pathogens
Alcoholism	*Streptococcus pneumoniae* (including DRSP), anaerobes, Gram-negative bacilli (possibly *Klebsiella pneumoniae*)
Chronic obstructive pulmonary disease/ current or former smoker	*Strep. pneumoniae*, *Haemophilus influenzae*, *Moraxella catarrhalis*, *Legionella* spp., enteric Gram-negatives
Residence in nursing home	*Strep. pneumoniae*, Gram-negative bacilli, *H. influenzae*, *Staphylococcus aureus*, anaerobes, *Chlamydophila pneumoniae*; consider *Mycobacterium tuberculosis*
Poor dental hygiene	Anaerobes
Bat exposure	*Histoplasma capsulatum*
Bird exposure	*Chlamydia psittaci*, *Cryptococcus neoformans*, *Histoplasma capsulatum*
Rabbit exposure	*Francisella tularensis*
Travel to south-west USA	Coccidioidomycosis, hantavirus in selected areas
Exposure to farm animals or parturient cats	*Coxiella burnetii* (Q fever)
Travel to South East Asia	*Mycobacterium tuberculosis*, *Burkholderia pseudomallei*, SARS virus
Suspected bioterrorism	Anthrax, smallpox, pneumonic plague
Endobronchial obstruction	Anaerobes
Post influenza pneumonia	*Strep. pneumoniae*, *Staph. aureus*, *H. influenzae*
Structural disease of lung (bronchiectasis, cystic fibrosis, etc.)	*Pseudomonas aeruginosa*, *P. cepacia* or *Staph. aureus*
Recent antibiotic therapy	Pneumococcus resistant to the class of agents to which the patient was recently exposed, enteric Gram-negatives

DRSP, drug-resistant *Streptococcus pneumoniae*; SARS, severe acute respiratory syndrome.

SPECIFIC ORGANISMS

Streptococcus pneumoniae

Streptococcus pneumoniae is the most common pathogen for CAP in all patient populations, possibly even among those without an etiology recognized by routine diagnostic testing. In one study, when no etiologic pathogen was defined by conventional testing, transthoracic needle aspirates, analyzed with polymerase chain reaction (PCR) probes, identified pneumococcus in half of the patients in whom the needle provided a diagnosis.[13] The organism is a Gram-positive, lancet-shaped diplococcus, of which there are 84 different serotypes, each with a distinct antigenic polysaccharide capsule, but 85% of all infections are caused by one of 23 serotypes, which are now included in a vaccine.

Infection is most common in the winter and early spring, which may relate to the finding that up to 70% of patients have a preceding viral illness. The organism spreads from person to person and commonly colonizes the oropharynx before it causes pneumonia. Pneumonia develops when colonizing organisms are aspirated into a lung that is unable to contain the aspirated inoculum. Patients at risk include the elderly; those with asplenia, multiple myeloma, congestive heart failure, alcoholism; after influenza; and in patients with chronic lung disease. Individuals with HIV infection develop pneumococcal pneumonia with bacteremia more commonly than in healthy populations of the same age.

The classic radiographic pattern is a lobar consolidation; however, bronchopneumonia can also occur and is a common pattern in some series. Bacteremia is present in up to 20% of hospitalized patients with this infection, and although the impact of this finding on mortality is uncertain, its presence probably does not lead to a worse outcome. Extrapulmonary complications include meningitis, empyema, arthritis, endocarditis and brain abscess.

Drug-resistant pneumococci (DRSP)

Since the mid-1990s, antibiotic resistance among pneumococci has become increasingly common in the USA and penicillin resistance, along with resistance to other common antibiotics (macrolides, trimethoprim-sulfamethoxazole, selected cephalosporins), is present in over 40% of these organisms, using older definitions of resistance. Fortunately, in the USA, a large number of penicillin-resistant organisms are of the sensitive and 'intermediate' type. In other parts of the world (e.g. the UK) rates of DRSP have remained low over the last decade. Recently, the definitions of resistance have changed for nonmeningeal infection, with sensitive being defined by a penicillin minimum inhibitory concentration (MIC) ≤2 mg/L, intermediate as an MIC of 4 mg/l and resistant as an MIC ≥8 mg/l.[18] While the clinical impact of resistance on outcomes such as mortality was hard to show using older definitions, with the new definitions of resistance very few pathogens will be defined as resistant; however, those that are may affect outcome. In fact, most experts believe that CAP caused by organisms with a penicillin MIC of ≥4 mg/l, still an uncommon finding, can lead to an increased risk of death.[19]

Later studies have shown that higher levels of resistance can affect outcomes such as mortality and the development of suppurative complications such as empyema. The relationship of resistance to illness severity is complex and in some studies severity of illness may be reduced in patients with resistant organisms, implying a loss of virulence among organisms that become resistant.

Resistance of pneumococcus has even been reported to the quinolones, which are ordinarily a reliable class of antibiotic for these organisms. In general, one important risk factor for resistance is repeated use of a given agent in the same patient. In fact, pneumococcal resistance to β-lactams (penicillins and cephalosporins), macrolides and quinolones is more likely if a patient has received the same agent in the past 3 months.[20] With these data in mind, new guidelines have

suggested that CAP patients not receive the same antibiotic as in the recent past, with the cutoff of defining this time interval being within the past 3 months.

Atypical pathogens

Originally the term 'atypical' was used to describe the nonclassic clinical features of infection with certain organisms, but recent studies have suggested that the term does not accurately describe a unique pneumonia syndrome related to specific pathogens. However, the term has been retained to refer to a group of organisms which includes *M. pneumoniae*, *C. pneumoniae* and *Legionella*, a group of organisms that cannot be reliably eradicated by β-lactam therapy (penicillins and cephalosporins) but must be treated with a macrolide, a tetracycline or a quinolone. The frequency of these organisms as CAP pathogens has varied in studies, with recent data from North America and elsewhere suggesting that they may be present in up to 60% of CAP episodes, and that they can serve as co-pathogens, along with bacteria, in up to 40% of patients.[14] When mixed infection is present, particularly with *C. pneumoniae* and pneumococcus, it may lead to a more complex course and a longer length of stay than if a single pathogen is present. In patients with severe CAP, atypical pathogens can be present in almost 25% of all patients, but the responsible organism may vary over time. While atypical pathogens have been thought to be most common in young and healthy individuals, some population data have shown that they are present in patients of all ages, including the elderly in nursing homes.[21]

Studies reporting a high frequency of atypical pathogens have made the diagnosis with serologic testing, which may not be as accurate and specific as culture and antigen identification. The importance of atypical pathogens has been suggested by a number of studies of inpatients, including those with bacteremic pneumococcal pneumonia, showing a mortality benefit from therapies that include a macrolide or quinolone, agents that would be active against these organisms.[22,23] Atypical organism pneumonia may not be a constant phenomenon, and the frequency of infection may vary over the course of time and with geography. In one study, the benefit of providing empiric therapy directed at atypical pathogens was variable, being more important in some calendar years than in others.[23] Differing views about the importance of atypical pathogens have led to disparate recommendations about whether they should be covered by empiric therapy, with some CAP guidelines recommending routine coverage, while others, particularly from the UK and Europe, suggest otherwise.

Legionella pneumophila

This small, weakly staining, Gram-negative bacillus was first characterized after an epidemic in 1976, and can occur either sporadically or in epidemic form. At present, although multiple serogroups of the species *L. pneumophila* have been described, serogroup 1 is the most commonly diagnosed and can be identified with a urinary antigen test. The other species that commonly causes human illness is *Legionella micdadei*. *Legionella* is a water-borne pathogen that can emanate from air conditioning equipment, drinking water, lakes and river banks, water faucets, saunas and shower heads. Infection is more common in the summer and early fall, and is generally caused by inhalation of an infected aerosol generated by a contaminated water source. When a water system becomes infected in an institution, endemic outbreaks may occur, as has been the case in some nursing homes and hospitals. In its sporadic form, *Legionella* may account for 7–15% of all cases of CAP, being a particular concern in patients with severe forms of illness.

The varying incidence of *Legionella* infection among admitted patients is a reflection of geographic and seasonal variability in infection rates, as well as the extent of diagnostic testing. For a serologic diagnosis, it is necessary to collect both acute and convalescent titers. The urinary antigen test is the single most accurate acute diagnostic test for *Legionella*, but is specific to serogroup 1 infection. In recent years, most cases have been diagnosed with urinary antigen and there has been less reliance on serology and culture.[24] With this increased reliance on urinary antigen testing, the case fatality rate of *Legionella* has fallen, possibly reflecting diagnosis of less severe illness than in the past.[24]

It is difficult to identify the microbial etiology of CAP on the basis of clinical and radiographic features and a unique presentation of *Legionella* is uncommon. The classic clinical syndrome is characterized by high fever, chills, headache, myalgias and leukocytosis, along with a history of preceding diarrhea, early onset of mental confusion, hyponatremia, relative bradycardia and liver function abnormalities. Symptoms are rapidly progressive, and the patient may appear to be quite toxic, so this diagnosis should always be considered in patients admitted to the intensive care unit (ICU) with CAP.

Mycoplasma pneumoniae

Mycoplasma pneumoniae can cause CAP year-round, with a slight increase in the fall and winter. All age groups are affected, and although it is common in those less than 20 years of age, it is also seen in older adults. Respiratory infection occurs after the organism is inhaled and then binds via neuraminic acid receptors to the airway epithelium. An inflammatory response with neutrophils, lymphocytes and macrophages then follows, accompanied by the formation of IgM and then IgG antibody. Some of the observed pneumonitis may be mediated by the host response to the organism rather than by direct tissue injury by the organism. Up to 40% of infected individuals will have circulating immune complexes.

Although *Mycoplasma* causes pneumonia, the infection is often characterized by its extrapulmonary manifestations such as upper respiratory tract symptoms, including sore throat and earache (with hemorrhagic or bullous myringitis). Pleural effusion is seen in at least 20% of patients although it may be small. Other manifestations include neurologic illness such as meningoencephalitis, meningitis, transverse myelitis and cranial nerve palsies. The most common extrapulmonary finding is an IgM autoantibody that is directed against the I antigen on the red blood cell and causes cold agglutination of the erythrocyte. Although up to 75% of patients may have this antibody and a positive Coombs' test, clinically significant autoimmune hemolytic anemia is uncommon. The extrapulmonary manifestations may follow the respiratory symptoms by as long as 3 weeks.

Gram-negative bacteria

The most common Gram-negative organism causing CAP is *H. influenzae,* an organism seen in the elderly and in those who smoke cigarettes, or who have a history of alcoholism or chronic bronchitis. *H. influenzae* is a coccobacillary rod that can be either a typeable (encapsulated) or nontypeable organism, and can lead to bronchopneumonia and rarely empyema. Encapsulated organisms require a more elaborate host response and thus are more virulent than unencapsulated organisms. However, several studies have shown that in adults, particularly those with chronic obstructive pulmonary disease (COPD), infection with unencapsulated bacteria is common. The encapsulated type of organism may cause bacteremic pneumonia in some patients, particularly in those with segmental pneumonias as opposed to those with bronchopneumonia.

Enteric Gram-negatives are generally not common in CAP unless the patients are elderly and have chronic cardiac or pulmonary disease, have HCAP or are alcoholic. In one study, the identified risk factors for Gram-negative CAP were probably aspiration, prior hospitalization, prior antibiotic therapy and pulmonary co-morbidity.[25] In these patients, organisms such as *E. coli* and *K. pneumoniae* can be found. Although *P. aeruginosa* is an uncommon cause of CAP, it can be isolated from patients with bronchiectasis, in those with severe forms of CAP and in patients with pulmonary co-morbidity and prior hospitalization.[2,25] Gram-negative CAP was often a severe illness, with septic shock and hyponatremia, and occurred especially in patients with malignancy, cardiac disease and a history of cigarette smoking.[26]

While the frequency of enteric Gram-negatives in CAP has been controversial, many of the patients at risk for these organisms would now be re-classified as HCAP. It is still important to identify patients at risk, since infection with a Gram-negative increased the chance of dying by more than threefold, with a mortality rate of 32% in one study.[25] These patients also need ICU admission and mechanical ventilation more often than patients infected with other organisms. Patients with HCAP severe enough to require mechanical ventilation, admitted from a nursing home and with risk factors for aspiration (intestinal or neurologic risk factors), are particularly at risk for infection with enteric Gram-negatives, more than any other pathogens, including anaerobes.

Anaerobes

These organisms have always been a concern in patients with poor dentition who aspirate oral contents, and those at risk have been patients with neurologic or swallowing disorders, as well as individuals who abuse alcohol and opiate drugs. As mentioned, these patients may also be at risk for infection with enteric Gram-negatives and in the study cited above, many of the aspiration-prone patients who had anaerobes recovered, had them along with aerobic Gram-negatives and their presence did not correlate with poor oral hygiene. Many of these patients received inadequate therapy for anaerobes, yet most recovered, raising a question about whether these organisms really need to be treated. These findings suggest that anaerobes may not always be pathogens but may be colonizers in the institutionalized elderly, including those with aspiration risk factors.

Staphylococcus aureus

Community-acquired pneumonia can also be caused by this organism, which can lead to severe illness and to cavitary pneumonia. This organism can also seed the lung hematogenously from a vegetation in patients with right-sided endocarditis or from septic venous thrombophlebitis (from central venous catheter or jugular vein infection). When a patient develops postinfluenza pneumonia, *Staph. aureus* can lead to secondary bacterial infection, along with pneumococcus and *H. influenzae*. In the past several years, community-acquired strains of methicillin-resistant *Staph. aureus* (CA-MRSA) have emerged, primarily in skin and soft tissue infections, but also as a cause of severe CAP. CA-MRSA is a clonal disease, emanating from the USA 300 clone of *Staph. aureus*, and is clinically and bacteriologically different from the strains of MRSA that cause nosocomial pneumonia.[17] In addition, it can infect previously healthy individuals, and the classic clinical presentation of this pathogen causing CAP is as a complication of a preceding viral or influenza infection. The illness is characterized by a severe, bilateral, necrotizing pneumonia. Since the pathogenesis of pneumonia due to this organism may be related to toxin production by the bacteria, therapy may need to involve both an antibacterial agent and an antitoxin-producing agent.[27]

Viruses

Although the incidence of viral pneumonia is difficult to define, during epidemic times influenza should be considered as it can lead to primary viral pneumonia or to secondary bacterial pneumonia. One careful study of over 300 nonimmunocompromised CAP patients looked for viral pneumonia by paired serologies and found that 18% had viral pneumonia, with about half being pure viral infection and the others being mixed with bacterial pneumonia.[15] Influenza (A more than B), parainfluenza and adenovirus were the most commonly identified viral agents.

Although influenza A and B are the most common causes of viral pneumonia, they can be prevented to a large extent by vaccination. There are also other viruses that can cause severe forms of pneumonia, as evidenced by the recent experience with SARS, which demonstrated the potential of epidemic, person-to-person spread of a virulent respiratory viral infection.

CLINICAL FEATURES

Symptoms and physical findings

Patients with an intact immune system who develop CAP generally have 'typical' respiratory symptoms such as cough, sputum production and dyspnea, along with fever and other complaints. Cough is the most common finding and is present in up to 80% of all patients, but is less common in those with impaired immune responsiveness, such as the elderly, those with serious co-morbidity or individuals coming from nursing homes (HCAP).[4] Pleuritic chest pain is also a common symptom in CAP and its absence has been identified as a poor prognostic finding.[28]

When pneumonia occurs in elderly patients, it can have a non-respiratory presentation with symptoms of confusion, falling, failure to thrive, altered functional capacity or deterioration in a pre-existing medical illness, such as congestive heart failure. Patients with advanced age often have a longer duration of symptoms such as cough, sputum production, dyspnea, fatigue, anorexia, myalgia and abdominal pain than younger patients. In addition, they have delirium or acute confusion more often than younger patients. Very few elderly patients with pneumonia are considered well nourished, with kwashiorkor-like malnutrition being the predominant type of nutritional defect and the one associated with delirium on initial presentation.[29]

Physical findings of pneumonia include tachypnea, focal crackles, rhonchi and signs of consolidation (egophony, bronchial breath sounds, dullness to percussion). Other physical findings can be signs of pleural effusion, metastatic infection (arthritis, endocarditis, meningitis) or extrapulmonary manifestations that can occur with *M. pneumoniae* or *C. pneumoniae*. One of the most important physical assessments in CAP is a careful measurement of respiratory rate, which can have both diagnostic and prognostic relevance. In the elderly, an elevation of respiratory rate may be the initial presenting sign of pneumonia, preceding other clinical findings by as much as 1–2 days.[30] In general, tachypnea is the most common finding in elderly patients; it is present in over 60% of all patients, but occurs more often in the elderly than in younger patients with pneumonia. Measurement of respiratory rate also has prognostic significance and the presence of a respiratory rate greater than 30 per minute is one of several factors associated with increased risk of mortality.

Typical vs atypical pneumonia syndromes

In the past, the clinical and radiographic features of CAP were characterized as fitting into a pattern of either 'typical' or 'atypical' symptoms which could be used to predict a specific etiologic agent. The typical pneumonia syndrome, attributed to pneumococcus and other bacterial pathogens, is characterized by sudden onset of high fever, shaking chills, pleuritic chest pain, lobar consolidation and a toxic-appearing patient with the production of purulent sputum. The atypical pneumonia syndrome, which is characterized by a subacute illness, nonproductive cough, headache, diarrhea or other systemic complaints, can be the result of infection with *M. pneumoniae*, *C. pneumoniae*, *Legionella* spp. or viruses, but bacterial pneumonia can present in this fashion if the patient has an impaired immune response.

Recent studies have shown that this approach is not highly accurate and there is only a weak relationship between clinical features and the etiologic pathogen, primarily because host, as well as pathogenic, factors play a role in defining patient symptoms. Clinical features have been shown to be only about 40% accurate in differentiating pneumococcus, *M. pneumoniae* and other pathogens from one another.[2,31] The limitations of clinical features in defining the microbial etiology also apply to evaluations of radiographic patterns.

Clinical assessment of pneumonia severity

Careful evaluation of illness severity is necessary to guide decisions about whether to hospitalize a patient, and if so, whether to admit the

patient to the ICU. Although a number of models have been developed to predict mortality, and they have been proposed to guide the admission decision, the decision to admit a patient to the hospital should be based on social as well as medical considerations and remains an 'art of medicine' determination. In general the hospital should be used to observe patients who have multiple risk factors for a poor outcome, those who have decompensation of a chronic illness or those who need therapies not easily administered at home (oxygen, intravenous fluids, cardiac monitoring).

Risk factors for a poor outcome include a respiratory rate ≥30/min, age ≥65 years, systolic blood pressure <90 mmHg, diastolic BP ≤60 mmHg, multilobar pneumonia, confusion, blood urea nitrogen (BUN) >19.6 mg/dl, Pa_{O_2} <60 mmHg (on room air), Pa_{CO_2} >50 mmHg, respiratory or metabolic acidosis, or signs of systemic sepsis.[2] The two best-studied and most widely used prediction rules for pneumonia severity are the Pneumonia Severity Index (PSI) and the CURB-65 rule, a modification of a prognostic model developed by the British Thoracic Society.[28,32] The PSI uses multiple demographic and historic findings, physical findings and laboratory data, each assigned a point score, and the total score is used to categorize patients into one of five classes, each with a different risk of death. Although this tool has worked well to define mortality risk, it has had variable success in predicting site of care and is limited by its complexity and its failure to always recognize the most severely ill patients, especially if they do not have underlying co-morbid illness.[33] The CURB-65 rule is simpler, using only five assessments: **C**onfusion (due to the pneumonia), blood **U**rea nitrogen >7 mmol/l, **R**espiratory rate ≥30/min, **B**lood pressure of <90 mmHg systolic or ≤60 mmHg diastolic, and age ≥**65** years. Each of the five criteria receives 1 point, and the score falls between 0 and 5, with mortality risk rising with the score.

In recent studies, both tools have worked equally well to identify patients at low risk of dying, but the CURB-65 has been more discriminating in recognizing patients who need ICU care (score of at least 3) and who have the highest risk of death.[34] On the other hand, the CURB-65 does not account well for patients with decompensated chronic illness that results from the presence of CAP. This is because the PSI weights advanced age and chronic illness very heavily, whereas the CURB-65 model includes age as only one of several risk factors and co-morbid illness is not measured, but instead most of the score is based on acute physiologic abnormalities. Neither prediction model includes 'social factors' and clearly these issues need to be included in patient assessment, paying attention to whether the patient has a stable home environment for outpatient care, an ability to take oral medications, the absence of acute alcohol or drug intoxication, and stability of other acute and chronic medical problems.

There is no specific rule for who requires intensive care, but in general ICU admission (in the USA) is associated with a mortality rate of at least 30%, compared to a mortality rate of 12% for all admitted patients and a 1–5% mortality rate for outpatients.[35] When the ICU is used early in the hospital stay, the mortality rate is lower than if patients are first admitted to the ward, then deteriorate and move to the ICU.[36] Earlier studies that suggested a limited benefit from ICU admission generally found that patients were admitted too late in the course of illness to benefit, thus emphasizing the need for accurately assessing mortality risk when the patient is first evaluated.

Radiographic abnormalities

Most patients are diagnosed and treated for CAP after the physician obtains a chest radiograph which shows the presence of a new infiltrate, although not all outpatients have access to this evaluation. However, even when the radiograph is negative, if the patient has appropriate symptoms and focal physical findings, pneumonia may still be present. When CT scanning has been used in patients with clinical signs and symptoms of CAP, it can demonstrate abnormalities in some patients with a negative chest radiograph and the abnormalities are generally more extensive on CT scan than on chest radiograph.[37] Thus, if a symptomatic patient has an initially negative chest radiograph, it should be repeated after 24–48 hours. It is uncertain why a chest film would initially be negative, but the idea that hydration, especially in the elderly, is the explanation is not proven and falls into the realm of anecdotal reports.

Numerous studies have documented that the pattern of radiographic abnormality cannot reliably be used to predict the etiology of infection. Pleural effusion may appear on the initial chest radiograph and if present, it is necessary to distinguish empyema from a simple parapneumonic effusion by sampling the pleural fluid.

DIAGNOSTIC TESTING

Recommended testing (Table 27.3)

History

Historic data should be collected to suggest the presence of specific unusual pathogens, in addition to the likely organisms[2] (see Tables 27.1 and 27.2). For example, if the presentation is subacute following contact with birds, rats or rabbits, then the possibility of psittacosis, leptospirosis, tularemia or plague should be considered. *Coxiella burnetii* (Q fever) is a concern with exposure to parturient cats, cattle, sheep or goats; *Francisella tularensis* is a concern with rabbit exposure; hantavirus with exposure to mice droppings in endemic areas; *Chlamydia psittacii* with exposure to turkeys or infected birds; and *Legionella* with exposure to contaminated water sources (saunas). Following influenza, superinfection with pneumococcus, *Staph. aureus* (including MRSA) and *H. influenzae* should be considered. The onset of respiratory failure after a preceding viral illness should lead to suspicion of a viral pneumonia. Endemic fungi, (coccidioidomycosis, histoplasmosis and blastomycosis) occur in well-defined geographic areas and may present acutely with symptoms which overlap with acute bacterial pneumonia.

Radiography

Once the clinical evaluation suggests the presence of pneumonia, the diagnosis should be confirmed by chest radiograph. Although a radiograph is recommended in all outpatients and inpatients, it may be impractical in some settings outside of the hospital. A chest radiograph not only confirms the presence of pneumonia, but can also be used to identify complicated illness and to grade severity of disease by noting such findings as pleural effusion and multilobar illness. As mentioned above, there is no specific radiographic pattern that can be used to define the etiologic pathogen of CAP but certain findings can be used to suggest specific organisms (see above).

Table 27.3 Recommended diagnostic testing for CAP

- Diagnose the presence of pneumonia with a chest radiograph and clinical data
- Look for specific pathogens that alter therapy, based on historic and epidemiologic clues
- Outpatient testing optional
- Blood cultures only with severe illness
- Sputum Gram stain and culture prior to therapy if good quality and rapid transport and processing in the laboratory (especially valuable if a drug-resistant or unusual pathogen is suspected, but not to narrow empiric therapy)
- *Legionella* and pneumococcal urinary antigen for severe CAP
- Endotracheal aspirate or sputum culture for severe CAP
- No routine serologic testing for atypical pathogens or viruses

Other testing

Even with extensive testing, at least half of all patients do not have an etiologic diagnosis established and thus therapy is usually empiric. In addition, recent studies have emphasized the mortality benefit of prompt administration of effective antibiotic therapy for those with moderate to severe illness, and therapy should never be delayed for the purpose of diagnostic testing. While several studies have shown that establishing an etiologic diagnosis does not improve the outcome of patients with severe CAP, diagnostic testing may have value for the purpose of narrowing and focusing therapy and for guiding management in the patient who is not responding to empiric therapy.[38]

Recommended testing for outpatients is limited to a chest radiograph and pulse oximetry, if available, with sputum culture being considered in patients suspected of having an unusual or drug-resistant pathogen. For admitted patients, current guidelines recommend that diagnostic testing should include a chest radiograph, assessment of oxygenation (pulse oximetry or blood gas, the latter if retention of carbon dioxide is suspected) and routine admission blood work. If the patient has a pleural effusion, this should be tapped and the fluid sent for culture and biochemical analysis. In addition, the patient should have blood cultures only in the presence of severe illness, while sputum Gram stain and culture have their greatest value when the sample is of good quality and can be transported to the laboratory rapidly. Culture is particularly valuable if the patient has risk factors for a drug-resistant or unusual pathogen. In the patient with severe CAP, an endotracheal aspirate should be obtained, along with urinary antigen testing for pneumococcus and *Legionella*.[2]

Although blood cultures are positive in only 10–20% of CAP patients, most often showing pneumococcus, they can be used to identify a specific pathogen and to define the presence of drug-resistant pneumococci. However, they should be limited to patients with a reasonable likelihood of having a true-positive result. If low-risk patients routinely have blood cultures, it is possible that the frequency of false-positives could exceed the true-positives and lead to inaccurate and unnecessary therapy. Thus, blood cultures are only recommended for patients who are severely ill, especially if they have not received antibiotic therapy prior to admission.[39]

The role of Gram stain of sputum to guide initial antibiotic therapy is controversial, but this test has its greatest value in guiding the interpretation of sputum culture and can be used to define the predominant organism present in the sample. The role of Gram stain in focusing initial antibiotic therapy is uncertain since the accuracy of the test to predict the culture recovery of an organism such as pneumococcus depends on the criteria used.[2] Even if Gram stain findings are used to focus antibiotic therapy, this would not allow for empiric coverage of atypical pathogens which might be present even in patients with pneumococcus as part of a mixed infection. However, Gram stain can be used to broaden initial empiric therapy by enhancing the suspicion for organisms that are not covered in routine empiric therapy (such as *Staph. aureus* being suggested by the presence of clusters of Gram-positive cocci, especially during a time of epidemic influenza).

Routine serologic testing for viruses and atypical pathogens is not recommended. However, in patients with severe illness, the diagnosis of *Legionella* can be made by urinary antigen testing, which is the single test that is most likely to be positive at the time of admission, but is specific only for serogroup 1 infection. Commercially available tests for pneumococcal urinary antigen have been developed and may have value to identify pneumococcus; however, as mentioned, the impact of a positive test on therapy choices is uncertain. Bronchoscopy is not indicated as a routine diagnostic test and should be restricted to immunocompromised patients and to selected individuals with severe forms of CAP.

THERAPY

Initial therapy should be focused on the administration of antibiotics and the use of supportive care. Since it is not possible to know the etiology of CAP on the basis of clinical and laboratory findings, and because of the need to administer therapy as quickly as possible, once the diagnosis is made antibiotic choice is empiric, focusing on the pathogens most likely to be present for a given type of patient. Supportive care includes oxygen if needed, hydration, control of hyperglycemia and possibly chest physiotherapy, as well as administration of bronchodilators and expectorants. For more severely ill patients, the management is similar to severe sepsis, as CAP is a common cause of this syndrome. This means evaluating the need for vasopressors in the presence of hypotension, the use of corticosteroids if relative adrenal insufficiency is suspected and consideration of the use of drotrecogin alpha in selected patients. In addition, the routine use of corticosteroids in patients with severe pneumonia is advocated by some, because of limited data showing a survival benefit with this anti-inflammatory intervention.[40]

In the past 15 years, a variety of professional societies have developed guidelines for the management of CAP and included in the recommendations are antibiotic choice, along with other strategies. Several studies have shown that when therapy is concordant with guideline recommendations, outcomes such as mortality and rate of treatment failure are improved, while for severely ill patients duration of mechanical ventilation is reduced.[41–43] However, the presence of a guideline by itself is not usually enough to lead to these benefits because the guideline requires an implementation strategy in order to be successful. In one study, the benefit of a guideline to reduce length of stay in the hospital was greatest when an implementation strategy was employed that relied on real-time intervention with case managers who identified variances from recommended management.[44]

Antibiotic therapy

Initial empiric therapy for CAP is selected by categorizing patients on the basis of place of therapy (outpatient, inpatient, ICU), severity of illness and the presence or absence of cardiopulmonary disease or specific 'modifying' factors that make certain pathogens more likely.[2] By using these factors, a set of likely pathogens can be predicted for each type of patient (see Table 27.1) and this information can be used to guide therapy. If a specific pathogen is subsequently identified by diagnostic testing, then therapy can be focused. In this scheme, it is also important to identify patients with HCAP and to exclude them from CAP management, as these patients require their own management approach.[2,3,16]

In choosing empiric therapy of CAP, certain principles should be followed. However, the principles that guide therapy in North America are not the same as those used in parts of Europe and the UK.[2,45–47] Although in general guidelines emphasize an empiric approach (without extensive microbiologic testing) and treatment in the community rather than in hospital, the North American guidelines suggest broad-spectrum agents reflecting a recommendation for routine atypical pathogen coverage. In contrast, the UK and European guidelines generally recommend an initial use of penicillins, avoid routine quinolone use and do not advocate therapy for both 'typical' and 'atypical' pathogens except in more severely ill patients. These regional differences reflect variability in the frequency and importance of atypical pathogens and of DRSP, and differing concerns about the importance of empiric broad-spectrum CAP therapy to the rise in resistant health-care-associated infection such as MRSA and *Clostridium difficile* infection. Table 27.4 highlights the principles for CAP management for the North American and UK approaches.

In North American guidelines, for outpatients with no co-morbid cardiopulmonary disease and no history of recent antibiotic use, therapy can be with an advanced macrolide (azithromycin or clarithromycin) or doxycycline. If the patient has co-morbid illness or a history of recent antibiotic therapy (in the past 3 months), then DRSP is a concern and therapy should be with a selected oral β-lactam (amoxicillin, amoxicillin-clavulanate, cefuroxime or cefpodoxime) combined with a macrolide or doxycycline.[2] Alternatively, these patients at risk for DRSP can be treated with an oral fluoroquinolone as monotherapy (gemifloxacin, levofloxacin or moxifloxacin). If the patient has received an

Table 27.4 Principles of antibiotic therapy for CAP: differences between North American and UK guidelines for treatment

Clinical situation	North American approach	UK approach
Timing of antimicrobials	Administer initial antibiotic therapy as soon as possible, after firmly establishing the presence of pneumonia	Antibiotics should be given as soon as possible and within 4 h of clinical diagnosis
Initial choice of antimicrobials	Treat all patients for pneumococcus (including DRSP) and for the possibility of atypical pathogen co-infection (if endemic rates in the community support a role for these organisms)	Treat all patients for pneumococcus. Other pathogens should be considered only in more severe cases or specific clinical situations
Initial antibiotic choice for adults hospitalized with low-moderate severity CAP treated in the community	Use either a macrolide alone (selected patients with no cardiopulmonary disease or modifying factors) or for those outpatients with cardiopulmonary disease or 'modifying factors': • use monotherapy with a quinolone • *or* the combination of a selected β-lactam (cefpodoxime, cefuroxime, high dose ampicillin (3 g/24 h) or amoxicillin-clavulanate) • with a macrolide or tetracycline. A macrolide alone should only be used in outpatients or inpatients with no risk factors for DRSP, enteric Gram-negatives or aspiration	Most patients can be adequately treated with oral antibiotics Oral therapy with amoxicillin is preferred When oral therapy is contraindicated, recommended parenteral choices include iv amoxicillin or benzylpenicillin, or clarithromycin
Initial antibiotic choice for adults hospitalized with moderate severity CAP	Provide initial therapy for hospitalized patients with an iv agent, or if oral only, use a quinolone because of its high bioavailability For inpatients at risk for DRSP: • use quinolone monotherapy • *or* the combination of a selected iv β-lactam (ceftriaxone, cefotaxime, ertapenem, ampicillin-sulbactam) • with a macrolide or tetracycline. Limit antipseudomonal therapy to patients with risk factors	Oral therapy with a combined β-lactam/macrolide regimen is recommended When oral therapy is inappropriate, parenteral amoxicillin or penicillin G are alternatives to oral amoxicillin, with clarithromycin, q12h, as the preferred macrolide for parenteral therapy Levofloxacin iv once daily or a combination of iv second- (e.g. cefuroxime) or third- (e.g. cefotaxime or ceftriaxone) generation cephalosporin with iv clarithromycin are also appropriate alternative choices
Initial antibiotic choice for adults hospitalized with severe CAP	If no pseudomonal risk factors use a selected β-lactam plus a macrolide or antipneumococcal quinolone New antipneumococcal quinolones, in order of decreasing antipneumococcal activity are: gemifloxacin (oral only), moxifloxacin (oral and intravenous), levofloxacin (oral and intravenous) In the combination regimens for severe CAP, consider a quinolone rather than a macrolide for suspected or proven *Legionella* infection For those with pseudomonal risk factors, use an antipseudomonal β-lactam *plus* either ciprofloxacin/high-dose levofloxacin *or* the combination of an aminoglycoside with *either* a macrolide or antipneumococcal quinolone (antipseudomonal β-lactams include cefepime, imipenem, meropenem, piperacillin-tazobactam) Never use monotherapy (including with a quinolone) for patients with severe CAP Empiric therapy for CA-MRSA should be confined to patients with severe pneumonia and evidence of necrotizing infection, particularly after a viral infection Consider using an antitoxin-producing agent with an antibiotic, either vancomycin combined with clindamycin or linezolid monotherapy	Patients with high severity pneumonia should be treated immediately after diagnosis with parenteral antibiotics An iv combination of a broad-spectrum β-lactamase stable antibiotic such as amoxicillin-clavulanic acid together with a macrolide such as clarithromycin is preferred Alternatively, in penicillin-allergic patients, a second- (e.g. cefuroxime) or third- (e.g. cefotaxime or ceftriaxone) generation cephalosporin can be used instead of co-amoxiclav, together with clarithromycin If *Legionella* is strongly suspected, consider adding levofloxacin

CA-MRSA, community-acquired methicillin-resistant *Staphylococcus aureus*; DRSP, drug-resistant *Streptococcus pneumoniae*.

antibiotic in the past 3 months, then ideally an agent from a different class should be chosen to avoid the risk of repeated use of the same agent, which can promote the emergence of pneumococcal resistance. In contrast to these recommendations, European and UK guidelines rely more on oral penicillins for these patients and place less emphasis on the need for macrolides, except for the more severely ill hospitalized patients, and discourage the routine use of quinolones (see Table 27.4).[45,46] As noted, this reflects different views on the frequency and importance of DRSP and atypical pathogens in the management and outcome of CAP.

The majority of inpatients will have cardiopulmonary disease or other risks for DRSP, and sometimes Gram-negatives, and North American guidelines suggest they should be treated with either a selected intravenous β-lactam (cefotaxime, ceftriaxone, ampicillin-sulbactam or ertapenem) combined with a macrolide or doxycycline; alternatively, they can receive monotherapy with an intravenous antipneumococcal quinolone (levofloxacin or moxifloxacin).[2] From the available data, either regimen is therapeutically equivalent, but an effort should be made to avoid repeating an agent in the same antibiotic class in the same patient, within a 3-month period. The antipneumococcal

quinolones are being widely used in North America because as a single drug, given once daily, it is possible to cover pneumococcus (including DRSP), Gram-negatives and atypical pathogens. In addition, quinolones penetrate well into respiratory secretions and are highly bioavailable, achieving the same serum levels with oral or intravenous therapy. There are differences among the available agents in their intrinsic activity against pneumococcus and, based on MIC data, these agents can be ranked from most to least active as: gemifloxacin (available only in oral form), moxifloxacin and levofloxacin. Some data suggest a lower likelihood of both clinical failures and the induction of pneumococcal resistance to quinolones if the more-active agents are used in place of the less-active agents.[2,20] UK and European guidelines do not recommend fluoroquinolones as initial therapy in severe pneumococcal CAP because of residual concerns regarding the clinical effectiveness of these agents, as well as concerns about their contribution to healthcare-associated infection (e.g. *Clostridium difficile*). Although oral quinolones may be as effective as intravenous quinolones for admitted patients with moderately severe illness, most admitted patients should receive initial therapy intravenously to be sure that the medication has been absorbed. Once the patient shows a good clinical response, oral therapy can be started.

In the ICU population, all individuals should be treated for DRSP and atypical pathogens, but only those with appropriate risk factors (see above) should have coverage for *P. aeruginosa* (see Table 27.4). In addition, no ICU-admitted patient should receive monotherapy with any agent, including a quinolone. While patients with severe CAP can receive either a macrolide or a quinolone as a second agent, several studies have shown a remarkably high efficacy of levofloxacin and moxifloxacin for documented *Legionella* infection, and thus a quinolone may be the preferred agent if this organism is suspected or proven.[48]

In addition to the therapy regimens discussed, some patients with severe CAP need added coverage for *Staph. aureus*, including MRSA. However, not all patients with severe CAP require this therapy, but most experts recommend that this organism be targeted empirically only in patients with severe necrotizing CAP following a viral illness, particularly influenza. Optimal therapy has not been defined and vancomycin alone may not be sufficient, having led to clinical failure, presumably since it is not active against the PVL toxin that accompanies community-acquired MRSA. For that reason, it may be necessary to add clindamycin to vancomycin or to use linezolid, with rifampin (rifampicin) in severe illness, since both of these latter agents can inhibit toxin production.[27]

Patients with HCAP are a heterogeneous group, with some at risk for MDR Gram-negatives and MRSA, and others not. As discussed earlier, the risks for drug-resistant pathogens in this population include recent antibiotic therapy and poor functional status, as well as severe illness.[16] An HCAP patient (from a nursing home, dialysis center or a patient who was recently hospitalized) who is not severely ill, and who has no or only one risk factor for resistance, can still be treated as non-severe CAP. Similarly, an HCAP patient with severe illness, but 0–1 risk factors for MDR pathogens can be treated with a severe CAP regimen.[16] On the other hand, a patient with severe HCAP and two risk factors for MDR pathogens should be treated for drug-resistant Gram-negatives, MRSA and other pathogens seen in those with nosocomial pneumonia. For these patients, the therapy should be with an aminoglycoside (amikacin, gentamicin or tobramycin) plus an antipseudomonal β-lactam (cefepime, imipenem, meropenem or piperacillin-tazobactam) plus linezolid or vancomycin[2,16] (see Chapter 28 for management of nosocomial pneumonia). If the severely ill HCAP patient has come from a nursing home that is known to have patients with atypical pathogen infection, then additional coverage of these agents is needed.

Other therapy issues

In addition to the general approach to antibiotic therapy outlined above, there are several other therapeutic issues in the management of CAP as highlighted in Table 27.4. These include the need for timely administration of initial antibiotic therapy, the findings of improved outcomes when pneumococcal bacteremia patients receive dual therapy rather than monotherapy, and the use of adjunctive therapies, especially in severely ill patients.

When a patient has CAP, administration of antibiotics as soon as possible has benefit for patient outcome and some retrospective data have suggested that mortality is reduced if the first dose of antibiotics is given within 4 hours of the patient's arrival to the hospital when compared to later administration.[49] These data led to widespread efforts in the USA to provide antibiotics as soon as possible to all CAP patients, with sometimes unintended consequences. While focus on this issue led to more patients receiving antibiotics sooner than before there was a focus on timely antibiotic administration, there was also more use of antibiotics before pneumonia was clearly known to be present, and in some instances patients without pneumonia were treated unnecessarily, occasionally leading to antibiotic-related complications such as *Clostridium difficile* colitis.[50,51]

As discussed, the antibiotic regimens used in North America provide routine therapy for atypical pathogens using either a macrolide or a quinolone, based on data that such an approach reduces mortality, especially in those with severe illness.[22,23] However, even in patients with documented pneumococcal bacteremia, the use of combination therapy (generally with the addition of atypical pathogen coverage to pneumococcal coverage) has been associated with reduced mortality compared to monotherapy.[52] In one study, the benefit of adding a second agent applied to those pneumococcal bacteremia patients who were critically ill, but not to other populations.[53] Rodriguez and colleagues found a benefit to adding a second agent for all patients with severe CAP and shock, and the benefit applied if the agent added was either a macrolide or a quinolone.[54]

For some patients, certain adjunctive therapies should be considered, including oxygen, chest physiotherapy (if at least 30 ml of sputum daily and a poor cough response are present), aerosolized bronchodilators and corticosteroids (if hypotension and possible relative adrenal insufficiency is suspected).

Response to therapy and duration of treatment

The majority of outpatients and inpatients will respond rapidly to empiric therapy, with clinical improvement usually occurring within 24–72 hours. Clinical improvement is measured by following the symptoms of cough, sputum production and dyspnea, along with documenting the ability to take medications by mouth and the presence of an afebrile status on at least two occasions 8 hours apart.[2] When a patient has met these criteria for clinical response, it is appropriate to switch to an oral therapy regimen and to discharge the patient, if he is otherwise medically and socially stable. Radiographic improvement lags behind clinical improvement and in a responding patient a chest radiograph is not necessary until 2–4 weeks after starting therapy.

There are few data about the proper duration of therapy in patients with CAP, especially those with severe illness. Even in the presence of pneumococcal bacteremia, short durations of therapy may be possible, with a rapid switch from intravenous to oral therapy in responding patients. Generally, CAP of unknown etiology and CAP due to *Strep. pneumoniae* can be treated for 5–7 days if the patient is responding rapidly, has been afebrile for 48–72 hours and has received accurate empiric therapy at the correct dose. The presence of extrapulmonary infection (such as meningitis and empyema) and the identification of certain pathogens (such as bacteremic *Staph. aureus* and *P. aeruginosa*) may require longer durations of therapy.[2] Traditionally, identification of *Legionella pneumophila* pneumonia prompted therapy for 14 days, although recent data have shown that quinolone therapy as short as 5 days with levofloxacin 750 mg may be effective.[48]

If the patient fails to respond to therapy in the expected time interval, then it is necessary to consider infection with a drug-resistant or unusual pathogen (tuberculosis, anthrax, *Coxiella burnetii*, *Burkholderia*

pseudomallei, *Pasteurella multocida*, endemic fungi or hantavirus), a pneumonic complication (lung abscess, endocarditis, empyema) or a noninfectious process that mimics pneumonia (bronchiolitis obliterans with organizing pneumonia, hypersensitivity pneumonitis, pulmonary vasculitis, bronchoalveolar cell carcinoma, lymphoma, pulmonary embolus). The evaluation of the nonresponding patient should be individualized but may include CT scanning of the chest, pulmonary angiography, bronchoscopy and occasionally open lung biopsy.

PREVENTION

Prevention of CAP is important for all groups of patients but especially the elderly, who are at risk for both a higher frequency of infection and a more severe course of illness. Appropriate patients should be vaccinated with both pneumococcal and influenza vaccines and cessation of cigarette smoking should be a goal for all at-risk patients. Immunization can be effective even for the patient who is recovering from CAP and hospital-based immunization is an effective and efficient way to promote vaccine utilization.

Pneumococcal vaccine

Pneumococcal capsular polysaccharide vaccine can prevent pneumonia in otherwise healthy populations, as was initially demonstrated in South African gold miners and American military recruits. The benefits in those of advanced age or with underlying conditions in nonepidemic environments are less clearly proven and have been demonstrated in case–control studies rather than in randomized trials. In immunocompetent patients over the age of 65, effectiveness has been estimated to be 75%, while it ranges from 65% to 84% in patients with chronic diseases including diabetes mellitus, coronary artery disease, congestive heart failure, chronic pulmonary disease and anatomic asplenia.[2,55] Its effectiveness has not been proven in immunodeficient populations such as those with sickle cell disease, chronic renal failure, immunoglobulin deficiency, Hodgkin's disease, lymphoma, leukemia and multiple myeloma. A single revaccination is recommended in patients over age 65 who initially received the vaccine more than 5 years earlier and were under age 65 on first vaccination. If the initial vaccination was given at age 65 or older, repeat is only indicated (after 5 years) if the patient has anatomic or functional asplenia or has one of the immunocompromising conditions listed above.

The available pneumococcal vaccine is generally underutilized and the 23-valent pneumococcal vaccine carries the serotypes causing the majority of clinical infection seen in the USA. A protein-conjugated pneumococcal vaccine has been licensed and appears more immunogenic than the older vaccine; however, it contains only seven serotypes and although recommended for healthy children, it has not yet been shown to be effective in adults. Nonetheless, the vaccine has had benefit for adults, even when given only to children, demonstrating a 'herd immunity' effect. More recently, however, children who have received the 7-valent pneumococcal polysaccharide vaccine have developed infection with strains not included in the vaccine, leading to a higher frequency of severe necrotizing pneumonia, especially with serotype 3.[56]

Influenza vaccine

The current vaccine includes three strains: two influenza A strains (H3N2 and H1N1) and one influenza B strain. Vaccination is recommended for all patients over age 65 and for those with chronic medical illness (including nursing home residents) and for those who provide health care to patients at risk for complicated influenza. When the vaccine matches the circulating strain of influenza, it can prevent illness in 70–90% of healthy persons over age 65. For older persons with chronic illness, the efficacy is less, but the vaccine can still attenuate the influenza infection and lead to fewer lower respiratory tract infections and the associated morbidity and mortality that follow influenza.

REFERENCES

References for this chapter can be found online at http://www.expertconsult.com

Chapter 28

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Hospital-acquired pneumonia

EPIDEMIOLOGY

Definition

Hospital-acquired pneumonia (HAP) is defined as pneumonia occurring at least 48 hours after hospital admission, excluding any infection incubating at the time of admission.[1] Ventilator-associated pneumonia (VAP) is a particular subgroup of HAP for which the incidence, etiology, investigation and outcome are somewhat different. VAP is excluded from this chapter and is discussed in Practice Point 16. Recently[1] there has been an increasing awareness of health-care-associated pneumonia (HCAP) as a variant of HAP. Patients at risk for HCAP include:

- those receiving home intravenous antibiotics, home nursing or home wound care;
- residents in a nursing home or long-term care facility;
- those who have been hospitalized for more than 2 days in the past 90 days; and
- those who have received dialysis or intravenous therapy at a hospital-based clinic in the past 30 days.

The importance of recognizing this group is that these patients have an increased risk for multidrug-resistant (MDR) organisms and therefore require management more closely akin to VAP than community-acquired pneumonia.

Incidence and size of the problem

Pneumonia is the second most common infection acquired in hospital after urinary tract infection. In a European multicenter study on 10 000 patients, the prevalence of nosocomial pneumonias was reported at 10%, representing 47% of the infections acquired in the intensive care unit (ICU).[2] There is a decreasing risk of acquiring pneumonia during a hospital stay. For example, the risk of developing VAP is 3% per day until day 5, decreasing to 2% per day until day 10 and 1% at day 15.[3] HAP adds 5–9 days to the hospital stay of survivors and billions of dollars to health-care costs.[4] The incidence varies with the type of hospital and ward and the age of the patient. The incidence is lowest in district hospitals and in general medical and pediatric wards, and higher in teaching hospitals (presumably because of the increased complexity of medical cases) and in patients over 65 years of age.[4]

Mortality rate

The mortality rate of nosocomial pneumonias ranges from 13% to 55% depending on the author.[4] This disparity is in large part linked to the types of patient studied (medical, surgical, traumatic) and to the heterogeneity of the diagnostic criteria employed. The severity of the underlying disease is an essential factor and is often poorly evaluated in studies reporting mortality rates. The cause and effect ratio is difficult to determine. Thus, the studies centered on the prevention of VAP, either by selective digestive contamination or by the choice of gastric protector, reach the conclusion that the number of cases of VAP has diminished but that the mortality is unchanged. Likewise, the occurrence of VAP during acute respiratory distress syndrome does not modify the mortality.[5]

Few studies have been solely dedicated to the prognosis of nosocomial pneumonia. Leu *et al*.[6] reported a retrospective paired study of 74 pairs of patients. The mortality in each group was comparable with a nonsignificant difference: 20% with the presence of nosocomial pneumonia and 14% without nosocomial pneumonia. A study[7] of the mortality of patients with VAP documented by protected specimen brush (PSB) reported mortality in patients presenting VAP that was double that of the control patients (54% vs 27%). An absence of surmortality was noted in another work[8] with a similar methodology with 85 pairs of patients with a diagnosis of VAP also documented by PSB and where the mortality was 41% in both groups. On close examination of the differences between these two studies, one can note that the principal difference is in the pairing of the diagnosis. The study which reported an attributable mortality for VAP[7] was not based on pairing according to the type of surgery or the underlying disease. Very recently, an international study of 2897 patients reported no surmortality linked to the occurrence of VAP.[9] Even in trauma patients with head injuries or in patients presenting a cerebrovascular accident, the occurrence of VAP did not seem to modify the prognosis. On the other hand, it did appear to worsen the prognosis of ventilated patients who presented chronic respiratory failure of an obstructive nature.[10] The role of VAP in lengthening the duration of ventilation and ICU stay is not debated but nevertheless remains difficult to quantify. Finally, the costs generated by the appearance of VAP are very high, ranging from $12 000 to over $40 000 (€8000 to €27 000) per patient.[11]

Overall, the appearance of VAP does not appear to be an independent factor for mortality (except for patients presenting chronic obstructive respiratory failure) but appears to be especially linked to the underlying condition on which the pneumonia occurs and to associated co-morbidities.

PATHOGENESIS AND ETIOLOGIC AGENTS

Pathogenesis

Hospital-acquired pneumonia is usually caused by the aspiration into the lungs of bacteria that colonize the upper respiratory tract. The oropharynx of a debilitated patient becomes colonized rapidly by enteric Gram-negative bacteria (EGNB). These bacteria are not normally present in the upper respiratory tract and the frequency of

colonization increases with the increasing severity of the underlying illness, the use of antibiotics and the duration of hospital stay. Microaspiration of pharyngeal secretions is usually clinically silent and even occurs in healthy subjects during sleep, but becomes very frequent in patients who have reduced consciousness.

In the healthy individual, aspirated secretions can be dealt with effectively by lung defenses, including mucociliary clearance and alveolar macrophages. When host defenses are impaired, bacteria are able to proliferate and cause pneumonia. Colonization of the lower respiratory tract is facilitated by changes in bronchial and alveolar epithelial cells that favor bacterial adherence. HAP is histologically characterized by the existence of infection sources made up of polymorphonuclear neutrophils present in the bronchioles and adjacent alveoli. A grading system of four successive stages based on histologic analysis of the lungs of 83 patients who were invasively ventilated and who died in the ICU has been proposed.[12] Owing to repeated seeding of the lower airways, these HAP lesions are very heterogeneous and can be associated with different lesional stages at the same time. It should be noted that the lesions are predominant in the right lung and in dependent lung segments. Less commonly, other mechanisms may be involved, including the hematogenous spread of infection to the lungs from a distant focus and inhalation of pathogens aerosolized either from contaminated respiratory equipment (e.g. ventilator or nebulizer equipment) or from the hospital environment (e.g. showers and water systems colonized with *Legionella* bacteria).

Causative organisms

The spectrum of potential pathogens associated with HAP differs from that of community-acquired pneumonia. The distribution of micro-organisms responsible for HAP (Table 28.1) is influenced by the existence of prior systemic antibiotherapy, by the type of patient (medical, surgical, or traumatic) and by the existence of co-morbidities.[4,13] The bacterial pathogens most frequently associated with HAP are EGNB and *Staphylococcus aureus*. Mixed infections are not unusual, particularly in VAP. The role of viruses has not been widely studied but is probably significant, not only at times of community outbreaks of viral infection, but also when staff and visitors may transmit viral infection to hospitalized patients. It has been admitted for 20 years that HAP is separated into early-onset pneumonia in patients who develop pneumonia early in their hospital stay (<5 days) and those who develop pneumonia later (≥5 days).[14] In general, the spectrum of causative organisms in patients who develop early pneumonia reflects community isolates – *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Escherichia coli*, nonresistant EGNB, methicillin-sensitive *Staphylococcus aureus* (MSSA) – rather than highly resistant nosocomial organisms. On the other hand, patients who develop late-onset nosocomial pneumonia are more likely to have resistant organisms – *Pseudomonas aeruginosa*, *Acinetobacter* spp., other resistant Enterobacteriaceae, methicillin-resistant *Staphylococcus aureus* (MRSA) and viruses. As for *Candida* spp., it has been clearly demonstrated that there are no sure criteria outside of those found on a lung biopsy[15] and that the positivity of directed or nondirected samples has no value.[15] *Aspergillus* spp. are in question, especially in immunodepressed patients and particularly in oncohematology, and are generally considered as unusual in immunocompetent patients.

Table 28.1 Micro-organisms responsible for hospital-acquired pneumonia[13]

Agent	Organism
Gram-positive cocci	*Staphylococcus aureus* *Streptococcus pneumoniae* Coagulase-negative staphylococcus
Gram-positive bacilli	*Corynebacterium* spp. *Listeria monocytogenes* *Nocardia* spp.
Gram-negative bacilli (lactose positive)	*Haemophilus influenzae* *Escherichia coli* *Klebsiella* spp. *Enterobacter* spp. *Proteus* spp.
Gram-negative bacilli (lactose negative)	*Pseudomonas aeruginosa* *Burkholderia cepacia* *Acinetobacter* spp. *Stenotrophomonas maltophilia*
Gram-negative cocci	*Neisseria* spp. *Moraxella* spp.
Anaerobes (cocci)	*Peptostreptococcus* *Veillonelia*
Anaerobes (bacilli)	*Bacteroides* spp. *Fusobacterium* spp. *Prevotella* spp. *Actinomyces* spp.
Intracellular micro-organisms	*Legionella* spp. *Chlamydia pneumoniae* *Mycoplasma pneumoniae*
Fungi	*Candida* spp. *Aspergillus* spp.
Viruses	Influenza, parainfluenza, adenovirus Respiratory syncitial virus Herpes simplex virus Cytomegalovirus
Other agents	*Pneumocystis jiroveci* *Mycobacterium tuberculosis* *Strongyloides stercoralis*

PREVENTION

General measures

Preferential use of noninvasive ventilation compared with first-intention invasive mechanical ventilation when possible reduces the incidence of nosocomial pneumonia.[16] The use of sedation and weaning protocols with noninvasive ventilation makes it possible to reduce the duration of invasive mechanical ventilation and therefore to reduce the incidence of HAP. Effective infection control measures include staff education, compliance with alcohol-based hand disinfection, and isolation to reduce cross-infection with MDR pathogens.[1]

Oral decontamination, selective digestive decontamination, antibioprophylaxis

Local oropharyngeal decontamination by antibiotherapy in the form of a paste or gel, or by chlorhexhidine[17] appears to be effective in the prevention of HAP. However, a recent multicenter American-European study examined the preventive effect of iseganan (an antimicrobial peptide active against bacteria and fungi) and concluded it was ineffective in the prevention of VAP.[18]

Selective digestive decontamination (SDD), given the coexistence of an oropharyngeal and a gastric colonization source, has long been proposed as a means to prevent VAP. It is generally made up of a combination of three anti-infectious agents with no or low systemic

diffusion (amphotericin B, polymyxin, aminoglycoside), applied on the buccal mucosa and administered in the digestive tract by nasogastric tube associated with short parenteral systemic antibioprophylaxis. It was initially proposed[19] to institute systemic antibioprophylaxis using intravenous cefotaxime until negativity of microbiologic samples is confirmed. The first studies were principally on polytraumatized patients in whom the major risk of inhalation before and after intubation was known, as well as their particular susceptibility to early *Staphylococcus aureus* pneumonia. Numerous studies have reported a reduction in the incidence of VAP but in practice it would appear that this method is little used and not formally recommended in the latest international consensus conferences.[1,20]

Certain problems that are inherent in the use of SDD have been advanced. The aspiration of antibiotics in the lower respiratory tract accounts for a great number of sterile cultures or cultures that are inferior to the recommended thresholds, given the partial but insufficient activity on these pathogens by antibiotics administered orally and the theoretic impact that the extensive use of SDD could have on the ecology of ICUs and the emergence of MDR bacteria. However, recent studies could renew interest in this preventive method. A randomized prospective study on a large population reported a reduction in the incidence of VAP and a reduction in mortality in the group benefiting from SDD with, in addition, a reduction in Gram-negative bacteria,[21] while a randomized study of ICUs showed a decrease in mortality with SDD.[22]

Systemic antibioprophylaxis used alone without local decontamination increases the risk of the development of MDR bacteria and the data vis-à-vis its efficacy in the prevention of VAP are contradictory.[23]

Stress-ulcer prophylaxis

The use of gastric protectors that increase the gastric pH (anti-H_2, antiacids) expose the patient to the risk of gastric microbial development. Although the results in the literature are contradictory, sucralfate appears to present several advantages:

- it does not modify the gastric pH;
- it presents proper antibacterial efficacy;
- it does not require manipulation of perfusion lines for its administration; and
- it is much less expensive than other gastric protectors.

Nevertheless, the largest randomized study comparing sucralfate with ranitidine reported that the latter is more effective in the prevention of gastric bleeding and, in addition, did not increase the incidence of VAP.[24] Finally, a recent observational study has shown the futility of systematically prescribing a gastric protector,[25] so it would be better to reserve it for patients at risk. The latest consensus conference went in the same direction by reserving gastric protection only for patients at risk and preferably with anti-H_2.[20]

Enteral nutrition

Enteral nutrition is preferred over parenteral nutrition to reduce the risk of complications related to central intravenous catheters and to prevent reflux villous atrophy of the intestinal mucosa which could increase the risk of bacterial translocation. However, its use increases the risk of VAP.

Antibiotic policy

It would appear to be logical, although unproven, that the controlled use of antibiotics in the ICU, following protocols based on established and recognized recommendations, could in part prevent the appearance of MDR infections.[26] Monitoring of ICU infections in order to identify and quantify endemic and new MDR pathogens as well as the preparation of timely data for infection control to guide appropriate antimicrobial therapy in patients with suspected HAP or other nosocomial infections are recommended.

Respiratory physiotherapy

One study[27] has reported that respiratory physiotherapy appears to be independently associated with a reduction in the risk of VAP but a randomized study is required to confirm it.

Patient posture

The semirecumbent position makes it possible to limit inhalation in patients receiving enteral nutrition. The only randomized study evaluating the role of the position of the patient on the development of VAP has shown the protective effect of the semirecumbent position at 45°.[28] It should be noted that no benefit in terms of mortality was demonstrated between the two groups.

In all, the principal measurement and the only one currently recommended is a positioning of patients in the semirecumbent position between 30° and 45°,[1,20,28] especially when enteral nutrition is administered.

CLINICAL FEATURES AND DIFFERENTIAL DIAGNOSIS

The diagnosis of pneumonia may be obvious in a hospitalized patient not presenting a respiratory illness on admission and who develops the classic symptoms of fever, malaise, cough, purulent sputum, localized chest signs and consolidation on the chest radiograph. All too often, however, the situation is less straightforward, with numerous differential diagnoses. Hospital-acquired pneumonia should finally be considered in the context of an illness developing after hospital admission and characterized by fever, leukocytosis, purulent sputum or tracheobronchial secretions and new or persisting infiltrates on the chest radiograph. However, the accuracy of a clinical diagnosis is poor compared with a microbiologic or pathologic diagnosis.

DIAGNOSIS

General investigations (hematology, immunology and radiology)

Patients with suspected HAP should have a full blood count. Neutrophilia may point to infection. Biochemical tests are often indicated to assess the impact of pneumonia on the underlying condition and to assess renal and hepatic function. Oxygenation should be assessed by pulse oximetry or arterial blood gas estimation. The chest radiograph will show new or worsening lung shadowing, although it is not usually diagnostic of infection. Cavitation is suggestive of infection, particularly by EGNB, anaerobes or fungi. Multiple studies of biologic markers of infection have attempted to find a noninvasive, rapid and accurate means of determining who requires antibiotics for presumed HAP. Unfortunately, the results have largely been disappointing (procalcitonin, for example). More recently, measurement of a soluble triggering receptor expressed on myeloid cells (sTREM-1) that is upregulated in the setting of infection has been shown to have potential to improve our ability to accurately diagnose pneumonia. When compared with post hoc physician consensus, measurement of sTREM-1 by mini bronchial alveolar lavage (BAL) was shown to be 98% sensitive and 90% specific for the diagnosis of pneumonia in mechanically ventilated patients.[29] Although promising, additional studies are required before this test can be widely recommended.

General microbiologic investigations

Blood cultures should always be obtained. A positive culture identifies the pathogen and is associated with a worse prognosis. However, only about 8–20% of blood cultures from patients who have HAP

are positive, indicating a low sensitivity.[1] Sources of the bacteremia, other than the lung, should always be considered. Pleural fluid should be sampled to identify impending empyema. Serologic tests for viral and atypical pathogens can be of some help. *Legionella* urinary antigen detection is a rapid, sensitive and specific test for nosocomial infection by *Legionella pneumophila* serogroup 1.

Special investigations and techniques to obtain lower respiratory tract samples

Ideally, there should be a widely available and accepted technique to obtain and culture uncontaminated secretions from the site of a lung infection that is simple, safe and inexpensive to perform, the results of which would differentiate infection from colonization and improve outcome. Although the past 25 years have seen a rapid expansion in the use of different techniques for sampling in HAP, none has fulfilled the above criteria and controversy continues among experts regarding the role of invasive and noninvasive investigations of HAP and VAP. The techniques can be broadly divided into noninvasive and invasive groups.

Noninvasive techniques

Expectorated sputum

The problems of sputum collection are well known and include contamination of the specimen by upper respiratory tract flora, making it unrepresentative of lower respiratory tract secretions. This is a particular problem in the nosocomial setting, when EGNB commonly colonize the upper respiratory tract and pathogens can be isolated with equal frequency in patients who have and do not have pneumonia. Only one-third to one-half of sputum cultures provide reliable information compared with blood cultures, transtracheal aspirates and PSB samples. Interest in tracheal aspiration in intubated patients with nonquantitative culture could be revived given the results of a recent North American multicenter study.[30] This study compared 741 patients with suspected VAP in a randomized and prospective manner, using quantitative BAL culture with nonquantitative tracheal aspirate culture, and demonstrated that mortality and targeted use of antibiotics were not different whatever the diagnostic strategy used. However, patients known to be colonized or infected with *Pseudomonas* spp. or MRSA were not included in this trial.

Bronchoscopic invasive techniques

Much has been published about the value of invasive techniques for managing HAP. However, the questions vis-à-vis in whom, when and how to perform these tests and the reliability of the results remain unresolved, as does the applicability of published results to everyday clinical situations. Fiberoptic bronchoscopy provides direct visual access to the lower airways. Bronchoscopic techniques are relatively simple to carry out in patients on mechanical ventilation. Invasive techniques could be considered in nonventilated patients who have moderate or severe HAP before antibiotic therapy is administered. In practice, immediate access to bronchoscopic techniques may be limited and the procedure itself could occasionally precipitate cardiorespiratory failure in a spontaneously breathing but hypoxic patient, necessitating ventilatory support earlier than anticipated.

Protected specimen brush

Protected specimen brush (PSB) consists of brushing the distal bronchial mucosa with a precision that requires the use of an endoscope. The low volume of secretions harvested (approximately 1 µl) explains a certain number of false-negatives as well as the difficulty of performing a direct examination and culture on the same brush. This technique, developed *in vitro* by Wimberley, was validated in ventilated patients by Chastre *et al*.[31] who studied the efficacy of this device in 26 ventilated patients who died in the ICU. Human histologic studies[32–34] have reported sensitivity of PSB ranging between 33% and 57%. The accepted threshold of 10^3 cfu/ml can explain a certain number of false-negatives. Repeating PSB in cases of a negative result associated with an evocative radiologic-clinical picture has been proposed.[35] In fact, when the first PSB was borderline ($\geq 10^2$ and $<10^3$ cfu/ml), a subsequent PSB turned out to be positive at a threshold of 10^3 cfu/ml in 35% of the cases. This could be attributed to the heterogeneity of the pneumonia lesions and the low volume of secretions harvested. Finally, beginning at the 12th hour after administration of an effective antibiotic, the PSB was negative in almost one-third of the cases,[36] justifying a new sample collection before administration of any new antibiotherapy.

Bronchoalveolar lavage

For BAL, the bronchoscope is wedged into a subsegmental airway and the bronchoalveolar area is lavaged. Volumes of over 100 ml sterile normal saline are required to reach the distal alveoli. The advantage of this technique is that a large part of the lung is sampled and the specimen allows microscopic analysis to assess the presence of intracellular bacteria, neutrophils and cytologic evaluation. This can be particularly useful if the diagnosis of pneumonia is in doubt. Contamination of the BAL fluid in the bronchoscopic channel is one drawback and careful technique is required to avoid this. After wedging the tip of the bronchoscope in the relevant subsegment, 50 ml normal saline is instilled. This aspirate samples airways rather than the alveoli, is best referred to as a 'bronchial wash' and is more likely to be contaminated with bacteria present in the suction channel. This sample should therefore be processed separately from the second, third and, if necessary, fourth aliquots of 50 ml, which should be pooled. The sensitivity and specificity of quantitative BAL cultures have ranged from 42% to 100% and 69% to 100%, respectively, using threshold concentrations of at least 10^4 cfu/ml. When compared with PSB performed at the same time, BAL has greater sensitivity but a marginally reduced specificity.

Percutaneous invasive techniques

Percutaneous lung needle aspirate

Percutaneous lung needle aspirate (PLNA) has been used successfully to investigate nonventilated patients who have lung shadowing. The technique can be performed at the bedside with minimal equipment. The use of a 25-gauge ultrathin needle reduces the chance of pneumothorax. The sensitivity of PLNA in HAP is lower than its specificity and the pneumothorax complication rate is lower than 5% in spontaneously breathing patients. When compared with histologic diagnosis of pneumonia on lung biopsy immediately postmortem as a 'gold standard', PLNA had the lowest sensitivity but the highest specificity of the invasive techniques discussed.

Transtracheal aspiration

Transtracheal aspiration is now rarely used due to the ready availability of fiberoptic bronchoscopy. However, its value has been demonstrated for the investigation of patients who have suspected HAP in general medical and surgical wards.

Influence of quantitative cultures on the management of HAP

The literature supports the view that PSB, BAL and tracheal aspirate specimens cultured quantitatively can provide useful and reliable information about the likely presence and cause of HAP in patients who have the clinical features of pneumonia, particularly in the ICU. Their value is considerably diminished if the patient is already receiving antibiotics, which is the usual situation. There is controversy as to whether these tests improve patient management and outcome in routine clinical practice. Randomized studies have shown that invasive

techniques lead to more antibiotic changes and increase costs, but do not affect mortality or morbidity.[37–39] Only one randomized study, from France, has shown a bronchoscopic strategy, including quantitative cultures of PSB and BAL specimens, to be superior to a clinical strategy using qualitative tracheobronchial aspirates in terms of 2-week mortality rate and overall morbidity.[40]

TREATMENT

Guidelines for empiric antibiotic therapy

The treatment of HAP is influenced by the availability of new anti-infectious agents, the development of micro-organism resistance and the willingness of clinicians to use therapies that have a reasonable cost-efficacy ratio. Since most cases of HAP are due to bacteria, empiric therapy or (even better) empiric antibiotherapy, oriented by the underlying condition, local microbiologic ecology and direct examination or the culture of routine tracheal aspirates, should be administered as soon as there are sufficient arguments to suspect a pneumonia. The selection of appropriate antibiotics for the initial management of HAP on the basis of time of onset of disease and risk for MDR pathogens as suggested by the American Thoracic Society/Infectious Diseases Society of America (ATS/IDSA) Consensus Conference is outlined in Tables 28.2 and 28.3. The key decision in initial empiric therapy is whether the patient has risk factors for MDR organisms. The adequate dosing of antibiotics for empiric therapy for MDR pathogens is summarized in Table 28.4. Ideally, this antibiotherapy must be administered after the performance of a series of hemocultures and respiratory samples (tracheal aspiration, PSB, BAL, etc.).[20] The recommended administration route is intravenous and, once the response to treatment is satisfactory, relayed by the oral route as soon as possible. Tracheal instillations or antibiotic aerosols are not recommended as a cure since the data in the literature are insufficient.[20] The latest ATS/IDSA Consensus Conference[1] proposed aerosols as a complementary treatment in cases of MDR Gram-negative bacilli if the systemic antibiotherapy appears to be insufficient.

Several observational studies have shown that administration of inappropriate initial antibiotherapy in patients with suspected pneumonia was associated with increased mortality.[41,42] This is not decreased by secondary adjustment of treatment after obtaining the results of microbiologic cultures.[41] The time period before antibiotherapy is administered is also important to consider. A period of 24 hours or more after the criteria for HAP have been collected is associated with increases in hospital mortality, duration of stay and cost.[43] The choice between mono- or bi-therapy has been discussed in only a few studies. The latest ATS/IDSA Consensus Conference[1] proposed a combination of antibiotics in cases of a multidrug-resistant pathogen and monotherapy, an approach mirrored by the British Society of Antimicrobial Chemotherapy.[44]

Finally, the choice of molecules is based on the suspected pathogen(s) depending on the underlying disease, the local microbiology, direct examination or cultures obtained in a systematic manner. After starting treatment, it is necessary to perform a careful clinical evaluation of the response to the treatment. Rapid deterioration or the absence of improvement after 72 hours of empiric treatment requires systematic re-evaluation. It should be noted that the absence

Table 28.2 Initial empiric antibiotic therapy for HAP in patients with early onset HAP and no known risk factors for multidrug-resistant pathogens

Potential pathogen	Recommended antibiotic
Streptococcus pneumoniae *Haemophilus influenzae* Methicillin-sensitive *Staphylococcus aureus* Antibiotic-sensitive enteric Gram-negative bacilli • *Escherichia coli* • *Klebsiella pneumoniae* • *Proteus* spp. • *Serratia marcescens*	Ceftriaxone or Levofloxacin, moxifloxacin or ciprofloxacin or Ampicillin-sulbactam or Ertapenem

Table 28.3 Initial empiric therapy for HAP in patients with late-onset disease or risk factors for multidrug-resistant pathogens

Potential pathogen	Recommended antibiotic
Pathogens listed in Table 28.1 and multidrug-resistant organisms	Antipseudomonal cephalosporin (ceftazidime, cefepime) or
Pseudomonas aeruginosa	Antipseudomonal carbapenem (imipenem, meropenem, doripenem)
Klebsiella pneumoniae (EBSL$^+$)*	or
Acinetobacter spp.	β-Lactam/β-lactamase inhibitor (piperacillin-tazobactam) plus
Methicillin-resistant *Staphylococcus aureus*†	Antipseudomonal fluoroquinolone (ciprofloxacin, levofloxacin)* or Aminoglycoside (gentamicin, tobramycin, amikacin) plus Vancomycin or linezolid

*If an ESBL$^+$ strain, such as *K. pneumoniae*, or an *Acinetobacter* spp. is suspected, a carbapenem is a reliable choice. If *L. pneumophila* is suspected, the combination antibiotic regimen should include a macrolide (e.g. azithromycin) or a fluoroquinolone (e.g. ciprofloxacin or levofloxacin) should be used rather than an aminoglycoside.
†If MRSA risk factors are present or there is a high incidence locally.

Table 28.4 Initial intravenous adult doses of antibiotics for empiric therapy of HAP in patients with late-onset disease or risk factors for multidrug-resistant pathogens

Antibiotic	Dosage
Ceftazidime	2 g q8h
Cefepime	1–2 g q8–12h
Imipenem	500 mg q6h or 1 g q8h
Meropenem	1 g q8h
Piperacillin–tazobactam	4.5 g q6h
Gentamicin	7 mg/kg q24h
Tobramycin	7 mg/kg q24h
Amikacin	20 mg/kg q24h
Levofloxacin	750 mg q24h
Ciprofloxacin	400 mg q8h
Vancomycin	15 mg/kg q12h
Linezolid	600 mg q12h

Table 28.5 Risk factors for multidrug-resistant pathogens causing HAP

- Antimicrobial therapy in previous 90 days
- Current hospitalization of 5 days or more
- High frequency of antibiotic resistance in the community or in the specific hospital unit
- Hospitalization for 2 days or more in the previous 90 days
- Residence in a nursing home or extended care facility
- Home infusion therapy (including antibiotics)
- Chronic dialysis within 30 days
- Home wound care
- Family member with multidrug-resistant pathogen
- Immunosuppressive disease and/or therapy

of a response to empiric treatment can be linked to a nonbacterial cause of infection (in particular viral) or to an extrapulmonary infection, or to the absence of an infectious problem requiring aggressive diagnostic measures which can go as far as a lung biopsy. In cases of favorable evolution, de-escalation of antibiotherapy is indispensable once the antibiogram is known. It is also necessary to know when to stop antibiotherapy that is useless owing to negative microbiologic samples (performed before the beginning of all antibiotherapy) if the patient is in a satisfactory clinical condition. Singh *et al.*[45] demonstrated that discontinuing antibiotics after 3 days of intubation in patients with a clinical pulmonary infection score <6 caused antibiotic usage to be reduced without compromising patient mortality rates. A French multicenter study has reported an equivalence in terms of efficacy between short antibiotherapy (8 days) and long antibiotherapy (15 days).[4] Antibiotherapy should be prolonged only in cases of *Pseudomonas aeruginosa* and bi-therapy maintained for at least the first 5 days. Antibiotic selection for each patient should be based on the risk factors for MDR pathogens summarized in Table 28.5.

REFERENCES



References for this chapter can be found online at http://www.expertconsult.com

Chapter 29

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Lung abscesses and pleural abscesses

INTRODUCTION

Lower respiratory tract infections (LRTIs) are a major indication for antimicrobial therapy in developed countries. Although many LRTIs are self-limiting, those caused by necrotizing organisms are invariably serious; they may lead to abscess formation in the lung and can spread to the pleural space.

EPIDEMIOLOGY

The etiologies of lung abscess and pleural abscess, or empyema, vary in different parts of the world. The common denominator is usually aspiration pneumonia, acquired either in the community or in the hospital. Nosocomial or health-care-associated pneumonia is a major cause of morbidity and lengthened hospital stay, with enormous economic impact. Aspiration pneumonia leading to a necrotizing pneumonia or lung abscess, with or without empyema, is a continuum; any stage or all stages may be encountered. Underlying diseases, associated trauma or surgery and the timeliness of appropriate therapy are the major factors in determining the clinical presentation and prognosis.

Lung abscess

A lung abscess is arbitrarily defined as a localized area of pulmonary necrosis caused by infection, with a solitary or dominant cavity measuring at least 2 cm in diameter. When cavities are multiple and smaller than 2 cm, the infection is usually referred to as a necrotizing pneumonia.[1,2] Most abscesses are suppurative bacterial infections caused by aspiration.

Primary lung abscesses typically present in patients who have no predisposing disease other than a predilection to aspirate oral secretions; they are more common in males than in females. Secondary lung abscesses occur in patients who have an underlying condition such as a partial bronchial obstruction or lung infarct, or in those who are otherwise immunocompromised because of chemotherapy, malignancy, organ transplantation or HIV infection. Lung abscesses may be termed nonspecific or putrid, referring, respectively, to the often unclear etiology and the offensive odor of the sputum.[3]

Over the past five decades, the incidence of bacterial lung abscess in the USA has diminished considerably and the mortality rate has decreased from 30–40% to 5–10%. Factors associated with a worse prognosis include advanced age, prolonged symptoms, concomitant disease, nosocomial infection and (according to some studies) larger cavity size. In the past, tuberculosis was responsible for a higher proportion of lung abscesses. In recent years, more lung abscesses have been associated with pulmonary malignancies or other underlying conditions.[4–6]

Empyema

A pleural effusion associated with pneumonia, lung abscess or bronchiectasis is referred to as a parapneumonic effusion. These occur in up to 40% of people who have bacterial pneumonia; they are the most common cause of exudative pleural effusions in the USA.[7] Empyema, or pleural pus, is an infected parapneumonic effusion with characteristic changes in the composition of the pleural fluid. It has been declining in frequency and changing in etiology.[8] Mortality ranges from approximately 2% to 50%, with the lowest rates in young, healthy people and the highest rates in the elderly and immunocompromised. The prognosis is poorer when pathogens are resistant to antimicrobial drugs or when appropriate treatment is delayed.[7,9,10]

PATHOGENESIS AND PATHOLOGY

Micro-organisms gain access to the lower respiratory tract by a variety of routes, including inhalation of aerosolized particles, aspiration of oropharyngeal secretions and hematogenous spread from distant sites (Fig. 29.1). Less frequently, infection occurs by direct extension from a contiguous site. Lung abscess is caused only by organisms that cause necrosis, but empyema can result from infection by any pathogen that reaches the pleural space.

Lung abscess

Of the inhaled respiratory pathogens, only the mycobacteria (see Chapter 30) and the dimorphic fungi (see Chapter 31) commonly cause lung abscesses. Bacterial abscesses are usually caused by aspiration of oropharyngeal secretions or, occasionally, by hematogenous seeding.[1]

Aspiration of small quantities of oropharyngeal secretions occurs intermittently in everyone, particularly during sleep. Despite the frequency of aspiration, the airways below the level of the larynx are normally sterile. Highly efficient clearing mechanisms are in place; these include cough, a mucociliary system that carries particles cephalad to be swallowed, phagocytosis by alveolar macrophages and neutrophils aided by opsonizing antibodies and complement, and lymphatic trapping with sequestration in regional lymph nodes. Risk factors for pneumonia after aspiration include conditions that increase the inoculum of pathogenic organisms in aspirated secretions, conditions that increase the likelihood of aspiration and conditions that increase the volume of the aspirate (Table 29.1). Under these circumstances, aspirated oropharyngeal secretions are more likely to cause chemical irritation and infection. When an anaerobic pleuropulmonary infection occurs in an edentulous patient, the diagnosis of bronchogenic carcinoma should be considered.[1–3,11]

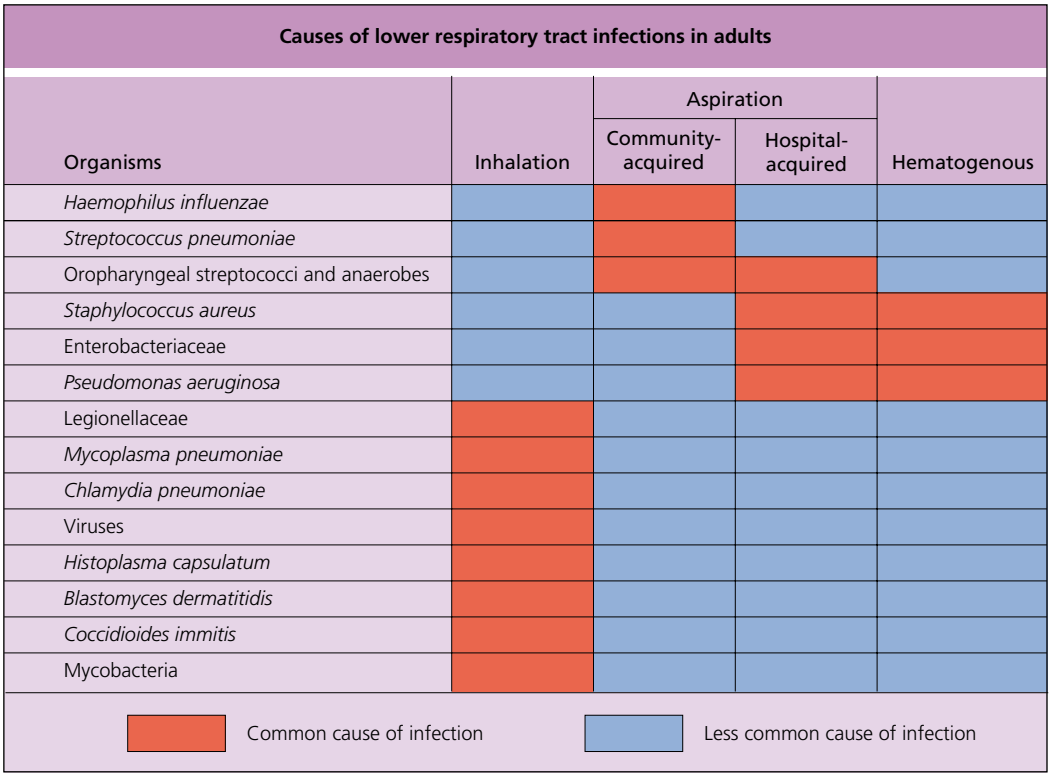


Fig. 29.1 Causes of LRTIs in adults. Oropharyngeal streptococci and anaerobes, *Staphylococcus aureus*, Enterobacteriaceae, *Pseudomonas aeruginosa*, the dimorphic fungi (*Histoplasma capsulatum*, *Blastomyces dermatitidis*, *Coccidioides immitis*) and mycobacteria frequently cause necrosis and subsequent abscess formation.

Table 29.1 Risk factors for aspiration pneumonia and lung abscess

Increased bacterial inoculum	Periodontal disease, gingivitis, tonsillar or dental abscess, drugs that decrease gastric acidity
Impairment of consciousness	Drugs, alcohol, general anesthesia, metabolic encephalopathy, coma, shock, cerebrovascular accident, cardiopulmonary arrest, seizures, surgery, trauma
Impaired cough and gag reflexes	Vocal cord paralysis, intratracheal anesthesia, endotracheal tube, tracheostomy, myopathy, myelopathy, other neurologic disorders
Impairment of esophageal function	Diverticula, achalasia, strictures, disorders of gastrointestinal motility, neoplasm, tracheoesophageal fistula, pseudobulbar palsy
Emesis	Nasogastric tube, gastric dilatation, ileus, intestinal obstruction

The composition of the oropharyngeal flora at the time of aspiration determines the potential etiologic agents for LRTIs. Those organisms that are most numerous or virulent proliferate and emerge as single or predominant pathogens. Although the classic non-necrotizing respiratory pathogens *Streptococcus pneumoniae* and *Haemophilus influenzae* can cause disease by this mechanism, normal oropharyngeal secretions contain many more streptococci of various species and more anaerobes (approximately 10^8 organisms/ml) than aerobes (approximately 10^7 organisms/ml). Some of the streptococcal species are microaerophilic (i.e. they require supplemental carbon dioxide to grow on artificial media).[12,13] The pneumonia that follows aspiration, with or without abscess formation, is typically polymicrobial with between two and four bacterial species present in large numbers. In general, 50% or more of these infections are caused by purely anaerobic bacteria, 25% are caused by mixed aerobes and anaerobes, and 25% or fewer are caused by aerobes only. Among hospitalized patients, progressive colonization with *Staphylococcus aureus*, Enterobacteriaceae and *Pseudomonas aeruginosa* occurs, and these aerobic organisms are frequent causes of health-care-associated aspiration pneumonia and lung abscess.[8,12,14,15] A retrospective evaluation of 90 adults with lung abscesses in Taiwan revealed that Gram-negative bacilli caused 36% of cases, whereas anaerobes accounted for 34% of cases. Twenty-five percent of the patients in this study had received antibiotics prior to culture, which could have made recovery of anaerobes even less likely.[16]

The anaerobic organisms that are associated with pleuropulmonary infection, using current nomenclature,[17,18] are shown in Table 29.2. The primary pathogens are *Streptococcus* spp., *Peptostreptococcus* spp. (now divided into four genera – *Anaerococcus, Finegoldia, Gallicola* and *Peptoniphilus*), *Fusobacterium nucleatum* and *Prevotella* spp. Additionally, *Porphyromonas* spp. are commonly associated with periodontal disease and may also be isolated. Although not consistently part of the normal oropharyngeal flora, members of the *Bacteroides fragilis* group of organisms are isolated from approximately 15% of patients.[2,11–14,17]

A variety of virulence factors associated with oropharyngeal streptococci and anaerobes have been identified. Properties that facilitate attachment include capsular polysaccharides, fimbriae, hemagglutinin and lectin. Tissue breakdown and the metabolic activity of organisms provide reducing substances and a low redox potential; these factors facilitate bacterial proliferation. Volatile fatty acids, sulfur compounds, indoles, amines and hydrolytic enzymes (hyaluronidase, chondroitin sulfatase and heparinase) produced by damaged tissue lead to subsequent abscess formation.[19]

Table 29.2 Anaerobic bacteria associated with pleuropulmonary infections

	Gram-negative bacteria	Gram-positive bacteria
Bacilli	*Bacteroides fragilis* group *Fusobacterium nucleatum* *Fusobacterium necrophorum* *Porphyromonas* spp.* *Prevotella* spp.	*Actinomyces* spp. *Bifidobacterium* spp. *Clostridium* spp. *Eubacterium* spp. *Lactobacillus* spp. *Propionibacterium* spp.
Cocci	*Veillonella* spp.	*Gemella morbillorum* *Peptostreptococcus* spp. *Streptococcus* spp.

*Note: *Porphyromonas* spp. include organisms previously named *Bacteroides melaninogenicus* subsp. *asaccharolyticus*, *B. endodontalis* and *B. gingivalis*. *Prevotella* spp. include organisms previously named *B. melaninogenicus* subspp. *melaninogenicus* and *intermedius*, *B. oralis* and *B. denticola*. *Gemella morbillorum* was previously named *Streptococcus morbillorum*. *Peptostreptococcus* spp. are now divided into four genera – *Anaerococcus*, *Finegoldia*, *Gallicola* and *Peptoniphilus*.

The pathology of aspiration pneumonia is characterized by alveolar edema and infiltration with inflammatory cells. Foci of aspiration pneumonia most commonly develop in the subpleural regions of the gravity-dependent segments of the lungs, particularly the superior segments of the lower lobes and the posterior segments of the upper lobes. The right lung is the more frequent location, presumably because of the less acute angle in the take-off of the right main stem bronchus. In general, the right upper and lower lobes are most commonly involved, followed by the left lower lobe and right middle lobe.[1,3,20,21]

The degree and rate of progression of aspiration pneumonia vary considerably. These infections may be acute, subacute or chronic, depending on differences in the etiology, size of inoculum and host factors. If the process is indolent, fibrosis limits the spread of infection. Abscesses typically communicate with a bronchus, producing the familiar air-filled cavity, often with an air–fluid level that can be seen on radiographs. These are usually not apparent until the infection has been present for 1–2 weeks, when multiple adjacent microscopic abscesses filled with necrotic material (pulmonary gangrene) slough to form a gross cavity (Fig. 29.2).[4]

Although less common than aspiration, hematogenous seeding of the lung, or septic emboli, may also result in lung abscesses or necrotizing pneumonia. There may be a solitary infiltrate or cavity or, more often, multiple bilateral lesions. The most common etiologic agents are the health-care-associated pathogens *Staph. aureus* and aerobic Gram-negative bacilli (see Fig. 29.1). When the primary focus is in the abdomen, anaerobes, particularly *B. fragilis*, may be present.

Infective endocarditis (predominantly right-sided), intravenous drug injection and indwelling right atrial catheters placed for vascular access are commonly associated with septic pulmonary emboli. Any organism that is part of the skin flora or contaminants in injected material may be responsible. *Staph. aureus* and streptococci are the most common pathogens, but *P. aeruginosa* and *Candida* spp. may also be responsible, causing serious infections.[22]

Some uncommon causes of lung abscess should be considered in appropriate circumstances. Inhaled micro-organisms such as *Legionella* spp., *Chlamydia* spp., *Mycoplasma pneumoniae* and viruses are rare causes of lung abscesses. *Cryptococcus*, *Aspergillus* and *Rhizopus* spp. occasionally cause disease in normal hosts but are more commonly opportunistic pathogens. Patients who have advanced HIV disease may have cavitary lesions caused by atypical mycobacteria, particularly *Mycobacterium kansasii*, and other organisms such as *Rhodococcus equi* and *Nocardia asteroides*.[23] *Burkholderia pseudomallei* is endemic to South East Asia, particularly Thailand, and typically causes upper lobe cavities. In endemic areas, the parasites *Paragonimus westermani* and *Entamoeba histolytica* may cause abscess by contiguous extension.

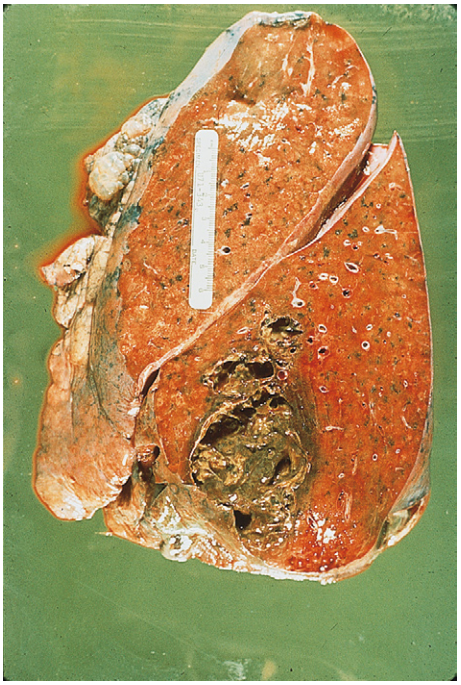


Fig. 29.2 Cross-section of a lung abscess.

Empyema

The pleural space is normally sterile. It is most commonly contaminated by direct extension from a contiguous focus of infection, usually pulmonary, or by direct inoculation at the time of trauma or surgery (Table 29.3).[7,24,25] The pleural space may also become involved through hematogenous seeding from a distant focus of infection, particularly in the presence of hemothorax or pleural malignancy.

The initial stage in the pathogenesis of empyema associated with pneumonia is the development of a sterile parapneumonic effusion, which has varying characteristics depending on its stage of evolution (Table 29.4).[7,25] The effusion is initially transudative but rapidly becomes exudative with an influx of leukocytes and increasing permeability of the visceral pleura. Neutrophils, lactate dehydrogenase

Table 29.3 Causes of empyema

Pulmonary infection	Pneumonia Lung abscess Bronchiectasis
Mediastinal disease	Tracheal fistula Esophageal perforation
Subdiaphragmatic infection	Subphrenic abscess Hepatic abscess
Skeletal infection	Paravertebral abscess Vertebral osteomyelitis
Direct inoculation	Trauma Thoracentesis
Postoperative	Hemothorax (infected) Pneumothorax (infected) Bronchopleural fistula

Table 29.4 Characteristics* of pleural fluid associated with bacterial LRTI

		EXUDATE		
Pleural fluid characteristic	**Transudate**	**Uncomplicated parapneumonic effusion**	**Complicated parapneumonic effusion**	**Empyema**
Appearance	Clear	Variable	Variable	Pus
White blood cell count (cells/ml)	<1000	Variable	Variable	>15 000
Differential cell count	Variable	Neutrophils	Neutrophils	Neutrophils
Protein (g/dl)	<3.0	>3.0	>3.0	>3.0
Glucose (mg/dl)	Same as serum	>60	40–60	<40
pH	Greater than serum	>7.2	7.0–7.2	<7.0
Lactate dehydrogenase (units/ml)	<200	<1000	>1000	>1000
Bacteria	Absent	Absent	Absent	Absent

*The values may overlap. Complicated pleural effusions are those with fluid characteristics that indicate the potential need for tube drainage. When the glucose is 40–60 mg/dl or the pH is 7.0–7.2, repeat thoracentesis may be helpful. When the glucose is <40 mg/dl or the pH is less than 7.0, a chest tube is indicated even if bacteria are not present by smear or culture.
Data from Light[7] and Sokolowski *et al*.[26]

(LDH) and protein increase, and glucose and pH decrease. Fibrin is deposited on the pleural surfaces and loculations may occur. With time, a final organizing stage occurs in which pleural fibroblasts produce an inelastic membrane or pleural peel that encases the lung and restricts inflation. Invasion with bacteria accelerates the fibropurulent reaction. Empyema fluid is relatively deficient in opsonins and complement and it becomes progressively more acidic as the infection ensues. Occasionally, an empyema may spontaneously drain through necrotic lung tissue into a bronchus (bronchopleural fistula) or communicate through the chest wall (empyema necessitans).[7,10,25]

The various respiratory pathogens have different propensities to cause empyema. *Streptococcus pneumoniae*, the most common cause of pneumonia, is frequently associated with a parapneumonic pleural effusion, yet pneumococcal empyema is relatively uncommon. Aspiration pneumonia more frequently progresses to empyema.[7] Empyema is also more common with organisms such as *Staph. aureus* and *P. aeruginosa* that produce potent extracellular enzymes that breach the integrity of the pleura, allowing penetration to the pleural space.

Overall, the relative frequencies of various organisms causing empyema have changed over time. Prior to the antibiotic era, most empyemas were caused by *Strep. pneumoniae* and, to a lesser extent, by *Staph. aureus* and *Strep. pyogenes*. Between 1955 and 1965, penicillin-resistant *Staph. aureus* was the predominant pathogen.[7,27] In the early 1970s, coinciding with a surge of interest in anaerobic infections, anaerobic empyemas were recognized more frequently (Table 29.5).[28,29] At that time, about 50% of empyemas were caused by aerobes, with Gram-positive cocci being more

Table 29.5 Frequency of organisms isolated from bacterial empyemas

	% OF ISOLATES		
Organism	**1971–1973[28] (n=214)**	**1969–1978[9] (n=93)**	**1973–1985[29] (n=343)**
Haemophilus influenzae	<1	0	3
Streptococcus pneumoniae	2	7	20
Other streptococci (including microaerophiles)	13	22	8
Staphylococcus aureus	8	8	17
Enterobacteriaceae	9	10	11
Pseudomonas spp.	5	9	3
Other aerobes	5	2	2
Bacteroides spp.	11	14	8
Anaerobic cocci	15	9	10
Fusobacterium spp.	7	8	6
Prevotella spp.	6	4	6
Other anaerobes	19	7	6

common than Gram-negative bacilli. About 25% were caused by anaerobes and about 25% were mixed aerobic-anaerobic infections. *Streptococcus pneumoniae* was most frequent in young ambulatory patients, anaerobes were most frequent after aspiration, and *Staph. aureus* and aerobic Gram-negative bacilli were most frequent after thoracotomy.[9,12,30,31] Because one-quarter of all empyemas are now associated with trauma or surgery, there has been a relative increase in the proportion of staphylococcal infections and a decrease in anaerobic infections.[7] With the widespread use of *H. influenzae* type b vaccination, empyema due to this organism, previously common in children, has become rare.

PREVENTION

Minimizing the risks of aspiration in people who are unconscious, undergoing anesthesia or subject to seizures will reduce the incidence of pneumonia with subsequent abscess formation or empyema. If pneumonia does occur, the timely administration of appropriate antibiotics reduces the likelihood of progression. Pleural effusions should be aspirated for diagnosis and drained, if indicated, to abort progressive suppurative complications.

CLINICAL FEATURES

Lung abscess

Aspiration is usually subtle and unrecognized but may lead to pneumonia and lung abscess. If overt, it may be followed by symptoms and signs such as choking, cough, wheezing, cyanosis or asphyxia, which are related to the particulate, liquid and chemical nature of the material aspirated. Within hours, there may be fever, tachypnea, diffuse rales and hypoxemia.[11,31] If pneumonia develops, patients usually present within 1 week with a productive cough, a temperature over 102°F (38.9°C) and a leukocyte count of more than 15 000 cells/mm^3.[31] Aspiration pneumonia is not readily distinguishable from pneumococcal pneumonia, although true rigors are uncommon in aspiration pneumonia and symptoms have usually been present for longer before presentation to medical care. A condition predisposing to aspiration is also more likely to be present. Among community-acquired cases, common conditions that predispose to aspiration are alcoholism, seizures and drug overdose. Among health-care-acquired cases, patients tend to have neurologic disorders, such as cerebrovascular accidents and brain tumors, or metabolic disorders that result in stupor or coma. Only a minority of patients who have aspiration pneumonia have putrid sputum, which, if present, tends to develop 1–2 weeks into the course when an abscess has formed.[8,12,14,31]

Lung abscesses may also present in a more indolent fashion with weeks to months of productive cough, malaise, weight loss, low-grade fever, night sweats, leukocytosis and anemia. The patient may become debilitated as if with tuberculosis. Findings that are suggestive of a suppurative lung abscess rather than tuberculosis include a shorter duration of symptoms, putrid sputum and leukocytosis. Lung abscess must also be distinguished from a necrotic neoplasm. Patients who have neoplasms often lack risk factors for aspiration, symptoms of respiratory infection, fever and leukocytosis. The possibility of tuberculosis or a noninfectious cause of a lung cavity (neoplasm, infarct or vasculitis) should be suspected in a patient treated for presumed lung abscess who does not respond to appropriate antimicrobial therapy.

In the pre-antibiotic era, lung abscess characteristically ran a chronic course with the potential for sudden, severe complications. These included brain abscess, massive hemoptysis, endobronchial spread to other portions of the lung, and rupture into the pleural space with the development of a bronchopleural fistula and pyopneumothorax. With modern antimicrobial therapy, these complications have become rare.

Empyema

Symptoms of empyema include fever, chills, cough, dyspnea and chest pain associated with a recent pulmonary or contiguous infection in the oropharynx, mediastinum or subdiaphragmatic area. The occurrence of persistent fever and leukocytosis with pleural effusion despite appropriate antibiotics should suggest the presence of empyema. There may also be severe constitutional manifestations such as shock, tachypnea, altered consciousness and respiratory failure. Typical findings on physical examination include diminished breath sounds, dullness to percussion and a pleural friction rub. Patients who have an empyema secondary to an aerobic pneumonia tend to present with an acute illness, whereas those who have an anaerobic pneumonia have subacute or indolent illness with findings such as putrid sputum to suggest the etiology.[1,4,31]

DIAGNOSIS

Radiography

Both lung abscess and empyema may be suspected from clinical symptoms but the chest radiograph is the primary tool for diagnosing these infections. Radiographs obtained soon after aspiration usually demonstrate localized or diffuse alveolar infiltrates within 1–2 days. There is nothing distinctive about the appearance of aspiration pneumonia except that infiltrates are usually in dependent segments of the lung. Multilobar involvement may suggest impairment of the host immune system.[32]

The characteristic appearance of a lung abscess is that of a density or mass with a cavity, frequently with an air–fluid level indicating communication with the tracheobronchial tree (Fig. 29.3). The time required for cavitation after a known episode of aspiration is about 1–2 weeks. With necrotizing pneumonia, multiple small lucencies in circumscribed areas of opacification may develop more rapidly.[1,4,33] Abscesses due to tuberculosis are less likely to have an air–fluid level and are more likely to have a dense fibronodular infiltrate that surrounds the cavities.[21] Those associated with a malignancy may be more sharply defined or have an eccentric-shaped cavity with a thick, irregular wall (Fig. 29.4).

A pleural effusion, visible on posterior-anterior and lateral upright chest radiographs, suggests the possibility of empyema. On a decubitus film, with the suspect side down, free pleural fluid can be visualized between the chest wall and the dependent lung. If the layer of pleural fluid is greater than 1 cm thick, it should be aspirated for diagnostic studies. A decubitus film with the suspect side up is also useful because it permits assessment of any underlying parenchymal infiltrate, less obscured by the effusion.[7,25]

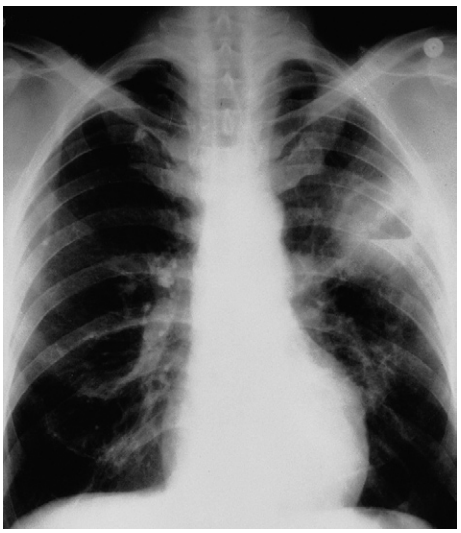


Fig. 29.3 A lung abscess showing an air–fluid level.



Fig. 29.4 A lung abscess associated with a multinodular bronchogenic carcinoma.

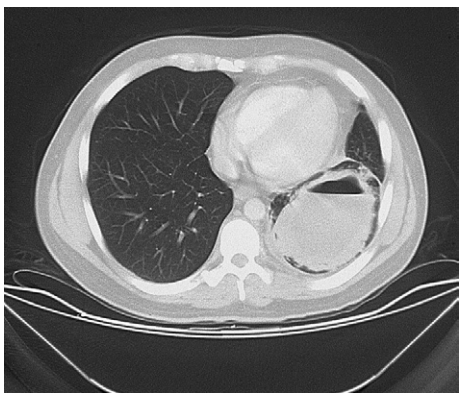


Fig. 29.5 CT scan of a lung abscess showing an air–fluid level.

Ultrasound and CT may be helpful in defining pleuropulmonary lesions. Ultrasound can define loculated collections of pleural fluid, and portable equipment is available for unstable or critically ill patients. CT can define abscesses that are not apparent on plain radiographs and can distinguish between parenchymal and pleural disease (Fig. 29.5). Loculated empyema with a bronchopleural fistula may resemble a lung abscess. Features on CT that tend to favor lung abscess are the presence of thick walls and lesions that are round or oblong, whereas empyemas have thinner walls with a smooth luminal margin and exterior.[34] Both ultrasound and CT may be used to guide aspiration of fluid from abscesses or the pleural space.

Investigations

The first step in determining the specific etiology of any LRTI is the evaluation of lower respiratory tract secretions by Gram stain. These are most likely to be useful if they are obtained before the administration of effective antimicrobial treatment. If stains of expectorated specimens show neutrophils and alveolar macrophages, without squamous epithelial cells (indicative of contamination with saliva), they are useful for defining the offending pathogen(s). In aerobic pneumonia there is usually a single predominant organism; in aspiration pneumonia there is usually a mixed flora, representing the diverse morphotypes of the oropharyngeal flora. Typically, there are various sizes of Gram-positive cocci and pleomorphic Gram-negative coccobacilli and bacilli, which may be tapered and are generally smaller and poorer staining than the Enterobacteriaceae (Fig. 29.6).[1,2,11,13,35] Although some of the individual organisms may resemble pathogenic aerobes such as *Strep. pneumoniae*, there is no predominant pathogen.

Invasive procedures, such as endotracheal aspiration and bronchoscopy, may be useful to obtain lower respiratory secretions for evaluation. Tracheal aspiration through the nose or the mouth is of limited use because these specimens are often contaminated with oropharyngeal flora. Bronchoscopy with bronchoalveolar lavage is useful

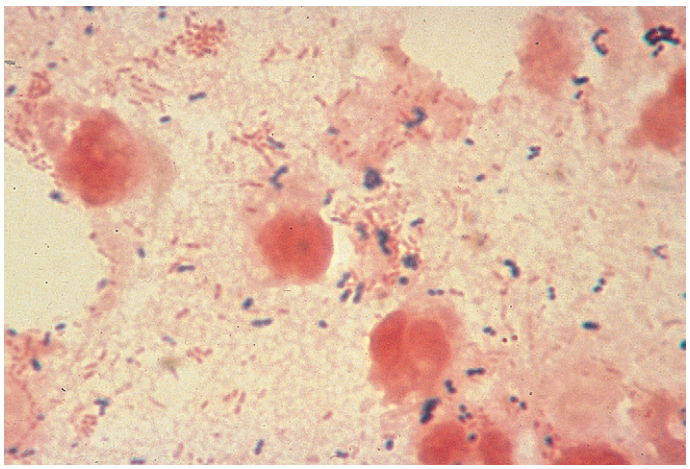


Fig. 29.6 Gram stain of lower respiratory tract secretions. The patient had a lung abscess caused by oropharyngeal streptococci and anaerobes.

because large samples can be obtained with relatively little contamination. They can be concentrated by cytocentrifugation, permitting multiple microbiologic and cytologic evaluations. The bronchoscopic techniques of protected catheter aspiration and protected specimen brushing reduce contamination considerably but they provide relatively scanty specimens and rely on quantitative cultures to help distinguish the significance of cultures. In general, counts that indicate more than 10^5 cfu/ml of respiratory secretions for an appropriate organism (see Fig. 29.1, Table 29.2) are indicative of infection.[1,4,15,24]

Specimens of lower respiratory tract secretions that have passed through the mouth should be cultured for aerobes but not for anaerobes. Growth of recognized aerobic pathogens is helpful for interpreting Gram stains and for providing isolates for susceptibility testing. The absence of aerobic pathogens in specimens from untreated patients should indicate the possibility of anaerobic infection. When specimens are contaminated by saliva, the streptococci and anaerobes that comprise the normal oropharyngeal flora will always grow in culture, whether or not infection is present, and they provide no insight into the pathogenicity of the organisms isolated.

The problem of contamination of respiratory tract secretions by saliva can be avoided if specimens of lower respiratory secretions are obtained by transtracheal aspiration. A catheter is passed through the cricothyroid membrane and specimens are collected by suction.[1,12,14] Although rarely used today, this technique established the role of anaerobes in suppurative pleuropulmonary infections in the early 1970s. Another method for obtaining uncontaminated material for culture is percutaneous transthoracic needle aspiration (percutaneous abscess drainage). Today, this procedure is more frequently performed under fluoroscopic or CT guidance for the diagnosis of malignancy than to obtain material for culture. It can be used, however, to aspirate peripheral abscesses, particularly if bronchoscopy does not provide an adequate specimen for microbiologic diagnosis.

If appropriate specimens from the lower respiratory tract are obtained for culturing anaerobes, they should be expeditiously transported to the laboratory, with minimal exposure to air, for proper processing. If tuberculosis or fungal infection is in the differential diagnosis, appropriate smears and cultures should be requested. If a malignancy is suspected, cytologic stains should be performed. An amplified *Mycobacterium tuberculosis* direct test (MTD) can be useful for detection of smear-negative pulmonary tuberculosis cases.

In addition to lower respiratory tract secretions, blood and pleural fluid, if present, should also be sent to the laboratory for microbiologic evaluation. In anaerobic lung infections, blood cultures are rarely positive. Pleural fluid, if present, requires analysis of protein, LDH and glucose, and determination of pH, as well as microbiologic evaluation. With empyema, the Gram stain usually indicates the pathogens.[7]

With the advances in molecular diagnostics, rapid and accurate identification of anaerobic bacteria by the polymerase chain reaction-based method is possible. Even though there is an increasing trend toward molecular diagnostics of anaerobes, identification of infections caused by these bacteria currently relies on clinical findings, appropriate transport of specimens, laboratory identification of the organisms by morphology and biochemical tests.[37]

MANAGEMENT

Lung abscess

In the past, penicillin G was the preferred drug for treating aspiration pneumonia and lung abscesses, as well as for all anaerobic infections above the diaphragm caused by oropharyngeal flora. In a study of over 70 patients hospitalized with lung abscesses in the 1960s, nearly all responded to intravenous penicillin G. Oral penicillin V in a dose of 3 g/day was also effective and those rare patients who failed to respond to penicillin could be satisfactorily treated with tetracycline.[8]

In the 1970s, when transtracheal aspiration was used to define the microbiology of pneumonia and lung abscesses, concern over the use of penicillin was raised because of occasional therapeutic failures and the isolation of penicillin-resistant *B. fragilis* from some patients.[1,14] However, in one study that compared clindamycin, a drug that is active against *B. fragilis*, with penicillin G in the treatment of aspiration pneumonia and primary lung abscess, there was no difference in rates of defervescence, radiographic clearing or ultimate outcome. Notably, seven patients from whom *B. fragilis* was isolated responded to penicillin.[33]

Subsequently, in a prospective study published in 1983 of 39 patients who had lung abscess, there were 0/19 failures with clindamycin and 4/20 failures with penicillin G. Resolution of fever and putrid sputum was more rapid with clindamycin (4.4 days) than penicillin G (7.6 days). Patients who did not respond to penicillin G ultimately responded to clindamycin.[38] In a 1990 study of 37 patients who had lung abscess or necrotizing pneumonia,[39] only 1/19 patients failed to respond to clindamycin, whereas 8/18 failed with penicillin G. In this study, patients underwent transtracheal aspiration or protected specimen brushing to culture for anaerobes; 9/10 of the penicillin-G-resistant strains were β-lactamase producers. It is now recognized that there has been a change in the susceptibilities of the oropharyngeal Gram-negative anaerobes since the 1970s. Many, in addition to *B. fragilis*, are now β-lactamase producers and resistant to penicillin. In a study of 449 isolates, which included *Bacteroides* spp. other than *B. fragilis*, *Fusobacterium* spp., *Prevotella* spp. and *Porphyromonas* spp. from 28 US medical centers, 57.9% of isolates were β-lactamase producers.[40]

Today, transtracheal aspiration and other invasive procedures are rarely performed to determine the microbiologic etiology of aspiration pneumonia and lung abscesses in nonimmunocompromised patients. Treatment is usually empiric and largely effective.[8] Clinical outcome for most anaerobic infections seems to correlate with *in-vitro* data as broadly applied, and detailed study of individual cases does not seem to be necessary. Monitoring trends in susceptibility patterns and detailed study in problematic individual cases suffices.[31]

The *in-vitro* spectrum and clinical utility of antimicrobials for the treatment of lower respiratory tract bacterial infections is summarized in Figure 29.7. For community-acquired infections that result from aspiration, and where oropharyngeal streptococci and anaerobes are the likely pathogens, penicillin G (or ampicillin or amoxicillin) remains an excellent foundation for treatment, but the addition of a β-lactamase inhibitor or metronidazole is advisable owing to the frequency of β-lactamase production among Gram-negative anaerobes.[31,40,41] Clindamycin is also a primary therapeutic agent, despite *in-vitro* resistance among some *Bacteroides* and *Fusobacterium* spp. Resistance rates vary significantly in different geographic regions, so surveillance of resistance is important in assessing the utility in a given area.[5,8,35,41,42] Metronidazole alone is not effective because of its inactivity against the aerobic and microaerophilic streptococci.[8,41,43]

For health-care-acquired infections, where *Staph. aureus* and aerobic Gram-negative bacilli are common components of the oropharyngeal flora, piperacillin or ticarcillin (rather than penicillin or ampicillin) with a β-lactamase inhibitor provide better coverage of likely pathogens. Appropriate alternatives are imipenem alone, clindamycin plus an aminoglycoside or ciprofloxacin, or an expanded spectrum cephalosporin such as cefotaxime, ceftizoxime or ceftriaxone plus metronidazole. In health-care settings with a high incidence of methicillin-resistant *Staph. aureus* (MRSA) pulmonary infection, the addition of intravenous vancomycin or linezolid should be considered. Many cephalosporins, for example ceftazidime, have little or no activity against anaerobes and should not be used unless the etiology has been defined and found to involve aerobic Gram-negative bacilli.[8,10,43]

Tetracyclines, which were used for lung abscess and empyema in the 1960s, are no longer recommended for the treatment of aerobic/anaerobic pleuropulmonary infections because of high rates of resistance. Chloramphenicol has an excellent spectrum of activity but is rarely recommended because of potential hematotoxicity and the availability of alternative agents.[8,43] The macrolides and azalides have inconsistent *in-vitro* activity against oropharyngeal anaerobes and there is little clinical precedent for their use.[44] Aminoglycosides and quinolones should be used only for their activity against aerobic Gram-negative bacilli and need to be combined with a drug that is active against streptococci and anaerobes. A newer quinolone, such as moxifloxacin or gatifloxacin, has greater activity than ciprofloxacin or ofloxacin against oropharyngeal streptococci and anaerobes, and is more useful.[45,46] In the treatment of aspiration-associated pulmonary infections, moxifloxacin appears to be clinically as effective and as safe as ampicillin-sulbactam.[47] Ertapenem, a once-daily carbapenem, can be used if the oral route is precluded and if the infection does not include *P. aeruginosa*.[48] Other agents that have suboptimal or no activity against oropharyngeal streptococci and anaerobes include aztreonam and trimethoprim-sulfamethoxazole. The antistaphylococcal penicillins and vancomycin should be reserved for documented staphylococcal infections.

The duration of antimicrobial therapy necessary to treat pleuropulmonary infections is variable: 1–2 weeks may suffice for simple aspiration pneumonia but necrotizing pneumonia and lung abscesses may require 3–24 weeks. Parenteral therapy is generally employed until the patient is afebrile (most are afebrile in 7 days) and able to have a consistent enteral intake.[31] For most community-acquired infections, amoxicillin-clavulanate and clindamycin are excellent oral drugs that can be used for continued treatment after initial parenteral therapy. A less costly oral alternative is penicillin V plus metronidazole. If aerobic Gram-negative bacilli are present, oral treatment is more problematic and must be based on the results of susceptibility tests; ciprofloxacin or ofloxacin plus a penicillin or clindamycin may be appropriate. Prolonged therapy is advisable, with treatment continued until the cavity is gone or until serial radiographs show considerable improvement or a small stable residual scar (Fig. 29.8). The time to cavity closure depends largely on the size of the cavity when treatment is initiated and the condition of the patient.

Surgical drainage of lung abscesses is rarely indicated because drainage occurs naturally via the tracheobronchial tree. If spontaneous drainage is not adequate, even with the aid of postural drainage and percussion, clinical improvement is impeded; CT-guided percutaneous abscess drainage may then be beneficial.[49] The drainage catheter can be left in place until there is clinical improvement and drainage has diminished, usually within several days to 1 week. This technique may also be used to reduce the risk of endobronchial spread of infection to other areas of the lungs as well as in patients who are too ill to undergo lung resection to remove necrotic tissue, persistent cavities or nonfunctional lung. Other indications for drainage include large abscess cavity (>8 cm), abscess caused by resistant organisms, obstructing neoplasm impeding drainage and failure of medical treatment. Endoscopic drainage, when performed by an experienced operator, can also be useful for patients who are considered to be poor surgical candidates.[50]

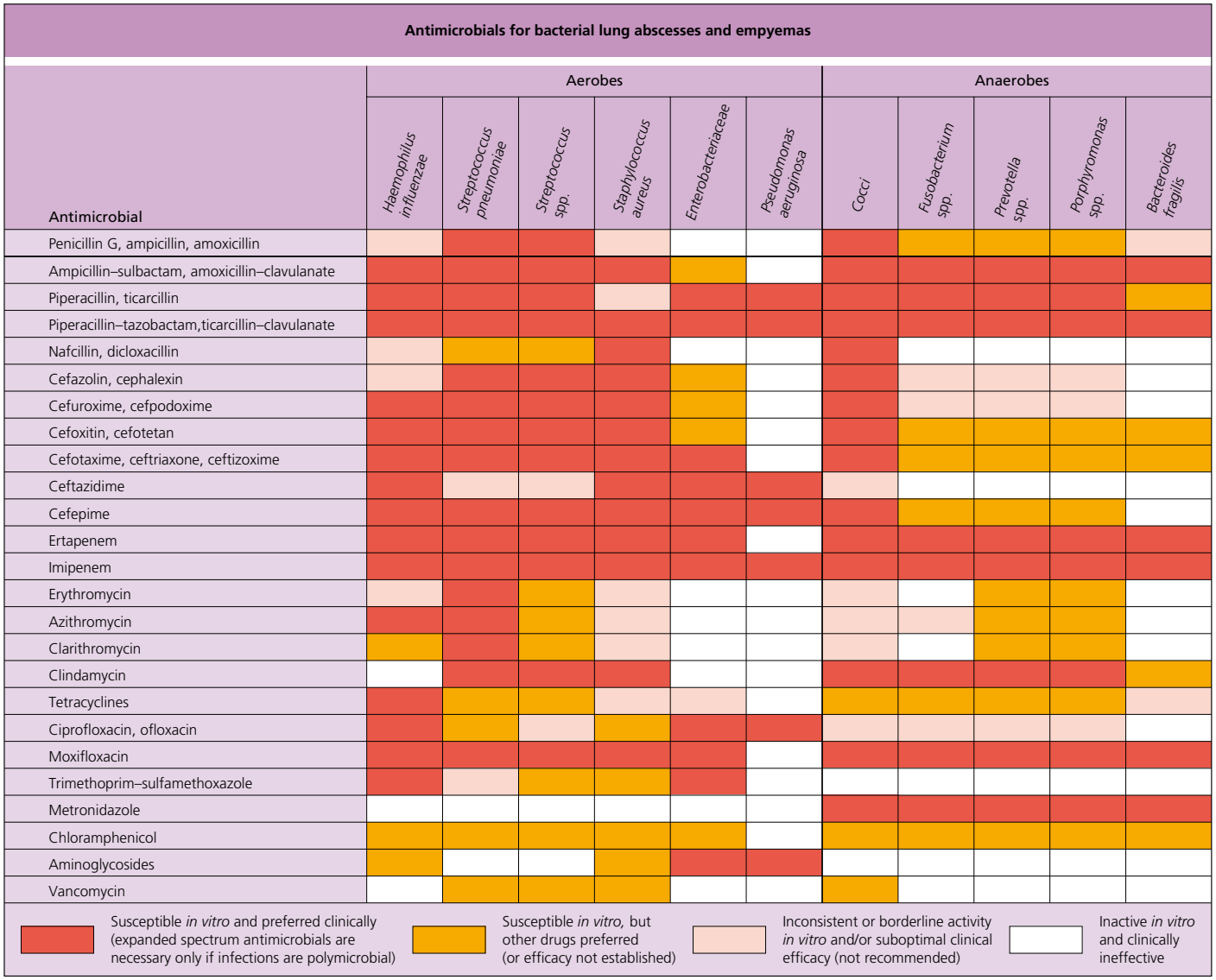


Fig. 29.7 Antimicrobials for bacterial lung abscesses and empyemas. Information for *Streptococcus pneumoniae* is for penicillin-susceptible strains – selected cephalosporins or vancomycin should be used for resistant strains. Information for *Staphylococcus aureus* is for methicillin-susceptible strains – vancomycin should be used for resistant strains. Vancomycin is the drug of choice for β-lactam-resistant Gram-positive organisms.

Empyema

The antimicrobial treatment of empyema is similar to that of aspiration pneumonia and lung abscess but single-organism infections with a defined etiology are more common, thus facilitating antibiotic choice. Antibiotics should be administered in full doses for 2–4 weeks. Therapy may need to be prolonged further, particularly if drainage is not optimal. Antibiotic levels in pleural fluid are comparable to those in serum, so standard systemic doses provide adequate pleural fluid levels.[25]

The proper assessment and management of parapneumonic effusions associated with LRTIs is critical for a successful outcome. Most small effusions clear with antimicrobial treatment and need not be drained. However, if fluid persists more than a few days or layers to more than 1 cm on a decubitus radiograph with the involved side dependent, it should be aspirated and analyzed. The characteristics of the fluid are used to determine the need for tube drainage (see Table 29.4).

Effusions are termed 'complicated' when characteristics indicate a potential need for tube drainage. Complicated effusions are termed 'empyemas' if they are frankly purulent and bacteria are present; tube drainage is mandatory. Chest tubes are placed using negative pressure until the lung is expanded, and then to an underwater drainage system until the fluid is scanty and clear, and then gradually withdrawn over a period of several days. Lack of prompt clinical improvement may indicate the need to reposition the tube to facilitate and continue drainage.

Chest tube drainage of complicated parapneumonic effusions is successful in most patients. It should not be delayed, because these effusions can progress from free-flowing to loculated fluid rapidly. When the fluid becomes loculated, drainage by repeated thoracentesis or tube insertion may not be adequate. The presence of loculated fluid should be suspected if the patient remains ill or febrile or has a persistent leukocytosis. After appropriate evaluation by ultrasound or CT, options for management include image-guided percutaneous drainage by an interventional radiologist[51] or instillation of thrombolytic agents (urokinase or streptokinase) through a catheter or chest tube.[52] If administered before fibrosis occurs, these agents attack the fibrin membranes causing the loculations. Successful therapy leads to an increase in the amount of drainage from the pleural space and can be administered for up to 2 weeks. Thoracoscopy to mechanically lyze adhesions and inspect the pleural cavity has also been used to assist in management.[7]

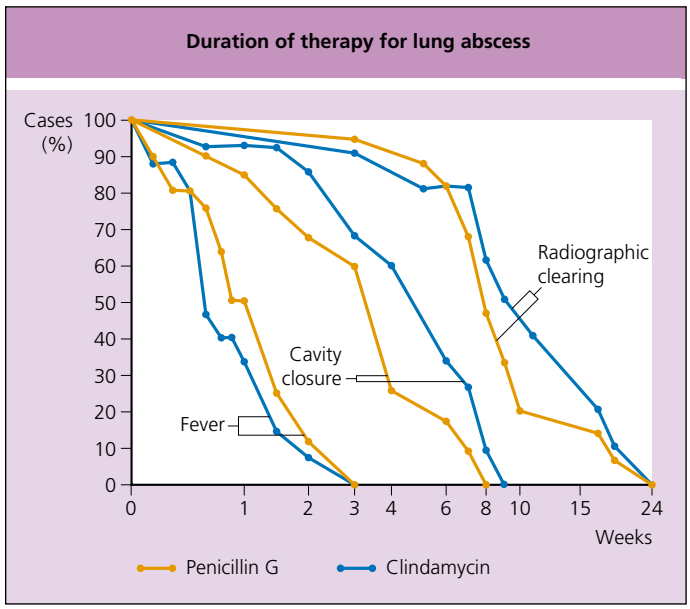


Fig. 29.8 Duration of therapy for lung abscess. Response of patients who had lung abscess to penicillin G (17 patients) and clindamycin (16 patients).

If patients do not respond to the above measures, contrast material can be injected into the chest tube to evaluate the empyema cavity. Open drainage, evacuation of all infected material and decortication of the pleura should be considered. This is a major surgical procedure that requires rib resection and may not be tolerated by debilitated patients. Open drainage, without decortication, may be better tolerated in these patients, but it is followed by a prolonged period of convalescence with an open chest wound.[7]

A serious complication of an empyema is a bronchopleural fistula. The presence of a peripheral air–fluid level radiographically suggests the presence of a bronchopulmonary fistula, although such an air–fluid level may occasionally be due to the presence of gas-forming bacteria. Adequate tube drainage is mandatory to minimize the spread of infection to other portions of the lungs. Empyema with a bronchopleural fistula after pneumonectomy is a disastrous surgical complication. The fistula often does not close with antibiotics, tube drainage and irrigation, and complex surgical procedures are usually necessary.[7]

REFERENCES

References for this chapter can be found online at http://www.expertconsult.com



Chapter 30

Jon S Friedland

Tuberculosis and other mycobacterial infections

Tuberculosis is the clinically most important of the mycobacterial infections and is the subject of the first section of this chapter; the second section covers other mycobacterial infections except for leprosy, which is dealt with in Chapter 103. Details of antimycobacterial drugs are to be found in Chapter 143. Tuberculosis and HIV co-infection is also covered separately in Chapter 93. The clinical microbiology of mycobacterial infections is discussed in Chapter 174.

Tuberculosis

Tuberculosis, a disease identified in skeletons over 6000 years old, remains the most prevalent infectious disease in the world. This chapter focuses on current understanding of pathophysiology, epidemiology and clinical aspects of tuberculosis.

EPIDEMIOLOGY

Worldwide incidence and prevalence

Mycobacterium tuberculosis is estimated to infect 1.6 billion people worldwide or approximately one-third of the world's population.[1] Usually infection is contained by the immune system so that about 14.4 million people have clinical disease at any one time. The World Health Organization (WHO) reported that there were approximately 9.2 million new cases and 1.7 million deaths from tuberculosis in 2006 (http://www.who.int/tb/publications/global_report/2008/en/index.html). Recent data on tuberculosis notification rates are shown in Figure 30.1. However, notification data may be incomplete with underreporting of cases. Confounding factors in global collection of incidence and prevalence data include effects of treatment, difficulties in identifying extrapulmonary disease and those associated with tuberculin testing.

Over 96% of tuberculosis-related deaths occur in the poorer nations of the world and the disease has huge social and economic costs. In wealthier nations, rates of tuberculosis have been falling over the last 50 years in part due to social improvements, development of effective treatments, active case finding and use of the bacille Calmette–Guérin (BCG) vaccine. Recently, this trend has been halted in some countries due to increased incidence of tuberculosis in high-risk population groups including poorer communities, migrants and patients with HIV infection. Levels of disease in homeless populations, intravenous drug users and prisoners in developed countries may also be high.[2] The worldwide prevalence of diabetes mellitus is increasing and increases the risk of tuberculosis.[3]

The impact of HIV

The WHO estimate that there were 709 000 new cases of tuberculosis associated with HIV in 2006. HIV-seropositive patients are more susceptible to infection by *M. tuberculosis*. Reactivation of tuberculosis occurs at least 10 times more frequently than in age-matched controls. The majority of people co-infected with tuberculosis and HIV live in sub-Saharan Africa (over 85%), the Indian subcontinent and South East Asia (Fig. 30.2). Patients tend to be sicker and in greater need of hospitalization. The relationship between HIV and tuberculosis is such that tuberculosis patients should be offered HIV screening. Diagnosis of dual infection may be difficult since HIV predisposes to atypical, nodal and extrapulmonary disease. The subject of HIV–tuberculosis co-infection is explored in depth in Chapter 93 and is not considered further here.

Spread of infection

Spread of infection is dependent on inhalation of aerosols from individuals with pulmonary infection. Proximity to and duration of association with an index case are critical factors. Up to 25% of household contacts of an index case may acquire infection although the extent to which individual genetic predisposition or immunologic impairment contributes to this is uncertain. Although contributory, the exact role of factors such as vitamin D deficiency and iron overload in the spread of tuberculosis is unknown. Spread of infection is separate to development of disease which occurs in less than 10% of infected persons and is significantly affected by impaired cell-mediated immunity. Congenital transmission of tuberculosis is not a significant factor in the natural spread of disease.

Transmission in closed institutions

Overcrowding contributes to the spread of tuberculosis amongst the poor. Close proximity to infected individuals is a significant issue in any closed institution and for health-care workers. Many countries have specific guidelines for tuberculosis control in institutions. In prisons, the situation is complicated by the fact that inmates have an increased incidence of HIV, are frequently moved to other prisons or back into the community with little warning and may be poorly managed in terms of health services. Since release of prisoners is often into poor circumstances and crowded hostels, the consequence of undetected or inadequately treated tuberculosis may be rapid spread of disease. Mass incarceration can lead to an increase in cases of tuberculosis and a rise in drug-resistant disease.[4] An effective public health program with an active community care component can overcome such problems.

Genetic techniques using restriction fragment length polymorphism (RFLP) analysis (often of the insertion sequence IS6110) to provide

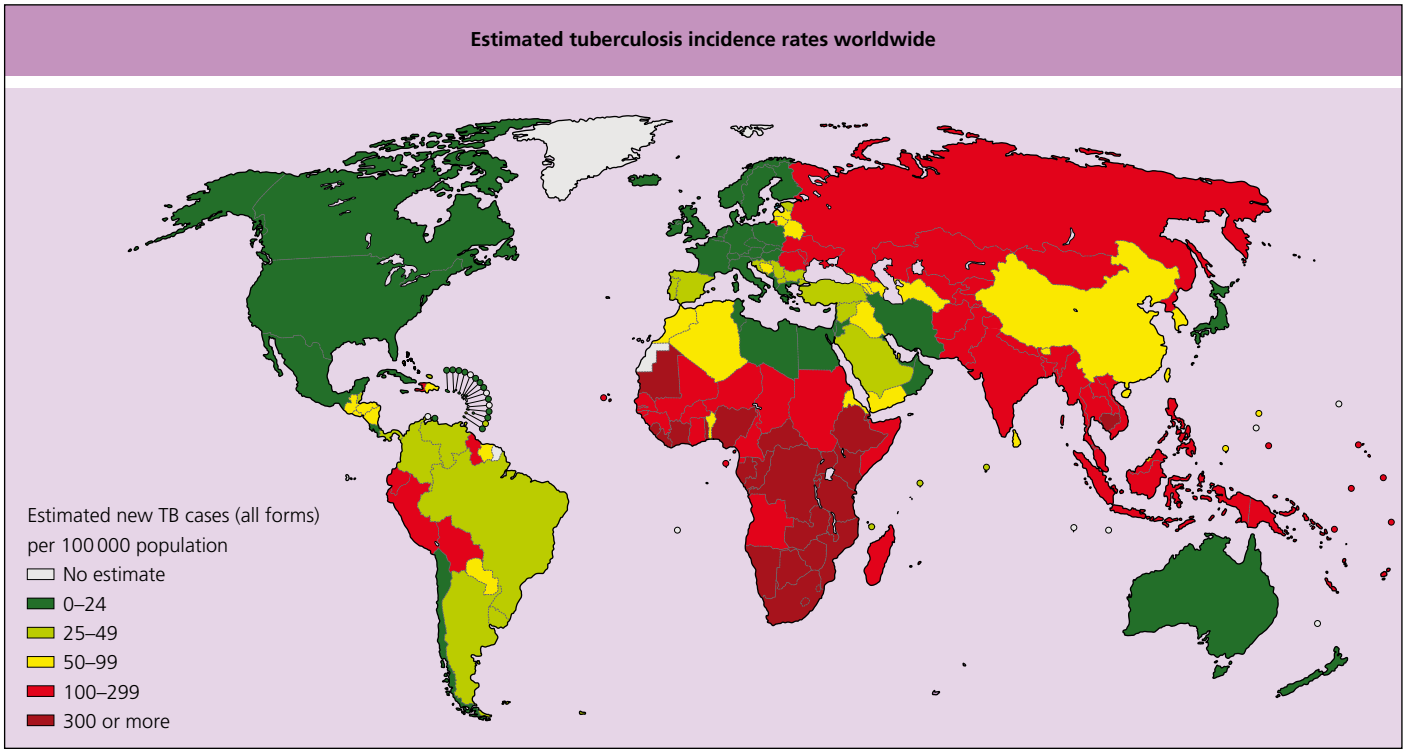


Fig. 30.1 Estimated tuberculosis incidence rates worldwide. Reproduced with permission from the World Health Organization, 2008.

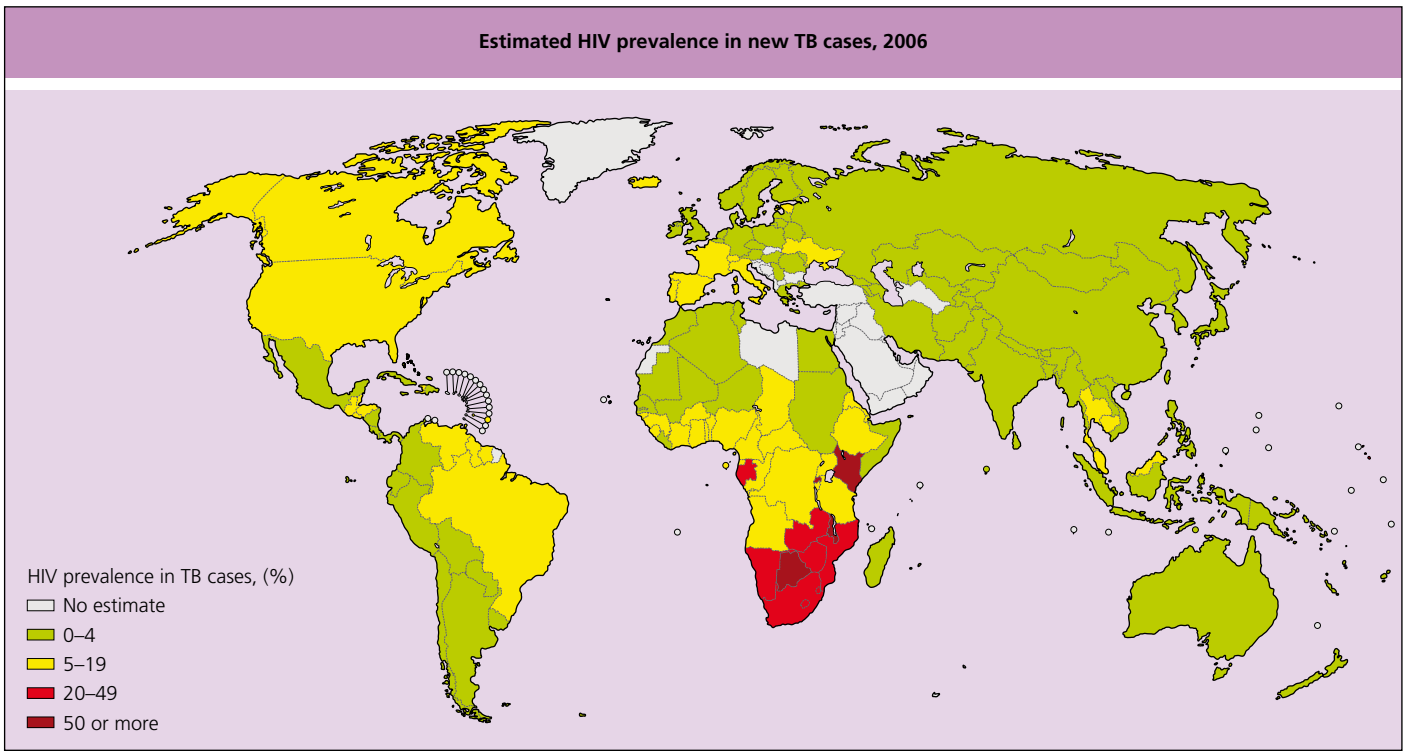


Fig. 30.2 Estimated HIV prevalence in new TB cases 2006. Reproduced with permission from the World Health Organization, 2008.

DNA fingerprints and spoligotyping have proved useful in documenting local outbreaks of tuberculosis including those involving drug-resistant organisms.[5] Recent analyses, using DNA sequencing, showed greater genetic diversity than had been previously recognized.[6]

PATHOGENESIS AND PATHOLOGY

The pathogen

Mycobacterium tuberculosis, discovered by Robert Koch in 1882, is characterized by a complex cell wall rich in mycolic acids, together with peptidoglycan and arabinogalactan, a complex polysaccharide molecule that surrounds the cell membrane. Many cell wall components, such as lipoarabinomannin, are of pathogenic significance. Mycobacteria contain diverse proteins associated with growth, virulence and intracellular survival. Amongst the most critical are the 10 kD, 65 kD, 70 kD and 90 kD families of heat shock proteins (HSPs) which are molecular chaperones involved in protein folding and assembly, and whose production is upregulated by environmental stress.

Host immune factors

The principal tissue immune response in tuberculosis is the formation of granulomas comprised of cells of the monocyte lineage including multinucleate giant cells, together with T lymphocytes and interdigitating stromal cells such as fibroblasts (Fig. 30.3). In the initial stages of the immune response, neutrophils are present whilst more advanced disease is characterized by caseous necrosis and eventually deposition of calcium. Granuloma formation is a consequence of a complex interaction between the organism, the immune system and local release of tissue factors and proteases. This process may be modulated by systemic or local production of hormones such as 1,25 dihydroxyvitamin D_3. Caseous necrosis, typical of mycobacterial granulomas, is probably due to the delayed type hypersensitivity response although it is not known why it is so very rare in nontuberculous granulomas.

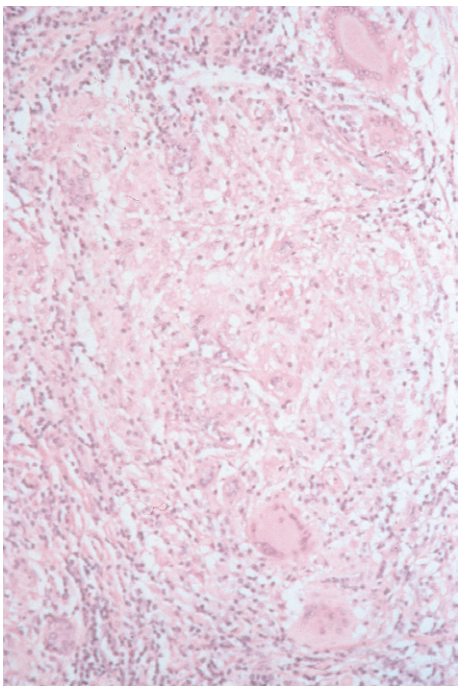


Fig. 30.3 Detailed histology of the tuberculous granuloma. Monocytic cells and smaller T cells are shown together with multinucleate giant cells.

Monocytes, macrophages and phagocytosis

Following inhalation, *M. tuberculosis* is phagocytosed by the alveolar macrophage; similar fixed tissue cells are present elsewhere in the body. Pulmonary surfactant protein may enhance the process of phagocytosis. The phagocytosing macrophage initiates the host immune response (Fig. 30.4). Phagocytosis of the pathogen may involve complement receptors CR1, CR3 and CR4 as well as mannose-binding and scavenger receptors and adhesion molecules. Intracellular *M. tuberculosis* partly inhibits phagolysosome fusion, arresting phagosome maturation and preventing acidification of the vacuole, thus maintaining a pH around 6.4.[7] Some mycobacterial components escape the phagosome, are detectable in the cytoplasm and are subsequently presented via major histocompatibility complex (MHC) class I molecules to T lymphocytes.

Phagocytosis and engagement with Toll-like receptors (TLRs) is a potent stimulus to gene expression and secretion of proinflammatory cytokines such as tumor necrosis factor (TNF), interleukin (IL)-1 and IL-6. TLR-2 and -4 are the principal pattern recognition receptors that have been associated with the proinflammatory responses in tuberculosis,[8] although additional data suggest involvement of TLR-6, -8 and -9.[9,10] The known consequences of TNF secretion include fever and cachexia, two prominent symptoms in tuberculosis. TNF has a pivotal role in granuloma formation and is found at sites of human infection. The clinical use of anti-TNF antibodies is associated with reactivation of disease in individuals with latent infection.[11] Macrophage-derived chemokines control cellular recruitment to the granuloma, regulate the extent of the proinflammatory response and may drive mycobacterial killing.[12] The granuloma in tuberculosis is well circumscribed and downregulatory cytokines such as IL-10 are involved in limiting inflammatory responses.

Infected macrophages drive the tissue damage characteristic of tuberculosis. Macrophages and stromal cells associated with granulomas secrete a number of proteases including collagenase, elastase and gelatinase which are matrix metalloproteinases (MMPs) whose action is normally opposed by tissue inhibitors of MMPs. Relatively unopposed MMP activity is found in tuberculosis and is associated with local tissue damage.[13] Other monocyte-derived proteins involved in tissue destruction include lysosomal proteases such as cathepsins which function best at acid pH and the plasminogen activator urokinase. Activation of plasminogen is followed by activation of the clotting system and laying down of fibrous tissue, a process in which macrophage-derived transforming growth factor beta (TGF-β) has a central role. The exact mechanisms by which monocytes and macrophages kill *M. tuberculosis* in man are not fully understood. Cathelicidins and similar molecules appear to have a central role and may be regulated in a vitamin D-dependent manner. Reactive oxygen may be involved in mycobacterial killing in man.

Lymphocyte responses in tuberculosis

Both αβ and γδ $CD4^+$ T cells are critical in immunity to tuberculosis. Patients with reduced T-cell function or numbers are at higher risk of clinical tuberculosis. *Mycobacterium tuberculosis* may impair macrophage antigen presentation to T lymphocytes by downregulating the co-stimulatory molecule B7-1. Cells of the T-helper 1 (Th1) subclass which secrete interferon gamma (IFN-γ) and IL–2 are central to control of infection in murine models. In human disease the situation is more complex, with Th1, Th2 (IL-4, -5, -6, -10 and -13 producing), Th17 and intermediate cell types detectable.[14] The dual presence of Th1 and Th2 cells may account for the fact that although tuberculosis is limited within the granuloma, the organism is not usually completely killed off by the immune response. IL-23, together with IL-12 with which it partly shares a receptor, are both involved in the protective IFN-γ response seen in tuberculosis.[15]

Other T-cell phenotypes involved in immunity to tuberculosis include $CD8^+$ cells and double-negative T lymphocytes lacking CD4 and CD8 which recognize mycobacterial lipoglycan antigens presented via CD1.[16] Many proteins, carbohydrates and lipids of *M. tuberculosis* contain epitopes that stimulate B lymphocyte antibody production. In terms of pathogenesis, it has proved difficult to identify specific antibody responses in sera of tuberculosis patients of importance in host defense.

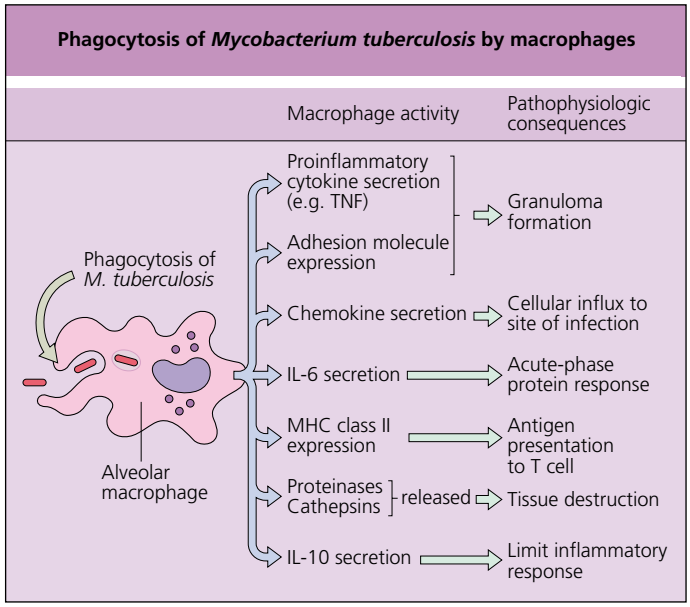


Fig. 30.4 Phagocytosis of *Mycobacterium tuberculosis* by macrophages. Phagocytosis initiates many critical pathways involved in host defense to infection.

Host genetic factors

There is a genetic component to host resistance to *M. tuberculosis*. Human leukocyte antigen (HLA) haplotypes associated with susceptibility to tuberculosis include A8, A10, B8, Bw15 and DR2. Non-HLA linked genes, such as the natural resistance-associated membrane protein, are a determinant of disease susceptibility in certain populations.[17] Certain families that are susceptible to mycobacterial infections have deletions in the IFN-γ receptor gene.[18] A milder phenotype of mycobacterial susceptibility is found in patients with mutations affecting IL-12 signaling.[19] It is likely that although many genetic polymorphisms which have been described in tuberculosis and that are encoded in diverse cytokine and endocrine (including vitamin D) genes influence susceptibility to infection to varying degrees, environmental factors have a greater effect on disease presentation.

PREVENTION

Public health measures

Strategies to prevent spread of tuberculosis aim first to identify and promptly treat infectious patients and secondly to prevent infection by vaccination. Many countries have a system, often legally enforced, of infectious patient notification to a central body which traces infected contacts of index cases. In addition, high-risk patients or communities such as intravenous drug users may be screened in order to institute definitive or prophylactic therapy. In its simplest form, case finding involves examining sputum smears although radiologic examination may be a useful adjunct. However, sputum smear microscopy will miss about 50% of culture-positive patients who are highly infectious. Routine screening for latent infection may be appropriate in countries with low rates of infection or in subgroups of patients particularly likely to reactivate disease such as those who are immunosuppressed, including renal dialysis patients[20] and those about to receive immunosuppressive therapy. Prevention programs may require incentives for successful implementation; they should be linked to educational initiatives and involve social services.

Infectious patients and those who are suspected of infection should be isolated until either tuberculosis infection has been ruled out or effective treatment established. In the USA and other affluent nations, laminar airflow and negative pressure ventilation rooms are used for known or suspected tuberculosis, particularly in patients with multidrug-resistant disease. Extensive guidelines have been produced in the USA relating to control of tuberculosis but these are not universally applicable.[21] The use of an efficient personal protective mask is key although even this is not available in many parts of the world where tuberculosis is prevalent. In many instances, basic patient isolation and possibly specified, ventilated rooms for procedures which generate aerosols is all that is feasible. Shortwave ultraviolet illuminators to kill organisms in clinics and shelters are potentially useful. Appropriate control measures should be defined in advance in high-risk procedure rooms (e.g. bronchoscopy suites), during patient transport and in all at-risk institutions which range from health-care facilities through to shelters for the homeless.

Testing for exposure

The tuberculin skin test

The Mantoux test is still the commonest test used to screen for tuberculosis exposure and depends on the intradermal injection of a specified quantity of an internationally standardized purified protein derivative (PPD) of tuberculin. PPD solution should not be left in syringes since it may be variably adsorbed to their surface. Tuberculin positivity manifests as induration at the site of testing after 48 hours. Induration less than 5 mm diameter following a standard injection of 5 units PPD is regarded as negative and greater than 15 mm diameter as positive. PPD may have a booster effect on immunologic memory particularly in older persons which can cause confusion if re-testing occurs after a few months when an apparently positive result is not the result of new infection.

Induration in the 5–15 mm diameter range may be indicative of current infection, previous treated infection, exposure to nontuberculous mycobacteria, vaccination or disease in the immunosuppressed patient, all of which have unpredictable confounding effects on Mantoux testing. In the USA, induration greater than 5 mm is taken as positive in patients with HIV, a close contact with tuberculosis or a fibrotic chest radiograph; induration above 10 mm is positive in any other at-risk groups. Patients who are immunosuppressed such as those with HIV (particularly if the $CD4^+$ T-cell count is below $400/mm^3$) or who have viral infections such as measles or sarcoidosis, have a strong tendency to anergy. Systemic illnesses, including miliary tuberculosis, are also associated with anergy. There is a poor relationship between general anergic responses to other antigens and tuberculin negativity. False-negative tests occur at the extremes of age, following use of inadequately stored tuberculin or due to poor injection technique.

The Heaf test involves placing PPD on the skin of a subject and then using a multiple puncture gun to piece the skin in six places. The response is then graded as described in Table 30.1. Heaf guns must

Table 30.1 The Heaf test grades

Heaf test grade	Response
0	No induration at puncture sites
1	Discrete induration at a minimum of four needle sites
2	Induration at needle sites merge to form ring but leave clear center
3	One large induration site seen (5–10 mm diameter)
4	Induration over 10 mm diameter

be resterilized between patients or a disposable head used. The Tine test is similar to the Heaf test except that the puncture needles (usually four not six) are precoated with tuberculin. There has been controversy as to the reliability of this technique and it has faded from general use.

Interferon gamma release assays (see also Chapter 174)

There are two main types of IFN-γ release assay (IGRA) in use and their main advantages are that they are not affected by previous BCG vaccination, are not confounded by boosting and do not require reading at 48 hours so can be arranged at a single clinic visit. Like skin tests, they cannot distinguish latent from active infection. They are relatively expensive compared to skin tests. IGRAs are stimulation assays and the current incarnations are based on immune responses to antigens encoded in the RD-1 region of the *M. tuberculosis* complex which make them specific for this organism. The first is a whole-blood enzyme-linked immunosorbent assay (ELISA)-based system and the second is an enzyme-linked immunospot (ELISpot)-based detection of IFN-γ.[22] Both use as antigens early secreted antigen target (ESAT)-6 and culture filtrate protein (CFP)-10.

The advantages of IGRAs mean that they are incorporated into national testing guidelines for screening and prevention of tuberculosis in a number of different countries including the UK (Clinical Guideline 33 from the National Institute for Health and Clinical Excellence; http://www.nice.org.uk/CG033) and the USA.[23] However, these tests do not necessarily replace the skin tests and have different sensitivities and specificities which may be population specific. There are many areas where their value is limited, unproven or confused, including diagnosis of active or early reactivating disease (when interferon responses can be suppressed) and use in immunosuppressed patients. There is a theoretic advantage in systems which use both skin tests and IGRAs although this still requires two clinic visits. IGRAs have use in excluding diagnosis of tuberculosis in children and in the context of occupational health screening. The negative predictive value of IGRAs is clinically useful. A negative test in a healthy patient excludes tuberculosis infection. However, as with other diagnostic tests, the positive predictive value depends on the prevalence of *M. tuberculosis* in the population that is being tested.

Chemoprophylaxis

Chemoprophylaxis is an important and arguably underused component of tuberculosis control programs which is misleadingly named since it is actually treatment of latent, nondividing organisms which can be undertaken with a single drug. Chemoprophylaxis may be primary in unexposed individuals or secondary in those exposed to *M. tuberculosis* who do not have clinical disease. Secondary prophylaxis is practiced most commonly and successfully in tuberculosis control programs in the USA. This approach, which is based on regular skin prick testing, is not possible where vaccination is common or resources inadequate. Isoniazid 300 mg daily for 6–12 months is the usual preventive regimen and is given with vitamin B_6 to prevent neurologic side-effects. Since patients are well, chemoprophylactic regimens with a high incidence of side-effects such as isoniazid and pyrazinamide[24] are not acceptable. Chemoprophylaxis is advocated in HIV-seropositive patients because of the increased incidence of clinical disease in patients exposed to *M. tuberculosis*. Chemoprophylaxis should also be considered in patients with latent infection prior to the initiation of TNF inhibitors and all such patients should be screened. However, if diagnostic facilities are poor, there is a significant chance of inadvertently treating established tuberculosis and increasing isoniazid resistance. In contrast, use of chemoprophylaxis in potential transplant recipients is established. Chemoprophylaxis should be deferred in pregnant women, a group more prone to isoniazid hepatitis (see also Chapter 143).

Vaccines

The live-attenuated BCG vaccine, developed by Albert Calmette and Camille Guérin, was first used in 1921. Subsequently, many strains have been used clinically although the current reference type is the Pasteur strain. Vaccination leads to a local immune response and ultimately scar formation (Fig. 30.5). Adverse reactions other than local irritation are uncommon and anaphylaxis extremely rare. Local abscesses or ulcers usually reflect poor technique although keloid formation may arise in susceptible individuals. Adenitis, sometimes suppurative, is the most important complication, the incidence of which depends on many factors including vaccine strain and immune status of the host. Lupoid reactions, infected osteitis and disseminated BCG disease are very rare, the latter two necessitating therapy with rifampin (rifampicin) and isoniazid (BCG is resistant to pyrazinamide).

Extent of immunity following BCG vaccination depends on the strain used, environmental factors and the genetics of the population being vaccinated as well as individual host factors such as age. There is a poor correlation between tuberculin reactivity after vaccination which develops to maximal levels within 3 months, and protection against disease. BCG vaccine efficacy is about 60–80%[25] and is most efficacious in preventing serious disease in children. Giving BCG to infants gives significant protection from tuberculosis in household contacts.

There is great interest in a number of approaches to developing new tuberculosis vaccines. The first is to improve the efficacy of the BCG vaccine by genetic modification or to develop a new live attenuated vaccine by deletion of specific virulence factors. The second approach involves using subunit vaccines often given as a booster to a BCG or modified BCG vaccine. A vaccine based upon *M. tuberculosis* antigen 85 is in field trials.[26] Different antigens may be linked in subunit vaccines. The third approach is to use naked DNA vaccines encoding for immunogenic mycobacterial antigens which has been successful in animal models.[27] New vaccines have potential both to prevent infection and to treat established disease.

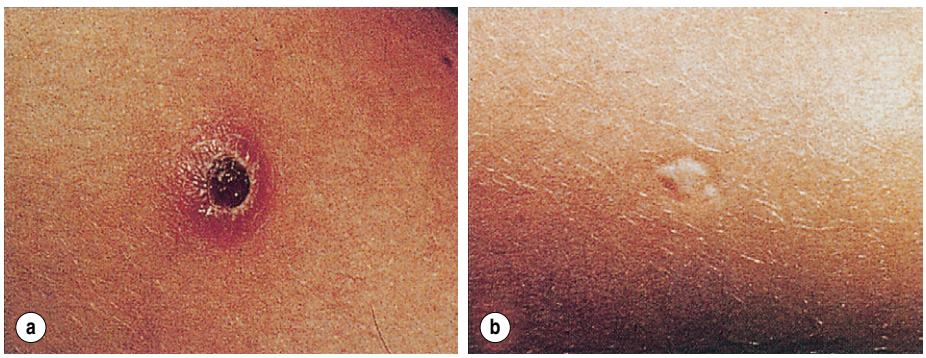


Fig. 30.5 BCG response at 6 weeks. (a) Clinical evidence of cell-mediated immune response is clearly apparent at 6 weeks. (b) The healed BCG scar. With permission from James DG, Studdy PR. A colour atlas of respiratory diseases, 2nd ed. London: Mosby; 1992.

CLINICAL FEATURES

In this section, the diverse clinical presentations of primary, pulmonary and miliary tuberculosis are reviewed, together with the characteristic changes found on radiologic examination. In addition, the principal extrapulmonary manifestations of tuberculosis are considered.

Primary and childhood infection

Primary tuberculosis is usually acquired by inhalation of infected particles in childhood, although in affluent countries the first encounter with tuberculosis may be as an adult. Although a single bacillus can cause disease, usually 50–200 organisms are required for development of active infection. Inhaled bacilli pass into the lung where damage is usually but not always confined to one segment with concurrent involvement of draining, frequently hilar, lymph nodes. This gives rise to the primary (ghon) complex. Clinical disease develops in less than 5% of people exposed to *M. tuberculosis*. After initial infection, the only sequela may be scar tissue which is often calcified and later identified on routine chest X-ray. After the first year of infection, there is a 3–5% lifetime chance of reactivation of disease in non-HIV infected individuals. Children aged 5–15 years develop active tuberculosis relatively infrequently.

Symptomatic patients present with cough with variable amounts of sputum and hemoptysis together with localized pleuritic chest pain and dyspnea. In addition, systemic features such as fever, night sweats, anorexia and weight loss occur. A minority of patients have retrosternal pain sometimes exacerbated by swallowing and increased by sternal pressure which is thought to relate to lymphadenopathy. Primary tuberculosis is seen as asymmetric hilar adenopathy and associated consolidation on chest X-ray. In children, isolated lymphadenopathy is more common. Less typical chest X-rays of primary infection include those which appear normal or have widespread disease, lobar consolidation and pleural effusions. An unusual complication of primary tuberculosis is bronchial obstruction due to pressure of a node on a main bronchus. This phenomenon, sometimes called epituberculosis, may lead to secondary bronchiectasis. Untreated primary disease may progress to involve the entire lung and disseminate. Symptoms are present at this stage and may be severe with continuous cough and sputum production, severe dyspnea, high fevers, drenching sweats and cachexia. Chest X-ray reveals widespread patchy consolidation with areas of collapse and cavitation.

Endobronchial tuberculosis is usually a complication of primary infection although it may occur during reactivation. It may follow adhesion of inflamed lung parenchyma or lymph nodes to bronchi or may arise via lymphatic or hematogenous spread of infection and even from direct seeding of inhaled bacilli. Endobronchial tuberculosis probably frequently goes undiagnosed since it was found in over 400 of 1000 consecutive autopsies. The classic clinical presentation is with a barking cough and wheeze but onset may be gradual, mimicking other respiratory diseases ranging from asthma to cancer. Sputum production may be exacerbated when the mucosa is breached and caseous material extruded. Parasternal pain, dyspnea, symptoms due to collapse and consolidation of distal lung tissue and systemic manifestations of tuberculosis may be found. The most important late complication of endobronchial infection is bronchiectasis.

Pulmonary infection

Risk factors for reactivation

The majority of cases of tuberculosis are due to reactivation of infection acquired years earlier. The stimulus to reactivation may be frank immunosuppression or may be more subtle when factors such as malnutrition and vitamin D deficiency may be involved. TNF inhibitors increase the susceptibility of patients to reactivation. The role of steroids in reactivation of tuberculosis has not been fully defined. High doses may cause reactivation which often presents atypically or is detected belatedly since the symptoms of infection may mimic symptoms of the disease for which steroids were being prescribed. Tuberculosis does not appear more common in asthmatics maintained on daily low-dose prednisolone. Other conditions associated with reactivation of tuberculosis are end-stage renal disease, diabetes mellitus, silicosis, gastrectomy and transplantation when disease is usually due to immunosuppression although it has been transmitted with the implanted organ. Although tuberculosis and lung malignancy may coexist and the symptoms of tuberculosis may mimic those of cancer, tuberculosis is not a risk factor for lung cancer. However, lung malignancy is a systemic risk factor for reactivation of tuberculosis and may invade and disrupt old tuberculous lesions leading to active infection. Some workers have detected mycobacterial DNA in patients with sarcoid but others have not and whether there is any link between these diseases is unresolved.

Clinical presentation

In one prospective study, symptoms of reactivation of pulmonary tuberculosis were cough in 78% of patients, weight loss in 74%, fatigue in 68%, fever in 60% and night sweats in 55%.[28] Some patients have only mild feelings of nonspecific malaise. Hemoptysis occurs in up to a third of patients. It may be massive from either enlarged bronchial arteries around tuberculous cavities (Rasmussen's aneurysms) or more frequently from erosions involving other bronchial or pulmonary arteries. Dyspnea suggests extensive disease and is a late symptom. Pleuritic chest pain indicates inflammation in and possibly infection of adjacent pleura.

Clinical examination may be misleading in the early stages of reactivation and radiologically apparent changes of consolidation and cavitation hard to detect. Noninfectious complications of tuberculosis may be present (see below). Some patients with advanced disease present in acute respiratory failure progressing rapidly to the adult respiratory distress syndrome (ARDS).

Chest X-ray shows disease localized to apical and posterior segments of the upper lobes of the lung in over 85% of cases with other sites often secondarily affected. The apical segment of the lower lobe is also frequently involved. Infiltration and cavitation secondary to caseous necrosis may be associated with air–fluid levels. On treatment, most cavities heal completely leaving residual scarring, often calcific. Tuberculous chest X-rays frequently show upper zone shadowing with fibrotic changes, lobar atelectasis, elevation of hilar nodes and deviation of the trachea (Fig. 30.6). A wide range of less common findings are described such as pneumonic consolidation (Fig. 30.7). It is notoriously



Fig. 30.6 Typical chest X-ray from a patient with tuberculosis showing upper lobe shadowing, elevation of hilar lymph nodes and deviation of trachea.

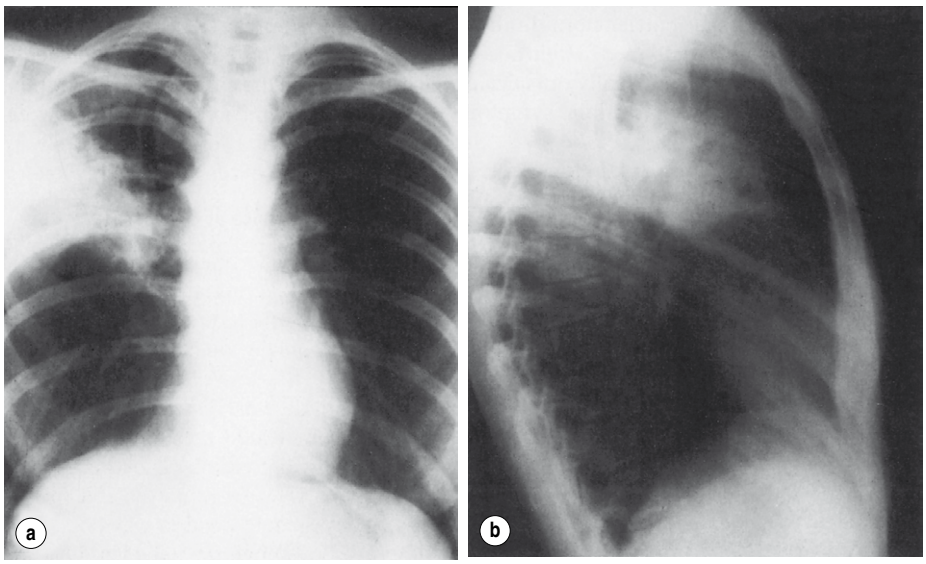


Fig. 30.7 Chest radiograph of pulmonary tuberculous pneumonia. (a) Posteroanterior and (b) lateral chest radiographs of a patient with TB presenting as a consolidation. Courtesy of Dr W Lynn, Ealing Hospital, UK.

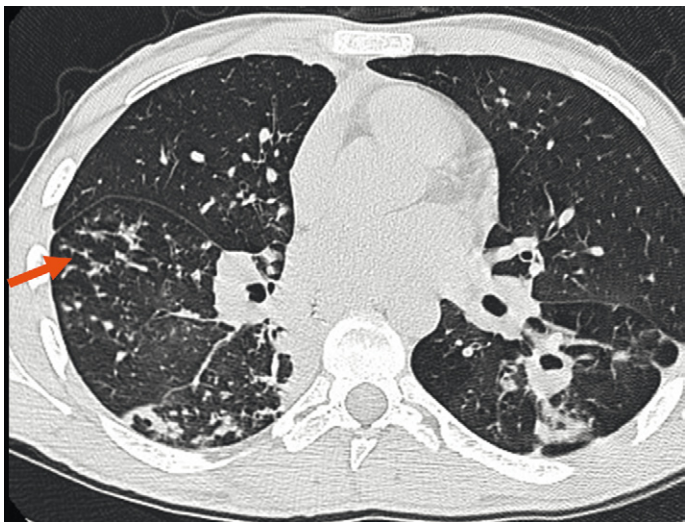


Fig. 30.8 CT scan of pulmonary tuberculosis showing tree-in-bud pattern (arrowed). Courtesy of Dr S Copley, Imperial College Healthcare NHS Trust, London, UK.

difficult to distinguish early reactivation from chronic healed lesions without follow-up. CT and MRI scan findings include cavitation with scarring as well as characteristic nodules and branching linear structures, sometimes referred to as a tree-in-bud pattern (Fig. 30.8).

Other investigations in pulmonary tuberculosis may reveal leukocytosis or more specifically but uncommonly a monocytosis. More commonly the leukocyte count is normal and more rarely, it is leukopenic. Anemia, generally normochromic and normocytic, is typical. An acute phase response is almost invariably present with elevated C-reactive protein (CRP) concentrations in plasma, raised erythrocyte sedimentation rate (ESR) and in more chronic cases, decreased serum albumin. Hyponatremia occurs in about 10% of patients due to antidiuretic hormone-like activity although may be a manifestation of concurrent extrapulmonary infection. A small number of patients have hypercalcemia, probably due to abnormal vitamin D processing by granuloma macrophages.[29]

Complications

The principal acute complications of pulmonary tuberculosis are hemoptysis (discussed above) and pneumothorax. Bronchopleural fistulae may heal spontaneously or require tube drainage and sometimes surgery. Prognosis is good because of formation of scar tissue in tuberculosis. Chronic complications of lung tuberculosis relate to parenchymal damage and scarring. Aspergillomas may develop within healed cavitating lesions in up to 20% of cases; patients typically present with hemoptysis. Localized bronchiectasis (see Chapter 26) may only become clinically significant years later and is best defined by high-resolution CT scanning. Tissue destruction may be so great as to cause respiratory failure.

Pleural tuberculosis

Pleural tuberculosis classically occurs 3–6 months after primary disease but onset may be delayed and pleural disease may be the first sign of reactivation. Pleural disease may accompany lung infection or be the predominant feature. Tuberculosis and malignancy are the principal differential diagnoses of massive effusions which are exudates (protein concentration greater than 3 g/dl (30 g/l)). Effusions are usually straw colored but may be blood stained and occasionally frankly bloody. Glucose concentrations in tuberculous effusions are frequently, but not always, below 40 mg/dl (2.22 mmol/l). Pleural fluid lysozyme, lactate and pH may be elevated in tuberculous effusions, as in bacterial infections. Elevated adenosine deaminase (ADA), derived from CD4 lymphocytes, is characteristic. Low ADA levels are against a diagnosis of tuberculosis but high concentrations are nonspecific. The leukocyte count of tuberculous effusions is usually raised and may be as high as 5000 cells/mm^3. Lymphocytes are generally the most prevalent cell type. Monocytes are also characteristic but mesothelial cells are scanty. Neutrophils may be present and even predominate in acute disease.[30]

Chest X-ray usually demonstrates a unilateral effusion, more often on the right. An effusion may be massive and bilateral effusions occur in approximately 10% of patients. Ultrasound may reveal loculated effusions containing fibrinous tissue reflecting activation of the fibrinolytic system. Ultrasound and CT or MRI scanning are useful to document underlying disease and to aid diagnostic and therapeutic aspirations.

Miliary tuberculosis

Miliary tuberculosis follows dissemination of *M. tuberculosis* and clinical presentation is varied. Factors involved in the development of miliary infection include delay in diagnosis, impaired immune responses, mycobacterial virulence factors, mycobacterial load and the number of organisms able to gain entry to the bloodstream. At least 50% of patients with miliary disease in developed countries are immunosuppressed, most commonly by alcohol. Diabetes, chronic renal failure,

underlying malignancies and immunosuppressive drugs are other risk factors. Disseminated infection due to *M. bovis*, BCG strain, may follow installation of the organism into the bladder when it is used as an adjuvant to chemotherapy for malignancy.

Miliary tuberculosis may present as pyrexia of unknown origin or with symptoms attributable to involvement of one or more organ systems. Commonly there are widespread pulmonary granulomas and CNS disease and rarely cardiac involvement (although pericardial, myocardial and endocardial manifestations have all been reported). Differential diagnosis is often wide and clinicians must be alert to the possibility of miliary tuberculosis. Acute miliary tuberculosis presenting with shock and ARDS has a mortality which may approach 90%. In more chronic cases, cachexia is prominent and localizing features may be few. Widespread macular and papular skin lesions (tuberculosis miliaris disseminata) are suggestive of miliary infection. Choroidal tubercles 0.5–3.0 mm in diameter are essentially diagnostic of miliary disease (Fig. 30.9).

The chest X-ray of miliary tuberculosis has well-defined nodules less than 5 mm in diameter throughout both lung fields (Fig. 30.10). X-ray changes may only develop after a patient has been admitted to hospital, so patients must be reassessed frequently. Larger nodules and a pulmonary focus occur in approximately one-third of patients. CT or MRI scanning may show smaller nodules not apparent on X-ray. Ultrasound scanning may show increased echogenicity and focal lesions in the liver but is not diagnostic. Hematologic investigations are similar to those found in tuberculous pneumonia but a neutrophilia may be seen and should not put the diagnosis in doubt or cause empiric therapy to be restricted to antibacterials. Rarer abnormalities include disseminated intravascular coagulation and the hemophagocytic syndrome. Sterile pyuria and organisms in the absence of urinary leukocytes have been reported but the exact frequency of such renal manifestations is uncertain. Delay in diagnosis contributes to mortality.



Fig. 30.9 Choroidal TB. Choroidal disease is a manifestation of TB which is highly suggestive of miliary disease. With permission from James DG, Studdy PR. A colour atlas of respiratory diseases, 2nd ed. London: Mosby; 1992.

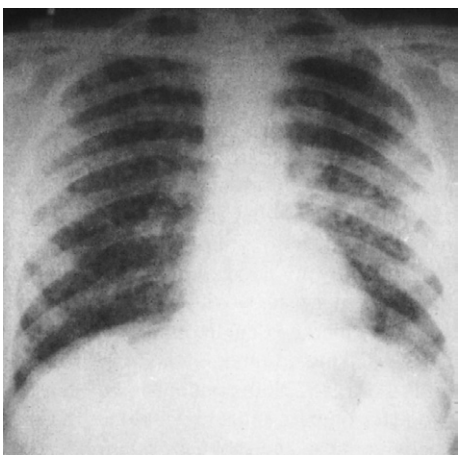


Fig. 30.10 Miliary TB. Chest radiograph of miliary TB showing characteristic mottled shadowing throughout both lung fields. Courtesy of Dr W Lynn, Ealing Hospital, UK.

Extrapulmonary tuberculosis

This section reviews extrapulmonary tuberculosis not considered in detail elsewhere and refers readers to other relevant chapters. The 2008 Annual Report of the Health Protection Agency (http://www.hpa.org.uk/web/HPAweb&HPAwebStandard/HPAweb_C/1225268885969) revealed that 44% of all cases of tuberculosis diagnosed in the UK was extrapulmonary. It is very likely that in resource-poor areas of the world, where the focus is on diagnosis of people who are potentially infectious, a large amount of extrapulmonary disease goes undetected.

Lymph node disease (see Chapter 14)

Lymphadenitis, also known as scrofula or the 'King's evil' (so called because in Europe the royal touch was thought to be curative in the Middle Ages), is a very common extrapulmonary presentation of tuberculosis. Peripheral nodes are usually infected following hematogenous spread of *M. tuberculosis* from the lung. The most commonly involved nodes are those in the cervical region, sometimes in association with axillary, inguinal or hilar lymphadenopathy. Patients may present with painless lymphadenopathy or with marked systemic symptoms. There may be associated sinuses and abscess formation. Hilar nodes may be associated with thoracic pain and untreated may infrequently result in esophageal erosion or a bronchoesophageal fistula. Unusual symptoms such as obstructive jaundice have been reported in specific association with anatomically defined local lymph nodes.

Tuberculosis of the head and neck

Aside from cervical node disease, tuberculosis of the head and neck is relatively uncommon and usually arises secondarily to pulmonary infection. Laryngeal tuberculosis may present with hoarseness, pain on speaking or swallowing, hemoptysis and respiratory obstruction. Cough may reflect lung disease or involvement of the superior laryngeal nerve. Untreated, widespread local tissue destruction may occur with secondary laryngeal stenosis. Chest X-ray may be suggestive but in one series radiographic changes were minimal in two-thirds of cases. In this study from a specialist center, coexistent squamous cell carcinoma of the larynx was present in 10% of cases. Therefore, all laryngeal lesions should be biopsied to exclude malignancy.

Tuberculosis rarely involves the middle ear when it may present with a local, painless discharge from which the organism may be cultured. There may be destruction of the ossicles, hearing loss and invasion of the facial nerve canal, sinuses and extension into the posterior cranial fossa. Secondary pyogenic infection may obscure the diagnosis. Tuberculosis of the nose, nasopharynx and adenoids is rare even in areas where infection is endemic and symptoms such as epistaxis reflect local tissue damage. Tuberculosis in the oral cavity generally presents as a solitary, often inflamed ulcer with irregular borders. There may be secondary infection of salivary glands.

Musculoskeletal infection

Vertebral infection (Pott's disease) is the commonest presentation of tuberculous osteomyelitis, accounting for about 50% of all cases of bony tuberculosis (Fig. 30.11). The male to female ratio is approximately 2:1. The thoracic spine is most frequently involved, followed by lumbar and then cervical regions. Presentation is usually with back or neck pain. Systemic symptoms tend to be less marked than in pulmonary disease. Neurologic symptoms such as weakness, numbness and disturbances of gait occur in about a third of cases. Some patients have an associated flank mass or other evidence of extraspinal



Fig. 30.11 Vertebral TB. Tuberculosis of the spine or Pott's disease. Kyphosis is secondary to anterior destruction of vertebral bodies resulting in wedging of adjacent vertebrae and loss of disk space clearly seen by radiography. Courtesy of Professor J Cohen, Brighton, UK.

tuberculosis. In more advanced cases, vertebral body collapse and gibbus formation lead to kyphosis of the spine. Destructive lesions with invasion of the joint space and deformity may be seen on plain X-rays, although CT or ideally MRI scanning is a better imaging modality for spinal pathology (Fig. 30.12).

Osteomyelitis is otherwise most frequently found in the metaphyses of long bones, although rib, pelvis and skull may be infected. In children in countries where tuberculosis is prevalent, osteomyelitis is a significant cause of crippling deformity. Direct extension from the ribs to the lung is rare, as is meningitis or tuberculomas in cases where there is skull involvement. Bony tuberculosis may be accompanied by sinus tracts or soft tissue masses. Diagnosis may be difficult since lesions can appear osteolytic or sclerotic on X-ray and malignancy may be suspected at first. Like X-rays, ^{99m}technitium bone scans have no pathognomonic features. Trauma does not predispose to tuberculous osteomyelitis.

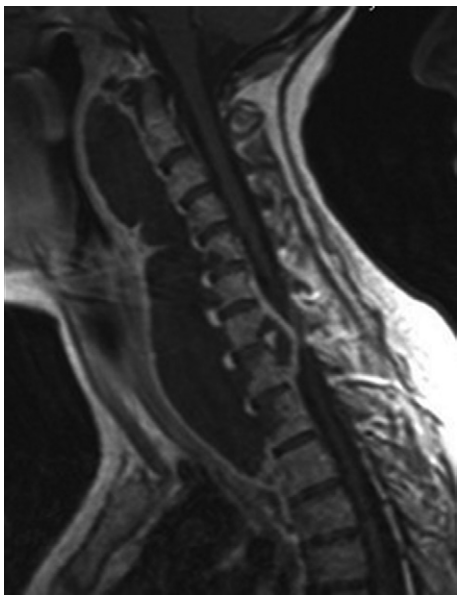


Fig. 30.12 MRI scan of cervical tuberculosis with massive paravertebral abscess which extends through the left C7 to T1 neural exit foramen causing an extradural collection compressing the spinal cord. Courtesy of Dr J Jackson, Imperial College Healthcare NHS Trust, London, UK.

Tuberculous arthritis most frequently presents in the hips and other weight-bearing joints although any joint may be involved. Polyarticular disease occurs in less than 20% of patients but evidence of tuberculosis elsewhere, generally the lung, is present in about 50% of cases. Synovial fluid is usually turbid with a high leukocyte count and up to 60% may be neutrophils rather mononuclear cells. Organisms are found in less than a quarter of cases although positive cultures are more frequent. Ideally a synovial biopsy should be examined histologically and microbiologically. Prosthetic joints may be infected with *M. tuberculosis* following either hematogenous spread or contiguous reactivation of infection. Tenosynovitis is rare and is generally associated with adjacent osteomyelitis.

Tuberculous abscesses may form in most soft tissues including muscle and may be multiple (Fig. 30.13). This is usually secondary to contiguous spread of infection but may follow hematogenous dissemination. The classic abscess site is in the psoas muscle, which can present with or without localizing signs, and a high index of clinical suspicion is necessary for the appropriate imaging to be arranged prior to diagnostic aspiration.

Abdominal infection

The abdomen is a frequent site of tuberculosis, especially in patients from the Indian subcontinent, where rates are up to 50 times higher than those in Europe, and those immunosuppressed with HIV. Disease is usually secondary to hematogenous spread of mycobacteria but can be secondary to local invasion or ingestion of organisms. Tuberculosis frequently mimics other pathologies such as gastrointestinal malignancy and diagnosis is often delayed. In the gut, tuberculosis most frequently affects the ileocecal region, then the small bowel and then the colon, with involvement of the duodenum occurring in less than 2.5% of cases and the stomach and esophagus in less than 1% of patients. Approximately one-third of patients have evidence of tuberculosis elsewhere, usually in the lung.

Symptoms reflect the site of involvement but may be nonspecific with fever, weight loss, chronic abdominal pain, nausea and anorexia.



Fig. 30.13 Abscess formation in TB. This patient has multiple TB abscesses particularly affecting the psoas and quadriceps muscle groups.

Diarrhea occurs in less than 20% of cases and reflects either secondary overflow due to obstruction or stimulation of the gastrointestinal tract by cytokines. Ileocecal tuberculosis may present as an acute abdomen secondary to either obstruction of the bowel lumen or appendix or following bowel perforation. Any tuberculous lesion, particularly those in the colon, may present with massive gastrointestinal bleeding but this is rare. Lesions may be ulcerative or hypertrophic and can be associated with fistulas which are probably due in part to secondary bacterial involvement. Unusual sites of intra-abdominal tuberculosis include the pancreas and the adrenal glands where disease presents very rarely as an adrenal crisis but more commonly with an insidious onset of symptoms that may be difficult to distinguish from those associated with infection. Tuberculous peritonitis is an important manifestation of disease and is discussed in Chapter 37. Hepatobiliary infection is considered in Chapter 39. Tuberculosis of the urogenital tract is specifically explored in Practice Point 28.

Pericardial infection

Tuberculous pericarditis is potentially fatal and may present in diverse ways. Onset is often insidious although acute pericarditis may occur. Common symptoms include breathlessness, chest pain and nonspecific changes such as fever and weight loss. Signs of cardiac tamponade such as raised jugular venous pressure, hepatomegaly, ascites and edema may be present, and in a minority of cases develops acutely requiring urgent pericardiocentesis. ECG may show low voltage or ST elevation consistent with acute pericarditis. An effusion may be seen on chest X-ray but is better characterized by echocardiogram or CT scanning. Pericardial calcification occurs late and in less than 25% of cases although data are limited. Diagnosis may be extremely difficult; failure to identify the organism and to detect granuloma on biopsy specimens does not rule out infection. Constrictive pericarditis may develop after active infection has resolved or been treated.

Central nervous system and eye disease

Tuberculous meningoencephalitis and central nervous system tuberculomas are extremely important, carrying a high morbidity and mortality. These topics are considered further in Chapters 18 and 20.

Ocular tuberculosis is relatively uncommon but important since, if overlooked, blindness may result. The commonest manifestation is choroidal disease (see Fig. 30.9) secondary to hematogenous spread in the context of miliary tuberculosis which may rarely spread to the retina. Conjunctival tuberculosis may be due to accidental self-inoculation by an infected person, spread from skin or very rarely by direct spread from another infected individual. Infection may be ulcerative or nodular although occasionally focal tuberculomas, sometimes appearing polypoid, are seen. Tuberculosis of the sclera and cornea are rare as is uveitis.

Dermatologic disease

Lupus vulgaris is the best documented manifestation of dermatologic tuberculosis and details of the dermatologic manifestations of primary and miliary infection are to be found in Chapter 12.

Clinical manifestations in HIV-positive patients

The clinical manifestations of tuberculosis in HIV-seropositive and -seronegative patients are often similar and are reviewed in Chapter 93. However, as the CD4 T-lymphocyte count falls, tuberculosis may be more widespread, have more atypical features and is more often extrapulmonary. Many relatively unusual presentations of tuberculosis have been reported, bronchoesophageal fistula being one example.[31] *Mycobacterium tuberculosis* bacteremia is more frequent in HIV-seropositive patients. Nonspecific systemic features of tuberculosis such as fever, malaise and a prolonged acute phase protein response are also characteristic of HIV infection.

Noninfectious complications

Erythema nodosum is the most common noninfectious complication of primary tuberculosis although it may occur in other granulomatous diseases (e.g. leprosy, sarcoid). It is particularly associated with primary disease. Arthritis occurs in up to 1% of patients with acute infection and may also complicate reactivation. Poncet's disease is a reactive polyarthritis associated with tuberculosis which resolves with antimycobacterial chemotherapy. Bazin's disease is a vasculitic skin reaction to tuberculosis which usually manifests as a purpuric rash on the lower extremities. Another important immunologic complication of tuberculosis is renal interstitial nephritis. Eales disease is the association of tuberculosis with retinal vasculitis with or without neurologic symptoms. More common in patients from the Indian subcontinent, the retinal manifestations may occur before or after the development of clinically apparent tuberculosis.

Bronchogenic carcinoma was thought for many years to be more frequent in patients with tuberculosis but this has not been borne out in careful studies. Hypertrophic pulmonary osteoarthropathy, principally associated with bronchogenic carcinoma, has been reported in tuberculosis[32] although some attribute the finding to undiagnosed lung malignancy. The syndrome of inappropriate antidiuretic hormone secretion is more common in pulmonary and miliary disease.

DIAGNOSIS

Clinical approach

The definitive diagnosis of tuberculosis requires identification of the pathogen in a patient's secretions or tissues. Therapy is often initiated before a definitive diagnosis has been made but this should always be pursued to determine the drug susceptibility of the organism which guides individual treatment regimens. Sputum microscopy and

culture has a high diagnostic yield, identifies the majority of infectious patients and is cheap to perform. Sputum should be collected on three separate occasions but additional specimens are not helpful.[33] Although induced sputum obtained in properly ventilated and isolated areas can be useful, bronchoscopy with alveolar lavage has the best diagnostic yield. *Mycobacterium tuberculosis* is infrequently seen on aspiration of pleural fluid and cultured in less than 50% of cases. Pleural biopsy reveals granuloma or results in culture of the pathogen in over 90% of cases. Thoracoscopy, in exceptional circumstances, may be indicated since it allows for visually guided biopsy of lesions. Elevated adenosine deaminase concentrations (in pleural fluid for example) support a diagnosis of tuberculosis but have limited specificity. 2-Fluorodeoxyglucose–positron emission tomography (FDG-PET) scans may be helpful in patients with tuberculosis but are also positive in other diseases such as malignancy (Fig. 30.14).

In extrapulmonary or miliary infection, appropriate body fluids and/or tissues must be obtained for microbiologic and histologic examination, with the aid of ultrasound, CT or MRI scanning if available. Liver biopsy and bone marrow aspiration are useful investigations in disseminated or occult disease.[34] An acid-fast stain of buffy-coat leukocytes may be diagnostic in the immunosuppressed. For tuberculous lymphadenitis, fine needle aspiration (FNA) is the initial investigation of choice since it is easy to perform and has a high specificity and sensitivity when both microbiologic and cytologic specimens are collected. FNA does not preclude subsequent lymph node biopsy. In pericardial tuberculosis, aspiration of pericardial fluid may recover the pathogen but is frequently nondiagnostic and pericardial biopsy is often indicated.

Specimens from potentially infected patients are normally analyzed in local laboratories but drug sensitivity testing and more specialized facilities are ideally concentrated in centralized laboratories. All laboratories must be arranged to deal with hazardous, possibly drug-resistant pathogens. Safety measures include containment areas, approved safety cabinets and centrifuges, as well as gowns, gloves, sinks and facilities for disposal of contaminated waste and treatment of spillages. Staff training is essential and may be legally required.

Usual principles of microbiologic safety apply to collection of specimens into sterile containers. Tissue biopsies should be divided and a fresh specimen sent to microbiology and one in formalin to histology. Tissue is better for culture than necrotic material and old pus which may contain acids toxic to mycobacteria, a fact to be considered when selecting biopsy sites. Blood and bone marrow specimens can be injected directly into BACTEC bottles. Examination of cerebrospinal fluid for *M. tuberculosis* is usually an emergency investigation with the clinician forewarning the laboratory. Specimens from gastric lavage should be rapidly transported to the laboratory since stomach acidity may kill mycobacteria. Feces are not useful in the diagnosis of *M. tuberculosis* in HIV-negative patients.

If no diagnostic results are forthcoming in patients in whom tuberculosis is a possibility, the clinician may reasonably resort to a trial of therapy. Improvement of symptoms and a decrease in the acute phase response (monitored by ESR, CRP, etc.) are sufficient indication to move from the diagnostic trial to full therapy. A diagnostic trial of therapy should last a minimum of 2 weeks and ideally 4.

Microbiologic diagnosis

The standard method for staining clinical specimens for *M. tuberculosis* is that of Ziehl–Neelsen (Fig. 30.15) which requires application of heat whereas a modification, the Kinyoun method, does not. Both methods utilize the fact that the stained cell wall is resistant to decolorization by acid alcohol although the biochemical reason for this is unknown. Auramine staining has facilitated rapid screening of diagnostic specimens and has greater sensitivity (Fig. 30.16). Mycobacteria are often scanty and specimens should be reviewed by an experienced operator. Diagnostic microscopy is most successful in patients with extensive disease and cavitation on chest X-ray but only diagnoses about 50% of cases positive by culture. Culture of the organism with subsequent testing for drug sensitivity from clinical specimens is the cornerstone of diagnosis and ultimate treatment; this is particularly important with the emergence of drug-resistant TB, as 'blind' therapy in the absence of sensitivity testing may increase the risk of multi-drug resistance. The clinical microbiology of tuberculosis is reviewed in Chapter 174.

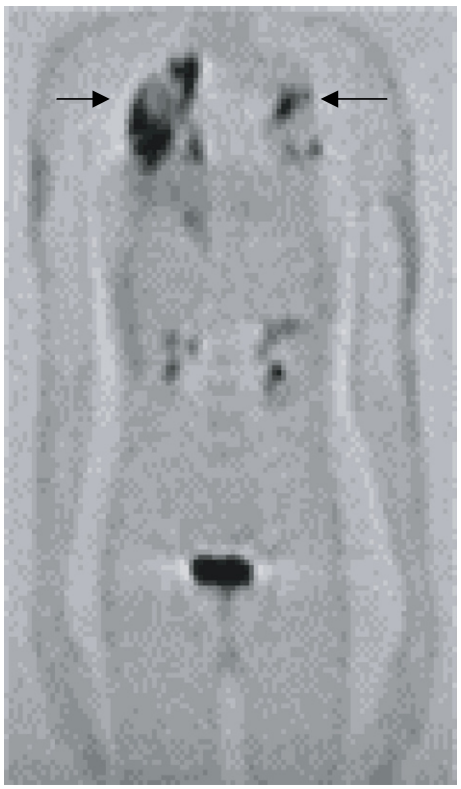


Fig. 30.14 2-Fluorodeoxyglucose–positron emission tomography (FDG-PET) scan in bilateral apical pulmonary TB (arrowed). Tracer is excreted via the renal tract and is seen in the bladder.

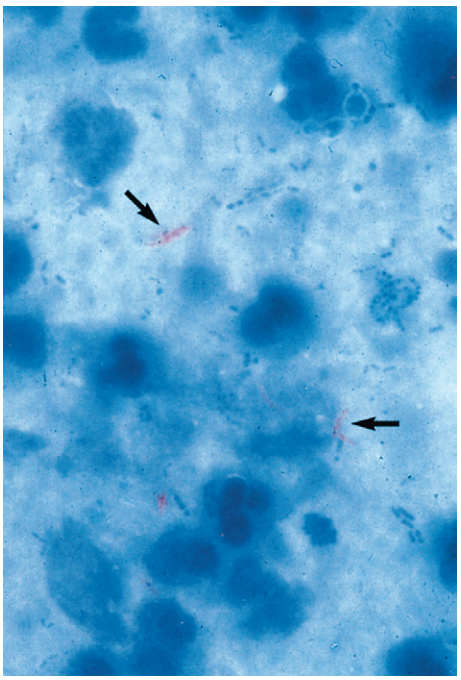


Fig. 30.15 Ziehl–Neelsen-stained sputum specimens containing *Mycobacterium tuberculosis*. Courtesy of Dr F Ahmed, Ealing Hospital, UK.

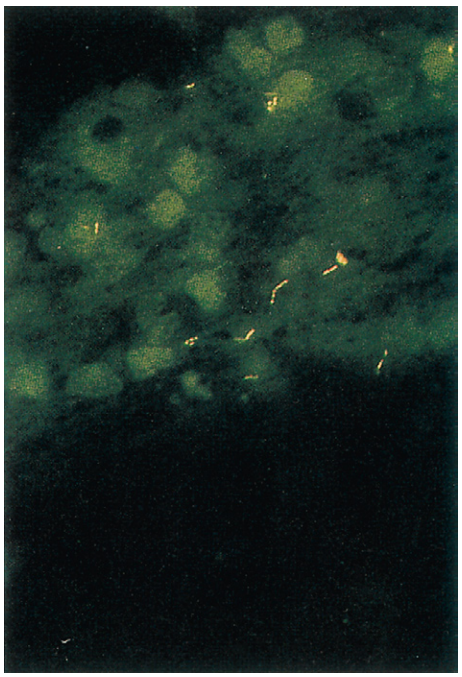


Fig. 30.16 *Mycobacterium tuberculosis* in sputum detected by auramine staining. Courtesy of Mr M Croughan, with permission from Edmond RTD, Rowland HAK, Welsby PD. A colour atlas of infectious disease. London: Mosby; 1992.

Genetic techniques

The polymerase chain reaction (PCR) has the potential to allow rapid detection of low levels of infection with the possibility of immediate probing for known drug-resistance genes. The usual target for DNA amplification is the insertion sequence IS6110 which is found in all the *M. tuberculosis* complex of organisms (*M. tuberculosis, M. bovis, M. africanum* and *M. microti*) or ribosomal RNA. Considerable methodologic problems with PCR have been encountered and sensitivity is still laboratory dependent outside recognized expert centers. PCR is potentially of most use in smear-negative culture-positive disease but sensitivity in pulmonary disease is no greater than culture. A problem is that PCR positivity in sputum may persist during treatment and after clinical cure.[35] PCR is potentially useful in diagnosing extrapulmonary tuberculosis but to date has been disappointing.

The major role of PCR is to determine drug sensitivities in culture and smear-positive specimens. In particular, detecting mutations in the *rpoB* gene which encode about 96% of resistance to rifampin is useful in identifying patients likely to have multidrug-resistant (MDR) TB. Line probe assay kits for detection of drug resistance (particularly to rifampin[36]) were approved by the WHO in 2008 for use in resource-poor countries although there is a severe shortage of well-resourced laboratories in many such countries.

Serodiagnosis

Serodiagnostic techniques do not perform well enough for routine use (http://www.stoptb.org/wg/new_diagnostics/assets/documents/jane_cunningham.pdf). Enzyme-linked immunoassays have been developed to detect a number of mycobacterial proteins and lipoarabinomannan but the sensitivity of antibody testing is not sufficiently high in ELISAs with adequate specificity. Local testing for antibodies at sites of infection such as in pleural effusions has also not proved to be useful. Antibody levels are often low in culture-negative individuals where alternative diagnostic approaches are needed. Alterations in antibody levels over time are not a useful guide to clinical response to treatment.

MANAGEMENT

First-line antimycobacterial drugs and their toxicities

This and the next section briefly outline the principal characteristics of the most important antituberculous drugs. These drugs are frequently used in fixed-dose regimens in various combination tablets in order to simplify regimens and improve patient compliance. More detail as to mechanisms, dosage regimens and complications are to be found in Chapter 143.

Isoniazid

Isoniazid (INH or isonicotinic acid hydrazide: $C_6H_7N_3O$) is a bactericidal drug (minimum inhibitory concentration generally <0.1 µg/ml) whose biologic activity resides in a pyridine ring and a hydrazine group. Isoniazid is well absorbed, minimally bound to plasma proteins, has a half-life of 1–3 hours depending on patient acetylator status and is excreted in urine. Concentrations achieved in most tissues are similar to those in serum with the important exception of the CNS where concentrations are approximately 20% of those in serum. Thus, the drug may be used at double the normal dose in CNS disease. Isoniazid enters *M. tuberculosis* by diffusion and by oxygen-dependent active transport where it inhibits mycolic acid synthesis, the nicotinamide adenine dinucleotide (NAD) pathway and interacts with the catalase–peroxidase enzyme system. Mutations in the *katG* gene encoding catalase–peroxidase confer resistance to isoniazid and account for approximately 25% of cases of drug resistance.[37]

The most important, potentially fatal side-effect of isoniazid is hepatotoxicity which is more common in patients older than 60 years, in the presence of coexisting liver disease and possibly in pregnancy. Asymptomatic, usually transient rises in transaminases occur in about 20% of patients. A greater than threefold increase in enzyme levels above the normal range is an indication to discontinue therapy. In practice, it is often impossible to separate isoniazid hepatotoxicity from that due to rifampin or pyrazinamide but interestingly, re-introduction of these drugs singly rather than in combination usually avoids a second episode of hepatotoxicity. The other major side-effect is a peripheral and rarely optic neuritis due to interference with niacin metabolism. This is prevented by concomitant administration of vitamin B_6 (pyridoxine 10 mg daily). Less serious side-effects include nausea, vomiting and arthralgia, while rare ones are hypersensitivity reactions, lupus-like reactions, cerebellar ataxia, convulsions, psychoses, hyperglycemia, agranulocytosis and in malnourished patients, pellagra.

Rifampin

Rifampin is a bactericidal drug which undergoes first-pass metabolism in the liver where it is deacylated, excreted into bile and then into the gut where there is a minor degree of enterohepatic circulation. Rifampin is a potent inducer of hepatic enzymes and therefore has many clinically significant interactions with other drugs (Table 30.2). Rifampin targets the RNA polymerase beta subunit blocking initiation but not elongation of mRNA transcripts.

Patients should be warned that rifampin turns all body secretions, including urine and tears, orange (Fig. 30.17). This is a useful side-effect in terms of monitoring compliance. Rifampin may cause hepatic injury, particularly in the presence of pre-existing liver disease. Gastrointestinal side-effects seldom necessitate stopping therapy and include anorexia, nausea, vomiting, abdominal pain and diarrhea. Pseudomembranous colitis due to *Clostridium difficile* toxin production is described. Immune-mediated problems associated with rifampin include rashes, urticaria, conjunctivitis and rarely hemolysis or thrombocytopenic purpura. A flu-like syndrome may

Table 30.2 Drug interactions with rifampin (rifampicin)*

Drug category	Example
Antibacterials	Chloramphenicol
Antifungals	Fluconazole
Antimalarials	Mefloquine
Corticosteroids	Prednisolone
Anticoagulants	Warfarin
Analgesics	Methadone
Immunosuppressive therapy	Ciclosporin
Ulcer-healing drugs	Cimetidine
Respiratory drugs	Theophylline
Cardiac drugs	
Beta-blockers	Propranolol
Calcium channel blockers	Diltiazem
Cardiac glycosides	Digitoxin (only member of class affected)
Antiarrhythmics	Disopyramide
Lipid-lowering drugs	Fluvastatin
CNS drugs	
Anti-epileptics	Phenytoin
Anxiolytics	Diazepam
Antidepressants	Tricyclic compounds
Antipsychotics	Haloperidol
Endocrine drugs	
Antidiabetics	Tolbutamide
Estrogens and progesterones	Combined and progesterone only contraceptive pill
Thyroid replacement	Thyroxine

*These are the principal classes of drugs for which metabolism is increased (plasma concentration decreased) when taken with rifampin (rifampicin). The examples are not exhaustive and clinicians should check interactions with related drugs in appropriate formularies. (Interactions with drugs used to treat HIV are considered elsewhere.)

be troublesome, particularly in intermittent therapy regimens, and very rarely may be severe with circulatory collapse and respiratory failure.

Pyrazinamide

Pyrazinamide, a structural analogue of nicotinamide, is a bactericidal drug which is well absorbed via the gut and distributed widely including in the CNS where concentrations are the same as those in serum in patients with tuberculous meningitis. Serum half-life is about 10 hours and excretion is urinary. Pyrazinamide principally acts intracellularly at relatively acidic pH although it may also have some cidal action on extracellular bacteria. The drug penetrates macrophages where an enzyme from *M. tuberculosis* converts it into active pyrazinoic acid. Some strains of *M. tuberculosis* are resistant to pyrazinamide due to production of the enzyme pyrazinamidase.

Hepatotoxicity ranging from elevation of liver transaminases to frank jaundice and liver failure is the principal side-effect of pyrazinamide. More common are mild gastrointestinal problems and an arthralgia associated with raised serum urate concentrations secondary to inhibition of tubular secretion of uric acid by pyrazinamide. Classic gout is rare. Sideroblastic anemia has been reported.



Fig. 30.17 Rifampin (rifampicin) urine testing. Patients should be warned that rifampin turns urine and other body secretions orange. This fact can be helpful in monitoring compliance with drug treatment. Courtesy of Dr W Lynn, Ealing Hospital, UK.

Ethambutol

Ethambutol is a bactericidal drug which is rapidly and well (over 80% of the dose) absorbed in the gut, with peak serum levels occurring 2 hours after a dose. It is then rapidly excreted in urine. Ethambutol appears to alter both *M. tuberculosis* RNA synthesis and transfer of mycolic acids into the cell wall. Changes in cell wall lipids have been noted in organisms resistant to the drug.

The most important complication of ethambutol therapy is retrobulbar neuritis manifest by impaired visual acuity, color blindness and restricted visual fields. Except in patients with pre-existing ophthalmic disease, optic neuritis is extremely rare when ethambutol is used at standard doses (15 mg/kg). In affluent countries, it is appropriate to have patients assessed by an ophthalmologist prior to starting treatment but lack of this facility should not prevent use of ethambutol. Patients should be warned to report symptoms of visual change immediately and ethambutol should generally be avoided in children. Very rarely ethambutol may cause a peripheral neuritis.

Streptomycin

Streptomycin, the first clinically useful drug discovered in the fight against tuberculosis, is an aminoglycoside that has to be given intramuscularly. Streptomycin penetrates cerebrospinal fluid and other remote tissues (e.g. prostate and eye) poorly. There is an immediate and a delayed pathway of excretion via the renal tract. The persistence of the drug at low doses is one factor in the development of side-effects. Streptomycin binds to the 30S ribosomal RNA subunit which results in decreased protein synthesis and misreading of mRNA. Mutations including those in the 30S subunit arise readily in response to isolated streptomycin therapy and lead to drug resistance. The principal side-effects of aminoglycosides are ototoxicity and nephrotoxicity. Rarer ones are neuromuscular blockade and hypersensitivity reactions with maculopapular rashes, fever and eosinophilia.

Second-line drugs

Second-line agents are becoming increasingly important with the rise in drug-resistant organisms. Basic information about such drugs is provided in Table 30.3. Quinolones such as levofloxacin and moxifloxacin are emerging as potentially valuable tools in the fight against tuberculosis and they are being investigated as agents that may reduce the duration of therapy required for treatment of drug-sensitive disease. The value of proven efficacious drugs such as ethionamide is limited by toxicity. Side-effects such as Stevens–Johnson syndrome have effectively eliminated the use of thiacetazone. The potential second-line role of clofazimine, used in the treatment of *M. leprae*, is uncertain. There is evidence to suggest that there may be a role for some drugs not normally considered as useful treatment for tuberculosis such as imipenem[38] and linezolid.[39] More details concerning second-line agents can be found in Chapter 143.

Treatment regimens

Many trials, notably ones involving the British Medical Research Council, have established short-course chemotherapy as the preferred treatment of pulmonary, pleural, nodal and most other forms of tuberculosis. Such regimens are dependent on using the potent antituberculous drugs isoniazid and rifampin throughout a 6-month course as well as 2 months of initial treatment with pyrazinamide. A fourth drug (e.g. ethambutol or streptomycin) is now routinely used in the induction period to ensure that single-resistant organisms are effectively treated. The aim of multidrug therapeutic regimens is to kill *M. tuberculosis* whilst preventing the spontaneous emergence of drug-resistant mutants.

One standard treatment protocol is given in Table 30.4. Alternative regimens are essential in the presence of drug resistance and are possible when drugs are in short supply. For example, 6 months of isoniazid and ethambutol can substitute for isoniazid and rifampin in the period following 2 months of quadruple therapy. For sensitive organisms, drug therapy in compliant patients is very efficacious with cure rates approaching 100%. However, it may be 2 weeks before clinical improvement becomes apparent which is important in both empiric trials of therapy and for appropriate patient expectations. Radiologic improvement lags further behind and it may take 3–5 months before all that remains is residual scarring on chest X-ray. Drug therapy must be linked with the public health measures discussed above.

The necessary duration of treatment for extrapulmonary tuberculosis is debated. Limited trials in osteomyelitis, regarded as a difficult site to treat, indicate that 9 months of therapy is effective providing both isoniazid and rifampin are used. In miliary disease, the 6-month regimens have been very successful. Complications may develop during treatment of disease, partly as a result of the influx of inflammatory leukocytes. The consequences of this depend on the site of infection and include pneumothoraces, expansion of intracerebral granulomas or discharge from subcutaneous nodes.

Directly observed therapy

Compliance with therapy is critical for successful treatment and to limit development of drug-resistant strains. Many social, personal, public health and economic factors influence patient noncompliance with treatments. The WHO and others strongly advocate directly observed therapy, short-course (DOTS) regimens although their efficacy in diverse clinical settings is not completely established.[40] When patients are in hospital, normal treatment regimens may be used but for patients in the community intermittent therapy (Table 30.5) is preferred. DOTS as defined by the WHO is a five-point strategy involving political commitment, increased case detection, standardized and closely supervised treatment, an effective drug supply and an established monitoring and evaluation system (http://www.who.int/tb/dots/en).

Treating multidrug- and extensively drug-resistant organisms

Multidrug resistance is defined as resistance to at least isoniazid and rifampin and has become a major international problem. In the fourth

Table 30.3 Second-line therapy in TB (see also text and Chapter 143)

Drug (chemically closely related drug)	Mechanism of action	Toxicity	Usual initial adult dose
Para-aminosalicylic acid (PAS)	Competes with mycobacterial dihydropteroate synthetase May decrease proinflammatory immune responses	Gastrointestinal intolerance, hypersensitivity, hypothyroidism, crystalluria	12 g q24h (divided doses)
Ethionamide (prothionamide)	Inhibits cell wall mycolic acid synthesis	Gastrointestinal intolerance, hepatitis, hypersensitivity, convulsions, depression, alopecia	0.5–1.0 g q24h (divided doses)
Moxifloxacin (levofloxacin, ciprofloxacin)	Inhibits topoisomerase II	Gastrointestinal intolerance, crystalluria, tremor, convulsions, rash, hepatitis, renal failure	400 mg q24h
Capreomycin (viomycin)	Binds 30S and 50S ribosomes	Nephrotoxic, ototoxic, hypersensitivity	1 g q24h
Kanamycin	Binds 30S ribosome	Nephrotoxic, ototoxic, hypersensitivity	15 mg q12h*
Amikacin	Binds 30S ribosome	Nephrotoxic, ototoxic, rash, neuromuscular blockade, eosinophilia	7.5 mg/kg q12h*
Cycloserine	Competitive D-alanine analogue	Seizures, psychoses, various CNS effects	250 mg q12h

*Peak drug levels should be less than 3 mg/dl (30 mg/l) and trough less than 1 mg/dl (10 mg/l).

Table 30.4 Short-course chemotherapy regimen for treatment of TB and necessity for drug dose modifications in renal impairment

Drug	Adult dose (orally)	Duration of treatment	Modification of drug dose in renal failure
1. Isoniazid*	5 mg/kg (maximum 300 mg)	6 months	No
2. Rifampin (rifampicin)*	10 mg/kg (maximum 600 mg)	6 months	No
3. Pyrazinamide*	30 mg/kg (maximum 2.0 g)	2 months	↓ dose or ↑ dosage interval
4. Ethambutol	15 mg/kg †	2 month	↓ dose or ↑ dosage interval
or			
Streptomycin	15 mg/kg im (maximum 1.0 g)	2 months	↓↓ dose or ↑↑ dosage interval

*Isoniazid and rifampin (rifampicin) are marketed as a single combination tablet ± pyrazinamide which may facilitate compliance.
†Some authorities use ethambutol at 25 mg/kg for 2 months only (longer courses should be at 15 mg/kg).

Table 30.5 Intermittent chemotherapy for TB

Drug	3 times/week dose
1. Isoniazid	10 mg/kg for 6 months (maximum dose 900 mg)
2. Rifampin (rifampicin)	10 mg/kg for 6 months (maximum dose 900 mg)
3. Pyrazinamide	40 mg/kg for 2 months (maximum dose 3.0 g)
4. Ethambutol	30 mg/kg for 2 months
or	
Streptomycin	15 mg/kg for 2 months

global report on drug resistance released in 2008 and available on the web, the WHO estimate that there are about 500 000 new cases of MDR tuberculosis worldwide, with the greatest burden of such disease in the Russian Federation, China and India. This is a consequence of multiple factors including poor prescribing practice by doctors, patient noncompliance with treatment and the endogenous mutation rate of *M. tuberculosis*. Patients should never be prescribed fewer than three drugs initially and seldom fewer than four if there is any chance of resistance or a history of previous therapy irrespective of the sensitivity of the organism initially isolated.

In 2006, a large cohort of HIV-infected patients in South Africa with extensively drug-resistant (XDR) tuberculosis (MDR tuberculosis plus resistance to at least a quinolone and one injectable agent) and a high mortality was described.[41] It has rapidly become apparent that this is a worldwide problem not restricted to HIV-positive individuals and approximately 50 countries have reported patients with XDR tuberculosis. This is an alarming trend.

Treatment protocols for MDR and XDR disease must be designed for the individual but the aim is use as many of the first-line drugs as possible before adding in second-line drugs. The WHO Green Light Committee provides second-line drugs in many parts of the world. A second-line therapeutic regimen should be continued for 18–24 months after cultures are negative. It is critical that affected patients are adequately isolated. Treatment of MDR tuberculosis can be difficult and reported success rates vary in different parts of the world[42,43] (Fig. 30.18). A concern is the rise of secondary drug resistance in other pathogens such as the *Pneumococcus* normally treated with second-line antituberculous agents such as quinolones.[44]

The use of steroids (see also Practice Point 13)

Steroid therapy is often advocated but seldom proven to be of benefit in tuberculosis. In pericardial tuberculosis, steroids have been shown to decrease acute mortality from pericarditis and reduce the need for pericardiocentesis.[45] They do not appear to influence the long-term

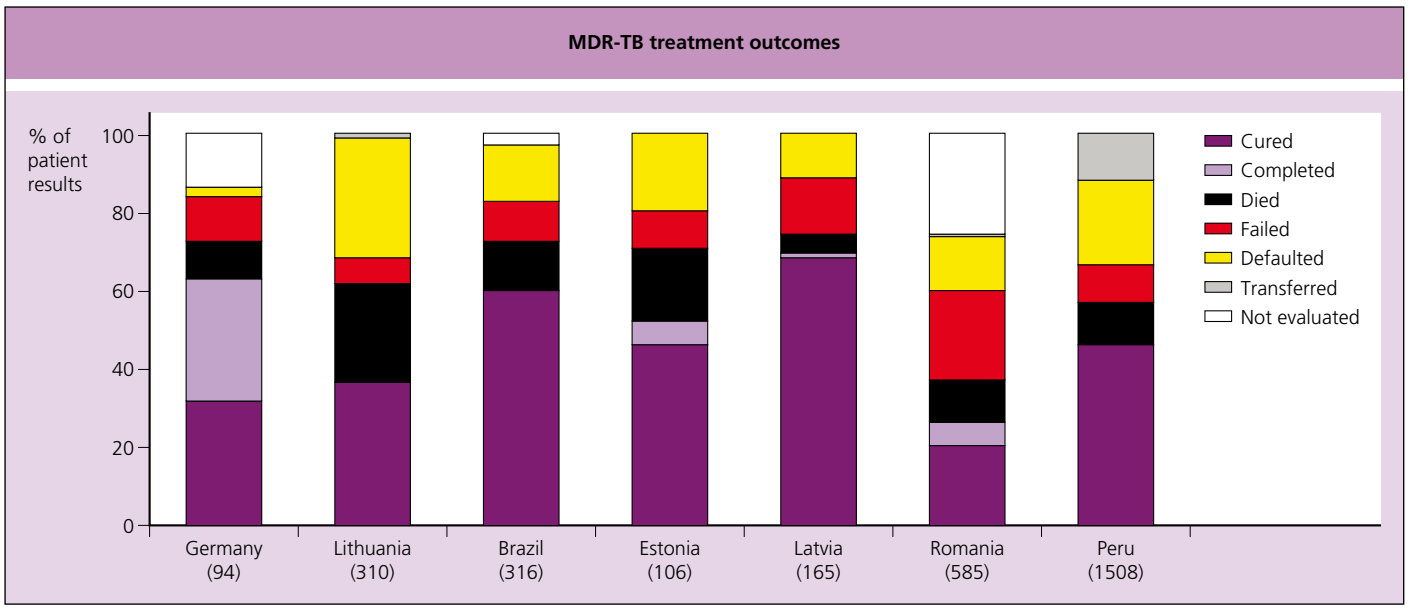


Fig. 30.18 MDR-TB treatment outcomes. Reproduced with permission from the WHO, 2008.

development of constrictive pericarditis. Steroids may have a role in pleural disease but further data are needed before they can be recommended.[46] Steroids are indicated during treatment of CNS tuberculomas to limit their expansion due to cellular influx and prevent raised intracerebral pressure and in tuberculous meningitis.[47] Retrospective studies investigating the role of steroids in miliary tuberculosis have shown no benefit. In all cases, steroid metabolism is increased by rifampin.

Pregnancy

Pregnancy probably does not alter the severity or reactivation rate of tuberculosis or responses to therapy.[48] However, there may be a significant increase in spontaneous abortions and labor difficulties. Congenital tuberculosis is very rare, presents with hepatosplenomegaly, respiratory distress and fever, and carries a high mortality.[49] Tuberculosis frequently requires treatment during pregnancy and this should not be deferred. Isoniazid, rifampin and ethambutol are not teratogenic but few data exist on pyrazinamide. Streptomycin is associated with fetal hearing loss and should be avoided. Little is known about second-line drugs in pregnancy except *para*-aminosalicylic acid (PAS) which appears to be safe. The presence of antituberculous drugs in breast milk is seldom a problem unless both mother and child are on treatment, in which case up to 20% more isoniazid than indicated may be taken by the child and bottle feeding is preferable. Breast-feeding children of mothers taking isoniazid require pyridoxine supplementation.

Surgery for tuberculosis

Surgical techniques such as artificial pneumothoraces, phrenic nerve paralysis, plombage and thoracoplasty are part of the history of tuberculosis before the era of chemotherapy. Now surgeons are most often involved in diagnostic rather than therapeutic procedures. However, resection of tissue may be very useful in patients with MDR or XDR tuberculosis and circumscribed disease.[50] Other indications include massive hemoptysis (after embolization has failed) and in the management of tuberculous empyema, draining sinuses and bronchopleural fistulae. Surgery has been widely used in the treatment of spinal tuberculosis but is only indicated in the presence of progressive neurologic abnormality and spinal instability and to drain large paravertebral abscesses where CT-guided drainage is not possible. Surgery may be required following destructive tuberculosis involving weight-bearing joints. Surgery may have an adjuvant role in nodal tuberculosis if a fluctuant mass persists. In pericardial disease, a pericardial window removes the need for repeated drainage. Pericardectomy may be required in constrictive disease which may develop in patients with previous or treated infection. The most appropriate timing of such surgery remains controversial. Success rates are greater if surgery is performed before extensive calcification develops. In laryngeal infection, temporary tracheotomy may be necessary and a few patients may require complex surgery such as partial laryngectomy.

Immunotherapy and the future

Drugs remain at the forefront in the treatment of tuberculosis but only a small number of new drugs are in development such as the diarylquinolines.[51] These may be insufficient to deal with the increasing amount and complexity of drug-resistant disease. The long-term aim must be to produce true short-course therapy of a few weeks' duration, rather than months, but study is hampered by inadequate biomarkers of disease. Increasing understanding of immune and inflammatory responses including the processes that drive tissue damage and development of cavitation may allow the development of novel therapies or treatment vaccines. Despite the increasing research funds being directed at tuberculosis, successes to date have been limited, in part due to inadequate *in vivo* models of this multifaceted disease of humans.

Nontuberculous Mycobacterial Infections

Nontuberculous mycobacteria (NTM) include all mycobacteria except for those in the *M. tuberculosis* group and *M. leprae*. This section briefly reviews the main pathogenic organisms focusing on clinical presentation and management.

EPIDEMIOLOGY

Nontuberculous mycobacteria are mainly environmental organisms although some are pathogens of other animal species. Many are found in soil and water including tap water. Transmission may be via the aerosol route as reported in *M. avium* complex infections or NTM may be ingested or infection may arise following direct exposure of broken skin. NTM are found worldwide. There are no good data on their incidence or prevalence as they are not notifiable diseases. Better diagnostic techniques are making their identification more frequent, rapid and accurate. Some NTM are more frequently though generally not exclusively reported from specific areas of the world such as *M. malmoense* from northern Europe and *M. fortuitum* complex from the USA. *Mycobacterium xenopi* which is relatively common in Europe is also frequently reported in Canada but is rare in the USA. *Mycobacterium ulcerans* is restricted to more tropical regions of Africa, South East Asia and Australia. In affluent countries, NTM infection, particularly by the *M. avium* complex, is associated with HIV (see Chapter 91) but this is not reported in sub-Saharan Africa, possibly in part because such patients die from other intercurrent illness including tuberculosis.

PATHOGENESIS

The noncaseating granuloma is the main host response to NTM. While there are many similarities, there are also subtle differences from the immune response to *M. tuberculosis* (see above). Granuloma formation may be poor to some NTM, particularly in the presence of immunodeficiency. There are diverse sites of infection, and in immunocompetent hosts with no structural abnormalities, the pathogen is often eliminated by the host response. NTM express virulence factors common to other mycobacteria whilst others seem relatively specific. Hemolysin has been identified as a virulence factor in disseminated *M. avium* infection.[52]

T lymphocytes have a key role in protection against NTM. NTM infection, particularly by the *M. avium* complex, is most common in HIV-seropositive patients when the $CD4^+$ T-cell count falls below 50/μl. *Mycobacterium ulcerans* infection is less strongly associated with HIV infection.[53] NTM infections are more common in patients with mutations in the IFN-γ and IL-12 pathways. TNF and granulocyte–macrophage colony-stimulating factor (GM-CSF) have both been implicated in driving the killing of NTM by monocytes and macrophages. There appear to be cross-immunologic responses between different types of mycobacteria but there is controversy as to whether infection by environmental NTM offers some protection against development of tuberculosis. In this context, it is interesting that repeated BCG vaccination in the Malawi population offered no protection against tuberculosis but decreased *M. leprae* infection rates.[54]

CLINICAL PRESENTATION

Mycobacterium avium complex

This complex includes the *M. avium* and *M. intracellulare* species. *Mycobacterium avium* infection may be disseminated in HIV-seropositive patients (see Chapter 91) whereas both species are associated with

more focal lung disease which tends to present in non-HIV-infected patients, although it is found in association with HIV.[55] Clinical signs may be nonspecific (e.g. malaise, fever and weight loss). Such symptoms in patients with HIV should alert the physician to the possibility of *Mycobacterium avium* complex (MAC) infection although the differential is wide and includes tuberculosis.

Pulmonary symptoms include cough and sputum production in the majority of patients while hemoptysis and dyspnea are rarer. MAC infection is well described in older men with chronic obstructive airways disease secondary to smoking or other underlying pulmonary abnormalities. MAC infection is more common in patients with a high alcohol consumption. Scoliosis, pectus excavatum and mitral valve prolapse are associated with pulmonary NTM including MAC infection but specific immune deficits are not well characterized.[56] Cystic fibrosis is associated with NTM including MAC infection and this may increase disease progression as well as susceptibility to other pathogens. MAC is also reported in children who are otherwise well. Bronchiectasis may both predispose to and be a consequence of infection and this may be more common in elderly nonsmoking women (Lady Windermere syndrome). Cavitatory disease is common in MAC infection which may mimic tuberculosis (Fig. 30.19) and multiple infiltrates are often found on CT scanning (Fig. 30.20). MAC aerosols can cause a hypersensitivity pneumonia (hot tub lung) presenting with dyspnea and dry cough, and ground glass shadowing and nodular change on CT scan.

MAC may cause lymph node disease in children.[57] Patients are usually under the age of 5 years and cervical nodes are most commonly affected. Other sites of MAC infection are rare but diverse. Tenosynovitis, septic arthritis and osteomyelitis are well described and occur mainly in the immunocompromised. Diverse skin manifestations have been reported.



Fig. 30.19 Apical cavitary infiltrates similar to those caused by pulmonary tuberculosis in a 60-year-old male cigarette smoker with *Mycobacterium avium* complex lung disease.



Fig. 30.20 *Mycobacterium avium* complex lung disease. Chest CT scan from a 52-year-old woman with MAC lung disease demonstrating three abnormalities that are common in MAC lung disease: bronchiectasis, a cavity and small (<5 mm) nodules.

Mycobacterium kansasii

Patients with *M. kansasii* usually present with respiratory symptoms that may be associated with night sweats, fever, weight loss and malaise. Presentation may be as insidious and prolonged exacerbation of underlying chronic obstructive airways disease. Hemoptysis may be present in up to a third of patients. Cavitation is frequent and disease is often located in the upper zones of the lung on radiologic examination of HIV-negative patients[58] (Fig. 30.21). Mediastinal lymphadenopathy and pleural effusions are very unusual. Respiratory or disseminated infection may be found in HIV patients. Lymph node disease occurs uncommonly, mainly in children.

Mycobacterium ulcerans

Mycobacterium ulcerans is the cause of Buruli ulcer which is a chronic necrotizing skin infection that is associated with stagnant water and the bugs residing within it. Inoculation is believed to follow minor trauma including bites. Initially a painless swelling develops which becomes a shallow ulcer with a necrotic base and characteristic undermined margins. Ulcers can become very large and involve joints or develop satellite lesions. This organism appears unique amongst NTM in that pathology is largely due to a secreted immunomodulatory cytotoxin called mycolactone.[59]

Other slowly growing atypical mycobacteria

Other slowly growing mycobacteria are a diverse group including *M. xenopi*, the *M. simiae* complex, *M. szulgai*, *M. malmoense*, *M. haemophilum*, *M. scrofulaceum*, *M. celatum*, *M. paratuberculosis* and

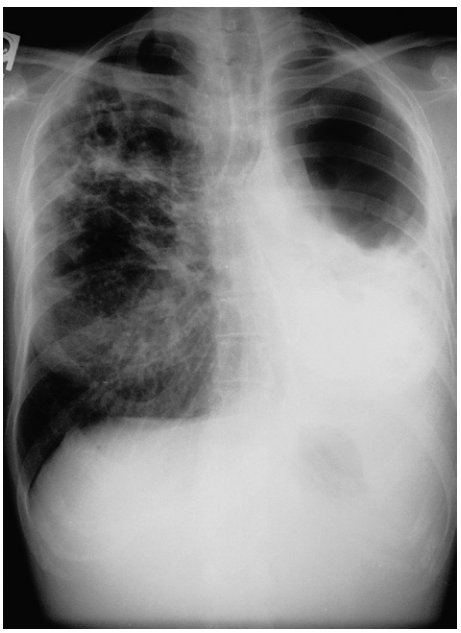


Fig. 30.21 Far advanced *Mycobacterium kansasii* bilateral ling disease in a 42-year-old male cigarette smoker with extensive cavity destruction of the left upper lobe.

M. genavense. *M. marinum* and *M. gordonae* which may grow somewhat faster are usually included within this group. Many members of this group grow optimally at lower temperatures, which for *M. marinum* is 30°C. This means that infection is usually localized to the skin, classically a single nodule on the skin which may spread along lymphatics to regional lymph nodes. Deep infections do occur but systemic spread and symptoms are rare. Minor trauma and exposure to water sports or hobbies may be found in *M. marinum* infections; this organism is also a fish pathogen.

In contrast, *M. xenopi* grows best at 43°C and has been recovered from hot water systems. It is a pulmonary pathogen in patients with underlying chronic lung disease. It may cause disease in patients immunosuppressed due to HIV or solid-organ transplantation. *Mycobacterium scrofulaceum* may cause lymph node disease but there is immunologic evidence that exposure to the organism is usually not associated with disease. Many of these organisms cause disseminated disease in HIV. They are also being increasingly recovered from patients with underlying lung disease although whether they are all of clinical significance is controversial. However, many would treat if there is evidence of increasing clinical symptoms with worsening changes on high-resolution CT scanning. Infection of skin, soft tissues, lymph nodes, indwelling plastic devices (including central lines) and bone, joint and tendon have all been reported. The number of mycobacteria associated with case reports of human disease, particularly in immunocompromised patients, is increasing and the list here is necessarily restricted.

Mycobacterium fortuitum complex and other rapid growers

The *M. fortuitum* group includes the named strain as well as *M. chelonae* and *M. abscessus*. There are many other rapidly growing mycobacteria, of which the *M. smegmatis* group (encompassing *M. smegmatis, wolinskyi* and *goodii*) is the best known. The *M. fortuitum* group of NTM is associated with both postsurgical infections and infections of indwelling catheters and shunts. They are also associated with skin infections or deep tissue infections which may be aggressive and disseminated. Infection can follow trauma including nail cutting.[60] These organisms are also more common in cystic fibrosis patients. *Mycobacterium fortuitum* has been found in deep CNS infections, sometimes following traumatic injury. *Mycobacterium smegmatis* organisms usually cause skin infections although lung infection and disseminated disease in a child with an IFN-γ receptor deficiency has been reported.[61]

DIAGNOSIS

The approach to diagnosis is similar to that in tuberculosis with the need to obtain a positive culture if at all possible. It is necessary for clinicians to be aware of the diagnostic possibility of NTM infection so that the correct samples are sent for the correct mycobacterial culture procedure. Alterations in high-resolution CT scans in patients with pulmonary disease may suggest the possibility of NTM and this is an important investigation in patients with potential NTM pulmonary disease. The diagnostic sample taken depends on the presentation of the infection and will range from sputum or bronchoscopic samples to lymph node biopsy. In childhood nodal disease fine needle aspiration is avoided to prevent the formation of discharging sinuses and scars. In systemic disease, particularly in the immunosuppressed, mycobacterial blood cultures are essential. NTM skin tests have no role. Genetic techniques are mainly used to determine whether smear- or culture-positive cases are due to NTM or the *M. tuberculosis* complex. PCR may be used to diagnose *M. ulcerans*. Drug sensitivity testing should be undertaken for NTM but not all laboratory breakpoints are well defined. In addition, there may be poor concordance between laboratory and clinical sensitivities.

In contrast to tuberculosis, the finding of a single positive sputum culture is not diagnostic of infection in the non-immunosuppressed patient but may represent contamination or colonization, although data concerning how and when colonization progresses to active infection are limited. In general, a single positive NTM sputum culture should be accompanied by progression of symptoms, ideally with worsening of findings on chest X-ray or high-resolution CT scan and exclusion of other diagnoses. Repeat sputum positives or growth of NTM from deeper bronchoscopic samples support a diagnosis of active disease. Expert advice should be sought on individual cases and even a positive diagnosis may allow an expectant strategy without initiation of therapy with toxic antimycobacterial drugs in some instances. A single positive diagnostic specimen in sites other than the lung is highly suggestive of active disease.

MANAGEMENT

Management of NTM is not well defined and in many areas, particularly for the less commonly detected organisms, there is a very weak evidence base. The situation is particularly obscure if two or more different mycobacteria are isolated. The threshold for active management may be influenced by co-morbidities such as cystic fibrosis. The American Thoracic and Infectious Diseases Societies have produced a useful consensus statement.[62]

Mycobacterium avium complex

Prophylaxis (see Chapters 90 and 91)

HIV-seropositive patients with a CD4 T-cell count less than 50/μl should be given clarithromycin 500 mg twice daily or azithromycin 1200 mg weekly. Rifabutin 300 mg weekly is an alternative, although the emergence of rifampin-resistant tuberculosis due an *rpoB* mutation in a patient taking rifabutin prophylaxis for *M. avium* complex infection raised concern.[63] Once the CD4 count rises above 100/μl prophylaxis may be stopped.

Treatment

MAC pulmonary disease is treated with 1 g clarithromycin or 500 mg azithromycin with 600 mg rifampin and 15 mg/kg ethambutol. In mild disease, alternate day or three times weekly regimens are sometimes used; in severe disease an aminoglycoside may be added. Treatment normally continues until 12 months after the last positive culture. Concurrent use of bronchodilators should be given as required but there are no data to support the use of steroids. In disseminated disease, therapy can be stopped when symptoms resolve and immune function is improved. Fluoroquinolones have good *in-vitro* activity against MAC but clinical data about their use are sparse. Additional positive cultures after a treatment course may represent infection by new strains rather than relapse. Macrolide-resistant organisms should only be treated in consultation with expert advice. Surgery has a very limited role in MAC infection. Successful management of the hypersensitivity syndrome requires removal of exposure to MAC antigens. Treatment of disseminated MAC infection in HIV disease is discussed in Chapter 91.

Mycobacterium kansasii

The best regimen and duration of therapy are not established. Ethambutol and rifampin are essential and isoniazid is normally added. A macrolide may be an effective alternative. The safest approach is to continue treatment until sputum cultures are negative for at least 12 months. Many isolates are sensitive to quinolones and amikacin but pyrazinamide resistance is the rule. Children with lymph node disease can be treated by excision alone.

Mycobacterium ulcerans

Surgical debridement is the main treatment for *M. ulcerans* infection which responds poorly to antimycobacterial drugs. Excision of pre-ulcerative lesions is advocated by some. Rifampin and streptomycin or amikacin for 2 months have been advocated for treatment or as an adjunct to surgery. Quinolones may also be active against *M. ulcerans*.[64]

Slowly growing atypical mycobacteria

The treatment for most members of this group is empiric, supported by available drug sensitivity data. Dual therapy often involving a macrolide is common and should continue for at least 3 months after resolution of symptoms. Lymph node disease in children may be treated by excision alone although a short course of clarithromycin has been advocated by some authorities. *Mycobacterium marinum* is usually treated by clarithromycin and ethambutol, with rifampin added for deeper infections. *Mycobacterium smegmatis* is typically resistant to isoniazid and rifampin. *Mycobacterium scrofulaceum* is frequently resistant to multiple antibiotics. *Mycobacterium xenopi* sensitivities may not transfer well to clinical responses but therapy with ethambutol, rifampin and a macrolide is one approach, with streptomycin and quinolones being potentially useful drugs. Surgical debridement or removal of focal infection should always be considered in such NTM. Treatment should involve physicians with an interest and expertise.

Mycobacterium fortuitum complex and other rapid growers

Mycobacterium fortuitum is relatively drug sensitive and may be treated with quinolones, amikacin, imipenem, doxycycline and macrolides according to sensitivities of a particular organism. However, as *M. fortuitum* (and *M. smegmatis*) may express an inducible macrolide resistance gene, therapy with clarithromycin is avoided. Removal of foreign bodies or infected lines and catheters is necessary and surgical debridement is often required (Fig. 30.22). *Mycobacterium chelonae* is sensitive to macrolides and 6 months of monotherapy with clarithromycin was effective in a study of 14 patients. In severe disease, a second agent such as tobramycin should be considered. *Mycobacterium abscessus* is relatively resistant to drugs other than macrolides which may be combined with intravenous amikacin. Surgical resection should always be considered for localized infection. Treatment should be for 12 months.

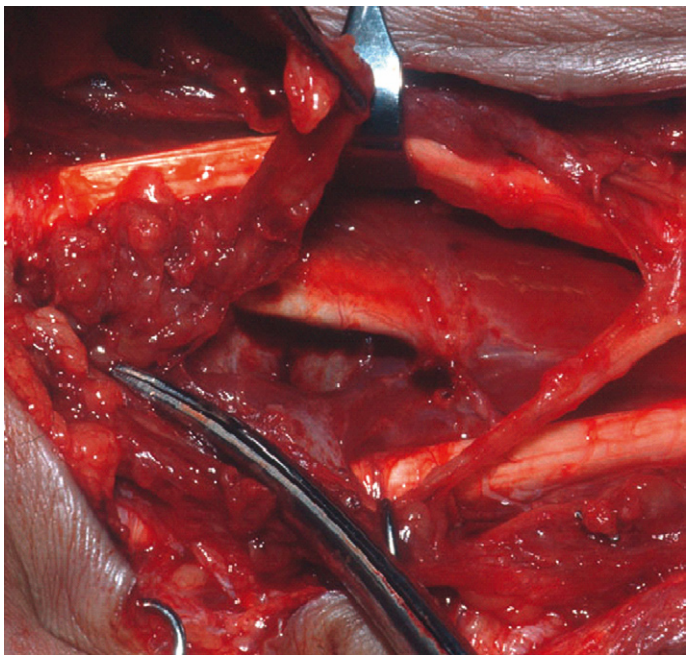


Fig. 30.22 Multiple small abscesses running along tendon sheaths caused by infection with *Mycobacterium fortuitum* following a fishing accident. Reproduced from Patel *et al.*[65] with kind permission of Springer Science and Business Media.

In general, susceptibility testing should be used to guide treatment in these rapidly dividing organisms. Linezolid may have useful activity against all members of the *M. fortuitum* complex although prolonged treatment is associated with side-effects.

REFERENCES

References for this chapter can be found online at http://www.expertconsult.com

Chapter 31

Carol A Kauffman

Fungal pneumonias

INTRODUCTION

This chapter will focus on the pulmonary aspects of the endemic mycoses, those fungi that are geographically restricted and that have the capability to cause infection in healthy hosts. Most infections with these fungi are initiated with inhalation of the conidia from the mold phase, which exists in the environment, and thus the lungs play a major role in the pathogenesis and clinical presentation of these infections. Infections with the endemic mycoses in immunosuppressed individuals are usually more severe and are more likely to be disseminated.

Histoplasmosis

MYCOLOGY AND EPIDEMIOLOGY

The causative agent of histoplasmosis is *Histoplasma capsulatum*, a dimorphic fungus that exists as a mold in the environment but converts to a yeast form in tissues at 37°C. There are two human pathogens, *H. capsulatum* var. *capsulatum* and *H. capsulatum* var. *duboisii*. The latter organism is endemic in Africa and will not be discussed further. In the mold phase, *H. capsulatum* var. *capsulatum* (hereafter referred to as *H. capsulatum*) develops characteristic macroconidia and microconidia. *H. capsulatum* is found primarily in North America in the Ohio and Mississippi River valleys and in many countries in Central America; localized foci exist in countries surrounding the Mediterranean Sea and in South East Asia.

The environmental niche for *H. capsulatum* is soil that is enriched by the nitrogen contained in bird and bat guano. Abandoned buildings and areas under trees that serve as bird and bat roosts and caves with high bat populations are especially likely to have high concentrations of *H. capsulatum*. In the endemic areas, exposure to *H. capsulatum* is common and it is estimated that hundreds of thousands of persons are infected yearly. Most infections are sporadic, acquired during the course of daily living, but dramatic outbreaks have been documented, including the large outbreak in Indianapolis that infected over a hundred thousand persons;[1] many smaller outbreaks have been traced back to spelunking, demolition of buildings or other activities that disrupt contaminated soil.[2]

PATHOGENESIS AND PATHOLOGY

Infection begins when the microconidia of *H. capsulatum* are inhaled into the alveoli. Neutrophils and macrophages phagocytize the organism, which triggers conversion to the yeast phase. The organism survives within macrophages, is spread to the hilar and mediastinal lymph nodes, and subsequently disseminates hematogenously. Asymptomatic hematogenous dissemination likely occurs in most patients who are infected. Specific cell-mediated immunity against *H. capsulatum* develops in a few weeks, leading to macrophage activation and, ultimately, killing of the organism. Cell-mediated immunity is crucial for control of the infection. It is thought that organisms can remain in a dormant state, similar to that which occurs with *Mycobacterium tuberculosis*, and then reactivate years later should cellular immunity wane.

The extent of disease is determined both by the inoculum of conidia inhaled and the immune response of the host. A small inoculum can cause severe infection in markedly immunosuppressed hosts, especially those who have AIDS or have been treated with anti-tumor necrosis factor (TNF) agents.[3,4] Conversely, healthy individuals can develop life-threatening infection when exposed to a large inoculum of fungi. Re-infection is possible in persons previously infected with *H. capsulatum* if they are exposed to a heavy inoculum of the organism.

CLINICAL FEATURES

It is important to emphasize that most patients who become infected with *H. capsulatum* remain asymptomatic or at most have a self-limited 'flu-like' illness. In those patients who do have acute pneumonia, symptoms are usually fever, fatigue, dyspnea, cough, mild chest discomfort, arthralgias and myalgias. The chest radiograph reveals a patchy lobar or multilobar infiltrate; hilar lymphadenopathy, when present, is a clue to the possibility of a fungal pneumonia.[5] The initial diagnosis in almost all patients is an atypical bacterial pneumonia, and almost all have received antibiotics to no avail before the possibility of histoplasmosis is entertained. If several patients who were involved with a particular outdoor activity have the same symptoms and see the same physician, then fungal pneumonia assumes a more prominent position in the differential diagnosis.

A small minority of patients with acute pulmonary histoplasmosis develop severe pneumonia that can progress to acute respiratory distress syndrome (ARDS). This occurs most often in patients who are immunosuppressed or in those who had an overwhelming exposure to the organism. Diffuse infiltrates that often have a nodular component are noted on chest radiograph (Fig. 31.1). In immunosuppressed patients, severe diffuse pulmonary involvement is often one component of widespread disseminated infection.

Chronic cavitary pulmonary histoplasmosis almost always occurs in older adults who have underlying emphysema and clinically mimics reactivation tuberculosis. Symptoms include fever, fatigue, anorexia, weight loss, cough productive of purulent sputum, and hemoptysis. Chest radiographs show unilateral or bilateral upper lobe infiltrates and thick-walled cavities, and fibrosis is seen in the lower lung fields[5] (Fig. 31.2).

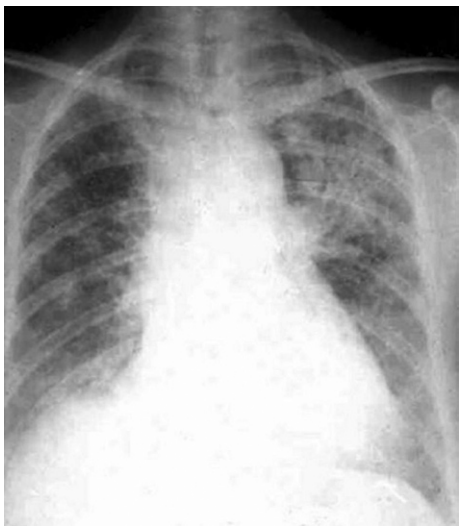


Fig. 31.1 Diffuse pulmonary infiltrates in a 40-year-old woman who had received a renal transplant 2 years before and who developed acute pulmonary histoplasmosis.

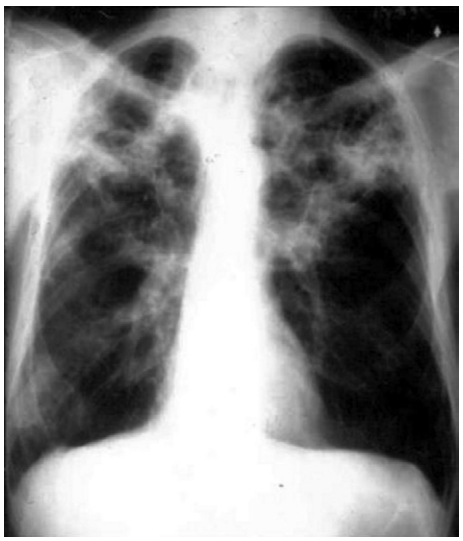


Fig. 31.2 Chronic cavitary pulmonary histoplasmosis in an elderly man who had severe underlying emphysema.

There are several complications of pulmonary histoplasmosis that can occur months to years after the initial infection. Mediastinal granuloma is characterized by persistent mediastinal and/or hilar lymphadenopathy.[6] Many patients are asymptomatic and the problem is discovered when a chest radiograph is performed for another reason. Others have symptoms of dysphagia, chest pain, dyspnea or nonproductive cough when the enlarged lymph nodes impinge on adjacent structures. CT scan usually reveals a confluence of lymph nodes that are surrounded by a fibrous capsule and that have central necrosis. Most patients have resolution of the lymphadenopathy, but fistula formation and compression of adjacent structures may require intervention. This syndrome does not progress to mediastinal fibrosis.

Mediastinal fibrosis is a rare complication of pulmonary histoplasmosis in which the host responds to histoplasmosis with excessive fibrosis that ultimately encases the great vessels and/or bronchi.[7] Obstruction of the superior vena cava and/or the vessels to one lung is most common. The disease is progressive and the symptoms include dyspnea, cough, wheezing and hemoptysis. CT scan reveals the extent of the fibrosis and angiographic studies pinpoint those vessels that are stenotic. When vessels to both lungs are involved, the disease is almost always fatal.

Many patients with disseminated histoplasmosis will have pulmonary manifestations. These may be subtle, as often occurs in older patients with chronic progressive histoplasmosis, but the pulmonary component may also be life threatening, which is noted most often in immunosuppressed patients. On presentation, some patients have chest radiographs that show diffuse pneumonia; in others, the primary manifestations are those related to other organ systems and pulmonary involvement is verified only later.

DIAGNOSIS

Histoplasmosis is definitively diagnosed by growth of the organism from samples taken from the infected site.[8] For pulmonary histoplasmosis, sputum, bronchoalveolar lavage (BAL) fluid, lung tissue or mediastinal lymph nodes are acceptable samples. *H. capsulatum* may take as long as 4–6 weeks to grow on Sabouraud's agar at room temperature in the mold form. Tentative identification can be made on seeing the characteristic tuberculate macroconidia; definitive identification is made with the use of a commercially available DNA probe that is highly specific for *H. capsulatum*.

Identification of the organism in tissue or fluid samples allows an early diagnosis while awaiting culture results. The organisms appear as uniform, 2–4 µm oval budding yeasts, most easily seen on tissue stained with methenamine silver or periodic acid–Schiff (PAS) stains or in smears or touch preparations stained with Giemsa stain (Fig. 31.3). For patients who have disseminated disease in addition to pulmonary manifestations, biopsy samples taken from bone marrow, lymph nodes or lesions on the mucous membranes or skin may reveal the organisms and avoid the need for bronchoscopy.

Serology plays an important role in the diagnosis of pulmonary histoplasmosis, especially chronic cavitary histoplasmosis and acute pulmonary histoplasmosis.[8] Both complement fixation (CF) and immunodiffusion (ID) tests are available. The ID test, which detects H and M antibodies, is slightly more specific than the CF test; however, the CF test has better sensitivity than the ID test. For patients with acute pneumonia, the initial studies may be negative but will show a fourfold rise over the subsequent few weeks. Antibody titers are almost always positive in patients with chronic cavitary histoplasmosis. In general, serology is not as useful in immunosuppressed patients who cannot mount an antibody response.

Antigen detection, using an enzyme immunoassay that measures a cell wall polysaccharide antigen of *H. capsulatum* in urine and serum, is useful in patients with acute pulmonary histoplasmosis, especially those with diffuse pneumonia, but is not useful for chronic cavitary histoplasmosis or mediastinal syndromes.[8] In those patients who

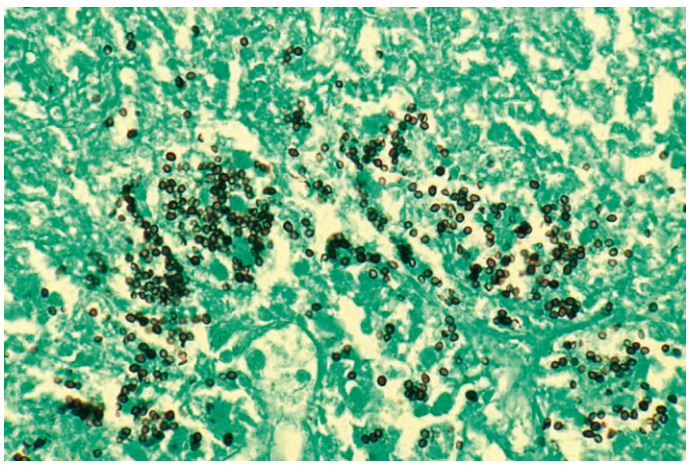


Fig. 31.3 Typical 2–4 µm yeast forms of *Histoplasma capsulatum* seen on a biopsy taken from an enlarged necrotic adrenal gland. The tissue is stained with Gomori methenamine silver stain.

have dissemination in addition to pulmonary involvement, the antigen assay has proved to be extremely useful. Cross-reactivity occurs with blastomycosis, coccidioidomycosis, paracoccidioidomycosis and penicilliosis.

MANAGEMENT

The Infectious Diseases Society of America has recently updated guidelines for the management of histoplasmosis.[9] Recommendations are based primarily on multicenter, nonrandomized, open-label treatment trials and case series from individual institutions. In general, patients with severe pulmonary histoplasmosis should be treated initially with an amphotericin B formulation. Lipid formulations are associated with fewer adverse effects, and in the only randomized treatment trial that was performed in AIDS patients who had severe disseminated histoplasmosis, liposomal amphotericin B was superior to amphotericin B deoxycholate.[10] For most patients, after a few weeks of amphotericin B therapy, step-down therapy to itraconazole is recommended.

Patients who have mild to moderate pulmonary histoplasmosis should be treated with itraconazole.[9] Fluconazole has proved to be less effective, and in a small number of AIDS patients resistance developed during fluconazole use.[11] Voriconazole and posaconazole both have *in-vitro* activity against *H. capsulatum*; however, there are only a limited number of case reports using these two azoles to treat histoplasmosis and they are currently reserved for therapy of patients who have failed therapy with other agents.[12,13] The echinocandins do not appear to have activity against *H. capsulatum* and should not be used.

Most patients with acute pulmonary histoplasmosis have self-limited infection and do not need to be treated with an antifungal agent. However, some patients remain symptomatic for weeks, and in these patients a short course of treatment with itraconazole 200 mg daily for 6–12 weeks is recommended.[9] Severe acute pulmonary histoplasmosis should be treated with lipid formulation amphotericin B, 3–5 mg/kg daily, or amphotericin B deoxycholate, 0.7–1.0 mg/kg daily. After the patient improves, step-down therapy to itraconazole can be initiated. Therapy should continue for at least 12 weeks and until all infiltrates have resolved. Many clinicians use intravenous methylprednisolone in addition to amphotericin B during the first 1–2 weeks in patients who are severely hypoxemic.[9]

Most patients who have chronic cavitary pulmonary histoplasmosis are chronically ill and can be treated with itraconazole, 200 mg twice daily for at least a year, and sometimes longer. The outcome is still dismal in many patients because of the degree of fibrosis and lung destruction that has occurred.

Patients with mediastinal granuloma do not require therapy with an antifungal agent. However, in symptomatic patients, many clinicians give a 6- to 12-week course of 200 mg itraconazole once or twice daily although there are no data showing this is effective.[9] Surgical resection of the granulomatous mass can be helpful in relieving compressive symptoms caused by encroachment on vital structures.

Mediastinal fibrosis should not be treated with an antifungal agent or with corticosteroids. Placement of stents in obstructed great vessels by an interventionalist who is experienced in the treatment of this rare disease has proved extremely useful in providing symptomatic relief.[14]

Patients who have pulmonary involvement as one manifestation of disseminated disease should always receive antifungal therapy. Patients who have mild to moderate symptoms can be treated with itraconazole, 200 mg twice daily for a year. Patients who have severe symptoms should be treated initially with lipid formulation amphotericin B, 3–5 mg/kg daily, that can be changed to itraconazole after a clinical response is noted.

Patients who have AIDS should receive chronic suppressive therapy with itraconazole, 200 mg daily as long as their CD4 counts remain below 200 cells/μl. It is safe to discontinue suppressive therapy if they have consistently taken antiretroviral agents and their CD4 count remains >200 cells/μl for at least 1 year (see also Chapter 91).[9]

Blastomycosis

MYCOLOGY AND EPIDEMIOLOGY

The causative agent of blastomycosis is *Blastomyces dermatitidis*, a dimorphic fungus that exists as a mold in the environment but converts to a yeast form in tissues at 37°C. The organism is found in North America, Africa and the Middle East. Most cases are reported from states that border the Mississippi River basin, the Canadian provinces of Ontario and Manitoba, and areas bordering the St Lawrence Seaway. There are occasional reports of cases that are associated with small microfoci that exist outside the typical endemic areas.

The environmental niche for this organism appears to be soil and decaying wood, especially along waterways. It is difficult to isolate the organism from the environment, but several outbreaks have been traced back to activities involving canoeing, camping and dismantling a beaver lodge.[15]

PATHOGENESIS AND PATHOLOGY

Blastomycosis begins with inhalation of the conidia of *B. dermatitidis* into the alveoli. The filamentous form converts to the yeast form in the lungs, and both neutrophils and cell-mediated immunity are important in the response to infection. It is likely that hematogenous dissemination occurs early in most patients, but clinical manifestations are uncommon. Weeks to months later, frequently after the pulmonary lesion has healed, patients can present with cutaneous or other organ involvement. It should be presumed that all extrapulmonary lesions represent hematogenous dissemination. The histopathologic picture is one of a mixed pyogranulomatous process. Blastomycosis tends to be more severe in those with cell-mediated immune dysfunction;[16,17] these patients have minimal granuloma formation and mount mostly a neutrophilic response. Reactivation of prior infection with *B. dermatitidis* has been noted, but appears to be less common than noted with *H. capsulatum.*

CLINICAL FEATURES

Most patients with acute pulmonary infection with *B. dermatitidis* remain asymptomatic or have a mild 'flu-like' illness that is never diagnosed as blastomycosis. Those who have acute pneumonia manifest fever, cough, mild dyspnea, myalgias and arthralgias, and are noted to have a localized pulmonary infiltrate on chest radiograph. Most patients are given a diagnosis of an atypical bacterial pneumonia and are treated with antibiotics. Only when the infection does not respond to this therapy is a fungal infection considered and further diagnostic studies undertaken.

A small proportion of patients with acute blastomycosis develop overwhelming pulmonary infection with ARDS[18] (Fig. 31.4). This occurs

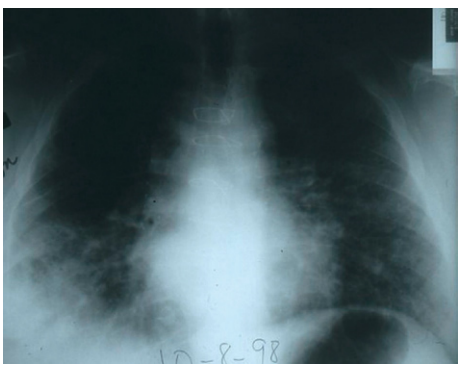


Fig. 31.4 Severe diffuse pneumonia with ARDS due to *Blastomyces dermatitidis* in a 50-year-old patient with no underlying illnesses.

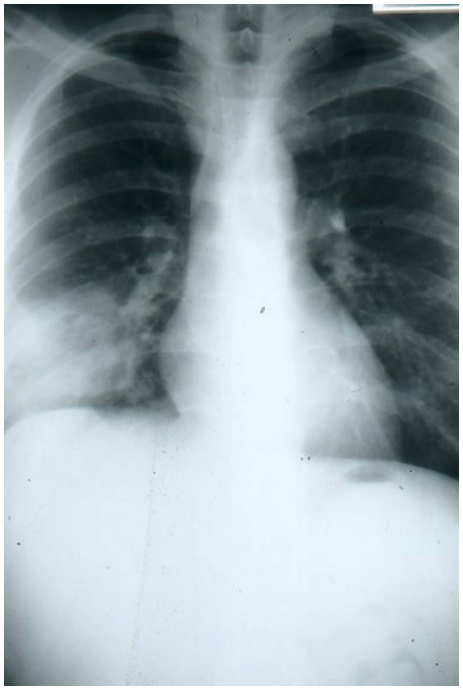


Fig. 31.5 Mass-like lesion in a 47-year-old man who was thought to have lung cancer until the biopsy revealed many granulomas and culture yielded *Blastomyces dermatitidis*.

more often in immunocompromised patients, but is also reported in previously healthy adults. The presumption in the latter group is that they inhaled an excessively large number of conidia that overwhelmed their immune response. The mortality rate is extremely high in this rapidly progressive form of pulmonary blastomycosis.

Pulmonary blastomycosis can also present as a mass-like lesion that is indistinguishable from lung cancer or with upper lobe cavitary lesions that resemble tuberculosis or histoplasmosis[19] (Fig. 31.5). Fever, night sweats, weight loss, fatigue, dyspnea and cough with purulent sputum and hemoptysis are often present for weeks before the patient seeks medical attention. Pleural effusions and hilar or mediastinal lymphadenopathy each occur in only about 20% of patients.

Cutaneous lesions are the most common manifestation of extrapulmonary blastomycosis; their appearance in a patient who has a nonresolving pneumonia can lead toward the diagnosis of blastomycosis. The lesions are frequently multiple and classically are well-circumscribed, plaque-like lesions that become verrucous and often show punctate draining areas in the center (Fig. 31.6). Less commonly, painful ulcerated lesions or multiple rapidly developing nodular lesions are noted.

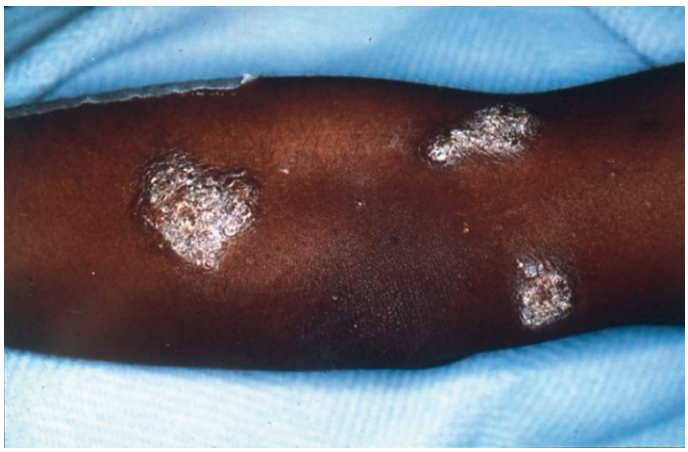


Fig. 31.6 Typical skin lesions of blastomycosis in a 24-year-old man. Courtesy of Dr Hector Bonilla.

Common noncutaneous manifestations of disseminated blastomycosis that can occur at the time the patient has pneumonia or after the pulmonary manifestations have resolved include osteomyelitis, septic arthritis and genitourinary tract infection, especially with prostatic involvement. Focal blastomycosis of other organs and central nervous system (CNS) infection manifested as chronic meningitis or brain abscess are well described.[20]

DIAGNOSIS

Blastomycosis is definitively diagnosed when the organism is grown on Sabouraud's agar from a sample taken from an infected site.[21] For pulmonary blastomycosis, sputum, BAL fluid and lung tissue are acceptable samples. *Blastomyces dermatitidis* takes several weeks to grow at room temperature in the mold form. Once growth occurs, identification can be made quickly with the use of a commercially available DNA probe that is highly specific for *B. dermatitidis*.

Prior to the growth of the organism, a preliminary diagnosis of pulmonary blastomycosis can often be made by examining sputum or BAL fluid treated with potassium hydroxide or calcofluor white or by examining a cytologic preparation stained with Papanicolaou stain.[22] The organisms are large (8–10 μm), thick-walled yeasts that have a single broad-based bud; this specific morphology distinguishes *B. dermatitidis* from other yeasts (Fig. 31.7).

If skin lesions are present in a patient with pulmonary infiltrates, biopsy should be performed and may yield a diagnosis of blastomycosis without having to perform bronchoscopy. The yeasts can be visualized in tissues with methenamine silver or PAS stains.

CF and ID antibody assays are neither sensitive nor specific for blastomycosis and are of little use for diagnosis. An enzyme immunoassay that detects a polysaccharide cell wall antigen of *B. dermatitidis* in urine and serum is commercially available and may prove useful for the diagnosis of blastomycosis.[23] There is a high degree of cross-reactivity between the antigen tests for *H. capsulatum* and *B. dermatitidis*.

MANAGEMENT

The Infectious Diseases Society of America has recently updated guidelines for the management of blastomycosis.[24] Recommendations are based on multicenter, nonrandomized, open-label treatment trials and case series from individual institutions. In general, all patients with symptomatic blastomycosis should be treated with an antifungal agent. Even patients who have acute pulmonary blastomycosis that

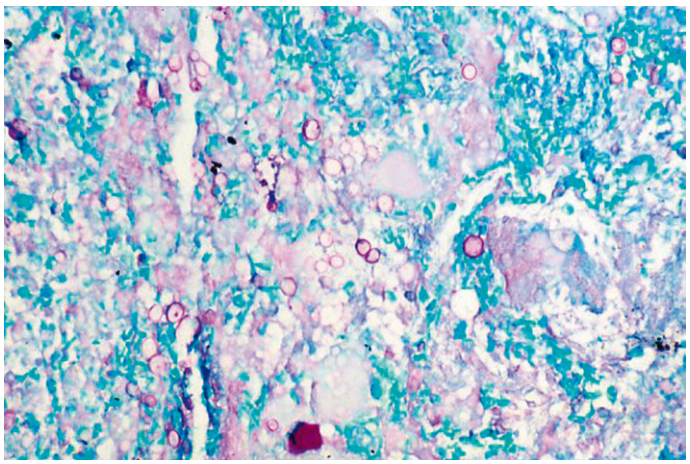


Fig. 31.7 Lung biopsy from a 54-year-old man who had a left upper lobe pulmonary infiltrate. Thick walled, broad-based, budding yeasts are seen with the periodic acid–Schiff stain.

appears to be resolving should be treated in order to decrease the risk for subsequent extrapulmonary infection.[24] Patients who have only a single cutaneous lesion or other focal manifestation should be treated because, by definition, they have disseminated blastomycosis. Mild-to-moderate blastomycosis should be treated with an azole agent; severe infection should be treated with an amphotericin B formulation as initial therapy.

Most cases of pulmonary blastomycosis can be treated with itraconazole.[24] The dosage is 200 mg once or twice daily. Second-line agents that can be used for patients who cannot tolerate itraconazole include high-dose fluconazole (800 mg daily), voriconazole (200–300 mg twice daily) and posaconazole (400 mg twice daily).[24–26] Fluconazole is not as effective as itraconazole for blastomycosis. *In-vitro* susceptibility studies show voriconazole and posaconazole to be active against *B. dermatitidis*, but there is little clinical experience with these agents to date. The echinocandins appear to have no activity against *B. dermatitidis* and should not be used.

Amphotericin B is reserved for those patients who have severe infection with *B. dermatitidis*; this includes those who have severe pneumonia, CNS involvement and widespread visceral infection, and most immunosuppressed patients. A lipid amphotericin B formulation, 3–5 mg/kg daily, or amphotericin B deoxycholate, 0.7–1 mg/kg daily, can be used.[24] After 1–2 weeks, if the patient's condition has improved, step-down therapy with oral itraconazole is recommended.

The length of time required for the treatment of blastomycosis depends on the extent of the infection and the clinical response of the patient. Most patients who have pulmonary blastomycosis should be treated for 6–12 months. If other sites of infection are noted, such as osteoarticular involvement, a minimum of 1 year of azole therapy should be planned. Immunosuppressed patients may require lifelong suppressive therapy with an azole.[24]

Coccidioidomycosis

MYCOLOGY AND EPIDEMIOLOGY

Coccidioides is a dimorphic fungus that exists as a mold in the environment and as a spherule *in vivo*. It differs from the other dimorphic fungi in that the dimorphism is not regulated by temperature. It has recently been verified that there are two species: *C. immitis* refers to isolates from California and *C. posadasii* to isolates from all other areas. *Coccidioides* species are generally found in the Lower Sonoran life zone, which is a desert environment that occurs in certain areas of South America, Central America and the south-western United States.

Most inhabitants of the endemic area are infected before they reach adulthood. An increasing number of infections are reported in older adults who moved to these warm climates at retirement and had never been exposed to this organism previously.[27] Catastrophic events, such as earthquakes, have led to the occurrence of coccidioidomycosis in areas beyond those normally seen, and environmental cycles of rain and drought in the desert are important in the natural history of *Coccidioides* spp.[28]

PATHOGENESIS AND PATHOLOGY

The mold form of *Coccidioides* develops arthroconidia that are easily dispersed and inhaled into the alveoli where the organism transforms into the spherule form. Spherules are large (20–80 µm), thick-walled structures that contain hundreds of endospores. The spherule ruptures when filled and releases many endospores, each of which is able to spread to form a new spherule.

The primary host defense against *Coccidioides* appears to be cell-mediated immunity. Neutrophils are present in most lesions, but they cannot eliminate spherules. It is likely that many patients experience silent hematogenous dissemination. *Coccidioides* species have the potential to reactivate years after the primary infection with the organism. For still unexplained reasons, dark-skinned races, especially African Americans and Filipinos, are at higher risk for dissemination and severe infection than white-skinned persons.[29] Pregnant women, especially those in the third trimester, are also at risk for severe disease.[30]

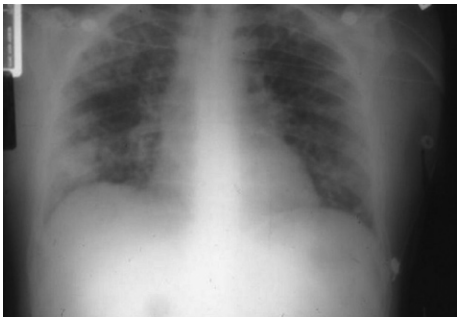


Fig. 31.8 Diffuse infiltrates in a patient with AIDS who developed pulmonary coccidioidomycosis.

CLINICAL FEATURES

Most persons infected with *Coccidioides* species have no symptoms or have mild symptoms suggesting a 'flu-like' illness. Patients with acute coccidioidal pneumonia have fever, fatigue, myalgias, arthralgias, dry cough, anterior chest pain and dyspnea. Erythema nodosum frequently occurs, especially in women, and should raise the possibility of coccidioidomycosis. Chest radiographs show patchy pneumonitis, with or without hilar lymphadenopathy patients[31] (Fig. 31.8).

In patients who are immunocompromised, especially those with AIDS, transplant recipients and those treated with anti-TNF agents, and in those who had exposure to a large number of arthroconidia, severe pneumonia with progression to ARDS can occur.[31,32] Chest radiographs reveal diffuse reticulonodular infiltrates.

Approximately 5–10% of patients have pulmonary complications following acute coccidioidal pneumonia. These include benign coccidioidomas, which are persistent asymptomatic pulmonary nodules, and solitary, thin-walled cavities that can persist for months to years. Although many cavities will resolve, hemoptysis or cavity rupture into the pleural space can occur in some patients.[31] Chronic progressive pneumonia is characterized by thick-walled cavity formation, fibrosis, purulent sputum, hemoptysis and dyspnea. This form of coccidioidomycosis occurs mostly in those who are older and have chronic obstructive pulmonary disease and/or diabetes mellitus.[33]

Less than 1% of patients with symptomatic coccidioidomycosis will develop symptoms of disseminated infection. Almost always, these patients are either dark-skinned or are immunosuppressed. The sites involved, in addition to the lungs, are most often skin, subcutaneous tissues, osteoarticular structures and meninges, but any organ can be affected. Meningitis, which is a dreaded complication, can be the sole manifestation of dissemination or it can be present along with dissemination to multiple different organs.

DIAGNOSIS

Coccidioidomycosis is definitively diagnosed when the organism is grown in culture from involved tissues or body fluids. *Coccidioides* species grow as a white mold within a few days on most standard media. It is important to warn laboratory personnel if coccidioidomycosis is a possibility as the mold form is highly infectious. *Coccidioides* is classified as a bioterrorism agent and must be handled using biosafety level 3 precautions, which are not present in most laboratories.

The large spherules are readily identified using KOH or calcofluor white preparations on sputum or BAL fluid. Smears from skin lesions show the spherules, which are readily seen on standard hematoxylin

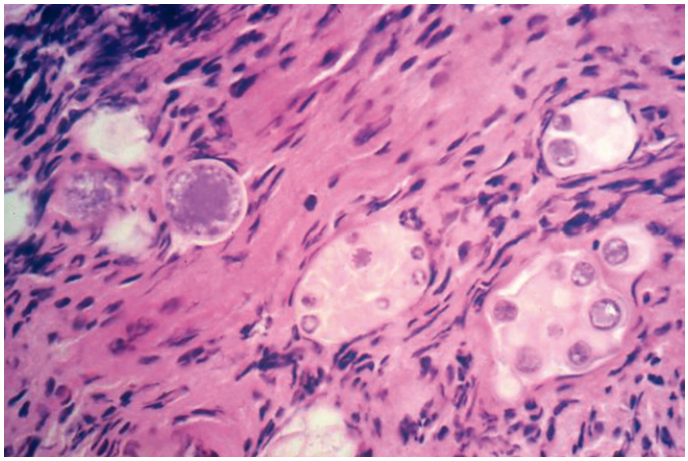


Fig. 31.9 Lung biopsy from the patient noted above showing several *Coccidioides* spherules containing endospores.

and eosin-stained tissue sections (Fig. 31.9). This is extremely helpful in establishing an early diagnosis before culture evidence is available.

Serology is helpful in the diagnosis of coccidioidomycosis, especially if a reference laboratory experienced in testing for coccidioidomycosis performs the tests.[34] Acute coccidioidomycosis can be diagnosed by finding IgM antibodies that are measured by an ID assay; IgG antibodies measured by CF appear later and persist longer. A positive CF antibody test for *Coccidioides* in CSF is diagnostic of coccidioidal meningitis.

MANAGEMENT

The Infectious Diseases Society of America has recently updated guidelines for the management of coccidioidomycosis.[35] Most of the recommendations are based on multicenter, nonrandomized, open-label trials and case series. There is one randomized, blinded comparative trial that showed an overall similar efficacy for fluconazole and itraconazole for the treatment of mild-to-moderate nonmeningeal coccidioidomycosis and a superior response with itraconazole for osteoarticular coccidioidomycosis.[36] There is little experience with the new azoles, but there are anecdotal reports of success with voriconazole[37] and several small series reporting on the use of posaconazole for patients with pulmonary and disseminated coccidioidomycosis.[38,39] The echinocandins appear to have no activity against *Coccidioides* species and should not be used to treat coccidioidomycosis.

Most patients with acute pulmonary coccidioidomycosis have a benign course and do not require therapy with an antifungal agent. However, patients who continue to have symptoms for 3–4 weeks with no improvement should be treated either with fluconazole, 400 mg daily, or itraconazole, 200 mg twice daily, for 3–6 months.[35] Patients who have underlying immunosuppression, those who are pregnant and patients who are African American or Filipino should be treated because of the high risk for dissemination in these groups.

Severe coccidioidal pneumonia should be treated initially with amphotericin B deoxycholate, 0.7–1.0 mg/kg daily, or a lipid formulation of amphotericin B, 3–5 mg/kg daily. After the patient has had a clinical response, therapy can be stepped down to itraconazole or fluconazole, given for 1–2 years.

Persisting thin-walled cavities can be observed, but surgical removal is reasonable for those that are adjacent to the pleura or that are noted to enlarge.[35] Chronic pulmonary coccidioidomycosis should be treated with itraconazole, 200 mg twice daily, or fluconazole, 400 mg daily, for 1–2 years. Persistent cavitary lesions that remain after adequate therapy should be evaluated for the feasibility of surgical removal.

Disseminated coccidioidomycosis is always treated. Severe infection is treated initially with amphotericin B deoxycholate, 0.7–1.0 mg/kg daily, or lipid formulation amphotericin B, 3–5 mg/kg daily.[32,35] After a clinical response is seen, therapy can be changed to an azole. Patients who have mild-to-moderate infection and are not immunosuppressed can be treated either with itraconazole, 200 mg twice daily, or fluconazole, 400 mg daily. The total treatment course is usually 1–2 years.

Coccidioidomycosis is the most difficult of the endemic mycoses to treat, and relapses are more common than with the other endemic mycoses. African Americans with disseminated coccidioidomycosis are at high risk for relapse, as are AIDS patients, and lifelong azole therapy is often required to prevent relapse.[32,35]

Paracoccidioidomycosis

MYCOLOGY AND EPIDEMIOLOGY

Paracoccidioidomycosis, or South American blastomycosis, is caused by *Paracoccidioides brasiliensis*, a thermally dimorphic fungus that in the environment is a mold and at 37°C, a yeast that has multiple buds. *Paracoccidioides brasiliensis* is endemic in humid areas in several countries in Central and South America; most cases are reported from Brazil.[40] The environmental niche is presumably soil and the disease is most often seen in middle-aged to elderly men who live in rural areas. Paracoccidioidomycosis is unique among the endemic fungi in that there is a strong sexual preference (male to female ratio 13:1). This imbalance can be partly explained by environmental exposure, but may also possibly be related to inhibitory effects of estrogens on the growth of the organism.[41,42]

PATHOGENESIS AND PATHOLOGY

Paracoccidioidomycosis is acquired by inhalation of conidia of *P. brasiliensis* into the alveoli where they are phagocytized by macrophages and convert to the yeast phase. It is likely that silent hematogenous dissemination occurs during most infections. Both neutrophils and cell-mediated immunity play a role in host defense although the latter is likely the more important. Patients who have deficient cellular immunity, especially those who have AIDS, have widespread disseminated infection and histopathologic examination reveals poorly formed granulomas.[43] Well-documented cases have occurred years after patients have left the endemic area, establishing reactivation of latent infection as a pathogenetic mechanism for this disease.[44] Many authors consider early childhood exposure the initial event and subsequent reactivation to be the cause of most cases of the chronic adult form of paracoccidioidomycosis.

CLINICAL FEATURES

Most cases of paracoccidioidomycosis occur in older men, are slowly progressive and are characterized as the chronic or adult form of the disease.[42] Pulmonary involvement is prominent; symptoms include fever, cough productive of purulent sputum, hemoptysis and dyspnea. Chest radiographs reveal nodular, interstitial or cavitary lesions that are more often in the middle and lower lung fields rather than the apices (Fig. 31.10). Progressive fibrosis is frequently seen, and is a major cause of death.[46] Most patients with this form of paracoccidioidomycosis have ulcerative or nodular mucous membrane lesions, primarily in the anterior nares and oral cavity, and papular, nodular or ulcerative skin lesions.

A less common form of paracoccidioidomycosis, the acute or juvenile form, also involves the lungs.[42] The hallmark of this form of paracoccidioidomycosis is widespread involvement of liver, spleen, lymph

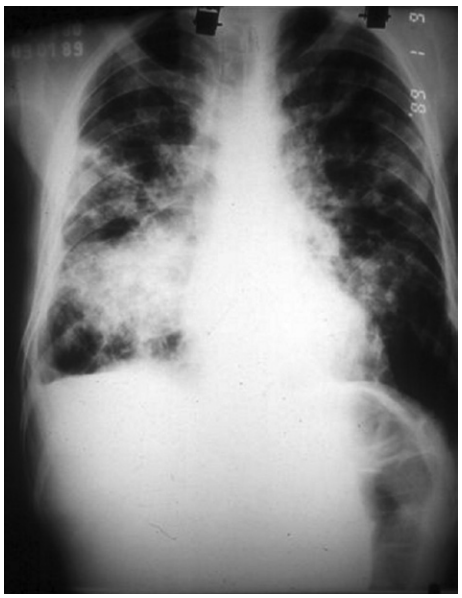


Fig. 31.10 Chest radiograph of an older man who had chronic paracoccidioidomycosis. Reproduced with permission from Kauffman.[45]

nodes, bone marrow, skin and lungs. Patients younger than 30 years of age, as well as immunosuppressed patients, especially those with AIDS, typically show this manifestation of paracoccidioidomycosis. The disease can progress rapidly and culminate with ARDS.

DIAGNOSIS

Growing *P. brasiliensis* from samples taken from infected sites, such as sputum, BAL fluid or lung tissue from those with pulmonary infection, establishes the diagnosis. The organism grows slowly in the mold phase at room temperature on Sabouraud's agar, and conversion to the yeast phase is necessary for firm identification.[40]

A presumptive diagnosis of paracoccidioidomycosis can be made if the organism can be visualized in smears with calcofluor white or KOH or in tissue sections stained with methenamine silver or PAS stains. The yeast cells are large (10–30 μm) and thick walled, and the budding daughter cells have a narrow base and remain attached around the circumference of the mother cell, creating a distinctive picture likened to a ship's steering wheel (Fig. 31.11).

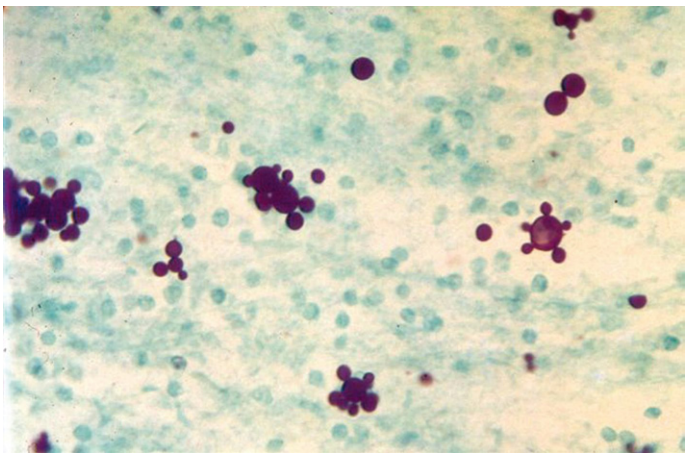


Fig. 31.11 Smear from a lymph node aspirate showing the typical captain's wheel configuration of budding daughter cells on the mother cell of *Paracoccidioides brasiliensis*. Reproduced with permission from Kauffman.[45]

Serology is less useful than culture techniques. Several different assays, including ID, counterimmunoelectrophoresis and CF, have been used, but none is commercially available and the sensitivity and specificity of each test has not been firmly established.

MANAGEMENT

Azole agents have assumed the primary role for the treatment of paracoccidioidomycosis. Itraconazole, 100 mg daily, for 6–12 months, is recommended most often.[47] Ketoconazole, 200–400 mg daily for 1 year, is still used because it is much less expensive than itraconazole; however, it is not as efficacious and has more adverse effects than itraconazole. Voriconazole appears to be efficacious and achieves superior levels in the CNS in those patients with disseminated infection.[48] Sulfonamides had been the treatment of choice used for years, but are less effective than the azoles and are used less frequently now.

For immunosuppressed patients who have widely disseminated *P. brasiliensis* infection, amphotericin B deoxycholate, 0.7–1.0 mg/kg daily is recommended;[42] there is little experience with lipid formulations of amphotericin B. After a clinical response is achieved, step-down therapy with itraconazole is appropriate. For patients who have HIV infection, lifelong suppressive therapy with itraconazole or trimethoprim–sulfamethoxazole is indicated.

Sporotrichosis

MYCOLOGY AND EPIDEMIOLOGY

Sporothrix schenckii is another dimorphic fungus that is a mold in the environment but assumes a yeast-like form at 37°C. Although found worldwide, most cases are reported from South America, North America and Japan. The organism can be found in soil, decaying wood and sphagnum moss, and outbreaks are described in association with exposure to contaminated moss or timbers.[49] Most cases are sporadic and related to exposure during activities, such as landscaping, farming, rose gardening or dirt biking. The organism can also be acquired from animals, with most cases being linked to cats that have the disease.[50,51]

PATHOGENESIS AND PATHOLOGY

In contrast to the other dimorphic fungi, *S. schenckii* causes disease in almost all cases by inoculation of the conidia through scratches or punctures from thorns, wood splinters or other sharp objects, rather than inhalation. It is likely that pulmonary sporotrichosis occurs when conidia are inhaled, but dissemination to lungs from skin lesions is also possible. The immune response to *S. schenckii* is a mixture of neutrophils and cell-mediated immunity. Patients with cellular immune deficiencies, such as AIDS, or those treated with immunosuppressive drugs are at risk of developing widespread dissemination, including pulmonary disease, when infected with *S. schenckii*.[52,53]

CLINICAL FEATURES

Sporotrichosis is primarily a localized lymphocutaneous infection and is discussed in depth elsewhere. Pulmonary sporotrichosis is rare and almost always occurs in patients with underlying chronic obstructive pulmonary disease and alcoholism.[49,54] The clinical picture is similar to tuberculosis or chronic cavitary histoplasmosis. Fever, night sweats, fatigue, cough with purulent sputum, hemoptysis and increasing dyspnea are noted in most patients. Chest radiographs show apical infiltrates with thick-walled cavities (Fig. 31.12). Even more uncommon,



Fig. 31.12 Chronic cavitary pulmonary sporotrichosis in a 54-year-old man who had emphysema.

disseminated sporotrichosis in markedly immunosuppressed patients can have diffuse pulmonary infiltrates associated with dyspnea and hypoxemia.[53]

DIAGNOSIS

The diagnosis of sporotrichosis is based on growth of the organism from samples taken from involved tissues. In the case of pulmonary sporotrichosis, sputum, BAL fluid and lung tissue are appropriate samples for culture. The organism grows in days to weeks on Sabouraud's agar at room temperature as a mold with conidia arranged in a distinctive bouquet pattern on thin hyphae. Seeing this allows for presumptive identification, but definitive identification requires conversion to the yeast phase at 35–37°C. The yeasts are rarely seen in sputum or BAL fluid. They can be found in tissue using methenamine silver or PAS stains, but are often difficult to visualize.

If a patient has cutaneous lesions in addition to pulmonary disease, sampling a skin lesion is a simple way in which to make the diagnosis. However, as pulmonary sporotrichosis is sufficiently rare that one must be sure that the pulmonary infiltrate is not due to another etiology, most patients have sputum or BAL fluid cultures performed as well.

Serology has not proved useful and is currently not available.

MANAGEMENT

The Infectious Diseases Society of America has recently updated guidelines for the management of sporotrichosis.[55] Recommendations are based mostly on case series from individual institutions and a few multicenter, nonrandomized, open-label treatment trials. None of these reports specifically addresses the issue of pulmonary sporotrichosis.

Most patients with pulmonary sporotrichosis should be treated initially with amphotericin B, either a lipid formulation, 3–5 mg/kg daily, or amphotericin B deoxycholate, 0.7–1.0 mg/kg daily.[56] After improvement is noted, step-down therapy to itraconazole, 200 mg twice daily, for at least 1 year is recommended. If the patient is not seriously ill, itraconazole at the dosage and length of treatment noted above is recommended.[55] If surgical removal of the area infected is feasible, this should be carried out. Unfortunately, most patients have severe underlying pulmonary disease and surgery is not an option.

Fluconazole failure rates for pulmonary sporotrichosis are high, voriconazole has no activity and should not be used, and posaconazole, although active *in vitro*, has not been used to treat sporotrichosis. Drugs that are used for lymphocutaneous sporotrichosis, such as potassium iodide and terbinafine, are ineffective and should not be used for pulmonary sporotrichosis.

Penicilliosis

MYCOLOGY AND EPIDEMIOLOGY

Penicillium marneffei is a mold in the environment and a yeast in the tissues at 37°C. The yeast divides by septation and not budding in contrast to most other dimorphic fungi. *Penicillium marneffei* is endemic in rural areas in most south-eastern Asian countries, including Thailand, Vietnam, Laos and southern China. The environmental niche is presumably soil. Bamboo rats in these same areas are also frequently infected, but there is no evidence of transmission from rats to humans.[56]

PATHOGENESIS AND PATHOLOGY

Penicillium marneffei is presumed to cause infection after the conidia are inhaled into the alveoli. Pulmonary manifestations are usually silent and hematogenous dissemination is the rule. The most important host defense against this organism is cellular immunity. Healthy individuals rarely have symptoms, but those who have deficient cellular immunity, especially persons with AIDS, develop disseminated infection.[57] The pathologic features include both neutrophilic infiltration and granuloma formation.

CLINICAL FEATURES

Pulmonary manifestations of infection with *P. marneffei* are almost entirely seen in patients who have widespread disseminated infection. Fever, weight loss, lymphadenopathy, hepatosplenomegaly and skin lesions are common presenting symptoms. Pulmonary symptoms other than dyspnea are uncommon.[57,58] The chest radiograph usually shows diffuse infiltrates.

DIAGNOSIS

The diagnosis of penicilliosis is established by growing the organism from a tissue sample or body fluids. Most patients will have a biopsy taken from a skin lesion, a lymph node or bone marrow, and not samples from the respiratory tract. The organism grows as a mold at room temperature after a few weeks; production of red pigment on Sabouraud's agar allows a presumptive diagnosis before conversion to the yeast phase is accomplished to confirm identification.

Smears of body fluids or from a bone marrow aspirate can establish an early diagnosis if the characteristic yeast form of *P. marneffei* is identified. The yeasts are oval to sausage-shaped and have a central septum, unlike other small yeasts, such as *H. capsulatum*.

MANAGEMENT

Penicillium marneffei is susceptible to amphotericin B, 5-flucytosine, ketoconazole and itraconazole. Most studies have been performed in patients with AIDS. Both amphotericin B and itraconazole have

been shown to be effective.[59] Amphotericin B is recommended for those patients who have severe illness; following a clinical response, step-down therapy with itraconazole, 200 mg twice daily, is recommended. For patients who have mild-to-moderate illness, itraconazole, 200 mg twice daily, is recommended. Long-term suppressive therapy with itraconazole is required to prevent relapse, and prophylaxis with itraconazole may also be useful in highly endemic areas.[60,61]

REFERENCES

References for this chapter can be found online at http://www.expertconsult.com

Practice point 10

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Investigation of pleural discharge/fluid

INTRODUCTION

The presence of pleural fluid, or parapneumonic effusion (PPE), is a common finding during bacterial pneumonia, occurring in 20–57% of cases. PPE usually consists of a small amount of fluid which disappears spontaneously with antimicrobial therapy. In 5–10% of patients, presenting late in the course of pneumonia or receiving inadequate antimicrobial therapy, the persistent eruption of bacteria into the pleural space leads to complicated PPE including thoracic empyema. The timing and indications for investigation of pleural fluid are crucial in the management of PPE in order to decrease the morbidity and mortality associated with the development of these complications.

PATHOGENESIS

Pleural effusion can be separated into three stages on the basis of the natural course of the disease.

1. Stage 1 (uncomplicated PPE) corresponds to an increase in production of interstitial lung fluid and capillary leakage which exceeds the pleural resorption capacity. At this stage, pleural fluid contains protein and neutrophils and is free from bacteria. This exudate usually resolves with pneumonia treatment and does not lead to pleural sequelae.
2. Stage 2 (complicated PPE) is related to the persistence of bacterial invasion within the pleural space and involves fibrinous adhesions. Neutrophil recruitment and lysis in the pleural space increases the lactate dehydrogenase (LDH) concentration and promotes the anaerobic metabolism of glucose with local acidosis.
3. Stage 3 corresponds to empyema and is defined by the presence of pus in the pleural fluid. Bacteria may be observed on a Gram stain but a positive culture is not required for diagnosis. Indeed, cultures are often negative due to inappropriate bacterial culture conditions, prior exposure to antibiotics or the presence of loculation.

Persistent inflammation of visceral pleura leads to fibrosis and lung entrapment requiring appropriate pleural drainage in all cases.

MICROBIOLOGY

All pneumonia-related pathogens can induce PPE, including typical bacteria, atypical bacteria and viruses. However, *Streptococcus* spp., *Staphylococcus* spp., Gram-negative bacilli and anaerobes are more likely to be associated with pleural effusion. The causative pathogens of empyema are aerobes in about 40% of cases, anaerobes in up to 30% and multiple organisms in around 30%.

PRESENTATION

Patients presenting with pleural effusion due to pneumonia do not differ clinically from those presenting without PPE. Seemingly, patients do not differ clinically according to the stage of pleural disease (complicated or not). Physical signs include unspecific fever, cough, shortness of breath, chest pain, chills and purulent sputum. Recrudescence or persistence of the symptoms of pneumonia should evoke the presence of empyema.

Chest radiography can suggest the presence of PPE. Ultrasonography is used to confirm and estimate the amount of liquid in the pleural space. It can also detect standing effusion or loculation, and be used for guided thoracocentesis. In the case of complicated pleural effusion, a thoracic CT scan with contrast is recommended to check for the presence of indirect signs of empyema, such as thickening of the parietal pleura, loculated pleural fluid and small air bubbles within the collection. It can be used to detect any underlying lung abnormalities (Fig. PP10.1).

PLEURAL FLUID ANALYSIS

Pleural fluid analysis should be performed early in the course of pneumonia, ideally before starting antimicrobial therapy, when pleural effusion is non-minimal (>10 mm on lateral decubitus), loculated or non-free-flowing. A specimen of at least 30 ml of pleural fluid should be collected. Appearance, biochemical (pH, LDH, glucose and protein), bacteriologic (Gram stain and culture) and cellular count analyses should be performed systematically (Table PP10.1). Pleural effusion for pH measurement should be collected using a heparinized syringe and pH measured with a blood gas analyzer.

To improve the yield of bacterial cultures, pleural fluid should be inoculated directly into bottle culture medium for aerobic and anaerobic bacteria, and on demand for mycobacteria, fungi and viruses. A cytologist should also examine the morphology and type of pleural cells in order to detect malignant effusion.

Depending on the appearance, pH, glucose, LDH and bacteriologic findings, the pleural effusion can be classified into three stages as summarized in Table PP10.1.

PROCEDURES FOR PLEURAL INVESTIGATION

Thoracocentesis is the simplest procedure to collect pleural fluid. It should be performed with patients sitting comfortably with their arms held straight upwards. The procedure should be explained to the patient and informed consent obtained. Sterile conditions should be maintained throughout the procedure.

Nosocomial pneumonia complicated by left lung abscess and empyema in an immunocompromised patient

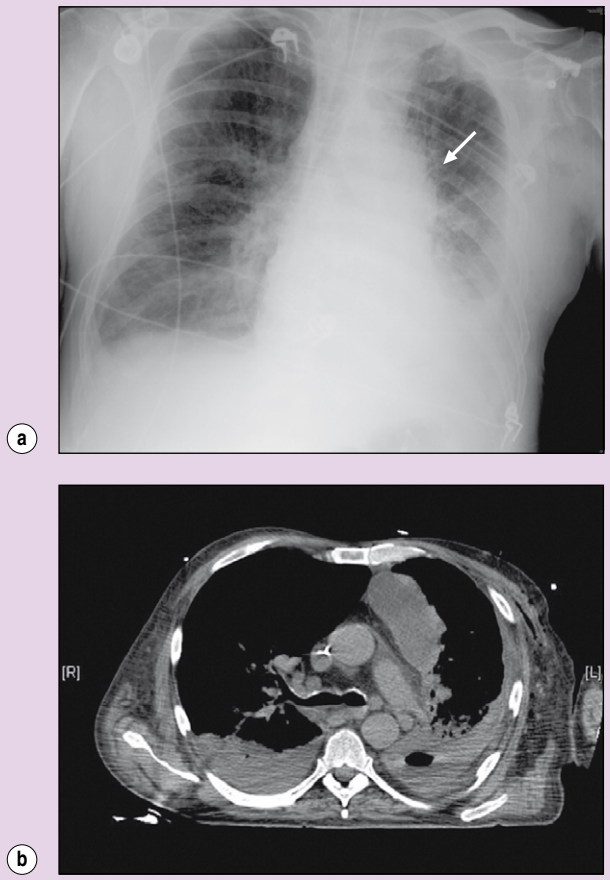


Fig. PP10.1 Nosocomial pneumonia complicated by left lung abscess and empyema in an immunocompromised patient. (a) Chest radiograph. (b) CT scan. White arrow indicates empyema.

The site of puncture is localized by percussion or ultrasonography and usually corresponds to the intersection between the hemithorax line and the sixth or seventh intercostal space. Local anesthesia should be administered to the upper side of the lower rib of the intercostal space until aspiration of pleural fluid into the syringe.

Insertion of a chest tube (from 16F to 28F) is recommended when a complicated PPE is diagnosed. Patients may be supine or semirecumbent. The ipsilateral arm should be placed behind the head. The safe zone for puncture corresponds to the intersection between the mammary and midaxillary line at the fourth to fifth intercostal space. The chest tube is introduced within the pleural space and directed towards the lower lobes. Aspiration of pleural fluid with a closed suction system is recommended at a depression range from –30 to –100 cmH_2O. Complications of chest tube insertion include pain, pneumothorax, hemorrhage and subcutaneous emphysema.

Small-bore catheters (from 8F to 16F) are more comfortable for patients and offer fewer complications. They should be placed using ultrasound or CT for the drainage of small or multiloculated effusion but they have also been used successfully for the management of empyema. These procedures should not be performed in patients presenting with coagulation abnormalities (for further details, see Dev *et al.* and Thomsen *et al.* in Further reading).

MANAGEMENT OF PLEURAL EFFUSION

Management of PPE is mainly guided by the results of pleural fluid analyses, which should be performed without delay (Fig. PP10.2). Delivering early and appropriate antimicrobial therapy will largely prevent the development of PPE or avoid the progression of uncomplicated to complicated PPE. In patients who already have complicated PPE, antibiotics alone are not sufficient to eradicate the pleural sepsis and pleural drainage is required for recovery. The risks for poor outcome situations requiring pleural drainage have been addressed by the American College of Chest Physicians. These include radiographic findings of a large effusion (>50% of hemithorax), a loculated effusion, an air–fluid level and pleural thickening. The characteristics of the pleural fluid also guide the need for drainage in the presence of pus or when one of the criteria for complicated PPE is present.

Pleural space drainage can be performed by repeat thoracocentesis, standard chest tube or small-bore catheter. Drainage failure may be related to misplacement or malfunction of the chest tube and loculation. In the setting of inappropriate pleural drainage, persistent pleural sep-

Table PP10.1 Pleural fluid analyses at different stages of parapneumonic effusion

Characteristics	Uncomplicated parapneumonic effusion	Complicated parapneumonic effusion	Empyema
Appearance	Slightly turbid	Cloudy	Pus
pH	≥7.2	<7.2	NA
Glucose, g/l	>0.6	<0.4	NA
LDH, IU/l	<700	>1000	NA
Ratio pleural:serum LDH	>0.6	>0.6	NA
Ratio pleural:serum protein	>0.5	>0.5	NA
PMN leukocyte count, cells/μl	<15 000	>25 000	NA
Gram stain and culture	Negative	May be positive	May be positive

LDH, lactate dehydrogenase; NA, not applicable; PMN, polymorphonuclear.

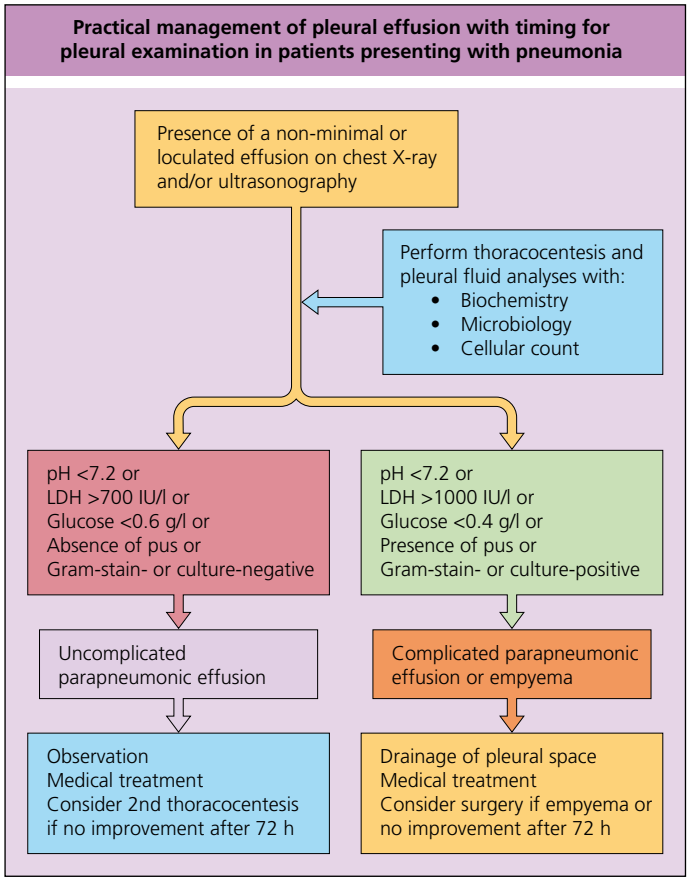


Fig. PP10.2 Practical management of pleural effusion with timing for pleural examination in patients presenting with pneumonia.

sis, multiple loculations or empyema, surgical options should be considered promptly. These include thoracoscopy, video-assisted thoracic surgery (VATS) and thoracotomy. VATS has been reported to improve the recovery rate in complicated PPE up to 86%. If VATS is unable to provide adequate pleural investigation, thoracotomy will be required.

Decortication is the optimal treatment, but this requires major surgery and cannot be performed in the most debilitated patients. It consists of stripping the visceral pleura, allowing the lung to fully expand into the parietal pleura. It is indicated in uncontrolled pleural sepsis or sometimes in restrictive ventilatory failure.

CONCLUSION

All patients with pneumonia should be screened to detect the presence of pleural effusion. If pleural effusion is more than minimal or loculated, thoracocentesis should be performed without delay. Examination of pleural fluid guides the management of PPE between observation, pleural drainage and surgery. It can discriminate uncomplicated from complicated PPE, providing information on the stage of the disease and the expected outcome. In nonpurulent effusion with pH <7.2, pleural drainage is recommended using either a small-bore catheter or a chest tube. Surgical options, starting with VATS, should be proposed in the presence of empyema or in ineffective pleural drainage. Decortication is a more invasive procedure which should only be performed in patients with uncontrolled pleural sepsis or unexpandable lung disease.

FURTHER READING

Further reading for this chapter can be found online at http://www.expertconsult.com

Practice point | 11 |

Marc Leone
Claude Martin

Managing postoperative fever

INTRODUCTION

Thermoregulation physiologists divide the body into core (deep chest and abdomen, central nervous system) and peripheral (arms, legs) thermal compartments. The core temperature is usually 2–4°C warmer than the peripheral compartments. Body temperature is tightly regulated by a complex system involving parallel positive- and negative-feedback loops. The system is located in nearly every part of the autonomic nervous system. Normal core temperatures typically range from 97.7°F to 98.6°F (36.5–37°C). Values less than 96.8°F (36°C) or greater than 100.4°F (38°C) are related to loss of control or a thermal environment so extreme that it overcomes thermoregulation defenses. In healthy individuals, the temperature varies by 0.5–1.0°C, according to circadian rhythm and menstrual cycle. In nursing home residents, the oldest are the coldest and fail to demonstrate a diurnal rise in body temperature.

DEFINITION

Fever is a rise of the normal core temperature exceeding the normal daily variation. The guidelines of the Society of Critical Care Medicine and the Infectious Diseases Society of America define arbitrary fever as a temperature of or above 100.9°F (38.3°C).[1] In neutropenic patients, fever is defined as a single oral temperature above 100.9°F (38.3°C) in the absence of an obvious environmental cause, or a temperature elevation above 100.4°F (38°C) for longer than 1 hour.

BRIEF PATHOPHYSIOLOGY

Fever is a normal adaptation in response to a pyrogenic stimulus resulting in the generation of cytokines and prostaglandins. Pyrogenic cytokines are released into the circulation by systemic mononuclear phagocytes that are activated by exogenous inflammatory agents. They are transported to the preoptic anterior hypothalamic area of the brain, where they act through prostaglandin E_2 activation.

In murine models of peritonitis, increasing core temperature from basal to febrile levels improves survival by accelerating pathogen clearance.[2] More conflicting results are found in models of pneumonia. The febrile response fails to improve survival despite affecting a profound reduction in pulmonary bacterial burden. Elsewhere, hyperthermia protects against pulmonary injury. Hence, antipyretic treatment should be administered in only a few situations where fever is irrefutably detrimental.

MEASURING BODY TEMPERATURE

Body temperature should be measured using the most accurate and reliable methods based on the clinical circumstances of the patient. The American College of Critical Care Medicine and the Infectious Diseases Society of America have provided guidelines for selecting the best sites and technologies for temperature measurement (Table PP11.1).[1]

Table PP11.1 Site and technology of temperature measurement

Order of sites accuracy	Limitations
Pulmonary artery thermistors	Pulmonary artery catheters not available
Urinary bladder thermistors	Costly Require urinary bladder catheter Should not prolong urinary bladder catheterization
Esophageal probe	Difficult placement Uncomfortable Risk of esophageal perforation
Rectal probe	Limitation due to patient position Spreading enteric pathogens Risk of trauma, perforation Perceived as unpleasant and intrusive
Oral probe	Requires alert and co-operative patients Local damage Should be avoided in neutropenic patients
Infrared ear thermometry	Conflicting results on accuracy Not accurate if auditory canal inflamed, tympanic membrane inflamed, external canal obstructed
Temporal artery thermometer	Impact of environmental temperature and sweating
Axillary thermometer	Lack of agreement with standard measurements
Chemical dot	Lack of agreement with standard measurements

ANESTHESIA- AND SURGERY-RELATED TEMPERATURE VARIATIONS

During anesthesia, patients are unable to activate behavioral responses.[3] Warm-response thresholds are elevated slightly, whereas cold-response thresholds are reduced. Manifestation of intraoperative fever is impaired by volatile anesthetics and muscle relaxants. Alfentanil reduces the febrile response to pyrogenic cytokines.[2]

The surgical procedure also interferes with postoperative fever incidence. Laparoscopic techniques have been associated with reduced incidences of postoperative fever, as compared with open techniques. Antibiotic prophylaxis and painkiller use are associated with reduced incidences of postoperative fever.

POSTOPERATIVE FEVER: INCIDENCE AND IMPACT

The incidence of postoperative fever ranges from 14% to 91%. Febrile patients are older, sicker, more likely to have undergone emergency surgery, more likely to develop organ dysfunction and more likely to die. On the first postoperative day after total hip arthroplasty, 44% of the patients are febrile. The number of febrile patients progressively decreases until the fifth postoperative day. The magnitude of postoperative fever is a determinant of mortality.

Fever is a nonspecific acute-phase response. An infectious cause is present in 40–60% of postoperative fevers. Fevers higher than 104.8°F (41°C) are more likely a result of noninfectious causes. Procalcitonin has been proposed to differentiate sepsis from other noninfectious causes of inflammation. Systematic reviews do not support the widespread use of the procalcitonin test in critical care settings.[4] One should note that a substantial proportion of infected patients may be euthermic or hypothermic, such as elderly patients, those with open abdominal wounds, patients with large burns, patients receiving extracorporeal membrane oxygenation or continuous renal replacement, and patients with congestive heart failure, end-stage liver disease or chronic renal failure.

STRATEGY FOR PATIENTS WITH POSTOPERATIVE FEVER

Fever occurring within two postoperative days does not need investigation beyond clinical assessment.[5] Surgical trauma is responsible for more than 70% of early postoperative fever episodes. Elsewhere, atelectasis (Fig. PP11.1) and medications are identified as causes of postoperative fever. β-lactam antibiotics, diphenylhydantoin and antiarrhythmics are commonly thought to induce fever in almost 10% of hospitalized patients. Other causes – including anesthetic drug effect on thermoregulation, transfusion of blood products, acalculous cholecystitis, local hematomas, deep venous thrombosis, pulmonary embolism, subarachnoid hemorrhage, fat embolism, hyperthyroidism and acute adrenal insufficiency (Fig. PP11.2) – can be responsible for postoperative fever. Thus, febrile patients should not be treated with antibiotics within two postoperative days. However, clinicians have to rule out pulmonary aspiration, myonecrosis and cellulitis. As suggested above, antipyretics should only be used if benefits exceed risks.

New or persistent fever occurring more than 72 hours after surgery suggests an infectious process. A systematic diagnostic approach helps to identify the potential source of infection (Fig. PP11.3).[5] Pneumonia is often suspected but remains diagnosed in less than 1% of postoperative patients. Clinical features, chest radiography showing new or progressive infiltrate, and blood and sputum cultures are the commonest components in diagnostic assessment (see Fig. PP11.1). Antibiotic therapy should be initiated early with a broad-spectrum β-lactam antibiotic. A glycopeptide can be added if methicillin-resistant *Staphylococcus aureus* is suspected.

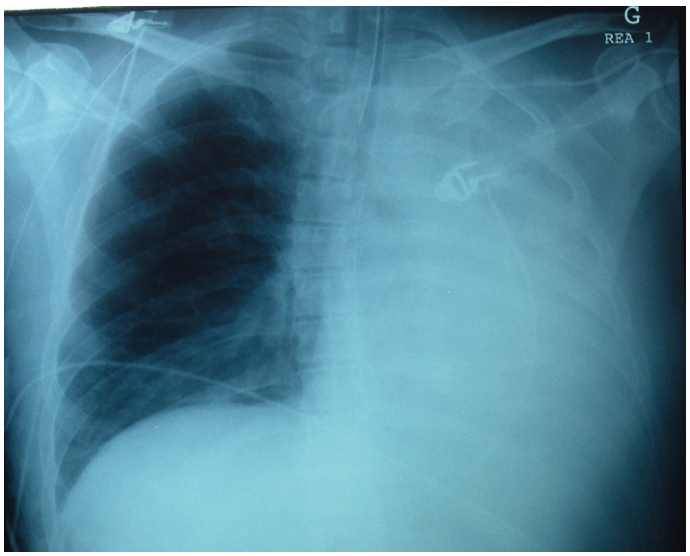


Fig. PP11.1 Chest radiograph showing complete opacification of the left hemithorax in a patient with postoperative fever. This suggests airway obstruction with whole-lung atelectasis. However, at first glance, ventilator-associated pneumonia cannot be ruled out. Fiberoptic examination will confirm this diagnosis. Another possible source is central line infection.

Urine cultures are positive in up to 30% of postoperative patients with a urinary bladder catheter *in situ*. However, as positive urine cultures are associated with sepsis in only 10% of patients, antibiotics should be used only after other causes of fever are ruled out. Wound infection is the second most common hospital-acquired infection. Subcutaneous abscesses are diagnosed by the presence of erythema, pus and tenderness of the surgical incision. Fascia and muscle can be involved. CT scan is useful in cases of deep infection (Fig. PP11.4). Deeper infections need early resuscitation, surgical debridement and antimicrobial therapy.

Catheter-related sepsis is another common cause of postoperative fever. The risk depends above all on the length of time since insertion. Central venous catheters inserted through the femoral vein are associated with a higher risk of infection than those inserted through the subclavian vein. Examination of catheter sites, blood cultures through the catheter and from peripheral venepuncture sites, and culture of suspected catheters lead to diagnosis. Removal of implanted sites should be discussed depending on the virulence of bacteria. Sinusitis, *Clostridium difficile* colitis, septic thrombophlebitis and fungal infection related to prolonged antibiotic use are less common causes of postoperative fever.

CONCLUSION

Fever is common after surgery and noninfectious causes are responsible for most episodes. Routine workup for early postoperative fever of low clinical yield adds cost. A rational approach is required for new or persistent fever arising more than 3 days after surgery. Among surgical patients with sepsis, fever-related infection seems protective, whereas excessive inflammatory response unrelated to infection predicts poor outcome.

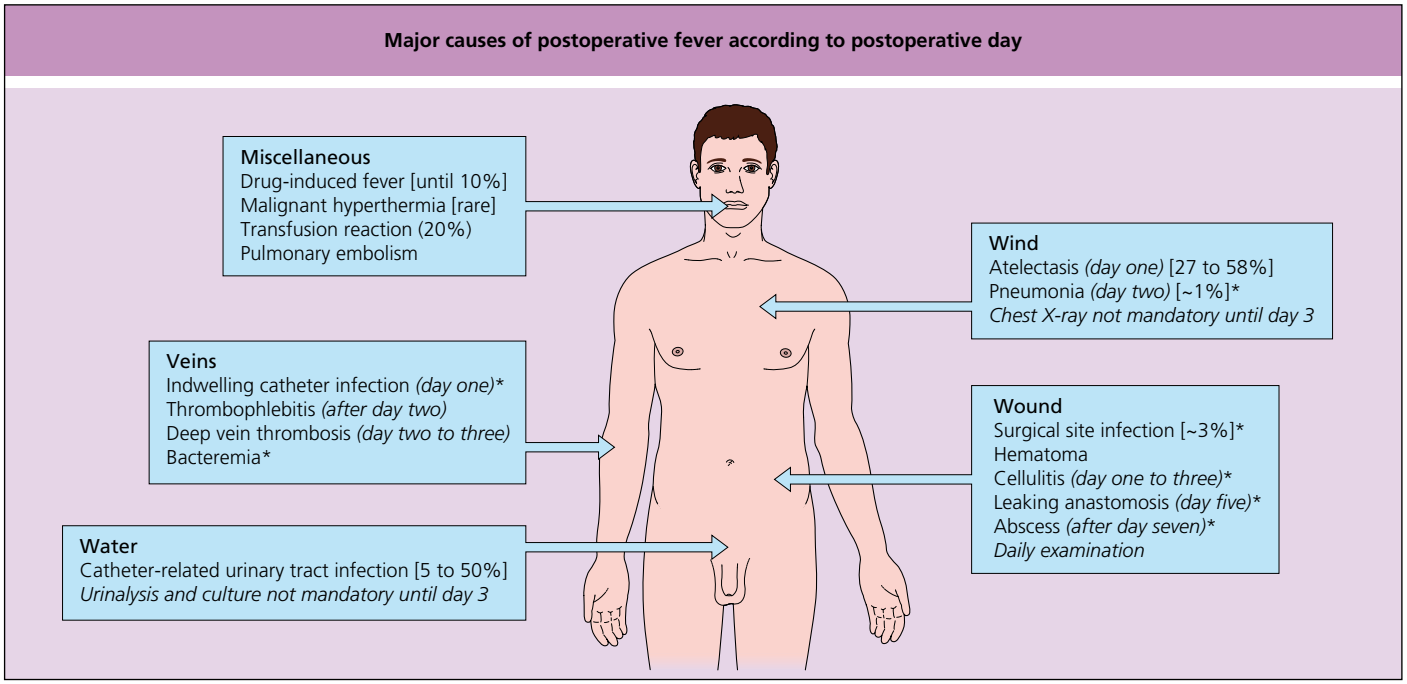


Fig. PP11.2 Major causes of postoperative fever according to postoperative day.

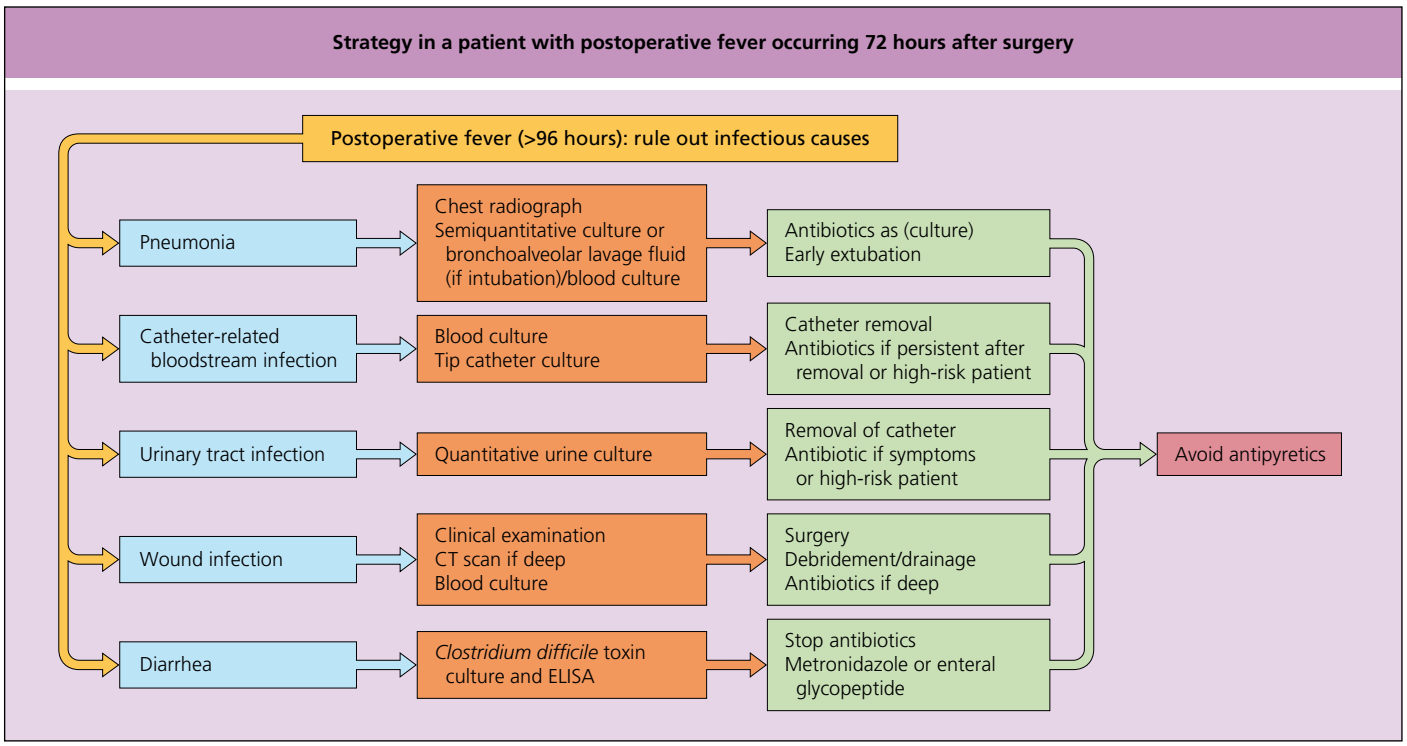


Fig. PP11.3 Strategy in a patient with postoperative fever occurring 72 hours after surgery. A systematic approach serves to rationalize the routine workups.

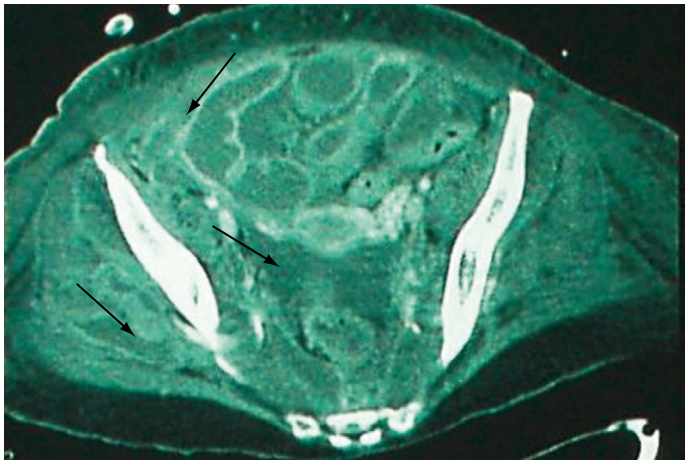


Fig. PP11.4 Persistent postoperative fever after gallbladder surgery. CT scan of the pelvic area shows several collections (arrows) requiring surgical drainage and prolonged antimicrobial therapy.

REFERENCES

References for this chapter can be found online at http://www.expertconsult.com

Practice point 12

Clarisse Rovery

Use of antibiotics in common respiratory infections

INTRODUCTION

Respiratory infections are the most common infections encountered. Although acute bronchitis usually does not require antibiotics, antibiotics are essential in the management of community-acquired pneumonia (CAP). Some patients with acute exacerbation of chronic obstructive pulmonary disease (COPD) might benefit from antibiotics. It is essential to make a correct diagnosis before prescribing antibiotics. Clinical distinction between these three syndromes is sometimes difficult and might require radiologic examination.

TREATMENT OF ACUTE BRONCHITIS

Acute bronchitis is one of the most common causes of antibiotic abuse, although it is generally caused by a virus. Most reports show that more than 70% of patients with acute bronchitis who seek care are given antibiotics. This has resulted in campaigns in most countries to dissuade physicians from this practice through the use of guidelines. In fact, multiple studies show that patients with acute bronchitis do not benefit from antibiotics.

TREATMENT OF ACUTE EXACERBATIONS OF CHRONIC OBSTRUCTIVE PULMONARY DISEASE

It is estimated that 50–60% of exacerbations of chronic obstructive pulmonary disease (COPD) are due to respiratory infections. Most exacerbations caused by respiratory infections are thought to be viral, rather than bacterial. *Haemophilus influenzae*, *Moraxella catarrhalis* and *Streptococcus pneumoniae* are the bacteria most frequently isolated during exacerbations of COPD. Even if bacteria are identified, it is uncertain whether they actually caused the exacerbation or whether they were present as part of the flora before the exacerbation. Patients who can clearly benefit from antibiotics are those with severe exacerbations; there is no evidence of benefit in patients with mild or moderate disease.

The optimal antibiotic regimen for the treatment of exacerbations of COPD has not been determined. The antibiotic chosen should target *H. influenzae*, *M. catarrhalis* and *S. pneumoniae*, while taking into account local patterns of antibiotic resistance. The recommended first-line agents (amoxicillin, trimethoprim–sulfamethoxazole, doxycycline) now have limited *in-vitro* activity against these pathogens. These days, broad-spectrum agents such as amoxicillin–clavulanate, azithromycin, telithromycin, cephalosporins, macrolides and fluoroquinolones are preferred (Table PP12.1).

TREATMENT OF COMMUNITY-ACQUIRED PNEUMONIA

Community-acquired pneumonia (CAP) is a common and serious illness with considerable mortality and morbidity. CAP is of particular concern for elderly patients with significant co-morbidities, and risk factors for drug resistance (see below) need to be sought. In fact, a new syndrome – health-care associated pneumonia (HCAP) – has recently emerged and has been shown to affect a significant number of patients. Treatment of HCAP is as for nosocomial pneumonia.

Indications for hospitalization

Determination as to whether a patient with CAP can safely be treated as an outpatient or requires hospitalization is essential before selecting an antibiotic regimen. Severity of illness scores have been developed to assist physicians in the decision of site of care. The Pneumonia Severity Index (PSI, Table PP12.2) and the CURB-65 are the two best evaluated scores. The PSI is better studied and evaluated, but requires a more complicated assessment. The CURB-65 uses five prognostic values:

- **C**onfusion;
- **U**rea greater than 7 mmol/l (blood urea nitrogen >20 mg/dl);
- **R**espiratory rate of 30 breaths per minute or greater;
- **B**lood pressure less than 90 mmHg systolic or diastolic blood pressure 60 mmHg or less;
- age **65** or older.

Patients with PSI <3 and CURB-65 <2 should be treated as outpatients.

Principles of antimicrobial therapy

The selection of antibiotics for empiric therapy is based upon a number of factors, including the most likely pathogens, clinical trials proving efficacy, risk factors for antimicrobial resistance, and medical co-morbidities that can influence the likelihood of a specific pathogen and may be a risk for clinical failure.

Drug resistance

Risk factors for infection with multidrug-resistant (MDR) pathogens include:

- treatment with intravenous antibiotics within the preceding 90 days;
- current hospitalization of >5 days;
- admission from a health-care-related facility (e.g. long-term care facility, dialysis unit);
- hospitalization for 2 days or more in the preceding 90 days;

Table PP12.1 Frequently used antibiotics for common respiratory infections

Antibiotic	Adults ≥60 kg	Adults <60 kg	Duration in CAP
Erythromycin (iv)	1 g q6–8h	30–40 mg/kg q24h in four equally divided doses (max 4 g)	Until afebrile for 2–3 days, minimum 5 days
Erythromycin (po)	500 mg q6h	30–40 mg/kg q24h in four equally divided doses (max 4 g)	Until afebrile for 2–3 days, minimum 5 days
Azithromycin (po)	500 mg day 1, then 250 mg q24h	5 mg/kg q24h (max 500 mg q24h)	5 days
Azithromycin (iv)	500 mg q24h	5 mg/kg q24h (max 500 mg q24h)	Until afebrile for 2–3 days, minimum 5 days
Clarithromycin (po)	500 mg q12h	7.5 mg/kg q12h (max 1 g q24h)	Until afebrile for 2–3 days, minimum 5 days
Telithromycin (po)	800 mg q24h	800 mg q24h	Until afebrile for 2–3 days, minimum 5 days
Doxycycline (po)	100 mg q12h	4 mg/kg q24h (max 200 mg) in two equally divided doses	7–10 days
Amoxicillin (iv/po)	1 g q8h	100 mg/kg q24h in three equally divided doses	Until afebrile for 2–3 days, minimum 5 days
Ampicillin–sulbactam (im/iv)	2 g ampicillin + 1 g sulbactam q6h	200 mg ampicillin/100 mg sulbactam/kg q24h in four divided doses (max 4 g sulbactam)	Until afebrile for 2–3 days, minimum 5 days
Amoxicillin–clavulanate (iv/po)	3–4 g q24h in two to four equally divided doses	80 mg/kg q24h in three equally divided doses (max 4 g/j)	Until afebrile for 2–3 days, minimum 5 days
Ceftriaxone (iv/im)	2 g q24h	50–75 mg/kg q24h (max 4 g q24h)	Until afebrile for 2–3 days, minimum 5 days
Cefotaxime (iv)	2 g iv q8h	150–200 mg/kg q24h in three or four equally divided doses (max 8–10 g q24h)	Until afebrile for 2–3 days, minimum 5 days
Gemifloxacin (po)	320 mg q24h	320 mg q24h	Until afebrile for 2–3 days, minimum 5 days
Levofloxacin (iv/po)	750 mg q24h	750 mg q24h	Until afebrile for 2–3 days, minimum 5 days
Moxifloxacin (iv/po)	400 mg q24h	400 mg q24h	Until afebrile for 2–3 days, minimum 5 days
Gatifloxacine (iv/po)	400 mg q24h	400 mg q24h	Until afebrile for 2–3 days, minimum 5 days

CAP: community-acquired pneumonia; iv, intravenous; po, per os.

- home infusion therapy;
- chronic dialysis;
- home wound care;
- family member with an MDR pathogen;
- immunosuppressive disease and/or therapy.

If one of these risk factors exists, pneumonia is no longer considered as community acquired, but as HCAP. Empiric treatment should be directed against MDR pathogens.

Treatment regimens for outpatients

No co-morbidities or recent antibiotic use

Two different approaches are used (Table PP12.3). The North American approach is to use first-line antibiotics that provide coverage for *S. pneumoniae* plus atypical pathogens. The macrolides, which are effective against atypical pathogens, are therefore recommended in the absence of significant risk factors for macrolide-resistant *S. pneumoniae* (e.g. recent use of macrolides). Erythromycin is not recommended due to its frequent side-effects. In Europe, due to the high level of resistance of *S. pneumoniae* to macrolides, amoxicillin and telithromycin are the recommended first-line empiric antibiotics. In fact, treatment failures have been demonstrated with the use of macrolides for macrolide-resistant *S. pneumoniae*. Despite *in-vitro* resistance, penicillin-resistant *S. pneumoniae* may respond to higher-dose β-lactams.

Benefit of initial coverage of atypical pathogens in outpatients is currently unclear. The use of fluoroquinolones in ambulatory CAP is discouraged since there is concern that widespread use of fluoroquinolones may promote fluoroquinolone resistance among bacteria. Concern has been expressed regarding liver toxicity of telithromycin, thus limiting its use.

Co-morbidities or recent antibiotic use

Where there are significant co-morbidities (e.g. chronic obstructive pulmonary disease, liver or renal disease, cancer, diabetes, chronic heart disease, alcoholism, immunosuppression) and/or use of antibiotics

Table PP12.2 Calculation of the Pneumonia Severity Index (PSI)

Factor	PSI score
Patient age	Age in years (male) or age –10 (female)
Nursing home resident	+10
Coexisting illnesses	
Neoplastic disease	+30
Liver disease	+20
Congestive cardiac failure	+10
Cerebrovascular disease	+10
Renal disease	+10
Signs on examination	
Altered mental state	+20
Respiratory rate ≥30 per minute	+20
Systolic blood pressure <90 mmHg	+20
Temperature ≤35°C or ≥40°C	+15
Pulse rate ≥125 bpm	+10
Results of investigations	
Arterial pH <7.35	+30
Serum urea level ≥11 mmol/l	+20
Serum sodium level <130 mmol/	+10
Serum glucose level ≥14 mmol/lL	+10
Hematocrit <30%	+10
Po_2 <60 mmHg or O_2 saturation <90%	+10
Pleural effusion	+10

Mortality within 30 days according to PSI risk class

Risk class	Score	Mortality (%)
I	Score not calculated	0.1
II	≤70	0.6
III	71–90	0.9
IV	91–130	9.3
V	>130	27.0

against infection with more resistant pathogens, the preferred regimens are combination therapy with a β-lactam effective against *S. pneumoniae* or a third-generation cephalosporin plus a macrolide or doxycycline; an alternative is a respiratory fluoroquinolone.

Treatment regimens for hospitalized patients

Not in the ICU

Combination therapy with a third-generation cephalosporin plus a macrolide is recommended; an alternative is a respiratory fluoroquinolone.

In the ICU

Intravenous combination therapy with a third-generation cephalosporin plus either a macrolide or a respiratory fluoroquinolone is recommended. In patients who may be infected with *Pseudomonas aeruginosa* (particularly those with bronchiectasis or COPD and frequent antimicrobial or corticosteroid use), therapy should include agents effective against *P. aeruginosa*, *S. pneumoniae* and *Legionella* spp. For severely ill patients, empiric treatment should include vancomycin or linezolid if there are risk factors for methicillin-resistant *Staphylococcus aureus* infection (e.g. recent influenza-like illness or a Gram stain of respiratory secretions suggesting *S. aureus*).

Table PP12.3 First- and second-line antibiotics for common respiratory infections

Syndrome	First-line antibiotics	Second-line antibiotics
Acute bronchitis	No antibiotics	
Community-acquired pneumonia		
Non-severe pneumonia, no co-morbidities (outpatient, PSI 1–2)	USA: macrolide Europe: amoxicillin or telithromycin	Respiratory fluoroquinolones
Moderately severe pneumonia (PSI 3) or significant co-morbidities	Amoxicillin–clavulanate or third-generation cephalosporin, plus macrolide	Respiratory fluoroquinolones
Severe pneumonia (PSI 4–5)	Third-generation cephalosporin, plus macrolide or respiratory fluoroquinolone	Antipseudomonal antibiotics if risk factors for *Pseudomonas aeruginosa* (bronchiectasis or chronic obstructive pulmonary disease and frequent antimicrobial or corticosteroid use), plus vancomycin or linezolid if risk factors for methicillin-resistant *Staphylococcus aureus* (prior influenza or Gram stain of respiratory secretions suggesting *S. aureus*)

PSI, Pneumonia Severity Index.

Health-care associated pneumonia

HCAP should be treated as for nosocomial pneumonia with broad-spectrum antibiotics directed against potential MDR pathogens, including *P. aeruginosa*.

Delay in administering the first dose of antibiotics has been associated with higher mortality and longer length of hospital stay. The first dose of antibiotics for the hospitalized patient should be administered within 6 hours of admission. Antibiotics should be modified when results of diagnostic studies indicate a specific pathogen. Intravenous therapy should be switched to oral therapy when patients are hemodynamically stable, demonstrate some clinical improvement and are able to take oral medication. Antibiotic therapy should be modified based on microbiologic results.

The recommended duration of therapy in CAP is 48–72 hours after clinical improvement (defined as no more fever) with a minimum of 5 days. This should be extended if infection is due to *Chlamydia pneumoniae* (21 days) or *Legionella pneumophilia* (10–14 days), or if the patient had necrotizing pneumonia or empyema (minimum 21 days).

FURTHER READING

Further reading for this chapter can be found online at http://www.expertconsult.com

Practice point | 13 |

Guy Thwaites

When to use corticosteroids in noncentral nervous system tuberculosis

INTRODUCTION

The use of corticosteroids in the management of noncentral nervous system tuberculosis is supported by much anecdote but little data from controlled trials. It has long been observed that the symptoms of tuberculosis often worsen after the start of treatment, a phenomenon that is believed to be caused by an exaggerated inflammatory response to dead or dying mycobacteria. It is hypothesized that corticosteroids suppress this response and thereby improve outcome. This hypothesis has never been clearly refuted or confirmed in any form of the disease, but remains sufficiently attractive to induce many physicians to start adjunctive corticosteroids, particularly when faced with a patient with severe or disseminated disease whose clinical condition deteriorates after the start of antituberculosis therapy.

EVIDENCE OF EFFICACY

The most convincing evidence for a beneficial effect of corticosteroids in noncentral nervous system disease exists for pericardial tuberculosis and stems primarily from two randomized controlled trials performed 25 years ago in South Africa.

- The first trial compared prednisolone with placebo in the treatment of 143 patients with active constrictive tuberculous pericarditis and showed that prednisolone increased the rate of clinical improvement, reduced the risk of death from pericarditis and reduced the need for pericardiectomy.
- The second trial studied 243 patients with tuberculous pericardial effusion and used a factorial design to compare the value of open complete surgical drainage with percutaneous pericardiocentesis as required; in addition, all patients were randomized to receive either prednisolone or placebo. Prednisolone reduced the risk of death from pericarditis and the need for repeat pericardiocentesis, but did not reduce the incidence of constrictive pericarditis. After 10 years of follow-up, the use of prednisolone was associated with a significant reduction in the risk of death from pericarditis of either form.

In the light of these data, treatment guidelines published in the UK and the USA recommended adjunctive prednisolone for the treatment of HIV-uninfected patients with pericardial tuberculosis. There is less certainty as to whether the same should apply for those with HIV infection, although a small randomized controlled trial from Zimbabwe suggested that 6 weeks of adjunctive prednisolone reduced the risk of death in adults with effusive tuberculous pericarditis and advanced HIV infection.

There is no convincing evidence that adjunctive corticosteroids benefits patients with other forms of noncentral nervous system tuberculosis, although few large randomized controlled trials have been conducted. Advocates of corticosteroids will point out that there is little evidence they do harm and appear to speed symptom resolution. Data from 12 controlled trials of corticosteroids for pulmonary tuberculosis support this view. Treatment with corticosteroids was associated with more rapid resolution of symptoms and chest radiographic appearances in many of the studies, although a reduction in the incidence of chronic restrictive lung disease or death was not observed. The beneficial effect on clinical condition was most pronounced in those with severe lung disease and it is probably only in these circumstances that adjunctive corticosteroids should be given for pulmonary tuberculosis. However, data from a large, well-conducted study published in 1983 strike an important note of caution: patients with bacteria resistant to two or more drugs who received prednisolone responded to treatment less well than those in the control group and bacteria could be cultured from the sputum for longer. Therefore, the risk of drug resistance should be carefully assessed before adjunctive corticosteroids are considered for any form of tuberculosis.

There is evidence that corticosteroids speed early symptom resolution in primary tuberculosis and tuberculous pleuritis and peritonitis, although their use does not appear to reduce the fibrotic complications of these diseases. Corticosteroids may also reduce mediastinal lymph node enlargement in primary tuberculosis and decrease the local obstructive complications, although the latter was not a recorded end point in the two published controlled trials. Unfortunately, there have been no controlled trials of the use of adjunctive corticosteroids in the treatment of cervical or inguinal tuberculous lymphadenitis, despite painful node enlargement occurring in one-third of patients after the start of treatment. Extrapolation of the effect of corticosteroids on mediastinal adenopathy suggests they might be of benefit and many physicians use them in patients with painful, swollen nodes that are threatening to rupture. A summary of the evidence and treatment recommendations for all forms of noncentral nervous system tuberculosis are presented in Table PP13.1.

USE OF ADJUNCTIVE CORTICOSTEROIDS IN TUBERCULOSIS AND HIV INFECTION

The safety and efficacy of adjunctive corticosteroids in patients with tuberculosis and HIV infection are unproven. HIV-infected adults with pericardial tuberculosis probably should receive prednisolone, but for all other forms of HIV-associated tuberculosis (with the exception of cerebral tuberculosis) there are no grounds to recommend their routine use. In addition, while there is little evidence that adjunctive corticosteroids are harmful to HIV-uninfected patients with drug-susceptible tuberculosis, this may not be true for those infected with HIV.

Table PP13.1 Summary of treatment recommendations and evidence for adjunctive corticosteroids in noncentral nervous system tuberculosis

Type of tuberculosis	Corticosteroids recommended by the National Institute for Health and Clinical Excellence (2006)	Corticosteroids recommended by the Infectious Diseases Society of America (2003)	Suggested corticosteroid regimens	Summary of the evidence from randomized controlled trials (RCTs)
Pulmonary	No	No		Twelve RCTs of variable size and quality suggest corticosteroids may speed resolution of symptoms and chest X-ray changes, but have no beneficial effect on long-term fibrotic complications or death. May be indicated in those with severe lung disease
Pericardial	Yes	Yes	Prednisolone 60 mg weeks 1–4 30 mg weeks 5–8 15 mg weeks 9–10 5 mg week 11. Then stop	Three RCTs (one involving HIV-infected adults) observed prednisolone was associated with faster resolution of effusions and lower mortality. Progression to constrictive disease not affected
Pleural	No	No		Four RCTs suggest prednisolone resulted in faster resolution of symptoms and effusion, but no impact on development of fibrosis, restrictive lung disease or death
Primary	No (except if evidence of bronchial obstruction)	No		Two RCTs suggest prednisolone may reduce mass effects of mediastinal lymphadenopathy, although one trial used very high doses (5 mg/kg) to achieve the effect
Lymph node	No	No		No evidence from controlled trials
Bone and joint	No	No		No evidence from controlled trials
Peritoneal	No	No		One RCT of 47 patients, using prednisolone 30 mg q24h for 3 months, reported no difference in symptom resolution between groups and a nonsignificant reduction in chronic fibrotic complications in the prednisolone group
Genitourinary	No	No		No evidence from controlled trials
Miliary	No	No		One RCT from China involving 55 patients (14 of whom also had meningeal disease) reported fewer deaths in the prednisolone group (not statistically significant)

A randomized controlled trial of prednisolone for HIV-infected adults with pleural tuberculosis in Uganda suggested the prednisolone-treated group recovered faster but suffered from a significantly increased incidence of Kaposi's sarcoma. Peripheral blood $CD4^+$ T-cell counts and viral loads increased in both treatment arms, but without any significant difference between them. Another study from the same centre of the immunoadjuvant properties of prednisolone in HIV-infected adults with pulmonary tuberculosis again observed a transient and worrying rise in peripheral blood HIV viral load in the prednisolone group that fell when the drug was discontinued. Unlike the previous study, there was no observed increase in Kaposi's sarcoma or other opportunistic infections in those treated with prednisolone, although they suffered more fluid retention, hypertension and hyperglycemia than the controls. The study was not powered to detect a difference in clinical outcome and few data regarding clinical and radiographic progress are given. However, the authors report a faster time to sputum sterility in the prednisolone group, an intriguing observation that replicates the findings of studies performed 40 years previously in HIV-uninfected patients with pulmonary tuberculosis.

There are no controlled trial data from patients receiving highly active antiretroviral therapy and studies involving such patients are urgently required (see Chapters 92 and 93).

FURTHER READING

Further reading for this chapter can be found online at http://www.expertconsult.com

Practice point | 14 | L Peter Ormerod

How to manage a patient on anti-TB therapy with abnormal liver enzymes

INTRODUCTION

Although isoniazid, rifampin (rifampicin) and pyrazinamide are all potentially hepatotoxic, when pyrazinamide is added to the other drugs there does not seem to be an increase in the incidence of hepatitis. However, in unselected cases outside clinical trials, pyrazinamide is the drug with the highest monthly rate of hepatitis.

The incidence of hepatic reactions to antituberculosis drugs depends on the drug itself, and the age and possibly sex and ethnic group of the patient. Hepatic reactions to antituberculosis drugs were reported in 4% of cases treated with isoniazid/rifampin with or without pyrazinamide in a UK trial, and in 3% in a large clinical series. The overall rate of adverse reactions increases with age. Liver function tests (LFTs), particularly serum bilirubin and transaminases – aspartate aminotransferase (AST) and alanine aminotransferase (ALT) – should therefore be checked before treatment. Pre-treatment liver function testing is not advised for children receiving treatment for latent infection (chemoprophylaxis) because of the very low incidence of reactions. This may, however, be required if rifampin/pyrazinamide for 2 months is used to treat latent infection in adults, a regimen not advised in the UK where rifampin/isoniazid for 3 months is the alternative to isoniazid.

TESTING OF LIVER FUNCTION

Regular monitoring of liver function is not required for those with no evidence of pre-existing liver disease and normal liver function pre-treatment. Patients and their family doctors should, however, be informed of possible side-effects and the indications for stopping medication and seeking medical advice, preferably in writing in their native language. Tests of liver function only need to be repeated (and treatment stopped) if fever, vomiting, jaundice, malaise or unexplained deterioration occur. In such circumstances virologic tests to exclude coexistent viral hepatitis (A or B) should also be considered.

MANAGEMENT OF ELEVATED TESTS OF LIVER FUNCTION

Modest elevations of hepatic transaminases (AST/ALT) are not uncommon in tuberculosis patients even without known liver disease. They are also to be expected in patients with chronic liver diseases, including alcoholism, chronic active hepatitis and cirrhosis, and in chronic carriers of hepatitis B or C. Monitoring of such patients should be as in Figure PP14.1. If the pre-treatment AST/ALT is more than twice normal, liver function should be monitored weekly for 2 weeks then 2-weekly until normal. If the pre-treatment AST/ALT is less than twice normal, liver function should be repeated at 2 weeks. If the transaminase levels have fallen further, repeat tests are only needed for symptoms. However, if the AST/ALT rises to above twice normal, management should be as above (see Fig. PP14.1).

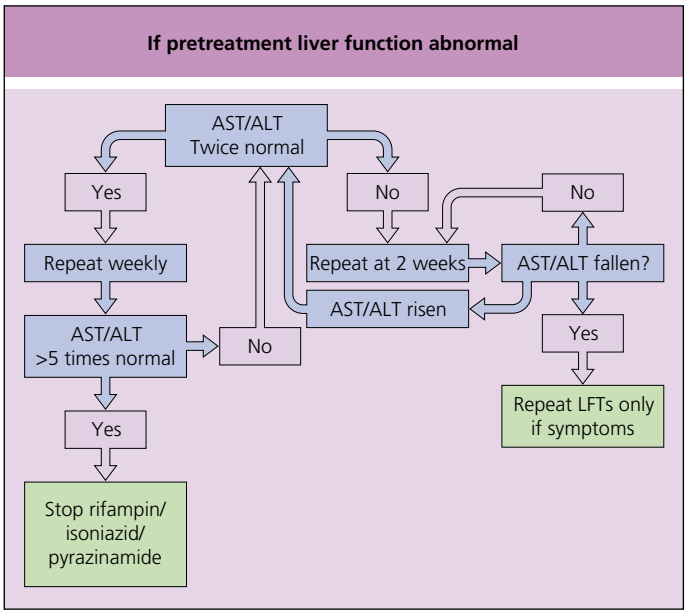


Fig. PP.14.1 Management of elevated tests of liver function if pre-treatment liver function is abnormal. Derived from Ormerod LP, Skinner C, Wales J. Hepatotoxicity of antituberculosis drugs. Thorax 1996;51:111–13, with permission from BMJ Publishing Group.

If the AST/ALT rises to above five times normal, or the bilirubin becomes elevated, then isoniazid, rifampin and pyrazinamide should be stopped. Immediate management then depends on the severity of the patient's illness and whether they are infectious as judged by positivity on sputum smear-microscopy within 2 weeks of treatment commencement (Fig. PP14.2). If the patient is not unwell and had a form of tuberculosis that is noninfectious, no treatment is needed until liver function returns to pre-treatment levels. If the patient is unwell or the sputum is smear-positive within 2 weeks of commencing a rifampin/isoniazid-based regimen, some form of drug treatment needs to be given until liver function returns to pre-treatment levels, preferably as an inpatient because of the monitoring required. In such cases a regimen of streptomycin and ethambutol with appropriate renal and visual checks is advised unless there are clinical

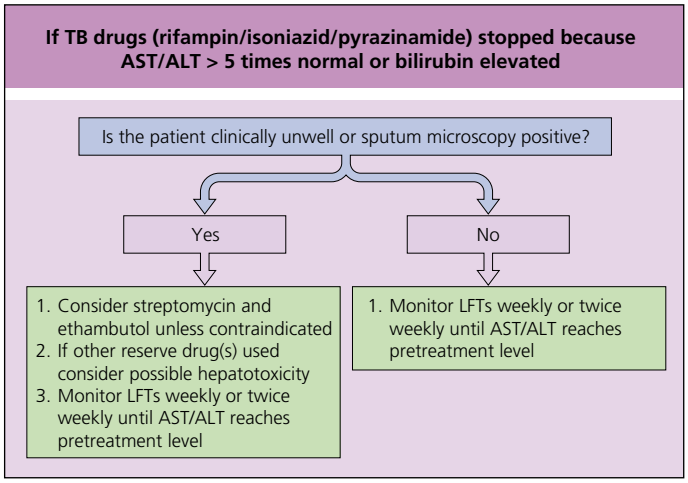


Fig. PP14.2 Management of elevated tests of liver function if antituberculosis drugs (rifampin/isoniazid/pyrazinamide) stopped because AST/ALT over five times normal or bilirubin elevated. Derived from Ormerod LP, Skinner C, Wales J. Hepatotoxicity of antituberculosis drugs. Thorax 1996;51:111–13, with permission from BMJ Publishing Group.

contraindications or drug resistance to these agents is known or suspected. If second-line drugs are used, any potential hepatotoxicity (e.g. with macrolides, quinolones, prothionamide or ethionamide) should be considered. Occasionally patients with serious liver disease and active tuberculosis have very abnormal LFTs, with raised bilirubin and AST/ALT over five times normal pre-treatment levels. In these circumstances a risk/benefit analysis needs to be made, and often the risk of death (and infectivity) from untreated tuberculosis significantly exceeds that of additional hepatotoxicity. Standard treatment may then be appropriate under corticosteroid cover with regular monitoring of liver function.

Once liver function has returned to normal or pre-treatment levels, challenge doses of the original drugs can be re-introduced sequentially (Table PP14.1). Such re-introduction is best carried out in parallel with treatment with ethambutol 15 mg/kg to prevent the possible emergence of drug resistance during the re-introduction. The patient's condition should be monitored daily together with liver function. Sequential re-introduction of isoniazid, rifampin and pyrazinamide is advised. Isoniazid should be started initially at 50 mg/day, increasing to 150 mg/day after 2–3 days and then 300 mg/day after a further 2–3 days if no reaction occurs, which is then continued. After a further 2–3 days without reaction, rifampin at a dose of 75 mg/day can be added, increasing to 300 mg/day after 2–3 days, and then to 450 mg (<50 kg) or 600 mg (>50 kg) according to patient's weight after a further 2–3 days without reaction, and then continued. Finally, pyrazinamide can be added at 250 mg/day, increasing to 1.0 g after 2–3 days and then to either 1.5 g (<50 kg) or 2.0 g (>50 kg). If there is no further reaction, standard chemotherapy can be continued and any alternative drugs introduced temporarily may then be withdrawn. Ethambutol may need to be continued in the initial phase if indicated by local drug-resistance profile and national treatment guidelines.

Table PP14.1 Order of re-Introduction* of antituberculosis agents following drug-associated hepatotoxicity

Start: Ethambutol (15 mg/kg) plus isoniazid 50 mg q24h for 2–3 days then plus isoniazid 150 mg q24h for 2–3 days then plus isoniazid 300 mg q24h for 2–3 days and continue
If no reaction then: Ethambutol (15 mg/kg) plus isoniazid 300 mg q24h plus rifampin (rifampicin) 75 mg q24h for 2–3 days then plus rifampin 300 mg q24h for 2–3 days then plus rifampin 450 mg q24h (under 50 kg) or rifampin 600 mg q24h (≥50 kg) for 2–3 days and continue
If no reaction then: Ethambutol (15 mg/kg) plus isoniazid 300 mg q24h plus rifampin 450/600 mg q24h and add sequentially: pyrazinamide 250 mg q24h for 2–3 days then pyrazinamide 1 g q24h for 2–3 days then pyrazinamide 1.5 g (<50 kg) or pyrazinamide 2 g (≥50 kg) for 2–3 days and then continue if no reaction
Ethambutol can then be withdrawn if not required on drug resistance probability grounds
*Re-introduction is carried out with daily monitoring of clinical condition and regular LFTs. Derived from Ormerod LP, Skinner C, Wales J. Hepatotoxicity of antituberculosis drugs. Thorax 1996;51:111–13, with permission from BMJ Publishing Group.

MANAGEMENT OF FURTHER REACTIONS FOLLOWING RE-INTRODUCTION OF THERAPY

If there is a further reaction during the re-introduction, the offending drug should be excluded and an alternative regimen constructed. If pyrazinamide is the offending drug, then a regimen of rifampin and isoniazid for 9 months, supplemented by ethambutol for 2 months, will be required. For other drugs an alternative regimen may need to be on the advice of an experienced TB physician. Sometimes, for example because of drug resistance, the choice of drugs is so limited that if reactions occur, desensitization and re-introduction of the offending drug may be necessary using conventional protocols.

Such densensitization may need to be carried out under the cover of two other antituberculosis drugs to avoid the emergence of drug resistance.

FURTHER READING

Further reading for this chapter can be found online at http://www.expertconsult.com

Practice point **15**

Daniel Rosenbluth

Management of the infected cystic fibrosis patient

DEFINITION OF THE PROBLEM

The airways of persons with cystic fibrosis (CF) are chronically infected with bacteria, usually *Staphylococcus aureus* and *Pseudomonas aeruginosa* (typically mucoid strains). This persistent infection and the accompanying inflammatory response result in mucus hypersecretion with obstruction of smaller airways, bronchiectasis and the symptoms of chronic cough, sputum production and dyspnea. While chronic symptoms are slowly progressive, intermittently patients may have a more accelerated decline associated with increases in the bacterial burden in the airways with additional symptoms, usually referred to as an acute respiratory exacerbation. Optimal management of the infected CF patient necessitates strategies to combat both the chronic airway infection and acute exacerbations.

TYPICAL CASE

Persons with cystic fibrosis typically exhibit symptoms of chronic cough, sputum production and dyspnea on exertion when they develop more advanced obstructive lung disease. Exacerbations of their lung disease are usually characterized by an increase in cough and sputum production, a change in the color (to darker or more green) or character (thicker and more viscous) of the sputum, dyspnea and worsening exercise tolerance. These exacerbations may also be accompanied by generalized fatigue, fevers, decreased appetite, weight loss and, in diabetic patients, worsening glycemic control. Acute exacerbations may be highlighted by massive hemoptysis, a true medical emergency (Table PP15.1).

Physical examination may demonstrate new clinical findings such as wheezes or crackles, use of accessory muscles of respiration, tachycardia, cyanosis and decreased oxyhemoglobin saturation. Chest X-rays may reveal a new infiltrate or evidence of increased mucus plugging on a background of chronic bronchiectatic changes. Many patients with acute exacerbations will not demonstrate any change in their radiograph. On occasion the onset of an exacerbation may be insidious and occur over weeks. Under these circumstances patients may not fully appreciate the magnitude of their decline unless questioned carefully. In these cases pulmonary function testing (discussed below) will usually detect a significant change from the patient's baseline.

DIAGNOSIS

Diagnosis of an acute respiratory exacerbation of cystic fibrosis-related bronchiectasis is best made by a clinician experienced in the care of patients with CF. It is usually made when a patient presents with a combination of some or all of the above-mentioned signs and symptoms, in association with a decrease in their forced vital capacity (FVC) and forced expiratory volume in 1 second (FEV1). These pulmonary function tests or spirometry are the best objective measure of a patient's status and during exacerbations will usually demonstrate worsening airway obstruction and a decline of at least 10%. A significant decline in FEV1 even in the absence of worsening symptoms should result in an intensification of the patient's therapy.

Table PP15.1 Signs and symptoms of acute exacerbations of CF-related lung disease

- Increase in cough and sputum production
- Change in the color or character of sputum
- Dyspnea and worsening exercise tolerance
- Fatigue
- Fevers
- Decreased appetite
- Weight loss
- Worsening glycemic control
- Massive hemoptysis
- New wheezes or crackles
- Use of accessory muscles of respiration
- Tachycardia
- Cyanosis
- New radiographic infiltrate
- Decrease in FEV1

MANAGEMENT OPTIONS

Optimal management of the infected patient with CF requires the identification of specific pathogens which infect the individual patient. Early in life infection with *S. aureus* and *Haemophilus influenzae* is common. While infection with oxacillin-sensitive and resistant *S. aureus* may persist as patients age, infection with *P. aeruginosa* and, less commonly, other Gram-negative organisms, such as *Achromobacter xylosoxidans, Stenotrophomonas maltophilia* and *Burkholderia cepacia*, predominates. Infections with *B. cepacia* can be especially difficult to treat, and have been associated with an accelerated decline in lung function in some patients and with epidemic outbreaks at some CF care centers. As this organism may not grow readily in culture, all respiratory samples from patients with CF should be cultured on media specific for this organism, in addition to the other organisms mentioned above. Increasing frequency of infection in CF patients with community-associated methicillin-resistant *S. aureus*, sometimes expressing the Panton–Valentine leukocidin (PVL) virulence factor, has recently been documented. This may be associated with acute invasive pulmonary infections and abscesses. When applied to lower respiratory tract secretions, techniques for molecular identification of pathogens may

identify pathogens (including anaerobes) which are not typically associated with airway infection in CF. The significance of these pathogens remains to be determined.

Many patients will be infected with multiple organisms or multiple colony types of the same organism that may have different susceptibility patterns. It is therefore recommended that all organisms and their differing microcolonies be identified in culture and have their susceptibility patterns determined to facilitate selection of the optimal antibiotic regimen.

When treating acute exacerbations (Table PP15.2), selection of specific antimicrobials should be guided by results of sputum culture and sensitivity. Mild exacerbations may sometimes be treated with oral and/or inhaled antibiotics if effective agents are available. Response to therapy should be closely monitored and if ineffective, a switch should be made to parenteral therapy. More severe exacerbations should be treated parenterally and in general two antipseudomonal agents are chosen, usually a third- or fourth-generation cephalosporin or antipseudomonal penicillin, along with an aminoglycoside. Therapy should be modified based on culture results. The antipseudomonal penicillins or carbapenems may be more active against *Achromobacter xylosoxidans*, and carbapenems such as meropenem may have increased activity against *B. cepacia*. Infections with *Stenotrophomonas maltophilia* are sometimes best treated with trimethoprim–sulfamethoxazole, minocycline or ticarcillin–clavulanate. Since patients with CF often exhibit high volumes of distribution and increased antimicrobial drug clearance, higher doses of antibiotics should be utilized. Aminoglycosides should be administered on an 8-hour dosing schedule with target peak levels of 8–12 μg/ml and trough levels less than 2 μg/ml. Some have reported successful therapy with single daily dosing regimens. Typically 2–3 weeks of parenteral therapy is required.

Therapy of the chronically infected CF patient who is not in the midst of an acute exacerbation may include inhaled suppressive antibiotic therapy. In clinical trials, a preservative-free formulation of tobramycin (TOBI®) has been shown to improve lung function and decrease the risk of hospitalization in patients colonized with *P. aeruginosa* who inhaled this medication for three cycles of twice-daily administration for 28 days followed by 28 days off medication. Patients are often treated with other inhaled antibiotics such as colistin; however, the use of these agents is less well studied or standardized. Additional inhaled antibiotics with novel and more convenient delivery systems are currently in all stages of drug development and some should be available for use in the near future. A study of oral cephalexin prophylaxis in infants and young children with cystic fibrosis failed to demonstrate a clinical benefit.

In addition to antimicrobial therapy, airway clearance is an essential component in the management of the infected airways of patients with CF. This may be accomplished via traditional chest percussion and postural drainage or with the assistance of various devices such as percussors, pneumatic compression vests or handheld devices which provide oscillatory positive expiratory pressure to the airways such as the Flutter and Acapella devices. Bronchodilators facilitate airway clearance and decrease airway obstruction, and are commonly used by patients with CF. Dornase alpha (Pulmozyme®) degrades extracellular DNA which is present in airway secretions of patients with CF as a result of the neutrophilic infiltration of the lung. Daily inhalation of dornase alpha decreases the resulting increased viscoelasticity of the CF sputum to allow improved airway clearance and has been shown to improve lung function and decrease the need for parenteral antibiotics in patients with CF. Recent studies have shown that inhalation of hypertonic saline (4 ml of 7% saline twice daily) improves lung function and decreases exacerbation rates in patients with CF. The mechanism of action is either via restoration of the airway surface liquid volume which is diminished in CF, or by enhancing cough-mediated airway clearance.

When administered three times per week to patients with CF who are chronically infected with *P. aeruginosa*, the macrolide antibiotic azithromycin has been shown to improve lung function and decrease exacerbations when compared to placebo. The mechanism of action is unclear but could be related to anti-inflammatory effects of macrolides or disruption of biofilm formation. Care must be taken to screen patients for nontuberculous mycobacterial infection to prevent emergence of macrolide-resistant infection in these patients. A study evaluating the utility of this therapy in CF patients without *Pseudomonas* infection is underway.

As many as 13% of CF patients in the USA may become infected with nontuberculous mycobacteria, most commonly *Mycobacterium avium* complex and *Mycobacterium abscessus*. Since some patients may acquire these organisms without a clear effect on clinical status, deciding which patients to treat can be problematic, as treatment can be difficult and eradication may not be possible. Patients who grow these organisms on repeated culture, do not respond as expected to courses of routine antibacterial therapy, have evidence of new cavitary disease on chest X-ray or CT scan, or evidence of progressive changes on CT scan compatible with mycobacterial infection, should be considered for specific therapy.

Other adjunctive therapies for the infected CF patient include regular exercise and aggressive nutritional support to address the increased metabolic needs and malabsorptive problems which these patients may exhibit. Since the inflammatory response in the CF lung may contribute to the morbidity of the chronic airway infection, anti-inflammatory therapy may benefit some patients. Short courses of corticosteroids may benefit some patients; however, while long-term corticisteroid use may slow the decline in lung function, their benefit is outweighed by side-effects of the therapy. High-dose ibuprofen may slow the decline in lung function in young patients with mild disease but this therapy should be closely monitored. Identification and evaluation of more specific and less toxic anti-inflammatory agents is currently being actively researched.

Optimal management of infected CF patients requires an effective infection control strategy to prevent patient-to-patient transmission of virulent organisms. This strategy should include both the inpatient and outpatient settings and conform to local infection control guidelines. Frequent handwashing is extremely important. Patients infected with *B. cepacia* should be segregated from other CF patients who are not infected with this organism. Since children infected with *P. aeruginosa* have decreased lung function and survival when compared with children only infected with *S. aureus*, some advocate segregating these groups of patients.

Table PP15.2 Management of acute exacerbations of CF-related lung disease

- Parenteral antimicrobial therapy based on respiratory culture results (typically two antipseudomonal agents)
- Enhanced airway clearance
- Bronchodilators
- Mucolytics
- Pulmonary rehabilitation
- Nutritional support

CONCLUSION

Management of the infected patient with cystic fibrosis requires strategies to address both the chronic infection and acute exacerbations of infection. This necessitates routine symptomatic and physiologic evaluation of patients, along with frequent microbiologic surveillance of airway secretions. These should guide aggressive but appropriate use of oral, inhaled and parenteral antibiotics, along with adjunctive therapies such as bronchodilators, mucolytics, airway clearance, pulmonary rehabilitation and nutritional support. Finally, an effective infection control policy should be in place to prevent patient-to-patient transmission of potentially harmful organisms.

FURTHER READING

Further reading for this chapter can be found online at http://www.expertconsult.com

Practice point | 16 |

Stéphane Y Donati
Laurent Papazian

Diagnosis and management of ventilator-associated pneumonia

A ventilator-associated pneumonia (VAP) appears, by definition, after at least 48 hours of invasive mechanical ventilation and is recognized as the first cause of nosocomial infection in the ICU.

CLINICAL, RADIOGRAPHIC, AND BIOLOGIC DIAGNOSIS

Diagnosis is based on an association of an infectious syndrome (fever or hypothermia/leukopenia or hyperleukocytosis), a lung infiltrate on chest X-ray (appearance or modification), purulent bronchorrhea and worsening gas exchange. The clinical pulmonary infection score (CPIS) proposed by Pugin *et al.*[1] uses six of these different criteria. Its sensitivity and specificity for a threshold at 6 are acceptable (Table PP16.1).

Table PP16.1 Clinical pulmonary infection score

Temperature	≥36.5° C and ≤38.4° C: 0 points ≥38.5° C and ≤38.9° C: 1 point ≤36° C or ≥39° C: 2 points
White blood cells	≥4 g/l and ≤11 g/l: 0 points <4 g/l or >11 g/l: 1 point If ≥0.5 g/l immature forms: + 1 point
Tracheal aspirations*	<14+: 0 points ≥14+: 1 point If purulence: + 1 point
***Pao*$_2$/*Fio*$_2$ ratio**	>240 or ARDS: 0 points ≤240 without ARDS: 2 points
Chest X-ray	Absence of infiltrate: 0 points Diffuse infiltrate: 1 point Localized infiltrate: 2 points
Semiquantitative culture of tracheal secretions (0, 1, 2 or 3+)	Pathogenic bacteria ≤1+: 0 points Pathogenic bacteria >1+: 1 point Same bacteria as the Gram: + 1 point

ARDS, acute respiratory distress syndrome.
*For each aspiration, the nurses estimate the quantity of tracheal secretions harvested and assign a semiquantitative score (increasing from 0 to 4+). The total estimation is obtained by adding up all of the pluses noted for 24 hours. The total score ranges from 0 to 12. A score >6 is in favor of a ventilator-associated pneumonia with a sensitivity of 93% and a specificity of 100% compared with bronchoalveolar lavage, and a sensitivity of 72% and a specificity of 85% compared with lung histologic assessment.

Nonrespiratory microbiologic samples

A large number of micro-organisms have been implicated in VAP (Fig. PP16.1).

Blood cultures are insufficient but are nevertheless always recommended before administering antibiotherapy.[2] Antigenuria by chromatographic techniques enables the detection of *Streptococcus pneumoniae* and *Legionella pneumophila* serogroup 1 antigens, usually performed on urinary strip (or even in the alveolar liquid) within the framework of community-acquired pneumonia and can be useful with VAP. Serology for intracellular bacteria and viruses is only of retrospective interest given the waiting period for seroconversion. Cytomegalovirus (CMV) viremia or antigenemia confirms extensive cytomegalic infection and makes it possible for CMV to attack the lungs. Hepatic cytolysis must lead the clinician to suspect an active CMV infection within the framework of late VAP (at least 5 days of mechanical ventilation).

Nondirected respiratory samples (Table PP16.2)

Thanks to the contribution of quantitative cultures, tracheal aspirate (TA) has found its place among diagnostic methods. The predominance of the right side and the fact that one is dealing with bronchopneumonia with a bronchial component undoubtedly explains the interest of this technique in the diagnosis of VAP. When TA at a threshold of 10^5 cfu/ml was compared with protected specimen brush (PSB) or bronchoalveolar lavage (BAL), the sensitivity and specificity were 92.8% and 80%, respectively.

A protected double catheter (double-plugged catheter), protected by a glycol polyethylene plug, makes it possible to perform 'blind aspiration brushing' through an internal catheter or mini-BAL in which 20 ml of liquid are instilled and whose semiquantitative culture has revealed a sensitivity of 80% and a specificity of 66%.

Directed respiratory samples (Table PP16.2)

Bronchoalveolar lavage (BAL) is performed by instilling and recovering sterile saline solution through the internal channel of the fiberscope which is positioned in a third- or fourth-generation bronchus where only distal bronchioles and alveoli are sampled. No consensus has been established on the quantity to administer by aliquot, on the number of aliquots or on preserving or eliminating the first aliquot representing the bronchial fraction of BAL. A meta-analysis of 23 studies revealed the sensitivity of BAL at 73 ± 18% and its specificity at 82 ± 19% when VAP was diagnosed.[3]

The interest in BAL is based on the possibility of examining for other pathogens such as intracellular pathogens and their nucleic acid thanks to polymerase chain reaction (PCR) amplification which appears to be

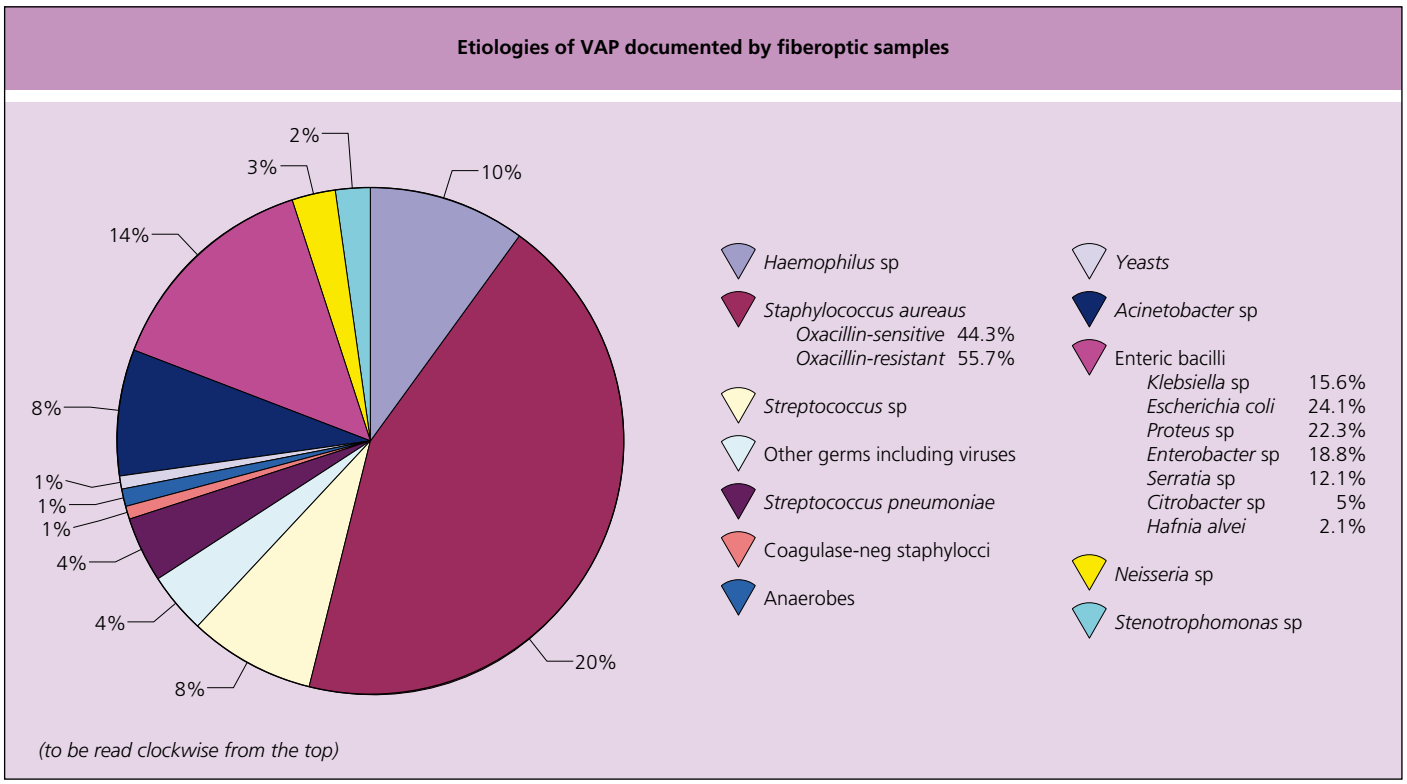


Fig. PP16.1 Etiologies of ventilator-associated pneumonia documented by fiberoptic samples. Results of 24 studies of 1689 episodes and 2490 pathogens.

Table PP16.2 Microbiologic diagnostic tools and thresholds of significance that are usually retained

- Blind tracheal aspirate $\geq 10^5$ or 10^6 cfu/ml
- Blind mini-bronchoalveolar lavage or blind protected double catheter brushing $\geq 10^3$ cfu/ml
- Protected specimen brush under fibroscopy $\geq 10^3$ cfu/ml
- Bronchoalveolar lavage under fibroscopy $\geq 10^4$ cfu/ml

more effective than cultures which are difficult to perform. A viral diagnosis (herpes simplex virus, cytomegalovirus) can also benefit from PCR amplification parallel to classic or rapid cultures and cytologic analysis.

Protected specimen brush (PSB) is a double catheter sealed by a glycol polyethylene plug that enables brushing of the distal bronchial mucosa following insertion of an endoscope. The low volume of secretions harvested (approximately 1 µl) explains a certain number of false-negatives as well as the difficulty in performing a direct examination and culturing on the same brush. This technique, developed *in vitro* by Wimberley, provides a sensitivity ranging from 33% to 100%.

No consensus conference has decided among the different types of sampling although the choice should favor quantitative techniques.

TREATMENT

Since the great majority of ventilator-associated pneumonias are due to bacteria, empiric therapy or (even better) empiric antibiotherapy – oriented by the patient's health status, local microbiologic ecology and direct examination (with TA, BAL, mini-BAL) – must be administered as soon as there are enough clinical arguments. The consensus from the American Thoracic Society and the Infectious Diseases Society of America[2] proposes broad-spectrum empiric double antibiotherapy in case infection with multidrug-resistant bacteria is suspected (Tables PP16.3, PP16.4) and double or even mono narrow-spectrum antibiotherapy in other cases (Table PP16.4). This antibiotherapy must begin after performing a series of hemocultures and respiratory samples (tracheal aspirate, PSB, BAL, etc.). Antibiotics are given intravenously, and when the response to treatment is satisfactory, relayed by oral or enteral administration if possible. Tracheal or aerosol instillations of antibiotics are not recommended as a cure can be but complementary in the treatment of multidrug-resistant Gram-negative bacilli if systemic antibiotherapy appears to be insufficient.[2]

Several observational studies have shown that appropriate antibiotherapy administered as soon as possible was associated with a reduction in the mortality of patients with suspected VAP.[4] The choice of therapy must also take into account recent antibiotherapy received by the patient so that a different class of antibiotic is used to initiate treatment. For this choice, the latest consensus conference has proposed that the recommended guidelines, regularly updated, adapted to local microbiology and the availability of molecules within the structure be systematically used. Another method to orient initial antibiotherapy can be proposed. It is to administer antibiotherapy as soon as BAL has

Table PP16.3 Risk factors associated with an increased incidence of multidrug-resistant bacteria

- Antimicrobial therapy in preceding 90 days
- Current hospitalization of 5 days or more
- High frequency of antibiotic resistance in the community or in the specific hospital unit
- Hospitalization for 2 days or more in the preceding 90 days
- Residence in a nursing home or extended care facility
- Home infusion therapy (including antibiotics)
- Chronic dialysis within 30 days
- Home wound care
- Family member with multidrug-resistant pathogen
- Immunosuppressive disease and/or therapy

Table PP16.4 Empiric antibiotherapy proposed by the ATS/IDSA Consensus Conference

Empiric antibiotherapy in case of early-onset VAP (<5th day) and in the absence of multidrug-resistant bacterium risk factors	Empiric antibiotherapy in case of late-onset VAP (≥5th day) and/or the presence of multidrug-resistant bacterium risk factors
β-Lactamine/inhibitor of β-lactamase Or ceftriaxone Or levofloxacin or moxifloxacin Or ertapenem	Cefepime or ceftazidime Or imipenem or meropenem Or piperacillin–tazobactam *plus* Ciprofloxacin or levofloxacin Or gentamicin or tobramycin Or amikacin *plus* Linezolid or vancomycin*

*If suspicion or risk factors of methicillin-resistant *Staphylococcus aureus* (corticotherapy, recent antibiotherapy, mechanical ventilation >6 days).

been performed by making use of the tracheal aspirate results systematically performed twice a week in all patients under invasive mechanical ventilation (Fig. PP16.2). A study has shown that this method improves the appropriateness of treatment compared with the use of the recommended guidelines.[5]

In any case, rapid deterioration or the absence of improvement after 72 hours of empiric treatment imposes systematic re-evaluation. It should be noted that the absence of response to empiric treatment can be linked to a nonbacterial infection, particularly viral, or to an extrapulmonary infection, or to the absence of an infectious problem (for example, during the fibroproliferative phase of the acute respiratory distress syndrome) requiring aggressive diagnostic measures which can go as far as a lung biopsy. In case of improvement, reduction or even discontinuation of antibiotherapy is mandatory once sampling results are known and appear to be coherent.

Short antibiotherapy (8 days) is currently recommended except in cases of *Pseudomonas aeruginosa* where at least 15 days of antibio-therapy appear to be necessary with bitherapy maintained for the first 5 days.[6] The administration of antiviral treatment (ganciclovir for CMV, aciclovir for herpes simplex virus, and amantadine, rimantadine, oseltamivir or zanamivir for respiratory viruses) and antifungal agents (with recently available molecules such as voriconazole and caspofungin) is conditioned by diagnostic difficulties. Finally, immunity-modulating treatments are not recognized and only a sufficient nutritional contribution and respiratory physiotherapy can be recommended as adjuvant treatments.

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References for this chapter can be found online at http://www.expertconsult.com

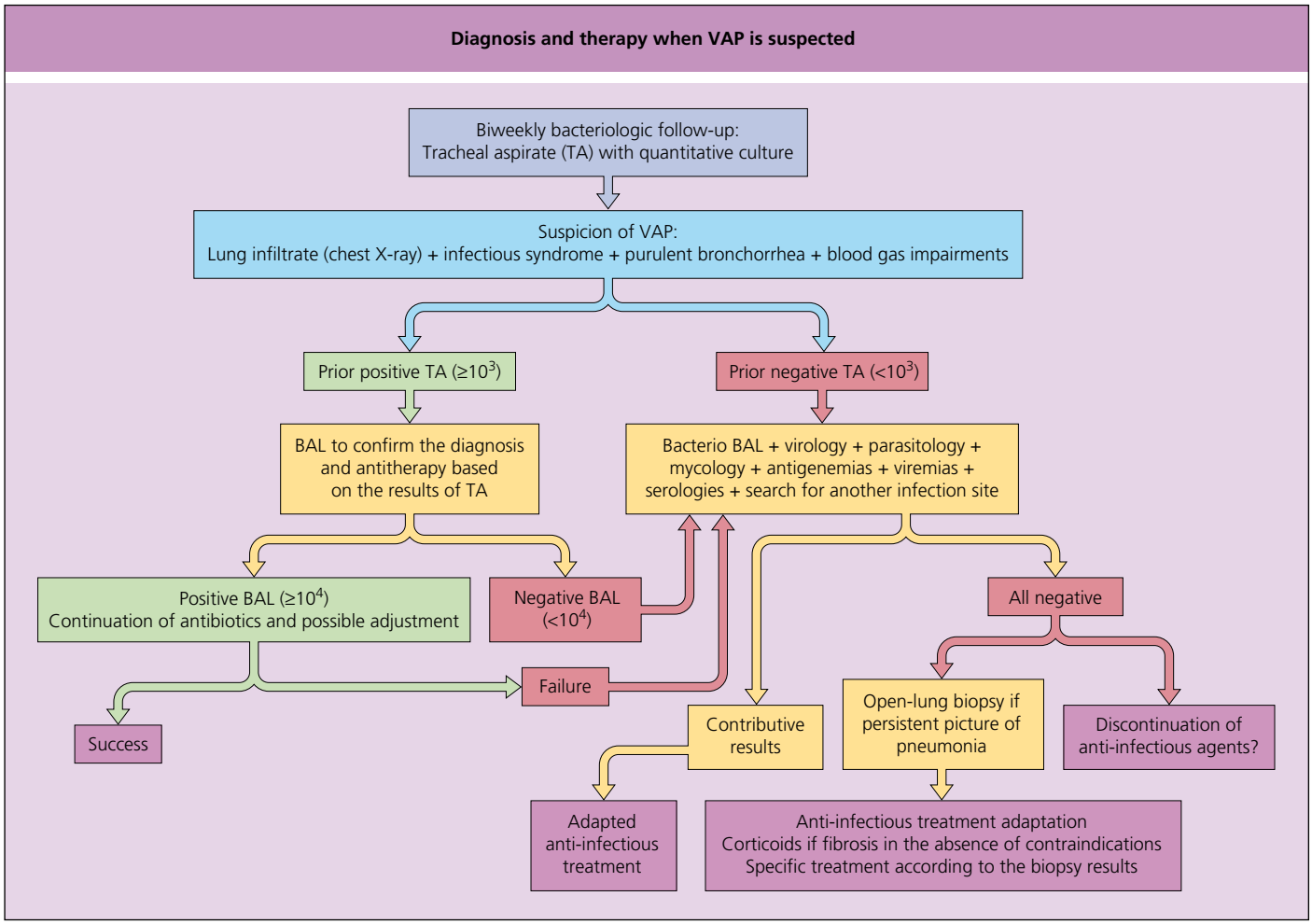


Fig. PP16.2 Decision tree proposed for diagnosis and therapy when ventilator-associated pneumonia (VAP) is suspected. BAL, bronchoalveolar lavage.

Chapter 32

Robert C Read

Orocervical infection

Infections of the oral cavity and neck include dental and periodontal infections, deep fascial space infections of the neck that are often odontogenic or caused by contiguous spread from pharyngeal foci, nondental oral infections, including ulcerative and gangrenous stomatitis, and infections of the salivary glands. Infections of the esophagus mostly occur in the context of severe underlying disease.

Dental and Periodontal Infections

EPIDEMIOLOGY

Dental caries is the commonest infectious disease in humans. Incidence is closely related to the use of derivatives of cane sugar and began to take on epidemic proportions in Europe in the 19th century. Dental caries first becomes evident in infancy and is most noticeable on the chewing surfaces of the molar teeth. The likelihood of dental caries is increased by high sugar intake, poor oral hygiene and any factors that reduce salivary flow – notably drugs (e.g. antidepressants).[1]

Periodontal disease, including gingivitis, is mainly related to poor oral hygiene and increasing age. Increased incidence of periodontal disease is also evident in diabetics and during hormonal disturbances, including puberty and pregnancy.[2] It is suspected that most periodontal disease arises from an inflammatory response to the accumulation of dental plaque in the gingival margin. Plaque contains mainly *Streptococcus* spp. and *Actinomyces* spp., which probably generate an early gingivitis, leading ultimately to periodontitis.[3] These processes occur over many years with incremental destruction of periodontal tissue.[4]

PATHOGENESIS AND PATHOLOGY

The indigenous oral flora includes a large number of aerobic and anaerobic bacteria and varies by site within the oral cavity. There are of the order of 10^{11} micro-organisms per gram wet weight of oral secretions, with the majority being obligate anaerobes. *Streptococcus* spp., *Peptostreptococcus* spp., *Veillonella* spp., *Lactobacillus* spp., *Corynebacterium* spp., *Bacteroides* spp., *Prevotella* spp. and *Actinomyces* spp. form the majority of oral flora.[5]

Dental caries is characteristically polymicrobial with no fixed pattern of microbial etiology, except that *Streptococcus mutans* has emerged as the only organism consistently isolated from carious teeth compared with normal teeth. In contrast, gingivitis has characteristic microbial specificity; the normal flora of the periodontium (i.e. *Streptococcus sanguis* and *Actinomyces* spp.) is replaced by anaerobic Gram-negative rods, notably *Prevotella intermedia*. With chronic gingivitis there is ulceration of the mucosa, loss of attachment of periodontal tissue, loss of enamel and necrosis of the dental pulp, and an increase in complexity of microbial flora with a preponderance of anaerobic Gram-negative rods, including *Porphyromonas gingivalis*.[2] When abscesses form, for example periapical abscesses, the flora is always polymicrobial with species such as *Fusobacterium*, pigmented *Bacteroides*, *Peptostreptococcus*, *Actinomyces* and *Streptococcus*.

In healthy individuals teeth are protected from decay by the cleansing action of the tongue and buccal secretions, the buffering effect of saliva and the acquired pellicle – the fluid microenvironment of the tooth surface. With poor dental hygiene the acquired pellicle is colonized and replaced by plaque that contains bacteria, notably *Strep. mutans*. This process is accelerated by intermittent exposure to carbohydrates and simple sugars. The presence of subgingival plaque leads to inflammation of the gingival epithelium and to destruction of the periodontium.[5] The normal resident polymicrobial flora provides an important defense against invasion of gingival epithelium by pathogenic bacteria. Normal saliva also protects the epithelium by irrigating it with enzymes, including lysozyme and lactoperoxidase, and other antimicrobial substances such as lactoferrin. Secretory IgA provides additional protection by aggregating organisms and preventing bacterial adherence. The importance of intact phagocytic defenses is reflected by the high prevalence of periodontal infections in patients who have cyclic neutropenia and chronic granulomatous disease.[3] Some oral micro-organisms involved in periodontal disease secrete IgA proteases.[6] Host factors credited with leading to periodontitis include psychosocial stress, diet, smoking, alcoholism and intercurrent disease.[7]

The usual cause of deep-seated odontogenic infection is necrosis of the pulp of the tooth, followed by bacterial invasion through the pulp chamber and into the deeper tissues.[8] If a pulp abscess is allowed to progress, infection will spread toward the nearest cortical plate (Fig. 32.1).

PREVENTION

Prevention of dental and periodontal infections includes interference with transmission and suppression of *Strep. mutans* colonization once it has occurred. There is a strong correlation between maternal salivary *Strep. mutans* infection and the presence of this organism in children. Acquisition of *Strep. mutans* by infants has been prevented by aggressive treatment of *Strep. mutans* infection in mothers.[9] Existing infections can be suppressed by regular cleaning with agents that include fluoride and antimicrobial substances such as chlorhexidine. Periodontal disease can be prevented by good oral hygiene and regular rinsing with chlorhexidine. Clinical trials of vaccines to prevent periodontitis have been conducted but vaccination has not yet entered current recommended practice.[10]

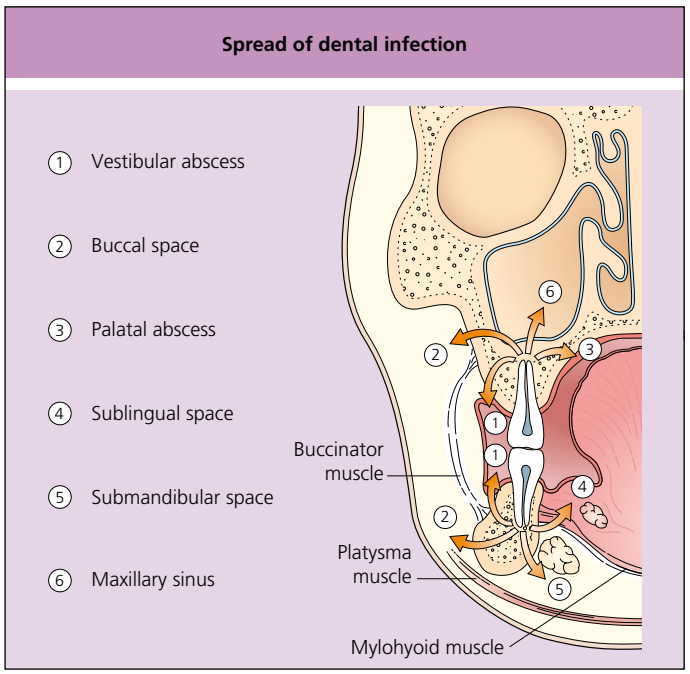


Fig. 32.1 Spread of dental infection. A spreading tooth abscess will encroach upon the nearest cortical plate and its subsequent spread depends on the relationship of that site to muscle attachment. Adapted from Peterson.[8]

CLINICAL FEATURES

Subgingival dental caries is asymptomatic, but destruction of enamel results in invasion of the pulp with subsequent necrosis, eventually leading to a periapical abscess. The tooth becomes sensitive to temperature and pressure once the enamel is penetrated, and toothache results.

In simple gingivitis there is usually discoloration of the gum margin with occasional bleeding after brushing of the teeth. There may be halitosis. If gingivitis is allowed to become chronic there may be destruction of periodontal tissue with loosening of the teeth. This may be relatively asymptomatic or the patient may have itchy gums, temperature sensitivity and halitosis.

COMPLICATIONS

Dental pulp infections can lead to involvement of the maxillary and mandibular spaces (see Fig. 32.1). Spread of infection from maxillary (upper) teeth most commonly leads to vestibular abscesses (Fig. 32.2).



Fig. 32.2 Painful vestibular abscess. Courtesy of Professor I Brook.



Fig. 32.3 Buccal space abscess originating from right lower molar infection. The buccal space lies between the buccinator muscle and the overlying skin and fascia. Courtesy of Professor I Brook.

Erosion of canine pulp abscesses can lead to canine space abscesses if the abscess points above the insertion of the levator labii superioris. This results in swelling lateral to the nose, which usually obliterates the nasolabial fold. Buccal space abscesses can result when pulp abscesses of the molar teeth erode above or below the attachment of the buccinator muscle; these point below the zygomatic arch and above the inferior border of the mandible (Fig. 32.3).

When infection spreads from mandibular (lower) teeth the commonest result is again vestibular abscess. Deeper abscesses may point into the sublingual and submandibular spaces. The sublingual space lies underneath the oral mucosa and above the mylohyoid muscle (see Fig. 32.1). Posteriorly, it communicates with the submandibular space. Infection within the sublingual space results in swelling of the floor of the mouth, which may spread to involve both sides and be sufficiently pronounced to lift the tongue. This space is involved if the infected tooth apex giving rise to the disease is superior to the insertion of the mylohyoid (e.g. premolars and first molars).

The submandibular space lies between the mylohyoid muscle and the skin. It becomes involved if the apex of the infected tooth is inferior to the insertion of the mylohyoid muscle (e.g. third molar). Clinically, infection in this space causes extraoral swelling (unlike sublingual space infections) that begins at the inferior lateral border of the mandible and extends medially to the digastric area. Occasionally the abscess may point spontaneously and rupture (Fig. 32.4).

Ludwig's angina refers to a severe cellulitis of the tissue of the floor of the mouth with involvement of the submandibular and sublingual spaces (Fig. 32.5). The source of infection is almost always the second and third mandibular molars. If the infection is allowed to continue there may be local lymphadenitis, systemic sepsis and extension of the disease to involve deep cervical fascia, with a cellulitis that extends from the clavicle to the superficial tissues of the face. Other potential complications include asphyxia, aspiration and mediastinitis. The disease is almost always polymicrobial, including α-hemolytic streptococci and anaerobes such as *Peptostreptococcus* spp., *Prevotella melaninogenica* and *Fusobacterium nucleatum*.[11]



Fig. 32.4 Submandibular abscess originating from a 2nd molar tooth infection. Courtesy of University of Sheffield School of Dentistry, UK.

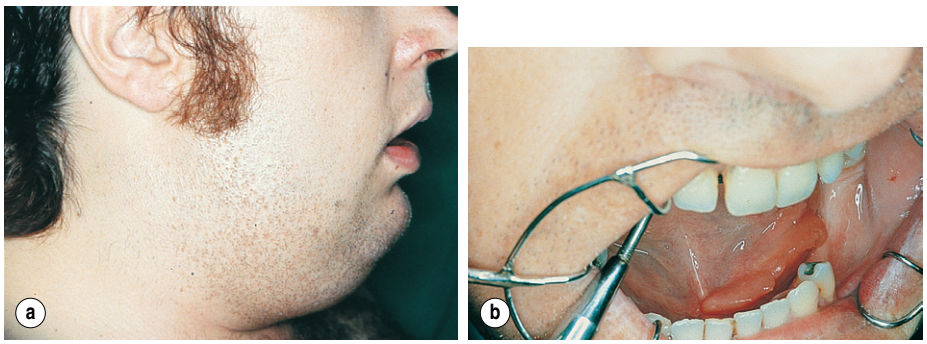


Fig. 32.5 Ludwig's angina. (a) This patient had painful cellulitis within the submandibular and sublingual spaces. (b) Brawny edema was present within the floor of the mouth, pushing the tongue upwards. Courtesy of University of Sheffield School of Dentistry, UK.



Fig. 32.6 Cavernous sinus thrombosis. A patient who displays evidence of severe orbital swelling caused by obstruction of orbital veins is shown. In this patient, the originating focus was infection of soft tissues of the nose. Courtesy of University of Sheffield School of Dentistry, UK.

Very rarely, spread of infection from maxillary teeth may cause orbital cellulitis or cavernous sinus thrombosis (see Chapter 20). The latter is distinguished by toxemia, venous obstruction within the eye and orbital tissues (Fig. 32.6), involvement of the III, IV and VI cranial nerves and meningismus.

MANAGEMENT

Treatment of dentoalveolar infections includes elimination of the diseased pulp and deep periodontal scaling or tooth extraction. Any dentoalveolar abscess present should be surgically drained. If drainage is not complete, antibiotic therapy is appropriate. Treatment of periodontal disease includes appropriate debridement and short-term antimicrobial therapy with oral metronidazole 400 mg q8h or oral phenoxymethylpenicillin 500 mg q6h. Periodontal and vestibular abscesses should be treated by drainage.

Treatment of maxillary and submandibular space infections should always be by surgical drainage of pus. Ludwig's angina is a life-threatening condition and the first aim of treatment is protection of the airway, if necessary by emergency intubation or occasionally tracheostomy. Intravenous antibiotics should be administered. Benzylpenicillin 1.2 g q4h plus metronidazole 400 mg q8h or clindamycin 450 mg q8h are appropriate.

Management of cavernous sinus thrombosis is by surgical decompression and high-dose intravenous antibiotics, the choice of which is influenced by whether the originating focus is dental or within soft tissues.

Deep Cervical Space Infection

Infections of the lateral pharyngeal space, the retropharyngeal space and the prevertebral space are uncommon but life-threatening problems. The lateral pharyngeal space is funnel shaped, with its base at the sphenoid bone at the base of the skull and its apex at the hyoid bone. It is bounded by the medial pterygoid muscle laterally and the superior pharyngeal constrictor medially. Posteromedially it extends to the prevertebral fascia and communicates with the retropharyngeal space. The carotid sheath and cranial nerves are within the posterior compartment of the space. The retropharyngeal space lies posteromedial to the lateral pharyngeal space, between the superior constrictor muscle and the alar portion of the prevertebral fascia. Superiorly, it extends from the skull base of the pharyngeal tubercle down to the level of C7 where the superior pharyngeal muscle and the prevertebral fascia fuse.[8]

Unlike the lateral pharyngeal space it has few contents apart from lymph nodes, but its importance as a site of infection relates to its proximity to the airway and to the contents of the superior mediastinum. The prevertebral space extends from the skull base inferiorly to the diaphragm. It is bounded by the two layers of prevertebral fascia: the alar and prevertebral layers.

EPIDEMIOLOGY AND PATHOGENESIS

Parapharyngeal infections can complicate peritonsillar abscess (see Chapter 24), but a larger proportion of infections are odontogenic or secondary to intravenous drug abuse. Rarer sources include parotitis, otitis and mastoiditis. The incidence of parapharyngeal infection has declined sharply in the antibiotic era and such infections now form less than 30% of all deep cervical infections.[11,12]

Infections of the retropharyngeal and prevertebral spaces most commonly result from lymphatic spread of infection in the pharynx or sinuses, with subsequent suppuration of the retropharyngeal lymph nodes. Retropharyngeal infections are therefore commonest in children, mainly because retropharyngeal lymph nodes are more numerous.[13] Occasionally retropharyngeal infections may be caused by accidental perforation of the pharynx, for example during emergency intubation. The bacteriology of deep cervical space infections reflects the microbial flora of the originating source. Thus, infections arising from the pharynx are often caused by *Streptococcus pyogenes*, whereas odontogenic infections are polymicrobial and include *Strep. mutans* and anaerobic pathogens such as *F. nucleatum*, *P. melaninogenica*, *Peptostreptococcus* spp., *Eikenella corrodens* and *Actinomyces* spp.

CLINICAL FEATURES

The characteristic feature of lateral pharyngeal space infection is severe trismus, which results from involvement of the pterygoid muscle and other muscles of mastication. There is also swelling of the lateral pharyngeal wall, which pushes the tonsil toward the midline. Occasionally there is lateral neck swelling below the angle of the mandible. The disease can be confused with peritonsillar abscess, although the latter should not produce trismus. The patient experiences fever, painful swallowing and pain that occasionally radiates to the ear. The infection tends to be severe and progresses rapidly. Posterior extension of the process into the carotid sheath can result in suppurative jugular thrombophlebitis, carotid artery erosion or interference with cranial nerves IX–XII. There is hyperacute sepsis, with rigors and high fever. There may be pain and swelling below the mandible, marked swelling of the lateral pharyngeal wall, torticollis and neck rigidity. There may be metastatic abscesses within the brain, lungs and bone. Suppurative thrombophlebitis of the internal jugular vein secondary to oropharyngeal infection was described by Lemierre and the syndrome bears his name.[14] The major organism associated with this complication is *Fusobacterium necrophorum*, which is usually obtained from blood cultures, but may require several days of anaerobic culture to grow.

Patients who have retropharyngeal abscess may present with fever and rigors that usually follow on from a streptococcal pharyngitis, but often there is no history of sore throat.[15] A child with a retropharyngeal abscess may be withdrawn and irritable. Adults may complain of sore throat, dysphagia, neck pain and dyspnea. The neck may be hyperextended, and there may be drooling and stridor. Examination of the throat by indirect laryngoscopy may reveal bulging of the posterior pharyngeal wall. Potential complications include upper airway obstruction as a result of anterior displacement of the posterior pharyngeal wall into the oropharynx, and spontaneous rupture of the abscess with aspiration pneumonia (which may complicate attempted insertion of an endotracheal tube). Other potential complications include purulent pleural effusion, pericardial effusion and posterosuperior mediastinitis.[16]

Patients who have AIDS, particularly intravenous drug users, have a higher incidence of deep neck infections, most commonly caused by *Staphylococcus aureus*, which is often methicillin resistant. In contrast to immunocompetent patients, there is often no leukocytosis.[17] Diabetics are also at increased risk of deep neck infections, and in addition to *Staph. aureus*, Gram-negative organisms, notably *Klebsiella* spp., may be isolated from these patients.[12]

DIAGNOSIS

If lateral pharyngeal space infection is suspected, the diagnosis is best confirmed by MRI or CT scanning. Plain radiographs are usually unhelpful. In contrast, a retropharyngeal space abscess can be diagnosed by a lateral radiograph of the neck (Fig. 32.7). The average width of the prevertebral soft tissue should be no more than 7 mm (average 3.5 mm) at C2 and no more than 20 mm (average 14 mm) at C6.[18] The neck should be fully extended during evaluation. The major clinical differential diagnosis of retropharyngeal abscess includes cervical osteomyelitis and meningitis. The latter can usually be discounted when there is obvious pharyngeal swelling, but cervical osteomyelitis may require MRI scanning of the cervical vertebral bodies for exclusion.

MANAGEMENT

In any patient with a suspected deep neck infection, maintenance of the airway is always the first consideration; up to one-third of patients who have retropharyngeal abscess will require tracheostomy. If pus is shown to be present, incision and drainage of involved spaces should be performed and intravenous antibiotic therapy should be administered promptly in order to produce rapid and complete resolution of the infection with minimal likelihood of complications.[12] Radiologic evidence of gas within soft tissues increases the urgency, because expansion of lesions containing anaerobes is usually rapid.



Fig. 32.7 Retropharyngeal abscess. Lateral radiograph of the neck in a patient who has a retropharyngeal abscess, showing gross expansion of prevertebral soft tissue. Courtesy of Mr R Bull.

Lateral pharyngeal and retropharyngeal abscesses can be drained by an incision along the anterior border of the sternocleidomastoid muscle followed by blunt dissection and drainage. Extensive surgery should be unnecessary if infections are treated promptly and high-dose intravenous antibiotics are used. Appropriate intravenous antibiotics include penicillin 1.2–1.8 g q3h plus clindamycin 300–600 mg q8h or metronidazole 400 mg q8h, plus ceftriaxone 2 g q12h. Alternatives include a carbapenem (e.g. imipenem or meropenem), or the combination of a penicillin (e.g. ticarcillin) and a β-lactamase inhibitor (e.g. clavulanate). Antimicrobial therapy can abort abscess formation if administered at an early stage of the infection.

Cervical Necrotizing Fasciitis

Cervical necrotizing fasciitis is a rare and extremely dangerous complication of odontogenic and deep cervical space infection. The disease is characterized by involvement of more than one neck space (usually bilaterally) and contiguously spreading necrosis of connective tissue, with cellulitis that extends below the hyoid bone to the chest wall, onto the face and into the mediastinum. Most cases are odontogenic, particularly after dental abscesses, but some cases follow on from tonsillar abscess or from surgical trauma to the oropharynx. Almost all cases are polymicrobial, often with a single aerobic isolate (e.g. *Streptococcus* spp.) plus two or more anaerobes (mostly *P. melaninogenica* and *F. nucleatum*), although any of the oral anaerobes can be involved.

The typical clinical presentation is usually with dental pain and submandibular swelling over a few days, followed by rapid evolution of fasciitis, which is extremely tender on palpation and usually associated with crepitus. Mediastinal extension can be clinically silent and detectable only by CT of the chest, but can lead to pericarditis, pneumonia or empyema. Predisposing conditions include diabetes mellitus, alcoholism and malignancy. Management includes surgical drainage via incision along the sternocleidomastoid muscle followed by blunt dissection of the neck.[19] Appropriate intravenous antibiotic therapy is benzylpenicillin 1.2–1.8 g q3h plus clindamycin 600 mg q8h, though rates of penicillin and clindamycin resistance of up to 40% of isolates from clinical cases have recently been shown.[20]

Actinomycosis

Actinomycosis is a chronic suppurative bacterial infection that principally affects the head and neck but can involve almost any system. It spreads directly through tissue, skin and bone, and therefore is able to form sinuses and fistulas.

EPIDEMIOLOGY AND PATHOGENESIS

The agents that cause actinomycosis are facultative anaerobic Gram-positive commensals of the mouth. *Actinomyces israelii* is the most common pathogen, but *Actinomyces naeslundii, Actinomyces viscosis, Actinomyces odontolyticus* and *Arachnia propionica* may also cause the disease. These agents commonly inhabit carious teeth, dental plaque and cavities and also the normal intestinal tract. Head and neck infection usually occurs in the context of dental disease or dentistry, during which the normal mucosal barriers are broken down. Thoracic involvement usually follows aspiration of infected oropharyngeal secretions in patients who have poor dentition. Lesions of actinomycosis consist of areas of acute inflammation surrounded by fibrosing granulation tissue. Such material contains 'sulfur granules' (colonies of organisms forming an amorphous center surrounded by a rosette of clubbed filaments); these usually contain associated organisms, including *Actinobacillus actinomycetemcomitans, Haemophilus* and *Fusobacterium* spp., which probably contribute to the pathogenesis of the disease.

Any age group can be infected, including infants and children. Males outnumber females by three to one.

CLINICAL FEATURES

The most common manifestation of actinomycosis is soft tissue swelling of the head, face or neck, usually over or underneath the mandible.[21] Occasionally the swelling is very extensive and waxes and wanes over many months, spreading to involve other parts of the head and neck, including the scalp, palate, eyes, larynx, salivary glands, middle ear and paranasal sinuses. Sinuses and tracts develop that open into the mouth and the skin (Fig. 32.8). Involvement of local bone (e.g. the mandible) can result in periosteal reaction or frank osteomyelitis.

DIAGNOSIS

The diagnosis is usually obvious in patients who have head and neck swelling, particularly in the context of poor dentition and discharging sinuses yielding sulfur granules. The granules can be trapped in gauze placed over the sinus opening or by injecting and aspirating saline from the sinus; by shaking the aspirate, the granules can be seen with the naked eye. Sulfur granules can also be seen in sputum on microscopic examination. Any material obtained can be cultured under anaerobic conditions. In formalin-fixed tissues, immunofluorescence can be used to identify species. There is no reliable serologic test; laboratory diagnosis depends on microscopy and culture of material from the patient.

MANAGEMENT

Most patients who have actinomycosis will respond to intravenous benzylpenicillin, 1.2–1.8 g q3h for 3–6 weeks, followed by oral penicillin V, 2–4 g/day for 6–12 months. Alternative treatments include intravenous amoxicillin or ampicillin, followed by oral amoxicillin. Chloramphenicol, erythromycin, tetracycline and clindamycin have also been used successfully. Prolonged treatment with penicillin results in complete resolution of the disease, although there may be some residual fibrosis or scarring (see Fig. 32.8). While intravenous benzylpenicillin has been the traditional treatment for this condition there have been reports of the use of intravenous agents that can be given in once-daily dosing for home therapy, including ceftriaxone, imipenem and fluoroquinolones.

Infections of the Oral Mucosa: Gangrenous Stomatitis

Acute necrotizing ulcerative gingivitis, or trench mouth, is an ulcerative necrosis of the marginal gingivae. The disease may spread to other oral structures, including the tonsils or pharynx, to cause Vincent's disease or may result in rapid necrosis and sloughing of facial structures, producing the classic features of cancrum oris (noma).

EPIDEMIOLOGY

The disease is mostly seen in developing countries in the context of severe debilitation and malnutrition. In addition, poor oral hygiene, HIV infection, measles, local irritation from food impaction and smoking are associated factors.[22]

PATHOGENESIS

Necrotizing gingivitis may begin as an aseptic necrosis secondary to mucosal capillary stasis. In infections in which the disease spreads superficially to involve the pharynx, it is most likely secondary to a combination of *F. nucleatum* and Gram-negative anaerobic organisms (*Bacteroides* subsp. *intermedius*). If the disease spreads deeper into facial tissues to cause cancrum oris, fusospirochetal organisms such

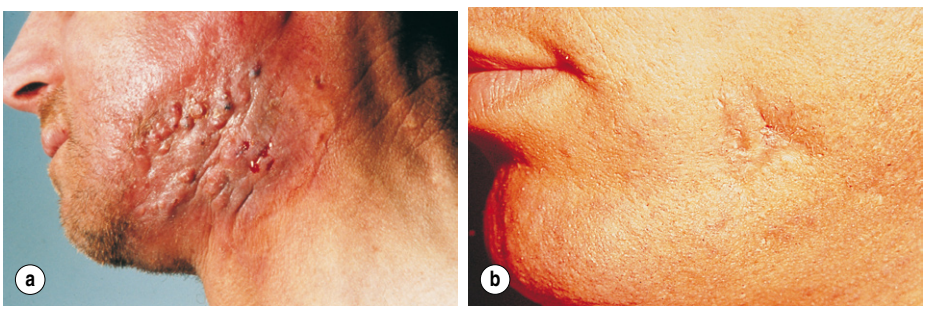


Fig. 32.8 Actinomycosis. (a) This patient had chronic disease over the mandible which (b) healed with several months of antibiotics, leaving a residual chronic sinus. Courtesy of Professor I Brook.

as *Borrelia vincenti* and *F. nucleatum* are consistently cultured. *Prevotella melaninogenica* may also be present. Biopsies of any advancing lesion often reveal a mat of predominantly Gram-negative, thread-like bacteria that cannot be positively identified.

CLINICAL FEATURES

The earliest feature is a small painful red lesion that may be vesicular on the attached gingiva and often in the premolar or molar region of the mandible, with sudden onset of painful gums (Vincent's disease; Fig. 32.9). The disease may then progress rapidly to produce halitosis and gingival bleeding. If there is involvement of the tonsils and pharynx (Vincent's angina) there is searing pain in the pharynx with high fever, regional lymphadenopathy and anorexia. If the disease spreads into deeper tissues (noma) a necrotic ulcer rapidly develops with painful cellulitis of the lips and cheeks, which often sloughs, exposing underlying bone, teeth and deeper tissues (Fig. 32.10).

DIAGNOSIS

Although the infection is usually polymicrobial, material should be obtained for Gram stain and aerobic and anaerobic culture. Debrided material is optimal for anaerobic culture. Gram stain may reveal fusospirochetal Gram-negative organisms as well as Gram-positive cocci and Gram-negative rods.

MANAGEMENT

In early acute necrotizing ulcerative gingivitis (Vincent's infection), treatment with oral penicillin V 500 mg q6h and metronidazole 400 mg q8h is usually sufficient. In patients who have noma, high doses of intravenous penicillin and metronidazole are required, with the dose being dependent on the age and size of the patient. An antibiotic to treat aerobic Gram-negative rods, such as ceftriaxone, may be necessary. Gangrenous tissues should be removed and loose teeth extracted. The patient should be carefully rehydrated. Once the infection has been controlled, reconstructive surgery is often necessary.

Infections of the Oral Mucosa: Primary Herpetic Gingivostomatitis

EPIDEMIOLOGY

Herpes simplex virus (HSV)-1 and HSV-2 can cause a primary infection of the oral cavity, although type 1 is much more frequently responsible. The disease can occur in infants, although this is becoming increasingly uncommon. Oral lesions caused by HSV-2 are seen in sexual contacts of patients who have genital herpes and are clinically indistinguishable from those caused by HSV-1.

CLINICAL FEATURES

The disease may be very mild, with a few painful ulcers and no systemic features, or it may be more severe with fever, sore throat, malaise, headache and regional lymphadenopathy. Oral lesions tend to appear 1–2 days after the onset of pain and lead to a painful, red gingiva or palate. These symptoms generally persist for approximately 2 days. The vesicles occur as 2–4 mm ulcers on a red background. When lesions coalesce they can resemble aphthous ulcers (Fig. 32.11). At this point the disease is highly infectious. The clinical course of unmodified primary herpetic gingivostomatitis usually lasts 2 weeks.



Fig. 32.9 Acute necrotizing gingivitis. Courtesy of Professor I Brook.



Fig. 32.11 Primary HSV-1 stomatitis.

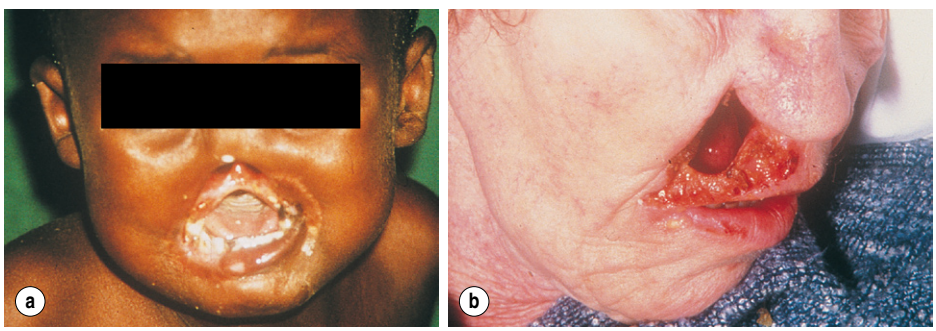


Fig. 32.10 Noma. This is a destructive process extending from oral structures, which is a sequel of necrotizing gingivitis and (a) is seen most commonly in patients in developing countries, although (b) occasionally it is seen in the elderly debilitated in developed countries. Courtesy of Professor I Brook.

DIAGNOSIS

The clinical differential diagnosis of oral herpetic gingivostomatitis includes herpangina, varicella, herpes zoster, and hand, foot and mouth disease. These diseases can usually be distinguished on the basis of concomitant cutaneous features. Primary herpes infection of the mouth can occasionally be recurrent and several other recurrent diseases have similar oral lesions – these include minor aphthous ulcers, Behçet's syndrome, cyclical neutropenia and erythema multiforme. A laboratory diagnosis of herpes can be verified by direct immunofluorescence or viral culture of material obtained by swabbing the ulcers.

MANAGEMENT

In primary herpetic gingivostomatitis oral aciclovir 200–400 mg q8h is appropriate therapy.

Other Infections of the Oral Mucosa

HERPANGINA

Herpangina produces characteristic oropharyngeal vesicles, generally at the junction of the hard and soft palates (Fig. 32.12). It primarily affects children and teenagers and generally occurs in epidemics during the summer. Several different coxsackieviruses, notably coxsackievirus A (types 1–10, 16 and 22) and less commonly coxsackievirus B (types 1–5), have been associated with this disease. Other enteroviruses, including echovirus, have been implicated.

Patients usually have mild disease, but they can complain of sudden fever, anorexia, neck pain, extremely sore throat and headache. The lesions are often more vesicular than herpetic, and consist of multiple small white papules with an erythematous base that appears less inflamed than that with herpetic lesions. These lesions usually spontaneously rupture within 2 or 3 days and seldom persist for more than 1 week. There may be cervical lymphadenopathy but this is unusual. A laboratory diagnosis can be obtained by culturing swabbed material from the lesions. Herpes simplex virus infection can usually be distinguished on clinical grounds, but can be rapidly excluded by direct immunofluorescence. Management consists of topical analgesia only.

HAND, FOOT AND MOUTH DISEASE

Hand, foot and mouth disease is caused by systemic infection with Coxsackie group A viruses (usually serotype 16) and primarily affects children, but occasionally adults. The disease consists of vesicular eruptions on the hands, wrists, feet and within the mouth. Lesions on the hands are almost always present, but oral lesions are present in 90% of patients and can occasionally be the only manifestation of the disease.[23] The oral vesicles are often on the palate, tongue and buccal mucosa and may range from a few isolated lesions to a marked stomatitis. In addition, patients may suffer fever, malaise, conjunctival injection, headache and abdominal pain and occasionally diarrhea. The lesions on the feet and hands are flaccid, grayish vesicles, most often on the sides of the fingers, instep and toes. If the disease is confined to the oral cavity it is almost indistinguishable from primary herpetic gingivostomatitis. Laboratory diagnosis of the disease can be confirmed by culture of feces or swabs obtained from the lesions.

Management is symptomatic. The disease is usually self-limiting and rarely persists for more than 2 weeks. However, hand, foot and mouth disease caused by enterovirus-71, associated with occasionally lethal encephalitis, has been occurring in outbreaks in South East Asia and China over recent years.[24]



Fig. 32.12 Herpangina in a teenager with severe throat pain.

APHTHOUS STOMATITIS

The cause of aphthous ulceration is unknown but a number of infectious agents, including viruses, have been implicated. It usually manifests as small ulcers of the buccal and labial mucosa, often affecting the floor of the mouth or the inferolateral aspect of the tongue, almost always within the anterior part of the oral cavity; the palate and pharynx are rarely involved. The ulcers are characteristically exquisitely painful, particularly during eating, and in the most severe form can lead to anorexia. Humoral and cytotoxic T cell-mediated immune responses to oral mucosa have been demonstrated in some patients, suggesting an autoimmune process. The lesions are usually raised and appear grayish yellow, but in severe cases they may be herpetiform with secondary bacterial infection and cervical lymphadenopathy. Major aphthous ulcers may persist for months, but minor lesions usually heal over 2 weeks. They often recur, with periods of remission lasting as long as a few years. Cultures of swabs from aphthous ulcers are negative on viral culture.

Treatment is usually symptomatic with mouth washes and anesthetic lozenges. Oral prednisolone has been used in some patients but is generally unhelpful. Severe aphthous ulcers have been successfully treated with oral thalidomide. The risk of teratogenicity precludes the use of this drug in women of child-bearing potential. Ulcers are particularly severe in HIV-infected patients.

PRIMARY SYPHILIS

Primary chancres can occur in the mouth approximately 3 weeks after oral sex. An ulcerating papule develops at the site of initial contact of *Treponema pallidum* with the oral mucosa. The papule is painless but is accompanied by significant regional cervical lymphadenopathy. At presentation patients are often seronegative, but darkfield microscopy of material obtained from the ulcer may reveal spirochetes, although care should be taken to avoid contamination of the material obtained with saliva because other *Treponema* species inhabit the mouth and may be easily mistaken for *T. pallidum*. Treatment is discussed in Chapter 57.

Candida Infections of the Mouth

Oral candidiasis is a common problem that usually signals local or generalized disturbance of host defenses.

EPIDEMIOLOGY

Most patients who have oral candidiasis are at the extremes of age, but any individual who has recently taken oral or inhaled steroids or broad-spectrum antibiotics is at risk. The disease is also seen in patients wearing dentures and patients who have diabetes mellitus.[25]

Between 1980 and 1989, rates of oropharyngeal candidiasis in hospitalized patients increased from 0.34 to 1.6 cases per 1000, caused mainly by the HIV epidemic.[26]

PATHOGENESIS

Yeasts are common colonizers of the oral cavity of healthy individuals. *Candida albicans* is the most common of oral yeast isolates (up to 50%).[27] *Candida albicans* can adhere to complement receptors, various extracellular matrix proteins and carbohydrate residues on oral epithelial cells and oral micro-organisms.[28] The organism exists in yeast and hyphal forms. Invasion of tissue is probably related to secreted hydrolytic enzymes and hyphal formation, each of which is possibly initiated by contact with epithelial cells. The immunopathology of mucosal candida infections is unclear, although suppression of normal oral microflora by antibiotics probably permits proliferation of yeasts. Salivary antibodies inhibit bacterial adherence to buccal epithelial cells and are protective in animal models.[29] Saliva also contains a number of antifungal proteins, including histatins and calprotectin, that protect the mouth in concert with local antibody and cell-mediated defense. A disturbance of cell-mediated immunity is partly responsible for overproliferation in patients who have HIV-1 infection and malignancy.

CLINICAL FEATURES

In patients using broad-spectrum antibiotics, or who suffer from candidiasis as a result of denture use, lesions are often erythematous with a burning sensation of the tongue, which displays diffuse redness of the entire dorsum. Most patients who have denture-related oral candidiasis are asymptomatic. Patients who have cell-mediated defects (i.e. diabetics, those on oral steroids or immunosuppressed patients) mostly have the characteristic syndrome of thrush, a pseudomembranous form of the disease in which there is a layer of white curd-like flecks of material that can be wiped off to leave an erythematous surface, beneath which there may be bleeding points.

DIAGNOSIS

The diagnosis is usually clinically obvious in patients who have thrush, but in patients who have erythematous lesions diagnosis can be made by scraping the mucosa and identifying characteristic ovoid yeasts with hyphal forms on microscopy. The organism can be cultured on Sabouraud's agar, but culture alone is insufficient to make the diagnosis since the organism can be recovered from the mouth of approximately 10% of completely normal individuals with no symptoms.

MANAGEMENT

In normal individuals the disease can usually be terminated by removing the cause – either inhaled steroids or broad-spectrum antibiotics – or by removing dentures at night. If necessary, patients can use 7–14 days of topical antifungal therapy, such as nystatin or clotrimazole, which is usually quite sufficient to ablate the infection. Immunocompromised individuals, particularly those with advanced immunosuppression, may require systemic therapy. For a full discussion of candidiasis in patients who have AIDS, see Chapter 91.

Other Oral Fungal Infections

Histoplasma capsulatum is endemic in the midwestern USA and Central and South America. The organism is generally associated with lower respiratory tract infection, but oral lesions can occur, particularly in elderly, debilitated patients who have disseminated disease. The lesions tend to appear as erythematous areas that may ulcerate.[30] Biopsy is usually required to establish a diagnosis. Because the infection is usually disseminated, systemic therapy with amphotericin B is generally required (see Chapters 31 and 178).

The dimorphic fungus *Paracoccidioides brasiliensis* is a major cause of systemic mycosis in Central and South America and should be considered in patients originating from these regions. Most patients have an oral mucosal ulcer with some surrounding edema. There may be perioral lesions that may be ulcerated or warty. Diagnosis can be made by smear and culture, and treatment with oral imidazole compounds is generally sufficient (see Chapters 149 and 178).

Oral Lesions in Patients Who Have Malignancy

A common problem among cancer patients undergoing chemotherapy or radiotherapy is severe mucositis and stomatitis that occurs approximately 1 week after the onset of chemotherapy.[31] At this point, destruction of oral epithelium is at its height with an accompanying disturbance of immune surveillance of oral mucosal micro-organisms. This leads to opportunist bacterial[32] or fungal infection. Patients nearly always complain of pain and tenderness in the mouth with or without formation of a pseudomembrane. Symptoms can persist long after chemotherapy has been terminated.

Management should include a vigorous search for a microbial etiology; a short course of metronidazole is sometimes helpful. Some prevention can be achieved by careful oral hygiene and effective management of xerostomia associated with chemotherapy. Once there is established mucositis, topical therapy with antiseptic and anesthetic preparations is indicated. Aluminum hydroxide gel can be used to provide symptomatic relief of painful inflammation.

HALITOSIS

Halitosis may affect up to 30% of the population. Oral malodor is the result of the production of volatile sulfur compounds by bacteria in the process of breaking down components of epithelial cells, salivary proteins and food debris. The main volatile compounds include methyl mercaptan, hydrogen sulfide and dimethyl sulfide. A wide range of oral anaerobes are associated with this, including *Porphyromonas* spp., *Prevotella intermedia*, *Treponema denticola*, *Fusobacterium nucleatum*, *Tannerella forsythensis* and *Eubacterium* spp. Most recently, halitosis has been linked to *Solobacterium moorei*.[33] Clinically, halitosis is mostly associated with periodontitis or with abnormal tongue coating.

Management is directed at reducing the bacterial load both in periodontitis and in tongue coating by oral hygiene measures, control of tongue flora by brushing or scraping, and occasionally the adjunctive use of antiseptic agents. Treatments have also been proposed to neutralize malodorous compounds by chemical agents such as zinc- and copper-containing compounds to mask the presence of the condition.[34]

Infections of the Salivary Glands

The most common cause of parotitis is mumps virus, but parotitis can occasionally be caused by bacteria or other viruses, including parainfluenza virus, coxsackievirus, echovirus, Epstein–Barr virus and HIV.

EPIDEMIOLOGY

The incidence of mumps has markedly decreased in the era of childhood measles, mumps and rubella (MMR) vaccination, which confers lifelong immunity. Despite this, mumps virus remains the most

common cause of parotitis. It is highly contagious by air-borne droplet transmission. Mumps infections occur in late winter and early spring; enterovirus infections, including parotitis, are mostly seen in mid to late summer. Before the introduction of the MMR vaccine in the UK in 1988, the annual incidence of mumps was approximately 5 per 100 000 population; however, in the postvaccine era this has declined to less than 0.5 per 100 000.[35]

Most patients who have primary bacterial parotitis are over the age of 60 years and are frequently debilitated because of chronic illness or have underlying diseases such as diabetes. Patients who are dehydrated, whatever the cause, are at greatest risk. Medications that lead to xerostomia include anticholinergic and occasionally diuretic agents. Poor oral hygiene increases the chances of reflux of bacteria into the salivary gland.[36]

PATHOGENESIS

Mumps virus is a paramyxovirus and gains entry via the respiratory tract. The subsequent viremia allows access of the virus to tissues for which it has tropism, including salivary gland tissue, gastrointestinal tissue such as pancreas, testicular tissue and the central nervous system. The incubation period is 18–21 days.

Bacterial infection of the salivary glands is normally prevented by constant salivary flow, which removes contaminants from the ductal systems. Dehydration, xerostomia or obstruction of the ducts can lead to bacterial proliferation within the salivary glands and subsequent parotitis.

CLINICAL FEATURES

The most common clinical manifestation is gradual onset of painful swelling of either one or both of the parotid glands, which occurs 14–21 days after contact with an infected individual. Pain within the parotid gland can be initiated by salivation during meals, and the glands are tender. Occasionally, submandibular salivary glands are involved, but inflammation of sublingual glands is extremely rare. Orchitis is present in approximately 10–20% of individuals and is bilateral in 5%, but there is no firm evidence that it causes male sterility. Mumps meningoencephalitis may occur in concert with parotitis, but patients who have mumps meningitis often do not have parotitis. In the pre-MMR era mumps was a relatively common cause of viral meningitis in children less than 15 years old in whom permanent unilateral deafness was a recognized complication. Pancreatitis is rare. On examination there is smooth tender swelling that obliterates the angle of the jaw and may raise the pinna. Rarely, the outlet of Stensen's duct may be inflamed. There may be generalized symptoms, including fever, arthralgia, malaise and headache, which generally persist for up to 1 week. Culturable virus is present in the saliva for up to 1 week after gland enlargement. Management is essentially symptomatic.

Recurrent episodes of glandular swelling, particularly of the parotid gland, can occur in children with a history of mumps. Clinical features include recurrent parotid swelling with general malaise and pain frequently after a meal. Viridans streptococci are usually cultured from exudate from the Stensen's duct.

In primary bacterial parotitis there is usually rapid onset of pain, swelling and induration of the involved gland (Fig. 32.13). Manual palpation of the gland is exquisitely painful and can result in discharge of pus from the duct. In addition, there are usually systemic features, including fever, rigors and a neutrophilia. The most frequently isolated organisms are *Staph. aureus, Strep. pyogenes*, viridans streptococci and *Haemophilus influenzae*.

HIV-associated salivary gland swelling most commonly occurs as a bilateral cystic enlargement of the parotid glands, occasionally in association with xerostomia, dry eyes and arthralgia. Salivary gland involvement can occur very early on in HIV infection but is most commonly seen in late disease. Histologically, there are numerous epithelium-lined cysts, some up to several centimeters in size, containing macrophages and lymphocytes. The commonest identified opportunist infection of salivary glands is cytomegalovirus (CMV); about 15% of post-mortem submandibular glands of all patients who have AIDS have evidence of CMV inclusion bodies.[37] In children, there is a strong association between HIV-parotid swelling and lymphocytic interstitial pneumonitis. Examination usually reveals smooth bilateral swelling. Uneven swelling should be biopsied because 10% of salivary gland disease in HIV-infected patients is caused by lymphoma.[38]

DIAGNOSIS

In mumps, this can be achieved by detection of salivary IgM or by culture of salivary washings or of viral throat swab. A convalescent rise in complement-fixing antibody occurs. In established viral parotitis there is elevation of serum salivary-type amylase. Rarely, there may be biochemical evidence of pancreatitis.

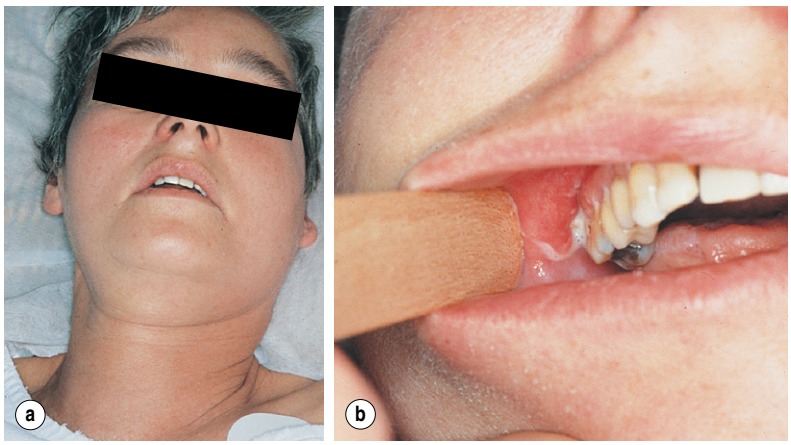


Fig. 32.13 Suppurative parotitis (a) in a diabetic patient who had a recent history of dehydration secondary to diabetic ketoacidosis. (b) Pus was manually expressed from Stensen's duct from which *Staphylococcus aureus* was cultured. Courtesy of Dr E Ridgway.

PREVENTION

The MMR vaccine consists of live attenuated measles, mumps and rubella viruses. Immunization provides protection for 90% of recipients for measles and mumps and over 95% for rubella. The antibody response to the mumps component is too slow for effective postexposure prophylaxis. After the first dose of MMR, malaise, fever or rash may occur about 1 week after immunization, although this syndrome usually self-terminates within 3 days. Febrile convulsions occur in approximately 0.1% of children between 1 and 2 weeks after administration of the vaccine (similar to the attenuated live measles vaccine), and by the fourth week parotid swelling is seen in approximately 1% of infants.[39]

MANAGEMENT

Management of viral parotitis is symptomatic. Bacterial parotitis can usually be managed by prompt fluid replacement and parenteral antibiotic therapy using amoxicillin–clavulanate 1.2 g q8h or intravenous cefuroxime 750 mg q8h. Drainage of the duct should be assisted by manual massage. Occasionally steroids are necessary to suppress inflammation and potentiate drainage. Surgical drainage of a salivary gland abscess is rarely necessary.

PAROTITIS CAUSED BY *MYCOBACTERIA* SPECIES

Nontuberculous mycobacterial infections of the parotid gland are now increasingly seen in children, in whom they present as unilateral painless indurated swellings that can be mistaken for neoplasm. Diagnosis can be made by fine needle aspiration with cytology and culture, which may reveal organisms such as *Mycobacterium scrofulaceum*, *Mycobacterium avium-intracellulare* or *Mycobacterium malmoense*. Management is conservative. *Mycobacterium tuberculosis* infection of the parotid gland is rare, but is one of the differential diagnoses of parotid tumor and should be rigorously excluded by histology of needle biopsy or fine needle aspiration cytology before unnecessary deforming surgery is undertaken.[40] The disease responds well to conventional antituberculous chemotherapy.

REFERENCES


References for this chapter can be found online at http://www.expertconsult.com

Chapter 33

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Andrew F Goddard

Gastritis, peptic ulceration and related conditions

INTRODUCTION

In 1983, Marshall and Warren described the bacterium now known as *Helicobacter pylori* (see Chapter 171) and suggested that it may be important in the pathophysiology of chronic active gastritis and peptic ulceration. In 2005, they received the Nobel Prize for Medicine for this discovery.[1] *H. pylori* infection is extremely common, affecting nearly half of the world's population. It invariably induces a chronic active gastritis, a histologically defined inflammation of the stomach which in itself is usually asymptomatic.[2] However, approximately 10–15% of infected people develop a duodenal or gastric ulcer in their lifetime and a much smaller proportion develop distal gastric adenocarcinoma or primary gastric lymphoma. *H. pylori* infection is now also thought to play a causal role in some cases of chronic anemia and possibly idiopathic thrombocytopenic purpura (ITP). At a population level, infection is protective against conditions caused by gastroesophageal reflux: reflux esophagitis, Barrett's esophagus and esophageal adenocarcinoma. Interesting but controversial recent reports suggest that it may also contribute to the 'hygiene hypothesis', i.e. that chronic infection may offer a degree of protection against conditions such as asthma, allergy and autoimmune diseases.

The term 'gastritis' is often erroneously applied to the macroscopic appearance of 'inflamed' (erythematous) gastric mucosa seen at endoscopy (Fig. 33.1a). However, these appearances correlate poorly with histologic inflammation, for which the term 'gastritis' should be reserved. Gastritis may be subtyped from the histologic appearance and distribution within the stomach, and these features often indicate etiology and risk of associated disease (Table 33.1). The main cause of gastritis is *H. pylori* infection. Two other helicobacters, namely *Helicobacter bizzozeronii* (formerly called *Helicobacter heilmannii* or *Gastrospirillum hominis*) and *Helicobacter felis*, are tight spiral bacteria present transiently in 2–6% of the population of a developed country and thought to be zoonotically acquired, as both commonly colonize domestic pets.[3,4] They usually cause mild gastritis and although there are case reports of associated disease and response to anti-helicobacter treatment, this is rare and a causal link is not well established.[3,4]

Peptic ulcers may be associated with some types of gastritis. A peptic ulcer is a macroscopic break in the gastric or duodenal mucosa with obvious depth and definite size (usually defined as greater than 0.5 cm; Fig. 33.1). Erosions are smaller breaks in the mucosal surface, which usually reflect the ulcer diathesis and should be managed similarly. Although gastric and duodenal ulcers share some characteristics, there are notable differences in their etiologies and pathogeneses (Table 33.2).

Gastric adenocarcinoma is the fourth most prevalent cancer in the world and the second most common cause of cancer deaths. The more prevalent distal form is now known to be caused by *H. pylori* infection, making *H. pylori* the leading infectious cause of human malignancy. Even the most conservative estimates place the attributable fraction of gastric adenocarcinoma to *H. pylori* at 60%.[5,6] Thus, primary prevention of *H. pylori* infection would more than halve gastric adenocarcinoma incidence.

EPIDEMIOLOGY

Prevalence and incidence

The age prevalence of *H. pylori* differs markedly between countries, but two broad patterns are found (Fig. 33.2).

- In group 1 countries (predominantly developing countries), there is a rapid rise in prevalence before 20 years of age, after which point prevalence stabilizes at above 80%, implying that *H. pylori* is acquired in childhood and persists throughout life.
- In group 2 (usually developed) countries, the prevalence of infection increases steadily with age at a rate of roughly 1%/year of life. Epidemiologic evidence suggests that this is largely the result of a birth cohort effect.[7] Thus, about 30% of 30-year-olds have acquired the infection in childhood, as compared with 60% of 60-year-olds, because of a changing incidence of infection in childhood over the past 60 years.

Associations

Aside from associations with age and geographic area, *H. pylori* is closely associated with socioeconomic conditions, particularly in childhood. This may explain the different prevalences of infection found in different ethnic groups within the same geographic area.[8] Markers of childhood socioeconomic status that have been correlated with prevalence of infection include general level of hygiene, water supply and sanitation, and level of crowding in the household. These associations further support the view that most *H. pylori* acquisition is in childhood, from when it persists throughout life in the absence of effective treatment.

Transmission

Helicobacter pylori is thought to be acquired by direct human-to-human contact in childhood. In developed countries it is usually acquired from the primary care giver but it may also be spread between children. Family members thus often, but not invariably, share the same strain, although this may evolve *in vivo* to change its pathogenicity. Although most infected adults became infected as children, there are well-documented examples of *H. pylori* being acquired de novo in adult life and several studies have suggested that crowding and poor sanitation are risk factors for this. However, marital status is only weakly associated with infection (and in some studies not associated), supporting the view that acquiring the infection *de novo* as an adult is rare.

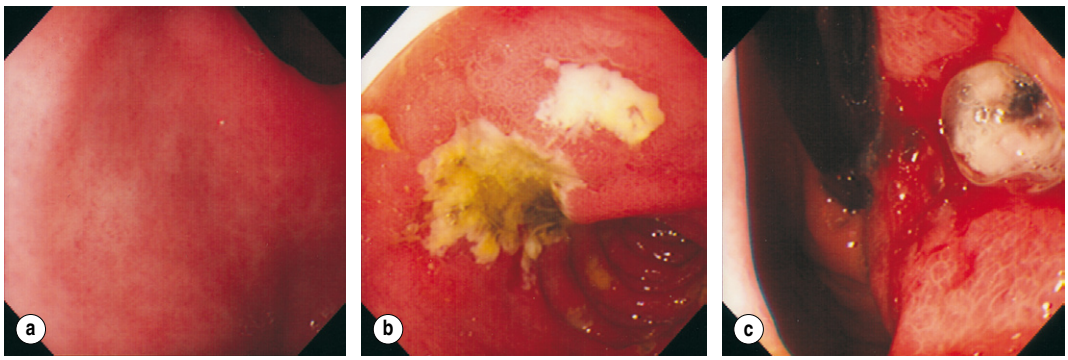


Fig. 33.1 Endoscopic pictures of the stomach and duodenum. (a) Erythema of the gastric antrum. This appearance correlates poorly with histologic gastritis and may be a normal finding. (b) Duodenal ulceration. (c) Gastric ulceration. Note the clot in the base indicating recent bleeding and high risk of rebleed and the endoscope in the stomach.

Table 33.1 Classification of chronic gastritis according to the updated Sydney system

Type of gastritis	Etiology
Nonatrophic	*Helicobacter pylori*
Atrophic Autoimmune Multifocal atrophic	 Autoimmunity *H. pylori* ± dietary and other environmental insults
Special forms Chemical Radiation Lymphocytic Noninfectious granulomatous Eosinophilic Other infectious gastritides	 Aspirin/NSAIDs, bile and possibly other agents Radiation Idiopathic, overt or latent celiac disease, drugs (ticlopidine), possibly *H. pylori* Crohn's disease, sarcoidosis, vasculitides, foreign substances, idiopathic Food sensitivity, possibly other allergies Bacteria other than *H. pylori* (particularly *H. bizzozeronii* or '*H. heilmanii*' and *H. felis*, mycobacteria and syphilis), viruses (particularly cytomegalovirus), fungi (particularly *Candida* spp., *Histoplasma capsulatum* and Mucoraceae)

Non-helicobacter infectious gastritides are very rare, usually occur in immunocompromised patients and are not discussed in this chapter. Data from Dixon *et al.*, with permission.[2]

Table 33.2 Causes of duodenal and gastric ulceration (with estimated proportions)

Cause		Duodenal ulcer (% of cases)	Gastric ulcer (% of cases)
Infection	*Helicobacter pylori*	80	60–70
Drugs	Aspirin and NSAIDs	10–20	25–30
Neoplasms	Zollinger–Ellison syndrome Lymphoma Gastric adenocarcinoma Other adenocarcinoma Leiomyoma	Rare Rare – Rare –	Rare Rare 2–5 Rare Rare
Others	Crohn's disease Systemic mastocytosis Severe systemic illness	Rare Rare Rare	Rare Rare Rare

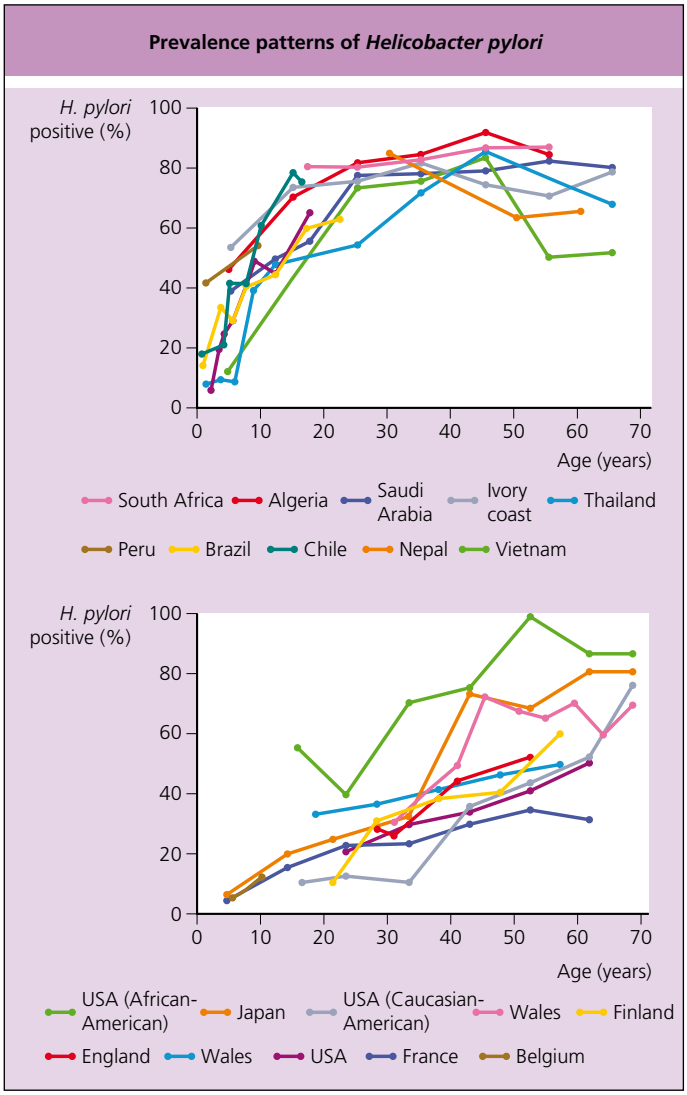


Fig. 33.2 Prevalence patterns of *Helicobacter pylori*. Prevalence of *H. pylori* infection in 10 developing countries (Group 1) and 10 developed countries (Group 2). Adapted with permission from Pounder & Ng.[7]

Although monkeys and domestic cats may become infected, these are not an important reservoir for human infection. Although *H. pylori* DNA has been isolated from drinking water supplies in developing countries, *H. pylori* has not been cultured consistently from these sources and most data do not support an environmental source of

infection. In transfer between humans, it is unclear whether *H. pylori* is transmitted by the fecal–oral or oral–oral route, or both. The bacterium has been cultured with difficulty from the feces of people who have *H. pylori* infection in both developing and developed countries, but it is more easily cultured from gastric refluxate into the mouth and from vomitus. However, it appears not to persist in the mouth as, although *H. pylori* DNA has been found in dental plaque, *H. pylori* culture from dental plaque is rarely successful. In past years, transmission of *H. pylori* has been documented following insufficient sterilization of endoscopy or gastric pH measuring equipment, although with adequate sterilization this is no longer a problem. Acute *H. pylori* infection by this route is thought to be the cause of the occasional outbreaks of epidemic acute hypochlorhydria[9] observed before the discovery of *H. pylori*.

PATHOGENESIS AND PATHOLOGY

Pathology and disease associations

Helicobacter pylori is primarily a gastric infection, although it may colonize areas of gastric metaplasia in the duodenum and esophagus, and rarely heterotopic gastric tissue elsewhere in the gastrointestinal tract. In the stomach, infection causes chronic active gastritis characterized by continuing neutrophil and lymphocyte infiltration, epithelial damage and thinning of the mucous layer (Fig. 33.3a). This is in contrast to gastritis caused by chemical agents, including nonsteroidal anti-inflammatory drugs (NSAIDs), which is characterized by regenerative epithelial changes and a paucity of inflammatory cells (Fig. 33.3b).

Most people infected with *H. pylori* remain asymptomatic and disease free throughout their lifetime, but some will develop an associated disease. These include:

- duodenal ulceration;
- gastric ulceration;
- gastric adenocarcinoma arising from the distal stomach; and
- primary gastric lymphoma.

There is controversy over whether *H. pylori* is the cause of symptoms in a subset of people with a microscopically normal upper gastrointestinal tract (so called 'functional dyspepsia'); treatment cures a small proportion of such patients. Longstanding *H. pylori* infection leads, in some people, to atrophic gastritis. This may be a precursor of gastric cancer, but also is increasingly recognized as an important cause, not just of vitamin B12 deficiency and its associated anemia, but also of iron deficiency anemia.[10] *H. pylori* is thought possibly to contribute to the reduced platelet count in a proportion of people with idiopathic thrombocytopenic purpura; treatment of *H. pylori* increases platelet count in some of these patients.[11] Finally, there is a weak association between *H. pylori* and atherosclerosis but it remains unclear whether this is a causal association or due to confounding, for example by social class.[12]

Less controversially, it is now clear that *H. pylori* infection, especially when associated with gastric atrophy, offers a degree of protection against complications of gastroesophageal reflux, including esophageal adenocarcinoma. Recent work also shows a negative association between *H. pylori* and autoimmune and allergic diseases, including asthma.[13] Again, it is unclear whether this is a causal relationship.

Pathogenesis

Whether an infected person develops disease is dependent on a combination of bacterial strain virulence, host genetic susceptibility and environmental co-factors.[14] Several bacterial virulence factors are found more prominently in ulcer- or cancer-associated strains than in nondisease-associated strains.[14] These include:

- the presence of the *cag* (cytotoxin-associated gene) pathogenicity island;
- production of an active vacuolating cytotoxin, VacA;
- the presence of other genes including the duodenal ulcer promoting gene A (*dupA*) and the outer inflammatory protein encoding gene A (*oipA*); and
- certain adhesins, most notably the blood group antigen binding adhesin A (BabA).

The *cag* pathogenicity island is a genetic region containing over 30 genes. The island encodes a type IV secretory apparatus, best thought of as a syringe, through which one *cag*-encoded protein, CagA, is 'injected' into epithelial cells where it stimulates a number of signaling pathways resulting in cytoskeletal changes and proliferation.[14] Soluble components of the peptidoglycan bacterial cell wall also leak across the syringe and are recognized by the intracellular receptor Nod1. Both pathways result in proinflammatory signaling.[15] CagA is also highly immunogenic and anti-CagA antibody detection can be used as a serum test for the presence of the *cag* island. About 70% of strains in the USA are cag^+ and these strains colonize the gastric mucosa more densely, cause more inflammation and are more likely to be associated with ulcers and gastric cancer than are cag^- strains.[14]

The vacuolating cytotoxin, VacA, is a pore-forming toxin that increases epithelial permeability and causes massive epithelial cell vacuolation *in vitro*. It is particularly suited to the stomach because it is activated by acid, then becoming acid and pepsin resistant.[16] Although only about 40% of strains isolated in the USA exhibit cytotoxin activity, all have *vacA*, the gene encoding the cytotoxin. However, only some *vacA* genotypes are associated with the toxigenic phenotype[17] and infection with strains of certain *vacA* genotypes is associated with increased prevalence of gastric cancer[18] and, in some reports, of peptic ulcer disease.

Host genetic susceptibility to disease amongst *H. pylori*-infected people is also becoming better understood. Polymorphisms in the interleukin-1β (IL-1β) gene which lead to more IL-1β expression in response to bacterial infections increase the risk of gastric cancer.[19] Many other polymorphisms in both cytokine genes and mediators of the innate immune response also affect disease risk. However, the risks confirmed by these polymorphisms differ in different worldwide human populations.

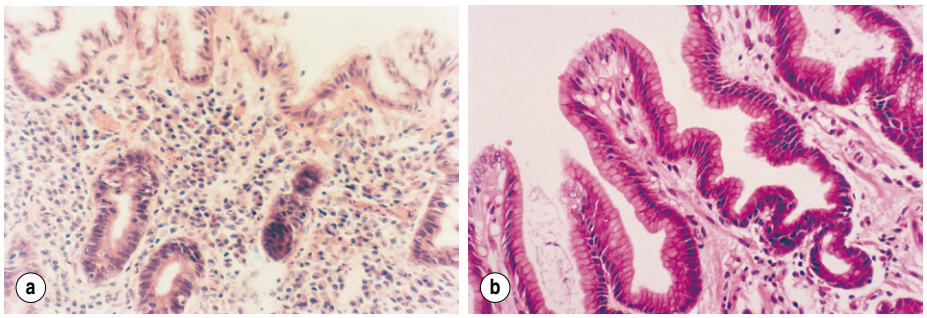


Fig. 33.3 Appearances of *Helicobacter pylori* and NSAID antral gastritis. (a) Antral gastritis in *H. pylori* infection with active (neutrophil) and chronic (lymphocyte) inflammation of the lamina propria and glands. The epithelial surface is typically ballooned. *Helicobacter pylori* organisms are not readily apparent on a hematoxylin and eosin stain. (b) Antral gastritis associated with NSAID use. Foveolar hyperplasia with a mild chronic inflammatory infiltrate and smooth muscle cells are seen in the lamina propria. Courtesy of Dr MM Walker.

Environmental co-factors are an important and underappreciated risk factor for disease. Amongst *H. pylori*-infected people, smoking is the most important determinant of whether duodenal ulceration will develop. Smoking and male sex are also important risk factors for gastric adenocarcinoma. High salt diets predispose to gastric cancer and diets high in fresh fruits and antioxidants are weakly protective.

Duodenal ulcer disease

The pathogenic link between infection in the stomach and ulceration in the duodenum is now reasonably well accepted. Duodenal ulcers arise in patients with antral-predominant gastritis. Infection of the gastric antrum leads to a reduction in somatostatin-producing D cells, resulting in hypergastrinemia, as somatostatin inhibits gastrin production.[20] Both inflammation and hypergastrinemia are more marked in infection with *cag*$^+$ strains. High gastrin levels lead to increased stimulated acid output from parietal cells in the gastric corpus, which is most marked when the corpus is relatively spared of inflammation. The resulting increased acid load entering the duodenum leads to the formation of adaptive gastric metaplasia. This can be colonized by *H. pylori* and local inflammation and release of toxic bacterial products can lead to ulceration. What remains unclear is why only some people have antral-predominant inflammation in response to *H. pylori* and so are at risk of duodenal ulceration. Aspirin and NSAIDs can cause ulcers independently of *H. pylori* infection and further increase the risk of ulcers in people with *H. pylori*.

Gastric ulcer disease

Helicobacter pylori-associated gastric ulcers usually arise in junctional mucosa between antral- and corpus-type tissue, typically on the lesser curvature. They usually occur in patients with pan-gastritis rather than antral-predominant gastritis and are not associated with increased stimulated acid output. Although *H. pylori* infection is the most common cause of gastric ulceration, the proportion caused by aspirin and NSAIDs is higher than for duodenal ulcers.

Gastric adenocarcinoma

The World Health Organization has classified *H. pylori* as a type 1 or causal carcinogen.[21] It is a risk factor for *distal* adenocarcinoma with a relative risk of 4–9. The relationship of *H. pylori* with *proximal* gastric adenocarcinoma is interesting. Cancers in this region appear to have two etiologies: some are related to *H. pylori* infection and others are related to gastroesophageal reflux and therefore negatively associated with *H. pylori* infection.[22] Thus overall in most populations there is no association between *H. pylori* and gastric cardia cancer because the opposing risks in different individuals cancel each other out. Gastric adenocarcinoma usually arises in patients with pan-gastritis or corpus-predominant gastritis; patients who have previous duodenal ulceration (usually with antral-predominant gastritis) are less likely than others to develop gastric cancer. Both *cag*$^+$ and cytotoxic strains are more likely to be associated with carcinoma than other strains.

The pathogenesis of gastric carcinoma is unclear and may differ between the two main types, intestinal and diffuse cancer. Intestinal-type gastric cancer is thought to occur by a step-wise process from superficial gastritis through atrophy to intestinal metaplasia, dysplasia and ultimately carcinoma.[23] One possibility is that the hypochlorhydria associated with pan-gastritis and with atrophy may allow survival of DNA-damaging oxygen and nitrogen free radicals produced by inflammatory cells or by other bacteria able to colonize the hypochlorhydric stomach. Hypergastrinemia may also be important in gastric carcinogenesis and is increasingly recognized as a risk factor for other gastrointestinal tract malignancies. Diffuse-type gastric cancer occurs in younger individuals and may arise more directly from simple *H. pylori*-induced gastritis.

Gastric lymphoma

Primary gastric lymphomas arise in lymphoid tissue; this is only present in the stomach following *Helicobacter* infection. Low-grade B-cell mucosa-associated lymphoid tissue (MALT) lymphomas are particularly interesting as they are driven by chronic stimulation by *H. pylori* antigens. A majority of low-grade lymphomas regress following *H. pylori* eradication, but some, particularly those with a t(11;18) chromosomal translocation, do not.[24] All patients need careful monitoring after treatment and although these low-grade gastric lymphomas are rather indolent, many physicians treat those that do not respond to *H. pylori* treatment with gentle chemotherapy or low-dose radiotherapy.

Protection against gastroesophageal reflux disease (GERD)

There is increasing epidemiologic evidence that people with *H. pylori* infection, especially those with pathogenic strains, are less likely to develop GERD and its sequelae – Barrett's esophagus and esophageal adenocarcinoma.[25] This is of considerable interest because the incidence of these conditions is increasing rapidly in developed countries. Evidence suggests that reduced risk is associated with pan-gastritis and reduced gastric acid production, presumably because this makes gastroesophageal refluxate less damaging. In people with *H. pylori* infection, its treatment may improve, worsen or not affect GERD symptoms and esophagitis. Thus the possibility of affecting or inducing GERD symptoms should not deter physicians from treating *H. pylori*, where an indication exists.

Other diseases

H. pylori has been associated with iron deficiency anemia in the absence of other causes of blood loss.[10] This may be due to recurrent peptic ulceration in some cases but is more frequently thought to be secondary to reduced iron absorption due to low acid production in the stomach. This is not entirely due to gastric atrophy, as several studies have shown that *H. pylori* eradication normalizes the blood count in some patients and gastric atrophy responds extremely slowly (if at all) to *H. pylori* treatment. More controversially, *H. pylori* has also been associated with idiopathic thrombocytopenic purpura (ITP).[11] *H. pylori* is more prevalent in patients with ITP than in controls and eradication leads to increased platelet counts in some ITP patients.

PREVENTION

Prevention of *H. pylori* infection is an attractive public health aim as it should lead to a vast reduction in the incidence of gastric adenocarcinoma and peptic ulceration. It is unclear to what extent it might lead to an increase in GERD and its complications, or more controversially to other diseases of modern life such as autoimmune and allergic diseases. However, most authorities consider that the balance lies firmly in favor of a large benefit from *H. pylori* prevention.

In developed countries, *H. pylori* incidence is falling steadily already, due to improvements in living conditions and public health, and possibly widespread antibiotic use. Thus specific preventive measures may not be necessary. As the wealth of developing countries improves, similar falls in *H. pylori* incidence are likely to follow. However, this will take time. A good solution in the meantime would be immunization against *H. pylori*. However, although vaccine research in animal models is encouraging, whether an effective human vaccine can be developed remains uncertain.

CLINICAL FEATURES

Acute *Helicobacter pylori* infection

The clinical features of acute infection in the community are unknown. However, where high doses of *H. pylori* have been self-administered, upper abdominal discomfort and pain occurred 3 days after dosing, followed by vomiting and finally a resolution of symptoms by the end of the week.[26] A similar pattern of symptoms was observed in patients with acute epidemic hypochlorhydria,[9] an illness that occurred in volunteers undergoing nasogastric intubation for acid secretion studies in the 1970s and now shown to be due to iatrogenic acute *H. pylori* infection. *Helicobacter pylori* is most commonly acquired in childhood, but whether initial colonization is usually symptomatic or asymptomatic is not known.

Chronic *Helicobacter pylori* infection

Chronic *H. pylori* infection is characterized by chronic active gastritis, but this condition is asymptomatic. Chronic infection is therefore only manifest symptomatically if complications develop, such as duodenal ulceration, gastric ulceration or gastric cancer.

DIAGNOSIS

For all tests, other than serology, proton pump inhibitors should be stopped for at least 2 weeks and antibiotics for at least 4 weeks before testing or false-negative results may occur. Diagnosis of *H. pylori* infection can be made by noninvasive tests or endoscopic biopsy-based tests. Noninvasive tests include serology, the urea breath test (UBT) and the stool antigen test. These tests are useful in primary diagnosis in young patients with dyspepsia. The urea breath test and stool antigen test (but not serology) are useful in assessing the success of *H. pylori* treatment. In patients undergoing endoscopy, these tests are unnecessary, because tests for *H. pylori* can be performed on gastric biopsy specimens.

Following *H. pylori* treatment, whether and how to retest for *H. pylori* depends on the treatment indication. If the treatment is for uninvestigated dyspepsia, most physicians do not retest unless symptoms recur, as modern treatment regimens have a very high efficacy. Following gastric ulcer treatment, it is usual to repeat endoscopy to check mucosal healing as occasionally a gastric neoplasm can masquerade as an ulcer. In this situation, biopsy-based tests can be repeated, but importantly these tests must be delayed for at least 4 weeks after finishing treatment. In cases of duodenal ulceration, treatment success can be assessed noninvasively by the urea breath test or stool antigen test. Some physicians do not do this routinely unless symptoms recur, but it is recommended for patients with complicated or large ulcers and our practice is to check treatment success in all patients with definite peptic ulceration. Serologic tests are not suitable for checking the success of treatment as specific antibody levels fall only slowly.

Many patients require endoscopy to assess indications for treatment, and so tests for *H. pylori* can be performed at this time. The choice of endoscopic-based test depends upon the information required, cost and convenience; usually only a biopsy urease test is used. As infection is very likely in non-NSAID-associated duodenal ulceration, some regard testing for *H. pylori* in this context as unnecessary. Our practice, however, is to make a positive diagnosis before prescribing potentially harmful multiple antibiotic treatment regimens.

Endoscopic tests for *H. pylori*

Endoscopic tests are based on mucosal biopsy specimens. Infection may be patchy, and so if possible two biopsies should be taken from the usually more uniformly infected antrum to minimize sampling error. In some situations, notably after treatment, during acid-suppressive therapy or when intestinal metaplasia and atrophy are likely (e.g. in the elderly), the infection may be more marked in the corpus and at least two additional biopsies should be taken from there.

Culture

Helicobacter pylori can be cultured from gastric biopsies, although sensitivity is often low compared with that of other tests. Biopsies should be put into a sterile solution and transferred as soon as possible to the laboratory. Methods for culture and identification are discussed in Chapter 171. Cultured bacteria can be tested for antibiotic sensitivities and the main indication for culture is previous failed treatment.

Histology

Chronic superficial gastritis seen on standard hematoxylin and eosin staining is strongly indicative of *H. pylori* infection, but unless specialized stains are used (e.g. modified Giemsa, Gimenez (Fig. 33.4), Warthin–Starry or Genta) the infection may be missed. Histology is expensive, but sensitive in experienced hands, and may provide other useful information, such as the presence of epithelial dysplasia.

Biopsy urease test

In this test, two gastric biopsies or one large biopsy are placed in a gel or solution containing urea and a pH indicator. If *H. pylori* is present, its urease enzyme catalyzes urea hydrolysis and a color change occurs. These tests can be performed in the endoscopy room and are sensitive, specific, cheap, convenient and quick. A positive result can be obtained in a few minutes, although for most commercial tests a 24-hour wait is necessary to ensure that the test is negative.

Nonendoscopic tests

Urea breath tests

Several protocols exist, but in essence the patient drinks urea solution isotopically labeled with either stable ^{13}C or, more unusually nowadays, ultra-low-dose radioactive ^{14}C. If *H. pylori* is present, the urea is hydrolyzed and labeled carbon dioxide can be detected in breath samples. Both isotopes are safe, although it is sensible to avoid ^{14}C in pregnant women and children. The best UBT protocols are as specific as biopsy-based tests and are perhaps more sensitive as sampling error is avoided. The UBT is the most widely used and appropriate test to check for treatment success in situations where repeat endoscopy is unnecessary, but must be performed at least 1 month after any antibiotic, bismuth or proton pump inhibitor treatment.

Stool antigen tests

These newly described simple stool tests are an alternative to UBTs and indications for use are the same.[27] The stool antigen test may be simpler and more cost-effective than the UBT for community use, and

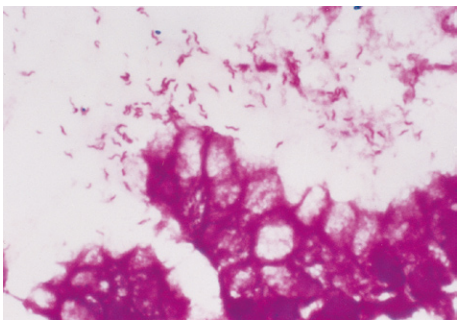


Fig. 33.4 *Helicobacter pylori* (Gimenez stain). Other special stains that can be used are the modified Giemsa stain or a silver stain such as Warthin–Starry stain. Courtesy of Dr MM Walker.

stool antigen tests are now accepted as appropriate in this setting.[6] However, the storage and handling of stool tests is crucial for their accuracy.

Serology

Although serology is cheap and convenient, a wide range of commercial tests are available and most of these are less accurate than the stool antigen test and UBT. Thus serology has rather fallen out of favor. However, serology should still be considered in patients where other tests may show a false-negative, such as patients with bleeding ulcers on proton pump inhibitors or patients with MALT lymphoma in whom other tests are negative. It may also be the best test for infection screening in primary care, provided appropriate cut-off values are employed and the best locally validated test is used.

MANAGEMENT

The clearest indications for *H. pylori* treatment are peptic ulcer disease (whether active or previous) and low-grade MALT lymphoma (unless it is known to be of the t(11;18) type). However, *H. pylori* treatment is now commonly given for other indications, including iron deficiency anemia (when no other cause is found) and more controversially for idiopathic thrombocytopenic purpura.

As discussed below, young patients with uninvestigated dyspepsia are often tested for *H. pylori* noninvasively and treated if they are positive.[28] Some physicians treat *H. pylori* in patients with dyspepsia even if an endoscopy shows no ulceration. This is because meta-analysis has shown that for 1 in 10–15 people symptoms will resolve following *H. pylori* treatment.[29]

Helicobacter pylori treatment is not normally recommended for the primary prevention of gastric adenocarcinoma, as treatment of established infection has not been shown to reduce cancer risk. However, in children and younger patients, successful treatment is likely to reduce cancer risk, so many physicians treat people in this age group if infection is found. Also, patients taking long-term proton pump inhibitors have an increased risk of developing accelerated atrophic gastritis, and although there is no evidence to date that this results in an increased risk of gastric adenocarcinoma, many physicians treat *H. pylori* in these patients.[6] Some also treat *H. pylori* where there is a family history of gastric malignancy.

Finally, in patients starting chronic or intermittent NSAID therapy (e.g. patients with chronic arthritis) there is good evidence that ulcer risk is reduced if *H. pylori* is treated. However, in high-risk patients, coprescription of NSAIDs with a proton pump inhibitor is even more effective in preventing ulcers.

Peptic ulcer disease

Eradication of *H. pylori* not only heals ulcers but also prevents their recurrence. Therefore, once an ulcer is diagnosed by endoscopy or barium meal, *H. pylori* infection should be sought and, if found, treated (Fig. 33.5). This is usually done immediately, but if there is a delay in performing diagnostic tests for *H. pylori*, ulcer healing can be started with acid-suppressing drugs and *H. pylori* treatment can be added when the infection has been confirmed.

Uninvestigated dyspepsia

In young patients with dyspepsia, most national and other guidelines advocate a 'test and treat' approach: noninvasive testing for *H. pylori* (thus avoiding endoscopy) and treatment if positive.[6,28,30] This reduces the number of endoscopies performed (and therefore cost), is more comfortable and convenient for patients, and is equivalent for successful management of symptoms. The reason for performing endoscopy in older people is to avoid delay in diagnosing gastric cancer. Thus the age cut-off for deciding who should undergo endoscopy depends on the local incidence and demographics of this disease; it is usually set at between 40 and 55 years. All patients with 'alarm' symptoms or signs such as weight loss, dysphagia, persistent vomiting, gastrointestinal bleeding, unexplained anemia, epigastric mass, previous gastric ulcer or gastric surgery should be referred for upper gastrointestinal endoscopy and/or other investigations regardless of age, both to exclude malignancy and to make a positive diagnosis.

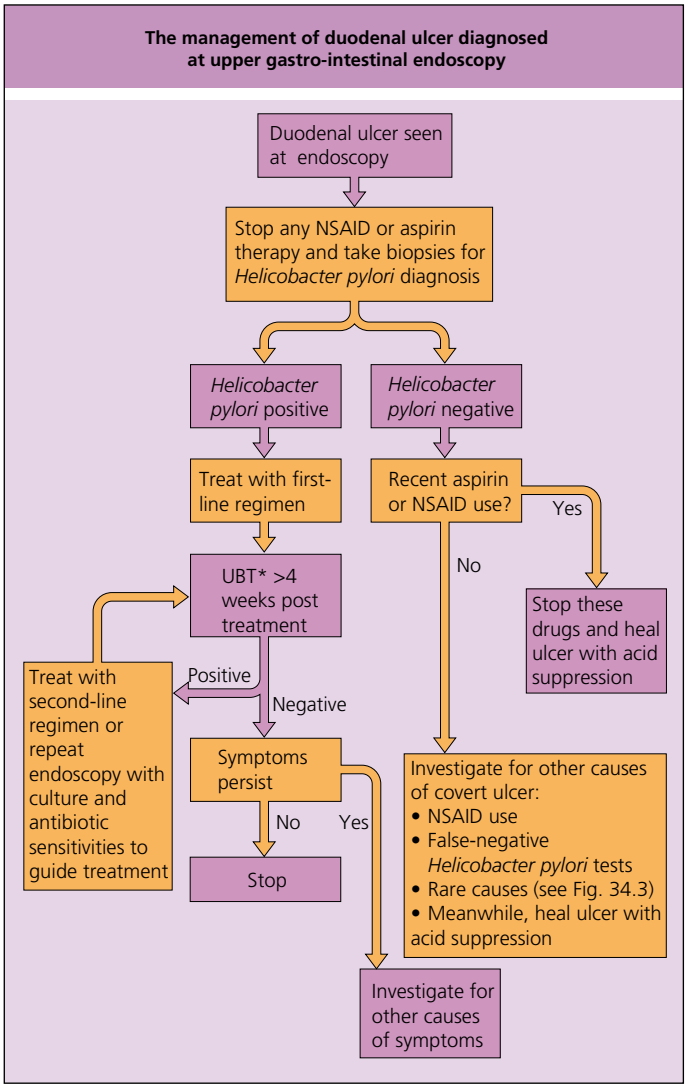


Fig. 33.5 Decision algorithm for the management of duodenal ulcer disease diagnosed at upper gastrointestinal endoscopy. UBT, urea breath test.

Helicobacter pylori treatment regimens

First-line treatment is with 'low-dose triple therapy' consisting of either a proton pump inhibitor, clarithromycin and metronidazole, or a proton pump inhibitor, clarithromycin and amoxicillin (Table 33.3). Although 1 week is usually recommended, meta-analyses show that 2 weeks of treatment is slightly more effective; whether it is cost-effective and worth the increased side-effects remains controversial.[6] Knowledge of local antibiotic resistance patterns is helpful in choosing optimal *H. pylori* treatment as regimens are less effective if *H. pylori* has *in-vitro* resistance to a component antibiotic. Metronidazole resistance is a partial phenomenon; metronidazole-containing regimens retain some efficacy. Metronidazole resistance in *H. pylori* is common in:

- developing countries;
- in many ethnic populations within developed countries; and
- in individuals who have previously taken metronidazole for concurrent illnesses, even in the distant past.

Table 33.3 *Helicobacter pylori* treatment regimens

First-line treatments	
Regimen 1	
Omeprazole 20 mg q12h*	7–14 days
Clarithromycin 500 mg q12h†	
Metronidazole q12h	
Regimen 2	
Omeprazole 20 mg q12h*	7–14 days
Clarithromycin 500 mg q12h	
Amoxicillin 1g q12h	
Second-line treatments‡	
Regimen 1	
Omeprazole 20 mg q12h*	14 days
Bismuth subcitrate§ 120 mg q6h	
Tetracycline HCl 500 mg q6h	
Metronidazole 400 mg q8h	
Regimen 2	
Omeprazole 20 mg q12h*	10 days
Levofloxacin 250 mg q12h	
Amoxicillin 1 g q12h	

*Other proton pump inhibitors are equally effective or, in regimens 1 and 2, ranitidine bismuth citrate 400 mg.
†Several studies show 250 mg to be as effective as 500 mg in this regimen.
‡Most physicians in North America and Northern Europe prefer the more complicated first of these second-line treatments. It is thought less likely to increase risk of serious *Clostridium difficile* infection. Also resistance to quinolones occurs easily in *H. pylori*
§Bismuth subsalicylate may be used instead of bismuth subcitrate.

Clarithromycin resistance is potentially more serious as it renders clarithromycin completely ineffective. It is relatively uncommon but increasing in many countries as the antibiotic becomes more commonly used for respiratory tract infections. Amoxicillin resistance is very rare.

Recently there has been interest in 'sequential' eradication therapy where a proton pump inhibitor is given with amoxicillin for 5 days before standard low-dose triple therapy for a further 5 days. Most studies show increased eradication success with these sequential regimens, but further large scale studies are needed before this becomes standard practice.[31]

For any *H. pylori* treatment regimen, patients should be warned of the importance of compliance and of potential side-effects. Providing written instructions may be the most effective strategy. Close compliance is important as failure of complete compliance is, after antibiotic resistance, the most common cause of treatment failure. Treatment failure is serious because unsuccessful treatment often induces antibiotic resistance in the colonizing strain, making further treatment more difficult.

If first-line treatment fails, several second-line treatments are available (Table 33.3). If second-line treatment fails, the usual approach is to use a third-line treatment, most usually the other second-line treatment given in the table. However, an alternative third-line strategy (which we prefer) is to perform endoscopy for *H. pylori* culture and sensitivity testing and to give appropriate therapy guided by known antibiotic sensitivities.

REFERENCES

References for this chapter can be found online at http://www.expertconsult.com

Chapter 34

Christopher P Conlon

Food-borne diarrheal illness

INTRODUCTION

Food-borne illness is defined by the World Health Organization (WHO) as a disease, either infectious or toxic in nature, caused by agents that enter the body through the ingestion of food; however, a more symptom-based case definition may be more useful.[1] Every person on the planet is at risk from food-borne illness but the risk varies geographically and also by age. Most food-borne illness results in vomiting and/or diarrhea and is commonly called food poisoning. In developed countries each adult consumes annually at least 500 liters of water and about 450 kg of meat and vegetables, along with other foodstuffs and liquids. Food production and distribution have become extremely complex in developed countries. It is now commonplace for food to be consumed long distances from its source and, often, a long time after its production. There is also an increasing tendency to eat food away from the home prepared by others. In the developing world consumption is less and food is usually produced and consumed locally. However, the lack of refrigeration and the problems of access to potable water create their own risks. This chapter will consider the causes of food-related diarrhea, its investigation and management.

EPIDEMIOLOGY

Food poisoning may be sporadic or may involve outbreaks; the latter are more common in developed countries because of the centralization of food production and the distribution of food over large distances. In the United States it is estimated that there over 76 million cases of food-borne illness per year, with as many as 300 000 hospitalizations and 3000 deaths. Laboratory-confirmed cases of food-related diarrhea in the United States occur with an incidence of 26 000 per 100 000 population and a mortality of 1.7/100 000.[2] In Europe, figures are 3400/100 000 in the UK and 1210/100 000 in France.[3] The French mortality rate is 0.9/100 000. The UK Food Standard Agency figures for the year 2000 showed 81 280 cases of culture-proven food-borne diarrhea, of which 62 209 were acquired in the UK and the rest abroad. Although responsible for much morbidity, the mortality from food-borne diarrhea is low in developed countries. The groups at the highest risk of death are the extremes of age, particularly the elderly. Studies in the United States suggest that *Salmonella* infections are most likely to lead to death compared with other causes of food poisoning.[4]

In developed countries, although most cases of food-related diarrhea occur sporadically, outbreaks from a point source are common and can affect many thousands of people. Ice cream contaminated with *Salmonella* affected 224 000 people in the United States in 1994.[5] *Salmonella* was also responsible for 19 deaths in the Stanley Royd Hospital outbreak in the UK in 1984 and the subsequent finding of the risk of infected eggs led to changes in legislation relating to food.[6] Although *Campylobacter* is now the most common cause of food-borne diarrhea, *Salmonella* is more likely to be the cause of outbreaks. This may be because *Campylobacter* does not multiply well in food. Increasingly, outbreaks are related to illness in food handlers and, particularly in the case of norovirus, there may be person-to-person spread from an initial food-poisoning episode.[7]

Figures from developing countries are much more difficult to ascertain as there are rarely any reporting regulations and no routine surveillance of food-borne disease.[8] The lack of data, compounded by increasing antibiotic resistance in bacteria, can make disease control very difficult in these settings. Estimates put the annual number of deaths from diarrhea (most of which is due to food-borne illness) at 2.5 million in the tropics. Most of the burden falls on children with about 3.2 episodes of diarrhea per child per year.[9] Diarrheal illness accounts for 21% of all deaths in children under 5 years old in developing countries.

PATHOGENESIS

Pathogens causing food-borne diarrhea include bacteria, viruses and protozoa (Table 34.1). In order to cause disease, they must overcome the natural defenses of gastric acidity, intestinal mucus, normal intestinal bacterial flora, intestinal motility and the immune system. Some organisms are more pathogenic than others and this is reflected in the inoculum needed to cause disease (Table 34.2). Diarrhea due to these organisms results from two common mechanisms:

- there may be excessive secretion of fluid and electrolytes by the small bowel, usually caused by enterotoxins, that overwhelms the absorptive capacity of the large bowel and leads to watery diarrhea; or
- there may be inflammatory damage to the intestinal mucosa, typically occurring in the distal small bowel or the colon, and the inflammatory process causes diarrhea, often with blood in the stools.

Bacterial causes of diarrhea

One factor that is important in the survival of pathogenic bacteria in the gut is achlorhydria.[10] The widespread use of proton pump inhibitors (PPI) and, to a lesser extent, H_2-antagonists has rendered many people more prone to food-borne illness. In general, once bacteria have successfully reached the small bowel they usually have to adhere to intestinal mucosa and elaborate toxins or they need to invade the mucosa.

Table 34.1 Characteristics of selected food-borne illnesses associated with diarrhea (arranged by incubation period)

Organism	Incubation period in hours median (range)	Vomiting	Diarrhea	Fever	Other symptoms	Common vehicles
Histamine fish poisoning (scombroid)	5 min to 1 hour	+	+++	–	Headache, flushing, urticaria	Tuna, mackerel, bonito, mahi-mahi, bluefish
Staphylococcus aureus	3 (1–6)	+++	++	–		Ham, poultry, cream-filled pastries, potato and egg salad
Bacillus cereus (emetic syndrome)	2 (1–6)	+++	+	–		Fried rice
Ciguatera	2 (1–6)	+	++	–	Paresthesias, myalgias, headache, arthralgia	Barracuda, snapper, grouper, amberjack
Bacillus cereus (diarrheal syndrome)	9 (6–16)	+	+++	–	Abdominal cramps	Beef, pork, chicken
Clostridium perfringens	12 (6–24)	+	+++	–	Abdominal cramps	Beef, poultry, gravy
Vibrio cholerae non-O1	11 (5–96)	+	+++	+++	Abdominal cramps, bloody diarrhea (25%)	Fish, shellfish
Vibrio parahaemolyticus	15 (4–96)	++	+++	++	Abdominal cramps, headache, bloody diarrhea (rare)	Fish, shellfish
Norwalk virus	24 (12–48)	+++	+++	++	Headache, myalgias	Water, ice, shellfish, salads
Shigella spp.	24 (7–168)	+	+++	+++	Abdominal cramps, bloody diarrhea	Lettuce, street food
Enterotoxigenic *Escherichia coli*	36 (16–72)	+	+++	+	Abdominal cramps, headache, myalgias	Ice, water, produce
Vibrio cholerae O1	48 (6–120)	++	+++	+	Dehydration	Shellfish
Salmonella spp.	36 (12–72)	+	+++	++	Abdominal cramps, headache, myalgias	Beef, poultry, pork, eggs, dairy products, vegetables, fruit
Campylobacter jejuni	48 (24–168)	+	+++	+++	Abdominal cramps, bloody diarrhea, myalgias	Poultry, milk
Clostridium botulinum	18 (6–240)	++	++	–	Dysarthria, diplopia, dry mouth, paralysis	Canned food, fermented seafood, garlic under oil, dried salted fish

–, rare symptom (<10%); +, infrequent symptom (11–33%); ++, frequent symptom (33–66%); +++, classic symptom.

Table 34.2 Doses of viable organisms responsible for producing diarrheal disease (ID_{25})

Enteropathogen	ID_{25}
Shigella spp.	10–100
Giardia and *Cryptosporidium parvum*	30–100
Shiga toxin *Escherichia coli* O157:H7	10–100
Norwalk-like virus	100
Salmonella	10^3–10^5
Campylobacter	10^3–10^6
Vibrio cholerae	10^6
Enterotoxigenic *Escherichia coli*	10^8

Adherence

In order to produce disease, bacteria need to attach themselves to mucosal epithelial cells and colonize the mucosa. Bacteria have developed a variety of mechanisms for adherence and such adhesins are often referred to as colonization factor antigens (CFA) which are usually encoded by genes on plasmids and, thus, transferable to other pathogens.[11] Details of the mechanisms by which bacteria adhere to enterocytes have been worked out in most detail for various types of *Escherichia coli* but *Salmonella* and *Shigella* strains use similar mechanisms.[12]

Enterotoxins

The elaboration of enterotoxins by bacteria accounts for most cases of secretory, or watery, diarrhea. The classic example of this mechanism is the cholera toxin, the details of which were clarified some years ago.[13] This toxin has five B subunits that bind to the enterocyte, allowing the active A1 subunit to enter the cell and activate adenylate cyclase and increase cyclic AMP (Fig. 34.1). The increase in cAMP results in increased secretion of chloride ions by crypt cells and also inhibition of absorption of coupled chloride and sodium ions. The net result is excessive secretion of fluid and electrolytes into the lumen of the bowel and an inability of the bowel to reabsorb this excess. Goblet cells are probably also affected by the cholera toxin as there is excessive mucus secretion as well. The mucus is responsible for the 'rice water' appearance of the stool.

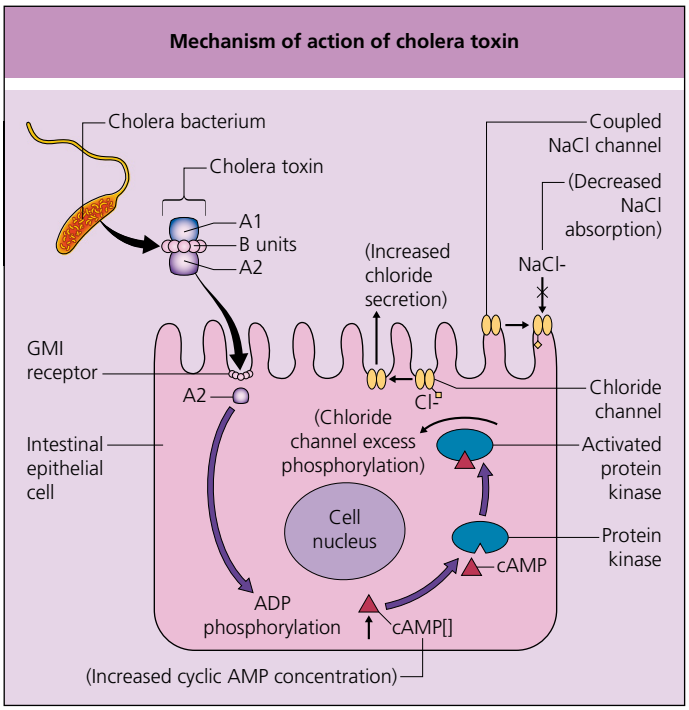


Fig. 34.1 Mechanism of action of cholera toxin. A similar mechanism occurs with enterotoxigenic *Escherichia coli* (ETEC) heat-labile (LT) toxin.

Enterotoxigenic *E. coli* (ETEC) produces a toxin that is heat-labile, often referred to as LT.[14] It is very similar to cholera toxin in both structure and mode of action. Other bacteria, such as *Salmonella* and *Campylobacter jejunii*, can also produce LTs but these are rarely the sole factor in the pathogenesis of these two organisms. ETEC also produces a heat-stable toxin (ST) which leads to an increase in cAMP in the enterocyte and also results in net secretion into the gut lumen. Similar toxins may be produced by *Yersinia*, *Citrobacter* and some non-O1 strains of *Vibrio cholerae.*

Cytotoxins

Whereas true enterotoxins act by stimulating secretions, enteric cytotoxins produce inflammation in the bowel mucosa, usually through effects on protein synthesis. Cytotoxins primarily act on the distal small bowel and colon, producing an inflammatory colitis that can mimic inflammatory bowel disease. The classic cytotoxin is the Shiga toxin that is produced by *Shigella dysenteriae*.[15] This toxin has a B subunit that binds to cells and an active A subunit that disrupts protein synthesis in the 60S ribosome. Vascular endothelium may be the target, explaining the capillary lesions seen in the mucosa.

Enterohemorrhagic *E. coli* (EHEC), such as O157:H7, causes disease by cytotoxin production. This toxin is closely related to Shiga toxin and is known as Shiga-like toxin or verotoxin. Its mechanism of action is similar to Shiga toxin and often the disease is indistinguishable from dysentery.[16] It is now recognized that other serotypes of EHEC, in addition to O157, cause a similar illness and can also result in hemolytic–uremic syndrome (HUS).[17] Other bacteria, such as *Clostridium perfringens* and *Salmonella* strains, also produce cytotoxins that are similar to verotoxin.

Invasiveness

The classic bacteria that invade the mucosa of the intestine are *Shigella dysenteriae* and enteroinvasive *E. coli* (EIEC). There is local invasion and destruction of mucosal cells and the resulting inflammation leads to dysentery. The invasiveness relates to adhesion and invasion proteins that are probably carried on plasmids.[18]

Salmonella typhi and *S. paratyphi* also invade, but usually through Peyer's patches. Although there is local inflammation, most of the disease caused by these organisms relates to bacteremia following invasion. Occasionally, nontyphoidal *Salmonella*, *C. jejunii* and *Yersinia* spp. do the same and bacteremia is then a complication of the initial diarrheal illness.

Neurotoxins

Some bacteria involved in food poisoning elaborate toxins that work primarily on the emetic center in the central nervous system and on the autonomic nerves. As these toxins are ingested preformed, symptoms develop quickly. Here, the primary symptom is vomiting although diarrhea is also common. Food poisoning by *Staphylococcus aureus* is mediated by this mechanism so that vomiting occurs soon after ingestion of the preformed toxin in the food.[19]

Botulinum toxin is also ingested preformed and although the primary symptoms are neurologic, diarrhea and vomiting also occur. This toxin affects neuromuscular transmission, inhibiting the release of acetylcholine at peripheral synapses.

Other organisms and pathogenic mechanisms

Viruses that cause diarrhea, such as rotavirus, probably cause disruption of the tips of the villi.[20] Animal studies show that the virus replicates in mature enterocytes near the villus tip. Disruption of the villus may lead to some malabsorption of sugars and proteins that can be osmotically active and lead to diarrhea. In addition, a viral nonstructural protein, NSP4, may have toxin-like activity. NSP4 has been shown to stimulate crypt cells to increase secretion and lead to diarrhea in mice.

Protozoa infections may cause diarrhea by similar mechanisms. *Giardia lamblia* attaches to enterocytes on the villi by a ventral disk and may disrupt the brush border enzymes, leading to malabsorption. In addition, crypt cells may increase in number in response to infection, leading to excess secretion.[21] Chronic giardiasis leads to villus blunting, reducing the surface area available for absorption. *Entamoeba histolytica* may cause disease by the cytopathic effect of secreted cysteine proteinases that damage enterocytes and cause inflammation.[22]

PREVENTION

Sanitation

The most important factor in reducing food-borne infections is the provision of safe water for washing and drinking, and adequate sanitation arrangements for the safe disposal of human waste. Modern water supplies and sewage systems have dramatically cut the incidence of diarrheal diseases in developed countries, a point recognized two centuries ago by John Snow in London. Unfortunately, the lack of such amenities in developing countries leads to the consumption of contaminated food and water and explains the high prevalence of diarrheal disease, particularly in children in the tropics.

Food hygiene

Education about and knowledge of the safe handling of food is another critical factor in reducing food-borne diseases. Ensuring that potentially infected raw foods, such as poultry, do not contaminate other foods or work surfaces is fundamental, as is the appropriate cooking temperatures and times to ensure potential pathogens are killed.[23] Adequate refrigeration is also important so that foodstuffs are kept safely. These basic domestic safety issues need to be reinforced in restaurants and other commercial food preparation outlets by national food regulations.[24] In the UK, the Food Safety Act compels organizations to give due regard to food safety. Regulation has to be coupled with proper surveillance and public health involvement to ensure compliance, and to be alert to problems related to food safety.

Vaccines

The majority of food-borne illnesses are not preventable by current vaccines. Early cholera vaccines were not very effective but new, recombinant vaccines can be protective.[25] However, they are rarely available in the parts of the world where cholera is endemic. Typhoid vaccines, both recombinant oral and parenteral polysaccharide varieties, offer protection for travelers but, again, are rarely used in endemic countries.

The relatively new rotavirus vaccine may well have significant impact on the incidence of diarrhea in children in developed countries but will be more difficult to roll out to developing countries where the need is greatest.[26] More recently a promising vaccine that uses LT from *E. coli* in the form of a transdermal patch was shown to provide protection from travelers' diarrhea.[27]

There is some evidence that antibiotics can be used to prevent travelers' diarrhea but generally the risks outweigh the benefits. It may be reasonable to use such prophylaxis in high-risk travelers such as the immunocompromised or those with inflammatory bowel disease. A recent study of the use of a nonabsorbable agent, rifaximin, showed promise but, again, the risks and benefits are not clear cut.[28,29]

CLINICAL FEATURES

Food-borne diarrhea can present in a number of ways and often the timing of onset and the accompanying symptoms might help to focus on a particular likely pathogen. Pathogens that primarily affect the small bowel usually cause watery diarrhea, whereas those that lead to inflammation of the colon cause diarrhea that often contains white and red cells or can lead to frank dysentery. One of the common features in food-related diarrhea is dehydration which may be severe enough to warrant hospitalization for fluid replacement.

Early onset vomiting and diarrhea

When vomiting and diarrhea occur within a few hours of a meal, the likely cause is the ingestion of preformed toxins from either *Staph. aureus* or *Bacillus cereus*. Vomiting is usually the predominant symptom and can lead to rapid dehydration in severe cases. The duration of symptoms is usually less than 24 hours.

Very similar symptoms, with predominant vomiting, occur with viral infections such as norovirus. Because the vomitus and stool contain large numbers of virus particles, secondary cases are common from aerosol spread or from fomites. Thus, outbreaks of vomiting and diarrhea with these small round-structured viruses are common.[30] Particular problems arise in hospital wards, nursing homes and, sometimes, on cruise ships.[31] The symptoms usually abate after 2–3 days.

Diarrhea within 24 hours of eating

Ingestion of food containing *Clostridium perfringens* or *B. cereus* leads to diarrhea after about 8–16 hours. This is the time it takes for the multiplying bacteria to generate toxins *in vivo*. The diarrhea is usually watery and is often accompanied by abdominal cramps. Vomiting is not a major feature, unlike some *B. cereus* infections when toxin has already formed in the food before ingestion.

Diarrhea 1–2 days after eating

A large number of cases of food poisoning result in symptoms 24–48 hours after eating the infected food. *Campylobacter jejunii* is the most common cause identified in sporadic cases, whereas *Salmonella* species, such as *S. enteritidis*, are more common in outbreaks.[32] More recently recognized as causes of diarrhea are *Listeria monocytogenes, Aeromonas* spp. and *Plesiomonas shigelloides*.[33–35] Most cases have diarrhea which can be watery and is often associated with abdominal cramps. However, more severe cases of *C. jejunii and S. enteritidis* may lead to more inflammatory disease. *Yersinia enterocolitica* and invasive *E. coli* infections may cause similar symptoms. Vomiting occurs in less than 30% of cases but fever is common. Abdominal pain can be severe and bloody diarrhea may occur so that the differential diagnosis of inflammatory bowel syndrome needs to be entertained.

Bloody diarrhea several days after eating

Unlike the syndrome described above, infections with EHEC, such as *E. coli* O157:H7, may take a few days to evolve. These cases are characterized by bloody diarrhea where there is little or no fever. These infections carry the risk of the development of hemolytic–uremic syndrome. Most cases are sporadic but large outbreaks have been recorded with significant morbidity and mortality, including one linked to hamburger meat, a continuing risk with ground beef.[36,37]

Diarrhea with neurologic symptoms

Although rare, cases of botulism continue to occur, often associated with home-canning of fruit and vegetables.[38] Botulinum toxin in the contaminated food is predominantly a neurotoxin leading to paralysis but in the early stages vomiting and diarrhea occur, probably as a result of effects of the toxin on the autonomic nervous system. Symptoms usually start 18–36 hours after ingestion of the toxin. The delayed onset of the neurologic symptoms differentiates botulism from some of the syndromes associated with the ingestion of seafood (see below).

Noninfective food poisoning

There are a variety of circumstances where ingestion of certain foods leads to vomiting and/or diarrhea due to toxins in the food that do not originate from microbial pathogens. Most commonly these syndromes relate to the ingestion of seafood or mushrooms but it should be remembered that poisoning with heavy metals, such as cadmium, can cause severe gastric irritation and produce vomiting and abdominal pain within an hour or so of ingestion.

Scombroid poisoning

The ingestion of certain oily fish, such as tuna and mackerel (Fig. 34.2) is associated with a syndrome of flushing, vomiting and diarrhea within an hour of ingestion.[39] The symptoms relate to excess histamine ingestion. Poorly cleaned or stored fish contain bacteria that cause the breakdown of fish muscle and the build-up of histamine. The histamine is not affected by cooking the fish and if enough is ingested the symptoms come on soon after consuming the contaminated fish.

Paralytic shellfish poisoning

A variety of neurologic symptoms can result from eating contaminated shellfish. Dinoflagellates (algae) that are fed on by various shellfish produce saxitoxins that are harmless to the shellfish but are neurotoxic if ingested by humans. The symptoms come on within an hour of ingestion and range from partial paralysis to amnesia. Although usually short lived, some of the neurologic impairment can, occasionally, be permanent. Diarrhea and vomiting can occur early on but are often absent.



Fig. 34.2 Mackerel and other oily fish may lead to scombroid poisoning, a noninfective cause of food-related diarrhea.

Ciguatoxin poisoning

Some tropical fish, such as barracuda, may contain a toxin, cigautoxin, which can lead to paraesthesia a few hours after ingestion. This toxin probably derives from dinoflagellates in the food chain and is heat stable; hence can be present in adequately cooked fish, even if previously frozen.[40] Early symptoms include abdominal cramps and diarrhea, with circumoral paraesthesia. Neurologic symptoms often take a few hours to develop and may remain for some days.

Mushroom poisoning

A variety of mushroom species may lead to illness if ingested by humans. Many of the more serious symptoms are neurologic, but nausea, abdominal pain and diarrhea often occur early on.[41] The most serious type of mushroom poisoning is that caused by *Amanita* spp. (e.g. *Amanita phalloides*). With these species diarrhea and abdominal cramps occur within a few hours; hepatorenal failure may occur a day or two later with significant risk of mortality.

SPECIAL CLINICAL SYNDROMES

Travelers' diarrhea

The most common infectious problem to befall travelers is gastroenteritis, particularly when travel is to less developed countries. Estimates are that attack rates of up to 50% occur in travelers and that as many as 11 million cases occur annually worldwide.[42] Although any of the pathogens described above may be the cause, the most common cause worldwide is ETEC.[43] This organism produces an enterotoxin which is structurally and immunologically related to cholera toxin. The result of infection is secretory, or watery, diarrhea. *Campylobacter jejunii* and *Salmonella* are responsible for a significant proportion of cases, as is norovirus in some settings.[44]

Risk factors include travel from a high income country to a less developed one, travel in the summer, staying in self-arranged accommodation, eating shellfish and gastric achlorhydria (usually resulting from the use of PPI drugs).[45] The highest risk regions of the world are South Asia, South East Asia, Central and South America and the poorer countries of sub-Saharan Africa.

Symptoms of travelers' diarrhea commonly start within 2–3 days of arrival and almost all cases occur within the first 2 weeks. In the majority of patients symptoms are short lived, lasting 3–5 days, but up to 40% may have to alter their itinerary due to diarrhea and around 1% require hospital treatment. Depending on the pathogen, most cases have watery diarrhea but some will develop dysentery with systemic features, such as fever. A small number of people may go on to have chronic diarrhea, some of which may be due to giardiasis. There is also a small risk (<10%) of irritable bowel syndrome developing after travelers' diarrhea.[46]

Diarrhea in those infected with HIV

The advent of highly active antiretroviral therapy (HAART) has dramatically altered the risk of gastrointestinal infections associated with HIV infection. In the pre-HAART era infectious diarrhea was a common problem, often with unusual pathogens.[47] Most of the disease occurs in those with CD4 lymphocyte counts <50 cells/mm^3. *Cryptosporidium parvum* was a particular problem, frequently causing cholera-like diarrhea which became chronic and debilitating. In some cases, this was associated with cryptosporidial cholecystitis and a sclerosing cholangitis clinical picture. Although less of a problem now in developed countries, in parts of the world where HIV is a major problem and where HAART is not readily available, *Cryptosporidium* remains a major problem.[48] Other protozoal parasites causing diarrhea, but usually less severe than *C. parvum*, are *Isospora belli*, *Cyclospora cayetanensis* and microsporidia species.[49]

Again, more of an issue in the pre-HAART era is infection with *Mycobacterium avium-intracellulare* (MAI). This usually harmless mycobacterium is often ingested with water and sometimes food, and multiplies in Peyer's patches before becoming disseminated. Although the dissemination and mycobacteremia cause the most symptoms, diarrhea is a frequent clinical feature as the intestinal mucosa becomes densely infiltrated with the organism. Patients may also develop cytomegalovirus (CMV) colitis, characterized by fever, bloody diarrhea and abdominal pain, but this is not food borne. Sigmoidoscopy reveals mucosal ulceration and typical CMV inclusions are found in histologic sections.

DIAGNOSIS

Clinical features

Although the symptoms of diarrheal illness are usually obvious, the history needs to be clarified to ensure that there is increased stool frequency. Diarrhea is probably best defined as three or more bowel movements in 24 hours in which the stool is loose and takes the shape of its container. Diarrhea that is watery is likely to be a result of infections affecting the small bowel and is more likely with ETEC, *Cl. perfringens* or viral infections. Bloody diarrhea, often with abdominal pain, usually reflects large bowel infection and inflammation and can be due to *C. jejunii*, *S. enteritidis*, EIEC, etc. However, none of these clinical variations is specific.

Radiology

Radiographs are not useful in clarifying the etiology of gastrointestinal infections but may identify those that are severe and should be done in patients with bloody diarrhea or marked systemic symptoms. Infectious diarrhea that inflames the colon can lead to toxic megacolon that can mimic severe inflammatory bowel disease and this will be identified with a plain abdominal radiograph. CT scanning may also show severe inflammatory changes.

Endoscopy

Upper gastrointestinal endoscopy is not useful in food-borne infections but flexible sigmoidoscopy can be helpful in cases admitted to hospital, particularly if bloody diarrhea is a symptom. Some infectious cases may be indistinguishable from inflammatory bowel disease on endoscopy, although histology may be helpful. Cases of dysentery due to amebiasis often show discrete ulcers separated by areas of normal mucosa (Fig. 34.3). Biopsies from the ulcers will often reveal trophozoites in the tissue.

Nonspecific laboratory tests

In cases of diarrhea due to food poisoning there might be nonspecific indicators of inflammation, such as a raised white cell count in the peripheral blood or an elevated serum C-reactive protein (CRP). With severe dysentery, the hemoglobin may drop and with more chronic diarrhea the serum albumin will fall, partly as a result of protein loss in the stool.

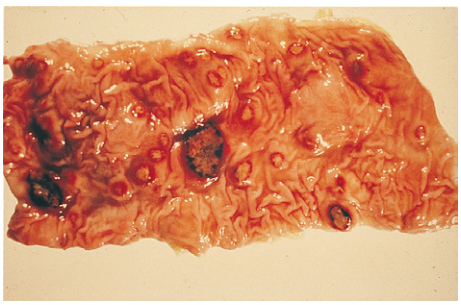


Fig. 34.3 Post-mortem specimen of colon showing discrete, 'flask-like' ulcers caused by amebic dysentery.

Stool microscopy and culture

Microscopy of stool samples in the investigation of infectious diarrhea is rarely diagnostic. The finding of white and red cells points to colonic involvement but their absence does not preclude a colonic source. Because the detection of fecal leukocytes can be helpful but often time-consuming, some studies have addressed the utility of testing stool for the presence of lactoferrin, a marker of leukocytes.[50,51] The assays are rapid and less labor intensive than microscopy but relatively expensive. However, there have been mixed reports concerning the usefulness of measuring lactoferrin and it is not commonly used. The main utility of stool microscopy is the identification of ova, cysts and parasites. *Giardia* trophozoites are rarely seen but cysts may be present. Although the ova of *Entamoeba* may be seen, some species, such as *Entamoeba dispar*, are nonpathogenic but indistinguishable from the pathogenic *E. histolytica*. The finding of *Entamoeba* trophozoites that have ingested red cells is, however, diagnostic of *E. histolytica*.

Stool culture is the mainstay of diagnosis of infectious diarrhea. Although bacteria remain the most common causes of food-borne diarrhea, cultures are positive in less than 40% of cases. Part of the reason for the low positivity rate is that various types of *E. coli* (EIEC, EHEC, ETEC, etc.) are not routinely identified in diagnostic laboratories. In the UK, since 2005, diagnostic laboratories have been required to screen stool specimens from cases of bloody diarrhea for *E. coli* O157:H7. This laborious process involves picking colonies from plates with selective media (MacConkey–sorbitol); this serotype, unlike most *E. coli* in the gut, does not ferment sorbitol. Identification by serologic or molecular tests confirms the diagnosis.

Blood cultures

Most cases of food poisoning lead to diarrhea without much in the way of systemic upset. However, about 1% of cases of nontyphoidal *Salmonella* infections are associated with invasion and bacteremia, particularly in the elderly.[52] It is sensible, therefore, to obtain blood cultures in those patients with diarrhea and fever and those with bloody diarrhea. Blood cultures are also mandatory in returning travelers with diarrhea and fever as enteric fever due to *Salmonella typhi*, although classically associated with constipation, can present with diarrhea.

Nonculture methods for detecting pathogens

These days, very few laboratories culture viruses routinely and although electron microscopy (EM) was used until recently to identify viruses causing food-borne illnesses (Fig. 34.4), molecular methods and antigen detection systems are more commonly used now.

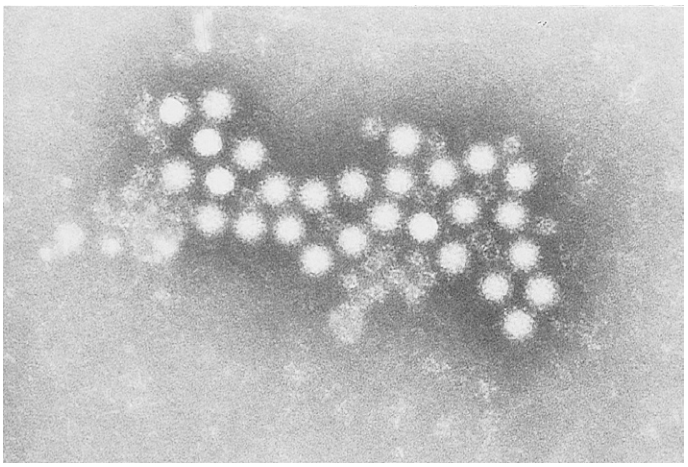


Fig. 34.4 Electron micrograph of norovirus, a common cause of diarrhea.

Because noroviruses are the most common viral causes of food-borne infections, many laboratories use a reverse transcriptase polymerase chain reaction (RT-PCR) assay to identify the virus rapidly.[53] Rotavirus detection is usually by latex agglutination tests or by enzyme immunoassay (EIA).[54]

There are still no readily available molecular methods for identifying bacteria from stool samples in cases of food-borne diarrhea although PCR-based methods are used to type *E. coli* in reference laboratories. Although light microscopy is still the mainstay of diagnosis for parasitic intestinal infections, there are EIA and other rapid antigen detection assays for *G. lamblia* and for *C. parvum*.[55]

MANAGEMENT

Hydration

As the most common problem in diarrheal illness is dehydration, attention must focus on the state of hydration of the patient and the maintenance of the circulation. In many cases rehydration can be safely and adequately achieved with oral fluids. Oral rehydration has been promoted by the WHO for the primary and secondary care of diarrheal illness in the tropics (Table 34.3). The recommended oral rehydration solution (ORS) contains sodium and potassium (as chlorides) to replace losses, glucose to facilitate active transport of sodium and, hence, water across the mucosa, and citrate to correct the acidosis resulting from diarrhea and dehydration.[56] The WHO also recommends zinc supplementation of ORS for children because of the large zinc losses in childhood diarrhea.[57] Studies have shown that, for adults, rice water-based ORS reduces diarrhea volumes and, more recently, the addition of L-histidine to rice ORS brings further improvement.[58]

In some severe cases, and often in hospitals in developed countries, intravenous fluid replacement is used (Fig. 34.5). The emphasis is on volume replacement with crystalloid solutions that provide sufficient sodium, potassium and glucose. However, the dangers of overhydration in the elderly and the risk of nosocomial line-related infections should be borne in mind before embarking on intravenous therapy. The success of fluid replacement should be monitored clinically by assessing blood pressure, urine output, skin turgor and, in some cases, central venous pressure measurement. Laboratory monitoring of renal function and plasma electrolytes should also be carried out.

Nutrition

Malnutrition secondary to diarrhea is a serious problem in the tropics and is responsible for considerable morbidity and mortality. Feeding in these settings is important to ensure both protein and calorie intake is adequate. In developed countries there are few trials of feeding in relation to food-borne diarrhea. Most clinicians recommend clear fluids orally initially, followed by a bland diet with starchy foods, such as rice or potatoes, until the diarrhea settles. Secondary lactose intolerance can be a problem after infections, such as giardiasis, that target the small bowel.

Table 34.3 Composition of oral rehydration solution recommended by WHO

Solute	Grams/liter	Mmol/liter
NaCl	2.6	Na 75; Cl 65
Glucose	13.5	7.5
KCl	1.5	K 20
Trisodium citrate	2.9	Citrate 10
TOTALS	20.5	245 osmolality



Fig. 34.5 Patients on a cholera ward in Peru receiving rehydration intravenously. (Courtesy of Dr J Sanchez.)

Antidiarrheal agents

There is considerable controversy about the use of antimotility agents in patients with food-borne diarrhea. An early study suggested that loperamide may increase the severity of illness in *Shigella* infections.[59] Although this has not been confirmed by other studies, this drug should probably be avoided if shigellosis is suspected or confirmed. Loperamide is the agent of choice, however, for adults with food-borne disease as it not only reduces intestinal motility but can also reduce secretion.[60] It has the advantage over codeine and other opiates in that it is not absorbed and thus is relatively free of adverse effects. Bismuth subsalicylate has also been shown to be effective, even in viral gastroenteritis.[61] Trials have also shown that zaldaride, a calmodulin antagonist, can be effective but this has not found widespread use, probably because of cost and limited availability.[62]

Antibiotics

The vast majority of food-borne infections causing diarrhea can be treated symptomatically and antimicrobial agents are not required. However, there are some clinical scenarios where antimicrobials should be used or, at least, should be considered. Sometimes this is because a specific diagnosis has been made and the pathogen isolated is best eliminated with antibiotics; in other situations, there may be justification for empiric antibiotic therapy in an attempt to reduce symptoms and complications.

Specific antibiotic therapy

Randomized, controlled trials have shown that antibiotic therapy will reduce the duration of illness due to *Shigella* species. Quinolones remain effective in many instances but azithromycin is an alternative.[63] Infections with *C. jejunii* can also be affected favorably; however, in this infection, the duration of illness will only be shortened if treatment is given within 4 days of the symptoms starting.[64] Because of the dramatic increase in quinolone resistance in *Campylobacter*, erythromycin is the drug of choice and has been shown to shorten the duration of excretion.[65]

Most nontyphoidal *Salmonella* infections do not require therapy as treatment may only reduce symptoms by a day or so. However, because of the risk of bacteremia in certain patient groups, treatment is warranted for those at risk. The risk groups include children under 1 year of age, adults over the age of 60 and those who are immunocompromised.

As with *Campylobacter*, quinolone resistance is increasing in *Salmonella* spp., so azithromycin may be a better choice than a quinolone.

Although fluid replacement is the mainstay of treatment, cholera responds to antibiotic therapy and tetracycline and doxycycline are the drugs of choice in adults.[66] In addition to killing the bacteria, these drugs also help to diminish toxin production via their effects on protein synthesis.

The protozoan parasites that cause diarrhea usually require specific treatment although some immunocompetent individuals may clear the infections spontaneously. Giardiasis is best managed by metronidazole or tinidazole. If the diarrhea has been chronic, it may also be helpful to give a cysticide and to recommend a lactose-free diet, as secondary lactose intolerance is not uncommon. Infections with *Cyclospora cayetanensis* and *Isospora belli* can be treated with co-trimoxazole (trimethoprim–sulfamethoxazole). Amebic dysentery responds to metronidazole but, as with giardiasis, it is often wise to give a drug active against the cysts, such as diloxanide furoate. Although *Cryptosporidium* is rarely a serious problem in the immunocompetent,[67] it can cause severe diarrhea in the immunocompromised. Recent studies have shown that nitazoxanide is effective.[68] There have been some studies to suggest that microsporidial diarrhea can be managed with albendazole.[69]

Although Shiga toxin-producing *E. coli* such as O157:H7 may be sensitive to some antibiotics *in vitro*, there are concerns that antibiotic therapy may worsen outcomes and increase the risk of hemolytic–uremic syndrome.[70] As a consequence, guidelines recommend withholding antibiotics if this infection is known or suspected.[71]

Empiric antibiotic therapy

Some clinicians advocate the use of empiric antibiotic therapy in adults with diarrhea and evidence of inflammation. A number of studies have shown benefit but, generally, the duration of diarrhea is only reduced by 1 or 2 days. The potential benefits of empiric therapy must be weighed against the risks which include drug side-effects, prolonging excretion of the organism and infection with *Cl. difficile*. Quinolones are often used as empiric therapy but over the past decade there has been increasing quinolone resistance in enteric pathogens and, more recently, quinolone therapy has been associated with colitis due to *Cl. difficile*.[72]

Travelers' diarrhea

This is one condition for which empiric therapy can still be considered. Studies have shown that a number of antibiotics given empirically can reduce the duration of symptoms. Bismuth subsalicylate is effective but its use is limited by the volumes that need to be given and by the risk of developing a blackened tongue due to the bismuth. Doxycycline has been shown to be effective, as has a single dose of ciprofloxacin.[73] However, the studies showing efficacy of these drugs were done before the more widespread antibiotic resistance of enteric pathogens worldwide was recognized. It may be that single dose azithromycin may be more effective.[74] Some studies have shown that rifaximin, a nonabsorbable rifamycin, may be useful in travelers' diarrhea.[75]

PUBLIC HEALTH MANAGEMENT

All cases of food-borne diarrhea should be reported to the appropriate public health authorities. This can allow suspect food sources to be investigated and might also point to outbreaks, sometimes even when cases are geographically separated. Ideally, stool specimens and food samples should be collected and detailed food histories obtained from affected patients. However, in practice, many instances of food-related diarrhea go unreported and even outbreaks are poorly investigated.[76] Public health issues are particularly important in the era of bioterrorism as the threat to the food supply is an important one and measures need to be in place to prevent attacks but also to recognize outbreaks secondary to malicious intent.[77]

CONCLUSION

Food-borne diarrhea is extremely common and costly, both in human and monetary terms. Better food safety regulations, improved hygiene and sanitation, and proper surveillance all contribute to containing the problem. Increased public understanding of the issues concerned should also help to reduce the incidence of disease in developed countries, whereas continued improvement in access to safe water remains a priority in the tropics.

REFERENCES

References for this chapter can be found online at http://www.expertconsult.com

Chapter 35

Michel Drancourt

Acute diarrhea

INTRODUCTION

There is an increasingly recognized array of bacterial, parasitic and viral organisms associated with infection of the intestinal tract which can profoundly disrupt intestinal function with or without causing acute diarrhea. Acute diarrhea is a syndrome that is frequently not differentiated clinically by specific etiologic agent. The wide spectrum of evolution varies from self-limited disease to death. Death is mainly due to dehydration and children in developing countries pay the highest tribute to acute diarrhea. This chapter examines the viral and bacterial causes of acute diarrhea, clinically defined by the emission of three or more loose or watery stools per day or a definite decrease in consistency and increase in frequency based upon an individual baseline lasting for less than 2 weeks. Parasitic infections of the gastrointestinal tract will be presented in Chapter 109 and diarrhea associated with food poisoning will be presented in Chapter 34. When diarrhea lasts for 14 days it can be considered persistent; the term chronic generally refers to diarrhea that lasts for at least 1 month (see Chapter 36).

EPIDEMIOLOGY

Prevalence

Acute diarrheal diseases ranked seventh in the causes of mortality in the low- and middle-income countries in the global disease burden series, 2001, with an estimated 1.78 million deaths (3.7%).[1] Most of these deaths occur in children under the age of 5 years in developing countries and diarrhea accounted for 15% of all deaths among children younger than age 5 years, as tabulated in 2001.[1] However, diarrheal diseases ranked fourth in the causes of life-years lost to disability and premature death, just after lower respiratory tract infections, HIV-related deaths and unipolar depression.[2] The incidence of acute diarrhea in the general population could be estimated by prospective studies such as those organized in the Foodborne Disease Active Surveillance Network (FoodNet) in the USA. The network observed that 6% of interviewed people reported an acute diarrheal illness during the 4 weeks preceding the interview, i.e. an annualized rate of 0.72 episodes per person-year. Rates of illness were highest among children younger than 5 years (1.1 episodes per person-year) and were lowest in persons aged ≥65 years (0.32 episodes per person-year).[3] A study in 2000 that estimated the economic burden of both infectious and noninfectious gastrointestinal and liver disease in the USA found that the most prevalent diseases were non-food-borne gastroenteritis (135 million cases per year) and food-borne illness (76 million cases per year).[4] A report from the Centers for Disease Control and Prevention (CDC) found that food-borne diseases account for approximately 76 million illnesses, 325 000 hospitalizations and 5000 deaths each year in the USA based upon surveillance data from multiple sources.[5] In the Netherlands, the incidence of gastroenteritis was 45 per 100 person-years in a prospective study.[6]

Surveillance

Not all enteric pathogens are notifiable, depending on the country. Moreover, report may not be timeless and a recent study in six US states indicated that multiple steps between onset of food-borne illness and its investigation by a public health agency could take up to 3 weeks.[7]

Sources of pathogens

Enteric pathogens are mainly food-borne and water-borne pathogens. Gastroenteritis is commonly contagious and may be transmitted by hand or saliva. While acute diarrhea occurs in most cases of food-borne illness, there are other causes of acute diarrhea that would not be captured in this type of survey. Water-borne outbreaks associated with recreational water (e.g. swimming or wading pools) are another source of acute diarrhea. The outbreaks were associated most frequently with *Cryptosporidium* (50%) in treated water sources and with toxigenic *Escherichia coli* (25%) and norovirus (25%) in freshwater sources. Swimming pools have been demonstrated as a source of norovirus gastroenteritis outbreak.[8] Travel is increasingly reported as a circumstance for acute diarrhea including enterotoxigenic *E. coli* infection. Cruising appears to be an emerging circumstance for food-borne pathogen outbreak such as reported for norovirus.[9] *Aeromonas* infections have been traced to aquarium water.[10] As for nosocomial enteritis, patients with actual infectious diarrhea are the main source of infection and health-care workers suffering the same condition are a second source. Contaminated food is a potential source of food-borne *Salmonella enteritidis* outbreak and a contaminated inanimate hospital environment is a source for *Clostridium difficile* nosocomial acute enteritis.

PATHOGENESIS AND PATHOLOGY

Diarrhea reflects an increased water content of the stool, whether due to impaired water absorption or active water secretion by the bowel. In severe infectious diarrhea, the daily volume of stool may exceed 2 liters. Dehydration and loss of potassium (hypokalemia) are two life-threatening consequences of severe diarrhea. Water is mainly absorbed in small bowel (for about 8 liters a day in an adult) and further in the colon. By the time the initial 8 liters of fluid reaches the ileocecal valve, only about 600 ml remain, representing an efficiency

of water absorption of 93%. By the time the remaining 600 ml of fluid reaches the anus, only about 100 ml of fluid remains, generally as formed feces. In the small intestine, water is absorbed by three basic mechanisms:

- 'neutral' sodium chloride (NaCl) absorption mediated by two coupled systems – one exchanges Na/H (cation exchanger) and the other exchanges Cl/HCO_3 (anion exchanger);
- 'electrogenic' sodium absorption where sodium enters the cell via an electrochemical gradient (this electrogenic sodium absorption mechanism is commonly damaged during acute enteric infection); and
- sodium co-transport.

Sodium absorption is coupled to the absorption of organic solutes such as glucose, many amino acids, and peptides. This co-transport mechanism remains intact during most acute diarrheal disorders. It is for this reason that oral rehydration is possible during acute diarrheal illness.

Osmotic diarrhea occurs when an absorbable solute, such as lactose, is not absorbed properly and retains water in the gut lumen. Infections that damage the intestinal epithelial cells either directly (rotavirus) or by a toxin (*Shigella* spp., *C. difficile*) can cause malabsorption and osmotic diarrhea. Secretory diarrhea results from an active, toxin-mediated secretion of water into the gut lumen. This is observed during cholera and infection by Shiga-toxin-producing *E. coli* and *Shigella* spp. Rotavirus also produces a viral enterotoxin, the nonstructural glycoprotein (NSP4).

Lastly, diarrhea can result from infection-mediated intestinal inflammation. After ingestion, an enteric organism colonizes the intestinal epithelium by adhering to an enterocyte. One of two pathways are generally followed depending upon the offending organism, either mucosal invasion or production of an enterotoxin.

ETIOLOGIES

As some enteric pathogens, such as *Vibrio cholerae*, are not ubiquitous, some pathogens are seasonal and some pathogens are responsible for epidemics, the prevalence of various pathogens responsible for diarrhea is variable. As for bacteria, the pathogens most frequently found are enteropathogenic clones of *E. coli*, *Shigella* spp., *Salmonella enterica* subsp., *Campylobacter* spp. and *Aeromonas* spp. As for viruses, the most frequent causes (outside local epidemics) include rotavirus, calicivirus (norovirus and saporovirus), astrovirus and enteric adenovirus. Less prevalent viruses include paramyxovirus, morbillivirus, rubivirus and reovirus. The prevalence of norovirus has recently been estimated to be 12% in children younger than 5 years hospitalized for severe diarrhea and 12% of mild and moderate diarrhea cases among patients of all ages.[11] These authors estimated that norovirus organisms were responsible for up to 200 000 deaths of children less than 5 years of age in developing countries.[11]

Nosocomial enteritis is due to *C. difficile*,[12] rotavirus and norovirus.[13] *Salmonella* enteritidis,[14] other Enterobacteriaceae pathogens and methicillin-resistant *Staphylococcus aureus*[15] are rare causes of nosocomial enteritis. In Canada, *C. difficile* enteritis has an incidence of 65 per 100 000 patient-days and a total attributable mortality of 5.7%.[12] The same incidence figure has been found in children with a median age of 4 years.[16]

CLINICAL FEATURES

Bacterial enteritis

Aeromonas infection

Aeromonas spp. are inhabitants of aquatic environments worldwide, including rivers and lakes as well as drinking water in plants and distribution systems. Also, most pathogenic *Aeromonas* spp. can be found in meat and dairy products. Some *Aeromonas* isolates encode enterotoxins, including an *alt* gene-encoded heat-labile and an *ast* gene-encoded heat-stable enterotoxin. *Aeromonas* enteric infection may range from, most commonly, an acute watery diarrhea to dysenteric illness. Symptoms may include abdominal cramps (70%), nausea (40%), vomiting (40%) and fever (40%). Infection is usually self-limiting and children may be rarely hospitalized because of dehydration. *Aeromonas caviae* is the most prevalent species. *Aeromonas veronii* can be associated with rare cholera-like illness and dysenteric diarrhea resembling shigellosis with bloody and purulent stools. One-tenth of patients are co-infected with a second enteric pathogen. Intermittent and persistent diarrhea may occur for years after initial infection. *Aeromonas* enteritis could be complicated by the hemolytic–uremic syndrome and kidney disease.[17]

Campylobacter infection

Campylobacter spp. comprise motile, Gram-negative, S-shaped, microaerophilic organisms responsible for zoonoses. Not only food animals such as poultry, cattle, sheep and pigs, but also domestic pets are reservoirs for worldwide human infections. Despite the incidence decreasing in the USA, *Campylobacter* spp. are still responsible for sporadic infections following improper manipulation of poorly cooked meat. Poultry is a major source of infection.[18] Unpasteurized dairy products and water have been found to be sources of limited outbreaks. The incidence of *Campylobacter* spp. infection is higher in developing countries than in developed ones and travelers to developing countries are at risk of *Campylobacter* infection.

The pathogenesis of *Campylobacter* infections is poorly understood. *Campylobacter jejunii* and *Campylobacter coli* are the most frequently encountered species responsible for diarrhea. Signs and symptoms vary from asymptomatic infections and may include fever, abdominal cramps and diarrhea with or without blood and fecal white blood cells. Although generally self-limited, relapses and chronic diarrhea are possible, as well as extraintestinal infection including bacteremia. *Campylobacter jejunii* infection is the most frequently recognized infection preceding the development of Guillain–Barré syndrome.[19] The mechanisms rely on the cross-reactivity between ganglioside-like motifs present in *Campylobacter jejunii* lipopolysaccharide and those of peripheral nerves. Also, this species has been associated with immunoproliferative small intestinal disease.[20] Species other than *Campylobacter jejunii* and *Campylobacter coli* are increasingly isolated from the stools of patients with diarrhea, including *Campylobacter fetus*, mainly isolated from extraintestinal sites, and *Campylobacter upsaliensis*. As both species are susceptible to cephalotin, an antibiotic usually incorporated in *Campylobacter jejunii*-selective media, their prevalence in stools and diarrhea may be underestimated by culture methods.

Clostridium difficile infection

Clostridium difficile is a sporulated Gram-positive anaerobe of the Firmicutes phylum. Spores are highly resistant in an inanimate environment which can be a source of infection. *Clostridium difficile* genome analysis indicated that this extremely variable genome encodes toxin A and toxin B[21] which are cytotoxic toxins implicated in the inflammatory intestinal lesions. Some *C. difficile* strains encode only toxin B and may be missed in laboratory tests detecting only toxin A. Also, emerging *C. difficile* clones have recently been identified as being responsible for both community-acquired and nosocomial severe enteritis due to the additional production of the iota toxin[22] or to the hyperproduction of toxin A and toxin B due to mutation in the regulator gene.[23]

Clostridium difficile is indeed responsible for episodes of community-acquired and nosocomial acute enteritis, some of them associated with the previous administration of a wide variety of antibiotics, with *C. difficile* being responsible for about 20% of antibiotic-associated diarrhea.[24] If the use of the offending antibiotic continues, *C. difficile* may cause severe enteritis and life-threatening pseudomembranous colitis.

Escherichia coli infection

Of the six *Escherichia* spp., *E. coli* organisms are common inhabitants of the intestinal tract of healthy people, yet a limited number of clones are responsible for acute diarrhea and extraintestinal infections. *Escherichia fergusonii* is frequently isolated from stools, yet its pathogenic role is unproven; *Escherichia albertii* is a possible agent of acute diarrhea.[25] There are five groups of *E. coli* organisms associated with acute diarrhea:

- Shiga-toxin producing *E. coli* (STEC), also termed enterohemorrhagic *E. coli* (EHEC);
- enterotoxigenic *E. coli* (ETEC);
- enteropathogenic *E. coli* (EPEC);
- enteroaggregative *E. coli* (EAEC); and
- enteroinvasive *E. coli* (EIEC).

STEC produce one or several Shiga toxins (also known as verocytotoxins) and are the most frequent *E. coli* organisms associated with acute diarrhea in developed countries. These organisms, comprising various *E. coli* O157 serotypes, are responsible for mild non-bloody and bloody acute diarrhea.[26] Non-O157:H7 STEC are associated with illnesses that differ from those caused by *E. coli* O157:H7. Most notably, they are found later and have a lower proportion of bloody diarrhea than in patients infected with *E. coli* O157:H7.[27] STEC are also responsible for an estimated 80% of hemolytic–uremic syndrome cases in about 4% of patients with enteric infection. Ground beef has been the major vehicle of transmission of O157 STEC, although other vehicles contaminated by bovine manure have been reported, including raw milk, sausage, apple cider, raw vegetables and nonchlorinated water supplies. Additionally, person-to-person transmission is responsible for outbreaks in communities.

ETEC produce heat-labile (LT) and heat-stable (ST) enterotoxins and are a frequent cause of acute diarrhea in developing countries, thus being a frequent cause of travelers' diarrhea. ETEC infection manifests as relatively mild watery diarrhea and abdominal cramps, but no vomiting or fever.

EPEC comprise organisms characterized by an adherence factor plasmid and the chromosomal locus of enterocyte effacement. These organisms are responsible for severe infantile diarrhea in developing countries associated with fever, vomiting and prolonged evolution. Chronic diarrhea may follow EPEC infection and be responsible for malabsorption, weight loss and growth retardation.

EIEC are responsible for an infection mimicking shigellosis. EAEC are responsible for worldwide, mild enteric infections with non-bloody diarrhea, abdominal pain and mild fever.

Salmonella infection

The genus *Salmonella* comprises motile enteric bacteria of problematic nomenclature. *Salmonella* comprises two species and the species *Salmonella enterica* comprises five subspecies. The vast majority of human infections are due to strains of *Salmonella enterica* subspecies I, also isolated from warm-blooded animals, while the other *Salmonella* organisms are isolated from the environment and cold-blooded animals. These organisms are further serotyped and serotype generally correlates with the food source of infection. Notably, *Salmonella enterica* serotype Typhi is the agent of typhoid fever. *Salmonella enterica* serotypes Enteritidis and Typhimurium are the most commonly isolated in developed countries. Salmonellosis outcome differs significantly with serotype.[28]

Nontyphoidal *Salmonella enterica* organisms are responsible for acute diarrhea with fever and abdominal cramps lasting for an average of 1 week. Rarely, these organisms are responsible for bacteremia and extraintestinal infections in immunocompromised patients. Contacts with animals and animal foods are the sources of infection, with the Enteritidis serotype being associated with chicken and egg products. Serotype Cholerasuis is adapted to pigs and serotype Dublin is adapted to cattle. Later serotypes harbor virulence traits in common with serotype Typhi, the typhoid fever agent. Typhoid fever is a life-threatening septicemia rarely observed in developed countries but still of public health concern in developing countries. The mortality rate in patients who did not receive appropriate therapy is more than 10% whereas it is less than 1% in patients who did receive appropriate antibiotic therapy.

Clinical presentation includes fever and headache without diarrhea and healthy carriers have been observed. Humans are the only known reservoir for serotype Typhi, which can be transmitted by direct person-to-person contact and contaminated water and food. A syndrome similar to typhoid fever is due to serotypes Paratyphi A, Paratyphi B and Paratyphi C.

Shigella infection

Although from a genetic standpoint, *E. coli* and *Shigella* spp. form a single bacterial species, four subgroups of *Shigella* have been taken as different species, i.e. *Shigella dysenteriae*, *Shigella flexneri*, *Shigella boydii* and *Shigella sonnei*.[29] Humans are the only known reservoir for *Shigella* spp. and transmission is by direct contact from person to person and by contaminated water and food. Sexual transmission has been observed in homosexual males. Most cases in developed countries are imported from developing countries. *Shigella* spp. are responsible for bacillary dysentery characterized by acute, bloody diarrhea accompanied by fever and abdominal cramps. Classic dysentery is characterized by the emission of scant stools containing mucus, pus and blood. Shigellosis is responsible for rectal and colonic ulcerations which do not develop beyond the lamina propria. Rare cases of septicemia have been observed but *Shigella* spp. are responsible for the hemolytic–uremic syndrome.

Vibrio infection

Vibrios are ubiquitously found in aquatic environments and are classified into more than 70 species responsible for trauma-related, extraintestinal infections and intestinal infections with diarrhea.[30] *Vibrio cholerae* is the etiologic agent of cholera. This is a motile, Gram-negative, facultative anaerobe bacterium which requires a small concentration of sodium for growth. *Vibrio cholerae* is primarily an aquatic inhabitant found in freshwater rivers and lakes as well as in estuarine and maritime environments. In these environments, *V. cholerae* is isolated from both inanimate environments and from plankton and various bivalves, crabs, shrimp and prawns. A viable-but-not-cultivable state has been described, the regulation of which may be phage-dependent.[31]

Vibrio cholerae comprises three major subgroups: *V. cholerae* O1, *V. cholerae* O139 and *V. cholerae* non-O1, widely distributed in tropical and subtropical areas, including the Gulf of Mexico for *V. cholerae* O1. The *V. cholerae* O1 chromosome contains a virulence cassette and pathogenicity islands, encoding virulence factors such as the pilus responsible for the attachment of *V. cholerae* O1 organisms to the intestinal epithelium and the cholera enterotoxin responsible for the large excretion of electrolytes and water in the intestinal lumen. Two biotypes, designated classic and El Tor, can be differentiated on the basis of simple laboratory tests including the hemolysis of sheep erythrocytes, the Voges–Proskauer test and the resistance to polymyxin, which are all positive in the El Tor biotype. The first six historical pandemics are though to be due to the classic biotype whereas the ongoing seventh pandemic (which started in 1961) is due to the El Tor biotype.

Vibrio cholerae O1 is the organism responsible for historic pandemics of cholera since 1816, including the current pandemics. Contamination may be hand transmitted through water drinking (and flies). Most patients contaminated with *V. cholerae* O1 have an asymptomatic or self-limited diarrhea (>75%), but massive contamination results in severe diarrhea with large volumes of 'rice water stools' and dehydration or infection in patients with neutral gastric pH. Clinical manifestations include loss of skin elasticity, watery eyes, painful muscle cramps and anuria. Dehydration leads to hypovolemic shock and death. Exceptional extraintestinal *V. cholerae* O1 bacteremia infections have been reported.

In 1992, cholera cases due to a new serogroup *V. cholerae* O139 (Bengal) were reported in India and Bangladesh and spread rapidly throughout Asia. The new serogroup probably resulted from the lateral gene transfer of novel capsule and somatic antigen genes to the El Tor strain. It causes disease similar to that caused by *V. cholerae* O1 except that adults are more frequently infected.

Some *V. cholerae* isolates do not agglutinate with anti-O1 and anti-O139 antisera and are therefore referred as *V. cholerae* non-O1 isolates. *Vibrio cholerae* non-O1 isolates do not produce the cholera enterotoxin and are responsible for mild watery diarrhea. Unlike *V. cholerae* O1 isolates, *V. cholerae* non-O1 isolates are responsible for extraintestinal infections, such as life-threatening septicemia, especially in patients with previous liver disease or hematologic malignancies. *Vibrio cholerae* non-O1 isolates have also been recovered from other anatomic sites.

Other *Vibrio* spp. responsible for diarrhea include *V. mimicus*, a species phenotypically related to *V. cholerae*, which is responsible for diarrhea after the consumption of raw seafoods; some strains harboring the cholera toxin can produce cholera-like symptoms. In Asia, *V. parahaemolyticus* is the leading cause of food-borne intestinal infections after the consumption of raw fish or shellfish. The species is responsible for watery diarrhea, rarely bloody diarrhea, and is particularly responsible for severe dehydration and death. The first pandemic clone, *V. parahaemolyticus* serotype O3:K6, emerged in 1996 in Taiwan and then spread throughout Asia to America, Africa and Russia. New serotypes emerged for a few years.

Vibrio vulnificus is primarily responsible for life-threatening septicemia and secondary skin infection with necrosis. It has an intestinal route of entry and is responsible for vomiting, diarrhea and abdominal cramps after the consumption of raw oysters in 95% of patients. Three biogroups have been defined in *V. vulnificus*, the vast majority of infections being due to biogroup 1. *Vibrio fluvialis*, *Vibrio furnisii* and *Grimontia* (*Vibrio*) *hollisae* cause sporadic cases of diarrhea worldwide.[32,33] *Vibrio alginolyticus* is seldom isolated from stools and there is little evidence for *V. alginolyticus* actually being responsible for intestinal infection and diarrhea.

Yersinia infection

Among the numerous members of the *Yersinia* genus, only *Yersinia enterocolitica* and *Yersinia pseudotuberculosis* have been associated with digestive tract infection. *Yersinia enterocolitica*, further divided into two subspecies *enterocolitica* and *paleartica* on the basis of 16S rDNA sequencing,[34] comprises more than 70 serotypes, five of them being associated with human infection. These strains encode for an enterotoxin and some strains harbor a chromosome-borne pathogenicity island which contains the yersiniabactin gene, providing the organisms with iron. *Yersinia enterocolitica* is primarily an environmental organism isolated from the gastrointestinal tract of numerous animals, most commonly swine, dogs and rodents. Its distribution is mostly in Northern Europe and northern states of the USA, reflecting its increased growth at cold temperatures. This species is responsible for gastroenteritis associated with the consumption of contaminated water and food, mainly poorly cooked pork.

The disease spectrum comprises self-limited, acute diarrhea to terminal ileitis and mesenteric lymphadenitis mimicking appendicitis. Prolonged shedding has been observed. Septicemia is an uncommon complication observed in patients with an increased iron pool such as thalassaemic patients in Western countries,[35] patients with liver disease or cancer and those undergoing steroid therapy. *Yersinia enterocolitica* is the bacterial organism most frequently associated with blood transfusion. Reactive arthritis is an uncommon sequel observed in HLA-B27 positive patients and immunocompromised patients; it is characterized by asymmetric involvement of multiple joints including the sacroiliac joint and the spine.

Yersinia pseudotuberculosis is rarely isolated as a cause of a self-limited acute diarrhea; it has been associated with outbreaks of gastroenteritis after consumption of contaminated fresh vegetables.[36,37] *Yersinia pseudotuberculosis* is also responsible for pseudoappendicitis, and reactive arthritis may develop after infection with *Yersinia pseudotuberculosis* O3.[38]

Miscellaneous bacteria

Arcobacter are *Campylobacter*-like organisms seldom isolated from the stools of patients with diarrhea, including *Arcobacter butzleri*[39] and *Arcobacter cryaerophilus* DNA group 1B.[40] Particular culture conditions are required for proper isolation of these fastidious organisms, thus limiting their detection to a few studies.

Listeria monocytogenes has only recently been recognized as an agent of acute enteritis, mainly transmitted by milk, but it has now been associated with several enteritis outbreaks.[41] Enteritis typically occurs after ingestion of a large inoculum and is self-limited after a few days' evolution.[42]

Klebsiella oxytoca is found in the environment but its principal reservoir is the human gastrointestinal tract; it has been associated with *C. difficile*-negative antibiotic-associated colitis.[43] *Klebsiella oxytoca* organisms cause experimental colitis and exhibit cytotoxicity against HEp-2 cultured cells.[43] Its culture is not routinely performed and requires a specific isolation agar medium.

Laribacter hongkongensis is a facultative anaerobic Gram-negative bacillus initially reported as being responsible for acute diarrhea in Asian patients.[44] A case-controlled study indicated that eating fish and travel were associated with *L. hongkongensis* acute diarrhea.[45]

Dysgonomonas capnocytophagoides (formerly CDC group DF-3)[46] are *Captocytophaga*-like organisms isolated from the stools of immunocompromised patients.[47–49]

Viral enteritis

Rotavirus infections

Rotaviruses are RNA viruses presenting as 70 nm particles with a wheel-like appearance (Fig. 35.1). Based on group-specific antigens of the major viral structural protein VP6, rotavirus can be classified into six groups, A–G. Groups A–C infect humans; the other groups are found in animals. Human rotaviruses are responsible for severe acute diarrhea with dehydration associated with childhood death in developing countries. In Europe, children with rotavirus-positive acute gastroenteritis were more likely to have lethargy, fever, vomiting and dehydration, and, therefore, more severe disease than were children with rotavirus-negative acute gastroenteritis. Dehydration was up to 5.5 times more likely in children with rotavirus-positive acute gastroenteritis than in those with rotavirus-negative acute gastroenteritis.[50] Acquisition of rotaviruses is likely from subclinical infection in parents or siblings but rotavirus infection can be a zoonosis. Rotaviruses are resistant in inanimate environments which may be implicated as a source of infection, including nosocomial outbreaks. Rotavirus infection is seasonal, with a peak incidence in winter/spring in temperate countries. Clinical symptoms include acute diarrhea for 2–3 days, fever, vomiting and anorexia.

Calicivirus infections

The family Caliciviridae comprises norovirus and saprovirus, both responsible for enteritis. These RNA viruses appear as <40 nm non-enveloped particles. Norovirus comprises five genotypes: genotypes I, II and IV are responsible for human infections, genotypes III and V are animal associated. Likewise, saprovirus comprises five genotypes; genotypes I, II, IV and V are responsible for human infections. These highly contagious human viruses reside in inanimate environments comprising contaminated surfaces, water and food. Direct evidence for animals as a reservoir for human infection is still lacking.

Noroviruses are the most prevalent cause of nonbacterial acute enteritis worldwide.[51] These viruses cause large outbreaks and provoke incapacity for a few days; they have therefore been included in List B

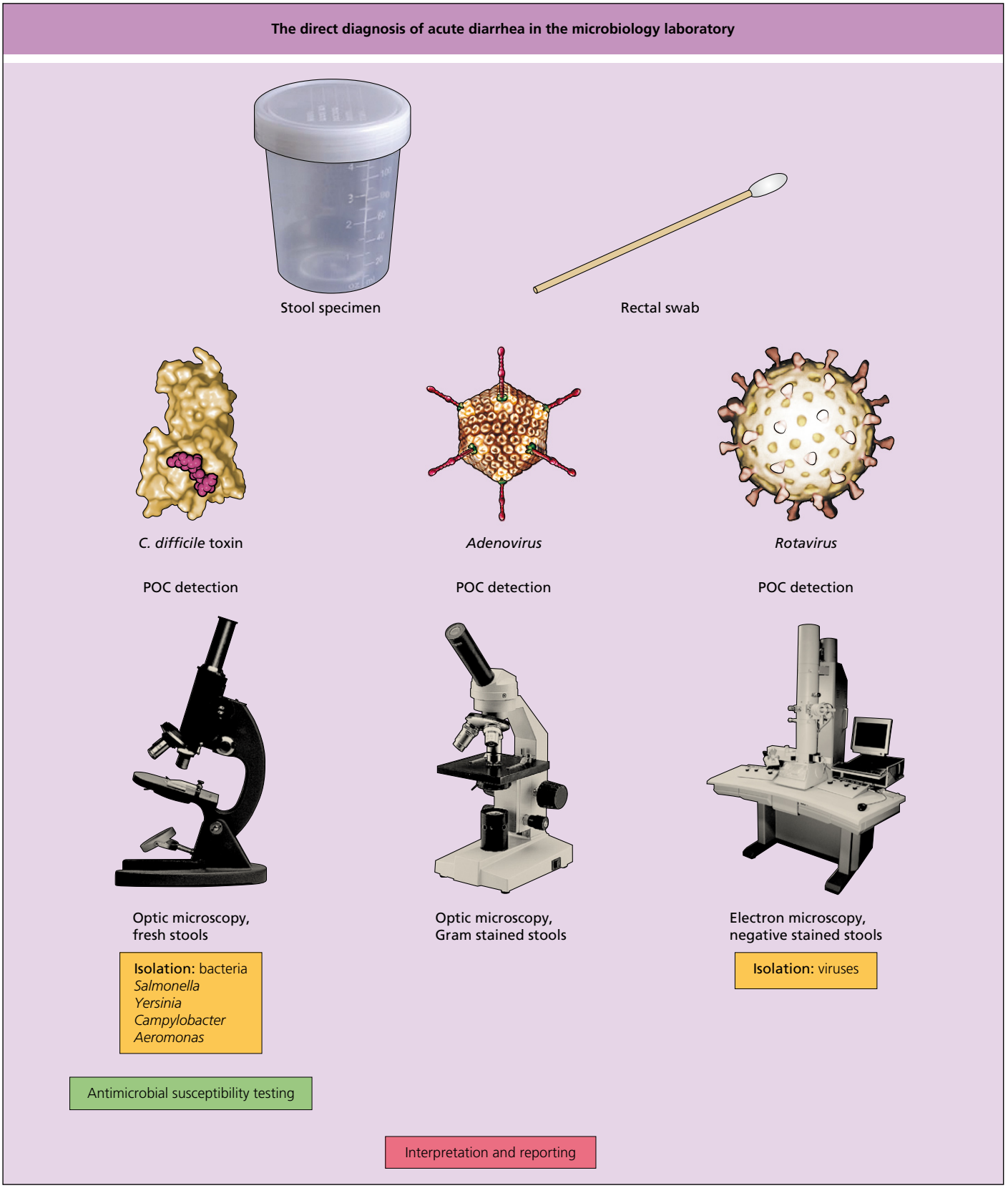


Fig. 35.1 Flow-chart for the direct diagnosis of acute diarrhea in the microbiology laboratory. POC, point of care.

of potential bioterrorism agents by the US National Institute of Allergy and Infectious Diseases (NIAID). Outbreaks mainly occur in institutions, health-care centers and cruise ships.

Astrovirus infections

These RNA viruses, averaging 30 nm in diameter, are mainly responsible for acute diarrhea in children, although outbreaks in military troops and hospitals have also been reported. These worldwide viruses are responsible for 2–10% of pediatric cases of acute diarrhea. Clinical signs and symptoms are nonspecific.[52]

Enteric adenovirus infections

Adenoviruses are nonenveloped, 100 nm round particles containing DNA and are responsible for human infections. Adenoviruses are divided into 51 different serotypes and six subgroups, of which only two serotypes – Ad40 and Ad41 (subgroup F) – have been clearly demonstrated to be agents of acute diarrhea. Clinical characteristics include a higher prevalence in children less than 4 years of age and a mean duration of disease of 5–10 days, i.e. longer than that caused by other viruses. Prolonged diarrhea has been observed in immunocompromised patients.[53]

Miscellaneous viruses

A few other viruses have been associated with acute diarrhea, yet their role remains to be firmly established. These include coronavirus, definite agents of diarrhea in animals and seldom visualized by electron microscopy and isolated in culture from the stools of patients with diarrhea. Likewise, toroviruses are responsible for acute human gastroenteritis and are responsible for nosocomial cases. Aichi virus, a member of the family Picornaviridae, has been characterized by reverse transcriptase-polymerase chain reaction (RT-PCR) during an outbreak of enteritis following the consumption of oysters in Japan.[54] Picobirnaviruses have been detected in stools of animals and humans; however, their significance remains to be established. Recently, cardioviruses closely related to Theiler's murine encephalomyelitis virus have been detected in stools in 1.2% patients with acute enteritis.[55]

Prevention

The global mortality from diarrhea declined from approximately 4.6 million annual deaths during the mid-1980s to 2.4 million deaths in 1990, and to the current estimate of 1.6–2.1 million.[1,56] The decline is generally attributed to global improvements in sanitation and the use of glucose–electrolyte oral rehydration therapy (ORT) which has dramatically reduced acute mortality from dehydration caused by diarrhea. In contrast to the fortunate decrease in mortality, morbidity remains as high as during the previous century. However, simple, cheap measures could be undertaken to make the incidence fall. A prospective study in India demonstrated that the promotion of hand washing with plain soap reduced by 53% the incidence of acute diarrhea (and of pneumonia and impetigo).[57] Indeed, hand washing with soap is effective against almost all enteritis-causing pathogens, whereas efficacy of alcohol against rotavirus and calicivirus remains controversial.[58] In developed countries, prevention relies on increased sanitary measures in collective sources of enteric pathogens such as swimming pools and recreational lakes for fishing and swimming, as well as better control over fresh foodstuffs. Patients should be isolated at home and children excluded from nurseries and school for the duration of the illness.

Another vaccine against ETEC, administrated as a patch, is being evaluated.[59] A live oral vaccine against rotavirus has recently been licensed after a few previous attempts and its safety and preventive effects have been carefully evaluated.[60] Cost-effectiveness of vaccine against rotavirus has been evaluated favorably in the Netherlands.[61]

As for travelers, pre-travel prophylaxis relies on vaccines. There is currently only one vaccine available that provides protection against diarrhea caused by *Vibrio cholerae* and by ETEC. This vaccine is licensed in only a few Western countries. Protective efficacy against cholera is 85%, while protection against the heat-labile toxin of ETEC reaches 67%. Current studies show a protective effect of up to 43%. Vaccination against cholera and ETEC should be recommended for at-risk travelers, in particular those with high exposure at their travel destination or high personal risks through fluid loss.[62] Typhim Vi is a conjugate vaccine aimed at prevention of typhoid fever, and its safety and effectiveness have been favorably evaluated.[63,64] During travel, systematic administration of antibiotics including fluoroquinolones, cyclines and co-trimoxazole is controversial. Prophylaxis may rely on the basic rules of boiling fresh water or drinking bottled water, and ensuring that meat is well cooked.

DIAGNOSIS

There is no recommended serologic test for the microbiologic diagnosis of enteric pathogens and the laboratory diagnosis of diarrhea relies solely on direct diagnosis. Serologic testing is useful for epidemiologic investigation of *Campylobacter* spp. infections.[65]

Fresh stools should be collected in a clean container with a tight lid. Alternatively, a transport medium incorporating buffered glycerol in saline could be used. A rectal swab is an alternative specimen in selected situations. It is well established that hospitalized patients who did not enter the hospital with diarrhea are unlikely to develop diarrhea caused by bacterial agents other than *C. difficile*. As such, stool culture will not be performed in patients hospitalized for more than 72 hours (the 3-day rule) and rapid detection of *C. difficile* toxins will be alternatively performed.[66] For routine purposes, testing a single stool specimen has acceptable sensitivity; however, testing a second specimen is mandatory when the first one was subject to a more than 2-hour delay in transport.[67]

Several techniques have been developed for the point-of-care diagnosis of diarrhea including the rapid (<30 minutes) agglutination-based detection of rotavirus and adenovirus as well as the detection of *C. difficile* toxins. The rapid detection of *C. difficile* toxins A and B should be routinely performed for both inpatients and outpatients. Point-of-care detection of Shiga toxin-producing *E. coli* in children using EIA has not been evaluated favorably.[68] However, a commercially available *Campylobacter* antigen detection kit has been favorably evaluated,[69] and a dipstick test for the rapid detection of *Shigella* is under appraisal.[70]

Further detection of the causative organism relies on stool examination in the clinical microbiology laboratory. Direct microscopic examination may yield motile bacteria such as *Vibrio* and *Salmonella* spp. and parasites. Although Gram-staining analysis of stool specimens may not be done routinely, it has demonstrated 66–94% sensitivity and >95% specificity for the rapid detection of *Campylobacter* species.[71] We therefore recommend microscopic examination after Gram staining in all specimens in addition to Ziehl–Neelsen staining and appropriate staining for microsporidia and other parasites in immunocompromised patients. Specimens should be examined by electron microscopy for the observation of enteric viruses within 4 hours of receipt in the laboratory. Negative staining is a rapid and easy procedure. Rotavirus and adenovirus are readily detected, which may not be the case for calicivirus and astrovirus[72] (Fig. 35.2).

Culture of stools will focus on frequent pathogens and the systematic search for less frequent bacterial pathogens will be guided by the local epidemiologic situation. Pathogens routinely detected by culture of diarrheal stools include *E. coli* O157, *Shigella* spp., *Salmonella enterica* serotypes, *Campylobacter jejunii*, *Campylobacter coli* and *Aeromonas* spp. Stools in O157, O111 and O26 serotypes of *E. coli* can be enriched by using specific, commercially available magnetic beads. Sorbitol MacConkey agar can be used for the isolation of O157 STEC as these organisms do not ferment D-sorbitol, contrary to the vast majority of

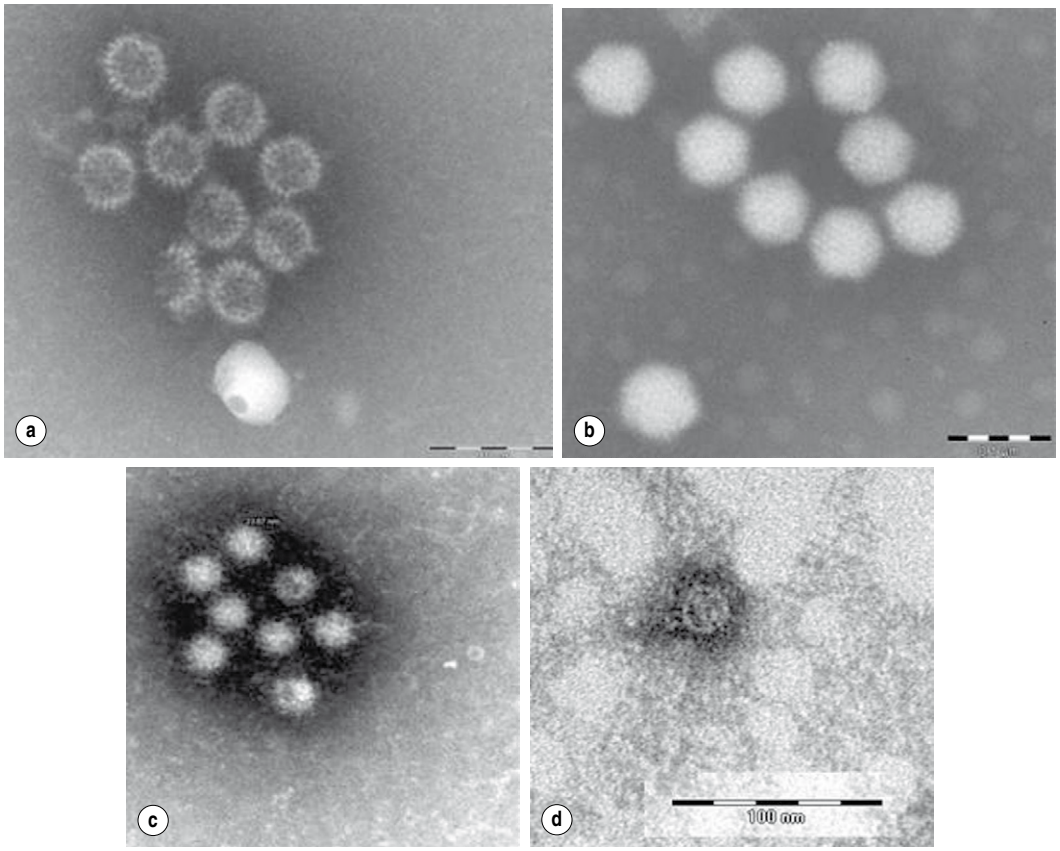


Fig. 35.2 Electron microscopy examination of diarrheal stools after negative staining shows the presence of enteric viruses responsible for acute diarrhea, such as (a) rotavirus, (b), adenovirus, (c) calicivirus and (d) enterovirus.

E. coli strains, and several chromogenic media have been developed for the selective isolation of O157 STEC. Although many rapid methods have been proposed for confirming the identification of O157 STEC, methods for the identification of ETEC, EPEC, EAEC and EIEC are most often available in reference laboratories only.

Salmonella enterica serotypes are better isolated by using an enrichment broth before plating onto selective media. Biochemical identification of *Salmonella* spp. and O (somatic), H (flagellar) and Vi (capsular) antigen serotyping should be performed in order to identify *Salmonella* enteritis Typhi (the typhoid fever agent, being capsular antigen Vi positive) and the most prevalent non-Typhi serotypes. The Vi capsular antigen is occasionally detected in non-Typhi, Dublin and Paratyphi C serotypes. O serotype determination is carried out by agglutination using pooled antisera while further H serotype determination is performed by tube agglutination tests using broth culture and testing the two phases of the flagellar antigens. MALDI-TOF mass spectrometry rapid analysis of *Salmonella enterica* isolates may resolve the serotype.[73]

Campylobacter spp. are recovered by using the filtration method in parallel to selective, blood-containing or non-blood-containing media and a microaerophilic atmosphere. Some *Campylobacter* spp. require 6% hydrogen in atmosphere. Incubation at 108°F (42°C) allows the growth of *Campylobacter jejuni* and *Campylobacter coli* but not all *Campylobacter* and *Aeromonas* spp. are recovered using blood agar incorporating 20 µg/ml ampicillin and produce β-hemolytic colonies. Further identification will be needed for oxidase-positive and indole-positive colonies. Modified cefsulodin-irgasan-novobiocin agar is also suitable for the isolation of *Aeromonas* spp. On this medium, *Aeromonas* colonies are undistinguishable from *Yersinia enterocolitica* colonies. Molecular identification should rely not only on 16S rDNA sequencing because of intragenomic heterogeneity and further identification based on *gyrB* and *rpoD* genes is mandatory. The interpretation of recovery of *Aeromonas* in stools must be cautious since there is no strong evidence that all *Aeromonas* isolates from stools are responsible for diarrheal infection.[74]

Blood cultures are mandatory for the diagnosis of typhoid fever as well as bacteremia due to non-Typhi serotypes of *Salmonella*.

The systematic search for other enteritis pathogens will depend on local epidemiology including *Yersinia enterocolitica*, *Vibrio* spp., *Klebsiella oxytoca*, *Listeria monocytogenes* and *Plesiomonas shigelloides*. Growth of *Yersinia enterocolitica* from stools is enhanced by incubation on selective media (e.g. pectin agar) at 95°F (35°C) and the search for *Listeria monocytogenes* could be routinely done in certain laboratories. *Vibrio cholerae* will be visible as very motile, Gram-negative, slightly curved bacilli cultivated using a thiosulfate citrate bile salts sucrose (TCBS) agar after enrichment. Yellow colonies of oxidase-negative bacilli could be identified after electron microscopy observation, by 16S rDNA sequencing. In parallel with the search for pathogenic bacteria, the search for viruses should be done using electron microscope observation after negative staining as well as detection of rotavirus.

Although identification based on the observations of phenotypic traits after bacterial growth is the routine approach to the identification of bacteria responsible for diarrhea, novel techniques are warranted in order to speed the identification process in a timely fashion. Rapid and cheap identification by using mass spectrometry analysis of entire organisms has already been reported for *Vibrio* spp.[75]

MANAGEMENT

Community-acquired enteritis

Management of acute diarrhea should include the clinical evaluation of the patient, including risk factors for specific etiology

and dehydration; rapid diagnosis of viral diarrhea; and treatment including rehydration, antibiotic therapy and symptomatic treatment. Rehydration is a major therapeutic measure; however, it should be borne in mind that patients on oral rehydration have a higher risk of paralytic ileus, and those on intravenous rehydration are exposed to risks of intravenous therapy. For every 25 children treated with oral rehydration one would fail and require intravenous rehydration.[76]

Rapid diagnosis of viral diarrhea is important in order to avoid unnecessary antibiotic treatment. Because of the absence of any antiviral drug effective against the viruses responsible for acute diarrhea, the management of viral diarrhea comprises the relief of symptoms and rehydration in cases of dehydration. Meta-analysis has confirmed that antibiotic treatment is useful to shorten the duration of signs and symptoms in travelers' acute diarrhea,[77] although most acute diarrheal episodes are self-limited and do not require antibiotic treatment. If an antibiotic is to be prescribed, fluoroquinolones are the drugs of first choice, including norfloxacin (400 mg q12h) or ciprofloxacin (500 mg q12h) and 1-day treatment is advocated except for *Campylobacter* and *Shigella* infection which should be treated for 3 days.[78] In the case of patients back from countries where fluoroquinolone resistance is prevalent,[79] such as *Campylobacter* spp. in Thailand, azithromycin (500 mg/day) could be used for 3 days.[80] Antimicrobial therapy for O157 *E. coli* enteritis or hemolytic–uremic syndrome remains a controversial issue because some studies reported a deleterious effect on the evolution of the latter syndrome. Antibiotic treatment of other serotypes by fluoroquinolones is advocated.

The increasing resistance of *Salmonella enterica* serotype Typhi to antibiotics, notably ciprofloxacin, makes the choice of first-line antibiotic treatment of typhoid fever more problematic. The majority of cases of *Yersinia enterocolitis* gastroenteritis do not require antibiotic treatment, contrary to systemic infection which could be treated using co-trimoxazole (no resistant strain reported) or fluoroquinolones, despite the fact that a few resistant strains have been reported.

Nosocomial enteritis

The main goal is to prevent nosocomial transmission of the etiologic agent in order to prevent and limit an extensive outbreak. The first step is to avoid the admission of patients diagnosed with diarrhea but without serious signs, and patients diagnosed at point-of-care as viral diarrhea. As for hospitalized patients, cohorting was not effective in the prevention of outbreaks. Patients suspected of infectious diarrhea should be treated in a single room, using standard precautions including hand washing and barrier protection using gloves and gowns. The duration of these precautions is for the duration of the illness.

Hand washing is the single most important measure in the control of nosocomial transmission of enteric pathogens. Because of concerns regarding the low virucidal activity of ethyl and isopropyl alcohols against naked viruses (rotavirus, norovirus, saporovirus)[81] and the lack of sporicidal activity (*C. difficile*), hand washing with soap in addition to alcohol is mandatory. The impact of improved hand hygiene using both soap and alcohol hand washing in decreasing nosocomial rotavirus infection has been proved.[82] Room environments should be carefully cleansed using sporicidal biocides in cases of *C. difficile* diarrhea.

There is no specific treatment for nosocomial viral enteritis. Cessation of the inciting antibiotic as soon as possible is the main management measure in case of *C. difficile* diarrhea. Patients should benefit from oral metronidazole (500 mg q8h) or vancomycin (125 mg q6h) for 10 days. The role of immunoglobulin for the treatment of severe *C. difficile* infection remains controversial.[83]

REFERENCES


References for this chapter can be found online at http://www.expertconsult.com

Chapter 36

Florence Fenollar

Chronic diarrhea

INTRODUCTION

Chronic diarrhea is a common symptom of many conditions with an estimated prevalence of 3–5%.[1–3] A current definition is the abnormal passage of three or more loose or liquid stools per day for more than 4 weeks and/or a daily stool weight greater than 200 g.[3] Indeed, a clinical definition of chronic diarrhea based on symptom reporting alone will lead to an overlap with functional bowel disorders such as irritable bowel syndrome, in which stool weight does not usually increase, and 'true' diarrhea. As irritable bowel syndrome affects 9–12% of the population, there is clearly the potential for inappropriate investigation of patients reporting diarrheal symptoms.[3] Conversely, new onset of diarrhea may reflect serious organic disease such as colonic neoplasia. Thus, chronic diarrhea continues to be a diagnostic challenge, largely because of the vast number of conditions included in its differential diagnosis as demonstrated in Table 36.1.

Most acute diarrheas are generally due to infectious etiologies, whereas symptoms persisting for longer than 4 weeks suggest a noninfectious etiology and occasionally might be due to microorganisms.[3] The main circumstances in which chronic infectious diarrhea is observed are in travelers, mainly in developing and tropical countries, or people living in such areas, in immunocompromised hosts, in hospitalized patients with *Clostridium difficile* and in patients with Whipple's disease.[4,5] In HIV-infected patients, chronic diarrhea is more common in patients with low CD4 lymphocyte counts (<200/mm^3, especially those <50 cells/mm^3).[6] The most essential component of any therapeutic strategy for an immunocompromised patient is restoration of the underlying immunologic defect such as highly active antiretroviral therapy (HAART) for HIV-infected patients.[6] Microorganisms observed are those which are capable of causing diarrhea in the immunocompetent population but also opportunistic such as *Cryptosporidium*, *Microsporidium*, *Cyclospora*, *Mycobacterium avium-intracellulare* and cytomegalovirus.[4,7]

In this chapter, bacterial, parasitic, fungal and viral causes of chronic diarrhea are reviewed and a general stepped strategy to explore chronic diarrhea (Fig. 36.1) is proposed. Obtaining a detailed medical history and stool characteristics should be the first step in defining a potential etiology and guiding appropriate choice of investigations. The second step should be based on the analysis of stool specimens, which is sufficient to establish the diagnosis of most infectious etiologies. Specific stepwise approaches for chronic diarrhea in travelers[8] (Fig. 36.2), HIV-infected patients[6] (Fig. 36.3) and patients suspected of Whipple's disease (Fig. 36.4)[9] are also proposed. Chronic (possibly infectious) diarrhea, such as tropical sprue and Brainerd diarrhea, is discussed, as well as perspectives to improve knowledge of chronic infectious diarrhea.

Table 36.1 Main causes of chronic diarrhea showing the place of infectious etiologies (shown in bold) among a broad spectrum of noninfectious etiologies

Colonic
Colon cancer, lymphoma
Ulcerative colitis, Crohn's disease
Microscopic colitis
Ischemic colitis
Radiation colitis
Gastrointestinal protozoans and helminths
Clostridium difficile
Ulcerating viral infections: cytomegalovirus and herpes simplex virus
Small bowel
Celiac disease
Crohn's disease
Whipple's disease
Other small-bowel enteropathies (tropical sprue, amyloid, intestinal lymphangiectasia)
Bile acid malabsorption
Disaccharidase deficiency
Mesenteric ischemia
Radiation enteritis
Lymphoma
Gastrointestinal protozoans and helminths
Small intestinal bacterial overgrowth
Pancreatic
Chronic pancreatitis
Pancreatic carcinoma
Cystic fibrosis
Endocrine
Hyperthyroidism
Diabetes
Hypoparathyroidism
Addison's disease
Hormone secreting tumors (gastrinoma, VIPoma, somatostatinoma, mastocytosis, carcinoid syndrome, medullary carcinoma of thyroid)
Others
Factitious diarrhea
Surgical causes (postresection diarrhea, internal fistula, postvagotomy diarrhea, postsympathectomy diarrhea)
Drugs
Alcohol
Autonomic neuropathy

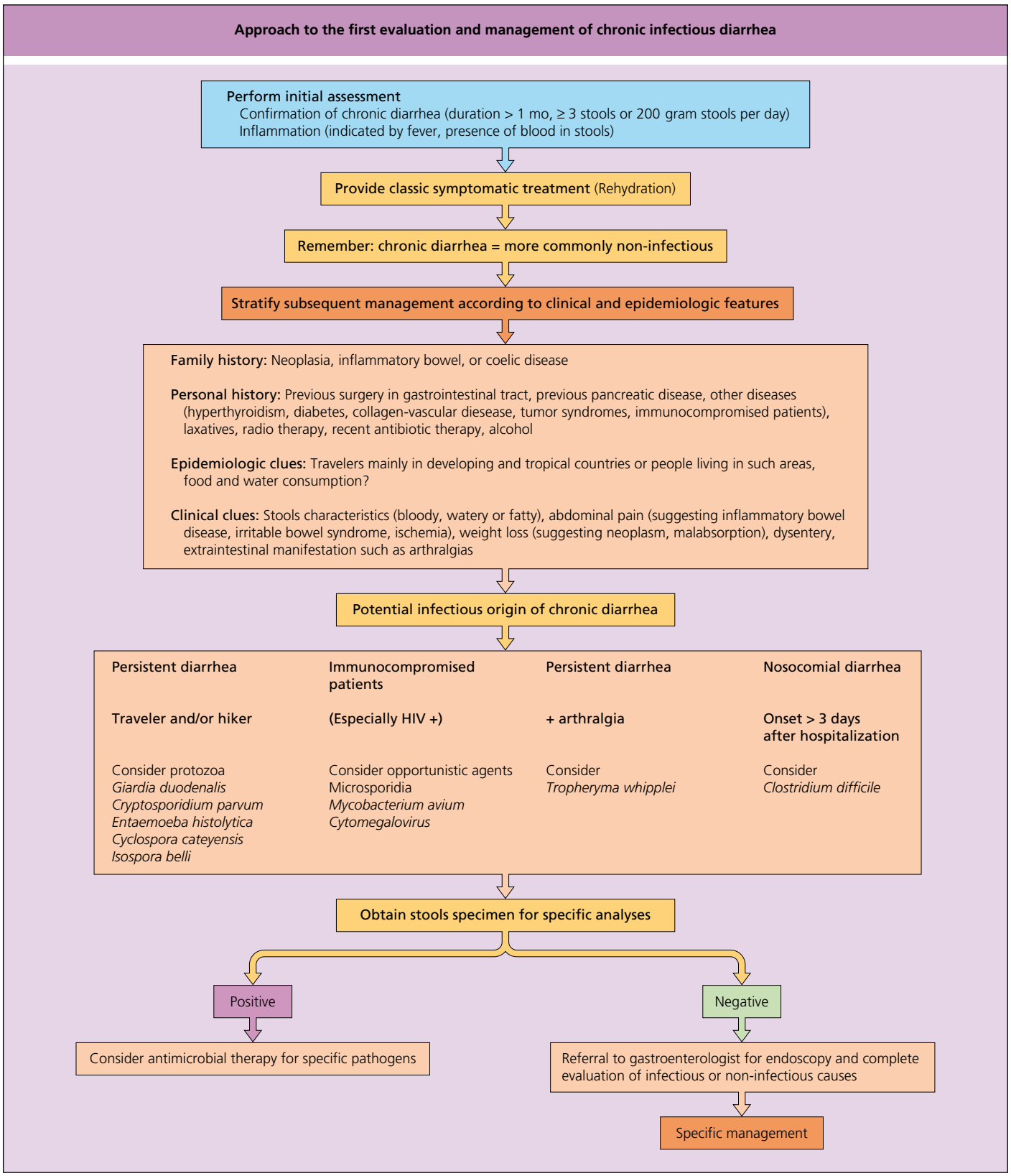


Fig. 36.1 Approach to the first evaluation and management of chronic infectious diarrhea.

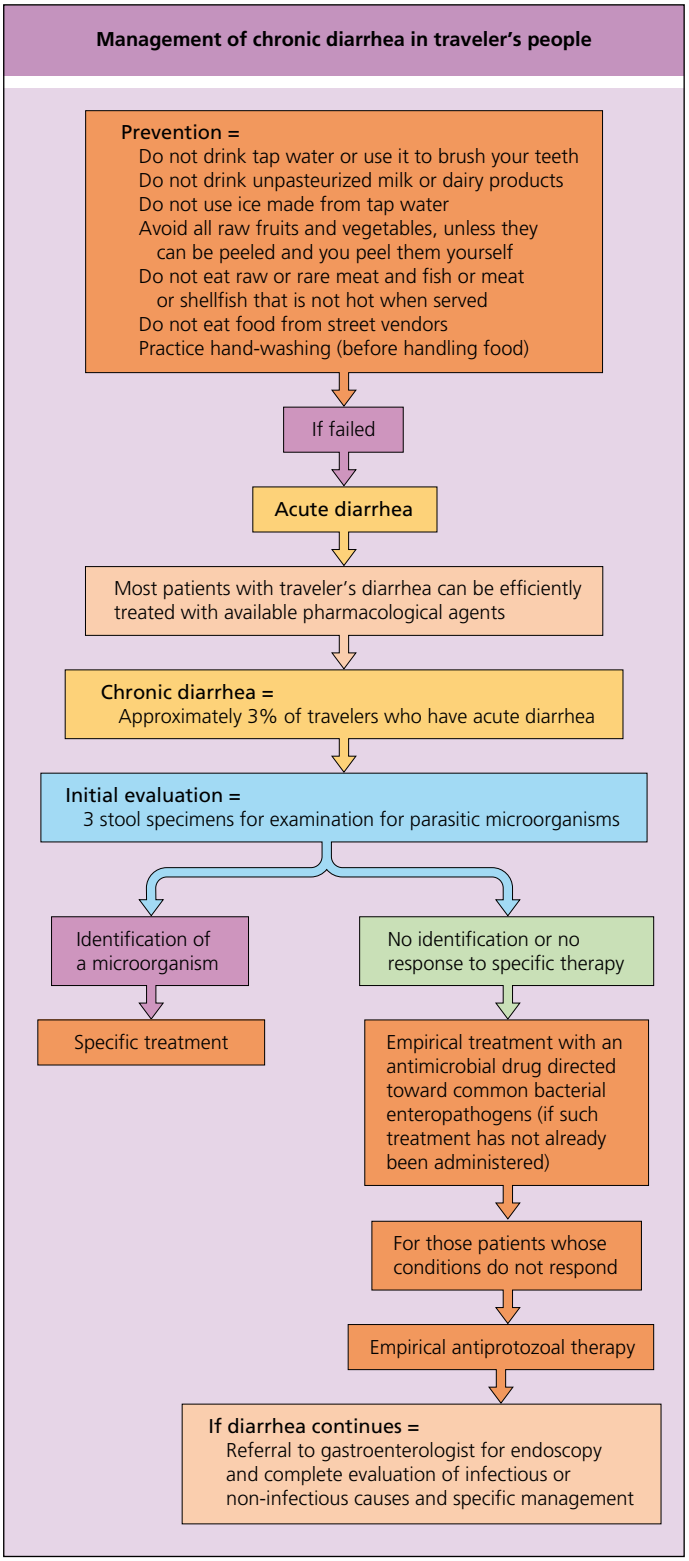


Fig. 36.2 Management of chronic diarrhea in travelers.

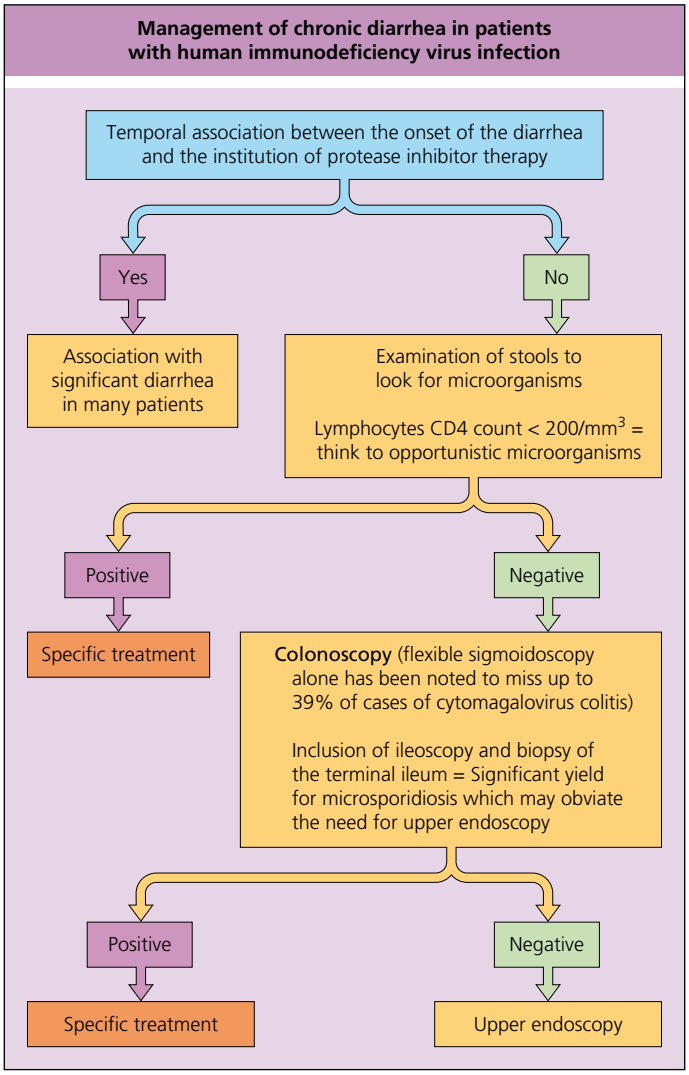


Fig. 36.3 Management of chronic diarrhea in patients with HIV infection.[6] Knowledge of CD4 lymphocyte count and type of antiretroviral therapy is useful in management of patients.

BACTERIAL CAUSES

Usual bacterial pathogens (*Salmonella* spp., *Yersinia enterocolitica*, *Campylobacter* spp., *Plesiomonas shigelloides* and *Vibrio parahaemolyticus*), which cause acute diarrhea in the immunocompetent population, produce more severe or prolonged infections in immunocompromised people.[7,10] Gastrointestinal tuberculosis remains a problem in impoverished areas, immigrants from developing countries and HIV-infected patients.[11] *Mycobacterium avium-intracellulare* is also involved in chronic diarrhea in HIV-infected patients, mainly those with CD4 lymphocyte counts >50/mm^3.[12] Clinical presentation, diagnosis and treatment for both mycobacteria are summarized in Table 36.2.

Other bacteria that are responsible for chronic diarrhea are *Clostridium difficile* and *Tropheryma whipplei*. Finally, small intestinal bacterial overgrowth is a condition that has been described for a long time but is still not clearly understood.[13,14]

Clostridium difficile infection

The anaerobic bacterium *Clostridium difficile* is an important nosocomial pathogen. Hospitalized patients may be rapidly colonized by *C. difficile* following admission.[15] It has been argued that any patient who develops diarrhea during hospital stay should be routinely tested for *C. difficile* infection.[15] Antibiotic therapy (mainly clindamycin, cephalosporins, quinolones) is the most significant risk factor for acquiring *C. difficile* infection.[15] *C. difficile* infection has a wide clinical range, from asymptomatic carriage to mild self-limiting diarrhea, chronic diarrhea and more severe pseudomembranous colitis.[16] Stools may be unformed or watery and

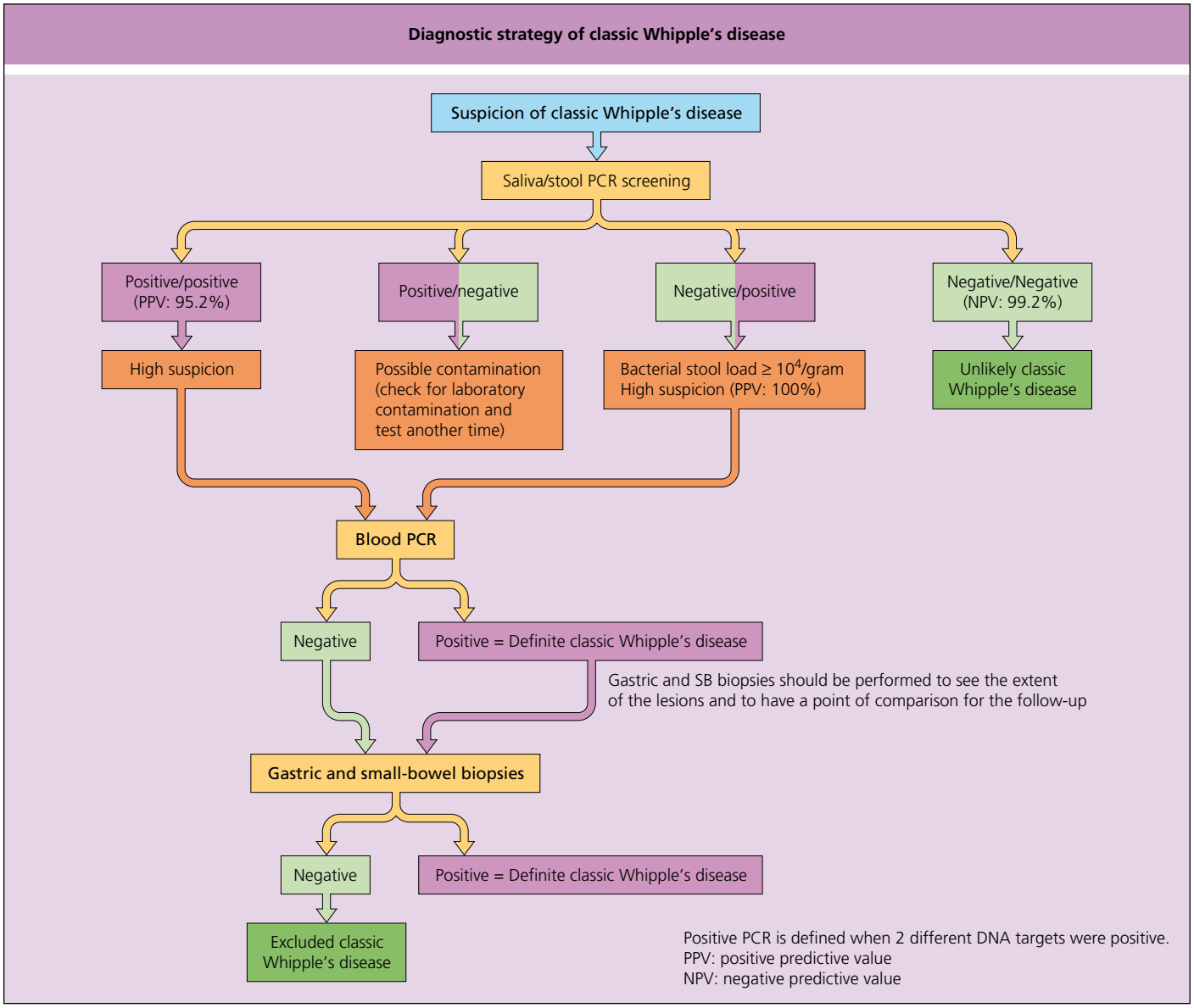


Fig. 36.4 Diagnostic strategy of classic Whipple's disease depending on quantitative real-time PCR results on saliva and stool specimens. (Adapted from Fenollar *et al.*[18])

rarely bloody.[15] Other common manifestations include abdominal pain and cramping, increased temperature and leukocytosis.

Many different tests are now available for the detection of *C. difficile* but most clinical laboratories use a commercial enzyme immunoassay for *C. difficile* toxin A or B, or both.[15] Colonic endoscopic examination may show classic pseudomembranes.[15] The most important therapeutic step is to immediately discontinue the agent responsible for provoking the disease.[15] In severe cases, or when the infection persists, metronidazole and vancomycin are the antibiotics of choice.[15]

Whipple's disease

Epidemiology

Whipple's disease (WD) is caused by the bacterium *T. whipplei*. The disease is considered as rare but there is no valid estimate of its actual prevalence.[17] The disease occurs worldwide; however, the typical patient is a middle-aged Caucasian male.[18] *T. whipplei* appears to be present in the general environment, though neither its source nor transmission is well established. Studies using polymerase chain reaction (PCR) have demonstrated *T. whipplei* DNA in sewage plant effluent.[18]

It has been speculated that *T. whipplei* might be acquired via fecal–oral transmission.[19] One hypothesis concerning pathogenesis of WD is that although many people in a given population are exposed to *T. whipplei*, only some of those with predisposing immune factors, as yet undelineated, subsequently develop the disease.[18]

Clinical features

WD is characterized by a prodromal stage, marked mainly by arthralgia/arthritis, and a much later steady-state stage typified by weight loss and/or chronic diarrhea, and occasionally by other manifestations, since nearly all organs can be involved. The time between the prodromal and the steady-state stage averages 6 years; however, in patients on immunosuppressive therapy a more rapid clinical progression may occur.[18] Roughly 15% of cases lack classic signs and symptoms.[18] Currently, the spectrum of infections caused by *T. whipplei* is more complex than previously suspected.

Table 36.2 Bacterial, viral and fungal opportunistic pathogens associated with chronic diarrhea: presentation, diagnosis and treatment

Microorganisms	Clinical presentation	Diagnosis	Treatment
Bacterial causes			
Mycobacterium avium-intracellulare	Chronic diarrhea, abdominal pain, weight loss, night sweats, fever	Blood: – specific culture Stools and colonic biopsies: – acid-fast stain – culture – PCR	Clarithromycin 1 g, ethambutol 15 mg/kg and rifabutin 300 mg, all po q24h for 3–6 months *Secondary prophylaxis in lack of immune reconstitution*: rifabutin 300 mg po q24h
Mycobacterium tuberculosis	Chronic diarrhea, abdominal pain, weight loss, fever, night sweats Only 15–20% of patients have concomitant active pulmonary infection	Stools and colonic biopsies: – acid-fast stain – culture – PCR	Rifampicin 10 mg/kg and isoniazid 5 mg/kg, both po q24h for 9 months, plus Pyrazinamide 20 mg/kg and ethambutol 15 mg/kg, both po q24h for 2 months
Viral causes			
Cytomegalovirus	Chronic diarrhea, cramps, tenesmus, fever, abdominal pain, weight loss	Blood: – antigenemia – viremia Stools: PCR Colonic biopsies: – H&E – PCR – viral culture – electron microscopy	Ganciclovir 5 mg/kg and foscarnet 90 mg/kg, both iv q12h for 2–3 weeks *Secondary prophylaxis in lack of immune reconstitution*: valganciclovir 900 mg po q24h and foscarnet 120 mg/kg iv q24h
Herpes simplex virus	Chronic diarrhea, proctitis, distal colitis, tenesmus, rectal pain	Colonic biopsies: – H&E – PCR – viral culture – electron microscopy	Aciclovir 10 mg/kg po q8h for 2–3 weeks and valaciclovir 1 g po q12h for 2–3 weeks *Secondary prophylaxis in lack of immune reconstitution*: valaciclovir 1 g po q24h
Fungal cause			
Histoplasmosis	Diarrhea, hematochezia, weight loss, fever, abdominal pain	Stools: NA Colonic biopsies: – H&E – PAS stain – silver stain – fungal culture	*Severe cases*: amphotericin B 0.7 mg/kg iv for 2–3 weeks *Mild or moderate cases*: itraconazole 200 mg po q8h for 2–3 weeks *Secondary prophylaxis in lack of immune reconstitution*: itraconazole 200 mg po q24h

H&E, hematoxylin and eosin; iv, intravenous; PCR, polymerase chain reaction; po, per os (oral).

We can describe classic WD as marked by histologic lesions in the gastrointestinal tract in association with diverse clinical manifestations, with typical histologic lesions on periodic acid–Schiff (PAS) stains of small-bowel biopsies, localized infections due to *T. whipplei* without histologic digestive involvement (blood culture-negative endocarditis, neurologic infection, uveitis, etc.) and asymptomatic carriers. The main clinical manifestations of classic WD are summarized in Table 36.3.

In asymptomatic carriers, *T. whipplei* exists in stool, saliva and duodenal biopsies.[9] In stools, the estimated prevalence is from 1 to 11% in the general population. In sewage workers, this rate is higher, from 12 to 26%. Carriage in saliva (0.2% in the general population and 2.2% in sewage workers) is present only in people who are also carriers in stools. In duodenal biopsies, a carriage of 0.2% has been reported. Finally, asymptomatic neurologic involvement of patients with classic WD has been demonstrated by detecting DNA of the microorganism in cerebrospinal fluid (CSF) using PCR.[18]

Diagnosis

Several nonspecific findings are observed in blood samples. Increasing acute phase reactants, anemia, leukocytosis, thrombocytosis and evidence of malabsorption may be present at the time of diagnosis.[18] Thrombocytopenia and eosinophilia have also been reported.[18] In cases of classic WD, the endoscopic examination might show that the duodenum and jejunal mucosa are pale yellow, shaggy, eroded, erythematous or mildly friable.[20]

The usual tool for diagnosing classic WD is the light microscopic finding of magenta-stained inclusions within macrophages of the lamina propria on PAS stain of small-bowel biopsies (Fig. 36.5A). Depending on clinical manifestations, other tissues might be sampled and stained with PAS stain.[20] However, it is important to note that PAS stain is not pathognomonic of WD.[20] For example, PAS-positive cells are also seen in patients with *Mycobacterium avium-intracellulare*.[19] The presence of noncaseous granulomas composed of epithelioid cells, PAS-negative in 40% of cases, may be observed mainly in the gastrointestinal tract and the lymphatic tissues.[18]

Immunohistochemistry using antibodies directed against *T. whipplei* allows the detection of the microorganism in various fixed tissue fragments (even retrospectively), in body fluids and on blood monocytes, providing direct visualization of the bacilli (Fig. 36.5B).[18,21] This technique, which offers increased sensitivity and specificity as compared to PAS staining, is not yet widely available.

Table 36.3 Main clinical manifestations of patients with classic Whipple's disease

Frequent manifestations (>50%)
Chronic diarrhea
Arthralgia
Weight loss

Less frequent manifestations (<50%)
Adenopathy (mainly mediastinal and mesenteric)
Melanoderma
Fever

Neurologic manifestations
Cognitive change
Supranuclear ophthalmoplegia
Hypothalamic manifestations
Myoclonus
Oculomasticatory or oculo-facial-skeletal myorhythmia

Cardiovascular involvement
Endocarditis
Pericarditis
Myocarditis

Pulmonary involvement
Pleural effusion
Pulmonary infiltration

Ocular involvement
Uveitis

Noncaseating epithelioid and giant cell granulomas, most often lymph node granulomas

PCR may be performed to detect *T. whipplei* in samples from a variety of tissue types and body fluids, including gastric and small-bowel biopsies and stools.[18] Currently, quantitative real-time PCR assay targeting repeated sequences of *T. whipplei* using fluorescent labeled oligonucleotide hybridization probes for specific identification is the best tool to establish the diagnosis of WD.[18] A diagnostic strategy for classic WD using this tool and depending on results of saliva and stool specimens has recently been proposed (see Fig. 36.4).[9] It is also critical to include positive and negative controls systematically to validate each PCR run. Cultivation of *T. whipplei*, using mammalian cell cultures and axenic medium supplemented by amino acids from various specimens, including small-bowel biopsies and stools, has been achieved. However, this technique is not generally available. Serologic diagnosis of classic WD is not available.

Treatment

WD was fatal before the advent of antibiotics. Moreover, the treatment is still empirical. Current recommendations, which include oral administration of trimethoprim (160 mg) and sulfamethoxazole (800 mg) twice daily for 1–2 years, usually preceded by parenteral treatment with ceftriaxone (2 g daily) or streptomycin together with penicillin G (2 g and 1.2 million units daily, respectively) for 2 weeks, are not based on therapeutics trials or the susceptibility of *T. whipplei* to antibiotics.[18] The reported relapse rate is as high as 23% after antibiotic cessation.[18]

With the recent culture of *T. whipplei*, susceptibility tests and full genome sequencing have been achieved. Many antibiotics, including doxycycline and trimethoprim–sulfamethoxazole, are active *in vitro*.[18] In axenic culture, ceftriaxone and levofloxacin are active; in cell culture, however, cephalosporins (including ceftriaxone) and fluoroquinolones are not active. Genomic analysis has pointed out that *T. whipplei* lacks the coding sequence for dihydrofolate reductase, a trimethoprim target. *In-vitro* tests confirm that trimethoprim is not active, whereas sulfonamide compounds, such as sulfamethoxazole and sulfadiazine, are active.[18] Interferon gamma (IFN-γ) has been proposed for treatment of recurrent WD, and success lasted for at least 1 year after IFN-γ therapy was stopped.[18] A combined regimen of oral doxycycline (200 mg daily) and oral hydroxychloroquine (200 mg thrice daily), the only successful bactericidal regimen against *T. whipplei in vitro*, has been proposed in patients with WD without neurologic symptoms and negative PCR on CSF. In cases with neurologic involvement, high-dose oral sulfamethoxazole or sulfadiazine must be added to the regimen described above.

There is no established marker to determine treatment duration at the present time. By analogy with other chronic infections,[18] it would seem reasonable to use this regimen for at least 18 months. Only a long-term follow-up of patients, ideally during all-life, will allow determination of the best management of WD.

Small intestinal bacterial overgrowth

Small intestinal bacterial overgrowth (SIBO) is a condition caused by an abnormal number of bacteria in the small intestine.[13,14] Related symptoms are malabsorption and diarrhea.[13,14] This entity was first described in patients in the context of abnormal or

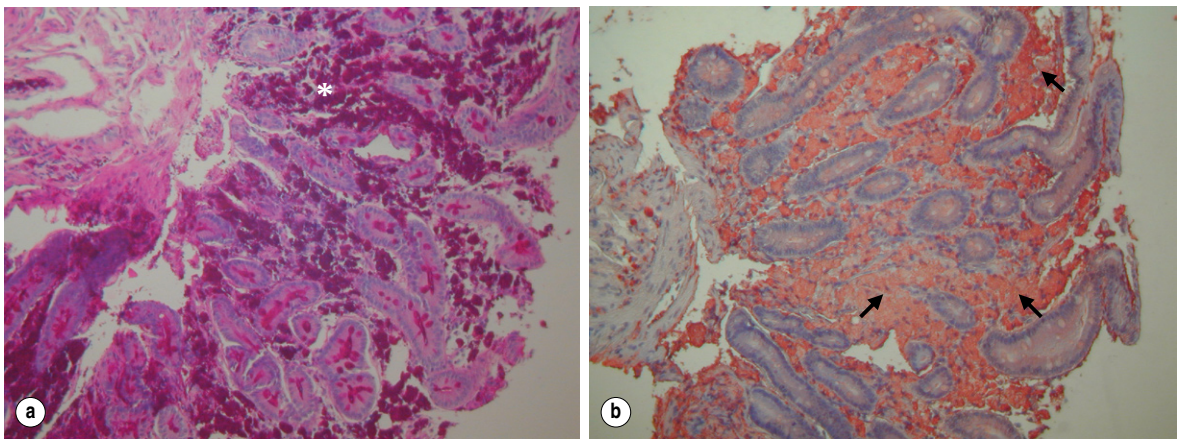


Fig. 36.5 Duodenal biopsies from a patient with classic Whipple's disease. (a) Duodenal biopsy specimen with reduced villous architecture and PAS-positive (asterisk) macrophages in the lamina propria. Periodic acid–Schiff stain. (b) Demonstration of *T. whipplei* by immunohistochemistry (arrows) in duodenal biopsy. Polyclonal rabbit anti-*T. whipplei* antibody used at a dilution of 1:2000 with hemalun counterstain. Courtesy of Hubert Lepidi.

postsurgical anatomy. The characteristic situation where SIBO was found to be most prevalent was the stagnant loop syndrome. Since then, there has been increasing suspicion of a bacterial overgrowth-like entity in many other diseases, adding to the confusion.[13,14] Indeed, currently, there is no adequately validated test for SIBO.[13,14] Although not widely accepted, the diagnosis may be suggested on small-bowel culture with a colony count $>10^5$/ml or on hydrogen breath test.[13,14] Thus, a better method for identifying SIBO accurately is needed.

The treatment of SIBO should address different aims: removal of the predisposing condition, nutritional support and suppression of the contaminating bacterial flora. Up to now, there has been no conclusive information on the most effective therapeutic approach.[22]

PARASITIC CAUSES

Parasitism and diarrhea are both endemic in areas with suboptimal sanitation.[23] Parasitic infections are mainly observed in travelers returning from developing countries and in immunocompromised people. The more frequent parasitic causes are *Giardia*, *Cryptosporidium* and *Entamoeba histolytica*.[5] In contrast to infection with intestinal protozoans, those with intestinal helminths are less common in returning travelers.[24]

To detect parasites, three or more stool samples should be examined. Special stains or concentration protocol may be required to look for some parasites that are also identified by appearance and size. Testing by means of enzyme immunoassay is considerably better than older methods based on microscopic examination.[4] Recently, real-time PCR assays targeting various protozoan or helminthic DNA sequences have become available.[25–28] Stool samples mixed with ethanol allow the samples to be stored and transported at room temperature to laboratories with the appropriate facilities for DNA extraction and detection. Obviously, PCR will not be appropriate as a routine diagnostic tool in clinical settings in endemic areas where laboratory resources are often limited. Serologic tests for intestinal protozoans and helminths are not really useful or helpful. If stool analysis does not reveal the cause of the diarrhea, additional tests may include endoscopy. Main parasitic causes of chronic diarrhea with their more relevant epidemiologic, clinical, diagnostic and therapeutic characteristics are summarized in Table 36.4.

Protozoan parasites

Giardiasis

Giardiasis is caused by the flagellated protozoan *Giardia duodenalis* (former *G. lamblia* or *G. intestinalis*). *G. duodenalis* is one of the major causes of parasitic diarrhea worldwide. In the developing world, giardiasis is pandemic, with peak prevalence rates of up to 20% in children less than 10 years of age.[23] It is an important cause of chronic diarrhea in travelers returning from developing countries, with an infection rate of 1–3% in short-term visitors to endemic areas.[23] Major routes of transmission are consumption of contaminated water and food or by

Table 36.4 Main parasitic causes of chronic diarrhea with their more relevant epidemiologic, clinical, diagnostic and therapeutic characteristics

Microorganisms	Modes of transmission and common epidemiologic settings	Clinical features	Diagnosis	Treatment
Protozoans				
Giardia duodenalis	Fecal–oral route, IgA deficiency	Most infections are asymptomatic Watery diarrhea/malabsorption Abdominal pain	Stools; if negative, small-bowel aspiration or biopsies: – ME – immunofluorescence, ELISA methods – PCR	Treatment recommended in symptomatic and asymptomatic disease to reduce transmission: metronidazole 250–750 mg po q8h for 7 days
Cryptosporidium parvum	Fecal–oral route, immunocompromised host	Watery diarrhea/malabsorption Immunocompetent host: usually asymptomatic or mild self-limited gastroenteritis, occasionally lasting more than 1 month Immunocompromised host: chronic, more often severe diarrhea, dehydration, weight loss	Stools; if negative, small-bowel aspiration or biopsies: – ME with modified acid-fast stain – immunofluorescence, ELISA methods – PCR	Generally not necessary in immunocompetent individuals Severe disease/ immunocompromised host: supportive therapy, no single effective therapy; nitazoxanide may be considered In patients with AIDS, HAART sufficient to achieve immunologic reconstitution is most effective
Cyclospora cayetanensis	Fecal–oral route, travel	Watery diarrhea/malabsorption	Stools; if negative, small-bowel aspiration or biopsies: ME with modified acid-fast stain	Trimethoprim–sulfamethoxazole (co-trimoxazole) 160–800 mg po q8h for 10 days

(Continued)

Table 36.4 Main parasitic causes of chronic diarrhea with their more relevant epidemiologic, clinical, diagnostic and therapeutic characteristics

Microorganisms	Modes of transmission and common epidemiologic settings	Clinical features	Diagnosis	Treatment
Entamoeba histolytica	Fecal–oral route Travel to tropical regions, recent emigration from such region	Asymptomatic colonization: ~90% Bloody diarrhea/dysentery	Stools; if negative, colonic biopsies: – ME – immunofluorescence, ELISA methods – PCR	Generally recommended for symptomatic and asymptomatic individuals to prevent transmission: metronidazole 750 mg po q8h for 5–10 days plus treatment for 7 days with paromomycin 500 mg po q8h
Isospora belli	Fecal–oral route, immunocompromised host	Watery diarrhea/malabsorption Immunocompetent host: acute-self-limited Immunocompromised host: chronic, occasionally severe diarrhea, dehydration, weight loss	Stools; if negative, small-bowel aspiration or biopsies: – ME with modified acid-fast stain – PCR	Trimethoprim–sulfamethoxazole (co-trimoxazole) 160–800 mg po q12h for 7–10 days
Microsporidia	Fecal–oral route, immunocompromised host	Watery diarrhea/malabsorption Immunocompetent host: rare cause of acute self-limited diarrhea Immunocompromised host: chronic diarrhea, dehydration, anorexia, weight loss	Stools; if negative, small-bowel aspiration or colonic biopsies: – ME with special stain – PCR	May be indicated in immunocompromised patients: albendazole 400 mg po q12h for 3 weeks In HIV-infected patients, HAART sufficient to achieve immunologic reconstitution is most effective
Balantidium coli	Fecal–oral route	Bloody diarrhea/dysentery	Stools: ME	Tetracyclines po for 10 days
Helminths				
Strongyloides stercoralis	Developing countries, immunocompromised host	Watery diarrhea/malabsorption Immunocompromised host (HTLV-1): chronic diarrhea	Stools; if negative, small-bowel aspiration or biopsies: ME	Ivermectin 200 µg/kg po q24h for 2 days
Trichuris trichiura	Fecal–oral route	Bloody diarrhea/dysentery	Stools; if negative, rectal biopsies: ME	Mebendazole 100 mg po q12h for 3 days Albendazole 400–600 mg po, single dose

ELISA, enzyme-linked immunosorbent assay; HAART, highly active antiretroviral therapy; ME, microscopic examination; PCR, polymerase chain reaction; po, per os (oral).

direct fecal–oral contact. Chronic infection has been associated with hypogammaglobulinemia, protein-calorie malnutrition, previous gastrectomy and use of immunosuppressive medication.[23] There is no particular increase in incidence among the HIV-infected population.[23]

The clinical spectrum of giardiasis ranges from asymptomatic carriage, through acute diarrhea to severe chronic diarrhea with intestinal malabsorption and growth failure in children.[29] Chronic infection is associated with profound malaise, lassitude, epigastric discomfort, headaches and steatorrhea.[23] Patients who develop recurrent diarrhea after treatment for giardiasis may have associated lactose intolerance rather than relapse of their infection.[23]

The most common method for diagnosis is microscopic examination of the stools for trophozoites using either wet mount or trichrome stain. Immunofluorescent- and enzyme-linked immunosorbent assay (ELISA)-based methods have been developed.[30] Highly sensitive and specific real-time PCR procedures have been validated for the detection of *G. duodenalis*.[25,26] In patients with negative stools, small-bowel aspiration or biopsies may be considered.

For treatment, the drug of choice is oral metronidazole.[23] The adult dose is 250 mg and for children 5 mg/kg three times daily for 7 days. Pregnant women may be treated with paramomycin.[23]

Cryptosporidiosis (see also Chapter 108)

Cryptosporidiosis in humans is most commonly due to *Cryptosporidium parvum*, a coccidian parasite. The number of cases has risen dramatically since the description of HIV infection. Cryptosporidiosis is described worldwide in both immunocompetent and immunocompromised hosts. When clinical stool specimens are examined for *Cryptosporidium* oocysts, approximately 1–3% are positive in North

America and Europe and 5–10% in developing countries. Risk factors for the acquisition of cryptosporidiosis include transmission via the fecal–oral route, contact with farm animals or pets, drinking contaminated water and eating contaminating food.[30]

The clinical manifestations depend on the host response. In immunocompetent hosts, the most commonly identified symptom is acute diarrhea, with watery stools that typically resolve in 10–14 days; prolonged disease is uncommon.[31,32] In immunocompromised hosts, disease onset is usually insidious. Watery diarrhea may gradually increase over weeks to months.[33] Weight loss, profound dehydration, abdominal pain and cramping are common. Symptoms typically persist unless there is improvement in the immunologic status.

Infection can be diagnosed by identification of oocysts in stools using modified Ziehl–Neelsen stain, immunofluorescence with monoclonal antibodies and commercially available ELISA.[30,34] Highly sensitive and specific real-time PCR procedures have been also validated for the detection of *Cryptosporidium* spp.[25,27] In patients with negative stools, small-bowel aspiration or biopsies may be considered.

Treatment of the immunocompetent host is supportive because cryptosporidiosis is self-limited.[30] Few options exists for HIV-infected patients in whom highly active antiretroviral therapy fails.[30] Monotherapy with spiramycin or paromycin has been reported to be active but efficacy has not been confirmed by controlled trials and a majority of patients relapse.[23,30] Nitazoxanide has been considered as a promising agent but clinical trials performed in the USA demonstrated little efficacy.[30]

Cyclosporiasis

Cyclosporiasis is caused by a coccidian parasite, *Cyclospora cayetanensis*.[23] Cyclosporiasis is described worldwide but more frequently in tropical countries. Its prevalence is unknown. Transmission occurs via the oral route, and local outbreaks have been traced to contaminated water or food supply.

C. cayetanensis is the cause of persistent diarrhea in immunocompetent and immunocompromised patients. Other common symptoms are abdominal pain, nausea, vomiting and anorexia. The duration of illness ranges from 4 to 107 days.[35] In HIV-infected patients, *C. cayetanensis* results in chronic diarrhea.[30]

Diagnosis is dependent on microscopic demonstration of morphologic characteristics, staining properties and autofluorescence under ultraviolet light.[35] In contrast to *Cryptosporidium*, staining is variable with the modified Ziehl-Neelsen stain. Measurement of oocysts is recommended to distinguish *C. cayetanensis* (8–10 µm) from *C. parvum* (3–5 µm).[30] In patients with negative stools, small-bowel aspiration or biopsies may be considered.

Trimethoprim–sulfamethoxazole (160–800 mg, twice daily) for 10 days is the treatment.[30]

Amebiasis (see also Chapter 110)

Amebiasis caused by *Entamoeba histolytica* and *Entamoeba dispar* is especially prevalent in Mexico, India, Africa and Central and South America. These species are morphologically indistinguishable but can be differentiated by monoclonal antibodies and DNA probes.[30]

Infections with *E. dispar* are characteristically asymptomatic, do not elicit a serologic response and are responsible for the majority of infections with *Entamoeba* species. In contrast, infections with *E. histolytica* result in symptomatic illness (80–98%) or invasive disease (2–20%) and the production of serum antibodies. In indigenous populations, asymptomatic carriage of *E. histolytica* is common. Major routes of transmission are consumption of contaminated water and food or by direct fecal–oral contact.[23] Individuals at highest risk for infection include persons who travel to developing nations, immigrants or migrant workers from areas of high endemicity, immunocompromised persons and persons housed in mental institutions.[23] *E. histolytica* is not a common cause of traveler's diarrhea but can be a cause of chronic diarrhea in travelers returning from developing countries.

Patients may be asymptomatic or present with colicky abdominal pain, frequent bowel movements and tenesmus.[23] Amebic dysentery is characterized by blood-stained stools with mucus occurring up to 10 times a day.[23] The duration of the dysentery can be very variable and may last for only a few days or for several months with concomitant weight loss.[23] Chronic amebic colitis is clinically indistinguishable from idiopathic inflammatory bowel disease.[30] Because corticosteroid therapy may result in perforation, stool examination for trophozoites should be performed prior to making a diagnosis of inflammatory bowel disease.[30]

Identification of *E. histolytica* cysts and trophozoites requires examination of a fresh stool sample and a trichrome stain.[30] New fecal antigen detection methods (monoclonal antibodies, ELISA) may also prove useful.[30,36] Real-time PCR procedures have been recently developed for the detection of *E. histolytica*.[25] In the presence of negative stool microscopy, colonic biopsies may sometimes be useful.

Invasive disease, such as severe colitis, should be treated with oral metronidazole (750 mg thrice daily) for 10 days, followed by an oral luminal agent such as diloxanide (500 mg thrice daily) or paramomycin (500 mg thrice daily) to prevent future invasion with any remaining cysts.[23]

Isosporiasis

Isosporiasis is caused by the coccidian parasite, *Isospora belli*. Infections have been described worldwide but with an increased prevalence in tropical and subtropical climates and in areas with poor sanitation.[30] Transmission is associated with contaminated water, although that route has not been proven.[30] Little is known about the prevalence of human infection. In a limited number of studies employing adequate techniques, 0.1–1.8% of stools were positive.[23] Isosporiasis is observed in HIV-infected patients with an incidence ranging from approximately 0.2 to 20% in tropical areas. *I. belli* has also been described in transplant patients (liver, renal) as well as in patients with lymphoblastic leukemia, adult T-cell leukemia, Hodgkin's and non-Hodgkin's lymphoma, either prior to or after chemotherapy, and in patients with human T-cell leukemia virus 1 (HTLV-1) infection.[37,38]

The predominant clinical symptoms are diarrhea-associated weight loss, abdominal pain and sometimes fever.[39,40] Stools are watery and foamy, suggesting malabsorption. While symptoms are usually self-limited in immunocompetent hosts, patients are often ill for 6 weeks to 6 months. In HIV-infected patients, isosporiasis presents with an insidious onset of diarrhea, weight loss and abdominal pain.[23]

Stool specimens frequently contain Charcot–Leyden crystals and fat. Peripheral eosinophilia is frequent.[30] Infection can be diagnosed by identification of oocysts in stools using acid-fast stains, such as the modified Ziehl–Neelsen method.[30] Real-time PCR has been recently developed.[28] If stool examination remains negative, even in cases of severe diarrhea, small-bowel aspiration or biopsies may be necessary to establish the diagnosis.

Isosporiasis responds promptly to oral trimethoprim–sulfamethoxazole (160–800 mg, four times daily) for 10 days in immunocompetent patients.[23] For HIV-infected patients, approximately half will relapse if not placed on secondary prophylaxis; therefore secondary prophylaxis should be maintained, using oral trimethoprim–sulfamethoxazole (160–800 mg) thrice weekly.[23]

Microsporidiosis

Microsporidiosis is caused by microsporidia, intracellular protozoan parasites belonging to the phylum *Microspora*.[23] Two species are associated with enteric infection, *Enterocytozoon bieneusi* and *Encephalitozoon intestinalis* (former *Septata intestinalis*). The former is more common. Little is known about the epidemiology of microsporidiosis. Serologic surveys suggest that infection with these organisms is widespread.[23] Spread of the organisms through environmental, person-to-person (fecal–oral, aerosolized respiratory secretions) and animal-to-person transmission have been postulated.[23] The prevalence of infection in selected groups of HIV-infected patients in different countries has a range of 1.7–30%.[30]

Microsporidia cause acute self-limiting diarrhea in immunocompetent persons and in patients who have immunodeficiency other than HIV infection.[41] In immunocompromised HIV-infected patients with CD4 lymphocyte counts <100 cells/mm^3, *E. bieneusi* is responsible for chronic watery diarrhea.[23,42] *E. intestinalis* is a less commonly recognized cause of chronic diarrhea.[23]

Spores of microsporidia can be detected in stools and in small-bowel and colonic biopsy specimens using Giemsa, Weber's trichrome or fluorochrome stains (Calcofluor, Uvitex 2B), but identification of the specific species requires electron microscopy or PCR.[30,43]

No real therapeutic options exist for HIV-infected patients in whom highly active antiretroviral therapy fails.[30] Preliminary data suggest that albendazole may be of use in the treatment of infections with *E. intestinalis* but not for *E. bieneusi*.[23]

Balantidiosis

Balantidiosis is caused by *Balantidium coli*, the only ciliate associated with human infection.[23] It has a widespread distribution, but is only rarely found in human stools (<1% in all surveys).[23] Many cases are asymptomatic.[23] A chronic form has been described with loose movements, abdominal pain, tenesmus, mucus in the stools and weight loss.[23] Diagnosis is based on demonstration of the trophozoites in stools.[23] Tetracyclines for 10 days are recommended as treatment.[23]

Helminths and chronic diarrhea

Strongyloidiasis (see Chapter 108)

Strongyloidiasis is caused by *Strongyloides stercoralis*, a helminthic parasite of worldwide distribution.[24] The prevalence is 5–10% in tropical countries. Humans are usually infected from moist soil by transcutaneous penetration. The clinical behavior of this disease is believed to be largely dependent on the host's immune response.

S. stercoralis can be associated with persistent watery diarrhea, cramping abdominal pain and weight loss with evidence of malabsorption, particularly when there is hyperinfection or disseminated strongyloidiasis.[24] This massive invasion by *S. stercoralis* can be observed in people debilitated by concurrent disease or malnutrition, patients treated with immunosuppressive drugs harboring *S. stercoralis*, patients infected with HTLV-1 but not in HIV-infected patients.[44] Eosinophilia is a prominent feature of this infection.

Diagnosis depends on demonstration of *S. stercoralis* larvae in stools, or in small-bowel aspiration or biopsies. Culture can be also undertaken. Ivermectin (200 µg/kg/day) for 2 days is the treatment of choice.[24] Albendazole can also be used, but may be less effective.[24]

Trichuriasis

Trichuriasis caused by the whipworm *Trichuris trichiura* is prevalent worldwide, with approximately 400 millions persons infected.[24] When few worms are present there is little damage, but heavy infections (more than 200 adult worms recovered after antihelminthic expulsion) cause a clinical syndrome characterized by chronic dysentery, predisposition to rectal prolapse, anemia and digital clubbing.[45] Reduced childhood growth rates, reduced food intake, iron deficiency and gastrointestinal protein loss are seen in heavy infections.[24] *T. trichiura* has been also described as an unusual cause of chronic diarrhea in a renal transplant patient.[46]

Most symptomatic cases can be readily diagnosed by routine microscopic stool examination.[24] The diagnosis is made when numerous worms are seen on the rectal mucosa. Treatment with mebendazole (100 mg twice daily) for 3 days is highly effective, as well as albendazole in a single dose of 400–600 mg.[24]

Trichinosis

Trichinosis caused by *Trichinella spiralis* is widely distributed throughout the world, except in Australia and parts of the South Pacific.[24] The occurrence of diarrhea of short duration following the ingestion of infected meat has been well described.[24] Intestinal symptoms typically resolve spontaneously. There is no reliable therapy.

A new clinical syndrome has been reported in the Inuit population in northern Canada.[24] These patients presented with chronic diarrhea, lasting up to 14 weeks. They also had only mild and transitory myalgias without fever. These clinical manifestations differ considerably from those of classic trichinosis, suggesting that this may be due to a distinct strain of parasite.

Capillariasis

Capillariasis is caused by a small nematode, *Capillaria philippinensis*, initially described in patients from the Philippines.[24] Since then, Thailand has been found to be endemic for the disease, and a few cases have been identified in Japan, Taiwan, Iran, Egypt and India.[24] Human infection results from consumption of raw fish. *C. philippinensis* can cause chronic diarrhea and malabsorption.[47]

The detection of *C. philippinensis* is based on the recovery of eggs, larvae and/or adult worms in the stool of the patients. However, small-bowel aspiration or biopsies may be necessary to confirm capillariasis. Oral treatment with mebendazole 200 mg twice daily for 20 days or thiabendazole 25 mg/kg/day has been used successfully.[24] However, relapse can occur. Albendazole (400 mg/day for 10 days) is currently the drug of choice since it appears to act on adult as well as larval stages of the parasite, thus reducing relapse.[24]

Schistosomiasis (see Chapter 112)

Colorectal schistosomiasis (usually *Schistosoma mansoni* infection) can present with chronic bloody diarrhea, due to the resulting granulomatous inflammation of the colon seen during the chronic phase in patients with heavy infections.[47]

Fungus

Histoplasmosis is caused by *Histoplasma capsulatum*, a fungus that is endemic in America and Africa.[12] It is a soil fungus that proliferates especially in soil enriched by bat and bird droppings.[12] Infection occurs by inhalation of conidia, and it is confined initially to the respiratory system. Severely immunocompromised patients are susceptible to disseminated histoplasmosis, which may involve the digestive tract and cause diarrhea.[12] An isolated colonic histoplasmosis infection was recently reported in a man who presented in end-stage renal disease, for which he was on chronic hemodialysis.[48]

Histologic examination of PAS-stained or silver-stained gastrointestinal biopsies allow detection of the fungus.[48] Treatment is based on amphotericin or itraconazole. Clinical presentation, diagnosis and treatment for histoplasmosis are summarized in Table 36.2.

VIRAL CAUSES

Usual viral microorganisms (rotavirus, astrovirus, adenovirus, norovirus) responsible for acute diarrhea are not involved in chronic diarrhea except perhaps in specific immunocompromised hosts. Indeed, rotavirus and astrovirus diarrhea may continue for months in children who have defects in T-cell function (e.g. DiGeorge syndrome, cartilage–hair hypoplasia syndrome).[49] Adenovirus is also a cause of chronic diarrhea in patients with immunodeficiency, mainly HIV-infected patients, in whom the infection is commonly systemic.[12,50] Light microscopic examination of colonic biopsies can identify adenovirus colitis which is significantly associated with chronic diarrhea, and in addition may facilitate gastrointestinal co-infection with cytomegalovirus.[12] Finally, norovirus and adenovirus have also been identified as responsible for chronic diarrhea in a transplanted child.[51]

Cytomegalovirus and herpes simplex virus are both specifically responsible for chronic diarrhea due to ulcerating chronic infection in immunocompromised patients, including HIV-infected patients and transplant recipients.

Cytomegalovirus

Cytomegalovirus is a herpesvirus and is the commonest viral cause of diarrhea in HIV-infected patients.[12] Cytomegalovirus is ubiquitous and endemic in humans. In individuals with normal immune systems, this organism is usually latent and asymptomatic. The occurrence of cytomegalovirus in the gastrointestinal tract, any part of which can be affected, is evidence of systemic infection and is related to failure of the dysfunctional immune system to maintain latency. It may cause enteritis, colitis, ulcers, appendicitis, ileocaecal obstruction, toxic megacolon, perforations and gastrointestinal hemorrhage.[12] Diagnosis and treatment are summarized in Table 36.2. (See also Chapter 91.)

Herpes simplex virus (HSV)

Although most humans are infected at some time in their lives by HSV-1 or HSV-2, diarrhea caused by these double-stranded DNA viruses are rare, virtually confined to individuals with impaired immunity.[12] Diagnosis and treatment are summarized in Table 36.2.

CHRONIC (POSSIBLY INFECTIOUS) DIARRHEA

Tropical sprue

Tropical sprue is associated with chronic diarrhea, malabsorption and nutritional deficiency in patients who live in or have visited tropical areas.[52] Pathogenesis is still unknown but an infectious cause is suspected.[52,53]

Tropical sprue starts with an acute intestinal infection (bacterial, viral or parasitic) which can affect predominantly the small or the large intestine.[52,53] Macrocytic anemia and hypoalbuminemia are present, together with progressive villus atrophy of the small intestine. Treatment with tetracycline, folic acid and nutritional support is generally effective but relapses after treatment are common.[52]

Brainerd diarrhea

A form of chronic diarrhea showing worldwide distribution and manifesting as outbreaks is Brainerd diarrhea from Brainerd, Minnesota.[54] The source is characteristically unpasteurized milk or untreated water. Despite intensive laboratory examinations, the cause of Brainerd diarrhea remains unknown. Extensive laboratory and environmental testing for bacterial, parasitic, mycotic and viral agents did not identify an etiologic agent.

Treatment with a wide variety of antimicrobial agents gives no significant clinical response. The prognosis is good, with diarrhea generally subsiding after 6 months to 1 year.

PERSPECTIVES

Research using modern techniques may be able to settle some of the unanswered issues and open new fields. For example, the advent of metagenomic sequencing has enabled systematic and unbiased characterization of microbial populations, including in children with acute diarrhea.[55] Thus, metagenomic approaches have the potential to define the spectrum of microorganisms, including novel microorganisms, present in stools during episodes of chronic diarrhea. The detection of novel or unexpected microorganisms would then enable investigations to assess whether these agents play a causal role in human chronic diarrhea.

REFERENCES


References for this chapter can be found online at http://www.expertconsult.com



Chapter 37

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Intra-abdominal sepsis, peritonitis and pancreatitis

INTRODUCTION

Intra-abdominal infection is a common clinical problem that contributes significantly to surgical, intensive care and laboratory workloads. The severity of infection may vary from mild appendicitis with minimal systemic upset to feculent peritonitis with multiorgan failure. Pancreatitis, by initiating an inflammatory cascade, can mimic peritonitis and although the inflammatory process is initially sterile, it may progress to fulminant, life-threatening sepsis. Management of peritonitis, intra-abdominal sepsis and pancreatitis remains one of the most challenging and resource-consuming activities of modern critical care. Therapy of intra-abdominal sepsis requires surgery or percutaneous drainage, antimicrobial therapy and resuscitation. Each pillar complements the other, and failure to meet an adequate standard in one may undo the benefit of the others.

ANATOMY

The peritoneum is the largest cavity within the body with a surface area that approximates that of the skin. The parietal peritoneum is lined by a surface layer of mesothelial cells supported by a lamina propria and loose connective tissue which covers the inner surfaces of the abdominal wall, the diaphragm and the pelvis, and is reflected onto the external surfaces of the abdominal organs as the visceral peritoneum. The visceral peritoneum has no somatic innervation whereas the parietal peritoneum overlies a well-vascularized subserosa with extensive lymphatic drainage and somatic innervation.[1] The peritoneal cavity is separated by the lesser or gastrocolic omentum into the greater and lesser sacs, while the greater sac is divided by the great omentum into supra- and infracolic compartments. The greater sac is functionally further divided by the viscera it contains into subphrenic, subhepatic, paracolic and pelvic spaces. The latter comprises the rectovesical pouch in males and the rectouterine pouch (of Douglas) in females. Knowledge of peritoneal anatomy is of importance to the surgeon and interventional radiologist in planning treatment of intraperitoneal pathology.

The peritoneal cavity normally contains a small volume (approximately 50 ml) of fluid which provides lubrication and facilitates peristalsis and other movements of abdominal viscera. The peritoneal fluid is secreted throughout the peritoneal cavity but is primarily absorbed through the pelvic and subphrenic peritoneum. This reflects the effects of gravity, capillary attraction and changes in intra-abdominal pressure occasioned by respiration. It explains the propensity for infected peritoneal fluid to cause subphrenic and pelvic collections and the incidence of transcelomic spread of malignant cells to these areas. The luminal surface of the mesothelial cells has numerous microvilli which act to protect the delicate surface by entrapping water and serous exudates.[2]

MICROBIOLOGY

In health, the low pH of the stomach maintains the number of organisms at less than 10^3 bacteria per mm^3, with a microflora similar to that of the oral cavity and upper respiratory tract. Organisms present are usually viridans streptococci, lactobacilli or *Candida* species, all of low pathogenic potential. Proton pump inhibitors or other causes of achlorhydria may allow the microflora of the stomach to approximate that of the small bowel, with increasing numbers of faculta tive Gram-negative bacilli, such as *Escherichia coli*, and strict anaerobes. Many intubated and ventilated patients in intensive care units have an increase in bacterial density in stomach fluid, with a shift towards aerobic Gram-negative bacilli, including *E. coli*, *Pseudomonas aeruginosa* and also *Candida*. Nasogastric and percutaneous endoscopic gastrostomy (PEG) feeding tubes, malignancy and bariatric surgery cause similar alterations.

The density of the normal microflora progressively increases from the stomach to the colon. In the proximal small bowel there are ~10^{4-5} bacteria per mm^3, whereas the terminal ileum contains 10^7–10^9 organisms per mm^3. In the presence of intestinal pathology such as scleroderma, celiac disease or other conditions causing stasis or diverticula, the microflora of the small intestine often more closely resembles that of the colon. A similar phenomenon occurs in patients with decompensated cirrhosis. The terminal ileum in health is a transition zone with equal numbers of strict anaerobes and facultative or aerobic organisms.

The highest absolute numbers of bacteria are found in the colon, with in excess of 10^{11} bacteria/gram of feces and more than 400 species. Strict anaerobes outnumber facultative and aerobic bacteria by a ratio of 1000 to 1 or greater. These include large numbers of various species of *Bifidobacterium*, *Propionibacterium*, *Fusobacterium*, *Lactobacillus*, *Veillonella*, *Eubacterium* and *Clostridium*. However, with the exception of *Bacteroides fragilis*, other members of the *B. fragilis group*, *Fusobacterium* species and *Bilophila wadsworthia*, they generally contribute little to clinical intra-abdominal infection.

The genus Bacteroides is now limited to the *B. fragilis* group, which numbers greater than 20 species. The most common pathogen within the *B. fragilis* group is the *B. fragilis* species itself, which although comprising a minority (1–10%) of the group within the normal colonic microflora, is the most common anaerobic pathogen (31%) and the most virulent of the group. Other species of the group commonly isolated in intra-abdominal sepsis are *B. thetaiotaomicron* (15.4%), *B. ovatus* (10.9%), *B. vulgatus* (9.4%), *B. uniformis* (4.9%) and *B. distasonis* (10%) (renamed *Parabacteroides distasonis* in 2006).

Most intestinal aerobic Gram-negative bacilli are Enterobacteriaceae, which as facultative anaerobes can survive and multiply in the relatively anaerobic environment of the colon. *E. coli* is the most common of the Enterobacteriaceae; others include species of *Klebsiella, Enterobacter, Serratia, Proteus, Providencia, Morganella, Citrobacter* and *Hafnia*. Other Gram-negative but strictly aerobic bacilli such as *Pseudomonas aeruginosa* and *Acinetobacter* species are usually transient in the normal gastrointestinal flora of healthy humans and in most populations are uncommon pathogens in community-acquired intra-abdominal sepsis. However, their frequency increases after prolonged hospitalization, especially with exposure to antimicrobial agents and requirement for intensive care. *Enterococcus faecalis* is present in feces in large numbers, as high as 10^{10}/g.

Studies from the 1970s and 1980s using aerobic techniques in patients with proven intra-abdominal infection show a mean of 3.8 organisms per patient, about half anaerobes and the others aerobic or facultative. *Pseudomonas* made up only about 6% of the aerobes. The most common aerobic pathogen was *E. coli*, accounting for 30% of the aerobes. Other aerobic Gram-negative bacilli also comprised 30%. Enterococci and Group D streptococci made up 12% of aerobic organisms. The *B. fragilis* group comprised 36% of the anaerobes, with other anaerobic Gram-negative bacilli comprising 20%. Clostridial species made up 18% and anaerobic Gram-positive cocci, 10%.[1] Animal models of intra-abdominal infection have also shown that the initially diverse complex inoculum from the colonic microflora is simplified to a limited set of pathogens. *E. coli*, other Enterobacteriaceae and the *B. fragilis* group are predominant; enterococci are found in a minority. Other anaerobic organisms, although dominant numerically in the fecal flora, are rarely pathogens.

The role of enterococci remains controversial. The balance of clinical and experimental evidence suggests that enterococci are unimportant in community-acquired intra-abdominal sepsis. In animal models, antimicrobial agents directed against Enterobacteriaceae prevent mortality, agents active against anaerobes reduced the incidence of abscesses, but failure to cover enterococci had no effect. Randomized controlled clinical trials of antimicrobial regimens in which one arm was active against enterococci and the other was not, have consistently shown no benefit from anti-enterococcal coverage, including most recently a large trial comparing ertapenem (32% susceptible) to piperacillin–tazobactam (97% susceptible).[3] However, isolation of enterococcus from the initial drainage has been associated with a higher treatment failure rate than in patients without enterococcus (28% versus 14%).[4]

HOST DEFENCE

The lymphatic drainage is of primary importance in defense against the initial inoculum. In the first few minutes of peritoneal contamination, organisms are absorbed directly by the lymphatics or phagocytosed by resident macrophages and dendritic cells and quickly removed by the lymphatics to the lymph nodes of the mediastinum, the thoracic duct and the bloodstream. This is the dominant host defense mechanism in the first few hours, but at a cost of bacteremia and systemic sepsis. The peritoneal polymorphonuclear leukocyte (PMN) response begins at 1–2 hours and is maximal at 24–72 hours. Lipopolysaccharide (LPS) released by Enterobacteriaceae is the major mediator and is recognized by cells of the innate immune system via the LPS receptor complex consisting of CD14, Toll-like receptor 4 (TLR-4) and the myeloid differentiation protein 2 (MD-2). Spontaneous diffusion of LPS is very slow due to its amphiphile structure and tendency to form aggregates in aqueous solution. LPS binding protein (LBP) is an acute phase protein, the systemic levels of which are greatly increased in the first few days of sepsis. Local LBP in the peritoneal cavity greatly enhances the transfer of LPS to the CD14/TLR-4/MD-2 complex and augments the immediate response to LPS up to 1000-fold. This causes a release of cytokines and chemokines, and initiates the inflammatory response.

Encapsulated strains of *B. fragilis* are necessary for abscess formation, as *B. fragilis* strains without a capsule generally fail to induce abscess. Extracted capsular polysaccharide from *B. fragilis* induces a sterile abscess, histologically identical to that induced by live organisms. Athymic or T-cell depleted models show that T lymphocytes are required for the formation of intra-abdominal abscesses and large numbers of $CD4^+$ T cells are present in the abscess wall. The proliferative response of T cells depends on amino acid (positively charged) and carboxyl or phosphate groups (negatively charged) on each repeating unit of the *B. fragilis* polysaccharide. These zwitterionic polysaccharides (ZPSs) are processed to low molecular weight carbohydrates by a nitric oxide-dependent mechanism in endosomes of antigen-presenting cells (APCs) and initially co-localize with HLA-DR MHC class II molecules on the endosomes and surface of the APCs. Co-stimulation via B7-2 and CD40 on the surface of the APC is required. This T-cell dependent abscess formation, unique to *B. fragilis, Staphylococcus aureus* and *Streptococcus pneumoniae* type 1, is an important exception to the general rule that the immune response to bacterial polysaccharide antigens is B-cell dependent and T-cell independent.[5,6] These models also demonstrated that adjuvant substances are critical through the induction of proinflammatory cytokines including tumor necrosis factor (TNF), interleukin (IL)-2, IL-18 and especially IL-17, produced by $CD4^+$ T cells in the abscess wall.

PATHOPHYSIOLOGY

Injury to the peritoneum causes desquamation of the mesothelial cells and exposes the underlying extracellular matrix causing an innate inflammatory reaction characterized by release of matrix-bound proinflammatory cytokines (transforming growth factor beta (TGF-β), TNF and IL-1), chemoattractants (IL-8 and monocyte chemoattractant protein 1 (MCP-1)) and growth factors (TGF-β, IGF-1 and platelet-derived growth factor (PDGF)). Expression of tissue factor, the major initiator of the extrinsic pathway of coagulation, is upregulated by macrophages and mesothelial cells.[7] Activation of the extrinsic pathway leads to a fibrinous exudate which is organized by activated fibroblasts. Contact with adjacent tissue results in formation of fibrin bands, ultimately leading to adhesion formation. The process resolves with ingrowth of mesothelium to cover the injured surface and fibrinolysis driven by the enzyme plasmin, derived from its precursor plasminogen, a process regulated by tissue plasminogen activator (tPA). In the first 24 hours of peritonitis, tPA increases in the peritoneal fluid as high as 65-fold, and is critical to host defense by enhancing the migratory response of polymorphonuclear leukocytes.

The greater the injury to the peritoneal surface, the greater is the inflammatory response. Activation of the adaptive immune system takes longer to develop. Cytokines, released as part of the innate immune response, activate dendritic cells which in turn function as APCs to lymphocytes.[8] Exposure to bacteria leads to activation of Toll-like receptors, the most characterized of which is TLR-4, which recognizes LPS.[8] At least 10 different TLRs have been identified in humans that respond to different pathogen-associated molecular patterns.[9] TLRs are widely present in extracellular matrix and cell surfaces, particularly peritoneal mesothelial cells. In addition, endogenous agonists, such as heparin sulfate, a biologically active saccharide released from cell surfaces and extracellular matrices, also activate dendritic cells via a TLR-4 response. Prior release from constitutive inhibition by exposure to extracellular proteases such as elastase is required for TLR-4 activation.[10] Once activated, TRL-4 stimulates proinflammatory transcription factors such as nuclear factor kappa B (NF-κB) with resultant upregulation of proinflammatory genes. The resulting cascade produces the local and systemic manifestations of the systemic inflammatory response syndrome (SIRS) or sepsis in the presence of bacterial infection.

Whether the inflammation is contained and eradicated depends on the nature of the contamination, the concentration and virulence of infecting organisms and the immune competence of the individual.

The local inflammatory response, fibrin deposition and secondary intestinal ileus act to confine the area of inflammation. The greater omentum frequently becomes adherent, a process facilitated by local ileus. Thus a visceral perforation may 'seal' or contamination be confined and an abscess develop, as for example in diverticulitis. In the absence of continuing bacterial contamination, the host response may be sufficient to resolve the process; more often surgical or radiologic drainage is needed.

CLINICAL PRESENTATION

The history and physical examination define the urgency of intervention and guide decisions regarding diagnostic techniques. The history should determine how long the patient has been ill, where the pain is located, whether it has changed location or character, whether it is associated with anorexia, vomiting or obstipation, and whether the patient has been aware of fever or chills. A pertinent past medical history should include recent hospitalization, medications, especially antimicrobial exposure, chronic disease diagnoses and prior operations.

INVESTIGATION

Imaging and laparoscopy

An erect chest X-ray (Fig. 37.1) or lateral decubitus X-ray of the abdomen may show free intra-abdominal air, indicating perforation of a hollow viscus. Ultrasound examination of the biliary tree and pelvis and CT scans have superseded other diagnostic methods in assessing intra-abdominal sepsis (Figs 37.2, 37.3). The urgency is dictated by the degree of hemodynamic instability present; however, CT is the single best modality for fully evaluating the extent of disease.

Increasingly MRI is of use, particularly in patients who have been exposed to considerable radiation dosage in the course of their disease. Ultrasound is quite versatile and portable, allowing procedures to be performed in the ICU. Ultrasonography is limited by bowel gas, body habitus and lower sensitivity for retroperitoneal processes and parenchymal infection. In suspected biliary sepsis, ultrasound rapidly provides information concerning gallstone disease and biliary obstruction. Laparoscopy is a valuable diagnostic and potentially therapeutic tool in the management of localized peritonitis, particularly in suspected appendicitis, pelvic inflammatory disease, diverticulitis and tuberculous peritonitis.

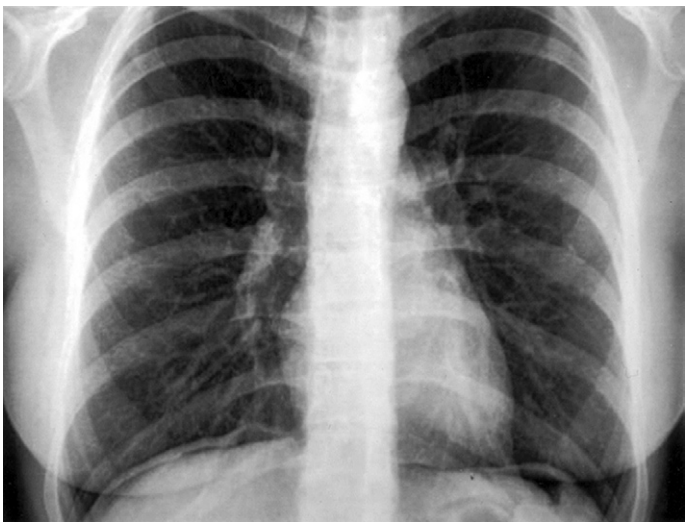


Fig. 37.1 Erect chest X-ray showing free intraperitoneal air.

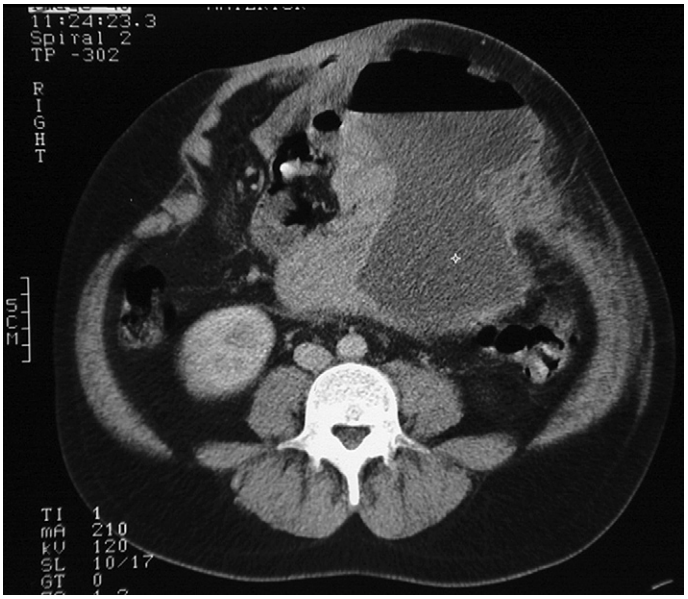


Fig. 37.2 CT abdomen showing a large intraloop abscess secondary to perforation from Crohn's disease.

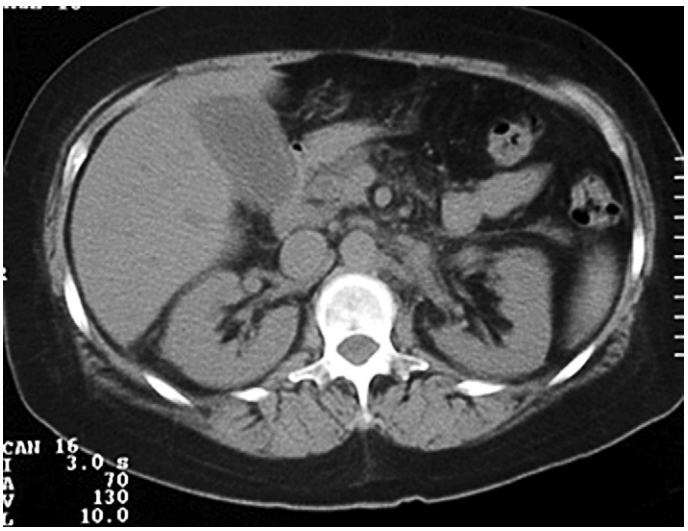


Fig. 37.3 CT abdomen showing thickened gallbladder with intramural air and pericholecystic fluid in a patient with biliary sepsis.

Microbiologic investigations

Blood cultures do not provide additional clinically relevant information for patients with community-acquired intra-abdominal infections. They are recommended in hospital-acquired complicated intra-abdominal sepsis, and when the patient presenting from the community is immunosuppressed or in shock, has a vascular access device, is an intravenous drug abuser or is otherwise at risk of bacteremia due to resistant organisms. With the exception of appendicitis, intra-operative cultures are recommended to detect resistant organisms, as failure rates are higher if therapy is not active against all Gram-negative facultative and anaerobic pathogens, and modifying the antibiotic regimen in response to the aerobic culture result improves outcome. A single sample of a least 0.5 cc of aspirated fluid or tissue is preferred to a swab. If anaerobic cultures are done, the specimen should be transported to the laboratory in an anaerobic transport system. If Gram staining is available in a timely manner

then it may be valuable in health-care associated intra-abdominal infection in defining the need for specific therapy for methicillin resistant *Staphylococcus aureus* (MRSA) or vancomycin resistant enterococci (VRE).

ANTIMICROBIAL THERAPY

General considerations

In choosing the optimal antimicrobial regimen there is always difficulty between the need to cover all possible pathogens so as to minimize morbidity and mortality and the need to avoid the adverse effects of excessively broad-spectrum regimens. The latter include not only greater risk of *Clostridium difficile* superinfection, direct toxicities and cost, but also selection of resistant pathogens in the individual unit, hospital and broader community. The imperative of wise antimicrobial stewardship therefore applies, tempered by knowledge that failure to provide therapy active against the subsequently identified pathogens is associated with increased mortality.[11]

Patients who present from the community with a low risk of mortality should receive narrower spectrum regimens, primarily active against facultative Gram-negative bacilli such as *E. coli*. Agents with activity against the *B. fragilis* group are required for distal small-bowel and colon-derived infections and for more proximal gastrointestinal perforations, when obstruction is present. Enterococcal coverage is not required. Prolonged hospitalization, previous antimicrobial therapy and postoperative peritonitis are associated with more resistant microflora. In this context, and in patients with septic shock, multiorgan failure or high APACHE score, antimicrobial regimens should be both broad spectrum and informed by local nosocomial isolation and susceptibility patterns. This often requires the use of multidrug regimens such as combinations of an extended spectrum penicillin–β-lactamase inhibitor or a carbapenem, with an aminoglycoside, quinolone, tigecycline, vancomycin or antifungal agent.[12]

Timing and duration

Antimicrobial therapy should be initiated without delay, as deterioration to severe generalized peritonitis, septic shock and multiorgan failure can occur over a few hours. Administration of effective antimicrobial agents to patients with septic shock within the first hour of hypotension is associated with a survival of 80%, with each hour of delay causing an increment in mortality of 8% per hour over the first 6 hours.[13] Delay should be overcome, if necessary, by the physician administering the first dose.

Bowel injuries repaired within 12 hours, due to penetrating, blunt or iatrogenic trauma with intraoperative contamination, should be treated with antimicrobial agents for less than 24 hours. Acute perforations of the stomach, duodenum and proximal jejunum in the absence of antacid therapy or malignancy should also be treated for no more than 1 day. Similarly, acute appendicitis, without evidence of gangrene, abscess, perforation or peritonitis requires only 24 hours of an inexpensive regimen.[12]

Antimicrobial therapy for established intra-abdominal infection should be continued until resolution of clinical signs of infection occurs, including normalization of temperature and white blood cell (WBC) count and return of gastrointestinal function. When clinical features of infection persist or recur 5–7 days after definitive surgical and antimicrobial therapy, a thorough diagnostic effort should be undertaken, directed at potential abdominal and extra-abdominal sources. Investigations should include CT or ultrasonographic imaging, and antimicrobial therapy effective against the organisms initially identified should be continued. If a patient has persistent clinical symptoms and signs, but no evidence of a new or persistent infection is uncovered after careful investigation, termination of antimicrobial therapy is warranted.

Antimicrobial susceptibility in the *B. fragilis* group (see also Chapter 173)

Most clinical laboratories neither fully identify organisms to the species level within the *B. fragilis* group nor determine antimicrobial susceptibilities, as results are typically only available after the infection has resolved. Susceptibility testing of anaerobes is recommended when there is persistent isolation of the organism, when bacteremia is present, and when prolonged therapy is needed. Antimicrobial agents for anaerobic infections are selected empirically, based on multicenter surveys of the *B. fragilis* group. The largest of these was reported from 10 centers in the USA, with over 5000 isolates, over 8 years.[14] This reported only one isolate resistant to metronidazole (0.02%) and none resistant to chloramphenicol. The carbapenems and piperacillin–tazobactam had rates of resistance of 1% or less. Ampicillin–sulbactam (2.6%) and tigecycline (4.3%) had acceptable rates for empiric use but cefoxitin (10.3%), clindamycin (25.6%) and moxifloxacin (34.5%) did not. Tigecycline has potential as monotherapy, as *in vitro* it is active against anaerobes, most aerobic and facultative Gram-negative bacilli (except *Pseudomonas* and *Proteus* species), along with MRSA and VRE. However, other reports of higher rates of resistance to tigecycline in the *B. fragilis* group (19%)[15] give cause for concern.

Choice of antimicrobial regimen

A variety of narrower spectrum choices are appropriate for lower risk patients with community-acquired intra-abdominal sepsis. First- or second-generation cephalosporins cover *E. coli* and most other community Enterobacteriaceae. Metronidazole must be added when the *B. fragilis* group is a concern. Ampicillin–sulbactam or amoxicillin–clavulanic acid are active against almost all community-acquired Gram-negative bacilli and against almost all the *B. fragilis* group. Quinolones such as ciprofloxacin, ofloxacin, levofloxacin and moxifloxacin will also cover community-acquired Gram-negative bacilli. Metronidazole should be added when a quinolone is used if the *B. fragilis* group is a concern.

For patients at higher risk, piperacillin–tazobactam, ticarcillin–clavulanic acid or the carbapenems (namely imipenem–cilastatin, meropenem, ertapenem and doripenem) are all appropriate as monotherapy. However, generally carbapenems should be held in reserve to avoid selection of resistance against this important class. A third- (cefotaxime, ceftriaxone, ceftizoxime, ceftazidime) or fourth- (cefepime) generation cephalosporin, or a monobactam such as aztreonam, are also appropriate, but all must be combined with metronidazole. For health-care associated intra-abdominal sepsis, additional coverage against MRSA, VRE or a second agent for resistant Gram-negative bacilli may be warranted. Ceftobripole and ceftaroline are new cephalosporins with excellent *in-vitro* anti-MRSA and anti-enterococcal activity (including VRE), while maintaining activity against Enterobacteriaceae, other than extended-spectrum β-lactamase (ESBL) producers and *P. aeruginosa*. Their role in combination with metronidazole for intra-abdominal sepsis awaits the outcome of clinical trials.

Because aminoglycosides have narrow therapeutic ranges and cause nephrotoxicity and ototoxicity, they are no longer recommended as primary agents to cover facultative Gram-negative bacilli. Ototoxicity is irreversible, and is generally unappreciated as a risk. When antimicrobial therapy directed at enterococci is required, a penicillin (including piperacillin–tazobactam, ampicillin–sulbactam, ticarcillin–clavulanic acid or amoxicillin–clavulanic acid) or vancomycin is bacteriostatic, but sufficient. The addition of a low-dose aminoglycoside is required to render the regimen bactericidal only when enterococcal bacteremia has been demonstrated, when endocarditis is thought possible or when prosthetic cardiac valves are present. Cephalosporins and monobactams are not active against enterococci, with the exception of ceftobripole and ceftaroline. All carbapenems, except ertapenem, are

active against *E. faecalis*. However, carbapenems are not active against other enterococcal species, such as *E. faecium*.

The extended-spectrum penicillins, when combined with a β-lactamase inhibitor (piperacillin–tazobactam or ticarcillin–clavulanic acid), are almost uniformly active against the anaerobic and facultative Gram-negative bacilli and enterococci, and are preferred choices as monotherapy for the sickest patients. Carbapenems should be chosen where ESBL-producing Gram-negative bacilli are recognized as a significant local problem. The chosen regimen should always cover enterococci for patients with septic shock, immunosuppression, prosthetic heart valves and postoperative peritonitis.

Intra-abdominal candidiasis

Candida is isolated from peritoneal samples in 20% of patients presenting in the community with acute perforation. However, this rarely progresses to invasive candidiasis so that specific antifungal therapy is unnecessary, unless the patient is immunosuppressed. Invasive intra-abdominal candidiasis is a significant complication of necrotizing pancreatitis and of postoperative peritonitis, especially in those with recurrent perforation or anastomotic leak. It carries a high mortality and manifests as peritonitis or abscess, with or without candidemia and disseminated candidiasis. It is difficult to confirm a diagnosis early, yet timely specific antifungal therapy is required in addition to drainage of abscess and surgical repair of perforation. Delay in antifungal therapy is associated with increased mortality.

The azole, fluconazole (800 mg load, 400 mg per day) is uniformly effective against *Candida albicans*. It is nontoxic, relatively inexpensive and effective by intravenous and oral routes. However, resistant non-albicans species such as *C. kruzei* and *C. glabrata* have become increasingly prevalent, especially in ICUs with considerable azole use. Fluconazole is the agent of choice for patients with mild to moderate disease, no previous azole exposure and not in a high risk group for *C. glabrata* (elderly, diabetics, cancer patients). In contrast to azoles, which are fungistatic, the echinocandins (caspofungin, anidulafungin, micafungin) are fungicidal, which is critical when cardiac or central nervous system involvement is suspected. Echinocandins may be superior to fluconazole and are clearly preferred as initial therapy for patients with moderate to severe disease and in those with recent exposure to azoles. In stable patients who are improving on an echinocandin, a switch to oral fluconazole should be considered when the isolate has been identified as a species susceptible to fluconazole (*C. albicans, C. paropsilosis, C. tropicalis*) or to oral voriconazole (*C. kruzei*). Fluconazole should be favored over echinocandins for *C. paropsilosis*, as resistance to echinocandins is a concern in this species. The duration of antifungal therapy is 14 days after resolution of clinical findings, drainage of abscess and correction of leaks.

Prophylaxis with fluconazole at 400 mg per day is justified in patients with necrotizing pancreatitis who are receiving broad-spectrum antibacterial therapy and in those with recurrent perforations or anastomotic leak. Empiric therapy with an echinocandin can be considered for critically ill patients with pancreatitis or intra-abdominal sepsis and no correctable cause of fever.

SURGICAL MANAGEMENT

The principles of surgical management of peritonitis are closure or removal of the perforated viscus and peritoneal decontamination, following appropriate preoperative resuscitation including antimicrobial therapy. In patients manifesting sepsis syndrome, resuscitation, prior to operative intervention, takes precedence over immediate laparotomy. The decision whether to re-anastomose following intestinal resection requires experienced assessment of risk factors. In general, anastomosis should not be undertaken in the presence of fecal peritonitis, hemodynamic instability or immunosuppression. Within these guidelines, primary anastomosis can be safely undertaken in patients with perforated diverticular disease, avoiding the substantial postoperative morbidity of a Hartmann's procedure and the likelihood of a permanent colostomy.

In extreme circumstances, a damage control laparotomy may be performed with the object of arresting hemorrhage and preventing further contamination. Devitalized tissues and perforated bowel are either removed or exteriorized. No attempt is made to perform anastomosis and the abdominal wound is left open pending more definitive exploration 24–48 hours later. It is essential to avoid blood loss and hypothermia, both of which can lead to irretrievable coagulopathy. Handling of inflamed viscera is minimized to reduce the cascade of inflammatory mediators that are inevitably released into the systemic circulation, causing further hemodynamic instability.

Relaparotomy

Scheduled or 'second-look' relaparotomy became popular in the 1980s as a means of treating diffuse peritonitis. This was based on the concept that repetitive laparotomy would allow abdominal irrigation and prevent recurrent abscess formation. However, as experience accumulated, it became clear that mortality was not decreased and that there was a substantial increase in the incidence of intestinal fistulas. Relaparotomy has, in the main, been superseded by CT scanning, when clinically indicated, with percutaneous drainage of collections. Second-look laparotomy is now rarely used, except in the management of patients with mesenteric ischemia in whom doubt remains at first laparotomy concerning the viability of significant portions of small intestine.[16]

Wound closure

Wound closure is particularly problematic in the presence of peritonitis. By definition the wound is 'dirty' with a risk of postoperative wound infection that approximates 30% and subsequent wound dehiscence of 5–10%. The risks are increased in the elderly, malnourished, obese and diabetic, and in the presence of sepsis syndrome. Surgical options are to close the fascial layer and leave skin and subcutaneous tissue as an open wound that can be closed 5–7 days later (delayed primary closure) or left to close by secondary intention. In the latter situation, closure may be accelerated by use of a vacuum-type dressing as long as the fascial closure remains intact. Occasionally, intestinal distention and edema, particularly of the small bowel, preclude safe closure. In such circumstances, fascial closure is unwise and may lead to abdominal compartment syndrome with intra-abdominal pressures exceeding 25 mmHg. A number of surgical techniques are available to 'silo' abdominal contents until it is safe to close the peritoneal cavity. Postoperatively, abdominal distention and falling urinary output should raise suspicion of abdominal compartment syndrome, which can be confirmed by measuring intravesical pressure.

Postoperative peritonitis

Peritonitis following recent abdominal surgery poses a particular diagnostic and therapeutic challenge. It is usually due to a leak from a suture line and is typically discovered only after some delay, as a rule between the fifth and seventh postoperative days. The symptoms are often insidious and may mimic a cardiorespiratory complication, most commonly pulmonary embolism. A high index of suspicion must exist after intestinal anastomosis, particularly in patients who have undergone anterior resection or resection for Crohn's disease. In this situation, CT scanning is valuable but may not be diagnostic. The presence of free air within the peritoneal cavity is sometimes wrongly attributed to residual air following recent laparotomy. A high index of suspicion should lead to laparotomy, even in the absence of definitive radiologic evidence of anastomotic leakage. Where anastomotic leakage is confirmed, conventional wisdom dictates takedown of the anastomosis with a proximal stoma

and closure of the distal stoma. Occasionally an experienced surgeon may choose to repair the defect and defunction the bowel proximally. Such a course can be considered only in a stable patient with minimal contamination.

Percutaneous drainage procedures

Percutaneous drainage and operative intervention are best viewed as complementary rather than competitive techniques. There are many situations for which percutaneous drainage is the definitive procedure of choice, others for which surgery alone is indicated and some for which both techniques are applicable, alone or in conjunction. Inflammation may manifest as a phlegmon (viable inflamed tissue), a liquefied abscess or infected necrotic (nonviable) tissue, or as a combination. Liquefied abscesses are drainable, whereas phlegmonous and necrotic tissue are not. Decisions regarding which modality to employ are largely based on CT findings.

Indications for percutaneous drainage have expanded significantly over the past decade and now include multiple and/or multiloculated abscesses, abscesses with enteric communication and infected hematomas. It is important that the drainage route does not cross a sterile fluid collection or other infected space due to the risk of cross-contamination. Crossing the pleural space for thoracic and upper abdominal drainage carries the risk of empyema formation. In most cases drainage is performed following fine needle (18–22 gauge) aspiration, with the aspirate being used to document infection, by Gram stain and culture and to determine the viscosity of the fluid. For most collections, a drain should be placed to ensure complete evacuation and minimize the chance of recurrence. The aspiration needle can be used for placement of a guide wire or as a guide for tandem insertion of the drain. If the patient is not already on antimicrobial therapy, this should be instituted prior to the drainage procedure to prevent bacteremia and minimize infection of sterile tissue.

The choice of catheter size is determined primarily by the viscosity of the fluid to be drained. In the majority of cases, 8–12F drains are sufficient. Larger drains may be needed for collections containing debris or more viscous fluid. Drains of larger caliber can be placed, if needed, by exchange over a guide wire. There is no limit to the number of drains that can be placed. While most abscesses can be drained with a single catheter, there should be no hesitation in placing as many drains as are needed to effectively evacuate the abscess(es).

Following catheter placement, the cavity should be evacuated as completely as possible and irrigated with saline until the fluid is clear. Initial manipulation of the catheter(s) and irrigation should be done as gently as possible to minimize the induction of bacteremia. Immediate imaging determines the need for repositioning of the catheter or for placing additional drains. For cavities that are completely evacuated at the initial drainage and for which there are no abnormal communications to viscera, simple gravity drainage is generally sufficient. For larger or more viscous collections and those with ongoing output due to fistulous connections, suction drainage with sump catheters is more effective. Presacral collections may be drained transrectally. Recent developments include endoscopically placed vacuum dressings mounted on a catheter delivery system.

Primary (spontaneous) peritonitis

Primary peritonitis is defined as infection of peritoneal fluid in the absence of causative intraperitoneal visceral pathology. It is almost always monomicrobial and bacterial, but herpes simplex virus and fungi, such as histoplasmosis and coccidioidomycosis, have also been described. Almost all cases occur in patients with ascites and cirrhosis and are then termed spontaneous bacterial peritonitis (SBP). Other underlying pathologies include nephrotic syndrome and congestive cardiac failure. The most common organisms cultured from SBP are *E. coli* and *K. pneumoniae,* which translocate from the bowel to the mesenteric lymph nodes and then to the ascitic fluid via to the bloodstream. An increasing proportion is now due to viridans streptococci, MRSA along with quinolone-resistant and ESBL-producing Gram-negative bacilli due to the use of prophylactic quinolones and cephalosporins in patients with severe ascites.

Historically, *Streptococcus pneumoniae* was the most commonly recognized cause of primary peritonitis and remains a significant cause in patients with cirrhosis, in whom it usually arises as a bacteremic complication of respiratory tract infection. Pneumococcal peritonitis can also arise by the ascending route through the fallopian tubes in both healthy women of reproductive age and in premenarchial girls. *Aeromonas hydrophila* has recently emerged as an important cause of SBP in East Asia. It is usually a summer infection, preceded by diarrhea, and may originate from shellfish consumption.

New onset abdominal pain and distention with evidence of systemic sepsis in a patient with chronic ascites suggests SBP, but this presentation is not typical. More usually the presentation is subtle, with acute deterioration in renal function, unexplained encephalopathy and borderline fever. A low threshold for paracentesis is thus appropriate in patients with chronic ascites, who have unexplained nonspecific alteration in their clinical state. Ascitic fluid will yield >250 neutrophils/mm^3; this, combined with a monobacterial-positive culture, defines SBP. An ascitic fluid pH of less than 7.35 or a blood–ascitic fluid pH gradient >0.10 supports the diagnosis. Inoculation of blood culture bottles at the bedside with ascitic fluid improves the culture yield.

Culture-negative ascites with >250 neutrophils/mm^3 is termed culture-negative neutrocytic ascites (CNNA); it responds to antimicrobial therapy. Patients with positive ascites cultures, but with <250 neutrophils/mm^3 (bacterascites), should be treated if symptomatic, as some will progress to SBP. An ascitic fluid protein >1 g/dl, a low ascitic fluid glucose (<50 mg/dl) or an ascitic fluid lactate dehydrogenase (LDH) greater than serum level and especially polymicrobial infection, all point to the possibility of secondary peritonitis and the need for emergency imaging to detect gastrointestinal perforation.[17] Early diagnosis and treatment significantly improve prognosis. A third-generation cephalosporin, a quinolone or amoxicillin–clavulanic acid are all appropriate choices for 7 days. Repeat diagnostic paracentesis is indicated at 48–72 hours if there is no clinical improvement.

Treatment failure (10%) is associated with a mortality of 50–80% and is either due to delay in treatment initiation or to resistant organisms. Albumin infusions (1.5 g/kg day 1, 1 g/kg day 3) have been shown to reduce acute renal failure and mortality in those with preexisting renal impairment and are recommended for all patients with SBP. Long-term secondary prophylaxis with a quinolone is recommended for all patients after an episode of SBP, despite the selection of quinolone-resistant Gram-negative bacilli, and higher rates of MRSA colonization and *C. difficile* diarrhea.

Short prophylaxis with a quinolone or a third-generation cephalosporin for up to 7 days in patients with variceal hemorrhage, irrespective of the presence of ascites, also improves patient survival. Survival benefit with long-term primary prophylaxis (before a first episode of SBP) has been demonstrated in two small studies,[18,19] which selected higher risk patients with low ascitic fluid protein. A low ascitic fluid protein of <10 g/dl and poor hepatic function, such as in those awaiting transplantation, identify a subset of patients who are at higher risk for developing SBP and in whom primary prophylaxis can be justified.

PERITONITIS ASSOCIATED WITH CHRONIC AMBULATORY PERITONEAL DIALYSIS (CAPD)

CAPD peritonitis carries a 1–6% mortality risk per episode, with an incidence that varies from 0.2 to 1.3 per patient per year.[20] Repeated bouts may result in sclerosing peritonitis, with resultant failure of dialysis and sometimes intestinal obstruction. Organisms gain entry most commonly by touch contamination, but a significant minority

are caused by a prior exit-site infection and possibly by translocation from the bowel. The causative organisms are coagulase-negative staphylococci (22%), *S. aureus* (15%), Gram-negative bacilli (28%), fungi (2.5%) or anaerobes (2.5%), with up to 20% culture-negative. There has been a decline in numbers due to *S. aureus* and coagulase-negative staphylococci, related to improved exit-site care and to newer techniques.

Diagnosis is suggested by a cloudy peritoneal effluent, usually with abdominal pain and fever, and confirmed by >100 WBC/mm^3 in the effluent, with greater than 50% polymorphonuclear cells. Initial empiric antimicrobial therapy should cover both Gram-positive and Gram-negative organisms. A combination of cefazolin and gentamicin is generally preferred, but a vancomycin–gentamicin combination is preferred in centers with significant rates of MRSA. Intraperitoneal administration is superior to the parental route and achieves equivalent blood levels. Duration is for 2–3 weeks.

Eradication of the infection and continuation of CAPD is usual in 80–85% of the episodes. Approximately 10–15% of patients require catheter removal and transfer to hemodialysis. Early removal of the peritoneal catheter should occur for episodes due to *P. aeruginosa*, fungi, mycobacteria and vancomycin-resistant enterococci. The catheter should also be removed in patients who fail to respond to appropriate therapy within 4 days and in those who develop recurrent peritonitis with the same organism.

TUBERCULOUS PERITONITIS

Tuberculous peritonitis is now rare in developed countries with just 136 cases reported in the USA in 1999 (compared with 26 800 cases of ovarian cancer in 1997). However, it is common in the developing world and in emigrant communities. In sub-Saharan Africa it is the most common cause of exudative ascites. In the anti-tuberculous therapy era, mortality has varied from 15% to 52%, depending on the proportion of patients with underlying conditions.[21]

It is the great pretender or mimic, because it presents with nonspecific, highly variable clinical findings. Investigations lack sensitivity and specificity with the exception of laparotomy or laparoscopy and possibly adenosine deaminase levels in ascitic fluid. Other conditions such as peritoneal metastatic malignancy and culture-negative neutrocytic peritonitis (CNNP) closely resemble it. Definitive diagnosis is often delayed due to the prolonged time required for mycobacterial culture. Furthermore, even culture of ascitic fluid may lack sensitivity, and although positive in the majority of modern reports, sensitivity in the past has been as low as 20%.

The proportion of all TB cases presenting as peritonitis has increased in some countries, in part related to a higher incidence amongst patients receiving CAPD and those with chronic ascites due to cirrhosis. Diagnosis in these groups is particularly challenging due to the difficulty in distinguishing TB peritonitis from the more common complications of CAPD peritonitis and spontaneous bacterial peritonitis. The triad of abdominal swelling, pain and fever is present in only 60% of patients, mainly due to a lack of fever in patients with advanced liver and renal failure.[21,22] The classic finding of doughy, tender abdominal masses occurs in only a small minority. Weight loss is common, but often obscured by weight gain from ascites. Peritoneal fluid is classically a lymphocytic exudate. However, especially in patients with cirrhosis or on CAPD, protein levels are commonly <25 g/dl. A significant minority of cases have >250 polymorphonuclear leukocytes/dl, suggestive of SBP in cirrhotic patients or of CNNP when bacterial cultures are negative.

Particularly in settings of high TB prevalence, care should be taken to exclude TB peritonitis using adenosine deaminase ascitic fluid levels (when available) or laparoscopy, before diagnosing CNNP. If measured, CA-125 levels can be markedly elevated in tuberculous peritonitis, leading to a false impression of ovarian carcinoma. Glove hypersensitivity, food-starch granulomatous peritonitis and pseudomyxoma peritonei are very rare conditions that may mimic TB peritonitis.

Although a tuberculin skin test (or QuantiFERON test) is often negative, a positive result in the presence of abdominal findings should heighten suspicion of tuberculous peritonitis. Abdominal CT scanning may show peritoneal or omental nodules or thickening, suggestive of carcinoma. Microscopy of ascitic fluid using Ziehl–Neelsen (ZN) staining is positive in zero to 6% of cases, while polymerase chain reaction (PCR) is positive in only a minority.[21] Adenosine deaminase is both highly sensitive and specific, when applied in countries of high prevalence. However, it had lower sensitivity (60%) when applied in a small study in the USA.

Direct inspection of the peritoneal cavity by laparoscopy, minilaparotomy or full laparotomy, with detection of tubercles macroscopically and granulomata microscopically, is diagnostic in patients presenting with ascites of unknown cause.[21,23] Uniform nodules of less than 0.5 cm with adhesions are virtually diagnostic, while hemorrhagic inflammation, with either adhesions or nodules, is highly suggestive of tuberculous peritonitis. Tissue samples free of formalin, along with ascitic fluid, should be sent for mycobacterial culture, which is especially important to detect multidrug-resistant TB (MDRTB). Empiric treatment for TB peritonitis should be considered while awaiting definitive diagnosis, using four drugs initially, consisting of rifampicin, isoniazid, pyrazinamide and ethambutol. In areas with significant rates of MDRTB, a quinolone and amikacin should be added while awaiting the results of full sensitivities. Delay in treatment is associated with greater mortality. (See also Chapter 30.)

A minority of survivors may have recurrent intestinal obstruction from multiple chronic adhesions. There is suggestive evidence that corticosteroids will diminish this morbidity, but by abrogating the host response, the patient's demise may be hastened in the event of MDRTB. Adjunctive corticosteroids should be considered in patients in whom the diagnosis of tuberculous peritonitis is certain and in whom there is little concern about MDRTB. If used, corticosteroid doses should be sufficient to abrogate the granulomatous response. The authors favor prednisone or prednisolone at 1 mg/kg/day for 2 months with a slow wean over the following 2 months. (See also Practice Point 13.)

BILIARY SEPSIS

Biliary sepsis is usually associated with gallstone disease, pancreatic or bile duct malignancy or previous biliary tract surgery that has resulted in bile duct obstruction with secondary infection. The organisms involved are usually Enterobacteriaceae. Enterococci are uncommon and anaerobes are involved only if there is a bile duct to bowel anastomosis or previous biliary tract surgery. Acute cholecystitis is often an inflammatory but noninfective condition. If infection is suspected because of clinical and radiologic findings, antimicrobial therapy should be directed solely at facultative Gram-negative bacilli. Enterococcal coverage is not required and anaerobic coverage is necessary only in patients with bile duct–bowel anastomoses and in patients who have had previous biliary tract surgery. Infection in the biliary tree (cholangitis) typically presents with a triad of fever, rigors and jaundice (Charcot's triad).

Ultrasound is the best modality to confirm dilatation of bile ducts and the level of obstruction, usually at the lower end of the common bile duct or intrahepatic ducts. Magnetic resonance cholangiogram is particularly useful for delineation of biliary anatomy. Treatment is with antimicrobial agents and urgent decompression of the obstructed system. The technique used to decompress depends on the site of obstruction. Endoscopic retrograde cholangiopancreatography (ERCP) with stent insertion is used for common bile duct lesions, whereas percutaneous transhepatic cholangiography (PTC) with stenting, with or without external drainage, is used for intrahepatic or hilar lesions. Occasionally, both techniques are used simultaneously in a so-called 'rendezvous procedure'.

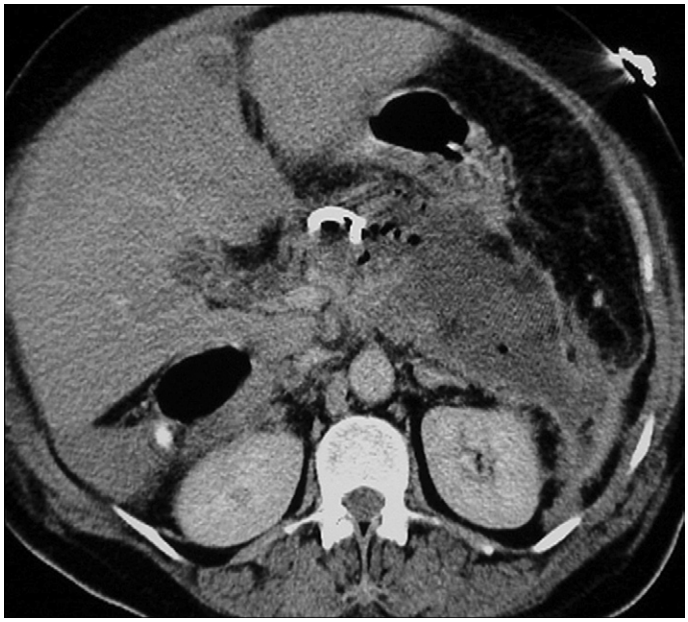


Fig. 37.4 CT abdomen showing extensive pancreatic necrosis.

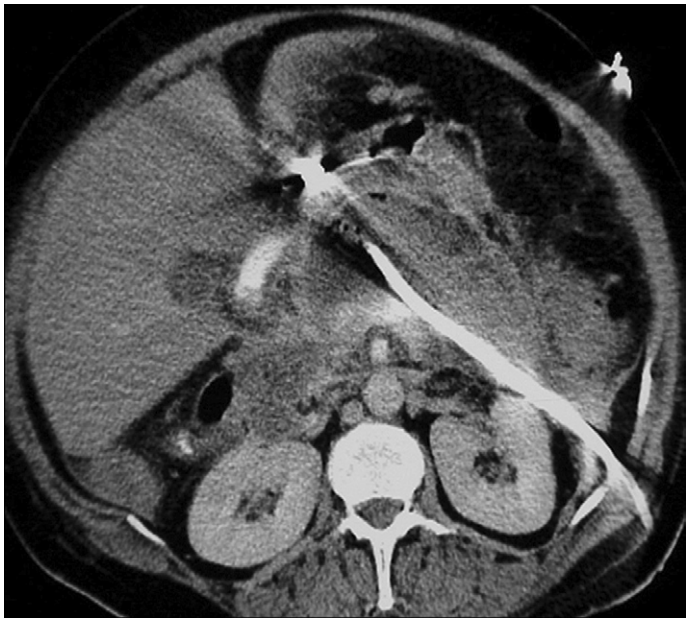


Fig. 37.5 CT drainage of pancreatic necrosis shown in Fig. 37.4.

PANCREATITIS

Severe acute pancreatitis occurs in about 25% of patients with acute pancreatitis. The severity of pancreatitis is related to development of SIRS, multiorgan failure and necrotizing pancreatitis.[24] The mortality in severe pancreatitis is bimodal, with a peak in the first week related to multiorgan failure, while a second peak occurs at 3–4 weeks related to pancreatic necrosis and retroperitoneal sepsis. Development of early multiorgan dysfunction and pancreatic necrosis (as determined by the presence of nonviable pancreatic tissue on CT scan) are highly predictive of subsequent later pancreatic and retroperitoneal sepsis. Infection complicating necrotizing pancreatitis is usually polymicrobial and resembles that occurring after colonic perforation.[25] There has been considerable surgical interest in whether early surgical intervention to debride the pancreas might favorably alter the course of necrotizing pancreatitis and reduce the incidence of late multiorgan failure, sepsis and death. Patients with extensive (>50%) sterile necrosis are at high risk of infection and progressive multiorgan failure.

Surgical debridement of necrotic pancreatic tissue is technically difficult and is associated with high perioperative complication rates. Frequently more than one exploration of the abdomen is required, leading to considerable difficulties in wound management and risks enteric and/or pancreatic fistula development. An aggressive early surgical approach advocated in many institutions has been replaced by a more conservative approach guided by the clinical course, sequential CT imaging (Fig. 37.4) and percutaneous wide bore catheter drainage (Fig. 37.5).[26] When a minimally invasive approach does not adequately evacuate the necrotic pancreatic and peripancreatic tissue, then either open or laparoscopic debridement may become necessary in the face of ongoing or worsening multiorgan dysfunction. Where possible, a retroperitoneal approach should be used to minimize risks of enteric injury leading to fistula.

There have been conflicting results concerning the role of prophylactic antimicrobial agents in preventing later pancreatic and retroperitoneal sepsis in patients with severe pancreatitis. Initially, a number of studies suggested a survival advantage and a decrease in pancreatic sepsis for the antibiotic treatment groups; however, a more recent meta-analysis[27] has shown no decrease in pancreatic sepsis but still a decrease in overall mortality was observed with treatment. However, a large multicenter, double-blinded trial of the use of intravenous meropenem in preventing or delaying pancreatic or peripancreatic infection in patients with a confirmed diagnosis of necrotizing pancreatitis has demonstrated no difference between treatment groups with respect to infection, mortality or requirement for surgical intervention.[25] This study leads to the conclusion that early prophylactic antibiotic use for patients with necrotizing pancreatitis is not supported.[26]

REFERENCES

References for this chapter can be found online at http://www.expertconsult.com



Chapter 38

Stephen D Ryder

Viral hepatitis

INTRODUCTION

The clinical syndrome of acute hepatitis has been recognized since antiquity and is characterized by jaundice, usually after a prodromal illness. Acute hepatitis may cause severe sequelae, including fulminant hepatitis, but the major impact on human health is chronic liver disease and hepatocellular carcinoma resulting from chronic infection.

The nomenclature of the hepatitis viruses is somewhat eclectic, being based on the time of discovery of new or putative agents rather than on any consideration of modes of transmission or clinical problems associated with that agent. There are at present five primary human hepatotropic viruses, A, B, C, D and E, which are well characterized and known to account for approximately 90% of acute and 95% of chronic viral hepatitis. Other viruses may cause human disease (see Chapter 154).

EPIDEMIOLOGY

Hepatitis A virus

Hepatitis A virus (HAV) is distributed throughout the world and causes outbreaks of infection, usually in association with direct fecal–oral contact[1] or contaminated water supplies.[2] Most food-related outbreaks of HAV infection are sporadic and due to poor food hygiene measures; however, contamination of shellfish caused by sewage pollution is well described[3] and represents a continuing problem because mollusks are able to retain and concentrate viruses from water. Homosexual men and those working with newly imported nonhuman primates are high-risk groups for HAV infection. In the 1980s the proportion of cases of hepatitis A spread by blood contact increased to 19% of reported cases in the USA; this appears to have been due to intravenous drug use.[4]

In the developed world, the proportion of people who have immunity to HAV has declined over the past two decades owing to improved sanitary conditions in childhood. Travelers from low-risk geographic areas to high-risk areas are at substantial risk of acquiring HAV infection.[5] In the developing world, infection rates are as high as 95% by the age of 16 years. Early infection usually produces a less severe clinical illness and immunity to future infection. The incubation period is short, ranging from 15 to 50 days (mean 30 days).

Hepatitis B virus

Hepatitis B virus (HBV) is one of the most common chronic viral infections in the world, with estimates of approximately 170–350 million chronically infected people.[6] Hepatitis B virus infection is relatively rare in developed countries, with an incidence of 1 per 200 population in the UK and North America. Even in areas like the UK, where acute infection is relatively uncommon, up to 2% of the population show evidence of past infection. In high-risk areas such as South East Asia and sub-Saharan Africa up to 20% of the population have evidence of previous infection.[7] A major mode of spread in high-endemicity areas is vertical transmission from carrier mother to child, and this may account for 40–50% of all HBV infections in such areas.[8] This mode of transmission is highly efficient; more than 95% of children of carrier mothers are infected and develop chronic viral infection themselves. In low-endemicity countries the mode of spread in acute HVB is predominantly by sexual transmission or blood-borne transmission through intravenous drug use, whereas in most of northern Europe immigration accounts for a substantial proportion of new chronic cases of hepatitis B infection presenting to the health-care services. Many migrants are from areas of the world where hepatitis B infection is very common. It is estimated that in the UK 90% of new hepatitis B diagnoses are in migrant populations with chronic infection acquired in childhood. Between 5% and 10% of acutely infected adults develop chronic viral infection. Certain groups in the Western world are known to be at higher risk of HBV exposure; these include intravenous drug users, hemodialysis patients, homosexual men and institutionalized people, particularly those with mental handicap. Males are more likely to become chronic HBV carriers than females, for reasons that are unclear. The incubation period ranges from 28 to 160 days (mean 80 days).

Hepatitis C virus

Hepatitis C virus (HCV) infection is common, with an estimated 350 million cases worldwide. Unlike infection with HBV, this infection is very common in the developed world, with 0.3–0.7% of the UK population infected. The virus is spread almost exclusively by blood contact. Since the introduction of screening of blood products in 1990/1991, intravenous drug use has become the almost exclusive mode of hepatitis C transmission in Northern Europe and North America. In the early 1990s, 35% of cases in northern Europe had a past history of blood transfusion and a further 40% had used intravenous drugs. Of new cases seen in the late 1990s, intravenous drug use accounted for over 75% of cases. Sexual transmission does occur, but is unusual; less than 5% of long-term sexual partners become infected.[9] Vertical transmission also occurs, but again is unusual. It is certainly not the predominant mode of spread of HCV, with the frequency of infection in children of viremic mothers less than 5%. The mode of delivery does not affect infection rates and breast feeding is safe.[10] The rate of infection in children of infected parents may rise in the first 10 years of life but it seems relatively difficult to acquire this viral infection from close household contact.

This leaves a substantial minority of those identified in whom no specific risk factor is present. This proportion may be up to 20% of cases. It has been postulated that this group may have acquired the

infection from medical interventions. This is based on the high prevalence of infection in areas such as southern Italy, where military vaccination programs were undertaken in the period immediately after the Second World War, and in Egypt, where up to 20% of the population has HCV markers in certain geographic areas and treatment for diseases such as schistosomiasis is commonly given by injection. Hospitalization for whatever reason appears to be a risk factor for HCV infection. The incubation period varies between 14 and 60 days (mean 50 days).

Hepatitis D virus

Hepatitis D virus (HDV) or delta virus is an incomplete RNA virus that uses hepatitis B surface antigen (HBsAg) to enable replication and transfer from cell to cell. Hence, its epidemiology is closely linked to that of HBV. There are, however, considerable differences in the frequency of delta infection or superinfection in different patient groups. Intravenous drug users have a relatively high incidence of HDV infection whereas homosexual men, a high-risk group for the sexual spread of HBV, have a low incidence of delta infection. The reason for this epidemiologic paradox is unknown. Transmission of HDV is parenteral,[11] either via transfusion or close personal contact. Screening of blood products for HBsAg effectively excludes HDV-positive donors. The commonest risk factor for the acquisition of HDV infection in the Western world is intravenous drug use, with between 17% and 90% of HBsAg-positive addicts also testing positive for HDV.[12] In developing countries, HDV infection generally parallels HBV infection, although there are exceptions, particularly in areas of Asia, where HDV is rare despite a high level of HBV carriage. In general, approximately 5% of people with chronic HBsAg carriage will be co-infected with HDV, giving an estimated worldwide figure in excess of 10 million. The incubation period is quite variable.

Hepatitis E virus

Hepatitis E virus is transmitted by the fecal–oral route. Its epidemiology correlates with the presence of contaminated water. It is responsible for outbreaks of epidemic-type hepatitis in the Indian subcontinent, but sporadic cases are seen throughout the world. The incubation period is short, ranging between 15 and 45 days (mean 40 days).

PATHOGENESIS AND PATHOLOGY

The molecular structure of the hepatitis viruses is considered in Chapter 154. The mechanisms of pathogenicity of the hepatitis viruses are complex and involve viral and host immune factors. The basis of any hepatitis is hepatocyte death, usually immunologically mediated in order to eliminate infected cells and to prevent further viral replication. It is highly likely that the mechanisms involved vary depending on the virus. Further differences in either the virus or the host response are involved in the development of chronic infection with HBV or HCV. Because these are the major cause of liver disease, these two viruses are considered in detail in this chapter.

Hepatitis B virus

Hepatitis B virus enters the hepatocyte by binding of determinants that are present on HBsAg. The virus replicates mainly in the hepatocyte (Fig. 38.1), although HBV DNA has been found in extrahepatic tissues, including skin, pancreas, kidneys, bone marrow and peripheral blood mononuclear cells. Hepatitis B virus has a highly unusual genetic structure, with a circular, partially ssDNA genome. When internalized in the hepatocyte, the genome is released and the negative strand is converted by ligation to a closed circular supercoiled form.[13] This form is present in the hepatocyte nucleus and forms the template for HBV RNA synthesis. Hepatitis B virus is almost unique in that its DNA is synthesized via an RNA intermediate; the same molecules are therefore used for protein synthesis and for reverse transcription to DNA.

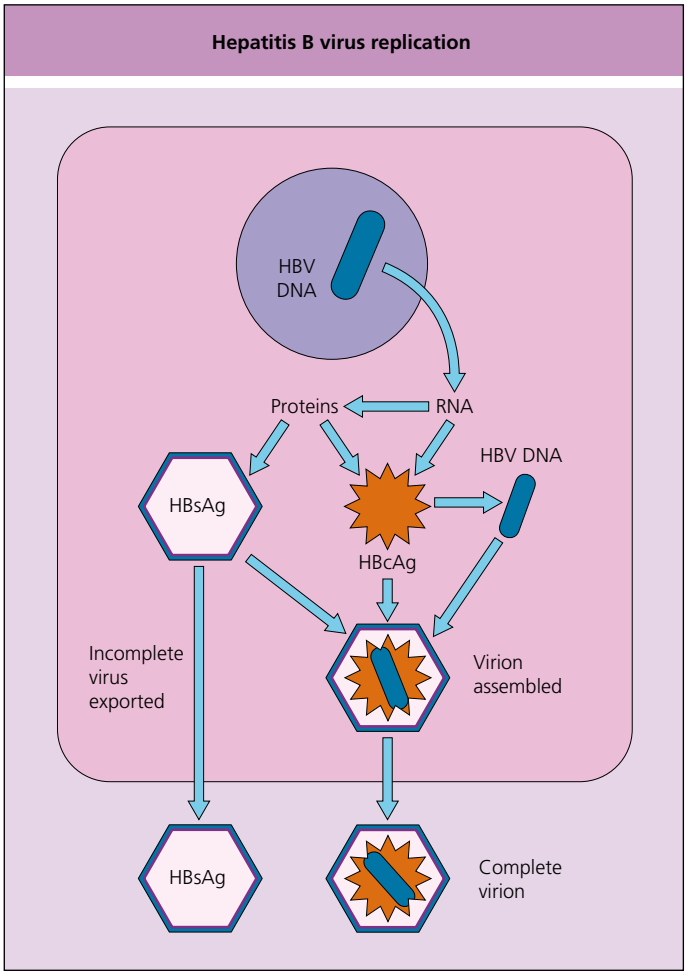


Fig. 38.1 Hepatitis B virus replication. Viral DNA present in the nucleus is transcribed to RNA, which then acts both as a template for protein synthesis (viral coat-HBsAg and viral proteins essential for infectivity and replication-HBcAg). Viral particles are then assembled and secreted from the cell cytoplasm. For every complete virion a large number of incomplete particles derived from HBsAg alone are exported. HBV, hepatitis B virus; HBsAg, hepatitis B surface antigen; HBcAg, hepatitis B core antigen.

The HBV RNA template is encapsulated in hepatitis B core antigen (HBcAg) particles and reverse transcribed to produce negative-strand DNA. This is then used to synthesize an incomplete positive DNA strand and the virion is encapsulated with HBsAg before excretion from the cell.

There are a number of host mechanisms deployed to prevent initial infection and then to remove infected hepatocytes. Hepatitis B virus is not thought to be directly cytopathic except in highly specific circumstances, such as fibrosing cholestatic hepatitis seen in re-infection of liver grafts, when the host is immunosuppressed. Liver cell damage in both acute and chronic HBV infection is thought to be immunologically mediated.

Cell-mediated and humoral immune responses occur in HBV infection, and both are probably important in limiting and eliminating infection. There is invariably a humoral immune response directed against HBcAg and usually against HBsAg,[14] but this response alone is not the cause of hepatitis as evidenced by liver disease in agammaglobulinemic patients. The responses of human leukocyte antigen (HLA) class I restricted cytotoxic T lymphocytes are thought to be the major mechanism of liver cell injury.[15] The fact that patients with production of HBsAg alone in hepatocytes usually have little inflammatory liver disease suggests that the target of this attack is likely to be core antigen.

Acute HBV infection can be self-limiting with complete clearance of the virus or it can develop into chronic infection with the potential for the development of cirrhosis and primary liver cell cancer.

Chronic HBV infection can be thought of as occurring in phases, depending on the degree of immune response to the virus. This is particularly true of patients infected in the first few weeks of life. If infected when the immune response is 'immature', there is initially little or no immune response to HBV. The levels of HBV DNA in serum are very high and the hepatocytes contain abundant HBsAg and HBcAg, but little or no ongoing hepatocyte death is seen on a liver biopsy because of the defective immune response. This state persists for a variable period of time; usually the degree of immune recognition increases over some years. When immune recognition starts to occur, the level of HBV DNA tends to fall and the liver biopsy shows increasing inflammatory liver disease. This inflammation and hepatocyte death produces hepatic fibrosis. Once this phase of infection is initiated there are two major possible outcomes: either the immune response is adequate and the virus is inactivated and then removed from the system or the attempt at removal results in extensive fibrosis, distortion of the normal liver architecture and death from the complications of cirrhosis.

A third phase of hepatitis B virus infection is now recognized. This occurs late in the natural history of the immune response against HBV. In situations where the wild-type virus is inactivated but not eradicated, characterized by hepatitis B surface antigen positivity, e antigen negativity and undetectable levels of HBV DNA, there is still virus present in hepatocytes. This virus will be present in a number of forms, with viral mutants commonly produced. It is now well recognized that some of these mutant species can replicate in the presence of an adequate immune response against wild-type virus. This leads to re-emergence of HBV DNA in serum and can lead to progressive liver injury (Figs 38.2, 38.3). These mutant stains may account for up to 60% of all replicating HBV infection in Europe. Recognition of this situation clinically is important because reactivation of HBV replication carries a significant risk of the development of hepatic fibrosis and cirrhosis and therapy is different from the usual 'wild type', e antigen-producing HBV. It is simple to recognize as abnormal transaminases and significant levels of HBV DNA are present in the serum in patients who are HBsAg positive but e antigen negative.

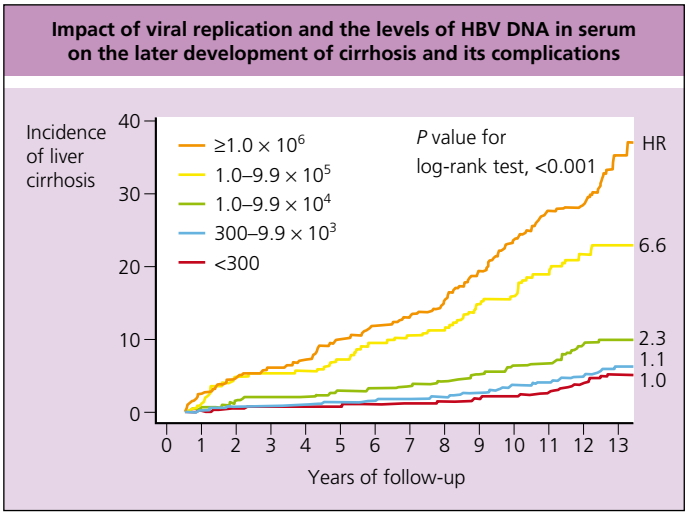


Fig. 38.3 Impact of viral replication and the levels of hepatitis B virus (HBV) DNA in serum on the later development of cirrhosis and its complications.

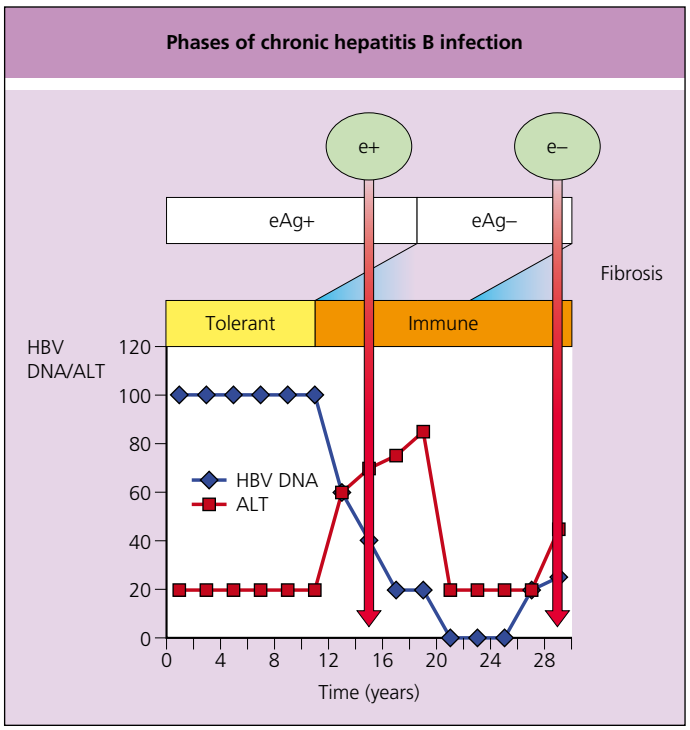


Fig. 38.2 Phases of chronic hepatitis B virus (HBV) infection. Initial infection is usually in childhood and has a long immune tolerant phase. Immune recognition then develops which can allow inactivation of HBV by either clearance of infected cells (with associated liver cell damage) or suppression of viral antigen expression on infected hepatocytes. This produces viral inactivation. In a significant proportion of patients where this has occurred, a third phase develops, where replication of HBV resumes due to viral escape mutants, leading to HBV DNA again appearing in serum and the risk of chronic liver disease. The two phases of liver injury where treatment may be required are indicated by the red arrows. ALT, alanine transaminase.

Hepatitis C virus

Chronic HCV infection has a long natural history, with most patients discovered in the presymptomatic stage. In the UK, most patients are now screened because of an identifiable risk factor (previous intravenous drug use or blood transfusion) or because of abnormal liver biochemistry.

The mechanism by which HCV causes human disease is unclear. A very high proportion (70–80%) of acute infections go on to become chronic and the prognosis from chronic infection is very variable. Some infected people have normal liver histology,[16] which indicates that HCV is not directly cytopathic for hepatocytes. Immune mechanisms are again thought to be important in determining outcome.

Hepatitis C virus is an RNA virus. It is highly variable, with six major genotypes.[17] Genotypes are frequently geographically restricted, such as type 4 in the Middle East, or may vary with time and mode of spread. This has been seen in Europe with genotype 1 occurring in older transfused individuals, with type 3 in younger drug users. There is some evidence that viral factors are important in the outcome of HCV infection. More severe fibrotic disease has been found in patients infected with genotype 1a or 1b than in those infected with other genotypes.[18] This is somewhat controversial as there are confounding factors, such as duration and mode of transmission, that make interpretation difficult.[19] The variability of the infecting HCV has also been postulated to affect outcome. The number of quasispecies present, a measure of genetic variability within a viral population, has been suggested as an important factor in evading the host immune response. Furthermore, quasispecies variability has been shown to be associated with increasing severity of liver disease,[20] whereas the number of quasispecies present in serum is higher in acute infection than in chronic infection.[21] More severe outcome appears to be correlated with selection of single species in the infecting HCV, which are presumably better adapted to survive in that particular immune environment.[22] The level of viremia in a patient with HCV infection can be determined using quantitative polymerase chain reaction (PCR) methods and is important in determining response to therapy.

Evidence suggests that the host immune response is important in determining the outcome of HCV infection. There are HLA associations with viral clearance as evidenced by positive antibodies and negative tests for circulating viral genome. In addition, patients with combined HCV and HIV infections and patients who were infected with HCV as a result of contaminated immunoglobulin given for hypogammaglobulinemia have more severe and more rapidly progressive liver disease.

PREVENTION

Strategies are available to prevent some, but not all, hepatitis virus infections; a detailed discussion is provided in Chapter 154.

CLINICAL FEATURES

Acute hepatitis

Anicteric disease

A large proportion of infections with any of the hepatitis viruses are asymptomatic or anicteric illnesses. Hepatitis A virus typically causes a minor illness in childhood, with more than 80% of infections being asymptomatic. In adult life infection is more likely to produce clinical symptoms, although only 30% of a cohort exposed to contaminated water experienced icteric illness.[2] Infections with HBV, HCV and HDV can also be asymptomatic. With HBV infection this again depends on the mode and time of transmission. Vertical transmission of infection from mother to child is almost always asymptomatic, although it produces chronic infection in the child. Transmission of HBV by other routes is much more likely to produce a symptomatic illness; about 30% of cases transmitted by intravenous drug use are icteric.

Clinically apparent acute hepatitis

Acute hepatitis presents with jaundice or elevated liver enzymes, usually preceded by a prodromal illness. The clinical features give little indication as to the likely etiologic agent. A history from patients who are suspected to have an acute hepatitis should be aimed at identification of specific risk factors for viral or other liver disease (Table 38.1).

Common symptoms in the preicteric phase include myalgia, nausea, vomiting, fatigue and malaise. There is often a change in the sense of smell or taste, and right upper abdominal pain is common. Coryza, photophobia and headache are often seen, and cough may be prominent in hepatitis A. Diarrhea with transient pale stools and dark urine may occur.

A serum sickness-like illness occurs in about 10% of patients who have acute HBV infection and in 5–10% of patients who have acute HCV infection.[23] This is characterized by an urticarial or maculopapular rash and arthralgia, typically affecting the wrist, knees, elbows and ankles. This illness is due to immune complex formation and rheumatoid factor is frequently positive. It is almost always self-limiting and usually settles rapidly after the onset of jaundice.

Table 38.1 Important points in the history of a patient who has suspected viral hepatitis

- Contacts with jaundiced patients
- Intravenous drug use
- History of blood transfusion
- Surgery or hospitalizations
- Family history of chronic liver disease
- Occupation



Fig. 38.4 Facial stigmata of chronic liver disease. This woman presented with serologically proven acute hepatitis A virus infection. She has multiple stigmata of chronic liver disease including facial spider nevi and was shown to have pre-existing cirrhosis.

Viral hepatitis may produce other clinical or subclinical problems. Acute hepatitis B is rarely associated with clinical pancreatitis in the acute phase of the illness, although elevation of amylase is present in up to 30% of patients and autopsy studies in patients who have fulminant hepatitis B show histologic changes of pancreatitis in up to 50%. Myocarditis, pericarditis, pleural effusion, aplastic anemia, encephalitis and polyneuritis have all been reported.

Physical examination in the preicteric phase is usually normal although mild hepatomegaly (10%), splenomegaly (5%) and lymphadenopathy (5%) may be seen. Stigmata of chronic liver disease should not be present in patients who have an acute illness, and their detection suggests either that the episode causing presentation is the direct result of chronic liver disease or that there has been an acute event superimposed on a background of chronic liver disease, as for example in HDV superinfection in an HBV carrier (Fig. 38.4).

The transaminase levels in acute hepatitis may reach 100 times normal.[24] Leukopenia is common, and in 10% of patients the white cell count may fall below 5000/mm^3.[25] Both anemia and thrombocytopenia are described. Immunoglobulin levels may be nonspecifically elevated in viral hepatitis, with levels usually returning to normal within 2 weeks. In a small proportion of patients who have acute viral hepatitis, a profound cholestatic illness may occur. This is most frequently seen in patients who have hepatitis A and it may be prolonged, with occasional patients remaining jaundiced for up to 8 months.

Death from acute viral hepatitis is usually due to the development of fulminant hepatitis. This is usually defined as hepatic encephalopathy with an onset within 8 weeks of symptoms or within 2 weeks of onset of jaundice.[26] The risk of developing fulminant liver failure is generally low but there are groups with higher risks. Pregnant women with acute HEV infection have a risk of fulminant liver failure of around 15%, with a mortality of 10–40%.[27] The risk of developing fulminant liver failure in HAV infection increases with age[28] and with pre-existing liver disease.[29] Fulminant hepatitis B is seen in adult infection but it is relatively rare.

The primary clinical features of acute liver failure are encephalopathy and jaundice. Jaundice almost always precedes encephalopathy in acute liver failure, and the onset of confusion or drowsiness in a patient who has acute viral hepatitis is always a sinister development. The degree of the rise in transaminase values does not correlate with the risk of developing liver failure. Prolongation of coagulation is the

biochemical hallmark of liver failure; it is caused by lack of synthesis of liver-derived clotting factors. Prolongation of the prothrombin time in acute hepatitis, even if the patient is clinically well without signs of encephalopathy, should be regarded as sinister and monitored closely. Hypoglycemia is seen only in fulminant liver disease, when it can be profound.

Chronic hepatitis

The agents that cause chronic hepatitis are HBV, HCV and HDV. Chronic hepatitis has been defined as abnormality of transaminase values persisting for more than 6 months. This has generally been a useful concept in patients who present with an acute illness. In clinical practice, the vast majority of patients will present with either an asymptomatic biochemical or serologic abnormality or the complications of cirrhosis, and it is reasonable to assume chronicity in these clinical settings at the time of initial presentation. Chronic viral hepatitis is characterized by the presence of inflammatory infiltrates in the liver associated with hepatocyte death.

The risk of developing chronic infection varies greatly with the virus implicated. Hepatitis A virus never causes chronic viremia or chronic liver disease. Hepatitis B virus causes chronic liver disease in a proportion of infected patients; this rate varies depending on the mode of transmission. Patients who are infected at or around the time of birth via a chronic carrier mother have infection rates of almost 100%, with the vast majority becoming chronic carriers of the virus and therefore at risk of long-term liver damage.[30] If HBV is acquired in later life, the risk of chronic infection falls considerably, with only 5% of such patients remaining HBsAg positive at 5 years.[31] The difference in the rate of chronic infection is probably related to the maturity of the host immune response.

Infection with HCV has, overall, the highest risk of chronicity. Post-transfusion studies indicate that at least 50% of patients with icteric disease develop chronically abnormal transaminase values, and most studies suggest that up to 80% of acutely infected people remain viremic.[32]

Hepatitis D virus can cause chronic liver damage and there is evidence to suggest that the combination of HBV and HDV carries a particularly poor outlook.

Most patients who have chronic viral hepatitis either present with a complication of their viral liver disease or are detected by screening, either for viral serology or for abnormal biochemistry.

Symptoms

The majority of patients who have chronic viral hepatitis are asymptomatic and patients are often unaware of the infection. In HCV infection a number of symptoms are frequently reported by patients in the absence of severe liver disease. These include lethargy, inability to concentrate and pain over the liver. It is unclear if these symptoms are the direct result of the viremia or are related to depression as a result of the diagnosis. These symptoms, whatever their origin, contribute to a reduced quality of life for patients with hepatitis C compared to patients with chronic hepatitis B.[33] Other symptoms are due either to associated diseases affecting organs other than the liver or to end-stage liver disease.

Extrahepatic manifestations

Chronic hepatitis B can be associated with polyarteritis nodosa, with vasculitic rash, fever and polyarthralgia. Circulating HBsAg and anti-HBs complexes can be demonstrated, as can cryoproteins and HBsAg in blood vessel walls.[34] Glomerulonephritis is now known to occur with both HBV[35] and HCV infections.[36] This is a relatively rare association thought to be mediated by the deposition of immune complexes. The renal lesion improves on treatment of the responsible virus. Up to 60% of patients who have mixed essential cryoglobulinemia are anti-HCV positive;[37] the condition is again thought to be the result of immune complex formation and antigen–antibody deposition in the vasculature. Successful therapy of the underlying hepatitis C produces remission.

Porphyria cutanea tarda is strongly associated with HCV infection, with up to 76% of Italian patients with porphyria cutanea tarda having antibodies to HCV.[38] Lichen planus is also associated with HCV infection.

Clinical signs

Patients who have decompensated cirrhosis caused by viral agents present with jaundice, encephalopathy, ascites or gastrointestinal bleeding as a result of portal hypertension (Fig. 38.5).

Cutaneous stigmata of chronic liver disease, spider nevi, leukonychia, gynecomastia, testicular atrophy and loss of body hair may be present. A late complication of chronic liver disease caused by HBV, HCV or HDV is hepatocellular carcinoma (Figs 38.6, 38.7).

Hepatocellular carcinoma may present as a sudden decompensation of previously stable chronic liver disease or as pain in the right upper quadrant of the abdomen, or there may be distant metastatic disease, with pulmonary or bone metastases being most common. Alpha-fetoprotein is often raised, although this is normal in up to 60% of small hepatomas.

All of these clinical signs occur late in the course of these viral infections and for most of the duration of infection no abnormal clinical signs will be present and the patient will be asymptomatic.

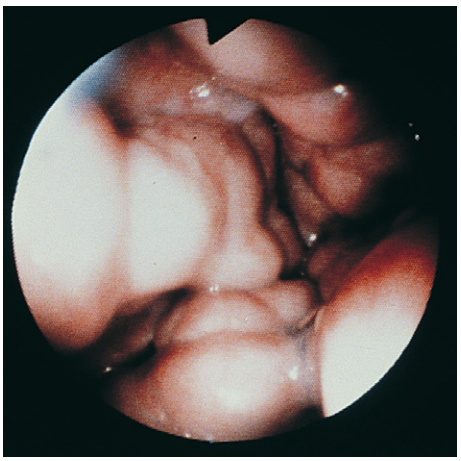


Fig. 38.5 Endoscopic view of esophageal varices.

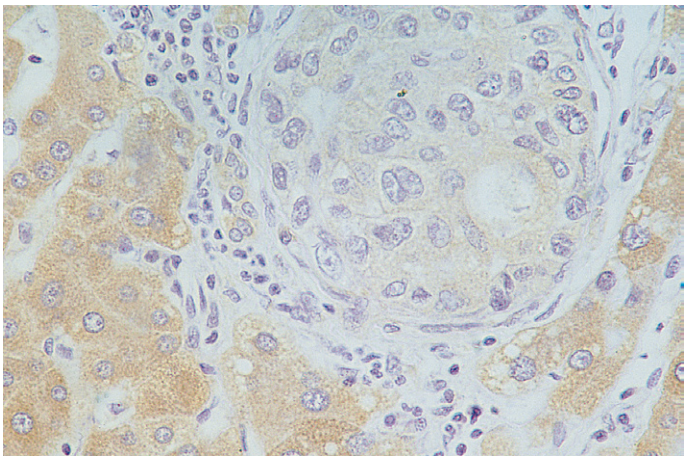


Fig. 38.6 Surgical histology of a nodule of hepatocellular carcinoma in a patient undergoing liver transplantation for hepatitis C cirrhosis.

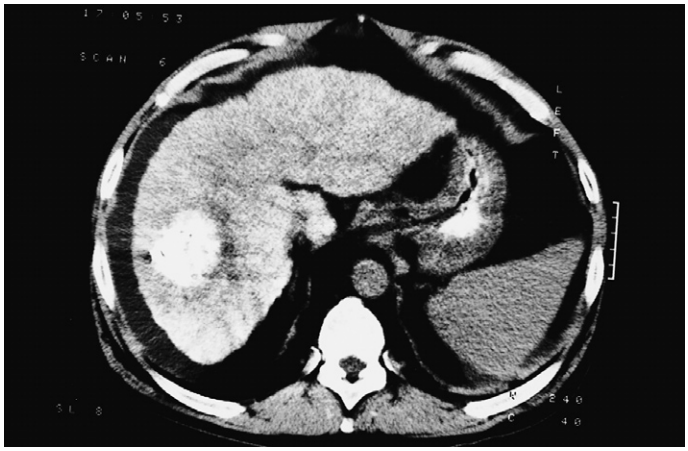


Fig. 38.7 CT scan following lipiodol injection into the hepatic artery showing hepatocellular carcinoma in the cirrhotic liver of a hepatitis B virus-positive male. Lipiodol is selectively retained in the tumor.

DIAGNOSIS

Acute hepatitis

Infection with HAV can be diagnosed by the presence of anti-HAV IgM with very high sensitivity and specificity.[39]

Infection with HBV is usually characterized by the presence of HBsAg. In acute HBV infection the serology can be difficult to interpret. The reason that an acute hepatitis develops is the immune recognition of infected liver cells, which results in T-lymphocyte-mediated hepatocyte killing. Active hepatocyte regeneration then occurs to replace those hepatocytes that have been lost. Because of the brisk immune response at the time of presentation with acute hepatitis B, viral replication may already have ceased and the patient may be HBsAg positive, hepatitis B e antigen (HBeAg) negative.

The diagnostic test is IgM antibody to HBcAg (IgM anticore; Fig. 38.8), which represents a sensitive and specific marker of acute HBV infection.[40]

Diagnosing past HBV infection or establishing immunity to vaccine is easy serologically. The levels of HBV-associated antibodies

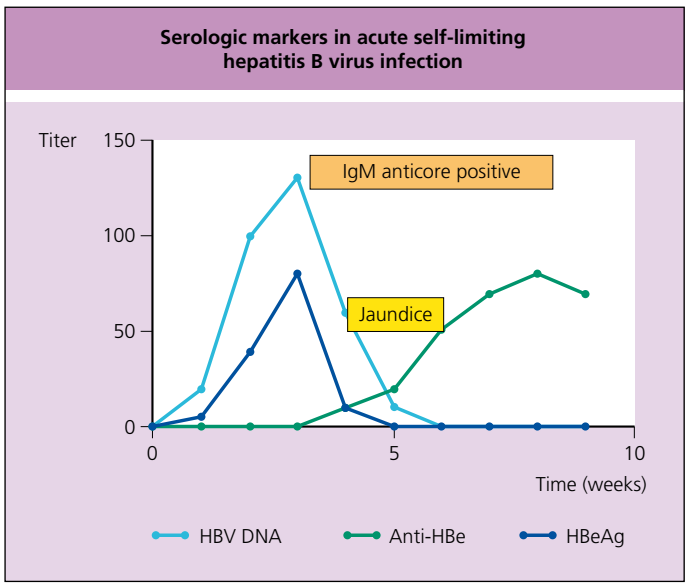


Fig. 38.8 Serologic markers in acute self-limiting hepatitis B virus (HBV) infection. HBeAg, hepatitis B e antigen. Measurements in arbitrary units.

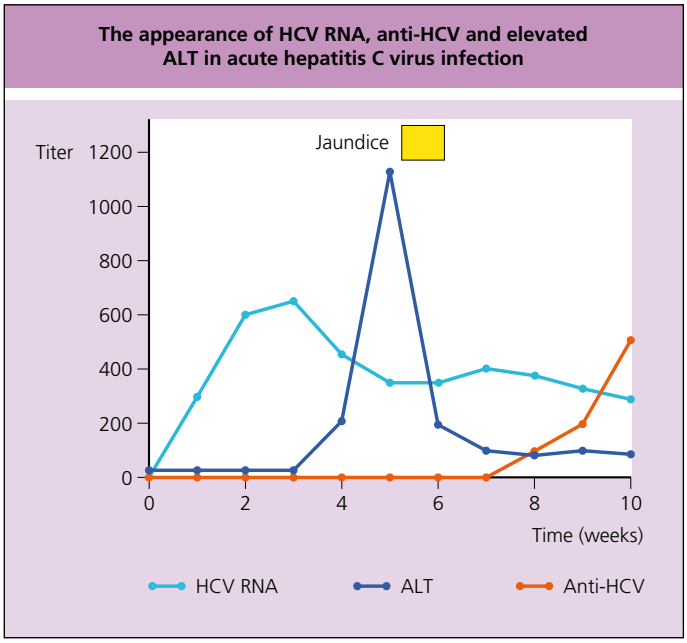


Fig. 38.9 The appearance of hepatitis C virus (HCV) RNA, anti-HCV and elevated alanine transaminase (ALT) in acute HCV infection. Measurements in arbitrary units.

decline with time. This is particularly true of anti-HBsAg. Despite this low level, immunity is still sufficient to prevent re-infection. Natural immunity is proven by the presence of IgG antibodies to HBcAg. These are a highly reliable marker of past infection and remain at detectable levels for a long period. Vaccine-induced immunity is directed purely against HBsAg epitopes, and hence a vaccinated person will have detectable levels of anti-HBsAg but no anti-HBcAg.

Acute hepatitis C cannot be reliably diagnosed by antibody tests because these frequently do not become positive for up to 3 months (Fig. 38.9).

Hepatitis C virus used to be the cause of more than 90% of all post-transfusion hepatitis in Europe and North America.[41] Acute HCV infection is now most commonly seen in intravenous drug users or in HIV-positive men who have sex with men where the risk of sexual transmission seems relatively high. If clinical suspicion is high and risk factors are present, testing of serum for HCV RNA is the only means of establishing the diagnosis (see Fig. 38.9). Identification of cases of acute HCV infection is important because there is strong evidence that early treatment with interferon alpha (IFN-α) may reduce the risk of chronic infection with HCV from around 80% to less than 10% with interferon monotherapy given for 6 months.[42]

Patients with HBV infection are at risk of delta virus infection either after HBV infection (superinfection) or simultaneously (co-infection). Co-infection is usually relatively benign; the determining factor for severity of illness is the HBV infection. As this is usually self-limiting, with loss of HBsAg after a relatively short interval, chronic infection with HDV is rare (about 2%).

Superinfection with HDV is a much more severe disease. The presence of large amounts of HBsAg allows rapid replication of the HDV and establishment of both acute and chronic infection. Chronic infection is common in this setting because the continued production of HBsAg allows the delta agent to continue replication. Diagnosis of delta hepatitis is by antibody testing and viremia is confirmed by the detection of HDV RNA in serum.[43]

A number of other viruses can cause hepatitis (Table 38.2). In the main, liver involvement is incidental and other features of the illness will suggest a diagnosis other than primary hepatitis. The exceptions are yellow fever, and disseminated herpes simplex and varicella-zoster infections in immunosuppressed patients, which in both cases may be associated with a severe hepatitis.

Table 38.2 Viral infections that may be associated with hepatitis	
CMV, EBV	Both these viruses cause an acute mononucleosis-type syndrome in the normal host. Mild elevations of the liver transaminases may be seen but are usually of little consequence
CMV, HSV, VZV	All may cause hepatitis in the immunosuppressed host as part of a disseminated infection. CMV is the least severe
Flaviviruses	Yellow fever causes severe hepatitis
Measles, rubella, rubeola, Coxsackie B adenovirus	Mild hepatitis occurs occasionally

CMV, cytomegalovirus; EBV, Epstein–Barr virus; HSV, herpes simplex virus; VZV, varicella-zoster virus.

Chronic viral liver disease

Chronic viral liver disease may present with abnormal liver biochemistry (elevated transaminases), by serologic testing in at-risk groups without symptoms or as a result of the complications of cirrhosis. In contrast to the situation in acute infection, the rise in the transaminases is characteristically only two or three times the upper limit of normal. In HCV infection, the gamma-glutamyl transpeptidase values are also often elevated. The degree of abnormality of transaminases has little relevance to the degree of underlying hepatic inflammation. This is particularly true of HCV infection, in which the transaminase values are often normal despite active liver inflammation.

Further investigation in patients who have a positive serologic test for a hepatotrophic virus is to exclude other concomitant liver disease and assess the severity of liver damage. Table 38.3 shows the required baseline serologic tests required. In HCV infection, autoantibodies are frequently positive, antismooth muscle antibodies being detected in about 10% of patients and low-titer antinuclear antibodies in 15–20%. These autoantibodies do not appear to play a part in pathogenesis and would only alter management if a high titer were present with a raised IgG, suggesting a primary autoimmune hepatitis, in which interferon therapy may be contraindicated.

Chronic hepatitis B

The vast majority of patients who have hepatitis B will be HBsAg positive. There are viral mutants that do not produce HBsAg detectable by the usual serologic tests (Table 38.4), but these are very rare except in patients treated with interferon or after liver transplant.

The traditional classification of those individuals who have chronic hepatitis B has been based on the presence of HBeAg. All patients who have HBsAg can be regarded as infected but HBeAg has been regarded as a marker of infectivity. Hepatitis B e antigen is produced as a truncated version of core protein (Fig. 38.10).

Table 38.3 Tests required in asymptomatic patients who have elevated transaminase levels

- Hepatitis B surface antigen
- Hepatitis C virus antibodies
- Antismooth muscle, antinuclear antibodies
- Immunoglobulins
- Copper studies (if patient >50 years)
- Ferritin
- α_1-Antitrypsin phenotype (if indicated)

Table 38.4 Tests required for diagnosis in hepatitis B virus infection

- Hepatitis B surface antigen
- Hepatitis B e antigen
- Hepatitis B anticore antibody (HB total anticore)
- Hepatitis B DNA
- Hepatitis B IgM anticore

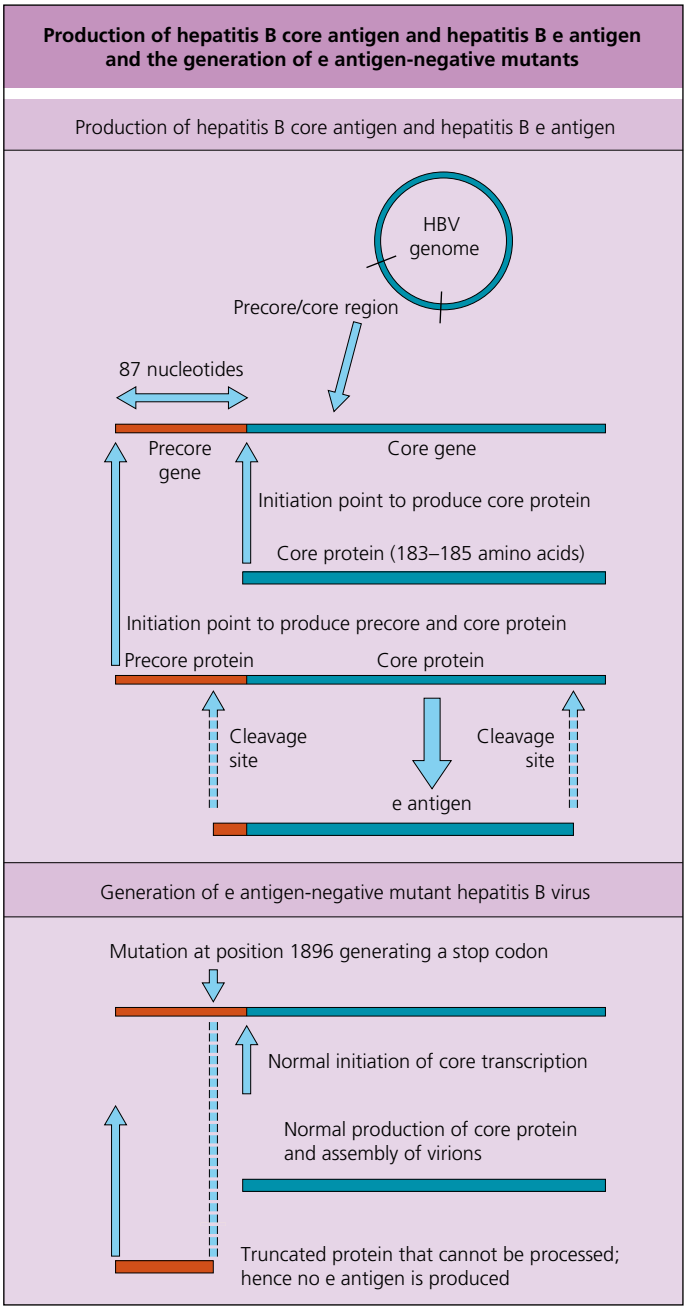


Fig. 38.10 Production of hepatitis B core antigen and hepatitis B e antigen and the generation of e antigen-negative mutants. Hepatitis B virus (HBV) has a closed circular genome that contains insufficient bases to produce all its required proteins. It therefore uses different start points for transcription, enabling it to use the same base sequence to produce different proteins. Two important proteins, e antigen and core antigen, are produced by transcription of the same region with overlap; e antigen is produced from the core protein by cleavage at a specific site.

Table 38.5 Investigations required in anti-hepatitis C virus-positive patients

Tests to assess hepatitis C virus	Tests to exclude other liver diseases
PCR for hepatitis C virus RNA Viral load Genotype	Ferritin Autoantibodies and immunoglobulins Hepatitis B serology Liver ultrasound

In the wild-type virus, HBcAg and HBeAg are transcribed together using overlapping bases. Hence HBeAg is an indirect marker of viral replication. The exact function of HBeAg is unclear but it appears to be involved in the downregulation of the T-lymphocyte response to HBV infection and may play a pivotal role in the development of the long phase of relative immunologic tolerance seen in chronic HBV infection acquired in childhood.

The discovery of HBeAg-negative mutant viruses has produced the need for a more specific marker of viral replication. The measurement of HBV DNA directly detects the circulating HBV genome.[44] This is the most sensitive measure of viral activity and is measured using PCR-based techniques, which can detect levels down to 40 IU/ml.

Patients who have persistence of HBsAg after 20 weeks of an acute episode can be assumed to have chronic infection. Spontaneous loss of HBsAg after this time is unusual, being seen in only 1–2% of patients a year. An apparently healthy person who is HBsAg positive can be regarded as chronically infected with HBV. The prognosis for that person is largely determined by the presence or absence of ongoing viral replication.

Chronic hepatitis C

Screening for HCV relies on antibody testing. Direct detection of viral RNA in peripheral blood using PCR provides evidence of ongoing viremia. Patients presenting with detectable antibodies to HCV should be investigated (Table 38.5) to exclude other causes of chronic liver disease and to assess the factors that will affect the outcome of therapy.

Chronic hepatitis D

Chronic infection with HDV has a high risk of producing severe liver disease. In the vast majority of cases of chronic infection, the HBV infection is nonreplicative as evidenced by HBeAg negativity and absence of HBV DNA.[45] This viral interference is seen in many combined viral infections (e.g. HBV and HCV infections) and it is rare to find patients with two replicating hepatotropic viruses. However, if the accompanying HBV is replicating, the prognosis is very poor, with rapid progression to cirrhosis over as little as 2 years.[45]

Diagnosis of chronic HDV infection is by antibody testing and viremia confirmed by delta RNA testing.

MANAGEMENT

Hepatitis B

Natural history of hepatitis B infection

Worldwide the majority of chronic HBV infections are in childhood. Infection in infancy with an immature immune system allows chronicity of infection. There are then two key factors in the outcome of that infection: viral replication and the inflammatory liver disease induced by the host in an attempt to clear infected hepatocytes from the liver. The virus is not itself cytopathic, other than in profound immunosuppression. The degree of immune recognition determines control of viral replication as well as causing liver injury.

There are a number of phases of infection with HBV determined by this immune response. Initially, there is high level viral replication in a host who has no immune recognition of the virus. At this stage there is no liver injury, hence alanine transaminase (ALT) is normal and if a liver biopsy were performed there would be no inflammatory liver disease. This phase of HBV infection lasts a variable time, from days to decades.

At some point, however, immune recognition of HBV-infected hepatocytes starts with the onset of liver injury. This phase of immune activation again is variable in its duration which is important in the risk of development of hepatic fibrosis, cirrhosis and its complications. If immune control is established rapidly it is likely that viral inactivation will occur (marked by the transition from e antigen to e antibody positivity) without the development of severe liver fibrosis; however, the opposite is also true, that a prolonged immune active phase may well result in the death of the infected patients from the complications of cirrhosis. Thus one of the key goals of therapy in e antigen-positive HBV is to reduce this phase of viral replication in the face of an immune response to reduce the risk of hepatic fibrosis. In most patients, with or without therapy, inactivation of HBV is marked by e antigen seroconversion and cessation of viral replication. With this control of viral replication liver injury also stops and transaminases return to normal. The patient remains infected with HBV and continues to be HBsAg positive. This was previously referred to as 'chronic carrier state' but this term is rather misleading and implies a benign process which is not always the case. The phase of immune control may last lifelong but is a dynamic situation, with the virus attempting to replicate and the host immune response providing control.

A final phase of HBV infection then ensues in approximately 40% of patients with the development of precore mutants of HBV which evade a previously adequate immune response and resume replication with concomitant liver injury.

Assessment of a patient who has chronic HBV infection should include ALT, e antigen and antibody status and HBV DNA levels. In patients who have no evidence of viral replication and normal liver enzyme levels and a normal liver ultrasound, liver biopsy is not usually required. Such patients have a very low risk of developing symptomatic liver disease or hepatocellular carcinoma. Reactivation of HBV replication has been described due to development of viral mutants which escape immune surveillance and patients who are HBsAg positive should be followed with yearly serology and liver enzyme estimations. The low risk of hepatocellular carcinoma does not justify screening in this group unless the patient is known to have cirrhosis. In patients who have abnormal liver biochemistry (even without detectable HBV DNA) or an abnormal liver texture on ultrasound, a liver biopsy is probably required, because such patients may either have superinfection with HDV or have had ongoing replication of HBV in the past, sustaining substantial liver damage in the process. It is estimated that around 5% of patients in developed countries who have only HBsAg carriage at presentation will have a posthepatic cirrhosis on liver biopsy. This finding is important because they are at risk of developing the complications of cirrhosis including variceal bleeding and hepatocellular carcinoma.

Patients with detectable HBV DNA in their blood are at risk of developing liver disease. There are a number of possible aims in HBV therapy, with the ideal being clearance of HBsAg. This is not commonly possible with current therapy and it is now generally accepted that the key aim is viral inactivation, with the hope that sustained suppression of viral replication will in time lead to viral eradication. There are two phases of HBV when therapy may be helpful: eAg positive immune-active phase and eAg negative reactivation (see Fig. 38.2).

Assessment of patients who have HBV replication is carried out by assessing the degree of liver inflammation and the stage of the underlying liver disease. Patients who are still in the tolerant phase of their infection usually have normal transaminase levels and high serum levels of HBV DNA (in excess of 10^8 IU/ml). As immune recognition increases, the HBV DNA levels fall, the degree of hepatic damage increases and the transaminases become abnormal. This is important in the selection of patients for therapy. Patients who have viral

replication and abnormal transaminases should have a liver biopsy. The need for liver biopsy in a patient who has high levels of HBV DNA and repeatedly normal transaminases is less clear because it is very unlikely that the biopsy will show advanced liver disease or considerable inflammatory activity. Age and gender are important in the risk of immune tolerance persisting and a male of 40 has a high risk of liver injury compared to a female of 20. The normal ranges for transaminases are also probably incorrect, not reflecting the variability with age. A normal ALT for a 20-year-old female is probably less than 20. For all of these reasons, liver biopsy has played an increasing role in assessment of patients with HBV infection.

Therapy for hepatitis B

When assessing the need for therapy in HBV infection the phase of the infection and the natural history should be taken into account. There is now compelling evidence that HBV DNA levels predict both the progression to cirrhosis and complications of liver disease (see Fig. 38.3) and the risk of developing hepatocellular carcinoma, particularly so in patients outside the immunotolerant phase.[46] There are differences in both treatment type and aims in HBV infection in the e antigen positive and negative phases.

There are two types of licensed drug treatment for hepatitis B: IFN-α and nucleoside/nucleotide analogues. Although the agents used to treat hepatitis B vary considerably in their mode of action, the basic aim of treatment is the same – to stop viral replication. The concept of 'viral eradication' is not possible in hepatitis B, as it is clear that viral DNA remains present in infected individuals over their lifetime. This is exemplified by reactivation of HBV in patients undergoing chemotherapy or other profound immunosuppression in whom the only marker of infection is antibodies to core antigen. It is, however, clear that almost all the major sequelae of infection with HBV are a consequence of active viral replication. Individuals who remain HBsAg positive but have no ongoing viral replication are not at risk of developing significant hepatic fibrosis and consequently have a dramatically reduced risk of developing decompensated liver disease compared to those with ongoing viral replication. The only serious complication of HBV infection which is not entirely dependent on viral replication is cancer development where a risk remains, particularly in those with established cirrhosis. Even there, the risk of developing hepatocellular carcinoma is substantially greater in patients where replication continues. Consideration of therapy is therefore only appropriate in patients where HBV is active as evidenced by detectable levels of HBV DNA. The choice of therapy is then determined by a number of factors: the stage of infection, the level of HBV DNA, the presence of mutant virus as the predominant strain (as evidenced by the presence or absence of e antigen), the degree of inflammatory liver disease and the degree of hepatic fibrosis.

Interferon therapy

Interferon alpha in eAg-positive HBV

Interferon alpha (IFN-α) was first shown to be effective for some patients who have HBV infection in the 1980s and it remains an effective therapy.[47] There are a number of commercially available variants of IFN-α including natural interferon, recombinant interferons and consensus interferons. In HBV infection there is little evidence that the type of IFN-α used has any substantial difference in effect or side-effect profile. Pegylated interferons are more convenient for patients because of once rather than three times weekly administration and have become the standard form of IFN-α used in HBV infection despite no evidence of improved effectiveness. There are a number of factors that can help predict the likelihood of response to treatment with IFN-α in e antigen-positive disease, and these help in selection of patients who have the best chance of response to therapy (Table 38.6).

Overall, in e antigen-positive disease the probability of response to interferon therapy in chronic hepatitis B is between 25% and 40%. A response to therapy is loss of e antigen in blood, accompanied by the development of antibodies to e antigen and the cessation of viral replication; only a small number of patients lose all markers of infection with HBV (2%) and HBsAg usually remains in the serum. There is now good evidence that successful therapy with interferon, which renders the HBV nonreplicative, produces a sustained improvement in liver histology and a decrease in the risk of developing end-stage liver disease. The risk of developing hepatocellular carcinoma also appears to be reduced but is not abolished in those who remain HBsAg positive. The likely reason why a number of patients with apparently successful responses to interferon therapy go on to develop significant liver disease is the emergence of mutant HBV strains which resume viral replication and hence liver cell injury.

Interferon therapy has a number of drawbacks: it is not effective in many patients, it is expensive and it has a large number of side-effects (Table 38.7). In general, about 15% of patients on interferon therapy have no side-effects, 15% cannot tolerate therapy and the remaining 70% experience side-effects but are able to continue therapy. Most patients are able to continue at work during therapy but many require substantial support. Depression can be a major problem and both suicide and admissions with acute psychosis are well described. Early use of antidepressants and close monitoring are essential, especially if there is a preceding history of depression.

Treatment of hepatitis B in patients who have significant fibrotic liver disease is rewarding because they have a relatively high probability of response and have most to gain from cessation of viral replication. Many patients have a substantial improvement in liver function if viral replication is stopped, but treatment in such patients does carry an increased risk. Interferon therapy produces viral clearance, at least in part, by inducing immune-mediated killing of infected hepatocytes, and hence a transient hepatitis can cause severe decompensation requiring liver transplantation.

Table 38.6 Factors indicating the likelihood of response to interferon in chronic hepatitis B virus infection

Factor	High probability of response	Low probability of response
Age	<50 years	>50 years
Sex	Female	Male
Hepatitis B DNA level	Low	High
Activity of liver inflammation	High	Low
Country of origin	Europe, North America, Australasia	Asia
Co-infection with HIV	Absent	Present

Table 38.7 Common side-effects of interferon-α therapy for viral hepatitis

Side-effect	Frequency (%)
Flu-like syndrome	80
Depression	20
Local inflammation at injection sites	25
Hypothyroidism	10
Arthralgia or arthritis	10
Hair loss	10

The optimal dose and duration of interferon for hepatitis B remains somewhat contentious, but most clinicians use pegylated interferons, with doses based on body weight for the 12 kDa molecule and a standard dose of 180 μg for the 40 kDa molecule for 6 months, even though trials with pegylated interferons were with 1 year's therapy. This practice is based on the extensive data using non- pegylated interferon where 6 months was as effective. No comparative data exist as to their relative effectiveness.

Patterns of response and special situations

The most frequent pattern of response to IFN-α is shown in Figure 38.11. The HBV DNA level falls rapidly after initiation of interferon therapy. This is followed by a marked rise in transaminase values. This represents immune-mediated clearance of virus, and HBV DNA levels quickly fall to undetectable levels. This is then followed within a few weeks by seroconversion to HBeAg-negative, HBeAb-positive status with complete normalization of transaminases. This type of response is seen in 25% of treated patients. Hepatitis B surface antigen usually remains positive, with a small proportion of patients clearing all markers of viral infection either during interferon therapy (2%) or many months after it (6%).

Delayed responses to therapy occur in 10–15%, usually within 9 months of completion of therapy

Interferon alpha in eAg-negative HBV

Interferon therapy for e antigen-negative patients is less effective. In this phase of the disease there is no e antigen seroconversion and therefore end points of studies are based on virologic suppression. Although a good rate of initial response to interferon with disappearance of HBV DNA from serum can be achieved, patients very frequently relapse after cessation of treatment (Fig. 38.12). Giving interferon therapy for longer in such patients may improve the rate of loss of viral replication. A regimen of 6 000 000 units of standard interferon three times a week for 24 months has been shown to produce a loss of viral replication in 30% of patients.[48] Pegylated interferons given in a 12-month treatment schedule were shown to be more effective than lamivudine[49] 6 months after stopping therapy; however, this is probably not a realistic trial as the use of nucleoside analogues would not be short term. The study did, however, show that a durable response to pegylated interferon could be achieved with suppression of viral replication below 400 IU/ml in 18% of patients after 6 months off therapy. This study also confirmed that the combination of interferon and lamivudine did not add to overall response rates to interferon (Fig. 38.13).

Clearly while confirming that pegylated interferon can be effective in a minority, the side-effects of interferon will make it second-line therapy for most patients and clinicians in e antigen-negative chronic hepatitis B.

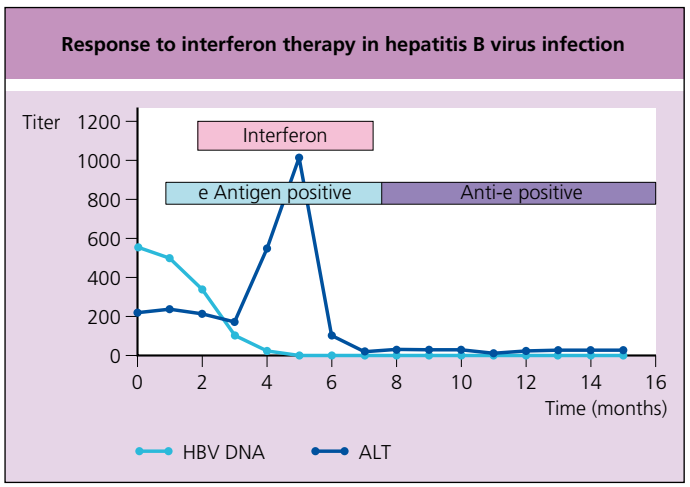


Fig. 38.11 Response to interferon therapy in hepatitis B virus (HBV) infection. ALT, alanine transaminase. Measurements in arbitrary units.

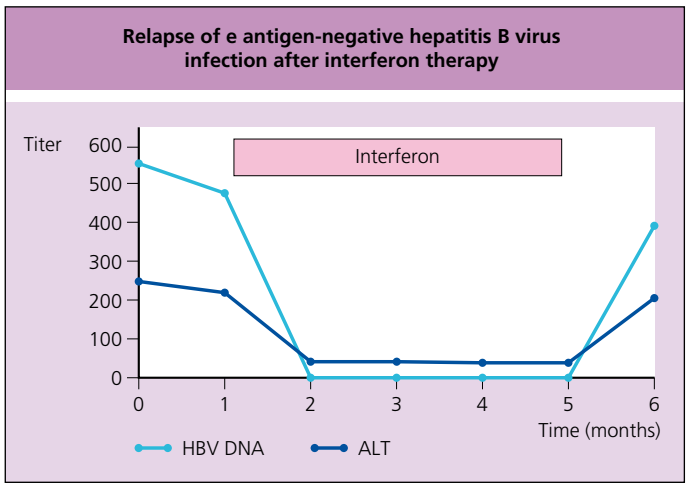


Fig. 38.12 Relapse of eAg-negative hepatitis B virus (HBV) after interferon therapy. ALT, alanine transaminase. Measurements in arbitrary units.

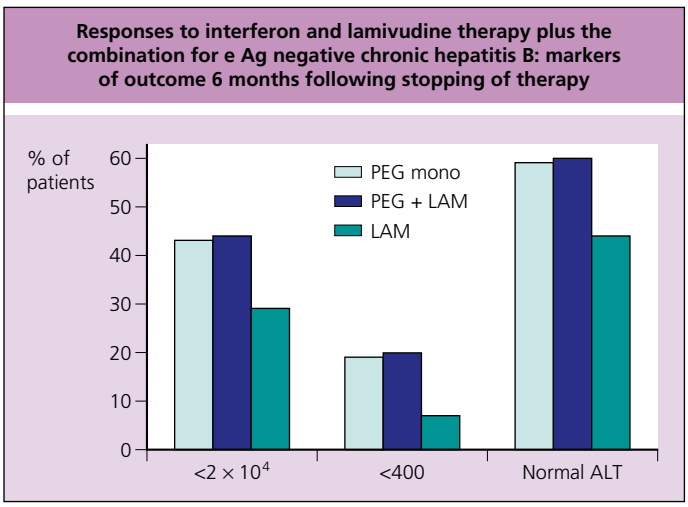


Fig. 38.13 Responses to interferon and lamivudine (LAM) therapy plus the combination for e Ag negative chronic hepatitis B: markers of outcome 6 months following cessation of therapy. The left columns are HBV DNA, either below a commonly accepted threshold for clinical significance or the limit of detection of the assay. ALT, alanine transaminase; mono, monotherapy; PEG, pegylated interferon. Redrawn from Marcellin *et al.*[49]

Nucleoside/nucleotide therapy

There are now a number of oral antiviral agents available for the treatment of HBV infection. The first of these, lamivudine, established the principle that these agents could improve the outcome in patients with serious liver disease by directly inhibiting HBV replication. A randomized study versus placebo in South East Asia showed a reduction in both progression of liver disease and cancer development in treated patients and hence no further placebo-controlled studies in HBV are ethical[50] (Fig. 38.14). Lamivudine also established the key feature of all oral antiviral agents active against hepatitis B, i.e. resistance. Initial descriptions of mutations in the tyrosine–methionine–aspartate–aspartate (YMDD) motif of the HBV DNA polymerase gene were rapidly confirmed and shown to result in reactivation of replication, albeit at a lower level, and resumption of liver injury as a result (Fig. 38.15).

Emergence of lamivudine-resistant HBV is increasingly common with prolonged treatment. Genotypic resistance is detectable in 14–32% of patients after 1 year and increases to 38%, 49% and 66%

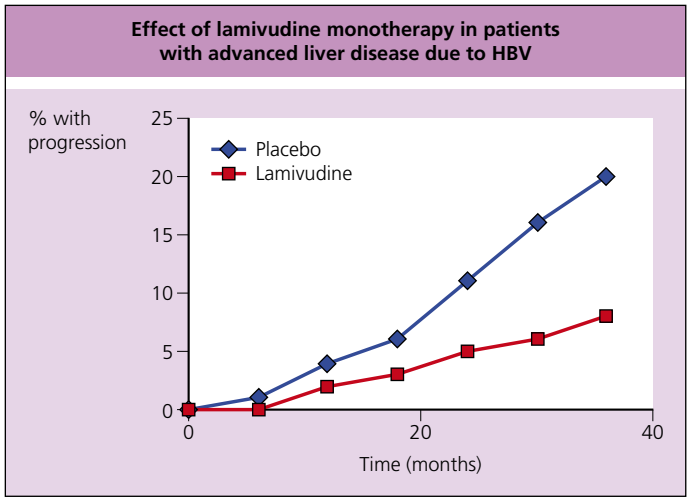


Fig. 38.14 Effect of lamivudine monotherapy in patients with advanced liver disease due to HBV. Progression was either points in Child–Pugh score or the development of hepatocellular carcinoma. Redrawn from Liaw *et al.*[50]

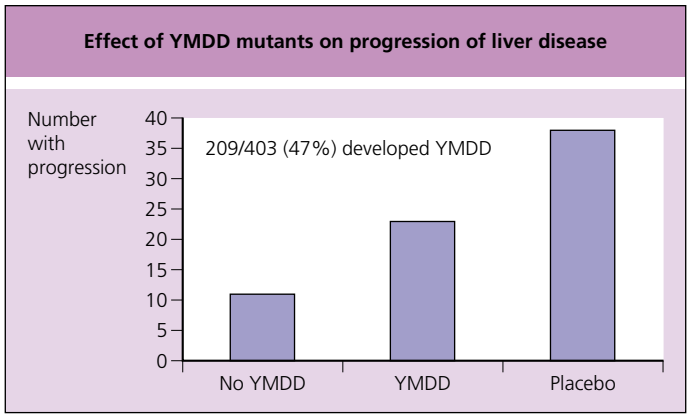


Fig. 38.15 Effect of tyrosine–methionine–aspartate–aspartate (YMDD) mutants on progression of liver disease, showing that resumption of viral replication leads to the development of progressive liver disease and confirms that YMDD mutant viruses are just as pathogenic. Redrawn from Liaw *et al.*[50]

after 2, 3 and 4 years, respectively.[51] There is no doubt that despite the lesser replication competence of YMDD mutant viruses as evidenced by the lower HBV DNA levels, they are still responsible for liver injury and once replication resumes liver disease resumes. This was demonstrated clearly in the study by Liaw and colleagues,[50] with patients with lamivudine resistance showing an increased risk of progression of their liver disease.

When assessing the oral agents now available there are key features that will predict their potency, i.e. the ability to rapidly and profoundly suppress viral replication. This is important as replication of the virus in the presence of drug is highly likely to select for mutant viral species which can survive in the presence of drug. The mutational resistance profile determines where changes in HBV DNA can select for drug resistance; thus if two drugs have a similar resistance profile then resistance to one is highly likely to predispose to resistance to the other. Currently available drugs and key resistance data are shown in Figure 38.16.

A small subgroup of patients with e antigen-positive chronic hepatitis B who would be eligible for short-duration therapy with oral agents are those who seroconvert from e antigen positivity to e antibody on

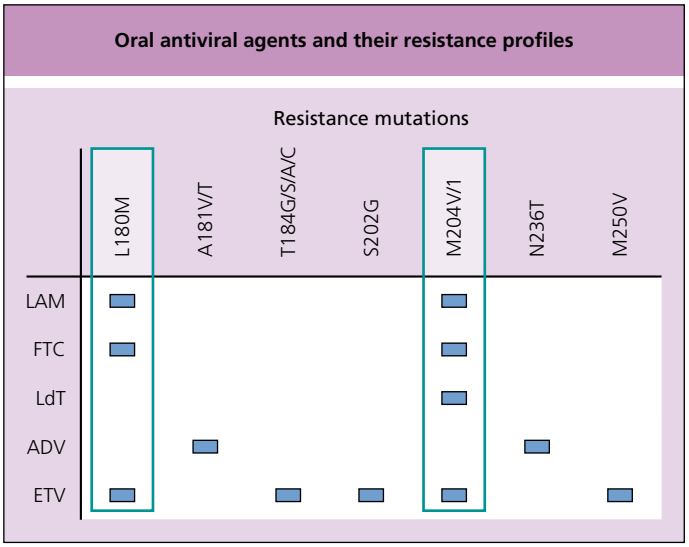


Fig. 38.16 Oral antiviral agents and their resistance profiles. The key feature is that most drugs share a resistance mutational profile with lamivudine (LAM) and cross-resistance is highly likely. Entecavir (ETV) shares this resistance but requires additional mutations for full resistance to develop. Adefovir dipivoxil (ADV) has a distinct resistance profile which it shares with tenofovir (not shown). FTC, emtricitabine; LdT, telbivudine.

therapy (see Fig. 38.17). A strategy of stopping drug when e seroconversion has occurred appears safe providing that sufficient time after seroconversion is given to consolidate the switch, probably 1 year. This is also with the caveat that HBV DNA should be completely suppressed. This may give the patient a significant time away from therapy but monitoring for reactivation remains mandatory. If the patient remains eAg positive after a year of therapy, then the drug therapy will have to be continued.

In Europe, up to 60% of all replicating hepatitis B infections now are in the late, e antigen negative, phase of infection. It is well described that these mutants are generally much less responsive to therapy with IFN-α and given the lack of a marker for immunologic control in this setting there will be a need for long-term therapy once initiated. Modeling of hepatocyte turnover suggests that with complete viral inhibition, and therefore a lack of new hepatocytes being infected, it may take more than a decade of continued viral suppression to clear all infected hepatocytes from the liver. The potential aims of therapy in patients on oral antiviral agents, both those with e antigen-positive disease who have not achieved short-term seroconversion and those with e antigen-negative disease, will be surface antigen loss. This will, however, require high levels of compliance with tablet taking over a decade or more to achieve – a significant challenge.

Lamivudine is not suitable for this task. Resistance rates are high and resumption of replication occurs in almost all patients given time. The newer agents are more effective, both in terms of potency and the genetic barrier to resistance. In terms of resistance profiles there are two classes of oral agent: lamivudine-like and adefovir-like (Table 38.8). There is no rationale for the use of sequential therapy with drugs of the same class; even entecavir, which requires a third mutation in the HBV DNA polymerase in addition to the two common ones which confer resistance to lamivudine, has been shown to have high rates of resistance if used in the presence of pre-existing lamivudine resistance mutants[52] (Fig. 38.17). This means that initial therapy must be with either a potent drug as monotherapy (entecavir or tenofovir), with the availability of a drug from the other class to act as rescue therapy if this fails, or with a combination of a drug from each class at the outset. National guidelines generally allow both strategies, and it will fall to individual clinicians and patients to decide, although the experience with HIV tends to suggest that combining drugs with differing resistance profiles may be the better strategy when 10 or more years of

Table 38.8 Markers of response to therapy including eAg seroconversion rates after 1 year of therapy with existing anti-HBV agents

	DRUG			
Outcome	**Lamivudine (%)**	**Adefovir (%)**	**Entecavir (%)**	**Pegylated interferon (%)**
HBV DNA negative	44	21	67	25
eAg/AB seroconversion	18	12	21	34
Alanine transaminase normal	55	48	68	39
Durability	50–80	91	82	82

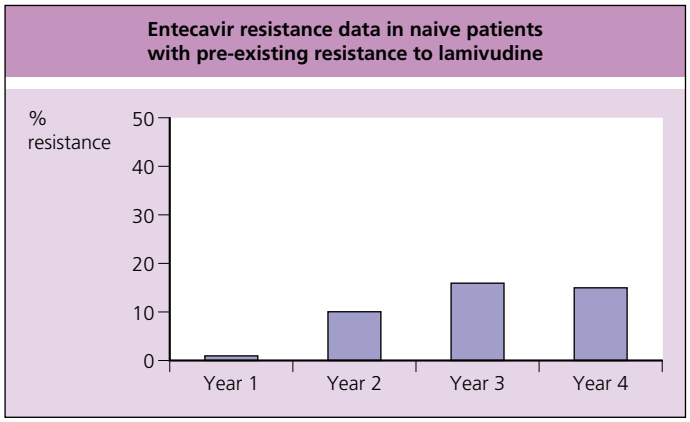


Fig. 38.17 Entecavir resistance (ETVr) data in patients with pre-existing resistance to lamivudine (LVDr). ETVr = LVDr (M204V & L180M) + T184, S2 and/or M250 substitutions plus virologic breakthrough (≥1 log increase from nadir) over 4 years of entecavir therapy in patients with prior lamivudine resistance. No resistance is seen at 4 years in non-lamivudine-resistant patients by comparison. Redrawn from Colonno *et al*.[53]

viral suppression is required to have any chance of cure. There are few significant side-effects of these agents, although renal impairment is a potential issue with tenofovir and monitoring is recommended.

Treatment of HIV and HBV co-infection (See Practice Point 44)

With the advent of improved antiretroviral therapy, patients with HIV infection have good immune reconstitution and excellent long-term survival. Co-infection with hepatitis viruses is common and liver disease is now a leading cause of death in HIV-infected people. Hepatitis B infection in this group is challenging to manage. Many patients have been exposed to lamivudine as part of their antiretroviral therapy and have high levels of replication with lamivudine-resistant HBV. In this setting interferon has only a limited role, as the altered immune environment rarely allows clinical response to this agent. In general the strategy in treatment-naive patients is to treat both HIV and hepatitis B. If the HIV requires therapy then it may be reasonable to give as part of that therapy two agents which will also suppress HBV, such as tenofovir and either lamivudine or emtricitabine (as Truvada in a single pill). If the HIV does not require therapy and the HBV does, a relatively unusual situation, the choices are difficult as most agents (including entecavir) do have activity against HIV which will incur resistance in that virus. In patients already on highly active antiretroviral therapy (HAART) for HIV who have HBV infection, significant HBV replication should lead to assessment of the liver and a change of therapy to cover HBV if significant liver injury is present.

Hepatitis C

Natural history of hepatitis C virus infection

In order to assess the need for treatment in a patient who has hepatitis C it is important to have a clear understanding of the natural history of this infection and of the factors that may predispose to more severe outcome. It is clear that HCV-related liver disease is usually slowly progressive, taking many years to produce significant hepatic fibrosis. The most definitive studies to date suggest that the average time from infection to the development of cirrhosis is 33 years.[54] The rate of progression is, however, very variable, with some 'rapid fibrosers' progressing to cirrhosis 11 years after infection and other 'slow fibrosers' who would take more than 40 years to progress to cirrhosis. The major factors associated with increased risk of progressive liver disease are age over 40 years at infection, high alcohol consumption and male sex (Fig. 38.18).

Therapy for hepatitis C

The current standard of care treatment for hepatitis C is a combination of pegylated IFN-α and ribavirin.

Interferon alpha was first used to treat non-A, non-B hepatitis in the 1980s. Since the identification of HCV in 1989, a large amount of information has become available about the effectiveness of interferon therapy in HCV infection and its drawbacks. These 'standard interferons' have been superseded by the advent of pegylated molecules, and these should now be regarded as the standard treatment for HCV infection when given in combination with ribavirin.

Defining response to therapy

There are three potential responses to interferon-based therapy in patients who have HCV infection:

- no response in virologic markers (nonresponders);
- a suppression of HCV RNA on therapy but relapse after cessation of therapy (relapsers); or

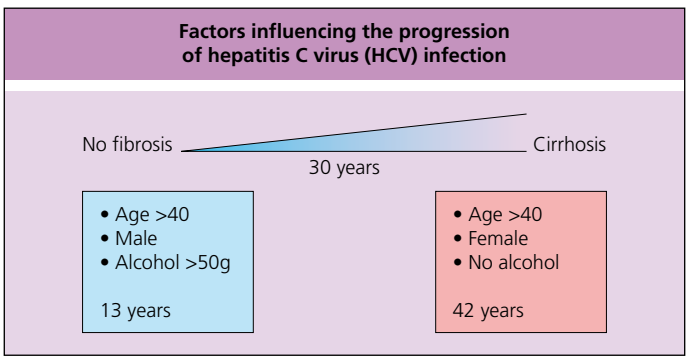


Fig. 38.18 Factors influencing the progression of HCV infection. Redrawn from Poynard *et al*.[54]

- a disappearance of HCV RNA that is maintained after stopping therapy (sustained responders).

In addition, the kinetics of viral disappearance in the responder groups also has prognostic significance, a rapid virologic response (RVR) being PCR negativity in serum using a sensitive assay (40 IU/ml) at week 4 of treatment, and early virologic response (EVR) being either PCR negativity or a 2 log drop in HCV RNA at week 12. The RVR is used as a predictor of high sensitivity to interferon-based therapy and may safely allow shortening of therapy and the EVR is used in a negative fashion, since failure to achieve this implies a poor response and early cessation of therapy.

Overall, the chance of sustained virologic response (SVR) to IFN-α as monotherapy in HCV infection was approximately 20%. Ribavirin (1-b-D-ribofuranosyl-1H-1,2,4-triazole-3-carboxamide) is an oral nucleoside analogue. Initial studies of ribavirin in chronic HCV infection showed a lowering of transaminase levels but no effect on viral load when given as monotherapy. A landmark study in 1998[55] showed that combining standard IFN-α (3 million units thrice weekly) with ribavirin (1–1.2 g/kg/day) increased overall cure rates from around 20% to 35–40%. This study in the USA and a parallel study in Europe[56] established the vital importance of hepatitis C genotype in the prediction of response to therapy, and these rules still hold true with the use of current standard therapy with pegylated interferon. Genotype (G) 1 or 4 infection is more difficult to treat and requires a longer duration of therapy to obtain the best results (Fig. 38.19).

While genotype of virus remains the major determinant of response to therapy, other factors continue to have an influence (Fig. 38.20). Ribavirin produces a hemolytic anemia in a substantial minority of patients treated. This effect is dose dependent, and it is possible to keep patients on therapy by the use of dose reduction (Fig. 38.21). The mechanism of ribavirin's effect on hepatitis C remains unknown. It appears that it acts predominantly by immunomodulation, rather than as a direct antiviral effect. Its major action seems to be to prevent patients with relatively interferon-sensitive hepatitis C infection relapsing once initial viral clearance has occurred.

Pegylated interferons have now superseded standard interferons. Peglyation involves the attachment of an inert polyethylene glycol molecule to the active molecule, in this case IFN-α. There are two pegylated IFN-α molecules available and they do have substantial differences in their chemistry, if not in clinical effect. Interferon alpha 2a (IFN-α_{2a}) has been pegylated to form a 40 kDa molecule whereas IFN-α_{2b} is pegylated into a 12 kDa molecule. The 40 kDa molecule is excreted in bile and to a lesser extent renally, whereas the smaller molecule is cleared exclusively by the kidney. Overall half-life in plasma is around 5–7 days with both molecules (Fig. 38.22).

Initial studies with these agents as monotherapy showed that effectiveness was enhanced compared to standard interferon monotherapy (Fig. 38.23).[57] These and other studies also established that the fibrosis seen in patients with hepatitis C infection could reverse with

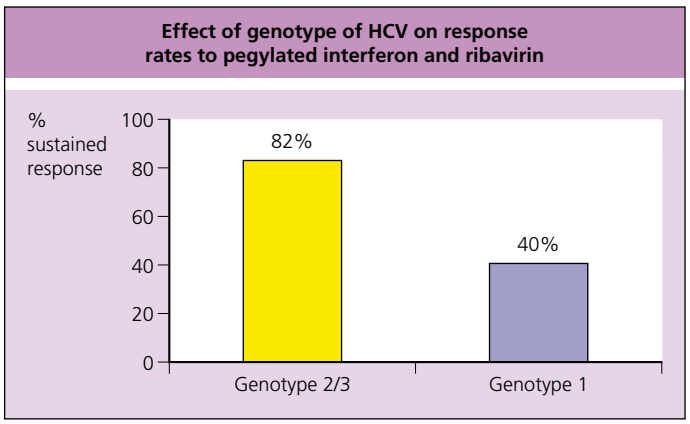


Fig. 38.19 Effect of genotype of HCV on response rates to pegylated interferon and ribavirin.

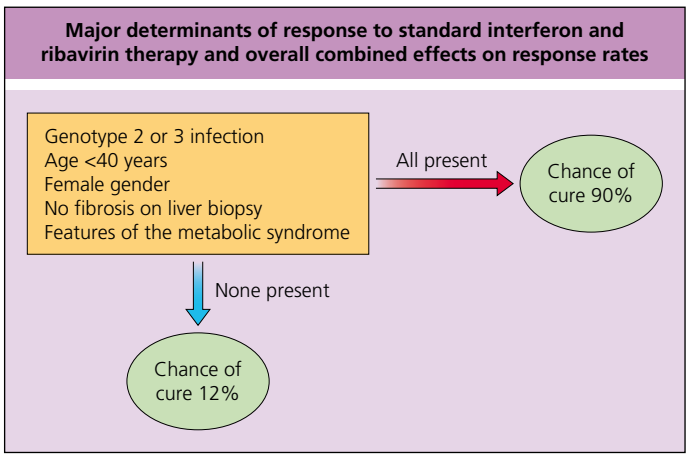


Fig. 38.20 Major determinants of response to standard interferon and ribavirin therapy and overall combined effects on response rates.

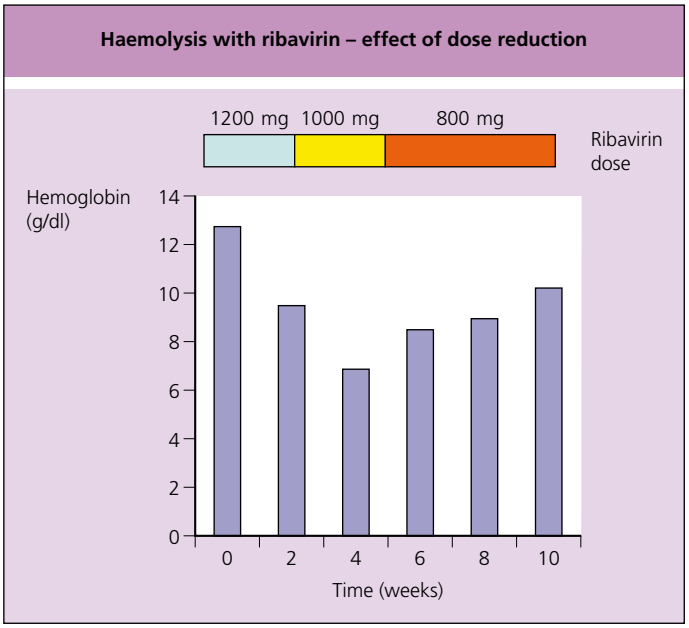


Fig. 38.21 Hemolysis with ribavirin – effect of dose reduction.

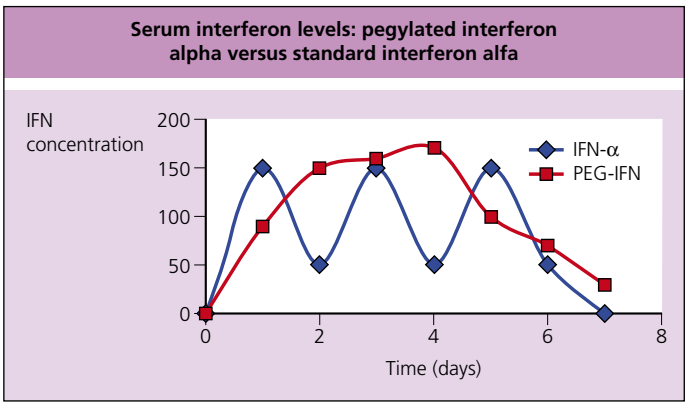


Fig. 38.22 Serum interferon levels: pegylated interferon alpha (PEG-IFN) versus standard interferon alpha (IFN-α).

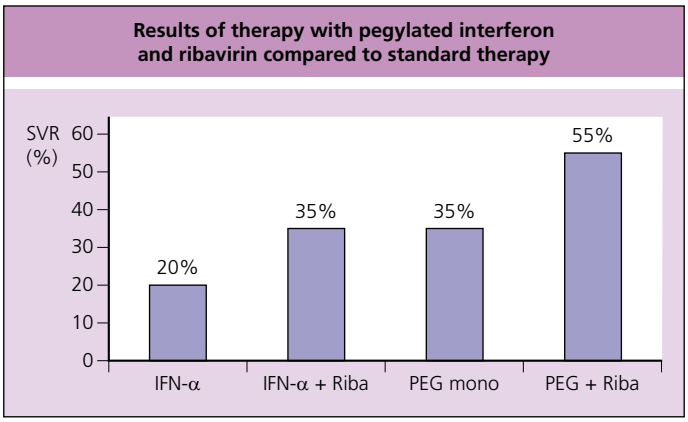


Fig. 38.23 Results of therapy with pegylated interferon (PEG) and ribavirin (Riba) compared to standard therapy. All based on 12 months duration of therapy. IFN-α, interferon alpha; mono, monotherapy.

successful therapy, underlining the fact that all patients with severe fibrotic liver disease should be considered for therapy.[58]

Both forms of pegylated interferon have been subjected to large trials and both seem equally effective, with sustained virologic responses seen in 55–60% of patients.[59,60] Response rates vary, with both host- and virus-related factors (Fig. 38.24) allowing reasonably accurate prediction of response rates in individual patients. Genotype remains the dominant response factor and cure rates in favorable genotypes (2 or 3) approach 80%. These high cure rates have markedly lessened the requirement for liver histology, although severe fibrosis remains a substantial factor predicting a negative outcome for therapy. The development of noninvasive markers of fibrosis, both in serum and using measures of liver stiffness (fibroscan), also allow reasonably reliable identification of patients with advanced fibrosis or those with no fibrosis.[61] These tests are less informative in the mid range of fibrosis and liver biopsy is still undertaken in many patients although most guidelines for therapy no longer include it as routine.

This great improvement in effectiveness of therapy has encouraged many patients to try treatment. The side-effect profile differs very little from standard interferon and ribavirin therapy, the only changes being

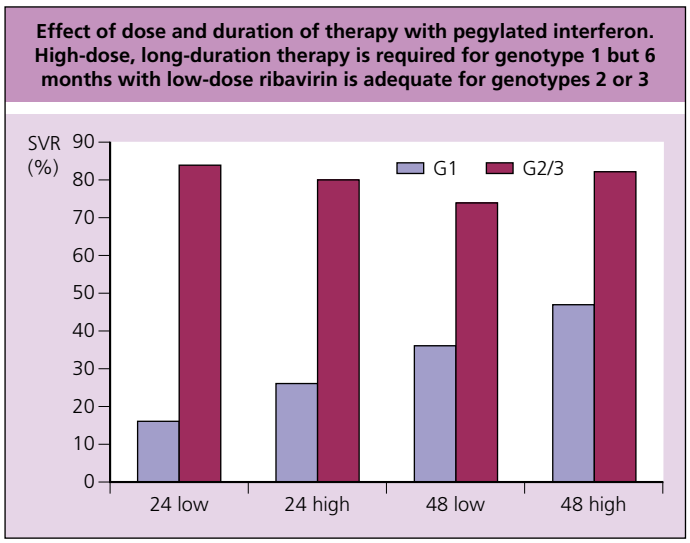


Fig. 38.24 Effect of dose and duration of therapy with pegylated interferon. High-dose, long-duration therapy is required for genotype 1 but 6 months with low-dose ribavirin is adequate for genotypes 2 and 3. SVR, sustained virologic response. Redrawn from Hadziyannis *et al.*[60]

a greater degree of injection site reactions. This remains a challenging therapy for patients to take. Compliance has been shown to be important. In an analysis of the 12 kDa molecule with ribavirin, patients who were able to comply with therapy (by taking 80% of their medication for 80% of the time[62]) had better response rates than those who did not. Hence encouraging patients to complete the intended duration of therapy is important. This is somewhat easier in patients with genotype 2 or 3 (G2/3) infection as they have a high chance of cure with only a 6-month course of treatment required. It is more difficult for genotype 1 or 4 infected patients who have to have 12 months of therapy to obtain best results. About 15% of patients will be nonresponders to pegylated interferon and ribavirin, and there is now good evidence to show that the results at week 12 of treatment are able to detect these individuals.

The viral kinetics of responders show an initial very rapid decline in viral load, often complete in the first week or two of therapy. In a proportion, this initial decline slows and a second phase of more gradual decline starts. With both pegylated interferons, if the virus has not disappeared from serum at week 12 or at least declined by 2 logs from pre-treatment samples, then the chance of a sustained response is almost nil (0% for the 12 kDa molecule, 2% for the 40 kDa molecule[63]). This allows patients who will not benefit from further therapy to be identified at the 3-month stage of treatment and therapy can be stopped. It also has the advantage of giving significant encouragement to patients who do make this cut-off, since if they have responded at this stage their overall chance of cure rises.

The high rates of response seen in G2/3 infected patients raised the possibility that shorter duration therapy may be effective based on viral kinetics. Patients with G2/3 who have a rapid virologic response have similar SVR rates with shorter therapy, 12 weeks for genotype 2 and 16 weeks for genotype 3.[64,65] It is important to stress, however, that all the short-duration therapy studies have been in patients without other patient-related adverse response factors, particularly advanced fibrosis. It seems prudent to restrict short-duration therapy to those without proven adverse response factors. The same RVR would theoretically apply to harder-to-treat patients, i.e. those with G1 infection. The registration studies of pegylated interferon and ribavirin showed that G1 low viral load patients do well with therapy, SVR rates of 70% being expected.[60] A study of 6 months' therapy in G1 low viral load patients showed that this was not sufficient, with SVR rates being below the expected; however, in the subgroup of G1 low viral load patients who had an RVR, response rates of 89% were achieved, showing this strategy can be successful.[66]

The dose–response relationship seen in G1 patients who are difficult to treat suggested that those patients who have unfavorable viral kinetics may benefit from extended-duration therapy. In genotype 1 patients with a 2 log drop at week 12 on therapy but who are not PCR negative, a trial has shown improved SVR with continuation of therapy for 72 weeks.[67] This remains a major undertaking for patients and motivation to continue such arduous therapy is clearly required.

In harder-to-treat populations there remains a need for improved therapy. Understanding of the HCV genome has allowed the identification of key components of the HCV replicative mechanism with the potential to act as targets for small molecule inhibitors. Most research has focused on the polymerase and protease genes of HCV in the NH3 and NS5 regions of the genome. Initial studies have established that these compounds have activity against HCV, with oral dosing leading to a drop in HCV RNA. When given as monotherapy, resistance rapidly developed with return to baseline HCV RNA levels in a period of 2 weeks, perhaps not surprising given the huge replication load in HCV infection where every base change compatible with viral viability will occur every day. The second issue limiting early drug development was toxicity, with a number of drugs falling at the preclinical or phase I stage. Phase II studies are now available for one agent which shows that in a group of genotype 1 high viral load patients treated with triple therapy (interferon, ribavirin and VX950) for 3 months, with a follow-on therapy of standard interferon and ribavirin, the SVR rate rose from 40% to 60% (Fig. 38.25), holding out promise that these agents

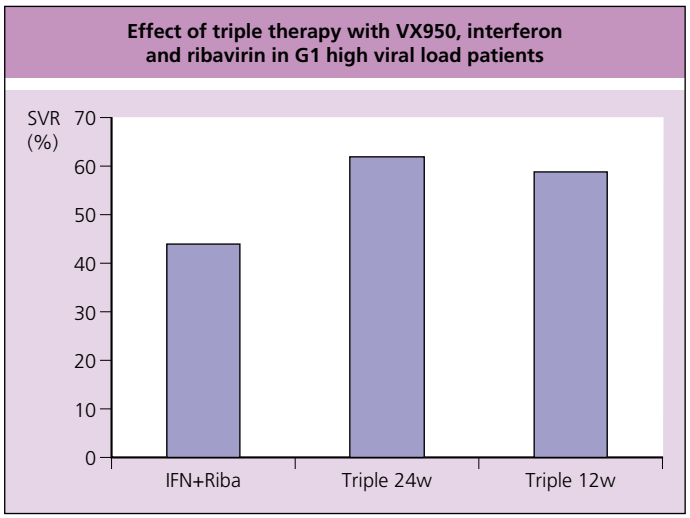


Fig. 38.25 Effect of triple therapy with VX950, interferon (IFN) and ribavirin (Riba) in G1 high viral load patients.

will improve the effectiveness of current therapies.[68] Other small molecule inhibitors of HCV are also in development as well as further modifications of interferon which have a longer half-life still and may allow dosing every 2 or even 4 weeks (albumin–interferon).

Special patient populations

Pegylated interferon–ribavirin in individuals co-infected with HIV shows a reduced SVR, on average some 10% lower than monoinfected patients. Immune reconstitution seems important in response; if CD4 counts are normal, response rates seem only slightly lower than non-HIV-infected patients but patients with CD4 counts below 200 do not respond well to therapy. The reduction in response rates is primarily due to the increased rate of side-effects on therapy, perhaps not surprising in a group already on HAART.[69] See Practice Point 46.

In renal replacement therapy units or after renal transplantation, treatment is difficult. Ribavirin is renally excreted and effectively contraindicated in this setting. Pegylated interferon monotherapy can be given and has a reasonable chance of success prior to renal transplant but carries significant risks of rejection if used post transplant.

Hepatitis D

Treatment of hepatitis D virus infection

Delta virus infection is uncommon and the mainstay of therapy is to inactivate HBV replication in those with active co-infection. For patients with negative HBV DNA, IFN-α therapy has been studied with only limited success. With standard interferon given at a dose of 3 million units three times per week, 29% will normalize ALT but most do not lose HDV RNA and relapse at the end of therapy. Higher dose treatment (9 million units three times per week) had a better biochemical and virologic response but again most patients relapsed.[70] It is clear that if interferon therapy is given for delta infection a long duration of therapy is required, which few patients can tolerate. There is a case report of resolution of HDV replication after 12 years on interferon,[71] but few patients will be so motivated. Studies of lamivudine[72] have shown no benefit. There are as yet no trials of pegylated interferon in delta hepatitis.

REFERENCES

References for this chapter can be found online at http://www.expertconsult.com

Chapter 39

Janice Main

Hepatobiliary and splenic infection

Liver Abscesses

PYOGENIC ABSCESS

Pathogenesis and pathology

Bacteria can reach the liver via the portal vein, the systemic circulation, the biliary tree, through the skin (following trauma or transhepatic procedures such as liver biopsy) or directly following perforation of a viscus with an ingested fish bone or other sharp object (Fig. 39.1).[1] Small animal bones may not be visible on a plain X-ray and can erode through the stomach wall. Leakage from the associated gastric perforation can impinge locally on the liver and lead to abscess formation. A similar phenomenon has been reported with the accidental ingestion of toothpicks or parts of toothpicks. However pyogenic liver abscesses are usually associated with biliary tree obstruction or gastrointestinal (GI) tract infection such as missed appendicitis, diverticulitis, perforated peptic ulcers, colonic carcinoma or following colonic surgery. The usual organisms are therefore from the GI tract or biliary tree. Liver abscesses are often polymicrobial with Enterobacteriaceae and enterococci predominating. *Streptococcus milleri* is increasingly seen in liver abscesses and staphylococci are not uncommon. There are reports in Taiwan and South Africa of an increasing incidence of infection with an invasive strain of *Klebsiella pneumoniae* with liver abscesses and endophthalmitis.[2] *Actinomyces* spp.,[3] *Bartonella henselae*[4] and *Capnocytophaga* spp.[5] are more unusual isolates from liver abscesses; in immunosuppressed persons infections by *Nocardia* spp.,[6] mycobacteria[7] and fungi such as *Aspergillus* spp. can cause abscess formation. Abscesses can be solitary or multiple and range in size from several centimeters in diameter to microabscesses identified histologically.

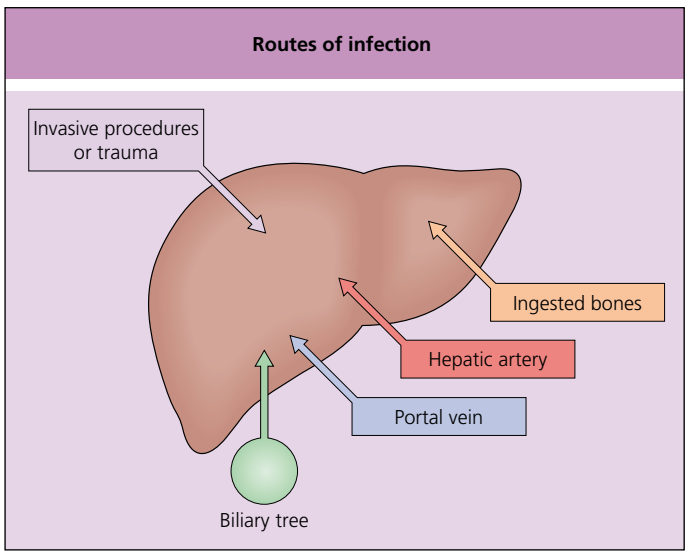


Fig. 39.1 Routes of infection.

Clinical features

Pyogenic liver abscesses are classically seen in elderly patients with underlying GI disease. The patient may complain of right hypochondrial discomfort that is insidious in onset or may present more acutely with feverishness or an acute confusional state. Interventions such as transarterial embolization and percutaneous laser ablation and other percutaneous approaches to hepatocellular carcinoma therapy can be complicated by liver abscess[8] and the diagnosis may be missed because fever and discomfort are not uncommon following the procedure. The differential diagnoses include cholecystitis and pyelonephritis. The clinical features of pyogenic and amebic liver abscesses are compared in Table 39.1.

Diagnosis

Abdominal ultrasound is the simplest way of making the diagnosis. Single or multiple abscesses may be present and the ultrasound may also help identify the original source of the sepsis, such as an

Table 39.1 Features of pyogenic and amebic liver abscess

	Pyogenic	Amebic
Patients	Elderly, underlying gastrointestinal or biliary tract disease	Much more common in males than females
Imaging	Single or multiple abscesses	Solitary abscess right lobe
Pathogens	Polymicrobial, Enterobacteriaceae, enterococci	*Entamoeba histolytica*
Other tests	Blood cultures	Amebic immunofluorescent antibody test
Treatment	Broad-spectrum antimicrobials, aspiration	Metronidazole, diloxanide ± aspiration

obstructed biliary tree. Smaller abscesses not detected on ultrasound may be visible on CT scanning,[9] which can also help determine the source of sepsis if this is not apparent on the ultrasound. Aspiration of the abscess yields useful microbiologic material and is an important part of therapy. Cytologic examination of smears from the abscess are important to exclude underlying malignancy.

Blood cultures may also help with the microbiologic diagnosis. The white count is usually elevated and liver function tests may be deranged with elevation of alkaline phosphatase. The presence of a microcytic hypochromic anemia in an elderly patient, for example, may point to an underlying colonic carcinoma. The patient should also be screened for diabetes and iron overload syndromes should be considered when *Yersinia* spp. are identified.[10]

Management

Drainage of the abscess is important and the favored approach is needle aspiration under ultrasound guidance. This may need to be repeated on several occasions, particularly if there are multiple lesions. Percutaneous catheter drainage is another approach, but is generally only practicable if there is one large and accessible abscess.

Abscesses are often polymicrobial and broad-spectrum intravenous antibiotics are generally recommended. If the abscesses are thought to have occurred as a result of biliary sepsis it is advisable to give antibiotics that achieve good concentrations in bile. Positive blood cultures and cultures from the abscess should help direct antimicrobial chemotherapy. Piperacillin–tazobactam and the carbapenems are useful and metronidazole can be added if there is concern about possible amebic infection.

Although liver abscesses are more commonly seen in hospital practice the mortality rate has fallen considerably over the past 40 years,[11] attributed to advances in imaging and antimicrobial chemotherapy.

AMEBIC LIVER ABSCESS

See Chapter 110.

Diffuse Parenchymal Involvement

The liver may be involved in many systemic infections. Viral infection has been described elsewhere (Chapter 38), but acute hepatitis has been described with several bacterial pathogens including meningococci, *Salmonella typhi*, *Listeria*, *Campylobacter*, *Borrelia* and *Brucella* spp.[12,13] Septicemia and other causes of circulatory collapse can also cause hepatic ischemia, which can be associated with high transaminase values.[14] Infections account for many of the main causes of granulomatous liver disease (Table 39.2). Liver involvement and symptoms of liver disease can be a major part of the following infections.

MYCOBACTERIAL INFECTIONS

Tuberculosis of the liver

The liver is generally involved in miliary tuberculosis and the liver biopsy may help establish a diagnosis of tuberculosis in a patient with unexplained fever.

Pathology

Tuberculosis of the liver may present as an abscess[7] or more diffuse disease and liver biopsy may reveal nonspecific hepatitis or granulomatous liver disease. Rarely tuberculomas may be present or enlarged intra-abdominal lymph nodes can cause compression of the biliary tree and the patient presents with features of biliary tract obstruction.

Table 39.2 Major causes of granulomatous liver disease

Bacteria Mycobacteria (*Mycobacterium tuberculosis*, *Mycobacterium avium-intracellulare*, *Mycobacterium leprae*) *Brucella* spp. *Listeria* spp. *Tropheryma whipplei* *Yersinia* spp. *Treponema pallidum*
Viruses Cytomegalovirus Epstein–Barr virus Hepatitis A and C viruses
Rickettsiae *Coxiella burnetii* *Rickettsia conori*, *Rickettsia typhi*
Protozoa *Leishmania* spp.
Worms *Schistosoma* spp. *Toxocara* spp.
Fungi *Histoplasma* spp. *Coccidioides* spp.
Drugs
Primary liver disease (e.g. primary biliary cirrhosis)
Neoplasms (e.g. lymphoma)
Diseases of unknown cause (e.g. sarcoidosis, inflammatory bowel disease)

Clinical features

Fever and weight loss are the usual symptoms. The patient may appear wasted. Hepatomegaly may be evident, but massive hepatomegaly is unusual. In severe tuberculosis infection the patient may present with liver failure.

Diagnosis

Elevated alkaline phosphatase levels are usually present and the albumin levels may be low if the disease has been longstanding. Anemia and elevation of the erythrocyte sedimentation rate and C-reactive protein are common. A chest radiograph is important to exclude active pulmonary disease, which is evident in a minority of cases.[15] The abdominal ultrasound may demonstrate intra-abdominal lymphadenopathy or evidence of peritoneal involvement. Liver biopsy may be diagnostic, but caseating granulomas with acid-fast bacilli are demonstrable in less than 10% of cases. The appearances may be nonspecific and it is important to culture some of the liver biopsy material. Recently available blood tests such as enzyme-linked immunospot assay (ELISpot) may be helpful in this setting[16] and there is an increasing use of polymerase chain reaction testing of biopsy material.[17]

Management

Standard antimycobacterial therapy should be given (see Chapters 30 and 143). Liver function tests should be monitored as with standard therapy. As the patient improves the elevated alkaline phosphatase gradually returns to normal. Elevation in the transaminase level may

be a sign of drug toxicity. Fever may persist for several weeks, but if the patient fails to improve then the possibilities of resistant *Mycobacterium tuberculosis* or atypical mycobacteria have to be considered.

Atypical mycobacteria

Atypical mycobacterial infection of the liver is usually seen in the setting of immunosuppression, particularly underlying HIV infection[18] or congenital immunodeficiency. Again culture of the biopsy material is important to establish the diagnosis, but the appearance of many organisms with poorly formed granulomas may be an important clue to atypical mycobacterial infection in an immunocompromised host.

SYPHILIS

The liver is involved with congenital, secondary and later stages of syphilis.

The standard screening tests usually confirm the diagnosis of congenital infection, but the diagnosis should also be considered in the setting of undiagnosed acute hepatitis in adults.[19] This is discussed in detail in Chapter 57.

LEPTOSPIROSIS (SEE ALSO CHAPTER 124)

Epidemiology

Several *Leptospira* species are associated with human disease, but *L. icterohaemorrhagiae* is specifically associated with Weil's disease. The main source of this pathogen is rat urine and it is therefore an occupational hazard for farm and sewage workers and those who use contaminated waters for recreational purposes such as rowing.

Pathology

The liver demonstrates cholestasis with swelling of hepatocytes, but very little necrosis. In classic Weil's disease renal disease is evident with acute tubular necrosis. There may be evidence of multifocal hemorrhage, particularly within skeletal and cardiac muscle.

Prevention

The use of protective clothing can help prevent occupational exposure and it has been recommended that sewage workers carry cards to remind clinicians of the possibility of leptospirosis should they become ill. Prophylactic antimicrobials such as doxycycline[20] have been suggested for those at risk of exposure through work or leisure activities such as canoeing, although this is not generally recommended.

Clinical features

The patient's occupation or leisure activities may point to the diagnosis.

In common with other spirochaetal infections there are several characteristic stages of the disease process. The severity varies from a minor influenza type illness to life-threatening multisystem failure. The incubation period ranges from 2 to 17 days.

The first (septicemic) stage is of a multisystem disease. It is abrupt in onset. Features may include:

- high fever
- myalgia (often severe)
- abdominal pain
- nausea
- vomiting
- severe headache and meningitis (cerebrospinal fluid leukocytosis and an elevated protein level)
- pneumonitis
- conjunctivitis
- hepatomegaly
- jaundice (said to be an ominous sign)
- bleeding (thought to be mainly related to capillary damage and leading to ecchymoses, gastrointestinal bleeding and on occasion intracerebral bleeding).

Urinalysis reveals proteinuria and bilirubinuria. The white count is usually elevated with mainly polymorphonuclear cells (it may be as high as 30 000/ml or 3×10^9/l). Thrombocytopenia may be present. The liver function tests demonstrate elevated levels of bilirubin and alkaline phosphatase. In contrast to fulminant viral hepatitis the transaminase values may be normal or moderately elevated.

During the second ('immune') stage the pyrexia generally resolves, but life-threatening disease may be evident. There may be signs of myocardial involvement with arrhythmias and nonspecific ECG changes. Blood tests confirm deteriorating liver function with evidence of muscle damage (elevated muscle enzymes). Worsening renal function is also a feature with elevations in the serum creatinine and proteinuria.

In the third (convalescent) stage a steady clinical and biochemical improvement occurs with resolution of the liver and renal failure and improved myocardial function. Minor relapses of symptoms can occur at this stage with further episodes of myalgia and spikes of fever.

Diagnosis

During the first stage of the disease *Leptospira* may be found on examination of the blood film or grown from blood cultures. By the second stage the blood cultures are likely to be negative. Leptospira may be evident on urine microscopy, but the diagnosis is more likely to be made from the serologic tests for anti-*Leptospira* antibodies, which are found in increasing titers in the convalescent stage of the disease.

At the time of the initial presentation, although the symptoms and jaundice may suggest a viral hepatitis, the high fever and leukocytosis would be unusual for viral etiology, but similar hepatic illnesses with or without renal failure have been described with hantaviruses.[21,22]

Management

Most patients with leptospirosis are not seriously ill, but for those with Weil's disease full supportive care may be required with support and hemodialysis in an intensive care unit. High-dose intravenous penicillin is the treatment of choice, but the dose may need modification according to the renal function. A febrile reaction, similar to the Jarisch–Herxheimer reaction, can occur.

Helminthic Parasites of the Liver

SCHISTOSOMIASIS (SEE ALSO CHAPTER 112)

Schistosomal ova can reach the liver via the mesenteric veins and portal system. Liver involvement is particularly seen with *Schistosoma japonicum* and *Schistosoma mansoni*, but can also occur with *Schistosoma haematobium*. In the early stages a granulomatous reaction is seen, but over years, if untreated, extensive collagen deposition can lead to portal fibrosis with the development of portal hypertension and splenomegaly (Fig. 39.2).

The diagnosis of schistosomal liver disease may only come to light when the patient presents with features of portal hypertension and, for example, has a variceal bleed.

Diagnosis

The diagnosis may be made from the serologic test – schistosomal enzyme-linked immunosorbent assay – or the histologic appearances of the granulomatous liver disease in the early stages and the fibrosis in the later stages. In the early stages a peripheral eosinophilia may be evident.

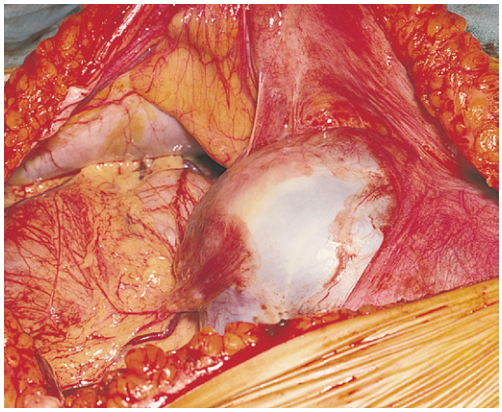


Fig. 39.2 Hydatid disease. Hydatid cyst of the liver.

Management

Praziquantel is the treatment of choice. Patients should be warned that an influenza-like illness can follow therapy. Supportive therapy is required for the patient with severe fibrosis and liver transplantation may be required in advanced cases.

HYDATID INFECTION (SEE ALSO CHAPTER 114)

Hydatid cysts can follow infection with the *Echinococcus* tapeworm.

Epidemiology

The disease is widespread and humans are typically infected by close contact with dogs which have become infected by the consumption of eggs from infected meat. Echinococcal infection is endemic in the main sheep farming areas of the world and is a particular concern throughout much of Europe, the Mediterranean littoral, Asia, South America and Kenya.

Pathology

The liver is the most frequent site for hydatid cysts (Fig. 39.3).

The ova enter the liver via the portal vein and lead to cyst formation within the liver parenchyma – classically involving the right lobe of the liver on the inferior surface. Rupture of a cyst, spontaneously or following trauma or surgical procedures, can lead to cardiovascular collapse. This is thought to be an inappropriate immune response to released hydatid antigens. Rupture of the cysts can also lead to infection in the biliary tree, the peritoneum, lungs and pleura.

Clinical features

Hydatid disease may be asymptomatic and liver cysts are found incidentally on ultrasound examination, as calcified lesions on plain abdominal radiographs or at autopsy. Hepatomegaly may be present. The cyst may be diagnosed after rupture or if secondary infection of the cyst occurs. Cysts can also be found within the brain, lungs, kidney and the heart (Fig. 39.4).

Diagnosis

The hydatid serology test is particularly useful although false-positive and -negative results can occur. A peripheral eosinophilia may be present. Several radiologic features can be helpful in making the diagnosis. Plain abdominal radiographs may reveal calcified spherical structures within the liver. Ultrasound examination can often reveal several forms of the cysts, which can be single, multiple, thin- or thick-walled. CT scanning can detect smaller lesions and the presence of calcification. It may difficult to differentiate a single cystic lesion from a tumor. Biopsy or aspiration of the cysts may be dangerous in view of the risk of antigen leakage and immune response and should be performed only when there is appropriate backup to treat circulatory collapse and laryngeal edema.

Management

The management of hepatic hydatidosis remains controversial and many approaches have been tried. It is recognized that by itself antimicrobial therapy with albendazole, mebendazole or praziquantel is generally ineffective and should be combined with a drainage procedure. Percutaneous drainage has the advantage of avoiding major surgery, but in open surgical procedures peritoneal contamination is thought to be minimized by packing the surgical field with povidone–iodine swabs, decompressing the cysts by aspiration and then removing the cyst contents. Some surgeons advocate the use of injecting formalin or hypertonic saline into the cysts following decompression. In one study,[23] 50 patients with hepatic hydatidosis were randomized to receive either percutaneous aspiration and albendazole or cystectomy. Similar efficacies were demonstrated in both treatment groups, but the open surgical procedure was associated with greater morbidity. Puncture, aspiration and the use of alcohol and polidocanol as sclerosing agents are being increasingly used.[24]



Fig. 39.3 Hydatid disease. Hydatid 'daughter cysts'.

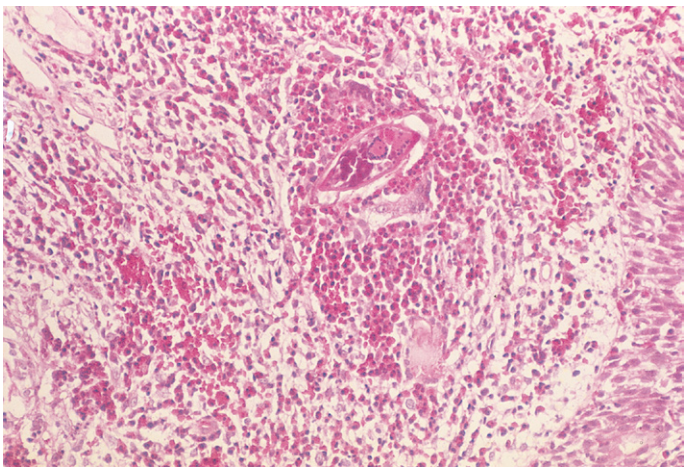


Fig. 39.4 A refractile schistosome ova is located in a portal tract and is associated with an eosinophil-rich granulomatous inflammatory reaction.

ASCARIASIS

Rarely the large *Ascaris* roundworm can migrate up through the bile duct and cause biliary obstruction. More commonly the liver is involved as a result of ova invading the liver via the portal vein and setting up a localized granulomatous reaction.

Liver involvement is usually asymptomatic unless rare complications such as biliary obstruction, hemobilia or liver abscesses develop.

Worms within the biliary tree may show up as motile linear lesions on ultrasound or endoscopic retrograde cholangiopancreatography (ERCP).

TOXOCARA

Toxocariasis is a further cause of hepatic granulomas. The recommended therapy is thiabendazole.

Liver Flukes

Liver flukes are thought to invade the liver from the peritoneal cavity and migrate through the liver parenchyma to the biliary tree where an inflammatory reaction develops.

CLONORCHIS SINENSIS

Infection with *Clonorchis sinensis* is usually associated with the consumption of raw or undercooked fish in Asia. The flukes live within capillaries of the biliary tree and the inflammatory reaction can cause obstruction and encourage cholelithiasis. Cholangitis is a common complication and the ongoing inflammation and fibrotic reaction is thought to predispose the patient to the development of cholangiosarcoma.[25]

Diagnosis

Flukes may be evident on radiologic imaging such as percutaneous transhepatic cholangiography or ERCP. This diagnosis must be considered when atypical cholangiopathy is diagnosed in patients of eastern origin. The diagnosis is confirmed by finding the ova on stool microscopy.

Management

Praziquantel is the treatment of choice. Surgical or endoscopic approaches may be required to deal with associated cholelithiasis and biliary obstruction.

FASCIOLA HEPATICA

Infection with this common sheep fluke can follow consumption of contaminated watercress and is recorded throughout Europe, South America, the Caribbean, Africa and China.

Pathology

The picture is usually of biliary tract infection and obstruction, but hepatic granulomatous reactions can also occur.

Clinical features

In the early stages of infection the patient may complain of fever and right hypochondrial pain. Hepatomegaly may be present. The migration of the flukes within the biliary tree can lead to inflammatory and fibrotic reactions. Cholelithiasis can occur and the clinical picture may be of a bacterial cholangitis. In common with bacterial cholangitis the alkaline phosphatase may be elevated, but the presence of an eosinophilia should point to the possibility of a fluke infection.

Diagnosis

The diagnosis may be suggested by a history of watercress consumption and features of cholangitis and an eosinophilia. Praziquantel is the therapy of choice and surgical and endoscopic intervention may be required to deal with biliary tree obstruction.

Protozoal Infections

LEISHMANIASIS (SEE ALSO CHAPTER 117)

Visceral leishmaniasis or kala-azar can lead to massive hepatosplenomegaly.

Epidemiology

Leishmanial infection is transmitted by sandflies and is reported particularly around the Mediterranean basin, in South America and Asia.

Pathology

Host factors are thought to be important in determining the disease outcome following leishmanial infection. It is not known why some patients develop only localized lesions and others develop more serious systemic disease. Underlying HIV infection is associated with multisystem disease and reactivation of previous infections.[26]

Clinical features

The patient may present with fever or anemia. Massive hepatosplenomegaly may be present.

Diagnosis

The presence of organisms may be detected by bone marrow examination, liver biopsy or splenic aspiration. Hypergammaglobulinemia is often present. Serologic tests are often useful, but antibody tests may be negative in patients with HIV infection or other types of immunodeficiency.

Management

Therapy of leishmaniasis is somewhat limited by the toxicity of many of the antimicrobial agents. Antimonial compounds, for example, can cause pancreatitis. Liposomal and other preparations of amphotericin are being increasingly used with success. In the setting of immunosuppression maintenance regimens are often required with regular administration of amphotericin B or intravenous pentamidine.

MALARIA

During acute malaria the parasitic load within the reticuloendothelial system and particularly the liver may be large and this may result in hepatic dysfunction. This is thought to predispose the patient with malaria to bacteremia. Mild elevation of the transaminase values may occur, but hyperbilirubinemia is mainly associated with the hemolysis of malaria rather than hepatic dysfunction.

TOXOPLASMA

Mild hepatitis or granulomatous liver disease has been reported with both primary and reactivation forms of toxoplasmosis infection.

Fungal Infections

The liver may be involved as part of a systemic fungal infection in a patient with candidemia.[27] Cryptococcal, histoplasmosis and pneumocystis infection of the liver are mainly seen in patients with HIV infection.

Aspergillus infection of the liver with abscess formation is mainly seen in patients with neutropenia as a result of chemotherapy.

Biliary Tree Infections

ACUTE CHOLECYSTITIS

Pathology

Gallbladder infections are usually the results of gallstone formation and impaction within the cystic duct[28] with impaired biliary drainage leading to infection, edema and compressive effects on the local blood supply, which can cause gangrene of the gallbladder. Suppuration within the gallbladder can lead to bacteremia, septicemia, cholangitis and liver abscess formation. Acalculous cholecystitis can follow infection with *Salmonella* or *Campylobacter* spp. or can occur in acutely ill patients following major surgery or burns. In the setting of HIV infection, infection with *Salmonella* or *Campylobacter* spp. may be implicated or the gallbladder disease may be a feature of HIV cholangiopathy,[29] which is often associated with cytomegalovirus infection, cryptosporidiosis, microsporidiosis or lymphoma.

Clinical features

The patient may describe right hypochondrial discomfort, which can occur in waves. Radiation to the right shoulder is common. Feverishness or rigors can occur and the patient may develop bacteremia and septicemia. Right hypochondrial tenderness is usually present and the gallbladder may be palpable in one-third of cases. A degree of jaundice may be present depending on the extent of biliary tree obstruction.

Diagnosis

The diagnosis is usually made clinically, but ultrasound of the liver and gallbladder may demonstrate gallstones with thickening of the gallbladder wall or dilatation of the biliary tree in the presence of obstruction. Laboratory findings include an elevated white cell count, hyperbilirubinemia and elevated alkaline phosphatase. There may be modest elevations of the transaminase values. Impaired biliary drainage because of pancreatic carcinoma can also cause dilatation of the biliary tree and ERCP may be required to delineate the anatomy and to facilitate drainage if required. Magnetic resonance cholangiopancreatography (MRCP) is a helpful, noninvasive imaging technique useful in visualizing the biliary tract.[30]

Management

The priorities are to facilitate drainage of the biliary tree and to treat infection. Cholecystectomy, when performed by the open technique, has a high mortality and morbidity rate in patients with acute cholecystitis and the usual practice was for conservative management of the acute illness and elective cholecystectomy some weeks later. This, however, required two hospital admissions and the emergency readmission rate was high for these patients.[31] Laparoscopic cholecystectomy is increasingly performed early in patients with acute cholecystitis[32] and appears successful, even when gangrenous cholecystitis[33] is present. A meta-analysis of four studies (375 patients)[34] concluded that early laparoscopic surgery resulted in a shorter total hospital stay but with significantly longer operation time. For frail patients who are unfit for surgery ERCP can be both life-saving and diagnostic, with endoscopic removal of stones by sphincterotomy, basket or balloon removal and stent insertion. Where ERCP is not available or not technically feasible, percutaneous biliary drainage can be performed.

Elective cholecystectomy can generally be performed subsequently, but emergency surgery may be required if the situation is complicated by a gangrenous gallbladder or poor response to antimicrobial therapy.[35]

Antimicrobial therapy is generally prescribed to cover Gram-negative bacilli, enterococci and anaerobes, although the importance of enterococcal infection is debated in this setting.[36] Piperacillin–tazobactam or ticarcillin–clavulanic acid is therefore useful. Carbapenems are increasingly used, particularly if the patient has had a prolonged inpatient stay or has undergone a number of invasive procedures. Aminoglycosides must be used carefully in this setting, particularly in the elderly because there is a high incidence of renal impairment in patients with biliary tree sepsis. Cephalosporins and quinolones do not adequately cover enterococcal infection. Antimicrobial prophylaxis is advised for procedures involving an obstructed biliary system.

CHOLANGITIS

Pathology

Bacterial cholangitis generally results from infection of an obstructed biliary tree and is most commonly caused by gallstones obstructing the common bile duct.[37] Other predisposing causes include congenital biliary tract disorders, sclerosing cholangitis, HIV cholangiopathy and bile duct strictures.

Clinical features

The symptoms and signs are generally similar to acute cholecystitis, but the patient may have no history of abdominal pain and present with septicemia.

Diagnosis

Bacteremia has been reported in half the cases of cholangitis. Blood cultures are important investigations. The alkaline phosphatase and bilirubin are usually elevated. Ultrasound may show dilatation of the biliary tree with stones, but the bile duct is often difficult to visualize and other imaging techniques, such as endoscopic ultrasound and MRCP[38] may be required. ERCP is useful for diagnosis when the patient has been stabilized.

Management

As with cholecystitis the priorities are supportive care for the patient with the appropriate antimicrobial chemotherapy and drainage of the obstructed biliary system.

CHRONIC CHOLECYSTITIS

Chronic cholecystitis is an unusual condition. Again gallstones are commonly seen. Chronic cholecystitis may be seen following infection with *Salmonella* or *Campylobacter* spp.

Management

Elective cholecystectomy is the treatment of choice. Laparoscopic cholecystectomy is increasingly used for gallstone disease and acalculous cholecystitis and is associated with lower morbidity and mortality rates.

SPLENIC INFECTION

Pathogenesis and pathology

Splenomegaly is present in many infections and may be a clue to the diagnosis. However, a large spleen is also evident in many inflammatory, infiltrative and malignant processes. Bacterial pathogens generally reach the spleen via the systemic circulation as part of a bacteremia but spleen infection can also occur contiguously from intra-abdominal sepsis or, more rarely, following percutaneous trauma. This may be obvious if there is a history of a percutaneous procedure or injury but blunt trauma may lead to hematoma formation with subsequent secondary infection and the diagnosis in this setting is often delayed. Splenic infarcts can be seen following septic emboli but can also occur with underlying hematologic conditions such as sickle cell disease and polycythemia. Vasculitides can also lead to splenic infarcts with risks of secondary bacterial infection and abscess formation. Rupture of an abscess can occur. Without appropriate intervention the abscess can rupture within the peritoneal cavity, with the development of peritonitis and often associated hemorrhage. More rarely, splenic abscesses rupture into the stomach, bowel, lung, kidney or, very rarely, externally.

Spleen involvement is common in viral infection such as Epstein–Barr virus infection and HIV. Protozoal infection, particularly leishmaniasis, can be associated with splenic enlargement. Splenic pneumocystis infection has been reported in patients with advanced HIV infection.

Clinical features of a splenic abscess

Only a minority of patients exhibit the classic features of left hypochondrial pain, fever and splenomegaly. Patients may complain of left shoulder pain or pleuritic pain. Left hypochondrial tenderness or, more rarely, a rub may be evident. More rarely there may be local swelling, erythema or tenderness. Splinter hemorrhages and a heart murmur suggest underlying endocarditis.

Diagnosis

The white blood cell count is usually elevated. A chest X-ray may demonstrate a left pleural effusion or elevation of the left hemidiaphragm. An ultrasound examination may be diagnostic but the most sensitive test is a CT scan which may also be useful in excluding underlying intra-abdominal sepsis. Blood cultures should be obtained and urine microscopy and an echocardiogram performed if there is suspicion of endocarditis.

Management

Blood cultures should help direct appropriate antimicrobial therapy. The presence of streptococci or staphylococci suggests seeding from bacteremia – particularly infective endocarditis – whereas polymicrobial infection is more usually seen when an abscess is secondary to local intra-abdominal infection as a result of a perforated viscus. Patients with splenic abscess are often elderly with underlying co-morbidities such as diabetes mellitus. Drainage of the abscess may be required but the spleen is highly vascular and the risks versus benefits of this approach, whether percutaneous[39] or open, have to be carefully considered for the individual patient. There should be close liaison with the radiologic and surgical teams and often splenectomy is required.

REFERENCES

References for this chapter can be found online at http://www.expertconsult.com

Practice point

17

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Travelers' diarrhea

INTRODUCTION

Travel-related diarrhea is the first cause of morbidity in travelers returning from Caribbean areas, Central and South America, South Central Asia and Africa. Ignorance about this risk appears to be the most crucial factor determining travel-related morbidity rates. Episodes of travelers' diarrhea are mostly benign. Nevertheless, associated dehydration can be severe. Prior education and simple advice are key to prevention of travelers' diarrhea.

DEFINITION, EPIDEMIOLOGY AND CLINICAL FEATURES

The definition of acute travelers' diarrhea (TD) is an abnormally increased frequency or decreased consistency of stools for less than 3 weeks. There are three different types of TD – classic, moderate and mild – defined as follows:

- Classic TD consists of three or more unformed stools in 24 hours with one of the following symptoms: nausea, vomiting, abdominal pain, fever or blood in the stools.
- Moderate TD consists of one or two unformed stools in 24 hours and one of the above symptoms, or more than two unformed stools in 24 hours.
- Mild TD consists of one or two unformed stools in 24 hours without any other sign.

Risk factors for developing TD are well known. The number of ingested organisms that reach the intestine alive will indicate the type of TD. People with a previous history of gastric surgery or abnormal digestive motility, or those treated for gastric ulcer with antihistaminic drugs (bacteria can reach the small bowel because of reduction of gastric acid), have an increased risk of developing TD. 'Adventure' trips, consumption of unclean food or water or travel in countries with poor hygiene standards are other risk factors for TD.

The etiology of TD is wide ranging (Table PP17.1). Bacteria, viruses, parasites and occasionally biologic toxins or fungal agents are responsible for TD. Up to 50% of TD is caused by bacteria. The most common agent identified in TD is enterotoxigenic *Escherichia coli* (ETEC). Other pathogens are reported in Table PP17.1. Although travelers with doxycycline malaria prophylaxis or treated by antibiotics can develop *Clostridium difficile*-associated diarrhea, it is a rare cause of TD. *Vibrio cholerae* O1 or O139 is an important cause of diarrhea in developing countries but not a common cause of TD. In a large study of 17 353 ill returned travelers, patients with bacterial TD came most commonly from South East Asia. *Campylobacter* spp. are the principal cause of TD.

Viruses are a less common cause of TD. Rotavirus is predominant (10–25% of TD). Norovirus as Norwalk agent is inconsistently reported.

Table PP17.1 Usual pathogens causing travelers' diarrhea and their clinical characteristics

Pathogen	Clinical features
Bacteria	
Enterotoxigenic *Escherichia coli*	'Turista': short incubation, fecal diarrhea without blood or pus (enterotoxin) Cholera-like diarrhea
Enteroaggregative *E. coli*	'Turista'
Campylobacter jejuni	Dysentery: bloody diarrhea, abdominal pain, tenesmus, fever (enteroinvasion) Fecal diarrhea
Salmonella spp.	Dysentery (enteroinvasion) Fecal diarrhea
Shigella spp.	Dysentery (enteroinvasion) Aqueous diarrhea (enterotoxin) Fecal diarrhea
Vibrio parahaemolyticus	Dysentery (enteroinvasion) or aqueous diarrhea (enterotoxin)
Aeromonas hydrophila	Aqueous diarrhea (enterotoxin) or dysentery (enteroinvasion)
Plesiomonas shigelloides	Cholera-like diarrhea
Yersinia enterocolitica	Dysentery (enteroinvasion)
Viruses	
Rotavirus	Vomiting, aqueous diarrhea
Enteric adenovirus	Vomiting, aqueous diarrhea
Parasites	
Giardia lamblia	Chronic diarrhea, predominant upper gastrointestinal signs
Cryptosporidium parvum	Children, chronic diarrhea
Cyclospora cayetanensis	Chronic diarrhea, predominant upper gastrointestinal signs
Microsporidium spp.	Frequent with AIDS, chronic diarrhea
Isospora belli	Frequent with AIDS, chronic diarrhea
Entamoeba histolytica	Chronic diarrhea or acute bloody diarrhea

Parasites are rarely implicated in TD. However, *Giardia lamblia*, *Cryptosporidium parvum*, *Cyclospora cayetanensis*, *Microsporidium* spp. and *Isospora belli* have been reported as causes of prolonged TD. Giardiasis was frequently reported among returned travelers from South Central Asia (proportional morbidity: 150 per 1000 returned travelers).

Contamination of seafood by toxins (e.g. ciguatera toxin from contaminated reefs in tropical areas) is occasionally responsible for TD.

Most episodes of TD begin between 4 and 14 days after people's arrival at their destination but the incubation period can be shorter if the inoculum is particularly prevalent or in cases of *Staphylococcus aureus* food poisoning (incubation period: 2–6 hours). The illness is frequently self-limiting and short. Only 20% of people suffering TD report needing bed rest for 1–2 days. Clinical characteristics depend on the cause (see Table PP17.1), the patient's age and their immune status. Location helps to focus on possible implicating agents.

DIAGNOSIS

For most cases of mild TD, laboratory tests on feces are unrewarding. Laboratory tests on freshly collected stools are necessary for identifying the etiologic agent in four conditions:

- persistent or severe diarrhea;
- dysentery (blood in feces);
- fever;
- severe abdominal pain.

A stool culture is nearly always necessary in a patient with fever and colitis. Clinical features suggest specific causes and help in the selection of biologic investigations. If upper gastrointestinal signs are predominant, stool examination for *G. lamblia* and *Cyclospora* must be undertaken. *C. arvum* or *Microsporidium* must be looked for if the symptoms persist for more than 14 days. Generally, three freshly passed stools must be collected on different days. Laboratories should be able to examine stools for *G. lamblia*, *Entamoeba histolytica*, *Dientamoeba fragilis*, *Blastocystis hominis*, *Cryptosporidium* spp., *C. cayetanensis* and *Isospora belli*. *Salmonella*, *Shigella* and *Vibrio* should be sought using routine enriched and selective media. For viral causes, latex tests for rotavirus are available.

MANAGEMENT

Most episodes of TD are self-limiting and require no specific treatment. Necessity to treat depends of the severity of the illness and the traveler's inconvenience. The three important points in TD treatment are fluid replacement, antibiotics and antimotility agents. Oral or intravenous fluid replacement is essential in moderate to severe TD. Fluids with sugar and salt are recommended for mild TD. Children must receive oral rehydration solution. Extreme ages of life (the very old and the very young), pregnant travelers or people treated by diuretics should be monitored carefully.

The issue of diet is controversial. It would appear that if the duration of TD is less than 3 days, no benefit is obtained with diet.

Antibiotics are indicated if TD consists of more than four stools in 24 hours, associated with fever, blood, mucus or pus in the stool. Travelers may have a prescription for antibiotics that can be taken over 3 days if such symptoms occur during the trip. In cases of severe TD (fever, more than eight stools per day), persistent diarrhea for more than 2 days and fragile status, or if antibiotic therapy fails after 2 days, medical consultation is required.

Table PP17.2 Infectious Diseases Society of America treatment recommendations for travelers' diarrhea

Drug	Dosage	Specific indications
Norfloxacin	400 mg q12 h	
Ciprofloxacin	500 mg q12 h	
Ofloxacin	200 mg q12 h	
Levofloxacin	500 mg q24 h	
Azithromycin	1000 mg q24 h	Children and pregnant women Fluoroquinolone resistance
Rifaximin	200 mg q8 h	

When the indication for treatment is confirmed, quinolone therapy should be started as soon as possible. Ciprofloxacin (500 mg twice daily) for 3 days is the treatment of choice. Azithromycin is safe to use in children and pregnant women. Quinolones are active against most of the strains of ETEC, *Campylobacter* spp., *Salmonella* spp., *V. parahaemolyticus* and *V. cholerae*. If there is resistance to quinolones (e.g. *Campylobacter jejuni* in South East Asia), azithromycin is helpful (single dose 1 g or 500 mg/day for 3 days). The Infectious Diseases Society of America treatment recommendations are summarized in Table PP17.2.

Antimotility agents are forbidden in cases of fever and bloody diarrhea. They must be stopped if abdominal pain increases or diarrhea persists for more than 2 days.

PREVENTION

Prevention consists of improving food and water selection, water purification and prior hygiene education. Specific vaccination against typhoid and hepatitis A must be carried out before traveling. Chemoprophylaxis for a short period (7 days) must be limited to high-risk travelers (e.g. AIDS, gastric surgery, chronic intestinal disease) or if digestive discomfort would cause considerable inconvenience (e.g. business, competitive sport).

FURTHER READING

Further reading for this chapter can be found online at http://www.expertconsult.com

Practice point | 18 |

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Management of persistent postinfectious diarrhea in adults

INTRODUCTION

The presence of diarrhea beyond 2 weeks of a confirmed or presumed infectious exposure is a useful working definition of persistent diarrhea as this helps to exclude most common acute bacterial and viral infections, although protozoal infections may persist for longer. Patients with persistent diarrhea after recent travel to a tropical or developing country can also be considered as having postinfectious diarrhea.

PATHOGENESIS AND CLINICAL FEATURES

Postinfectious irritable bowel syndrome

In a prospective study, we found that 25% of previously healthy patients developed a diarrhea-predominant type of irritable bowel syndrome (IBS) (Table PP18.1) after an episode of acute infectious diarrhea. Psychologic disturbances such as anxiety and stressful recent life events were important predictors for the development of IBS. Further studies support the presence of subcolitic inflammatory changes in this subset of IBS. Other potential pathogenic factors are small intestinal bacterial overgrowth, changes in the colonic microflora, lactose malabsorption, alterations in colonic motility and sensitivity, and bile acid malabsorption. Latent or potential celiac disease may also be present in a subset of patients with IBS as evidenced by the presence of serologic markers.

Table PP18.1 Points to note in the history and physical examination of patients who have persistent diarrhea

- Onset of diarrhea in relation to confirmed or presumed infectious illness
- Travel to or residence in tropics or developing countries
- Weight loss
- Nature of the stools: frequency, consistency, estimated volume, steatorrhea
- Bowel symptoms suggesting irritable bowel syndrome: abdominal pain relieved by defecation, constipation, passage of mucus, feeling of incomplete evacuation, bloating
- Other abdominal symptoms: flatulence (suggesting giardiasis, lactose malabsorption), blood in the stool (suggesting colitis, dysentery)
- Risk factors for HIV if appropriate
- Examination for evidence of weight loss, anemia, malabsorption, abdominal masses, lymph nodes

Inflammatory bowel disease

Some patients who have inflammatory bowel disease (IBD) in their initial presentation have a positive microbial finding. However, symptoms may persist or recur despite the eradication of the inciting organism. Commonly implicated pathogens are *Entamoeba histolytica*, *Shigella* spp., *Salmonella* spp., *Aeromonas* spp. and *Clostridium difficile*. Possible explanations for this association include bacterial infection added on to previously unrecognized IBD, and the precipitation of IBD by an altered intestinal microflora or by an immunopathogenic effect of bacterial products of inflammation.

Human immunodeficiency virus-associated enteropathy

HIV-associated enteropathy may present with chronic diarrhea and significant weight loss or malabsorption. It is believed that in the majority of HIV-infected patients who have chronic diarrhea, a potential pathogen can be detected.

Postinfective malabsorption

Tropical sprue presents with chronic gastrointestinal symptoms and malabsorption following an acute diarrheal episode that is usually contracted in a tropical country (although it has also been described in travelers to the Mediterranean area). Although a specific microbial agent has not been identified, the typical response to broad-spectrum antibiotics suggests an infectious pathogenesis. Intestinal infections with parasites, especially *Giardia lamblia*, may also present similarly.

Persistent intestinal infections

Occasionally infective colitis associated with *E. histolytica*, *Cryptosporidium* spp., *Campylobacter jejuni* or *Salmonella* spp. may persist beyond 6 weeks (Table PP18.2). The clinical spectrum of *G. lamblia* infection ranges from asymptomatic cyst excretion through chronic diarrhea with marked flatulence to intestinal malabsorption. *Cyclospora cayetanensis* is a newly recognized protozoal parasite that has been reported in many countries, but is most common in tropical and subtropical areas. It causes prolonged watery diarrhea in a characteristic relapsing cyclic pattern even in immunocompetent patients. Although cases of chronic diarrhea associated with *Blastocystis hominis*, *Aeromonas* spp. and *Plesiomonas shigelloides* have been reported, their pathogenic potential remains uncertain. In a middle-aged white man presenting with diarrhea, abdominal pain, weight loss and joint pains, Whipple's disease should be considered. Recently *Tropheryma whipplei*, the bacterium implicated in Whipple's disease, has been cultivated and its genome sequenced and analyzed.

Table PP18.2 Organisms that may be involved in chronic diarrhea and their antibiotic treatment

Organism		Antibiotic	Suggested dosage
Bacteria	*Shigella* spp.	Quinolones, e.g. ciprofloxacin,	500 mg q12h for 5 days
	Salmonella spp.	norfloxacin	400 mg q12h for 5 days
	Campylobacter spp.		
	Aeromonas spp.		
	Plesiomonas shigelloides		
	Spirochetes	Metronidazole	400 mg q8h for 10 days
	Clostridium difficile toxin-positive colitis	Metronidazole	400 mg q8h for 10–14 days
		Vancomycin (oral)	125 mg q6h for 10–14 days
		Colestyramine	4 g q8h for 10–14 days
Protozoa	*Giarida lamblia*	Metronidazole	2 g q24h for 3 days
		Tinidazole	2 g q24h for 1 day
		Albendazole	400 mg q24h for 5 days
	Entamoeba histolytica	Metronidazole	800 mg q8h for 5 days
		Tinidazole	1 g q12h for 3 days
	Cyclospora cayetanensis	Trimethoprim–sulfamethoxazole	2 tablets q12h for 7 days
	Isospora belii	Trimethoprim–sulfamethoxazole	2 tablets q6h for 14 days
	Blastocystis hominis	Metronidazole	800 mg q8h for 10 days
Helminths	*Strongyloides stercoralis*	Thiabendazole	25 mg/kg (maximum 1.5 g) q12h for 3 days
		Albendazole	400 mg q12h for 3 days (repeat at 3 weeks if required)
	Trichuris trichiura	Mebendazole	100 mg q12h for 3 days
	Capillaria philippinensis	Mebendazole	100 mg q6h for 20–30 days
	Mixed infection	Mebendazole	200 mg q12h for 5 days
		Albendazole	400 mg q24h for 3 days
Tropical sprue		Tetracycline plus	250 mg q6h for 30 days (minimum)
		Folic acid	5 mg q12h for 90 days

DIAGNOSIS

Patients can be stratified to an appropriate level of testing on the basis of the points listed in Table PP18.1.

Level 1 tests are:

- stool studies – microscopy, culture, ova, cysts and parasites, and tests for *C. difficile* toxin;
- blood studies – full blood count, erythrocyte sedimentation rate, C-reactive protein, biochemistry (electrolytes, protein, albumin) and studies for the detection of HIV antibodies; and
- sigmoidoscopy.

In a patient who has no history of travel to or residence in a tropical or developing country and no significant weight loss or blood in the stools, level 1 tests would usually be sufficient. To optimize the yield from stool examinations, three specimens, preferably when stools are loose, should be collected on separate days and processed rapidly. In places where facilities are readily available, sigmoidoscopy is the most expedient way to detect colitis and determine its cause through the biopsy of endoscopically visible lesions. Biopsies taken late in an infective colitis often show changes that are difficult to distinguish from IBD. When there is doubt about the diagnosis, a follow-up biopsy 6–8 weeks later is helpful, as infective colitis usually reverts spontaneously to normal. The decision to test further should be based on clinical indications and the results of the level 1 tests. For instance, in the presence of relevant exposure, or the finding of lymphopenia, the issue of testing for HIV should be addressed.

Level 2a tests are:

- lactose and fructose tolerance tests or a 2-week trial of dietary exclusion; and
- serology for IgA antigliadin, antiendomysium and anti-tissue transglutaminase.

For patients whose presentation is consistent with IBS (see Table PP18.1), level 2a tests can be considered, depending on the clinical circumstances and the facilities available.

Level 2b tests are:

- serum folate, vitamin B12, ferritin;
- gastroscopy with duodenal biopsy.

If there is a suspicion of malabsorption, evidence for this should be obtained as indicated for level 2b tests. Where there is positive evidence, and if stools are negative, endoscopy with duodenal biopsy is the next test of choice. Histologic examination contributes to the diagnosis of conditions such as tropical and celiac sprue through the diagnosis of villous atrophy, as well as helping to isolate *Giardia* spp., trophozoites and other infectious and parasitic agents that reside on enterocytes. Duodenal biopsy is also the first test of choice for the diagnosis of Whipple's disease which is characterized by the presence of foamy macrophages that stain positively to periodic acid–Schiff.

Level 3 tests are:

- colonoscopy;
- multislice CT scan;
- capsule endoscopy; and
- enteroscopy.

If the diagnosis remains uncertain, level 3 tests help to exclude Crohn's disease, colonic tumors, small intestinal diverticula (which may give rise to bacterial overgrowth) and intestinal lymphoma. Where the facilities are available, multislice CT scan has largely superseded barium studies for radiologic imaging of the small intestine. Capsule endoscopy may be helpful to identify lesions in the jejunum or ileum beyond the reach of the standard endoscope. Tissue diagnosis may be obtained subsequently by means of an enteroscopic technique.

MANAGEMENT

If malabsorption or weight loss is present and a specific infective diagnosis can be made, the indication for antimicrobial therapy is clear. If the diagnosis remains uncertain after thorough investigation, a chemotherapeutic trial of metronidazole or tetracycline appears justified. In the presence of HIV, a persistent search for an enteric pathogen is important because it is recognized that a potentially treatable pathogen can often be detected. Nutritional supplements may occasionally be required.

Patients who have a positive stool isolate but no malabsorption, weight loss or dysenteric stools pose a therapeutic dilemma. Many of these patients are persistent excretors or carriers of the organism and are suffering from postinfectious IBS; antibiotic treatment will either not improve the diarrhea or will produce a temporary improvement, possibly as a nonspecific effect of antibiotics on colonic flora. However, occasionally bacterial pathogens may give rise to protracted diarrhea and, as long as the pathogenic potential of an organism remains uncertain, a trial of antimicrobial chemotherapy can be justified. Repeated courses of antibiotics should not be pursued if the patient shows no improvement.

In patients who have postinfectious IBS, symptomatic treatment with loperamide, colestyramine and low-dose tricyclic antidepressant agents may be helpful. Clinical evidence of efficacy is now beginning to emerge for treatment with probiotic bacteria such as lactobacilli and bifidobacteria. Patients who have positive serology for celiac-associated antibodies should undertake a gluten-free diet with repeat serology after 3 months. IBS patients with lactose or fructose intolerance should modify their diet as appropriate.

FURTHER READING


Further reading for this chapter can be found online at http://www.expertconsult.com

Practice point | 19 |

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Approach to liver abscesses

INTRODUCTION

Liver abscess is an important, potentially life-threatening disorder. The two most frequent varieties of liver abscess are pyogenic abscess and amebic abscess. Etiology, epidemiology and treatment have changed over time. Historically, pyogenic liver abscess (PLA) was described as primarily affecting young men secondary to suppurative appendicitis and pylephlebitis; surgery was rarely recommended. At present, source and treatment differ radically.

EPIDEMIOLOGY

In recent studies, the incidence of liver abscess (LA) in Europe was estimated at 18 cases per 100 000 hospital admissions and 2–3 cases per 100 000 population. This differs from countries such as Taiwan where the incidence of LA is 446 per 100 000 hospital admissions. Recent reports have noted that the average age of patients has increased substantially. Nowadays, the mean age is 56.4 years. A recent study showed that diabetes mellitus is a strong independent risk factor for PLA but is not a factor for a worse prognosis.

SOURCE

The prevailing causes of PLA have changed over the past few years. Recent studies have shown an increase in the proportion of cases with underlying biliary disease which is often the most common identifiable cause of PLA. Other underlying conditions include intra-abdominal infections, malignancy, cardiovascular disease, alcohol abuse, cirrhosis, diverticulitis and inflammatory disease.

Hematogenous seeding is not the most usual pathway for the development of LA but if a single micro-organism (typically *Streptococcus* or *Staphylococcus* spp.) is isolated from an LA, a distant source must be sought. The usual pathways for the development of LA include contiguous spread from the gallbladder, ascending biliary obstruction with gallstones, or surgical or traumatic wounds (notably perforation of the gastrointestinal tract by a foreign body). Almost 80% of abscesses are solitary. Most liver abscesses are right sided.

Amebic LA is prevalent in tropical countries; in temperate areas, it is usually observed in patients who have been staying in endemic countries.

MICROBIOLOGY

Most liver abscesses are polymicrobial. Gram-negative bacilli and anaerobes are predominantly isolated in culture. Some streptococcal species are specific for LA with simultaneous metastatic infections at others sites. *Klebsiella pneumoniae* has been described in some studies as an emergent organism that can cause primary and secondary LA. In New York in 2004, *K. pneumoniae* became the predominant etiology of PLA (23%). In Taiwan, *K. pneumonia* is recovered in 82% of cases. Moreover, *K. pneumoniae* isolates from primary LA have an increased number of virulence factors (especially *K. pneumoniae* genotype K1) which confer a risk of metastatic infections with ocular and neurologic complications.

Other organisms can be recovered from patients with LA. *Candida* species represent 22% of LA in some series. It usually occurs in immunocompromised patients with neutropenia. Gram-positive pathogens have been reported in patients who underwent embolization for hepatocellular carcinoma. Tuberculous LA is rare but must be considered if no other organism is recovered. Amebic LA should be considered in patients who traveled in endemic areas. Useful features in distinguishing pyogenic from amebic LA are outlined in Table PP19.1.

CLINICAL MANIFESTATIONS

Fever is the most common finding at presentation, occurring in almost 90% of cases. Other frequent symptoms are right upper quadrant pain (72%), chills (69%) and nausea (43%). The absence of right upper quadrant pain or hepatomegaly does not exclude the diagnosis of LA. Isolated fever can be the only complaint in elderly patients. Other manifestations include vomiting, weight loss, jaundice and diarrhea.

A recent study showed that the mean duration of fever was 4.6 days and the mean duration of hospitalization was 19.6 days, with no significant difference in length of stay between patients undergoing different treatments.

IMAGING STUDIES

Chest X-ray may show elevation of the right hemidiaphragm, right basilar infiltrate, pleural effusion, atelectasis or consolidation of the right base. A recent study showed that chest X-ray was abnormal in 33% of cases. Rarely, abdominal X-ray may show gas within a liver abscess.

Table PP19.1 Useful features in distinguishing pyogenic from amebic liver abscess

	Pyogenic liver abscess	Amebic liver abscess
Age >50 years	x	
Diabetes mellitus	x	
Chest pain	x	x
Rale/ronchi/ cough	x	
Jaundice	x	x
Low albumin	x	
Elevated bilirubin	x	x
Elevated alkaline phosphatase	x	x
Male predominance		x
Abdominal pain	x	x
Diarrhoea		x
Hepatomegaly		x
Abdominal tenderness	x	x
High indirect hemagglutination assay titers		x
Solitary abscess		x
Right lobe abscess		x



Fig. PP19.1 Contrast-enhanced CT of a low-attenuation lesion in the right liver lobe: shape is regular, septa appear inside.

Ultrasonography and CT are the imaging methods of choice. CT shows that abscesses typically appear with low attenuation. Shape is regular or irregular, with or without septa inside (Figs PP19.1, PP19.2). A wall is sometimes present. A gas-containing sign is specific for pyogenic abscess although it is uncommon (Fig. PP19.3). There is no differential diagnosis for a typical simple cyst. However, the differential diagnosis of atypical cysts includes benign tumors and malignancy. Both imaging studies and clinical features may help to distinguish an atypical cyst from a liver abscess.

NONSPECIFIC LABORATORY FINDINGS

The most common laboratory nonspecific abnormalities are increased polymorphonuclear cell count, elevated erythrocyte sedimentation rate, decreased albumin level and elevated alkaline phosphatase levels. Serum bilirubin and aspartate aminotransferase are elevated in 50% of patients.

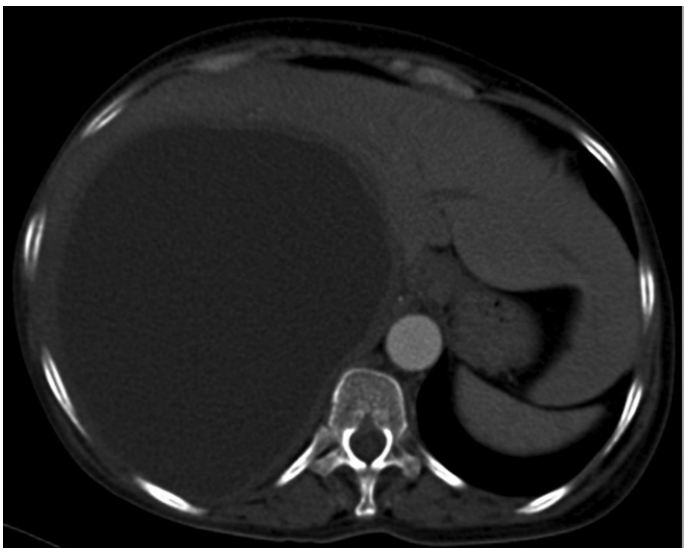


Fig. PP19.2 CT of a large, round, low-attenuation liver lesion with a smooth edge. The wall is thick and enhanced and localization is subcapsular. The appearance is evocative of amebic abscess.

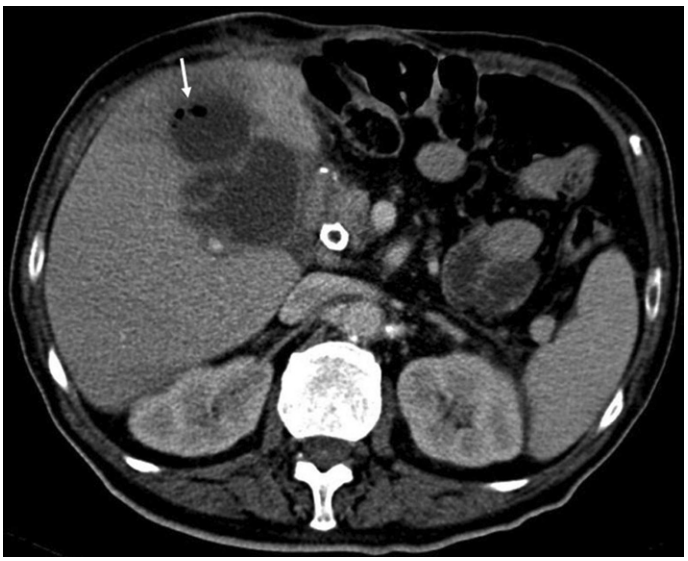


Fig. PP19.3 Contrast-enhanced CT of two low-attenuation lesions. Gas-containing sign (arrow) is specific for pyogenic abscess.

MICROBIOLOGIC DIAGNOSIS

Diagnostic aspiration of the lesion is required to confirm a pyogenic abscess. Samples should be sent for Gram stain and aerobic and anaerobic culture. Blood cultures are necessary because 24% of patients with PLA have bacteremia. Amebic serology is necessary because amebic LA cannot be distinguished from PLA by imaging studies alone. Moreover, amebic serology is positive in 95% of cases of amebic abscess. High indirect hemagglutination assay (IHA) titers are observed with or without amebic cysts in stools.

Identifying the underlying source of a liver abscess is the second approach to diagnosis. In a recent study, a primary abnormality was present in 92% of cases. Possibilities include a contiguous biliary tract infection, peritonitis, pylephlebitis, colon cancer or hematogenous seeding of the liver.

Foreign body ingestion may lead to gastrointestinal perforation and subsequent migration of the foreign body in the liver, causing a migrated foreign body left-sided liver abscess.

TREATMENT

Successful treatment usually requires both antibiotic therapy and drainage. In contrast to PLA, drainage of amebic LA is not usually necessary. Three drainage methods exist. Percutaneous drainage guided by imaging is most often used as compared to surgical drainage. Efficacy of repeated percutaneous needle aspiration versus percutaneous drainage is always controversial. There is no significant difference in morbidity, mortality or time to resolution between percutaneous versus surgical drainage. Complications after one or the other technique differ: patients who underwent surgical drainage have complications of bleeding, wound infection and intra-abdominal abscess; patients who underwent percutaneous drainage have complications of peritonitis, catheter blockage and catheter dislodgement.

Nevertheless, surgical drainage must be used when there is no positive clinical outcome after 4–7 days of percutaneous drainage and when there are multiple, large or loculated abscesses, there is a risk of the catheter being plugged by viscous pus and if there is an underlying condition which requires surgical intervention (e.g. biliary tract lesion). For this last eventuality, some authors propose endoscopic retrograde cholangiopancreatography.

Antibiotic therapy includes initial parenteral treatment with one of the regimens outlined in Table PP19.2. There is no randomized study describing the optimal duration of therapy. Parenteral antibiotics are often prescribed for the first 2–3 weeks or until there is a favorable clinical outcome. The remainder of the course is completed with oral drugs such as metronidazole and a fluoroquinolone.

Table PP19.2 Antibiotic therapy of pyogenic liver abscess

	First-intention therapy	Alternative therapy
Monotherapy		
β-Lactam/β-lactamase inhibitor	Ampicillin–sulbactam 3 g q6h	Piperacillin–tazobactam 3 g q4h
Carbapenem	Imipenem 1 g q8h	Ertapenem 1 g q24h
Bitherapy		
Metronidazole plus cephalosporin/ fluoroquinolone	Metronidazole 500 mg q8h plus ceftriaxone 1 g q24h	Metronidazole 500 mg q8h plus ciprofloxacin 500 mg q12h

Specific therapy for amebic liver abscess is oral metronidazole 500–750 mg, three times a day for 7–10 days.

FURTHER READING

Further reading for this chapter can be found online at http://www.expertconsult.com

Chapter 40

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Infective and reactive arthritis

INTRODUCTION

Infective arthritis is an inflammation of the joint space caused by invasion of different micro-organisms. Hematogenous seeding of the joint is the most common mechanism of infection in native joints. The incidence of infective arthritis in adults caused by bacteria other than *Neisseria gonorrhoeae* is relatively low, but these infections can cause major morbidity as a result of pain, immobility and loss of joint function. Successful treatment requires prompt drainage of the joint, using multiple arthrocenteses or open arthrotomy, and prolonged antimicrobial therapy to achieve sterilization of the joint space as well as a satisfactory functional result. This chapter discusses infective arthritis in adults, with the major emphasis on bacterial infective arthritis. Viral and reactive arthritis are discussed briefly. Infective arthritis caused by *Borrelia burgdorferi* is discussed in Chapter 43 and mycobacterial arthritis in Chapter 30.

Bacterial Arthritis

EPIDEMIOLOGY

In 2005, according to the Centers for Disease Control and Prevention (CDC), there were an estimated 24 000 cases of pyogenic arthritis that were hospitalized in the USA; 58% of the patients were male, and 33% were 65 years of age or older.[1] The mean duration of hospitalization of patients who had a primary diagnosis of pyogenic arthritis was 7.8 days. These data are in agreement with other published incidence rates of bacterial arthritis in the general population.[2,3] Disseminated gonococcal infection with associated gonococcal infective arthritis is the leading cause of hospital admission due to infective arthritis in the USA, with an estimated incidence rate of 2.8 per 100 000 person years (Chapter 168).[4]

RISK FACTORS

Rheumatoid arthritis, diabetes mellitus, malignancy, old age, the use of systemic steroids and prior surgery on the joint suppress the immune system and, as such, are risk factors for acquiring bacterial arthritis.[5–8] Novel therapies such as etanercept and infliximab, and targeting tumor necrosis factor for the treatment of rheumatoid arthritis have come into widespread clinical use. These agents have been shown to be implicated as a possible risk factor for severe polyarticular infectious complications in this patient population.[9] Situations that increase the risk of bacteremia, such as injection drug use, indwelling intravenous catheters and skin infection, and situations that allow direct inoculation of micro-organisms, such as intra-articular injection or arthroscopy, also predispose the joint to infection. Several cases of infective arthritis due to *Clostridium* spp. were reported to the CDC. All these cases underwent tissue allograft reconstruction surgery. Anaerobic cultures of the non-implanted donor tissue yielded *Clostridium* spp. in selected cases. It is believed that the donor tissue was hematogenously seeded by bowel flora and that the aseptic processing techniques used for the implicated donor tissues did not eliminate spores of *Clostridium* spp.[10] Individuals with rheumatoid arthritis and/or receiving disease-modifying antirheumatic drugs are at increased risk of *Staphylococcus aureus* arthritis.[11] Emerging cases of methicillin-resistant *Staphylococcus aureus* arthritis are being reported from the community and the health-care setting.[12]

Disseminated gonococcal infection is more common among sexually active, menstruating women, although it can also occur during pregnancy and the peripartum period.[4] The male:female ratio is approximately 1:4. Often the microbiologic etiology of infective arthritis can be predicted based on the specific risk factor predisposing to infection (Table 40.1).[13]

PATHOGENESIS

Nongonococcal bacterial arthritis most often results from hematogenous seeding of the joint space as a result of bacteremia. Synovial tissue has a rich vascular supply but no basement membrane, factors that favor ingress of blood-borne organisms.[8,13] The bacteremia can be primary or secondary to an infection elsewhere in the body (e.g. pneumonia, cellulitis) or to injection drug use.[5] An identifiable focus of infection can be found in approximately 50% of cases.[8,14]

Direct inoculation of micro-organisms into the joint space due of trauma, arthrotomy, arthroscopy or diagnostic and therapeutic arthrocenteses is another mechanism of infection. The risk of septic arthritis after arthrocentesis has been reported to be 0.002–0.007%; after arthroscopy it is reported to be 0.04–0.4%.[15] Infection of the joint space as a result of contiguous soft tissue infection or periarticular osteomyelitis is much less common.

Once bacteria have entered the joint space there is ingress of polymorphonuclear leukocytes, which results in hydrolysis of proteoglycans and collagen through stimulation of locally synthesized cytokines and release of enzymes such as gelatinases.[1,16] If left untreated, destruction of the articular cartilage eventually occurs, leading to irreversible joint damage.[17–19]

Staphylococcus aureus is the most common etiologic agent of infective arthritis in adults (Table 40.2).[8,20–23] In young sexually active persons, *N. gonorrhoeae* is the predominant pathogen. Infection in patients who have rheumatoid arthritis is due to *S. aureus* in as many as 80% of patients. Group B streptococcal infection is more likely to occur in patients who have diabetes mellitus. Coagulase-negative staphylococci

Table 40.1 Epidemiologic and clinical features associated with specific etiologic agents of infective arthritis

Likely etiologic agent	Clinical or epidemiologic setting
Bacteria	
Staphylococcus aureus	Rheumatoid arthritis, injection drug use, arthroscopy, arthrotomy, polyarticular arthritis
Coagulase-negative staphylococci	Arthroscopy, arthrotomy, foreign material
Neisseria gonorrhoeae	Young, sexually active, history of sexually transmitted disease or unsafe sex, menstruation, pregnancy, multiple skin lesions
Pseudomonas aeruginosa and other aerobic Gram-negative bacteria	Injection drug use, elderly
Anaerobes	Human and animal bites, orthopedic allograft infection with allograft without sporicidal sterilization, anaerobic infection elsewhere in body
Usual oral flora	Human and animal bites
Eikenella corrodens	Human bite
Pasteurella multocida	Cat or dog bite
Streptobacillus moniliformis	Rat bite
Neisseria meningitidis	Multiple purpuric lesions
Other	
Mycoplasma spp.	Common variable hypogammaglobulinemia
Borrelia burgdorferi	Resident in endemic area, known or suspected tick exposure, history of erythema chronicum migrans
Troypheryma whipplei	Other manifestations of Whipple's disease
Mycobacteria	
Mycobacterium tuberculosis	Positive tuberculin skin test, resident in country where disease is endemic, known exposure history
Mycobacterium marinum	Exposure to aquatic environment
Mycobacterium avium intracelulare	HIV, T-cell suppression
Mycobacterium leprae	Resident in country where disease is endemic
Fungi	
Sporothrix schenckii	History of trauma with exposure to colonized soil (e.g. sphagnum moss)
Candida	Prior known or suspected candidemia or culture-negative central infection, neutropenia
Aspergillus	Prior known or suspected invasive aspergillus infection, prolonged neutropenia
Blastomyces dermatididis	Resident in area where disease is endemic, exposure to beaver dams
Coccidiomyces immitis	Resident in area where disease is endemic

Data from Smith & Piercy.[13]

Table 40.2 Etiologic agents of nongonococcal bacterial arthritis in adults

Micro-organisms	Cases (%)
Staphylococcus aureus	68
Streptococci (including β-hemolytic streptococci, viridans group streptococci and *Streptococcus pneumoniae*)	20
Haemophilus influenzae	1
Aerobic Gram-negative bacilli	10
Polymicrobial and miscellaneous	1
Unknown	<1

Data from Roberts & Mock.[23]

may cause infection following arthroscopy and other medical procedures, including intra-articular injections. Infective arthritis due to Gram-negative bacilli is more common in the elderly and in patients who have co-morbid illnesses.[24]

Anaerobic infection is uncommon except in the setting of septic arthritis occurring after human or animal bite injuries or diabetic foot infections. Among injection drug users, *Pseudomonas aeruginosa* and *S. aureus* are common pathogens.[25,26] Hypogammaglobulinemia is a risk factor for infective arthritis due to *Mycoplasma* spp.[27]

PREVENTION

Prevention of infective arthritis is obviously preferable to treatment of established infection. Examples of efforts to decrease the incidence of infective arthritis include the promotion of public health measures

to prevent the acquisition of *N. gonorrhoeae*, measures to decrease the incidence of animal bites, prophylactic foot care in patients who have diabetes mellitus and eradication of injection drug use. Rapid and effective treatment of antecedent infections that may cause joint infections, such as catheter-associated bacteremia due to *S. aureus* or skin and soft tissue infection in patients who have rheumatoid arthritis and diabetes mellitus, as well as the administration of vaccines against *Haemophilus influenzae* and *Streptococcus pneumoniae*, are also effective preventive measures.[28] Eradication of the *S. aureus* carrier state with the use of topical mupirocin and chlorhexidine body washes in patients at risk for infective arthritis might play a role in reducing the risk of infection prior to joint surgery.[29]

CLINICAL FEATURES

Nongonococcal infective arthritis

Nongonococcal infective arthritis is typically monoarticular and has an acute presentation. Patients complain of pain and limitation of motion in over 90% of cases.[13] In one study, fever was present in 78% of patients within 24 hours of hospitalization, although it was rarely above 102°F (39°C).[19] Chills were uncommon. Physical examination usually reveals a large effusion and a marked decrease in active and passive range of motion of the joint. However, these findings may be minimal or absent in those patients who have rheumatoid arthritis, and they may be difficult to discern in infections of the hip or shoulder.

The knee is the most commonly involved native joint in adults. In a case series from the Netherlands, the percentage of cases involving a particular joint was: knee 55%, ankle 10%, wrist 9%, shoulder 7%, hip 5%, elbow 5%, sternoclavicular joint 5%, sacroiliac joint 2% and foot joint 2%.[3] Sacroiliac or sternoclavicular joint infection is more common among injection drug users and may be difficult to diagnose. Polyarticular infection occurs in approximately 15% of patients and is often due to *S. aureus*.[30] Thus, a polyarticular presentation does not always imply the presence of gonococcal, viral, reactive or noninfectious arthritis. It is more common among patients with rheumatoid arthritis and patients who have other co-morbid illnesses.

The case fatality rate for patients with nongonococcal bacterial arthritis is estimated to be between 10% and 16%, and as many as 50% of patients who survive their infection will have some degree of permanent loss of joint function.[8,20–22] Morbidity and mortality are dependent on a number of factors, including age, presence of rheumatoid arthritis, infection in the hip or shoulder, duration of symptoms before treatment, the presence of polyarticular arthritis, persistently positive joint fluid cultures after appropriate therapy, the presence of bacteremia and the virulence of the infecting organism.[2,8,31,32]

Gonococcal arthritis

Disseminated gonococcal infection presents with two distinct clinical entities.[4] Early after dissemination from mucosal surfaces such as the cervix or urethra the patient presents with bacteremia, fever, polyarthralgia, tenosynovitis (typically of the hands and fingers) and multiple maculopapular, pustular, vesicular or necrotic skin lesions. Asymmetric joint involvement is common. The knee, elbow, wrist, metacarpophalangeal and ankle joints are the most commonly involved. This presentation accounts for 60% of patients who present with disseminated gonococcal disease, and it has been described as the dermatitis–arthritis syndrome. If left untreated, the patient will present later with monoarticular arthritis, usually without tenosynovitis or skin lesions. Co-infection with HIV often leads to infection of unusual joints and an aggressive course.[33]

The outcome of disseminated gonococcal infection is almost always excellent (see Practice Point 29).

DIAGNOSIS

Although the history and physical examination can lead to a high index of suspicion for infection, a synovial fluid culture that yields a causative micro-organism is the only definitive method for diagnosing bacterial arthritis. Fever and rigors in the setting of an inflammatory arthritis have a low positive predictive value for bacterial arthritis and have been reported in crystal-induced arthropathy.[15] The erythrocyte sedimentation rate (ESR), C-reactive protein and leukocyte counts are elevated in the majority of cases, although again the positive predictive value of these tests in the setting of a monoarticular inflammatory arthritis is low. A rise in the ESR may help in the differential diagnosis of new joint pain and effusion in those patients who have rheumatoid arthritis.[13,34] Blood cultures are positive in up to 70% of all patients, and more often than this in patients who have polyarticular involvement.[22,30]

In disseminated gonococcal infection the majority of patients have an elevated ESR, and only 50% will have an abnormal leukocyte count. Anemia and abnormal liver function tests also may occur but these findings are usually transient.[4]

In approximately 80% of patients who have disseminated gonococcal arthritis there is a positive culture or *N. gonorrhoeae* DNA can be identified by polymerase chain reaction (PCR) or ligase chain reaction in samples from the cervix, urethra, rectum, pharynx or urine. Skin lesions yield *N. gonorrhoeae* in 30% of cases and blood cultures in 5%.

The diagnostic procedure of choice for bacterial arthritis is an arthrocentesis. This should be done immediately once the diagnosis of joint infection is suspected so as not to delay appropriate medical or surgical therapy. If synovial fluid cannot be obtained by blind needle aspiration (e.g. in the case hip joint infection), then aspiration should be done with the help of a radiologist. If necessary, an open arthrotomy should be performed to make a definitive diagnosis.

Synovial fluid is often cloudy or purulent in appearance. The synovial fluid should be routinely examined for uric acid and calcium pyrophosphate crystals, and a leukocyte count and differential should be obtained on each specimen. The leukocyte count is usually greater than 50 000/mm^3 and often greater than 100 000/mm^3, with more than 75% polymorphonuclear leukocytes. These findings can also be seen in patients who have inflammatory arthritis and crystal deposition arthritis. The sensitivity and specificity of a synovial fluid leukocyte count of $>2 \times 10^9$ for the presence of inflammatory arthritis have been estimated to be 84% and 84%.[35] For a differential count of >75% polymorphonuclear leukocytes, the sensitivity and specificity are 75% and 92%. Although many clinicians order synovial fluid glucose and protein levels, the results of these tests have been shown to be less informative than the leukocyte count and differential.[35] The synovial fluid lactic acid and lactate dehydrogenase levels are often elevated in patients who have infective arthritis, but elevation of these tests can be seen in other inflammatory joint disorders as well.[8]

Synovial fluid should be cultured for both aerobes and anaerobes and other organisms, depending on the clinical circumstances. A recent study performed at the Mayo Clinic showed superior performance of the BACTEC Peds Plus/F bottle over the conventional agar plate method for the detection of clinically significant microorganisms from synovial fluid specimens.[36]

The synovial fluid culture will be positive in 90% of cases of nongonococcal arthritis, assuming that antibiotic therapy has not been started before the sample has been collected.[19] The Gram stain is positive in 50% of patients.[8]

In patients who have disseminated gonococcal infection, the synovial fluid cultures are positive in 25–30% of all patients and 50% of patients who present with monoarticular arthritis. The role of PCR in detecting bacterial pathogens in patients who have infective arthritis has not yet been well defined, although the technique seems a promising tool for the detection of infectious arthritis due to *N. gonorrhoeae*,

Borrelia burgdorferi and *Tropheryma whipplei*.[37] PCR can help to distinguish disseminated gonococcal infection from other inflammatory arthropathies such as Reiter's syndrome.[38]

Synovial tissue cultures are indicated only for chronic infective arthritis when mycobacterial or fungal arthritis is suspected or when synovial fluid cultures cannot be obtained by less invasive techniques.

Periarticular soft tissue swelling is the most common abnormality seen on plain radiography in patients who have bacterial arthritis. Periarticular erosions and osteoporosis as well as joint space narrowing due to cartilage destruction do not occur for several weeks. Thus, plain radiographs are not usually helpful in making a diagnosis of bacterial arthritis. Occasionally, in longstanding infection, periarticular osteomyelitis will be visible on plain radiograph (Fig. 40.1). It is often difficult to distinguish infection from inflammatory arthritis using radiographic methods in the setting of rheumatoid arthritis, but the development of a rapid destructive arthritis in one or two joints suggests infection. CT scans and MRI are more useful than plain radiographs for identifying concomitant periarticular osteomyelitis, soft tissue abscesses and joint effusions, but they are expensive and most often are not necessary (Fig. 40.2). Sacroiliac or sternoclavicular joint disease is optimally evaluated with these modalities, as well as with radionuclide studies. ^{111}Indium scans may be useful for identifying relatively asymptomatic septic arthritis in immunocompromised patients who have one or more known septic joints in whom there is a high index of suspicion for polyarticular infection.

In adults, the differential diagnosis for patients with an acute onset of fever, chills and an inflammatory arthritis of one or more joints includes bacterial arthritis, gout, pseudogout, rheumatic fever, reactive arthritis and rheumatic illnesses such as rheumatoid arthritis and psoriatic arthritis. Gout and pseudogout are the most common noninfectious inflammatory arthritides that need to be differentiated from bacterial arthritis.[8] They should be suspected when there is a history of previous episodes or there is chondrocalcinosis on plain film. Synovial fluid analysis using polarized light microscopy is the most useful diagnostic test for gout or pseudogout.

The articular manifestations of rheumatic fever occur in approximately 75% of first episodes, last for several weeks and do not cause permanent joint damage. Typically there is development of a migratory polyarthritis involving the knees, elbows, ankles and wrists that occurs within 1–5 weeks of the antecedent streptococcal pharyngitis. Joint symptoms can range from arthralgia without obvious physical findings to inflammatory arthritis that is indistinguishable from infective polyarthritis. Often more than six joints are affected. The diagnosis of rheumatic fever is dependent on satisfying the updated Jones criteria (see Chapter 166).[39]

Bacterial arthritis should be suspected in those patients at increased risk of infection. Fungal and mycobacterial infection is usually monoarticular but their presentation is usually over weeks to months instead of hours to days. Diseases that must be distinguished from disseminated gonococcal infection include viral and reactive arthritis, rheumatic fever and secondary syphilis.

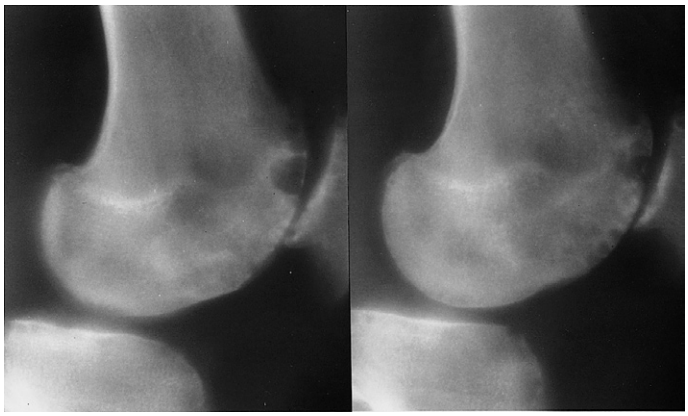


Fig. 40.1 Tomogram of right knee of a patient who has *Staphylococcus aureus* septic arthritis and periarticular osteomyelitis. Note the mixed sclerosis and lytic changes suggestive of osteomyelitis.

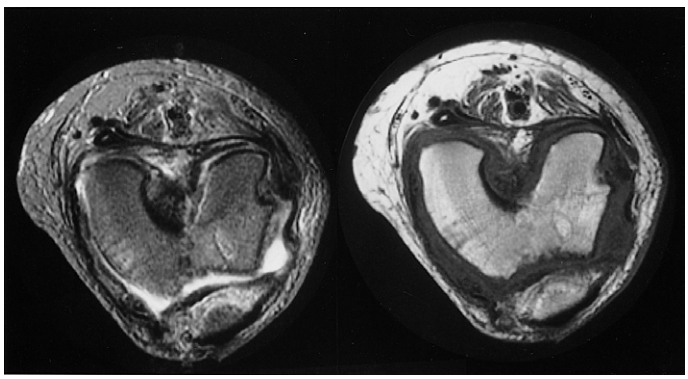


Fig. 40.2 MRI scan of right knee of a patient who has *Staphylococcus aureus* septic arthritis. Note the soft tissue inflammation and a joint effusion.

MANAGEMENT

The keys to the management of infective arthritis are:

- drainage of the purulent synovial fluid;
- debridement of any concomitant periarticular osteomyelitis; and
- administration of appropriate parenteral antimicrobial therapy.

Experimental models of septic arthritis suggest that early drainage and antimicrobial therapy prevent cartilage destruction.[40] Local antimicrobial therapy is unnecessary and may cause a chemical synovitis.[8] Joint immobilization and elevation is useful for symptomatic relief of pain early in the course of the disease, but early active range of motion exercises are beneficial for ultimate functional outcome.

Synovial fluid drainage

The optimal method of drainage of an infected joint remains controversial, in part because no well-controlled randomized trials exist to guide therapy and because the therapy of each patient should be individualized.[41] Most adults who have septic arthritis have been managed with repeated joint aspirations instead of surgical debridement.[19] Patients who have disseminated gonococcal infection rarely require repeat joint aspirations, arthroscopy or arthrotomy.[4]

The use of arthroscopy has expanded in recent years because of the minimal morbidity of the procedure and the improved ability of arthroscopy to adequately drain purulent material from the joint compared with joint aspiration.[42–47] Large multicenter, randomized trials are needed to evaluate the comparative efficacy of these modalities.

Debridement

Recommended indications for surgical debridement have included effusions that fail to resolve with 7 days of conservative therapy and inability to adequately drain the infected joint by aspiration or arthroscopy, either because of location (hip and shoulder) or loculations of pus[8,13] (Fig. 40.3).

Antimicrobial therapy

Antimicrobial therapy should be administered as soon as the diagnosis is suspected and synovial fluid cultures obtained. Any delay in the administration of antimicrobial therapy may result in significant

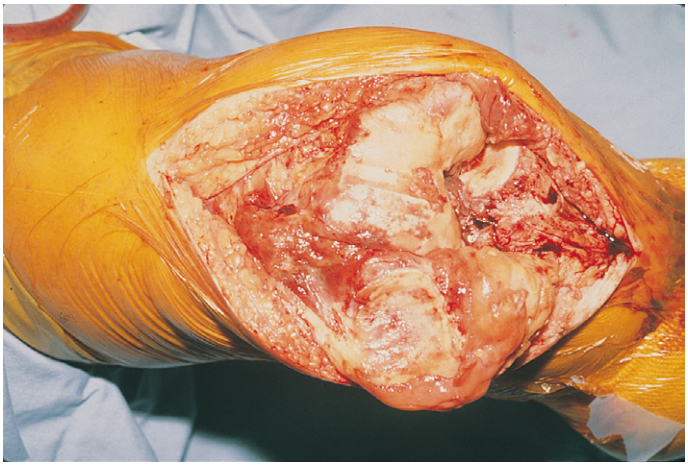


Fig. 40.3 Intraoperative photograph of right knee of a patient who has *Staphylococcus aureus* septic arthritis. Note the damaged joint and dark brown, boggy and hyperemic synovium.

cartilage loss and functional limitation of the affected joint. To date there are no randomized studies to help guide the clinician in the antimicrobial therapy of septic arthritis. Initial antimicrobial therapy should be based on the results of the Gram stain and the specific clinical and epidemiologic setting. If no micro-organisms are seen on the Gram stain, then empiric therapy for *S. aureus*, streptococci and gonococci (in young sexually active adults) should be given. Antimicrobials with anaerobic activity should be added to this regimen if septic arthritis occurs after the use of an allograft.[10] Administration of vancomycin or an antimicrobial with MRSA activity should be considered empirically in patients at risk for MRSA or when methicillin resistance is high in the community. Most experts administer 2–4 weeks of intravenous antimicrobial therapy for the treatment of nongonococcal septic arthritis.[8,13,48] In most cases this therapy can be administered on an outpatient basis after an initial period of hospitalization.

Suggested antimicrobials for specific pathogens causing infective arthritis are shown in Table 40.3.

Oral antimicrobial therapy with an effective agent with excellent bioavailability, such as ciprofloxacin or linezolid, is also acceptable, particularly if compliance with oral therapy can be assured.

Table 40.3 Antibiotic therapy of infective arthritis in adults for selected bacterial micro-organisms

Micro-organisms	Antibiotic therapy	Alternative therapy	
Staphylococcus aureus	Methicillin-sensitive strains	Nafcillin *or* oxacillin 1.5–2.0 g iv q4h *or* Cefazolin (or other first-generation cephalosporins in equivalent dosages) 1 g iv q8h	Vancomycin 15 mg/kg iv q12h, not to exceed 2 g in 24h unless serum levels are monitored
	Methicillin-resistant strains	Vancomycin 15 mg/kg iv q12h, not to exceed 2 g in 24h unless serum levels are monitored	Consult a specialist in infectious diseases Linezolid 600 mg iv/po q12h
Penicillin-sensitive streptococci *or* pneumococci with an MIC ≤0.1 μg/ml		Aqueous crystalline penicillin G 20 × 10^6 U iv q24h either continuously *or* in six equally divided doses *or* Ceftriaxone 2 g iv or im q24h *or* Cefazolin 1 g iv q8h	Vancomycin 15 mg/kg iv q12h, not to exceed 2 g in 24h unless serum levels are monitored
Enterococci *or* streptococci with an MIC ≥0.5 μg/ml *or* nutritionally variant streptococci (all enterococci causing infection must be tested for antimicrobial susceptibility in order to select optimal therapy)		Aqueous crystalline penicillin G 20 × 10^6 U iv q24h either continuously *or* in six equally divided doses, plus gentamicin sulfate 1 mg/kg iv or im q8h *or* Ampicillin sodium 12 g iv q24h either continuously or in six equally divided doses	Vancomycin 15 mg/kg iv q12h, not to exceed 2 g in 24h unless serum levels are monitored
Neisseria gonorrhoeae		Ceftriaxone 1 g im *or* iv q24h for 24–48h after clinical improvement *followed by* Cefixime 400 mg po q12h for 1 week *or* Ciprofloxacin 500 mg po q12h for 1 week *or* Ofloxacin 400 mg po q12h for 1 week	Ciprofloxacin 400 mg iv q12h for 24–48h after clinical improvement *or* Ofloxacin 400 mg iv q12h for 24–48h after clinical improvement *or* Spectinomycin 2 g im q12h for 24–48h after clinical improvement *followed by* Ciprofloxacin 500 mg po q12h for 1 week *or* Ofloxacin 400 mg po q12h for 1 week
Enterobacteriaceae *Pseudomonas aeruginosa*, *Enterobacter* spp.		Ceftriaxone 2 g iv q24h *or* Ciprofloxacin 750 mg po q12h (based on *in-vitro* susceptibility)	Levofloxacin 500 mg po q24h

Dosages recommended are for patients who have normal renal function.

The therapy of gonococcal septic arthritis would require the use of ceftriaxone as the initial drug of choice, followed by oral therapy with a cephalosporin or a quinolone after initial clinical improvement (see Table 40.3). Given the increasing incidence of quinolone-resistant *N. gonorrhea*, however, we would advocate avoidance of empiric use of a quinolone pending the results of the *in-vitro* sensitivities.[49]

Viral Arthritis

Arthritis is a common complication of infections with hepatitis B virus, erythrovirus B19 (formerly paravovirus B19), rubella and alphaviruses, and is relatively rare with mumps virus, enteroviruses, adenoviruses and herpesviruses. The most common mechanism by which viruses cause arthritis is by invasion of the joint during the period of viremia. Other postulated mechanisms include immune complex deposition, insertion of the viral genome into the host DNA, thus promoting autoimmunity through an 'altered self', and direct viral infection of the immune system, thus altering the immune response.[50–53]

Typically, viral arthritis occurs during the prodromal stages of viral infection and is associated with a rash. Polyarticular involvement, including the small joints of the hands, is typical. There is no specific pattern of joint involvement that is unique to a given viral etiology. Diagnosis is based on historic and clinical clues (Table 40.4) and diagnostic testing specific for each individual virus, the details of which are discussed in the relevant chapters. Recently, an emerging form of acute and chronic distal joint arthritis related to chikungunya virus has been reported in the Far East.[54] Viral arthritis is usually self-limiting but may progress to a chronic arthropathy in certain instances. It is important that viral arthritis is distinguished from rheumatic fever and the initial presentation of autoimmune disorders, including rheumatoid arthritis.

Treatment is discussed in the chapters devoted to specific viruses. Prevention of viral arthritis is dependent on vaccination against the specific pathogen causing arthritis (e.g. mumps virus, rubella, hepatitis A virus, hepatitis B virus and varicella-zoster virus) (see Chapters 152, 154, 161 and 163).

Reactive Arthritis

Reactive arthritis describes the acute onset of an inflammatory arthritis soon after an infection elsewhere in the body in which microorganisms cannot be cultured from the synovial fluid. In some

Table 40.4 Clinical or epidemiologic features of infective arthritis caused by selected viruses

Viral agent	Epidemiologic features	Clinical characteristics	Outcome
Rubella (including rubella vaccine)	No prior vaccination; 51–61% of rubella cases; 0–14% of patients receiving vaccination. Less common with new vaccine. Ratio of women:men – 9:1.	Symmetric arthritis of the metacarpal and proximal phalangeal joints, wrist, elbow, ankle, knee; onset variable in relationship to rash. May mimic rheumatoid arthritis. Post vaccination disease less symptomatic	Spontaneous resolution in days to weeks but may be chronic or recur.
Erythrovirus B19	60% of adult cases, females > males. Child care providers or school teachers; unusual in children	Sudden severe polyarticular arthritis in small joints. May mimic rheumatoid arthritis	Spontaneous resolution most common. Chronic arthritis can occur
Hepatitis A	10–14% of cases of hepatitis A; typical risk factors for acquisition of hepatitis A	Often associated with rash	Resolves spontaneously
Hepatitis B	10–25% of cases of hepatitis B; typical risk factors for acquisition of hepatitis B	Severe arthritis of sudden onset, symmetric, polyarthritis involving hand and knee; morning stiffness is considerable; skin rash may be present including urticaria	May last 1–3 weeks. Typically resolves during the preicteric phase. Chronic arthritis may occur with chronic hepatitis B infection
Hepatitis C	Typical risk factors for acquisition of hepatitis C	Can occur with acute or chronic infection. Sudden onset; joint pain in hands, wrists and shoulders often greater than physical findings. May mimic rheumatoid arthritis. Distinct from cryoglobulinemia with hepatitis C	May resolve spontaneously. Has been reported to persist for months and recur
HIV	Typical HIV-associated risk factors. Approximately 8% of HIV infected patients affected	Most cases are monoarticular but monoarticular and polyarticular presentations occur. Distinct from Reiter's syndrome or psoriatic arthritis which also occur in HIV-infected individuals. Occurs in multiple stages of HIV infection	Usually resolves in several weeks. May persist for months
Arthropod-borne Chikungunya O'nyong-nyong Sindbis virus Ross River agent Barmah Forest virus	 East Africa, India, South East Asia, Philippines East Africa Sweden, Finland, Russia Australia, New Zealand, New Guinea Australia	May occur in epidemics. May be associated with rash. Constitutional symptoms may be present	Spontaneous resolution is typical. Chronic arthritis is unusual except with Sindbis virus

Data from Smith & Piercy,[13] Siegel & Gall,[50] Veno *et al.*,[58] Espinosa,[59] Naides.[60]

instances genetic material may be found in the joint using molecular diagnostic techniques. Reiter's syndrome (the classic triad of arthritis, urethritis and conjunctivitis) is a common example of reactive arthritis. Many patients who develop reactive arthritis are HLA-B27-positive. The micro-organisms that have been associated with reactive arthritis are detailed in Table 40.5.[55] A recent study that evaluated the risk of reactive arthritis following a large outbreak of O157:H7 and *Campylobacter* species found an incidence of arthritis of 17.6% and 21.7% respectively in patients who developed moderate or severe gastroenteritis symptoms.[56]

Typically, reactive arthritis begins several weeks after an antecedent infection. The initial clinical presentation is usually asymmetric oligoarticular arthritis without prominent constitutional symptoms. The syndrome also occurs without any identifiable symptoms of infection, however, particularly in the case of *Chlamydia trachomatis*. In the case of Reiter's syndrome, extra-articular manifestations are also present.

Laboratory abnormalities are nonspecific and include mild elevations in the leukocyte count, ESR and C-reactive protein. Radiographs usually show only soft tissue swelling in early disease, but juxta-articular osteoporosis and erosion may also be seen.

Reactive arthritis is normally a self-limited disease, but chronic arthritis and sacroiliitis can occur in up to 15–30% of patients. Treatment is with anti-inflammatory agents. The role of antibacterial therapy is controversial. Randomized trials evaluating the efficacy of antimicrobials in patients with reactive arthritis have been limited by small numbers of patients. In one study there was a trend towards efficacy for acute reactive arthritis due to *Chlamydia* spp.[57] Prevention of infection is reliant on effective prevention and treatment of precipitating antecedent infections.

Table 40.5 Micro-organisms associated with reactive arthritis

Definite association
• *Chlamydia trachomatis*
• *Shigella flexneri*
• *Salmonella enteritidis*
• *Salmonella typhimurium*
• *Yersinia enterocolitica*
• *Yersinia pseudotuberculosis*
• *Campylobacter jejuni*

Data from Hughes & Keat.[55]

REFERENCES

References for this chapter can be found online at http://www.expertconsult.com

Chapter 41

Anthony R Berendt

Acute and chronic osteomyelitis

INTRODUCTION AND EPIDEMIOLOGY

The osteomyelitic left fibula of the largest articulated fossil specimen of *Tyrannosaurus rex*, 'Sue', on display in the Field Museum of Natural History in Chicago,[1] vividly illustrates the ancient relationship between vertebrate bones and microbial pathogens, and the highly conserved biologic responses that have evolved to protect the host from this interaction. These responses, at their most successful, will prevent septicemic death and contain infection within a small part of the bone, either arresting and eradicating the infection, or creating a situation in which the infected portion of bone can be expelled from the body. It is common, however, for the eradicative responses to be incomplete, leading to chronic suppurative infection associated with pain, poor function and ill-health, and sometimes with progressive disability. This morbidity is as debilitating for the survivor of modern trauma, military conflict or sepsis developing osteomyelitis today, as it will have been for 'Sue' over 65 million years ago.

The formidable clinical reputation of osteomyelitis is perhaps well earned[2] but not inevitable with currently available treatments. Indeed, one way to make a poor outcome much more certain is to succumb to a nihilistic view that this is a condition characterized by intractability, recurrence and failure. Experiences from well-organized and skilled multidisciplinary teams suggest that with a positive approach, high rates of arrested infection and restored function can be achieved.[3] Difficulties in delivering success for all patients are partly due to variation; in the interplay of microbial virulence and host biology in each patient, in the extent of pre-treatment damage to bone stock and soft tissues, and in the clinical team's judgments and technical skill. The latter is unsurprising given the relative lack of good evidence to direct treatment, as a result of which most recommended management strategies are based on expert experience and opinion, not on randomized controlled trials.

The epidemiology of acute osteomyelitis has been studied in the northern and southern hemispheres[4–6] (Fig. 41.1). In all locations hematogenous osteomyelitis is a disease of children and adolescents and is much less common in adulthood. Incidences have been shown to be greater in the southern hemisphere. High rates are seen in Polynesians and in New Zealand, in Maori children compared to Caucasians,[4,5] but the relative importance of microbial, genetic, socioeconomic and cultural factors is unknown. In the northern hemisphere, rates of acute and subacute hematogenous osteomyelitis in children have been declining over decades,[6] compounded by the elimination of *Haemophilus influenzae* osteomyelitis[7,8] through immunization. A second peak in incidence occurs in older adults[9] and this includes vertebral osteomyelitis with an incidence of approximately 2 per 100 000 in both France and Sweden.[10,11]

Recent studies have stressed a growing role for community-acquired strains of methicillin-resistant *Staphylococcus aureus* (CA-MRSA) in acute hematogenous osteomyelitis,[8,12] the incidence of which may therefore rise once more.

The epidemiology of chronic osteomyelitis is less well documented, due as it is to a variety of different insults and manifesting in a variety of sites and ways. Cases are more frequently due to old trauma or instrumentation than to prior hematogenous infection. The enormous burden of war and civil unrest, the expansion and aging of human populations, and the rapid increases in automobile transport (and associated trauma) are all likely to have increased the prevalence of chronic osteomyelitis. A single example is the pandemic of type 2 diabetes. It is estimated that up to 25% of persons with diabetes will have a foot ulcer at some time during their life[13] and approximately 20% of patients attending foot ulcer clinics have osteomyelitis complicating the ulceration.[14] With global numbers of persons with diabetes at some 250 million, this suggests that around 12.5 million of today's current diabetics will have had, or will develop, osteomyelitis of one or more foot bones. The health-care burden of this is very substantial.

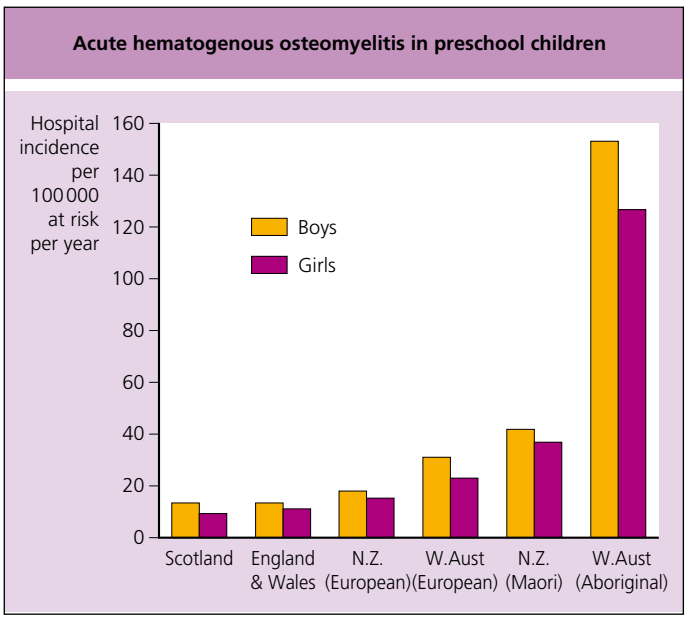


Fig. 41.1 Acute hematogenous osteomyelitis in preschool children. Data from Gillespie.[4]

PATHOGENESIS, PATHOLOGY AND CLASSIFICATION

Microbial factors

The pathogenesis of osteomyelitis begins with access and attachment[15] of organisms to bone either via hematogenous spread or from a contiguous focus of infection. Hematogenous spread may be from an obvious or an inapparent focus of infection or bacteremia. The contiguous focus may be infected soft tissue, a joint, orthopedic instrumentation or an area of chronic ulceration overlying bone.[16]

Staphylococci are the most common agents of bone infection. *Staphylococcus aureus* possesses numerous cell-wall-associated adhesins mediating specific attachment to a wide variety of extracellular matrix proteins found in bone[17] (Figs 41.2, 41.3). These include fibronectin, laminin, osteopontin, bone matrix sialoprotein and collagen. Yet other adhesins mediate attachment to components of thrombus such as fibrinogen and thrombospondin. Patients with hematogenous osteomyelitis commonly report some form of antecedent blunt injury to the affected part. This could expose bone matrix proteins (by microfracture of their mineral covering) and produce local thrombus to which staphylococci can attach. Where bone is directly exposed, necrosis of the outer surface provides an ideal substrate for bacterial attachment and subsequent invasion.

Post-attachment events include the elaboration of toxins that directly contribute to necrosis of tissues. In *S. aureus* the synthesis of these is controlled by quorum sensing mechanisms[18] and global virulence regulators.[19] Adherent bacterial growth leads to the formation of a biofilm, an adherent consortium of micro-organisms enmeshed in an exocellular polysaccharide. Biofilmed organisms manifest different phenotypes from planktonic ones, being much less susceptible to antimicrobial agents and to host defenses, and hence capable of prolonged survival.[20]

Small colony variants are another potential mechanism for persistence.[21] Altered gene expression affects metabolic pathways, growth rates, expression of virulence factors and antibiotic susceptibility. Small colony variants have been shown capable of persisting intracellularly including in endothelial cells, fibroblasts and osteoblasts, and have been recovered from clinical cases of chronic osteomyelitis.[22]

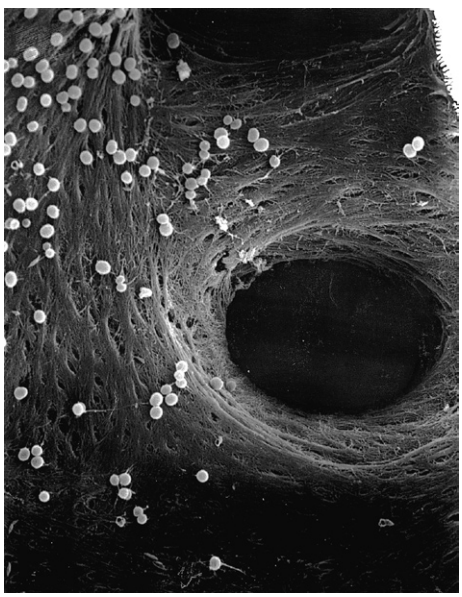


Fig. 41.2 Endosteum of bone showing staphylococci near the endosteal Haversian canal. *In-vitro* incubation of bone chips with *Staphylococcus aureus* interrupted at 48 hours (scanning electromicrograph). From Norden CW, Gillespie WJ, Nade S. Infections in bones and joints. Blackwell Scientific Publications; 1994, with permission.

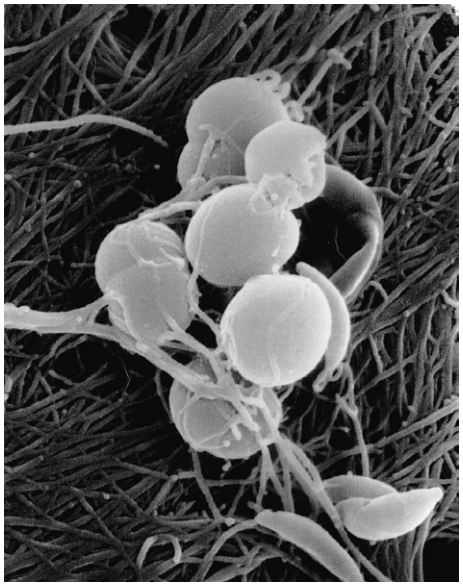


Fig. 41.3 Staphylococci enmeshed in glycocalyx near the Haversian osteum. *In-vitro* incubation of bone chips with *Staphylococcus aureus* interrupted at 48 hours (scanning electromicrograph). From Norden CW, Gillespie WJ, Nade S. Infections in bones and joints. Blackwell Scientific Publications; 1994, with permission.

Host factors

In hematogenous infection the pathogen most commonly localizes to the metaphysis of a large long bone just beneath the growth plate. The commonest sites are therefore femur, tibia, humerus, radius and ulna, but any bone can be involved and periosteal or epiphyseal seeding can occur. Extension across the growth plate is impeded in children; following epiphyseal fusion this route becomes possible and the joint can be involved. This is also the case in neonates since the joint capsule extends past the growth plate and encompasses some of the metaphysis as well as the epiphysis.

Infection triggers an acute inflammatory response, which in the rigid mechanical microenvironment of bone causes raised intraosseous pressures contributing to vascular thrombosis. Infection may be contained, in the resulting region of bone death, by the surrounding inflammation.

If infection is arrested at an early stage the effects on bone may be minimal. If infection progresses, pus may track to other areas of the bone along the medullary canal or through the Haversian systems in cortical bone from the medulla to the outer surface of the cortex. Here it may strip the periosteum (especially in the young where it is less adherent) and form a subperiosteal abscess. This may contribute to a bacteremia and major septicemic illness, or it may track out into the soft tissues and eventually form abscesses or a sinus, draining to the outside world.

Establishment of chronicity

Dead bone acts as an inert surface for biofilm formation and is surrounded by acute inflammation and suppuration. Both the inflammatory cytokines and mediators released during infection, and in some cases bacterial products themselves, can trigger bone resorption either by osteoclast activation or by stimulating phagocytic cells to take on a bone-resorbing phenotype.[23,24] Hence bone is lost from around the dead area, potentially ultimately separating it from the surrounding living bone to form the sequestrum. At the same time, where periosteal stripping has occurred, the resulting periosteal reaction produces a shell of new bone, the involucrum, around the dead bone. This will not be seen where the periosteal tissues themselves have been destroyed by infection or by pre-existing ulceration. Pus drains via one or more cloacae in the involucrum or from cortical breaches. This situation is compatible with life and function since the draining infection assumes a less

aggressive nature and the mechanics of the bone can be preserved by a well-developed involucrum. The patient does, however, suffer ongoing bone pain, drainage, chronic debility and ill-health, and potential flares in infection that can affect the soft tissues, the bone or a neighboring joint, or lead to abscess formation or bacteremia.

Microbiology

The earliest descriptions established the primacy of *S. aureus* as a cause of osteomyelitis[25] and this remains the case today, accounting for over 50% of cases in pure or mixed growth.[26,27] In recent years there has been an increased importance of MRSA strains,[12] with community-acquired strains (CA-MRSA) of increasing importance in many locations worldwide. Where environmental contamination has been prominent, as in open fractures or blast injuries, or where infection arises from chronic wounds with their attendant complex polymicrobial flora, a wider variety of pathogens is seen with a rise in the importance of the Enterobacteriaceae[28] and of *Pseudomonas aeruginosa*. The latter is also associated with specific situations and risk factors, often involving initial interaction with a cartilaginous area adjoining bone, such as:

- puncture injuries to the foot through the soles of sneakers (training shoes) causing osteochondritis of the metatarsophalangeal joint;[29]
- malignant otitis externa involving the junction of the external auditory meatus and the skull base;[30]
- hematogenous seeding to vertebral end-plates in intravenous drug users and renal dialysis patients; and
- as a cause of chronic long bone osteomyelitis after open fracture and multiple procedures.

Anaerobes can play primary or synergistic roles and there is some evidence that their prevalence rises with increased efforts to isolate them.[31] Low-virulence organisms such as coagulase-negative staphylococci or enterococci are common in mixed infections, especially in diabetic foot osteomyelitis[32] or where infection arose in association with orthopedic instrumentation (even if metalware has subsequently been removed). In these situations they sometimes appear to be capable of acting as pathogens in their own right, being isolated in pure growth from reliable specimens.

Worldwide, infection with *Mycobacterium tuberculosis* remains important[33,34] and in appropriate geographic areas, *Brucella* spp. are important causes of spondylodiscitis.[35] *Salmonella typhi* and non-typhoidal *Salmonella* spp. have been identified as important in Africa, both in and outwith the context of sickle cell anemia or coincident HIV infection; various unusual *Salmonella* spp. have also been described in temperate zones from animal contact, such as with pet reptiles. Also of geographic importance are endemic fungal diseases including coccidioidomycosis, histoplasmosis and blastomycosis, all of which can produce bone lesions, as can late stage mycetomas. *Kingella kingae* has been shown to play an important role in childhood infections in Israel and France; for unclear reasons, this is not the case worldwide.[27] It would not be wrong to say that there are few surprises in the microbial 'small print' of causative pathogens, including atypical mycobacteria, *Nocardia*, melioidosis, yeasts, actinomycetes and even classic pathogens such as *Treponema pallidum*. Where chronic bone infection has been associated with multiple treatment attempts, multidrug-resistant organisms (MDROs) are common.

Pathology

The histologic appearances of osteomyelitis mirror the physiologic processes. The hallmarks are acute inflammatory cells within bone, and bone death, which may be associated with acute inflammation in the adjoining soft tissues, ulceration or purulence. Bacteria or fungi may rarely be demonstrable on special staining. As chronic infection develops, chronic inflammatory cells are admixed to the acute picture, and enhanced osteoclastic activity can be seen alongside new bone formation. In the case of a number of infections, including tuberculosis, granuloma formation occurs.

Classification

Osteomyelitis can be classified in several ways. *Temporal classifications*, namely acute, subacute and chronic, help to articulate the urgency and priorities of treatment and the prospects of cure. *Pathogenetic classifications*, namely hematogenous and contiguous focus, can help to direct attention to the likely spectrum of causative pathogens. Furthermore, this scheme also indicates whether the disease is 'inside-to-out' (hematogenous) and so effectively chronic in the bone by the time infection drains to the soft tissues, or 'outside-to-in' (contiguous focus), where there is often a substantial soft tissue problem to manage at the earliest point that bone is involved. *Anatomic schemes* can helpfully draw attention to specific challenges linked to regional anatomy; certainly the epidemiologies and treatments of skull base, long bone, diabetic foot and spinal osteomyelitis are different.

Most helpful of all, however, is the eponymous *anatomic-physiologic staging system* developed by Cierny & Mader[36] (Fig. 41.4). This details both the extent of bony involvement and key local and systemic factors that may affect prognosis. The staging system also inherently suggests

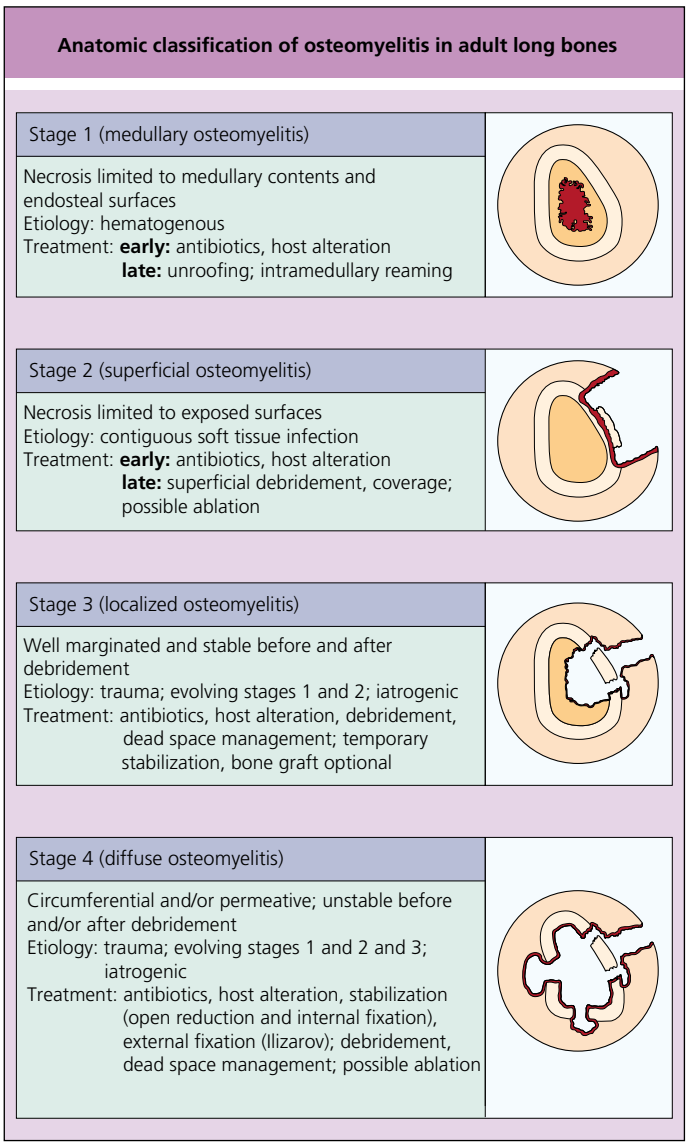


Fig. 41.4 Anatomic classification of osteomyelitis in adult long bones. Adapted with permission from Mader JT, Calhoun J. Osteomyelitis. In: Mandel G, Bennet J, Dolin R, eds. Infectious diseases. New York: Churchill Livingstone; 1995:1039–52.

different surgical strategies to precede antibiotic therapy and in some cases also predicts the reconstructive approach likely to be required.

PREVENTION

Since bone infection is relatively rare, an extensive literature on its prevention is lacking. Effective treatment of soft tissue infection, and careful attention to the prevention of health-care-associated infections (including orthopedic surgical site infections), may prevent a handful of cases of osteomyelitis but the data may never be forthcoming to prove this. Appropriate treatment of open fractures with debridement, antibiotics, stabilization and where needed, plastic surgery for soft tissue cover does have a demonstrated role in reducing infection and the subsequent risk of chronic osteomyelitis.[37] Finally it is clear that careful attention to pressure offloading can lead to primary healing and the secondary prevention of ulceration in the diabetic foot and other pressure situations.[38] It is highly likely that the systematic application of, and adherence to, these measures will prevent a substantial burden of diabetic foot osteomyelitis. Similarly, pressure sore risk assessment and care is essential in the prevention of decubitus ulcers and the osteomyelitis that sometimes complicates them.

CLINICAL FEATURES

Acute osteomyelitis

The classic descriptions are of an acute septic illness associated with agonizing pain localized to a bone.[2] Small children unable to localize or verbalize their pain may instead refuse to move a whole limb. There is high fever, prostration and systemic upset. Bacteremia complicates up to 50% of cases.

As the infection progresses, worsening sepsis is accompanied by increasing pain and bony tenderness together with soft tissue edema. Intra- or extraosseous abscesses may form. Without treatment, the outcome is either septicemic death or a prolonged febrile illness that resolves when pus drains spontaneously via a sinus.

Subacute osteomyelitis

The decline in acute hematogenous osteomyelitis of childhood appears to have been accompanied by a greater proportion of cases presenting with longer and more insidious histories, and with less systemic or local upset.[39] Often the major findings are that early radiologic changes are visible at presentation, not the case for classic acute osteomyelitis (see below). These painful bone lesions may mimic tumors[40] and hence are usually managed with biopsy or complete excision, often before they are recognized as being infective, and sometimes therefore without cultures being sent. Prognosis is, however, usually good.[41]

Chronic osteomyelitis

There is rarely a fever unless the infection is undergoing an acute flare. A degree of insidious ill-health is common, often noticed by the patient only after successful treatment. The most consistent features are bone pain, continuous or intermittent wound drainage, and impaired function; this may be profound (Figs 41.5, 41.6). Draining sinuses may alter in location and number over time through spontaneous remissions and relapses, healing and reopening via old or new routes. Where soft tissue loss preceded bone infection, continuous drainage or exposure of bone leads to persistent wounds. The abnormal soft tissue envelope is often confounded by scars from previous surgery, which can form preferential routes for sinus formation.

Progressive bony changes can include the expulsion of fragments or the whole of a sequestrum leading to self-arrest of infection. Relapse can occur decades after initial injury and infection.



Fig. 41.5 Chronic osteomyelitis. The patient is a 30-year-old man who was born in Pakistan and who, as a child, had chronic osteomyelitis caused by *Staphylococcus aureus*. He is asymptomatic now except for occasional pain in the hip and a limp. The radiograph shows destruction of the femoral head and acetabulum, chronic changes in the femoral shaft and fusion of the right hip joint. Courtesy of Dr Joseph Mammone.

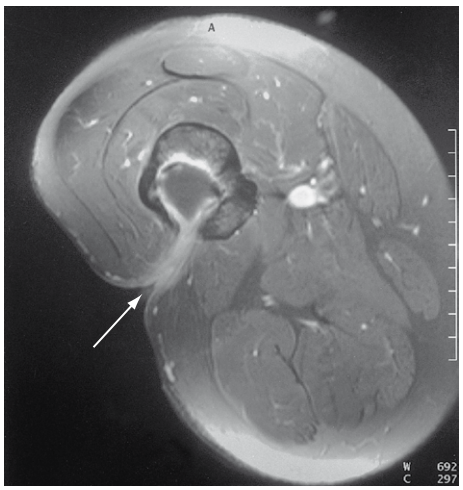


Fig. 41.6 Chronic active osteomyelitis in the femur. This case of osteomyelitis was secondary to a fracture and open reduction and internal fixation 30 years before. This axial, contrast-enhanced, fat-suppressed T1-weighted MRI scan shows cortical thickening and a focal intraosseous fluid collection with an enhancing rim, communicating via a sinus tract to the surface of the thigh (arrow).

Pathologic fracture of the osteomyelitic bone may occur, especially if a mechanically sound involucrum has failed to form. Rarely, after decades of continuous drainage, chronic osteomyelitis can be complicated by the development of squamous cell carcinoma or sarcoma in the soft tissue or bone. This may be presaged by increased pain, swelling, drainage or changes in the character of the ulcer tissue.

Osteomyelitis in specific anatomic situations

The accounts above relate particularly to long bone osteomyelitis but other important manifestations exist that are worthy of comment.

Vertebral osteomyelitis

The entity of infective discitis is recognized in childhood but is of particular significance in adults, especially the elderly.[42] Disc space and spinal infections may be postsurgical, in which case there is a greater likelihood of coagulase-negative staphylococci or other low virulence organisms being responsible; the majority of infections are, however, of hematogenous origin and due to *S. aureus* or the Enterobacteriaceae. It is suggested that the latter find a route, via the venous system, from sites of infection in bowel or urinary tract via retrograde flow into Batson's venous plexus which envelops the vertebrae. Seeding just beneath the vertebral end-plate is rapidly followed by involvement of the disc and the other adjoining vertebrae, setting up a pattern of a disc-space-centered infection. This process may occur at multiple separate or contiguous levels and is often accompanied by paraspinal or psoas abscess formation. Infection may track to form anterior or posterior epidural abscesses. These, retropulsion of disc space contents or mechanical instability of the spine from bone destruction, can cause cord compression or cord infarction, and paraplegia that may be irreversible (Figs 41.7–41.9). Nerve root irritation or compression at the lateral foraminae can lead to radicular symptoms and hence presentations with chest or abdominal pain rather than back pain. Tuberculosis of the spine manifests in similar ways.

In addition to the classic picture described above, isolated infections involving facet joints, posterior spinal elements and even the odontoid peg are well recognized. Severe back pain is an almost universal feature and with or without fever, should always raise the possibility of spinal infection.



Fig. 41.7 Vertebral osteomyelitis. A sagittal, contrast-enhanced conventional spin echo MRI scan (T1-weighted) demonstrates a posteriorly located epidural abscess at the L4–L5 vertebral level with an enhancing rim and displacement of the nerve roots anteriorly. Courtesy of Dr Joseph Mammone.



Fig. 41.8 Vertebral osteomyelitis. A sagittal, turbo spin echo MRI scan (T2-weighted) from the same patient as the scan in Fig. 41.7. Courtesy of Dr Joseph Mammone.



Fig. 41.9 Vertebral osteomyelitis. A myelogram showing posterior compression of the spinal cord by an inflammatory mass. Note the involvement of adjacent vertebral end-plates and the intervertebral disc. Courtesy of Dr Joseph Mammone.

Diabetic foot osteomyelitis

The peripheral neuropathy of diabetes has sensory, autonomic and motor components. Motor neuropathy causes differential loss of function of the distally innervated intrinsic foot musculature, resulting in changes to the biomechanics of the foot with clawing of toes and an equinus deformity. This leads to sites of excessive pressure,

notably under the metatarsal heads, the heel, on the dorsal aspects of the interphalangeal joints and on the ends of the toes. In the context of loss of protective sensation and reduced skin compliance due to disordered sweating and lubrication, together with changes in collagen cross linking from excessive nonenyzmatic glycosylation, the result is ulceration. Alternatively, in dense neuropathy, sensation may be so impaired that foreign objects can accidentally fall inside footwear and be unnoticed, or be trodden on barefoot, and cause injury.

Ulceration may involve the periosteum or a joint capsule and hence allow infection to gain access to bone directly or via a chronic septic arthritis. Diabetic foot osteomyelitis therefore most commonly involves a metatarsal head (extending variably into the shaft and metatarsophalangeal joint), the phalanges at the interphalangeal joints or the terminal tuft, or the calcaneum. Infections of the bones of the midfoot may arise from soft tissue spread or because Charcot neuro-osteoarthropathy has led to collapse of the plantar arch and a 'rocker bottom' foot with plantar ulceration. Subsequent progression of infection is as for other sites, with the frequent development of spreading cellulitis, abscesses (that may be in the deep compartments of the foot) and necrosis. Fever is by no means universal, even with significant deep infection, and when present indicates severe disease with possible accompanying bacteremia or necrotizing fasciitis. Pain is substantially less than would normally be expected because of neuropathy; as a result presentation is delayed, especially in patients who are poorly educated, unmotivated or inadequately resourced to access health care promptly for foot problems (Fig. 41.10).

Osteomyelitis complicating decubitus ulcers

Decubitus ulcers occur where prolonged loading due to immobility interacts with poor quality soft tissue due to debility, especially if also complicated by neuropathy or cognitive impairment. Bone pain is a variable complaint; bone is often visible or palpable in large pressure ulcers, and fragments may regularly be exfoliated into the drainage or dressings. The most common sites are the calcaneum, the femoral greater trochanter, the sacrum or the ischial tuberosity, depending on the areas of maximal pressure ulceration. Lesions may be bilateral. The soft tissue component is a dominant feature of the clinical problem; where the patient becomes systemically unwell this often reflects the development of abscesses, spreading soft tissue infection and/or bacteremia, rather than being directly due to the osteomyelitis. However, extensive involvement of bone is possible, particularly in the pelvis, and possible involvement of the hip joint should be borne in mind in patients with very advanced ulceration involving the structures around the hip.



Fig. 41.10 Osteomyelitis in a diabetic patient. Diabetic patient with osteomyelitis and destruction of proximal second phalanx and metatarsal as well as second metatarsal-phalangeal joint. Courtesy of Dr Joseph Mammone.

Skull-base osteomyelitis

This condition is most common in elderly diabetic males and commences as a malignant otitis externa that then involves the petrous temporal bone.[43] It is also recognized in some postneurosurgical situations when midline involvement of the skull base is possible. Causative agents include *P. aeruginosa*, staphylococci or Enterobacteriaceae, though it may be hard to persuade surgeons to take biopsies for culture because of the complex anatomic relationships of the bones of the skull base to major neurovascular structures. Severe headache with referred ear and facial pain can occur, as can cranial nerve palsies. These are caused when nerves are compromised by inflammatory tissue in their anatomic course through the involved bone. Overall, significant morbidity and mortality still results and prolonged treatment is required.[44]

Mandibular osteomyelitis

Infections of mandibular reconstructions manifest as swelling and drainage from surgical wounds that resolve only with surgical debridement and targeted antibiotics. Infection of the native mandible is uncommon and is largely dealt with in dental practice. Localized infections associated with carious teeth manifest as pain, loosening of the tooth, and possibly a dental sinus draining beneath or lateral to the jaw. More challenging are cases with pain and diffuse sclerosis of the mandible without a realistic target for the dental or maxillofacial surgeon to resect; these are frequently culture negative but anaerobes may play an important role.

Chronic relapsing multifocal osteomyelitis (CRMO)

Another uncommon but important condition, CRMO is a term given to a culture-negative form of chronic bone inflammation (as seen histologically on bone biopsy) that does not produce the bone destruction typical of pyogenic osteomyelitis, and that arises apparently hematogenously.[45] Classically lesions are self-limiting, with different sites being involved at different times; there is a greater tendency for involvement of the sacrum, pelvis, spinal column and clavicle than is common in pyogenic hematogenous osteomyelitis. Pain is the dominant symptom, of insidious onset and therefore chronic at the time of presentation; wound drainage is not a feature. Some patients have other clinical manifestations making up the SAPHO syndrome (*s*ynovitis, *a*cne, *p*almoplantar pustulosis, *h*yperostosis, *o*steitis);[46] others have some but not all of these features, or other conditions such as sacroiliitis or inflammatory bowel disease; and yet other patients have nondestructive, culture-negative chronic osteomyelitis of similar appearances in single bones. Many accounts have emphasized a resolution of symptoms as the patient reaches adulthood, but a proportion of patients definitely have persistent pain and evidence of ongoing inflammation.[47] It is not clear whether this entity is a single condition that varies in extent and number of bones involved, or whether multiple conditions are being categorized as one. A genetic basis has been identified for a related condition, Majeed syndrome,[48] in which CRMO is accompanied by a dyserythropoietic anemia; a mutation in the LPIN2 gene (of unknown function)

is strongly associated.[49] In the mouse, a chronic multifocal osteomyelitis condition is known to be due to an autosomal recessive gene mapping to chromosome 18[50] and since identified as likely to be a missense mutation in the *pstpip2* gene;[51] again, the human homologue PSTPIP2 is of unknown function.

The particular importance of this condition lies in its combination of diffuse bone involvement (offering no defined target for curative surgery) with unpredictable responses to medical therapies. These have included anti-inflammatory antibiotics such as tetracyclines and macrolides, nonsteroidal anti-inflammatories and bisphosphonates. Because there is no surgical solution for most cases and no clear medical therapy to offer, the prognosis can sometimes be worse than that predicted for a pyogenic chronic osteomyelitis.

DIAGNOSIS

Clinical features

Osteomyelitis should be suspected whenever a patient presents with bone or limb pain and fever. Differentials include soft tissue abscess (which does not rule out osteomyelitis), necrotizing fasciitis, pyomyositis and occasionally tumor. Pointers to chronic osteomyelitis include draining sinuses or chronic wounds that will not heal despite appropriate wound care, especially if in the context of risk factors for pressure ulceration. Visible or palpable bone is virtually pathognomonic of bone involvement, as is the expulsion of bony sequestra; a sterile metal probe has been shown to be useful for palpating bone in the diabetic foot, with a high specificity for underlying osteomyelitis.[52–54]

Blood tests

Elevations of inflammatory markers such as C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR) are common, and in the absence of other causes have good specificity;[55,56] however, in a proportion of cases, levels will be falsely normal.[57] Biochemical and serologic tests are of limited value.

Radiology

Plain radiographs

Plain radiologic appearances are normal in early acute osteomyelitis,[58] becoming abnormal after 10–14 days when sufficient bone resorption has taken place to be visible on radiographs, and when periosteal reaction has laid down enough new mineral to be opacified.[59] Subcortical lucency is often seen when chronic septic arthritis leads to osteomyelitis of the subarticular bone. Areas of bone death are visible as denser parts of bone once the osteopenia of disuse and inflammation has reduced the mineral density of the surrounding viable bone. Over time, sclerotic areas and mature involucrum develop through the laying down of new bone. Fragmentation of sequestrum or of the infected segment of bone, including cortical breaches and pathologic fracture, occurs later in the disease.

As a diagnostic tool plain radiology is limited by its time dependence for the development of typical changes. Although the combination of destructive and reparative responses with evidence of bone death is relatively pathognomonic, particularly when serial films allow assessment of the timescale of changes (infection leads to rapid change compared to tumor), in some locations plain films are relatively unhelpful due to poor anatomic definition (e.g. in the spine) or because neuro-osteoarthropathy leads to abnormal appearances that can be confused with infection (as in the diabetic foot).[60]

Ultrasound

Ultrasound can be useful for the detection of soft tissue collections and also for examining the periosteum. It can show periosteal edema and elevation with subperiosteal pus, which can be aspirated under image guidance.[61] It can demonstrate sinus tracts and cortical breaches. In all these respects it is limited by the specification of the machine, the build of the patient and the skill of the operator.

Isotope scanning

Isotope scanning consists of several different modalities of different value. Triple phase bone scans are sensitive but nonspecific and while a negative scan largely rules out infection, the need to use other tests as well limits cost-effectiveness (Table 41.1, Figs 41.11, 41.12). White cell scans, despite being much more complex to perform and having inherent risks (including the rare but catastrophic injection of heterologous labeled white cells from another patient due to laboratory error), have a greater specificity and so can be useful. Fluorodeoxyglucose-positron emission tomography (FDG-PET) appears to be promising in studies to date.[63]

Computed tomography

CT has a place in surgical planning for the assessment of non-unions and complex bony architecture, and is well suited for identifying sequestra and soft tissue abscesses. It is easily affected by the presence of metalware, involves a significant radiation dose, and does not provide physiologic information about the intraosseous environment though it will identify cavities within bone. Other than for assessment of union and when MRI is contraindicated or unavailable, MRI scanning is preferred to CT.[64]

Magnetic resonance imaging

This provides information on soft tissues including sinus tracts, bone anatomy, and intraosseous edema and abscess formation. It is the most valuable of the imaging modalities in osteomyelitis,[64] including in diabetic foot osteomyelitis where this is required,[65–68] with high

Table 41.1 Tests for osteomyelitis.
Sensitivity, specificity, positive predictive values and negative predictive values of tests used to diagnose infection of bone

	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)
Three-phase bone scan	95	33	53	90
Gallium scan	81	69	71	80
Indium-labeled white blood cell scan	88	85	86	87
MRI	95	88	93	92

Adapted from White *et al.*[62]



Fig. 41.11 Twenty-four-hour bone scintigram of the hands. The patient is a 50-year-old diabetic with a draining ulcer at the top of the right thumb (arrow). A biopsy grew *Staphylococcus aureus*. There is intense uptake in distal first phalanx and in multiple neuropathic joints. With permission from Jacobson AF, Harley J, Kipsky B, Pecoraro R. Diagnosis of osteomyelitis in the presence of soft tissue infection and radiologic evidence of osseous abnormalities. AJR Am J Roentgenol 1991;157:807–12.

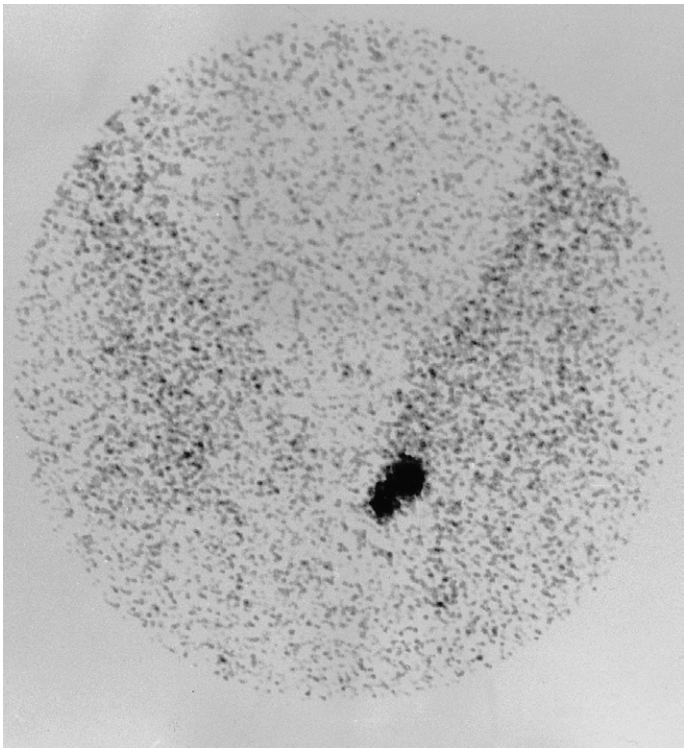


Fig. 41.12 Leukocyte scintigram of the hands. This scan is from the same patient as the scan in Fig. 41.11. Again there is intense uptake in the distal first phalanx, but there is no accumulation of leukocytes in the multiple neuropathic joints. With permission from Jacobson AF, Harley J, Kipsky B, Pecoraro R. Diagnosis of osteomyelitis in the presence of soft tissue infection and radiologic evidence of osseous abnormalities. AJR Am J Roentgenol 1991;157:807–12.

sensitivity and if reported by an expert, specificity (Fig. 41.13). It is prone to postsurgical and postinstrumentation artifacts, with signal voids being created by metalware and by microscopic metallosis persisting even after metalware removal. Slow resolution of changes makes MRI of uncertain value in monitoring response to treatment, but it has proved very valuable in primary diagnosis and in surgical planning.

Bone biopsy

The criterion standard for diagnosing osteomyelitis is the culture of microbes from reliably obtained samples of bone, accompanied by inflammation in histologic samples. Bone biopsy may be performed safely either surgically or as a percutaneous procedure[69–71] under CT or fluoroscopic guidance. This is particularly useful in situations where there is a desire to avoid surgery, or when surgery is not always required for success (e.g. in the spine[72] and diabetic foot[70]). Bone samples or those of adjoining soft tissue are recommended in preference to superficial swabs or biopsies of sinus tracts, which have a greater range of organisms present that usually do not reliably represent the deep flora.[70,73] Obtaining multiple deep samples, and using separate instruments to avoid cross-contamination, increases sensitivity and specificity of culture in revision arthroplasty[74] and by extrapolation, in chronic osteomyelitis too. A range of conditions including use of broth enrichment culture is advised to maximize the chance of isolating the causative pathogens, despite which some 15–20% of chronic cases are culture-negative. Molecular diagnostic methods[75,76] are still not in routine use as a solution to this problem.



Fig. 41.13 T1-weighted image of the foot. The scan reveals forefoot amputation and a normal signal in distal tibia, talus and posterior calcaneus. The interior portion of the calcaneus has edema. The remainder of the tarsal bones have been destroyed and replaced by a low signal inflammatory mass.

MANAGEMENT

Acute osteomyelitis

Initial actions

The priority is to start antibiotics promptly in order to reduce the risks of bacteremia and of progressive bone death and destruction. Acute osteomyelitis should therefore be diagnosed clinically, prompting blood cultures and any other rapid cultures (e.g. aspiration of a collection) and initiation of empiric treatment without undue delay.

Immediately following on from this, a more full clinical and laboratory assessment can be carried out. Hematologic and biochemical testing provide a broader picture of the degree of systemic upset, plain radiology helps to rule out fracture and define pre-existing disease and a definitive confirmatory test such as MRI or white cell scanning can be organized.

Antibiotic therapy

Intravenous antibiotics should be commenced empirically and must include an agent active against *S. aureus*; the need to include activity against MRSA should be considered carefully based on risk factors and local prevalence. Once culture results are available, therapy can be rationalized (Table 41.2). There is very extensive experience, especially in children, of treating for 48–72 hours with intravenous antibiotics and then switching to oral therapy provided the clinical response has been prompt, the organism is susceptible to highly bioavailable oral therapy and adherence to the agreed regimen can be assured.[77–79] In acute osteomyelitis without complications, 4 weeks of therapy is generally adequate; this may need to be extended for longer periods if bone has already been killed but not yet removed.

The role of surgery

Surgery is not necessary in every case but orthopedic review is essential if available. Most surgeons no longer favor routine drilling of the bone.[80] Surgery should be reserved for clinical or imaging evidence of abscess formation, necrosis, or if infection fails to respond to appropriate antibiotics.

Prognosis

Acute osteomyelitis, diagnosed and treated promptly with appropriate antibiotics, carries a good prognosis with several studies showing cure rates of over 90% without major sequelae.[79] However, when diagnosis or treatment is delayed, and in a minority of cases despite appropriate therapy, serious sequelae can occur. Secondary septic arthritis can have a serious impact on longer term function in neonates or adults, and growth plate disturbance adjacent to a metaphyseal focus can lead to limb length discrepancy or angular deformity.[81] Chronic infection is highly likely if significant bone death occurs.

Chronic osteomyelitis

Initial actions

Whereas time is of the essence in acute osteomyelitis, this is not generally the case in chronic infection. Here, a process of patient-centered goal setting is required to ensure that realistic treatment aims are defined. As long as the patient remains medically stable, imaging and other diagnostics should usually be undertaken before antibiotics are commenced, planning for the surgery that will be necessary in most situations. While there is evidence that diabetic foot osteomyelitis may respond to antibiotics (and podiatry) alone,[82] and whilst most spinal infection can be treated with prolonged antibiotics without surgery,

Table 41.2 Antimicrobial therapy for infections of bone

	ANTIMICROBIAL AGENT OF CHOICE	
Organism	**Agent**	**Alternative agents**
Staphylococcus aureus (methicillin-sensitive)	Nafcillin or oxacillin 2 g q6h iv	Cefazolin, vancomycin, clindamycin
Staphylococcus aureus (methicillin-resistant)	Vancomycin 1 g q12h iv	Trimethoprim-sulfamethoxazole plus rifampin
Streptococcus pneumoniae	Penicillin G 5 × 10^6 U q6h iv*	Cefazolin, vancomycin, clindamycin
Group A β-hemolytic streptococci	Penicillin G 5 × 10^6 U q8h iv	Cefazolin, vancomycin, clindamycin
Enterococci	Ampicillin 2 g q4h iv†	Vancomycin
Haemophilus influenzae (β-lactamase-negative)	Ampicillin 2 g q4h iv	Trimethoprim-sulfamethoxazole, ceftriaxone
Haemophilus influenzae (β-lactamase-positive)	Ceftriaxone 1 g q12h iv	Trimethoprim-sulfamethoxazole
Klebsiella pneumoniae	Ceftriaxone 1 g q12h iv	Ciprofloxacin, piperacillin, imipenem
Escherichia coli	Cefazolin 1g q8h iv	Ciprofloxacin, ceftriaxone, imipenem
Pseudomonas aeruginosa	Ciprofloxacin 400 mg q12h iv or ceftazidime 1 g q8h iv	Pipercillin plus aminoglycoside, aztreonam
Serratia marcescens	Ceftriaxone 1 g q12h iv	Imipenem, trimethoprim-sulfamethoxazole, ciprofloxacin
Salmonella spp.	Depends on sensitivity test; choose between ampicillin, ceftriaxone, imipenem, ciprofloxacin	
Bacteroides spp.	Clindamycin 600 mg q8h iv	Imipenem, metronidazole

*If susceptible or intermediate resistance. If high level resistance, use ceftriaxone 1 g q24h or vancomycin 1 g q12h.
†If susceptible. If ampicillin resistant but vancomycin susceptible, use vancomycin. If resistant to both, check susceptibility to tetracyclines, chloramphenicol or experimental agents.

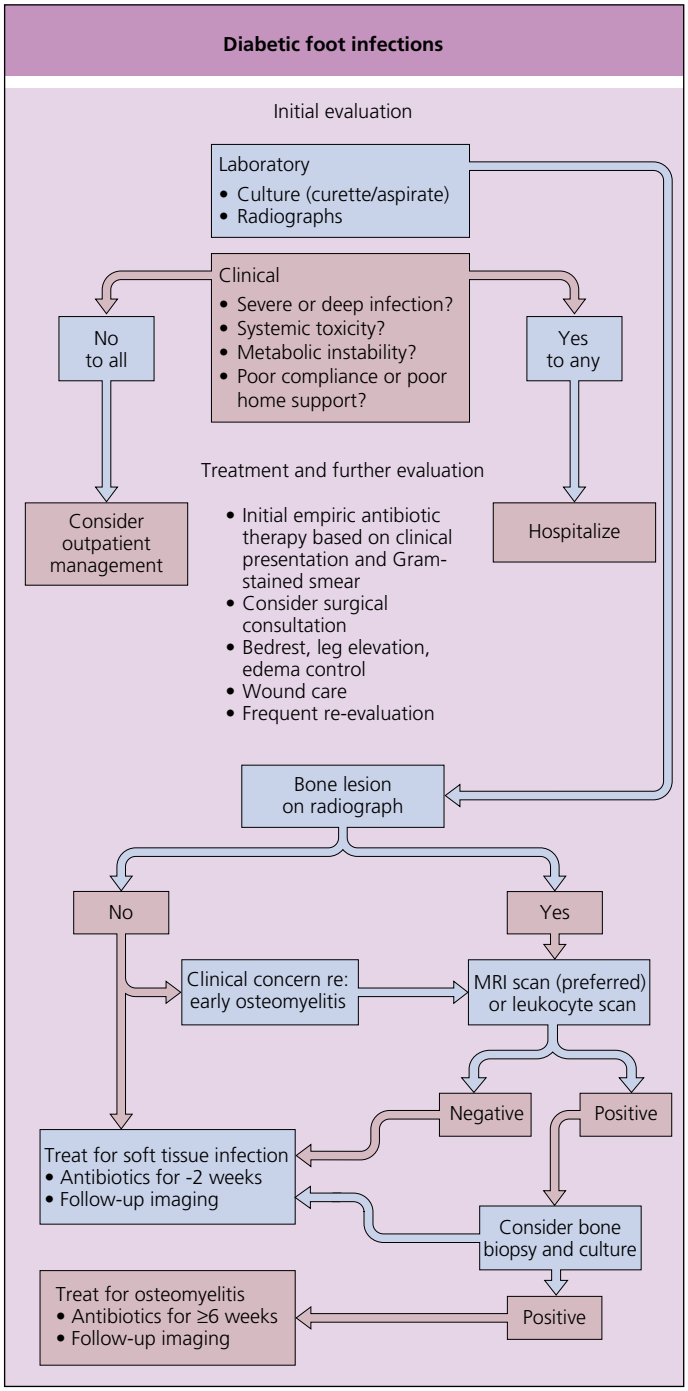


Fig. 41.14 Diabetic foot infections. Algorithmic approach to diagnosis and management. Adapted with permission from Lipsky BA, Pecoraro RE, Wheat LJ. The diabetic foot. Soft tissue and bone infection. Infect Dis Clin North Am 1990;4:409–32.

achieving reliable arrest of infection in the pelvis or long bones generally requires an operation (Figs 41.14, 41.15).

The role of surgery

There is a range of surgical debridement methods and reconstructive techniques, all geared towards control of infection, removal of the sequestra, skeletal stabilization and healing, and restoration of healthy soft tissue cover. Procedures include intramedullary reaming, limited cortical debridement, cortical windowing to access large and complex sequestra and endomedullary cavities, and segmental resection for extensive disease.[83] In some cases conservative and reconstructive techniques are inappropriate and amputation provides the quickest and most acceptable route back to health. Plastic surgical techniques have proved exceedingly important in permitting postoperative soft tissue closure without excessive tension, largely through free muscle flaps with microvascular anastomosis. Advanced orthopedic methods, such as the Ilizarov circular frame,[84] allow the management of segmental defects and their reconstruction, using distraction osteogenesis to regenerate bone, or stabilizing the defect to accept a vascularized bone transfer such as a free fibula graft.[85] These technologies allow the surgeon to be much more radical in removing dead and infected tissue, thereby reducing the likelihood of recurrence.

While the role of surgery has been stressed here, it must be recognized that for some patients there are no surgical options that leave the patient with a better quality of life than the disease itself imposes. This group where 'cure is worse than disease' is joined by those who decline technically possible but high-risk or high-morbidity treatment, even if a successful outcome would be predicted to give good results. Finally, some conditions respond well enough to prolonged culture-directed antibiotic therapy and conservative wound care, with optimization of co-morbidities, that surgery can be deferred until the results of this strategy are clear. Many infections of spine, skull base and the diabetic foot fall into this category and a patient-centered approach is always important. In the case of diabetic foot osteomyelitis, it is not clear in which circumstances surgery is essential;[82] many cases can be managed successfully with antibiotics alone.[86]

Antibiotic therapy

Given the wide range of potential pathogens and the increasing prevalence of multidrug-resistant organisms (MDROs), empiric therapy should, whenever possible, be avoided until reliable deep samples have been obtained for culture. It is safe in the vast majority of patients to stop antibiotics at least 2 weeks before planned surgery or biopsy, provided the patient's prior history of infection and antibiotic response is considered carefully and mechanisms for urgent review are put in place. Patients must understand the rationale for the antibiotic-free period and know what to do if infection is flaring in advance of planned definitive diagnosis or treatment.

Once samples are obtained, empiric therapy can begin. As for acute osteomyelitis the choice of regimen must include activity against staphylococci, but given the higher prevalence of methicillin-resistant *S. aureus* and coagulase-negative staphylococci, and the increased likelihood of isolating Gram-negative pathogens, initial cover is frequently broader in spectrum. Regimens will need to balance the benefits of early appropriate therapy for all likely pathogens (favoring a broader spectrum) against risks of *Clostridium difficile* diarrhea and selection of MDROs in the patient flora and hospital environment (favoring a narrower spectrum).

Definitive antimicrobial regimens should, when possible, be based on culture and sensitivity results. There is experience in using prolonged intravenous therapy for up to 6 weeks including via outpatient parenteral antibiotic therapy (OPAT) programs,[87] in using switch regimens to move much earlier to oral therapy,[88] in relying largely on locally delivered treatment using antibiotic-loaded bone cement[89,90] or even calcium sulfate (plaster of Paris),[91,92] and in some situations (especially when surgical treatment is not undertaken) in using entirely oral regimens. A systematic review of available literature found no evidence of superior outcomes with any particular antibiotic choice or route of administration in all types of bone and joint infection;[93] more recently, a systematic review specifically of diabetic foot osteomyelitis reached similar conclusions.[82] However, adequately powered, randomized studies to answer these important questions directly are still lacking.

Duration of antibiotic therapy in chronic osteomyelitis varies substantially between and within centers. Where it is possible to have confidence in the judgment of the surgeon regarding the completeness of resection of dead bone, a logical scheme is to give 4–6 weeks of therapy following complete resection (and potentially less for more localized disease), and much more prolonged therapy when

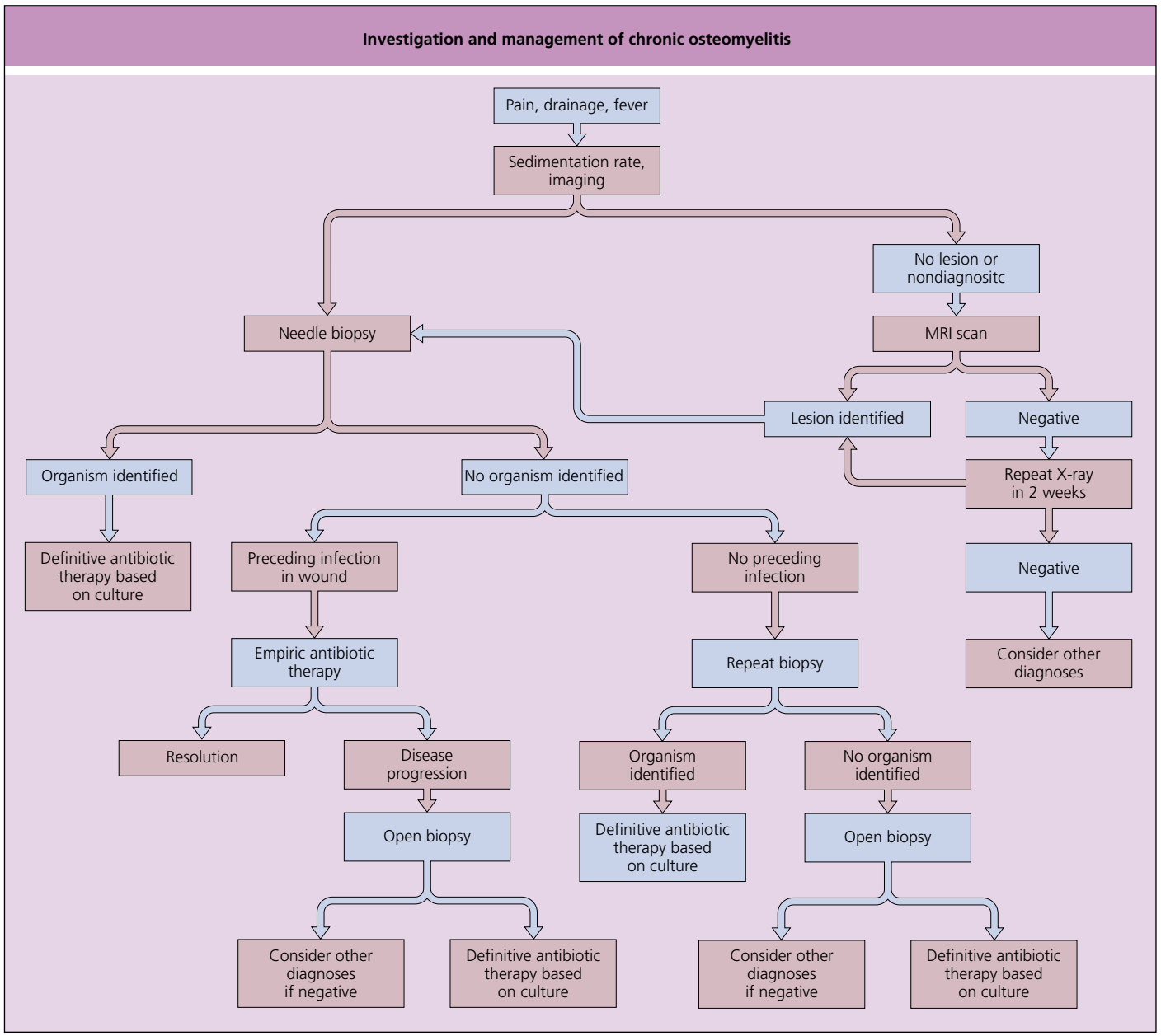


Fig. 41.15 Investigation and management of chronic osteomyelitis.

dead bone is known to persist.[14] The precise duration of this can be tailored to the specific circumstances of the patient, recognizing that relapse is much more likely in this situation. The goal therefore becomes to deliver a disease-free period while on therapy, during which important biologic events may occur (expulsion of a sequestrum, consolidation of an involucrum, healing of a fracture or nonunion) or even key personal milestones important to the patient's quality of life. If amputation has been necessary then the duration of postoperative antibiotic treatment can be based on the degree of infection in the remaining soft tissue and bone.

Soft tissue management

The soft tissues are critical as barriers to re-infection and as a delivery vehicle for host defenses and antibiotics. Their importance is often underestimated. Effort must be made to ensure the removal of chronically scarred and indurated soft tissues, surgical wound closure without dead spaces or excessive tension, coverage of debrided bone with healthy tissue, and for chronic wounds not appropriate for surgery, an optimized wound-healing environment. This includes fastidious pressure offloading in decubiti and diabetic foot ulcers,[38] assessment and optimization of local vascularity (arterial and venous) and of systemic factors (hematocrit, oxygenation, nutrition and the minimizing of drugs that affect healing).

Adjunctive factors

Careful attention to co-morbidities as listed above is important for bone as well as for soft tissue healing. Of other specific adjunctive therapies used, virtually none has an established evidence base. A systematic review of diabetic foot osteomyelitis found no evidence to support any specific adjunctive therapies in diabetic foot osteomyelitis.[82] In particular, the use of hyperbaric oxygen, in widespread use in many centers, has not yet been validated for

osteomyelitis in large-scale, appropriately blinded, randomized controlled trials. The topic remains a controversial one with advocates on both sides.[94,95]

CONCLUSIONS

Osteomyelitis remains a fascinating and challenging condition requiring more basic and translational research. The development of consensus definitions and the widespread adoption of standardized classification and staging systems is an essential step in enabling multicenter studies to develop the most cost-effective diagnostic and therapeutic protocols. These will need to be developed and implemented by multidisciplinary teams that together can provide the surgical, medical and psychological expertise that patients with osteomyelitis so frequently need if they are to achieve the best possible outcomes. Development of competencies in these teams is perhaps the greatest priority of all so that more patients can experience, now, the best of what current treatments can offer.

REFERENCES



References for this chapter can be found online at http://www.expertconsult.com

Chapter 42

Anthony R Berendt

Infections of prosthetic joints and related problems

INTRODUCTION

Implant technology is one of the cornerstones of modern orthopedics. Of the few clouds on the extensive horizons that orthopedic instrumentation has opened up, infection is without doubt one of the darkest. Thus while hip replacement is one of the most cost-effective health-care interventions ever developed,[1] the chronically infected hip replacement is one of the most challenging and costly conditions for an orthopedic surgeon to treat.[2] Similarly, modern technology now permits a much greater likelihood of salvaging a functional limb after severe trauma, but if infection develops the limb may ultimately be lost after all, with much greater suffering and cost in the interim.

While both serious systemic sepsis leading to mortality and necrotizing soft tissue infections are relatively uncommon following orthopedic instrumentation, infection nonetheless causes significant morbidity. This may take the form of continuous wound drainage, implant failure from loss of mechanical fixation to the bone, progressively worsening pain and resultant disability, and chronic morbidity affecting physical and mental health parameters. Patients with chronic orthopedic implant infections have multiple dimensions to their problems, many of which lie outside the traditional 'comfort zone' of many orthopedic surgeons.

Perhaps for this reason, allied to the relative scarcity of these problems, the field remains bedeviled by a paucity of high-quality evidence. This has not prevented the establishment of the principles of good practice that form the basis of much of this chapter, nor a considerable degree of consensus among many different expert centers. Such expert consensus is the basis of forthcoming clinical practice guidelines for prosthetic joint infection that are expected to be produced by the Infectious Diseases Society of America in 2010. One key principle on which all are agreed is the necessity for these problems to be treated by a multidisciplinary team or via a co-ordinated multidisciplinary care pathway, with expertise in the whole range of skills required to treat and rehabilitate these patients optimally.

EPIDEMIOLOGY

Rates of infection following hip and knee replacement are cited as between 0.5% and 2% in large series from the Scandinavian joint replacement registries or specialist centers.[3,4] Rates of infection of less common arthroplasties such as shoulder, elbow and ankle are less well defined. Substantial datasets from large-scale surveillance schemes as in the UK suggest infection currently complicates 0.5% of hip replacements, 0.25% of knee replacements, 2.5% of hemiarthroplasties for fractured neck of femur and 1% of fixations for long bone fracture.[5] Ascertainment is a particular issue given the increasing trend for early discharge after surgery. There are also issues of definition: while the Centers for Disease Control and Prevention (CDC) definitions for organ/space infection are clear in the event of a prosthesis being present,[6] diagnostic difficulties (see below) and the very slow evolution of infections with low-grade pathogens in some cases may reduce early recognition; so too may the effect of denial or a lack of diagnostic rigor from the surgical team. Finally, different approaches are required to diagnose a superficial compared to a deep infection; it remains to be seen whether the trend for health-care commissioners not to reimburse complications such as infection constitutes a perverse incentive that drives clinicians to avoid diagnosing infection in the early postoperative period.

PATHOGENESIS AND PATHOLOGY

Pathogenesis

Prosthetic joint infection is a paradigm of device-related infection and of the issues involved in treating complex surgical infection. There are three elements which come together:

- the effect of implants on host biology (affecting susceptibility and response to infection);
- the effect of implants on microbial biology (including susceptibility to host defenses and antimicrobials); and
- the effect of chronic infection on the biology of bone and soft tissue.

All foreign implants increase susceptibility to infection, as the work of Elek and Conen demonstrated, using human volunteers to assess the inoculum of *Staphylococcus pyogenes* required to cause a wound infection in the presence or absence of a piece of suture material.[7] Complement is depleted in the vicinity of an implant and white cell function is abnormal. The effect of these changes is to permit relatively low-virulence organisms to survive in the microenvironment of the prosthesis, as well as making infection with a higher-grade pathogen more likely. Finally, the act of implanting orthopedic instrumentation causes a degree of direct injury not only to the soft tissues but also to the bone. In addition to the sawing and drilling that is commonly necessary to prepare and anchor some implants, the use of polymethylmethacrylate bone cement leads to the death of bone at the cement–bone interface, since the cement generates considerable local heat as it cures.

Implants also profoundly affect the biology of microbes, in the first instance by providing substrates to support adhesion. This is mediated by a number of different mechanisms, including hydrophobic interactions between implant and microbial cell surface, and specific receptor–ligand interactions between implant surfaces conditioned with host proteins such as fibronectin and their cognate ligands on the bacterial cell surface.[8]

There is a substantial body of evidence that many micro-organisms will establish very large communities of adherent cells, involving one or more species, usually linked together with an exocellular polysaccharide that may be sufficiently abundant to be visible experimentally as 'slime'. This structure, known as a biofilm, can be demonstrated to have a complex internal structure, the nature of which will depend upon environmental factors including rates of flow of fluid across the biofilm and availability of key nutrients to the enormous numbers of microbes constituting the biofilm[9] (Fig. 42.1). The molecular basis for biofilm formation and the intercellular signaling events that occur are increasingly well understood[10] and are described in detail elsewhere.

Biofilms appear to be important in the persistent nature of implant-related infections. The mechanisms for this are not fully understood; while there is some evidence that local immune function is impaired,[11] there is also evidence that white cells can penetrate biofilms and kill at least some of the microbes present within them.[12] In the same way, the notion that antibiotics cannot penetrate biofilms now has little sway, but what is clear is that within a biofilm is a population of cells that do indeed phenotypically resist antibiotic action and will recover rapidly once antibiotic pressure is lifted.[13] These 'persisters' are of considerable interest, but still poorly understood. They may relate to small-colony variants, which have been shown to exist in the context of the gentamicin-loaded bone cement commonly used in joint replacement, and to constitute a mechanism for intracellular persistence and phenotypic antimicrobial resistance.[14,15]

The host mobilizes acute inflammatory responses to combat infection, even though these are not usually curative (Fig. 42.2). The result is a continuous process of acute inflammation which frequently leads to the formation of pus around the implant. The development of a sinus is a common response to this: by allowing pus to drain, it has the effect of decompressing the infection and, as a result, of averting the most harmful aspects of an infected collection. Patients with draining sinuses may remain in relative health, and relative comfort, for considerable periods of time. Sinus blockage may trigger acute flares of soft tissue or systemic infection as the pathogens switch to more invasive phenotypes through quorum sensing mechanisms. Chronic sinuses may produce such extensive effects, in particular loss of soft tissue, so that primary closure of subsequent surgical incisions cannot be achieved.

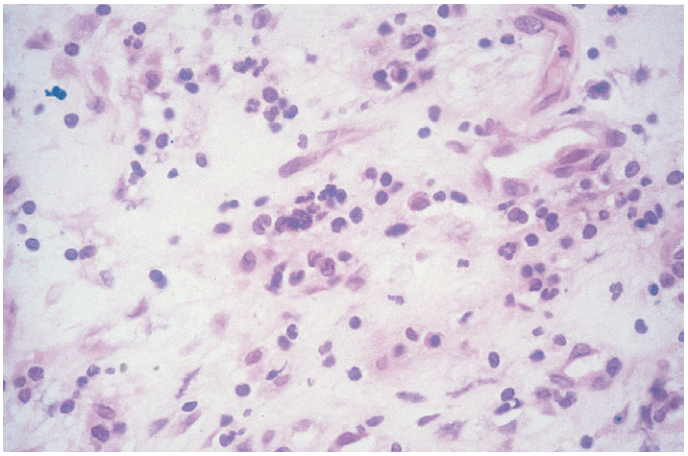


Fig. 42.2 Histologic features of infection. Periprosthetic tissue from a clinically infected total hip replacement from which multiple specimens grew an indistinguishable organism. Numerous neutrophils are present in the tissue.

Continuous inflammation does, however, have undesirable effects on bone biology in that it leads to the activation of osteoclasts and the recruitment of other cells that take on a bone-resorbing phenotype.[16] This activity is cytokine–directed and so is focused in the immediate vicinity of the implant, the effect being that bone is lost at the bone–cement or bone–metal interfaces. This ultimately leads to implant loosening; in the context of joint replacement the result is a progressively more painful, ultimately failing joint; in a fracture fixation or fusion construct, the result is mechanical instability that jeopardizes union and appears to make infection harder to control. The ultimate effects are hence a mechanically failed, infected arthroplasty or an infected non-union (Fig. 42.3).

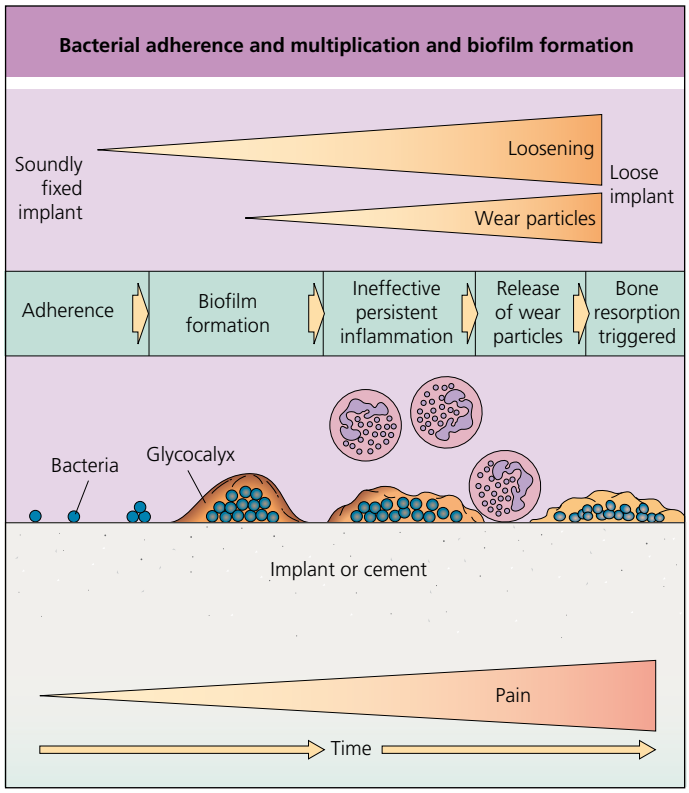


Fig. 42.1 Bacterial adherence to the implant, bacterial multiplication on the surface and biofilm formation. An ineffective host response triggers bone resorption, contributing to loosening, which is accelerated by wear particles and the host response to them.



Fig. 42.3 An acutely infected knee replacement. The site was washed out but the infection failed to resolve. At reoperation the implant was found to be loose and it needed to be removed. *Staphylococcus aureus* was grown from deep specimens.

An additional feature that is not unique to infection is that once the loosening process begins, wear particles – from metal, high-density polyethylene used for some bearing surfaces or bone cement – accumulate at an increased rate. These particles trigger chronic inflammation that also contributes to bone loss;[17] the effects on immune function are unclear.

Pathology

The pathologic hallmark of infection is the presence of acute inflammatory cells in periprosthetic tissues[18,19] (see Fig. 42.2). By contrast, chronic inflammation is associated with aseptic loosening and uninfected non-union. The only situation in which a neutrophil infiltrate is not pathognomonic of infection is when an inflammatory arthritis is present. In addition, mycobacterial infections generally cause typical appearances of granuloma formation in the context of chronic inflammation.

Microbiology

The majority of orthopedic device-related infections are caused by skin commensals gaining access to the implant or to periprosthetic tissue at the time of surgery (Table 42.1). For this reason the majority of infections are due to aerobic Gram-positive cocci, with staphylococci pre-eminent.[5,20,21] Some infections are produced via hematogenous spread, mostly with *Staphylococcus aureus* and β-hemolytic streptococci, but rarely from oropharyngeal, bowel or urinary tract, or other causes of bacteremia.

The preponderance of staphylococci, including coagulase-negative staphylococci in equal numbers to *S. aureus*, also includes an important role for methicillin-resistant *S. aureus* (MRSA) in some centers (Fig. 42.4). This has also included infections with community-associated strains (CA-MRSA) increasingly prevalent in many urban environments in the USA, which on some occasions have been demonstrated to be introduced into, and transmitted within, the hospital environment.[22]

The very wide range of different pathogens described in the literature includes not only the full gamut of skin commensals but also less common pathogens including *Mycobacterium tuberculosis*, *Campylobacter* spp. and even *Clostridium difficile*, all in prosthetic joints. It is sensible for the infectious diseases physician to remember, and to remind orthopedic colleagues, that the range of possible pathogens and antimicrobial resistance patterns is significant, so that microbial diagnosis is a key element of successful treatment in many situations.

Table 42.1 Bacteria associated with infected implants presenting at different stages

Early infection (up to 3 months)	*Staphylococcus aureus* Coagulase-negative staphylococci Aerobic Gram-negative rods β-Hemolytic streptococci
Delayed infection (4–12 months)	Coagulase-negative staphylococci Other skin commensals *Staphylococcus aureus*
Late infection, including hematogenous seeding (more than 12 months)	Coagulase-negative staphylococci Other skin commensals *Staphylococcus aureus* Aerobic Gram-negative rods Anaerobes *Mycobacterium tuberculosis*



Fig. 42.4 A sinus tract discharging from an infected total hip replacement. *Staphylococcus aureus* was grown from deep specimens. Note the Koebner phenomenon; this patient's psoriasis was probably a significant risk factor for infection.

PREVENTION

The great difficulties and costs currently associated with the diagnosis and treatment of orthopedic implant infections fully justify increased attention to preventive measures. These can be considered in the pre- and perioperative, the immediate postoperative and the late postoperative stages.

Pre- and perioperative

At a system level, there is some evidence that separation of elective from emergency workstreams, including introducing 'ring-fenced' beds for orthopedic surgery, reduces infection rates.[23] Furthermore, the advent of care bundles has been a significant advance, and the systematic implementation of surgical site infection (SSI) care bundles has been highly successful in some institutions. Several guidelines for prevention of SSIs have been produced based on systematic reviews of the literature.[24,25]

Studies have demonstrated that certain patients are at greater risk of prosthetic joint infection, namely those who are obese, diabetic, taking steroids or of advanced age,[26,27] and in a multivariate analysis of a case-control group, with histories of malignant disease, prior surgery on the joint, or one or more factors from the National Nosocomial Infections Surveillance Score (NNISS) which integrates wound status, duration of surgery and American Society of Anesthesiology score as a simple indicator of co-morbidity.[28] While the possession of such risk factors is not an acceptable reason to withhold joint replacement from a deserving patient, it makes sense to identify patients at higher risk both to ensure preventive measures are reliably implemented and to trigger a heightened index of suspicion for infection if there are postoperative concerns.

It is standard orthopedic practice to attempt to identify and remove all sources of actual and potential sepsis, both local and distant, such as poor dentition, leg ulcers or exfoliative skin conditions, prior to arthroplasty. It is also sensible to ensure that treatable co-morbidities like diabetes are optimally controlled. In keeping with SSI prevention bundles and guidelines, preoperative shaving of the surgical site should be avoided if possible; if unavoidable, it should only be undertaken with an electrical clipper on the day of surgery.[24,25] There are no real options to undertake these measures in acute trauma where, instead, thorough wound toilet and debridement of devitalized tissues around the fracture are critical actions in reducing the infection rate.[29]

Good sterile technique remains of paramount importance and failings in this cannot readily be compensated for by other measures including prophylactic antibiotics. Surgical teams should maintain sterile technique at a high standard; however, if infection rates rise, all elements of practice should be observed for breaches of best practice. This also applies to theater discipline and the avoidance of unnecessary interruptions and human traffic in the operating room.

Historical data indicate that excessive use of diathermy for the control of bleeding or for incising tissue may increase risk of infection by creating a substantial volume of devitalized tissue. This risk must be balanced against the risk from hematoma formation, which has itself led to a long-running controversy over the use of suction drains, seen either as reducing the risk of infected hematoma or as increasing risk of infection by providing a portal of entry into the deep tissues. Good tissue handling, dead space management and effective wound closure are all important factors in preventing the access of pathogens to the deeper tissues in the postoperative period. In lower limb trauma (particularly tibial fractures) this attention to the soft tissues includes early plastic surgical intervention to ensure early wound closure with healthy tissue.[30]

The theater environment is also important, with a high level of cleanliness desirable. The use of ultraclean air was pioneered by Charnley when he developed hip replacement and it continues to be a mechanism that can greatly reduce the number of bacteria able to access the wound from the air.[31] Despite this the limitations of ultraclean air must be understood, including the turbulent airflows that can be induced by the presence of theater personnel and the entrainment of air, contaminated with skin scales shed by them, into the operative field or over exposed trays of surgical instruments.

The landmark Medical Research Council trials conducted by Lidwell demonstrated the importance of antibiotic prophylaxis though this was of equal (and independent) value to that conferred by ultraclean air.[32] A single dose of antibiotic may be sufficient, though it is considered good practice to redose if short half-life antibiotics are used and the wound remains open for over 2 hours. However, the data from Scandinavian arthroplasty registers suggest, based on historical controls, that infection rates may be reduced if 24 hours of prophylaxis is given. This is reflected in guidelines,[24,25] leading to obvious tension with the efforts to reduce to a minimum the selection pressures for MRSA and *C. difficile*. Prophylactic regimens must take into account local sensitivity patterns among key pathogens and there should be a process of periodic review and local guideline updating.

Early postoperative

In the early postoperative period the implant is surrounded by hematoma and lies at the bottom of a substantial soft tissue wound, albeit one that has usually been closed in layers to reconstitute anatomic barriers to externally derived infection. While it is too late to influence the events that have taken place in the operating room, efforts can still be made to minimize infection from external sources. Care should therefore be taken to comply with all other infection control care bundles in the postoperative period and to optimize wound care with strict non-touch, aseptic techniques. The infection specialist has an important leadership role in emphasizing the importance of all these measures.

Late postoperative

It seems self-evident that infections that might constitute sources of bacteremia should be treated particularly promptly in individuals with orthopedic instrumentation. However, it is important to understand that the microbiologic data suggest that the vast majority of cases of orthopedic device-related infection do not originate from distant sources but are acquired during the initial surgery.[20] Nonetheless hematogenous seeding of prosthetic joints (perhaps more so than static implants for fracture fixation or fusion) does without doubt occur, and it therefore makes sense to act as if it is a real possibility, ensuring timely, appropriate management of suspected or proven distant infection.

If the role of distant infection is uncertain, the need for prophylaxis associated with dental treatment is yet more problematic. Very small numbers of infections are demonstrably due to the kinds of organism typical of the orogingival flora. Most large centers will have anecdotal experience of such infections and in some such cases patients recall recent dental work. Theoretical considerations remind us that large numbers of oropharyngeal bacteria are released into the circulation not only during dental treatment but also during much more regular activities such as dental brushing or flossing. Thus, while much expert and pragmatic guidance currently favors the administration of prophylaxis within 2 years of implantation of a joint replacement or for any high-risk patient,[33] the evidence base for this remains scanty and the guidance therefore controversial.[34] There is consensus that prophylaxis for dental treatment is not indicated for fixed metalware, i.e. pins, plates and screws.[33]

CLINICAL FEATURES

Time to presentation

The clinical features of infection are often related to the time after implantation on the assumption that infections with high-grade pathogens present early, while lower virulence pathogens take correspondingly longer to achieve a sufficient burden to trigger a clinically evident inflammatory response. Thus early infections are defined as within 3 months of implantation, delayed as 4–12 months, and late infections, often thought to be hematogenous in origin, occur over a year after implantation. As originally described, early infections were thought to have a preponderance of virulent Gram-positive pathogens, delayed as being dominated by coagulase-negative staphylococci, and late being once again due to virulent causes of bacteremia and hematogenous seeding. At least one large study of cases of infection treated with debridement and implant retention at a range of times after implantation found no such relationships between pathogens and time after surgery.[20] It is possible that the dogma in the existing literature is biased by clinical management behaviors in some centers, lower grade infections taking longer to diagnose in the absence of a highly standardized clinical protocol for dealing with persistently draining wounds or those displaying signs of mild, apparently superficial, infection.

Acuity of presentation

Another commonly employed scheme is to describe the acuity of presentation. Acute infections are those with typical features of wound purulence and drainage, spreading soft tissue erythema and tenderness, systemic features such as fever, and short histories. In the context of a healed wound, acute infections also have the appearances of a septic arthritis or of a soft-tissue abscess associated with the implant. In the case of spinal instrumentation this can even, rarely, take the form of an epidural abscess.

These features would be contrasted with those typical in chronic infections where progressive pain, intermittent or continuous sinus drainage, and implant failure are much more likely to be present. In a small minority of cases a chronic infection is so indolent that it is an incidental finding at revision arthroplasty or implant removal for painful loosening (provided, that is, there is a routine of performing culture and histology on all cases of revision joint surgery or metalware removal). Very late-diagnosed infections of posterior spinal instrumentation, caused by coagulase-negative staphylococci or propionibacteria, have been recognized by the use of this rigorous diagnostic approach.[35]

Both the temporal and the acuity-based schemes for describing infections have their limitations if used alone. In combination, the two describe the timing of onset of symptoms in a way that is of some value even though it appears less effective at predicting the microbiology than was earlier supposed.

DIAGNOSIS

The particular difficulty surrounding the diagnosis of infection is the variability in the clinical presentation, in the ability to culture the causative pathogens, and in the density of neutrophils within periprosthetic tissues (as measured by the numbers seen per high power field

in histologic analysis). In addition, the fact that most infections are caused by skin commensals raises significant issues of culture contamination and diagnostic specificity. As a result, no consensus criteria for defining infection have yet been established, and most diagnostic tests or protocols have been assessed against criteria variably defined by the investigator.

Crucial in making the diagnosis of infection is the maintenance of clinical vigilance. In the early postoperative period, persistent wound problems or complaints of pain should raise suspicions, even if typical features of wound infection are lacking. After the first few months, patients who complain that the implant was 'never right' should receive careful attention, especially if this is their first joint replacement or other orthopedic metalware. Patients who have known an uninfected implant and then receive an infected one are likely to be much more demanding since they know what to expect (an inapparent, normal-feeling joint after a few months). The danger with the 'first-timer' who develops infection is that they do not realize just how asymptomatic a good joint replacement or fracture fixation should be.

In the context of an acute infection, investigations are undertaken both to assess the degree of systemic upset and to evaluate the implant. Blood cultures may demonstrate an ongoing bacteremia; measurement of inflammatory markers provides a useful baseline for the assessment of clinical response to treatment; plain radiography demonstrates if the implant is soundly fixed, assesses the bone stock and any bony reactions, and excludes fracture or mechanical failure. If the patient is acutely unwell, ultrasound may help in assessing the presence of joint fluid or peri-implant collections for aspiration, with Gram stain and culture of the material obtained. Ultimately, the diagnosis can be made through surgical exploration, in part visually (though if explored promptly, an acute early infection may show only hematoma and tissue edema, and not frank pus), but in particular through the ability to obtain multiple specimens for culture and histology.[36]

In chronic infection, certain clinical features, such as sinus tract formation, are to all intents and purposes pathognomonic; however, the diagnostic problem is much greater with loose implants and with well-fixed but chronically painful implants (see Fig. 42.4). Plain radiographs are a standard part of assessment, allowing planning of further surgical options, examination for progressive loosening or implant failure, and assessment of the bone for chronic responses that may be due to infection (Fig. 42.5). However, features linked to infection, such as periosteal reaction, endosteal scalloping or extensive bone resorption, may in fact be caused by noninfective processes such as large diameter cementless implants, giant cell granulomas and stress shielding, respectively.

In the chronic situation, inflammatory markers are less helpful than in acute disease. When elevated in the absence of other causes of inflammation they may be of value;[37] however, it is well recognized that patients may have normal markers despite an unequivocal diagnosis of infection.

Aspiration of joint fluid for culture has been shown to have high sensitivity and specificity compared to cultures and histology obtained at revision, particularly if selectively performed in patients with an elevated erythrocyte sedimentation rate and/or C-reactive protein.[38–40] However, for those infections associated with lower numbers of neutrophils in periprosthetic tissue and with organisms isolated only on enrichment cultures, aspirates perform less well. The use of needle biopsy may enhance recovery of pathogens by providing a tissue sample and by allowing histologic analysis as well as culture, especially if multiple passes are made.

Exploration permits visual inspection of the prosthesis and the periprosthetic tissues. Purulence around the implant is diagnostic of infection and periprosthetic tissue samples are readily obtained, especially if the implant is being removed. Taking multiple independent samples increases the sensitivity of microbiologic diagnosis, which is also rendered more specific by requiring two or more samples to yield an indistinguishable organism and by histologic examination of periprosthetic tissue.[19,36]

Tissue samples should be sent in preference to swabs, but an increasing body of evidence suggests that bacteria adherent to the prosthesis can be dislodged with sonication,[41] allowing culture or other examinations and producing a test that may be at least as sensitive as the multiple sampling method and possibly of more value if there has been recent antibiotic therapy.[42] This, with molecular methods, may increase our understanding of the range of organisms that can infect orthopedic instrumentation. Despite some concerns,[43] an increasing number of reports suggest that polymerase chain reaction can yield causative pathogens,[41,44–46] though information on a full panel of antimicrobial sensitivities may require further work in contrast to what can be obtained with a cultured isolate. It may even be that gene expression profiling can help make the diagnosis of infection by characterizing distinctive patterns of gene expression in infected individuals.[47]

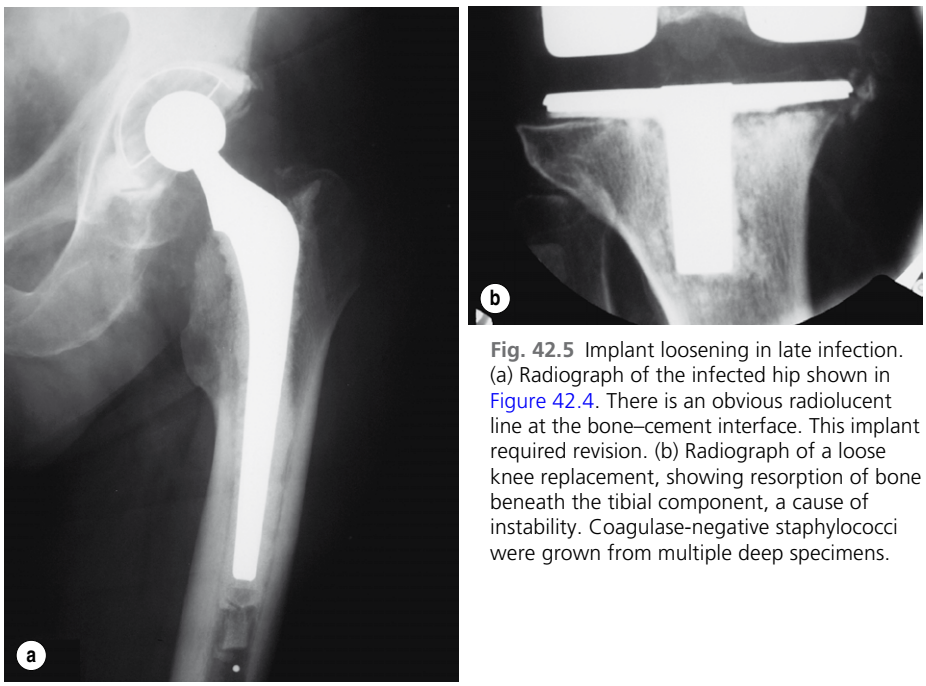


Fig. 42.5 Implant loosening in late infection. (a) Radiograph of the infected hip shown in Figure 42.4. There is an obvious radiolucent line at the bone–cement interface. This implant required revision. (b) Radiograph of a loose knee replacement, showing resorption of bone beneath the tibial component, a cause of instability. Coagulase-negative staphylococci were grown from multiple deep specimens.

MANAGEMENT

The cornerstone of successful management is the setting of realistic, patient-centered goals that take into account the specific symptoms and the options for reconstruction. The paramount aim is usually to control infection or sinus tract drainage, but for some it is the relief of intractable pain. In acute presentations there is a significant risk of serious sepsis and decision-making needs to be urgent; in chronic situations there is rarely this urgency and careful planning is important for best outcomes.

Assessment

At presentation, patients should initially be assessed for their systemic state as for any acute infection, with urgent treatment indicated for any sepsis syndrome. It is then appropriate to assess implant function and stability in order to establish whether an attempt should be made to salvage the existing implant or to revise it. This decision will also be related in part to the bone stock, which may set technical limits on possible reconstruction if the implant is indeed removed. Also of importance is the quality of the soft tissue envelope, defects or extensive scarring, which may point to the need for a plastic surgeon to be part of the treating team. It is likely that one or more co-morbidities will need to be addressed (given that medical co-morbidities are risk factors for infection); these can also include significant anxiety, depression or anger from chronic pain and disability, altered body image (from chronic wounds), social isolation, the iatrogenic nature of many infections, and the threat of permanent disability including the possibility of limb loss.

Treatment options

As stated above, a key decision to make is whether or not to attempt to salvage the implant. The rationale for attempting this is when the implant is soundly fixed and functioning well. Implant retention may be attempted with curative intent when there is a short history (within 2 weeks of onset of symptoms) and the pathogens are susceptible to both a fluoroquinolone and rifampin (rifampicin). Using this combination for 3–6 months, following a formal debridement and retention procedure in which all infected and necrotic soft tissues are removed and all modular components exchanged, leaving a stable implant covered by healthy soft tissue, cure rates of around 80% or more have been described.[48,49] Where antibiotic regimens are required that do not make use of this combination, there may be some grounds for optimism[50] (e.g. with β-lactam-sensitive streptococci other than enterococci),[51] but there is less published experience.

The alternative is to give longer term antibiotic therapy without necessarily expecting cure.[52] In this situation infection might be expected to recur if antibiotics were stopped because of the length of time it had been established, or because of significant biologic factors that are likely to favor recurrent infection such as cancellous bone graft or a large revision implant. Suppression is also appropriate when the implant is not required in the long term but has a temporary function to stabilize a healing fracture or a fusion procedure. In that situation the infection should be suppressed until bony union, followed by removal, bone and soft tissue debridement, and further antibiotic therapy. If implant removal is expected to carry significant risk, it may be reasonable to treat for 3–6 months after union and then discontinue antibiotics, or to decide to treat indefinitely.

Implant removal (without reimplantation) is an option when a temporary implant has fulfilled its role; however, in the case of prosthetic joints it can only be considered in certain locations, notably the hip where a Girdlestone's pseudarthrosis forms over time after excision arthroplasty. In most joints there is an unacceptable level of pain from the lack of an articular surface, and in fusions and fractures metalware removal prior to union results in considerable pain from instability. Excision arthroplasty delivers cure rates in excess of 90% but just as when reimplantation is planned, it is essential that all foreign material including any bone cement has been removed.[53]

It is therefore much more common to remove prosthetic joints in the context of revision surgery, aimed at restoring function through reimplanting a prosthesis in the future. There is uncertainty as to whether an immediate reimplantation (a one-stage revision) is as effective as a delayed reimplantation (two-stage revision) which theoretically gives an opportunity to treat the infection without the presence of a prosthesis.[54–58] In many joints the bones are kept apart using some form of spacer, which may be fashioned from antibiotic-loaded bone cement to allow passive joint motion. Spacer devices have the additional advantages of allowing maintenance of limb and muscle length, management of any dead space and can act as a vehicle for the delivery of antibiotics impregnated into the cement. Special forms of spacer have been designed to cope with massive bone loss and allow for subsequent reconstruction with tumor-type endoprostheses.[59] Selected results of one- and two-stage revision with the use of bone cement are broadly similar, but a randomized controlled trial has not been carried out to test the question fully.

As described in relation to excision arthroplasty, it is not always possible to offer revision surgery and joint reconstruction. In some situations as at the knee joint, surgery to carry out fusion may be a limb-sparing technique that a patient will opt for. There are technical difficulties, however, because by the time fusion is carried out, there have often been several revision attempts and bone stock may be poor. A range of techniques is possible and it may be desirable to cover such a treatment plan with antibiotics, especially if infection is active when the fusion is undertaken. Amputation lies at the extreme end of treatment but has to be considered in cases of intractable infection with unreconstructable soft tissues and poor bone stock.[60]

Antibiotic duration and route of administration

It is essential that antibiotics achieve high levels at the site of infection. Three routes of administration are possible, with little good evidence to inform choices between them.[61]

Locally, antibiotics can be delivered via antibiotic loaded acrylic (bone) cement (ALAC). The bulk of the data and published experience relate to the use of aminoglycosides but other antibiotics that are heat stable (e.g. vancomycin) can be incorporated. Elution of antibiotic occurs over at least 1–2 weeks during which time there are very high local, but negligible systemic, levels. ALAC can be fashioned into beads on chains, spacers, cylinders or poured into molds to generate controlled shapes. In some studies it seems to play an important role in success.[59]

Systemic antibiotics can be delivered in two ways, either orally provided highly bioavailable drugs are used, or parenterally mainly via the intravenous route. Cost and convenience favor oral therapy with a somewhat greater risk of gastrointestinal intolerance, poor adherence to the treatment protocol and poor absorption if there are co-morbidities. Intravenous therapy ensures delivery but at the risk of vascular catheter infection and this is only sustainable for prolonged treatment if there are well-supervised outpatient parenteral therapy (OPAT) programs.[62] This can include self-administered outpatient parenteral antibiotic therapy (S-OPAT) which has advantages over health-care-worker administered therapy (H-OPAT).[62]

There are no proven advantages for durations of intravenous therapy beyond 6 weeks except on an exceptional basis for the treatment of a multidrug-resistant organism (MDRO). Total durations of therapy of 3–6 months have been associated with good outcomes,[49] though where there is doubt about the likelihood of cure, much longer periods of treatment are used.[52] Choices of antibiotic must take into account convenience (if OPAT is undertaken), side-effects and interactions, bioavailability and the antimicrobial resistance profile of the organism(s).

CONCLUSIONS

We have seen how prosthetic joint infection constitutes a major challenge to clinicians, scientists and indeed, to managers responsible for the organization of care. Prevention is of great importance, but equally we must stress that good outcomes are often possible with teamwork, skill and knowledge. There are still key questions to resolve, but the future of care will likely continue to include a specialist multidisciplinary team approach, with attention to careful patient assessment regarding goals, meticulous diagnostic and surgical debridement techniques, careful selection of antibiotics including duration, choice and route, and an understanding that in many circumstances, cure of infection, together with re-establishment of pain-free mechanical function, is often achievable.

REFERENCES

References for this chapter can be found online at http://www.expertconsult.com

Chapter 43

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Lyme disease

HISTORY

Lyme borreliosis is caused by the tick-borne spirochete, *Borrelia burgdorferi*, which is distributed throughout the Northern Hemisphere. The cutaneous manifestation, acrodermatitis chronica atrophicans (ACA) was first described in Germany by Buchwald in 1883. Afzelius subsequently described erythema migrans (EM) in Sweden and speculated that the rash was associated with tick bites. French physicians Garin and Bujadoux reported the first case of a neurologic manifestation, meningoradiculo-neuritis.[1–3] The disease was first treated with antibiotics in 1949 by Thyresson. In 1975, Steere and colleagues delineated the disease in the USA, while Burgdorfer and colleagues isolated and cultivated the causative agent.[1,2]

EPIDEMIOLOGY

Lyme disease is the most common vector-borne disease in the USA[4] and occurs widely throughout Europe and Asia.[5] The infecting organism, *B. burgdorferi*, is maintained in and transmitted by ticks of the *Ixodes ricinus* complex, including *Ixodes scapularis* in north-east and central USA, *Ixodes pacificus* on the west coast of the USA, *Ixodes ricinus* in Europe and *Ixodes persulcatus* in Asia (see Chapter 11).

In Europe, three species of the *B. burgdorferi* sensu lato complex are pathogenic, including *B. burgdorferi* sensu stricto, *Borrelia garinii* and *Borrelia afzelii*. *Borrelia burgdorferi* is the only pathogenic species in North America. The relative distribution of these species from region to region throughout Europe and Asia may account for the relative variability of disease syndromes associated with Lyme disease. Additional borrelial species *B. valaisiana* and *B. lusitaniae* have been identified by polymerase chain reaction (PCR) in skin biopsies or blood from a few patients with EM, ACA and with flu-like illnesses. However, these organisms have not yet been cultured from these lesions and their role in human illness remains unclear.[1,5,6]

Lyme disease is increasing in both incidence and recognition. The known endemic range is expanding, but the precise incidence and geographic spread are uncertain due to difficulties in establishing widely accepted diagnostic criteria. A National Surveillance Case Definition was adopted in the USA in 1990 to establish uniform diagnostic criteria for surveillance (Table 43.1). Over 90% of cases in the USA have been reported by 10 states in the north-east, upper midwest and Pacific coastal regions.[3] Even within these regions local distribution is highly variable. The recent upsurge in cases in the USA is a result of reforestation of land used for farming a generation ago, creating environments suitable for the deer reservoir,[7] and the outward migration of people from cities to rural areas.

Individuals who are exposed to natural outdoor habitats in the spring and early summer months are at greatest risk as this is the time when the tick vector is most active.[7,8] Erythema migrans is the sentinel clinical feature for most patients with Lyme disease. Those who do not manifest this skin lesion may come to medical attention months later with one or more symptoms of disseminated disease.

The seasonal variation of onset in temperate climatic zones follows the ecology of the predominant tick vectors. The life cycle and feeding habits of *I. scapularis* are best understood (Figs 43.1, 43.2). This tick has a three-stage life cycle (larva, nymph and adult) that spans 2 years. Larvae hatch from fertilized eggs in late spring and feed once for 2 or more days in midsummer. Preferred hosts are rodents and other small mammals. The next spring they molt into nymphs, and feed again for 3 or 4 days, with the same host range. Humans usually acquire Lyme disease from infected nymphs. After this second blood meal, the nymphs molt into adults. Adult *I. scapularis* has a narrower host range, with a preference for deer. Mating occurs on deer, and the female deposits her eggs and the cycle begins anew.[9]

In endemic areas, 30% or more of nymphs may be infected with *B. burgdorferi*; the rate of infection in adult ticks may be even higher, but infection rates in unfed larvae are less than 1%.[10] This pattern suggests that ticks acquire *B. burgdorferi* from reservoir hosts rather than transovarian transmission. The white-footed mouse, *Peromyscus leucopus*, is the primary reservoir host for *I. scapularis*.[11] As long as the organism, ticks, mice and deer are all present in the environment, the enzootic cycle will continue.

Variation in vector–host relationships provide the primary explanation for wide regional variation in the incidence of Lyme disease in California,[12] south-east USA[13] and elsewhere in North America. A primary determinant of transmission risk after a known tick bite is the duration of tick attachment before removal.[14,15] A tick attached for less than 24 hours has a low likelihood of transmitting *B. burgdorferi*.

Other modes of transmission have been postulated including transfusion of infected blood products[16] and biting flies,[17,18] but evidence strongly favors ixodid ticks as the primary and, most likely, exclusive vector of Lyme disease. Congenital transmission has been reported,[19,20] but the evidence regarding clinical disease resulting from transplacental transfer is inconclusive.

Borrelia burgdorferi sensu stricto strains likely differ in their host specificity and the degree of human pathogenicity.[9] A specific restriction fragment length polymorphism type of intergenic spacer (IGS) sequence and genotypic variants of the *OspC* gene are associated with hematogenous dissemination in patients with early stage Lyme disease.[20,21] Variations in *OspC* clonal types and risk of invasive disease are found in other pathogenic genospecies such as *B. afzelii* and *B. garinii*.[22,23]

Molecular analysis found a close relationship and overlapping genotypes between the European and North American populations.[24–26] A phylogeographic approach to the population history of Lyme disease

Table 43.1 Lyme disease: a summary of the US National Surveillance Case Definition

Definition	A systemic, tick-borne disease with protean manifestations: dermatologic, rheumatologic, neurologic and cardiac abnormalities. The initial skin lesion, erythema migrans, is the best clinical marker (occurs in 60–80% of patients)
Case definition	1. Erythema migrans present *or* 2. At least one late manifestation and laboratory confirmation of infection
General definitions	
1. Erythema migrans (EM)	• Skin lesion typically beginning as a red macute/papule and expanding over days or weeks to form a large round lesion, often with partial central clearing • A solitary lesion must measure at least 5 cm; secondary lesions may also occur • An annular erythematous lesion developing within several hours of a tick bite represents a hypersensitivity reaction and does not qualify as erythema migrans • The expanding EM lesion is usually accompanied by other acute symptoms, particularly fatigue, fever, headache, mildly stiff neck, arthralgias and myalgias, which are typically intermittent • Diagnosis of EM must be made by a physician • Laboratory confirmation is recommended for patients with no known exposure
2. Late manifestations These include any of the opposite *when an alternative explanation is not found*	Musculoskeletal system • Recurrent, brief attacks (lasting weeks or months) of objective joint swelling in one or a few joints, sometimes followed by chronic arthritis in one or a few joints • Manifestations not considered to be criteria for diagnosis include chronic progressive arthritis not preceded by brief attacks, chronic symmetric polyarthritis, or arthralgias, myalgias or fibromyalgia syndromes alone Nervous system • Lymphocytic meningitis, cranial neuritis, particularly facial palsy (may be bilateral), radiculoneuropathy or, rarely, encephalomyelitis alone or in combination • Encephalomyelitis must be confirmed by evidence of antibody production against *Borrelia burgdorferi* in CSF, shown by a higher titer of antibody in the CSF than in serum • Headache, fatigue, paresthesia or mildly stiff neck alone are not accepted as criteria for neurologic involvement Cardiovascular system • Acute-onset, high-grade (2nd or 3rd degree) atrioventricular conduction defects that resolve in days to weeks and are sometimes associated with myocarditis • Palpitations, bradycardia, bundle-branch block or myocarditis alone are not accepted as criteria for cardiovascular involvement
3. Exposure	• Exposure to wooded, brushy or grassy areas (potential tick habitats) in an endemic county no more than 30 days before the onset of EM • A history of tick bite is not required
4. Endemic county	• A county in which at least two definite cases have been previously acquired or in which a tick vector has been shown to be infected with *B. burgdorferi*
5. Laboratory confirmation	• Isolation of the spirochete from tissue or body fluid *or* • Detection of diagnostic levels of IgM or IgG antibodies to the spirochete in the serum or the CSF *or* • Detection of an important change in antibody levels in paired acute and convalescent serum samples • States may separately determine the criteria for laboratory confirmation and diagnostic levels of antibody • Syphilis and other known biologic causes of false-positive serologic test results should be excluded, when laboratory confirmation is based on serologic testing alone

reveals recent migration events and recombinant genomic types. In fact, a highly pathogenic clone seems to have spread rapidly in recent years to infect a broad range of host species in both continents.[27]

CLINICAL FEATURES

Although the most recognizable feature of Lyme disease is EM, it is a complex systemic illness with protean clinical manifestations. Like syphilis, Lyme borreliosis is a 'great imitator'; its symptoms range from cutaneous to musculoskeletal, cardiac to neurologic. It is interesting to note the differences in manifestations between North American and European disease arising from the endemicity of particular genomospecies in those regions.[5] Species and strain differences induce differing immunologic responses, rendering clinical and serologic diagnosis a formidable challenge.[21] Reported seropositivity depends upon the borrelial strain employed as antigen. Further confounding this diagnostic dilemma is the issue of co-infection with other tick-borne illnesses (i.e. ehrlichiosis, flaviviruses and babesiosis).

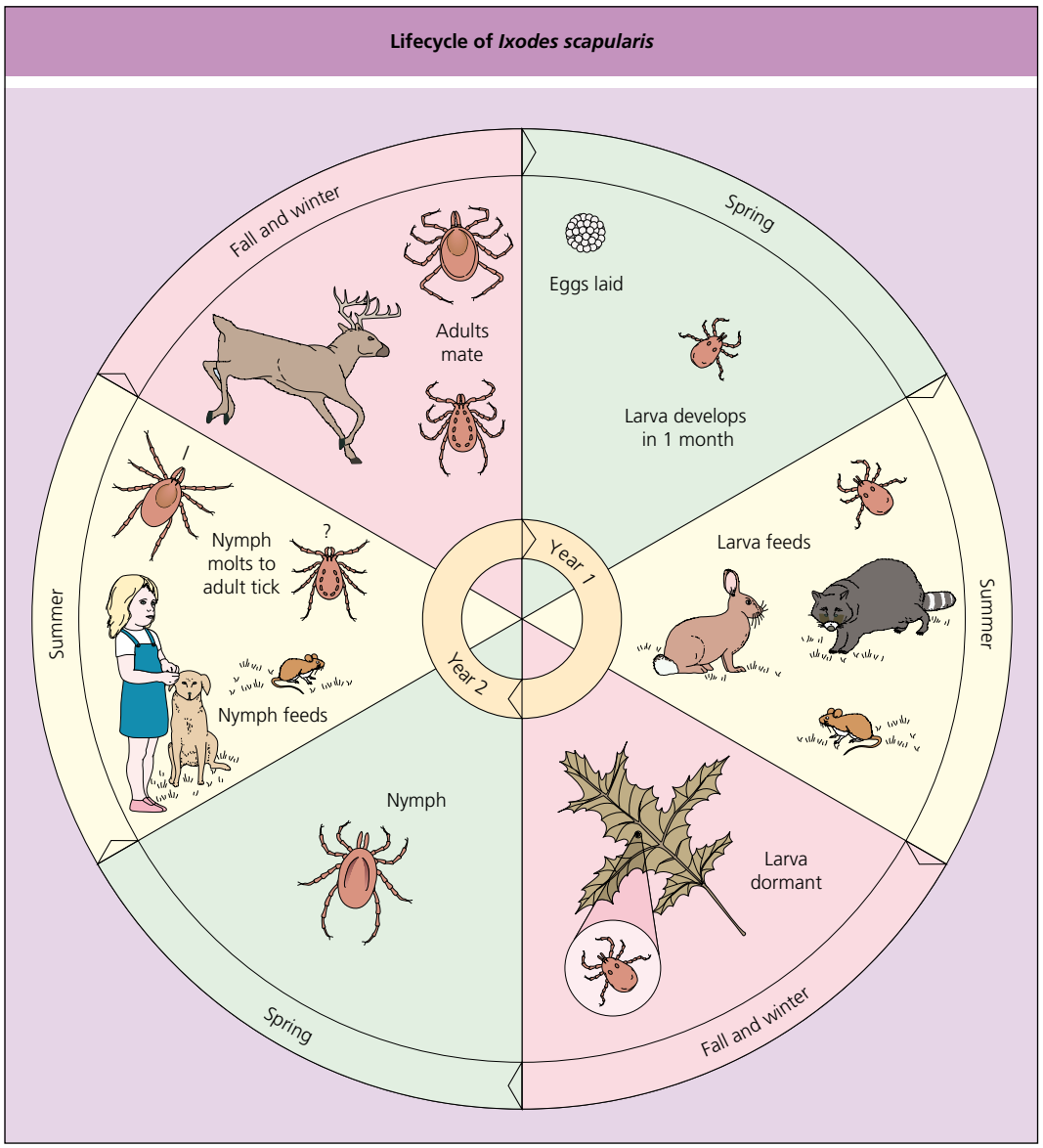


Fig. 43.1 Life cycle of *Ixodes scapularis* (also known as *Ixodes dammini*). The life cycle spans 2 years. Eggs hatch in the spring; six-legged larvae develop and feed once in the summer, acquiring *Borrelia burgdorferi* from their preferred host, the white-footed mouse. Next spring, the larvae molt into eight-legged nymphs, which feed once; mice are the preferred host, humans not being necessary for the ticks' life cycle. The nymphs molt into adult male and female ticks; mating often occurs while the female feeds on a deer, and the male may remain on the deer, the female falling off and then laying eggs. Adapted, with permission, from an illustration by Nancy Lou Makris in Rahn & Malawista.[101]

Lyme disease, human granulocytic ehrlichiosis and babesiosis not only share a common tick vector (*I. scapularis*), but also exhibit overlapping clinical features.[28] Despite these similarities recognition of co-infection is crucial to guide choice of antibiotic therapy.

Localized early disease

Lyme disease begins with EM at the site of a tick bite (Figs 43.3–43.6). In approximately one-third of cases, this skin lesion is missed or absent and patients present with symptoms of flu-like illness or disseminated disease. The interval between tick bite and appearance of EM varies from a few days to a month (median 7 days). The lesion begins as an erythematous papule and expands over several days to achieve a median diameter of 15 cm; favored sites are the groin, buttock, popliteal fossa and axilla. The Centers for Disease Control and Prevention (CDC) has determined 5 cm (in largest diameter) as the minimum diagnostic criterion for EM to distinguish this lesion from other diagnoses.[29] Transient and localized inflammatory reaction at the bite site after tick attachment is common; this resolves spontaneously and is not associated with an infection. Another useful differential point is that EM lesions tend to occur at sites not commonly seen in community-acquired cellulitis such as the axilla, popliteal fossa, back, abdomen and groin. Cellulitis lesions are typically tender to palpation and pruritic while EM lesions can be remarkably asymptomatic, despite their often striking appearance.[30] Although lesion size more than 5 cm is a useful diagnostic criterion, it should not be used to exclude EM when clinical and epidemiologic characteristics are suggestive of Lyme disease as early treatment is correlated with clinical response.[30–32]

The lesions of EM are generally annular with a sharply demarcated outer border and an erythematous or bluish hue. They are warm to the touch, flat and minimally or non-tender. Lesions may show partial central clearing, but can be indurated or even necrotic. This cutaneous lesion is the best clinical marker of Lyme disease, yet an



Fig. 43.2 *Ixodes scapularis*. Larva, nymph, adult male and adult female. Courtesy of Pfizer Central Research.



Fig. 43.3 Erythema migrans. A typical annular, flat, erythematous lesion with a sharply demarcated border and partial central healing. Courtesy of Dr Steven Luger, Old Lyme, Connecticut, USA.

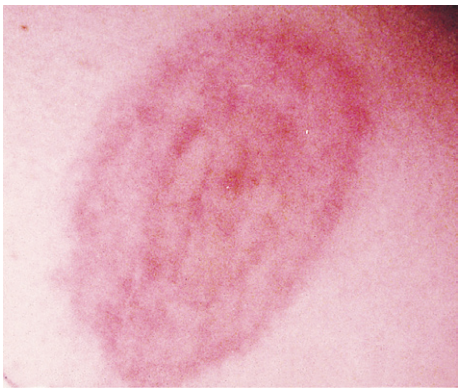


Fig. 43.4 Erythema migrans. A lesion with variation in color and a target-like appearance. The bite site is visible in the center. Courtesy of Dr Steven Luger, Old Lyme, Connecticut, USA.

annular erythematous lesion following a tick bite is not sufficient evidence for a definitive diagnosis of Lyme disease, especially in nonendemic areas.[8]

A lesion that may be mistaken for Lyme disease is known as southern tick-associated rash illness (STARI) which results from a bite of the *Amblyomma americanum* tick (also known as the lone star tick), not known to be able to transmit *B. burgdorferi*.[31] STARI can occur concomitantly with



Fig. 43.5 Erythema migrans. A lesion with a dusky center, a common variant. Courtesy of Dr Steven Luger, Old Lyme, Connecticut, USA.

human monocytic ehrlichiosis (HME) which is transmitted by the same tick vector. *Amblyomma americanum* ticks were originally thought to be restricted to southern states of the USA but have expanded northward over the past 30 years to as far north as Maine.[31,33]

Early disseminated disease

Within several days of the appearance of EM, many patients develop disseminated infection with the appearance of prominent systemic symptoms, the occurrence of multiple secondary skin lesions, or both. Malaise, fatigue, lethargy, headache, fever and chills, arthralgia and myalgia are particularly common, each occurring in one-half or more of patients. Symptoms may fluctuate rapidly and vary from a flu-like syndrome to a meningitis-like illness.

Secondary skin lesions may occur anywhere on the body and resemble primary lesions but are usually smaller, show less expansion with time and lack indurated centers. These manifestations of early disseminated infection appear to be more common in the USA than in Europe and may reflect biologic differences in infecting organisms. Erythema migrans, secondary skin lesions and associated symptoms resolve, even without antibiotic therapy, a median of 28 days after onset. Recurrent crops of evanescent lesions may occur, and fatigue, intermittent musculoskeletal pain and headaches may persist for months.

Late disseminated disease

After resolution of the signs and symptoms of early disease, most untreated patients (>50%) will develop some form of long-term sequelae from Lyme disease.[22] Predominant complications are involvement



Fig. 43.6 Multiple erythema migrans lesions. Lateral (a) and posterior (b) views of the same patient with multiple erythematous macules of EM. Secondary lesions result from hematogenous spread. They may occur anywhere in the body. Secondary lesions are usually of uniform color and lack induration. Courtesy of Dr Steven Luger, Old Lyme, Connecticut, USA.

of the heart, nervous system and joints, but case reports have described involvement of multiple organs, including the liver,[23] subcutaneous tissue,[24] muscle,[25] eye structures[25] and spleen.[27] More than one organ system can be affected simultaneously or sequentially in an individual patient.

Carditis

Less than 10% of patients with untreated early Lyme disease develop carditis, generally a few weeks to a few months after EM. However, palpitations from an arrhythmia or unexplained syncope from high-degree atrioventricular block may be the presenting manifestation of Lyme disease.[34] The primary clinical manifestation of Lyme carditis is heart block, which may fluctuate from first-degree to complete heart block over minutes to hours and generally resolves spontaneously within a few weeks, even in untreated patients.[35] Although temporary pacing is frequently required, permanent pacing is rarely needed in the treated patient.[34]

Rarely, congestive heart failure has been linked to Lyme disease. A case report suggested that Lyme carditis might be a cause of chronic dilated cardiomyopathy.[36] Endomyocardial biopsy and electrophysiologic testing have revealed direct spirochetal invasion of cardiac muscle and widespread abnormalities of cardiac conduction.[37]

Neurologic manifestations

Frank neurologic abnormalities, including cranial neuropathy (particularly Bell's palsy), meningitis, radiculoneuropathy, myelopathy and encephalopathy occur in about 15% of patients. Neurologic symptoms generally begin a few weeks after EM (median 4 weeks), although some patients can initially present with neurologic manifestations alone.[8,38]

Subtle neurologic complaints without objective deficits (headache, irritability, paresthesias, photophobia and lethargy) may accompany early disease dissemination and represent the mildest end of the spectrum of neurologic Lyme disease. Multiple neurologic abnormalities may coexist or occur sequentially. The most common neurologic complication of Lyme disease is seventh nerve palsy and bilateral involvement may occur.[8] Occurrence of Bell's palsy in young people in the summer months in Lyme-endemic areas is highly suggestive of Lyme disease. Peripheral nerve abnormalities may involve sensory and motor nerve roots, plexi and motor and sensory nerve fibers.[39,40] Meningitis and radiculoneuropathy often wax and wane for weeks to months but ultimately resolve spontaneously.

Borrelia burgdorferi has been isolated from the cerebrospinal fluid (CSF) of a patient whose only complaint was tinnitus,[41] and has been demonstrated by PCR in patients with symptoms limited to headache, Bell's palsy or paresthesias, indicating that the symptoms associated with central nervous system (CNS) infection may be minimal.[42] The emergence of subtle neurologic manifestations years after onset of Lyme disease also supports the possibility of latent persistent infection in the CNS (see Late neurologic syndromes, below). Rarely, cases of demyelinating encephalopathy mimicking multiple sclerosis have been reported.[43,44]

Arthritis

Most people who have untreated Lyme disease develop arthritis. Brief, intermittent attacks of migratory musculoskeletal pain commonly begin early in Lyme disease and may persist for months before the appearance of overt arthritis. Frank arthritis occurs in 60% of patients in the USA, at a median of 6 months after EM, but, as with other symptoms of disseminated disease, arthritis may be among the presenting manifestation of Lyme disease.[45] Although the clinical expression varies, patients most often experience brief recurrent attacks of monoarticular or oligoarticular inflammatory arthritis involving large joints, particularly the knee.[46,47] Effusions may be massive (100 ml or more), causing popliteal cysts, which may rupture and result in a pseudothrombophlebitis syndrome.

Attacks last a few days to a few weeks and over time (months to years) decrease in severity, frequency and duration.[47] Arthritis has been reported to be milder in children than in adults.[48,49] Polyarthritis is decidedly uncommon. Synovitis becomes chronic in 20% of cases (see Chronic arthritis, below).

Late (persistent) disease

After the disseminated stage of Lyme disease, symptoms can vary substantially from self-limited inflammation that resolves spontaneously, only to recur years later and give rise to the chronic manifestations of disease. This has been called 'late Lyme disease'. Late Lyme disease may involve the CNS, the joints and the skin (particularly in Europe). Information is still emerging on this patient group. It is as yet unclear whether the clinical manifestations of late Lyme disease result from persistent sterile inflammation or persistent infection.

Late neurologic syndromes

In Europe, radiculoneuritis is the predominant late neurologic manifestation of *B. burgdorferi* infections in adults, with more frequent occurrence of meningitis in children.[2] Other reported CNS abnormalities include lymphocytic meningitis, peripheral neuropathy and cranial neuritis, collectively known as Garin–Bujadoux–Bannwarth (MPN–GBB) or Bannwarth's syndrome.[50] Bannwarth's series of 26 patients led to the discovery of the link between these neurologic syndromes and tick bite/EM.[2] Pfister and colleagues found 73% with radicular pain within the dermatomal distribution of the prior arthropod bite or EM.[50]

Both a mild, predominantly sensory, peripheral neuropathy and subtle encephalopathy may occur months to years after the onset of Lyme disease.[51,52] The diagnosis of Lyme encephalopathy hinges on the presence of cognitive deficits involving primarily short-term memory and concentration, and serologic or PCR evidence of antecedent infection with *B. burgdorferi*. Chronic fatigue, headaches and sleep disturbance frequently accompany these abnormalities and can be very problematic. Rarely, severe encephalomyelopathy has occurred with impairment of higher cortical function, seizures and spinal cord lesions. Chronic neuropathy in patients with ACA has been described. The most common manifestations include peripheral neuropathy with sensory deficits in a patchy distribution.[53]

Despite extensive study, the post-Lyme disease or chronic Lyme disease syndrome remains an enigmatic process. Its pathogenesis is not understood and even its clinical validity as an infectious disease has been questioned. The diagnosis is often based solely on clinical judgment rather than on well-defined clinical criteria. Four randomized placebo-controlled studies[54–57] have been performed, each with different entry criteria and with varying results. The results have varied from some improvement in fatigue and neurocognitive function to others that have shown no effect. In all of these studies, antibiotic therapy offers no sustained benefit to patients who have post-Lyme disease syndrome. These studies also showed a substantial placebo effect and a significant risk of treatment-related adverse events. To complicate our understanding of post-antibiotic Lyme disease, recent animal studies have demonstrated persistent infection in mice treated with a prolonged course of antibiotics.[58,59] Further research to elucidate the mechanisms underlying persistent symptoms after Lyme disease and controlled trials of new approaches to the treatment and management of these patients are needed.

Chronic arthritis

Arthritis becomes chronic in 20% of patients who have untreated Lyme disease, resulting in a syndrome that is clinically indistinguishable from other forms of monoarticular or oligoarticular inflammatory arthritis. Chronic Lyme arthritis is preceded by recurrent brief attacks of joint inflammation. The knee is by far the most commonly affected joint. Immunogenetic factors, particularly human leukocyte antigens (HLA-DR2 and DR4), may predict which people are at highest risk of developing chronic Lyme arthritis.[60] Even chronic Lyme arthritis may eventually remit spontaneously. Only a minority of patients develop radiographic evidence of erosions of cartilage or bone.[61] There have been no unequivocal cases of symmetric, peripheral, polyarticular, inflammatory arthritis with joint destruction resulting from Lyme disease. Differentiation from rheumatoid arthritis is rarely a problem.



Fig. 43.7 Acrodermatitis chronicum atrophicans. Typical inflammatory bluish-red lesions of acrodermatitis chronicum atrophicans. Lesions usually occur on acral portions of extremities. Courtesy of Dr. Eva Asbrink.

In the diagnosis of persistent Lyme arthritis, culture from synovial fluid has generally been unsuccessful. PCR of synovial fluid has been shown to successfully detect *B. burgdorferi* DNA with an estimated sensitivity of approximately 80–85%.[62] There is concern that PCR positivity due to dead spirochetes may prevent its use as a means of determining whether infection is persistent, and reports of positive PCR in patients after antibiotic therapy exist.[58,59] However, as most studies have found that PCR is negative in patients receiving more than 1–2 months of antibiotic therapy,[63] *B. burgdorferi* is likely no longer present in the joints of most patients who have chronic antibiotic-refractory arthritis. Many clinicians now use PCR positivity from synovial fluid as a prerequisite before additional courses of antibiotics are given for persistent arthritis after a standard course of antibiotics.

Chronic skin involvement

Chronic skin involvement (acrodermatitis chronicum atrophicans) as a late manifestation of Lyme disease occurs primarily in Europe.[64] It usually occurs in an acral distribution and is characterized by violaceous discoloration and swelling at a site where EM occurred years earlier. The lesion eventually becomes atrophic (Fig. 43.7). Other clinical manifestations include fibrotic nodules, ulnar bands, sensory disturbances, muscular weakness, myalgias, arthralgias and tenderness on impact over bony prominences. In a study of 50 consecutive patients with ACA in Sweden, elevated antispirochetal antibody titers were found at indirect immunofluorescence and enzyme-linked immunosorbent assay (ELISA), and histologic biopsies demonstrated dermal lymphocytic infiltrates with plasma cells and telangiectasia.[64,65] Acrodermatitis chronicum atrophicans is thought to result from local persistence of *B. burgdorferi*, which has been isolated from a lesion 10 years after onset.[65] Lichen sclerosis et atrophicus or morphea-like lesions have also been described.

DIAGNOSTIC INVESTIGATIONS

Lyme disease is a diagnostic challenge by the clinician and the laboratory alike. The clinical presentation is highly variable with a myriad of possible outcomes depending on host factors and pathogen characteristics. Currently available diagnostic assays are not always precise and are subject to bias in their interpretation. The National Surveillance Case Definition of Lyme disease developed in the USA (see Table 43.1) requires either the presence of EM or a definite late manifestation of Lyme disease combined with laboratory confirmation of infection in a person who has had opportunity to be exposed to the causative agent. Definitive laboratory confirmation requires isolation of the causative organism from clinically involved tissue, but this is

rarely achievable in clinical practice. Demonstration of specific serologic immune responsiveness against *B. burgdorferi* has been accepted as a substitute for bacteriologic isolation. Unfortunately the sensitivity and specificity of many tests has been poor.[66–68] For 75% of patients, a serum sample obtained 2 weeks after the initial sample will be positive, but prompt antibiotic treatment may prevent seroconversion.

Western blot confirmation of all positive and equivocal screening results is recommended.[69] The predictive value of laboratory tests used for the diagnosis of Lyme disease improves dramatically when used in patients with characteristic clinical features of the illness. Laboratory testing should only be used for confirmation; it should not be used for screening asymptomatic patients.

Detection of a specific immune response to *B. burgdorferi* remains the best means of confirming the diagnosis of Lyme disease. The first immune response in Lyme disease is mediated by T cells and is directed against a variety of epitopes.[70,71] This T-cell response subsequently localizes preferentially to involved tissues.[70,72] Unfortunately, tests to measure this response are not standardized, are technically demanding, require live cells and have varied in sensitivity and specificity.[73] For these reasons, measurement of the T-cell response cannot be recommended routinely for diagnosis.

The serologic response to *B. burgdorferi* has been well characterized (Fig. 43.8).[74] Specific IgM antibody, directed initially primarily against the flagellae of the organism, is detectable a few weeks after disease onset. The response broadens to include additional antigens over time and peaks by 3–6 weeks. Generally IgM antibody falls to within the normal range by 6 months, but occasionally it may remain elevated for much longer. Specific IgG antibody is detectable a few weeks after IgM and is directed against the same antigen, but IgG antibodies may not peak until many months after disease onset.

Both immunofluorescence assay (IFA) and ELISA have been used to detect this antibody response; in general, ELISA is preferable because of better sensitivity, objectivity and reproducibility, and because of its adaptability to automated systems. Regrettably, serologies for Lyme disease may be falsely positive or negative for various reasons.

Both IFA and ELISA may yield false-positive results because of epitopes in the test antigen preparations that cross-react with other bacteria.[68] False-positive results may also occur in conditions associated with polyclonal B-cell activation. Specificity is increased by a two-step approach in which all positive or equivocal results are confirmed by Western immunoblotting. This two-step approach was recommended by participants in a national conference on serologic diagnosis of Lyme disease held in 1994 under the sponsorship of the CDC.[69] A true positive ELISA is associated with immunoreactivity against polypeptides specific for *B. burgdorferi*, reactivity that is absent in the various conditions associated with false-positive ELISA results. The currently recommended criteria for a positive Western blot are given in Table 43.2.

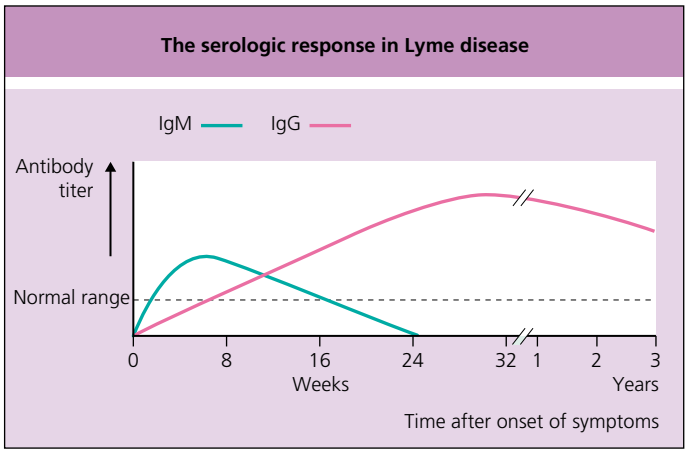


Fig. 43.8 The usual serologic response in Lyme disease. Specific IgM becomes detectable 1–2 weeks after symptom onset and the appearance of erythema migrans. The later appearance of IgG is frequently concurrent with systemic manifestations. IgG is nearly always elevated with late disease. Typically, and even in untreated patients, IgM falls over 4–6 months; persistence for longer than this predicts later manifestations.

Table 43.2 Western blot criteria. Centers for Disease Control and Prevention Working Group recommendations for Western blot positivity

- All serum specimens found to be positive or equivocal by a sensitive enzyme immunoassay or immunofluorescent assay should be tested by a standardized Western blot procedure
- When Western immunoblot is used in the first 4 weeks of illness, both IgM and IgG procedures should be performed
- After the first 4 weeks of illness, IgG alone should be performed
- An IgM blot is considered positive if two of the following three bands are present: 24 kDa (OspC), 39 kDa (BmpA) and 41 kDa (Fla)
- An IgG blot is considered positive if five of the following 10 bands are present: 18, 21 (OspC), 28, 30, 39 (BmpA), 41 (Fla), 45, 58, 66 and 93 kDa

Recommendations of the Second National Conference on Serologic Diagnosis of Lyme Disease sponsored by the Centers for Disease Control and Prevention, the Association of State and Territorial Public Health Laboratory Directors and the Michigan Department of Health.

Osp, outer surface protein; Fla, flagella.

One special circumstance deserves mention. Antibiotic therapy administered early in the course of Lyme disease may result in a negative serology by curing the infection and eliminating antigen before systemic immune challenge.[68] One series indicated, however, that infection may persist after early antibiotic therapy despite persistently negative serologies.[71]

In a study of 17 patients with acute Lyme disease who received prompt treatment with oral antibiotics and subsequently developed 'chronic' illness, it was shown that none of them had diagnostic levels of antibodies to *B. burgdorferi* on either a standard ELISA or IFA.[71] On Western blot analysis, the level of immunoglobulin reactivity against *B. burgdorferi* was no greater than that of normal controls. These patients had a vigorous T-cell proliferative response to whole *B. burgdorferi* at levels similar to that of 18 patients with chronic Lyme disease with detectable antibodies. The T-cell response of both groups was greater than that of a control group. The investigators concluded that the presence of chronic Lyme disease cannot be excluded by the absence of antibodies against *B. burgdorferi* and that a specific T-cell response to *B. burgdorferi* is evidence of infection in seronegative patients with suggestive clinical parameters.[71]

This apparent dissociation between T- and B-cell immune responses may be attributed to the administration of antibiotics for EM and, therefore, the presence of low or undetectable levels of anti-*Borrelia* antibodies. Antibiotic therapy may have resulted in the elimination of most spirochetes at a critical early stage of the immune response, thereby hindering the development of a sustained B-cell response.[71] Persistent disease activity may result from harboring of *B. burgdorferi* in sites such as the CNS, which may not receive adequate concentrations of antibiotics to reach the minimum inhibitory concentrations (MICs) required against the majority of strains of *B. burgdorferi*. In support of this observation is the discovery of local production of anti-*Borrelia* antibody in the CNS in those suffering from neurologic symptoms of Lyme borreliosis in the absence of diagnostic serum antibodies levels.[75,76]

The concept of antigenic variation displayed by other borrelial species may represent an alternative explanation for the apparent absence of a specific antibody response in seronegative Lyme disease.[77] Classically, immunodominant 'variable major proteins' are replaced periodically by variant forms, enabling the organism to elude the host's antibody. Although *B. burgdorferi* does not precisely exhibit marked antigenic variation, antigenic diversity has been noted among strains found in humans, animals and ticks.[77]

Other laboratory findings

Lyme meningitis is characterized by lymphocytic pleocytosis (<300 wbc) with elevated protein and usually normal CSF glucose (although hypoglycorrhachia is found on occasion). In protracted cases the IgG:albumin ratio can be found with oligoclonal banding of CSF proteins.[43,44]

Attacks of arthritis are accompanied by an inflammatory joint fluid containing a few hundred to 50 000 white blood cells, mostly polymorphonuclear, elevation in protein and normal glucose.[45] Radiographs of affected joints most often show only soft tissue swelling, but when joint inflammation has persisted for many months, there may be pannus formation and erosions of underlying cartilage and bone.[47] Some patients develop enthesopathy with calcifications of tendon and ligament attachment sites.

Cognitive deficits associated with late neurologic involvement may be quantified by neuropsychologic testing, a very helpful modality in the formal assessment of cognitive complaints.[51,52] A depressive overlay is often present, but findings should also reveal an organic encephalopathy primarily affecting short-term memory. Peripheral neuropathy and radiculoneuropathy cause abnormalities on electromyography and, less often, abnormalities of peripheral nerve conduction;[52] sensory fibers are more frequently affected than motor fibers. Although these findings on tests of CNS and peripheral nervous system function are not specific for Lyme disease, abnormal findings indicate a definite disorder of the nervous system, and the combination of central and peripheral nerve abnormalities characteristic of Lyme disease is uncommon in other diseases.

Specific laboratory confirmation of *B. burgdorferi* infection is possible with culture of affected tissue, or by PCR on skin, urine, joint fluid, blood or CSF. Although the causative organism has been cultured from a variety of affected tissues[78] and joint fluid,[79] this technique is not readily available in routine clinical laboratories. The PCR assay is a valuable adjunctive study[80,81] but is not considered a routine diagnostic test for Lyme disease.

Despite all the problems with diagnostic assays, most patients, even those with longstanding symptoms, can be adequately managed and effectively treated. At present, the diagnosis of chronic Lyme disease is loosely applied to a constellation of symptoms including fatigue, arthralgias, aches, pains, memory disturbances and impairment in concentration. Referring to these patients as having chronic Lyme disease when there is no clinical or laboratory evidence of *B. burgdorferi* infection is misleading and does a disservice to the patients and other physicians alike. Confusion over this designation has created misunderstanding and promotes phobias regarding the stigma of 'chronic' or lifelong disease.

An accurate diagnosis is usually readily achievable by a careful history of exposure and a history of EM with suggestive clinical features. Serologic assays interpreted under these circumstances remain the best screening methods for exposure followed by confirmation with immunoblotting techniques. A lack of response to antibiotic therapy in presumed Lyme disease is most likely due to incorrect diagnosis, thereby warranting a continued search for the cause of illness.[82,83]

Measurement of immunoreactivity in CSF is a useful adjunct in the diagnosis of CNS Lyme disease.[84] To have diagnostic value, a CSF index should be calculated comparing the ratios of specific antibody to total immunoglobulin in CSF and serum. A specific antibody response in CSF is not always detectable with CSF Lyme disease, but is helpful when positive.

DIAGNIOSTIC PITFALLS IN THE DIFFERENTIAL DIAGNOSIS

Erythema migrans is the classic skin lesion of Lyme disease and, in its typical appearance, can be confused with little else. However, atypical presentations may lead to confusion[85] and caution must be exercised when making this diagnosis in nonendemic areas.[86,87]

In later stages, Lyme disease may mimic a variety of cardiac, neurologic and arthritic disorders. Carditis can be confused with subacute bacterial endocarditis or acute rheumatic fever, but valvular lesions do not occur in Lyme disease. Blood cultures are negative.[88]

Bell's palsy caused by Lyme disease is indistinguishable from idiopathic Bell's palsy, but Lyme disease is one of very few causes of bilateral Bell's palsy (a helpful clue if present) and, by this stage of disease, patients are typically seropositive. Lyme meningitis presents a clinical picture similar to that of viral meningitis, but there is often an antecedent history of EM, the course is more protracted and Lyme meningitis is characterized by relapses and remissions. The peripheral neuropathy associated with the late neurologic manifestations of Lyme disease mimics other sensory neuropathies; however, a history of previous non-neurologic involvement is usually present and serologies can be helpful in the differential diagnosis. The rare patients who have demyelinating encephalopathy must be distinguished from patients who have multiple sclerosis; positive serology (particularly if present in CSF) or CSF PCR, combined with an exposure history, is very helpful in this circumstance.[88]

The typical patient with Lyme arthritis who has monoarticular inflammatory arthritis of a knee can be confused with reactive arthritis, crystal-induced arthritis or septic arthritis. At initial presentation, bacterial infection must always be excluded. The pattern of brief, recurrent attacks is more common in Lyme disease than other common forms of acute arthritis. Furthermore, patients who have Lyme arthritis are virtually always seropositive.

It is particularly important to differentiate fibromyalgia from 'chronic' Lyme disease because the prognosis and response to therapy are quite different.[83,88]

PATHOGENESIS AND PATHOLOGY

Borrelia burgdorferi has been unequivocally established as being the cause of Lyme disease. Clinical isolates differ in their outer surface protein expression[89] and genetic composition.[90] All three genospecies – *B. burgdorferi*, *B. garinii* and *B. afzelii* – have been isolated from patients who have Lyme disease and it is likely that disease expression in humans may vary depending upon the genospecies.[91]

Unlike *Treponema pallidum*, *B. burgdorferi* can be grown in culture although a specialized medium is required. Serial passage in culture alters surface protein expression with loss of pathogenicity in mice,[92] but the specific factors conferring pathogenicity have not yet been identified. *Borrelia burgdorferi* can penetrate endothelial monolayers and survive intracellularly in cultured fibroblasts.[93] Work is in progress to understand the mechanisms by which this organism can cause persistent infection despite a vigorous immune response.

The organism may evade eradication through an initially delayed and ineffective immune response. It disseminates preferentially to certain target organs where it engenders an immunologically mediated host response, with tissue injury occurring as a result of the inflammatory response. Why this lesion becomes persistent in some people and whether live organisms persist in all cases of chronic disease remain to be elucidated. An important question is how *B. burgdorferi* avoids destruction in the presence of the vigorous specific T- and B-cell responses that are usually apparent within a few weeks of onset of disease. In humans, the early immune response is directed primarily against a flagellar antigen. Vaccine studies in mice have shown that specific antibodies against this antigen do not protect against subsequent infection. Vaccination with outer surface protein (Osp) A, an immunodominant surface antigen to which antibodies appear much later in human disease, is protective.[94] It may be that, in human Lyme disease, *B. burgdorferi* is able to establish infection because of an ineffective initial immune response focused primarily against flagellae. Later, when the immune response broadens, infection may already be established and disease may be perpetuated through other mechanisms such as survival in sequestered, intracellular sites.[95]

HLA-DR4 predisposes to the development of persistent Lyme arthritis and DR4 is associated with a lack of response to antibiotics.[60] Patients who are HLA-DR4 positive and who have treatment-resistant Lyme arthritis have also been shown to have a strong immune response to an epitope on OspA that cross-reacts with human lymphocyte function antigen 1 (LFA-1), which may serve as an autoantigen.[96] This evidence suggests a possible autoimmune mechanism for chronic Lyme arthritis through a mechanism involving molecular mimicry between OspA and LFA-1 in HLA-DR4-positive individuals. No predictors of chronic neurologic disease have been described as yet. Histologic studies of affected tissues have provided evidence for immunologically mediated inflammation. Stains of EM lesions reveal a perivascular mononuclear infiltrate and fibrin deposition in the dermis, without epidermal changes except at the site of the bite.[8] Endomyocardial biopsies have revealed similar changes in the heart, with a focal perivascular infiltrate of mononuclear cells and fibrin deposition in both the endocardium and myocardium.[36] Biopsies of affected nerves show inflammatory infiltrates around endoneurial and perineurial vessels without vessel necrosis.[97] Both myelinated and unmyelinated fibers may be affected. Synovial biopsies from involved joints have revealed synovial lining cell hyperplasia and hypertrophy, vascular proliferation and lymphocytic infiltration of the subsynovial areas. The intensity of the infiltrate varies, and fibrin deposition may be pronounced. Aggregates of T and B cells, often with lymphoid follicle formation, are common and may be concentrated in perivascular areas with obliteration of vessels but without vessel necrosis.[47] Levels of interleukin (IL)-1,[98] prostaglandin E_2 and collagenase[99] in joint fluid are elevated, similar to the situation in rheumatoid arthritis. Spirochetes have been visualized in skin lesions,[63] heart tissue[36] and synovium,[95] but not in peripheral nerves, where it has been postulated that an autoimmune mechanism accounts for the inflammatory lesions.[100]

MANAGEMENT

The primary goals of therapy for Lyme disease are the control of inflammation and the eradication of the infection. Lyme disease is most responsive to antibiotic therapy early in the course of the disease. As with syphilis, some later disease manifestations do not seem to improve after administration of antibiotics.

Treatment regimens are based in part on data from controlled clinical trials and in part on clinical experience.[101] *In-vitro* antibiotic sensitivity testing does not reliably predict clinical response. Loose criteria for diagnosis accepted without critical review have led to widespread antibiotic use for presumptive Lyme disease in patients who almost certainly have other explanations for their symptoms. In one report, the leading reason for failure to respond to antibiotic therapy for Lyme disease was incorrect diagnosis.[102] In addition, the appropriate end point of antibiotic therapy is often not clear because of the difficulty of proving when the infection has been eradicated and because of the common persistence of symptoms long after treatment. Current treatment recommendations (Table 43.3) represent a distillation of available evidence and will no doubt be refined in time.[83]

Early localized or early disseminated disease

If antibiotic therapy is initiated early in the course of Lyme disease, EM typically resolves promptly and later stage disease is prevented.[83,103] Early localized infection, limited to a single skin lesion, with mild or no systemic symptoms, is uniformly responsive to short-course oral antibiotic therapy with a number of agents. Of the antibiotics studied to date, amoxicillin (500 mg q8h), doxycycline (100 mg q12h) and cefuroxime axetil (500 mg q12h) have been the most effective for this stage of disease.[104,105] Although the optimal duration of therapy is unknown, most clinicians currently recommend 2–3 weeks for both early localized and early disseminated disease.

The appearance of systemic symptoms (fever, arthralgias, fatigue) and secondary skin lesions reflects dissemination of the organism beyond the site of inoculation. As long as no neurologic symptoms are present, 3 weeks of oral therapy is sufficient for this group of patients. In a carefully performed prospective study, 10% of patients with a single EM lesion and no systemic symptoms had a positive PCR on blood. This was interpreted as demonstrating that clinically silent bloodstream invasion may be relatively common early in the course of infection.[80]

Some experts have recommended the addition of probenecid to amoxicillin, but this combination has been associated with a relatively high frequency of rashes and is not known to be superior to amoxicillin alone. The pediatric dose range of amoxicillin is 30–40 mg/kg per day in three divided doses. Herxheimer-like reactions, with intensification of fever and arthralgias, may occur shortly after initiation of therapy. If possible, doxycycline should be avoided in children under the age of 9 years and during pregnancy because of the possibility of staining of the teeth. Tetracyclines must be used with caution during summer in all patients because they may predispose the patient to sun-sensitive rashes or severe sunburn. Penicillin-allergic young children can be treated with cefuroxime or erythromycin, but results with macrolide antibiotics have been less satisfactory than those with penicillin, amoxicillin or tetracyclines.[83] Azithromycin has been studied systematically and found to be less effective than amoxicillin.[105]

Regardless of which agent is chosen, some patients experience a delayed resolution of systemic symptoms (headache, musculoskeletal pain, fatigue) which may persist as long as 3 months after completion of therapy. These symptoms usually resolve spontaneously and do not indicate continued infection requiring further antibiotic therapy.[103] A seemingly self-perpetuating fibromyalgia syndrome may develop in response to Lyme disease; this too is unresponsive to antibiotic therapy.[106] The likelihood of delayed resolution of symptoms is greatest in patients who have prominent systemic symptoms or a delay in diagnosis before institution of antibiotics.[83]

Disseminated disease

With the possible exception of arthritis, which usually responds to oral therapy, disseminated infection with target organ involvement should generally be treated with intravenous antibiotics. Carditis, meningitis, cranial neuropathy, radiculoneuropathy and arthritis are discussed separately.

Carditis

Although carditis resolves spontaneously, observational data suggest that resolution of heart block may be hastened by treatment with salicylates, corticosteroids, oral penicillin, oral tetracyclines and intravenous ceftriaxone and penicillin.[37,83] This response to either anti-inflammatory therapy or antibiotics suggests that the proximate cause of the heart block is the inflammatory reaction engendered by the Lyme spirochete rather than direct tissue destruction resulting from the infection. Control of the inflammatory response leads to resolution of clinical manifestations.

Heart block caused by carditis may progress suddenly, necessitating a temporary pacemaker, but permanent pacing is rarely necessary. Hospitalization with cardiac monitoring is prudent while antibiotic therapy is instituted. Although no comparative trials have been conducted, treatment with intravenous penicillin or a third-generation cephalosporin for a minimum of 2 weeks to eradicate systemic spirochetal infection is recommended. Salicylates or non-steroidal anti-inflammatory agents may hasten symptom resolution. Systemic corticosteroids may be dramatically effective in reversing heart block, but they are rarely needed during antibiotic therapy for Lyme carditis.[83]

Table 43.3 Suggested antibiotic regimens for Lyme disease

Early disease	• Doxycycline, 100 mg po, q12h for 21 days, or • Amoxicillin (with or without probenecid) 500 mg, q8h for 21 days, or • Erythromycin, 250–500 mg po, q6h for 21 days, or • Azithromycin 500 mg daily for 7 days, or • Cefuroxime axetil, 500 mg po, q12h for 21 days Shorter courses (14 days) may suffice for localized early disease. Erythromycin and azithromycin less effective than other choices
Lyme arthritis	Initial treatment: • Doxycycline, 100 mg po, q12h for 30 days, or • Amoxicillin and probenecid, 500 mg each po, q6h for 30 days If initial treatment fails: • Penicillin G, 20 × 10^6 IU iv, daily in divided doses for 14 days, or • Ceftriaxone sodium, 2 g iv, daily for 14 days
Neurologic manifestations	For facial nerve paralysis alone: • Doxycycline, 100 mg po, q12h for 21–30 days, or • Amoxicillin, 500 mg po, q8h for 21–30 days
Additional signs (e.g. Lyme meningitis, radiculopathy, encephalitis)	• Ceftriaxone, 2 g iv, daily for 30 days, or • Penicillin G, 20 × 10^6 IU iv, daily in divided doses for 30 days Possible alternatives: • Cefotaxime sodium, 2 g iv, q8h for 30 days, or • Doxycycline, 100 mg po, q12h for 14–30 days, or • Chloramphenicol, 1 g iv, q6h for 14–30 days
Lyme carditis	• Ceftriaxone, 2 g iv, daily for 14 days, or • Penicillin G, 20 × 10^6 IU iv, daily in divided doses for 14 days Possible alternatives: • Doxycycline, 100 mg po, q12h for 21 days, or • Amoxicillin, 500 mg po, q8h for 21 days
During pregnancy	Localized, early disease: • Amoxicillin, 500 mg po, q8h for 21 days Other manifestations: • Penicillin G, 20 × 10^6 IU iv, daily in divided doses for 14–30 days, or • Ceftriaxone, 2 g, daily for 14–30 days

Neurologic manifestations

Data on the treatment of neurologic manifestations are derived primarily from clinical experience. The tendency for spontaneous resolution of Bell's palsy, the fluctuating course of meningitis, the clinical variation of neurologic syndromes and the delayed emergence of the subtle deficits associated with late neurologic Lyme disease must all be considered in evaluating the clinical response to antibiotic therapy.[38,50,104] The emergence of chronic neurologic impairment years after remission of acute neurologic symptoms highlights the danger of complacency about the potential consequences of incomplete eradication of CNS infection.

Bell's palsy

Historically, Lyme-associated facial palsy has been treated with oral antibiotics, whereas other neurologic syndromes have been treated intravenously. It is unclear whether this distinction is warranted. Facial palsy itself resolves completely or almost completely in nearly all patients (121 of 122 patients in one series).[107] Patients who have facial palsy should undergo a careful neurologic evaluation, including a CSF examination. Cerebrospinal fluid invasion has been demonstrated by PCR in patients who have minimal CNS complaints and facial palsy, most of whom have clinically silent CSF pleocytosis.[40] If facial palsy is the only clinical abnormality and CSF is normal, current practice is to administer oral antibiotics for 21–30 days, a practice that has resulted in favorable outcomes. Long-term follow-up of this group is important and, if CSF examination is not possible, the preferred course at present is to administer intravenous antibiotics.

Meningitis

Intravenous penicillin for 10 days has been shown in clinical trials to be effective treatment of meningitis. In one small series, intravenous ceftriaxone for 14 days was superior to a 10-day course of penicillin.[108] Most experts prefer a 30-day course of treatment, however, because of the occasional occurrence of late neurologic relapses after shorter courses of therapy. It is not necessary to document clearing of all CSF abnormalities before discontinuation of therapy because clearing of inflammation may lag behind bacteriologic cure.[83] The co-occurrence of encephalopathy or encephalomyelopathy does not change this approach.

Radiculoneuropathy

Radiculoneuropathy is less clearly responsive to antibiotic therapy. Intravenous penicillin has not been shown to hasten resolution, and the response to ceftriaxone is unpredictable.[40,41] Current practice, however, is to administer intravenous antibiotic therapy based on favorable long-term outcome with this approach and the belief that radiculoneuropathy is driven by systemic infection. Resolution of neuropathy may be very gradual (taking place over months) and, with chronic involvement, this response may be incomplete. Doxycycline has been used orally and intravenously as an alternative to ceftriaxone

or penicillin, but experience with this agent is limited.[109] One patient who had severe neurologic involvement, unresponsive to penicillin, responded favorably to chloramphenicol.[110]

Arthritis

Arthritis may respond to either oral[111] or parenteral[112] antibiotic therapy, but antibiotic failures occur with either approach. Amoxicillin plus probenecid given orally for 4 weeks cures the majority of patients and those who fail oral therapy do not appear to respond to intravenous therapy. In a carefully carried out PCR study, no joint fluids were PCR positive in patients who had received at least 8 weeks of antibiotic therapy. The optimal duration of therapy is unknown but an initial course for 4 weeks is recommended.

The role for parenteral therapy for Lyme arthritis is unclear. Patients who have concurrent neurologic involvement should receive intravenous treatment. In one randomized study, intramuscular benzathine penicillin given weekly for 3 weeks cured less than one-half of patients, but intravenous penicillin for 10 days cured a higher percentage.[112] Ceftriaxone given for 14 days has been found to be more effective than 10 days of penicillin.[83] The primary reason for selection of intravenous therapy is concurrent neurologic involvement, in which case ceftriaxone or cefotaxime for 2–4 weeks are probably the agents of choice.

Resolution is commonly delayed, with synovitis persisting for months after completion of antibiotic therapy before eventually resolving. It has been suspected that administration of intra-articular corticosteroids during or before antibiotic therapy increases the risk of antibiotic failure, but a single intra-articular injection after completion of antibiotic therapy may hasten resolution. Patients who do not respond to a first course of therapy may respond to a repeat course, but there is no known rationale for a course of longer than 8 weeks. Adjunctive treatment measures should include evacuation of large effusions and limitation of weightbearing during acute attacks. Chronic inflammatory arthritis may occur through an autoimmune mechanism rather than as a result of persistent infection.

Late Lyme disease

Late Lyme disease, a designation reserved for those patients who have symptoms that persist for longer than 1 year, generally involves persistent inflammation in the CNS, joints or skin (ACA). Generally, patients who have ACA respond to oral penicillin. Late neurologic and arthritic involvement, however, are less predictably responsive. In one report, only one-half of patients who had late neurologic symptoms showed either resolution or sustained improvement after 6 months of follow-up after a 2-week course of ceftriaxone.[52] Those who did not respond, however, did not show progressive worsening. Long-term follow-up of this patient group is essential. In a European trial ceftriaxone and penicillin for 2 weeks were both effective for late neurologic involvement.[113]

Persistent arthritis after antibiotic therapy occurs most often in people who have HLA-DR4.[60] They are usually PCR negative and may be treated satisfactorily with arthroscopic synovectomy after failing to respond to either oral or intravenous antibiotics, suggesting that the pathogenesis of antibiotic-resistant arthritis may involve mechanisms other than persistent infection.[114] A recent study has provided strong evidence of an autoimmune mechanism for chronic Lyme arthritis.[96] Comparative trials of different antibiotics and varying durations of therapy are currently in progress for the treatment of late manifestations of Lyme disease, but there are no controlled data involving treatment periods longer than 4 weeks.

Pregnancy

Lyme disease acquired during pregnancy represents a special category because the health of the fetus must also be considered. Case reports have provided convincing evidence that *B. burgdorferi* can cross the placenta. Stillbirth[115,116] and neonatal death[19,20] have been attributed to *B. burgdorferi* transmitted from mother to fetus in utero, but the evidence to support this conclusion is still incomplete. The vast majority of pregnancies complicated by maternal Lyme disease have normal outcomes. *Borrelia burgdorferi* has not been linked statistically to congenital anomalies,[103] and no increased risk of an adverse outcome of pregnancy has been associated with asymptomatic seropositivity[117] or history of previous Lyme disease. It is appropriate to maintain a lower threshold for institution of aggressive antibiotic therapy for suspected Lyme disease during pregnancy, but women should be reassured that no cases of fetal Lyme disease have occurred with currently recommended antibiotic regimens. Doxycycline and other tetracyclines should be avoided during pregnancy.

Previous controlled clinical trials indicate that antibiotic therapy offers no sustained benefit to patients who have post-Lyme disease syndrome. Further research to elucidate the mechanisms underlying persistent symptoms after Lyme disease and controlled trials of new approaches to the treatment and management of these patients are needed.[115,118]

PREVENTION

If possible, it is preferable to prevent Lyme disease by personal protection and avoidance of prolonged tick attachment if a tick bite occurs. One obvious issue with regard to prevention is whether an individual with a known ixodid tick bite in a Lyme disease endemic area should be treated prophylactically with antibiotics. This question has been studied in a Lyme disease endemic area in Connecticut. A randomized, double-blind trial of amoxicillin therapy for tick bites[15] showed that, although the tick infection rate approached 15%, the risk of Lyme disease in untreated people was so low – 1.2% (95% CI 0.1–4.1%) – that prophylactic therapy (although probably effective) was not warranted. In a recent survey of pediatricians in an endemic area, 26% (70/267) routinely administered prophylactic antibiotics after tick bites.[119]

Nadelman *et al.*[120] advocated a single dose of oral doxycycline (200 mg) within 72 hours after removal of an *I. scapularis* tick. They found this to significantly reduce the incidence of Lyme disease (1/235 in the doxycycline group vs 8/247 in the placebo group). Many clinicians argue that this practice is unwarranted and may promote the development of antibiotic resistance.[121,122] Prophylactic antibiotics may be cost-effective in endemic regions if the risk of transmission following tick bites is greater than 0.01.[122] Personal protection including protective clothing measures such as wearing light-colored clothing to provide a background that contrasts with the ticks is often recommended.[83] The use of insect repellent containing *N,N*-diethyl-M-toluamide (DEET) and the prompt removal of ticks reduce the risk of Lyme disease.

Another area of active research involves efforts to develop a Lyme disease vaccine. A recombinant vaccine based on OspA, Lymerix, was approved for use in the past but was subsequently withdrawn from the market.[123] Efficacy was reported to be 79%[124] after three doses. New recombinant-based vaccines currently under development are expected to have a broad range of specificity against the variants of *B. burgdorferi* distributed throughout the world.[125,126]

REFERENCES

References for this chapter can be found online at http://www.expertconsult.com

Practice point 20

Barry D Brause

Postoperative infections in a patient with a prosthetic joint

INTRODUCTION

The major clinical issues involved in caring for an infected patient with an indwelling joint prosthesis are difficulties encountered in establishing the anatomic extent of involvement (i.e. is the prosthetic joint involved in the infection?), difficulties in selecting an appropriate therapeutic option once prosthetic joint infection has been diagnosed and difficulties in confirming the adequacy of the therapeutic approach chosen. Many of these clinical problems can be resolved by employing both evidenced-based judgments and clinical observations to select an appropriate option to satisfy the patient's orthopedic and infectious disease needs.

EXTRA-ARTICULAR INFECTIONS (AFTER IMPLANTATION OF A JOINT PROSTHESIS)

Postoperative suprafascial wound infections (such as suture abscesses, cellulitis and infected extra-articular hematomas) should be treated promptly by debridement (if needed) and antibiotic therapy, often intravenously, at least in part, for up to 2 weeks.

Urinary tract infections and pneumonia should also be treated promptly and for durations appropriate for the rapidity of the patient's clinical response. If the patient has blood culture evidence of bacteremia, then a course of intravenous antibiotic therapy for a minimum of 2 weeks should be administered. If the patient appears to have had a bacteremia (such as the presence of true rigors with fever or the presence of an intravenous site infection), this should be treated with at least a 2-week course of intravenous antibiotics.

PROSTHETIC JOINT INFECTIONS

Diagnosis

Prosthetic joint infection produces the cardinal symptoms of inflammation with a wide spectrum of severity. The frequencies of these presenting symptoms are listed in Table PP20.1. If a painful prosthesis is accompanied by a fever or purulent drainage from overlying cutaneous sinuses, infection may be presumed, pending further confirmatory tests. However, in most cases, infection must be differentiated from aseptic and mechanical problems (such as hemarthrosis, gout, bland loosening, dislocation, metallic debris-induced synovitis, osteolysis) which are more common causes of pain and inflammatory symptoms in these patients. Joint pain is nearly universally present. Constant joint pain is suggestive of infection, whereas mechanical loosening commonly causes pain only with motion and weight-bearing.

Table PP20.1 Presenting symptoms of prosthetic joint infection

Symptom	Frequency (%)
Joint pain	95
Fever	43
Periarticular swelling	38
Wound or cutaneous sinus drainage	32

Radiologic abnormalities are found in 50% of septic prostheses. They are generally related to the duration of infection because it may take 3–6 months for such changes to appear. If both distal and proximal components of a prosthetic joint demonstrate radiographic pathology, sepsis is more likely than simple mechanical loosening. However, all of these radiographic changes are nonspecific for infection as they are also seen frequently with aseptic processes.

Abnormal uptakes on radioisotopic scans (technetium bone scan, sequential technetium–gallium bone scanning, indium-labeled leukocyte scanning) are also too nonspecific or too variably sensitive to be able to carry the burden of establishing the diagnosis of prosthetic joint infection. Increased technetium diphosphonate uptake on bone scans is seen routinely around normal prostheses for 6 months after arthroplasty. A normal or negative technetium scan can be considered evidence against the presence of infection, but a positive radioisotopic scan, of any type, is not definitive in establishing the diagnosis of arthroplasty infection. Elevated peripheral or joint space leukocyte counts, erythrocyte sedimentation rates and C-reactive protein levels, although suggestive, also are inadequate to diagnose prosthetic joint sepsis.

The single observation that delineates the presence of implant infection is isolation of the pathogen by aspiration of joint fluid or by surgical debridement. Arthrocentesis demonstrates the pathogen with a sensitivity of 86–92% and a specificity of 82–97% in the larger surveys. Gram staining is positive in only 32% of cases. Fluoroscopic guidance and arthrography are useful to document accurate needle placement for prosthetic joint aspiration. If difficulty is encountered in obtaining intra-articular fluid, irrigation with sterile normal saline solution, without antiseptic preservative additives, can be used to provide the necessary fluid for culture. Since fastidious micro-organisms, including anaerobes, may be causative agents in prosthetic arthroplasty infections, multiple specimens should be obtained and rapidly cultured in appropriately supportive media. If initial cultures reveal a relatively avirulent organism (such as coagulase-negative staphylococci, corynebacteria, propionibacteria or *Bacillus* species), a second aspirate should be considered to re-confirm the bacteriologic diagnosis and to substantially reduce the possibility that the isolate is artifactual.

Table PP20.2 Diagnostic value of the number of positive operative cultures (for the same organism) when three to six specimens are examined

Number positive	Probability of infection (%)
3 or more	94.8
2	20.4
1	13.3
0	3.4

From Atkins BL, Athanasou N, Deeks JJ, *et al*. Prospective evaluation of criteria for microbiologic diagnosis of prosthetic joint infection at revision arthroplasty. J Clin Microbiol 1998;36:2932–9.

As operative debridement cultures are also used to definitively diagnose prosthetic joint infection, the patient should not receive antimicrobial therapy for several weeks prior to surgery and no antibiotic therapy should be given preoperatively. Optimally several (between five and seven) specimens of tissue, purulence and fluid should be submitted for culture. The diagnostic value of the number of positive operative cultures for the same organism has been calculated (Table PP20.2). If at least three specimens are submitted to the microbiology laboratory, then a finding of one positive culture represents a 13.3% probability of a prosthetic joint infection, two positive cultures represent a 20.4% probability and three positive cultures a 94.8% probability. The results of these microbiologic techniques should confirm the presence and nature of the infection and allow for optimal treatment.

In the uncommon circumstance in which the clinical suggestion of sepsis is strong but the cultures are sterile, fastidious micro-organisms, particularly anaerobes, should be suspected. Mycobacteria and fungi should also be considered as possible etiologic pathogens in appropriate clinical circumstances and specific cultures for them should be obtained. To design the most efficacious and least toxic antimicrobial therapy, the patient's infecting strain of bacteria must first be available for *in-vitro* evaluation.

Therapeutic options

The most predictably effective treatment for prosthetic joint infection involves complete removal of all foreign materials (metallic prosthesis, cement and any accompanying biofilm). Explantation and re-implantation can be accomplished in a single stage (exchange procedure) or in a two-stage approach. The most successful protocol incorporates standardized antimicrobial therapy with a two-stage surgical method:

1. Removal of the prosthesis and cement followed by a 6-week course of bactericidal antibiotic therapy chosen on the basis of quantitative *in-vitro* susceptibility studies.
2. Re-implantation at the conclusion of the 6-week antibiotic course.

Use of methylmethacrylate cement impregnated with an antimicrobial agent (usually tobramycin or gentamicin with or without vancomycin) has not been subjected to controlled trials but is commonly practiced during re-implantation if a cemented prosthesis is employed. The antimicrobial agents leach from the hardened plastic (cement) to produce variable but high initial release and protracted diffusion of antibiotic into surrounding tissues at the bone–cement interface (at high levels for approximately 2 weeks). Antibiotic-loaded polymethylmethacrylate is also commonly used in cement spacers inserted after prosthesis removal to maintain the structural anatomy and to facilitate subsequent re-implantation. With this protocol, a 90–96% success rate has been achieved in total hip replacement infections and a 97% success rate in total knee replacement infections. The success of this regimen relies on thorough debridement techniques and effective antimicrobial therapy.

Systemic antibiotic potency against the infecting pathogen is standardized by using the serum bactericidal test. Blood is drawn at a time representing 25% of the interval between doses:

- at 1 hour post-dose for drugs given every 4 hours;
- at 1.5 hours post-dose for drugs given every 6 hours;
- at 2 hours post-dose for drugs given every 8 hours;
- at 3 hours post-dose for drugs given every 12 hours; and
- at 6 hours post-dose for drugs given every 24 hours.

The serum bactericidal test is performed with a goal of producing a minimum 1:8 titer against all infecting pathogens. In this manner, both Gram-positive bacteria (including methicillin-resistant *Staphylococcus aureus* and enterococci) and Gram-negative bacilli (including *Pseudomonas aeruginosa*) can be eliminated if the specific sensitivity of each isolate allows eradication. The empirical selection of a 6-week duration of antibiotic therapy may be critical for efficacy. Others have employed a similar approach to therapy but with only a 2-week course of antibiotic treatment before implantation of the new prosthesis. With this shorter protocol, the pathogen was eradicated in only 79% of cases with only 35% of the patients obtaining good function with the new prosthesis.

The single-stage (exchange procedure) treatment involves extraction of the metallic joint and cement with immediate implantation of a new prosthesis. Antibiotic-loaded methylmethacrylate cement is used during this implantation. The protocol is effective in 70–83% of cases. If the exchange operation is repeated (in those for whom the first replacement failed), and antibiotic-loaded cement is used, the success rate can be increased to 90%. It has been suggested that this mode of therapy is applicable only to infections with the less virulent micro-organisms, because high failure rates are observed when the pathogen is *Staphylococcus aureus* or a Gram-negative bacillus. Systemic antibiotics are administered rarely and without standardization in this regimen. Moreover, the customary selection of an aminoglycoside as a component in the re-cementing phase of these operations may not reflect the susceptibility of the pathogen being treated. Currently, surgeons have the additional option of including vancomycin with the aminoglycoside in loading the cement. This should increase the effectiveness of this local, depot administration of antibiotic therapy.

Although the two-stage removal and re-implantation approach is more effective, the single-stage procedure is often used for elderly or infirm patients who might not tolerate protracted bed rest and a second major operation. Patients chosen for the single-stage approach should also have infecting organisms which are very sensitive to aminoglycosides with or without vancomycin since these are the antibiotics that can be used most successfully when incorporated into the polymethylmethacrylate cement used in this procedure.

In contradistinction to the previously described therapeutic approaches, treatment with retention of the infected prosthesis can be a consideration in specific clinical circumstances. In patients with prosthetic joints infected with penicillin-susceptible streptococci, with symptoms for 10 days or less, 89.5% were successfully treated without implant removal. Early postoperative infections (<1 month after implantation) and early hematogenous infections (<1 month of symptoms) treated without prosthesis removal have had reported success rates of approximately 71% in small numbers of patients receiving a single debridement followed by 4–6 weeks of antibiotic therapy. Some of these cases may have had false-positive cultures in this setting, which could inflate the success rate. Other studies of similar patient populations have had very low success rates, such as 23% of those who had symptoms for <2 weeks (100% failure rate in those with symptoms for >2 weeks) and 38% and 36% success rates in two additional studies. When the pathogen is *S. aureus*, the success rate was 50% if symptoms were present for 2 days or less; the success rate was 10% if symptoms were present for >2 days.

Success with long courses (3–6 months) of antibiotic therapy using rifampin (rifampicin) in combination with other antimicrobial agents has been reported in selected patients with retained orthopedic implants. Most of these infected implants were fracture-fixation devices and not septic total joint prostheses; infections of the latter

appear to be more difficult to cure. Nonetheless, rifampin appears to have an enhanced capacity to penetrate membranes and biofilms and therefore may be a critically important antibiotic in the treatment of infected prosthetic articulations without removal of the implant.

Deciding which therapeutic approach is appropriate in individual patients is highly dependent on isolating the pathogen. Knowledge about the infecting micro-organism is important in systemic antibiotic selection, in selecting antibiotics to be incorporated into polymethylmethacrylate spacers (or deciding not to use a spacer if the pathogen is resistant to incorporated antibiotics) and in deciding whether to use a single-stage or a two-stage therapeutic approach. The etiologic bacteria also must be available to measure the bactericidal potency of designed therapy (using the serum bactericidal test).

In those circumstances in which the clinical suggestion of sepsis is strong but the prosthetic joint cultures are sterile, anaerobic bacteria (such as *Peptostreptococcus*, *Bacteroides*, *Propionibacterium acnes*) should be suspected and empiric antibiotic regimens should be designed to include anaerobic coverage. Consideration should also be given to the presence of other fastidious pathogens such as mycobacteria and fungi. In addition, the possible masking effect of any prior antibiotic therapy (particularly within 6 weeks of the diagnostic cultures) should be borne in mind when designing the necessarily empiric antimicrobial therapies in these circumstances.

Suppressive antibiotic therapy

Suppressive oral antimicrobial therapy may be of value in selected cases in which:

- prosthesis removal is not possible;
- the prosthesis is not loose;
- the pathogen is relatively avirulent
- the pathogen is exquisitely sensitive to an orally absorbed antibiotic; and
- the patient can tolerate an appropriate oral antibiotic.

Successful retention of a functioning hip arthroplasty was observed in 63% of patients when all of the above five criteria were fulfilled. When similar therapy was employed in total knee replacements infected with a variety of micro-organisms (both virulent and avirulent), successful joint function was only variably maintained in 26–82% of patients. The suppressive approach is not without risk. Serial radiographs are needed over the course of treatment to monitor for progressive bone resorption at the bone–cement interface, which could reduce the success of any future revision surgery. Secondary resistance can emerge and, despite continual antibiotic therapy, the localized septic process can extend into adjacent tissue compartments or become a systemic infection. Moreover, the patient is subjected to the potential side-effects of chronic antibiotic administration.

Prevention

In view of the catastrophic effects of prosthetic arthroplasty infection, prevention of these septic processes is of prime importance. In anticipation of elective total joint replacement surgery, the patient should be evaluated for the presence of pyogenic dentogingival pathology, obstructive uropathy and dermatologic conditions that might predispose to infection and bacteremia. The use of prophylactic antibiotics in anticipation of bacteremic events (i.e. dental surgery, cystoscopy, surgical procedures on infected or contaminated tissues) has been suggested on the same empirical basis on which endocarditis prophylaxis is recommended. This approach to prevention is controversial, and no data are available with which to determine the adequacy or the cost-effectiveness of such measures. The American Dental Association and the American Academy of Orthopedic Surgeons have jointly advised that a single dose of prophylactic antibiotic be given to selected patients undergoing dental procedures associated with significant bleeding. Similarly the American Urologic Association and the American Academy of Orthopedic Surgeons have jointly advised that prophylactic antibiotics be considered in patients undergoing urologic procedures with higher bacteremia risk. Clinical decisions regarding prophylactic antibiotics for expected bacteremias in patients with prosthetic joints should be made on an individual basis.

FURTHER READING


Further reading for this chapter can be found online at http://www.expertconsult.com

Chapter 44

William A Lynn

Sepsis

This chapter examines the consequences of the systemic effects of infection, broadly referred to as sepsis.

EPIDEMIOLOGY

Definition and nomenclature

Physicians have long recognized that infection can lead to generalized circulatory collapse and death. In the 1990s Bone initiated a debate on the terminology of sepsis, identifying key clinical criteria and definitions.[1] Bone recognized that similar physiologic changes could occur in noninfective inflammatory processes, such as pancreatitis, and introduced the term the systemic inflammatory response syndrome (SIRS) to define a common set of diagnostic criteria for infected (sepsis) and noninfected patients.[1] The term SIRS has been criticized for being too imprecise[2] but the concept and definition of sepsis has remained.[3] It is important to remember that sepsis is a heterogeneous condition resulting from the interaction of host, pathogen and environmental factors. Some authors have questioned the validity of grouping all such patients together, particularly in research studies of new therapeutic agents.[4]

Severe sepsis (Table 44.1) may be defined simply as 'sepsis plus sepsis-induced organ dysfunction or tissue hypoperfusion'.[5] The challenge for clinicians is to recognize the presentation of sepsis/severe sepsis (Table 44.2) and intervene early.

Incidence and prevalence of sepsis

Host and environmental factors interact in the development of sepsis with varying rates in different patient populations. Furthermore, variation in the organization of health care in different medical systems makes comparisons between countries difficult. Angus and co-workers examined the epidemiology of sepsis in the USA.[6] Using existing databases they analyzed discharge records of over 6 million patients from seven US states during 1995 and extrapolated the results for the whole US population. Data collected in this way should be viewed with some caution but the size of this study demands attention. They estimated an annual incidence for severe sepsis of 3 cases per 1000 (2.26 cases/100 hospital discharges), equivalent to 751 000 cases each year. Incidence rose from 0.2/1000 in childhood to 26/1000 over the age of 85. Severe sepsis was estimated to cost around $16 billion per

Table 44.1 Definitions of infection-related syndromes

- *Bacteremia*: viable bacteria cultured from bloodstream – patient may or may not have evidence of sepsis/severe sepsis
- *Sepsis*: infection, documented or suspected, plus systemic manifestations (see Table 44.2)
- *Severe sepsis*: sepsis-induced organ dysfunction or tissue hypoperfusion (see Table 44.2)
- *Septic shock*: sepsis-induced hypotension not responding to fluid resuscitation

Table 44.2 Clinical signs and laboratory parameters in the diagnosis of sepsis

Infection – documented or suspected, plus some of the following:	
Clinical signs	Fever temperature >110.4° F (38.3° C) or Hypothermia temperature <96.8° (36° C) Heart rate >90 beats/min Hypotension SBP <90 mmHg, MAP <70 mmHg, SBP fall of >40 mmHg from baseline Prolonged capillary refill time or tissue mottling – indicates poor tissue perfusion Tachypnoea: respiratory rate >20 breaths/min or arterial CO_2 tension <32 mmHg (4.3 kPa) Altered mental state from baseline
Inflammatory markers	WBC >12 000 cells/mm^3, <4000 cells/mm^3 or >10% immature forms (bands) Plasma C-reactive protein >2× normal range Procalcitonin >2× normal range
Organ dysfunction	Metabolic acidosis: elevated base deficit ≥5, increased blood lactate Hypoxia: Pao_2/Fio_2 <300 Oliguria: urine output <0.5 ml/kg for >2 h despite fluid resuscitation Creatinine rise from baseline >40 µmol/l Coagulopathy: INR >1.5 in absence of anticoagulation, platelet count <100 × 10^9/l Hyperbilirubinemia: bilirubin >4 mg/dl or 70 µmol/l Hyperglycemia or hypoglycemia in nondiabetic

INR; international normalized ratio; MAP, mean arterial pressure; SBP; systolic blood pressure; WBC, white blood count.
Adapted from Dellinger *et al*.[5]

year. In a separate study, Martin *et al*. reported similar figures and also that the incidence of sepsis in the USA was rising at around 9% per year, reaching 240/100 000 in 2000.[7] A review of 330 million emergency department attendances in the USA showed that 0.69% were for severe sepsis.[8]

In Europe Van Gestel *et al*. performed a point prevalence study in 47 intensive care units (ICU) across the Netherlands, showing that 29.5% of patients fulfilled severe sepsis criteria.[9] Extrapolating from these data indicated that severe sepsis was responsible for 0.61% of all hospital admissions but 11% of admissions to intensive care in The Netherlands. Similar data showed a point prevalence for severe sepsis in ICU admissions of 28.7% in the UK[10] and 27% in France.[11] In Australia and New Zealand severe sepsis has been estimated in 0.77 per 1000 of the population and is responsible for 11.8/100 ICU admissions.[12]

Morbidity and mortality rates

The mortality of sepsis varies because of variations in infection prevalence, case definition, ICU facilities and patient populations. In an analysis of four large sepsis trials, 14-day mortality averaged 26% and 28-day mortality 42%.[1] In the Angus study cited above overall mortality was 28.6%, equivalent to 215 000 deaths per annum in the USA.[6] In this study mortality in childhood was around 10%, rising to 38.4% in those over age 85.[6]

Factors associated with early death include the number of organ systems involved,[13] acidosis, and shock.[14] Late deaths are associated with medical co-morbidities, underlying illness and multiple sources of infection. *Pseudomonas aeruginosa*, *Candida* spp. and mixed infections have a higher attributable mortality than other agents. Patients with sepsis often have serious co-morbidity, but even when this is taken into account the attributable mortality from severe sepsis is still 25%,[15] increasing to over 60% if four or more organs are failing.[13] Long-term mortality is high after an episode of severe sepsis.[16] Some studies have suggested that while the incidence of severe sepsis continues to increase, there has been a recent improvement in mortality, possibly due to the introduction of a more systematic approach to recognition and therapy.[10,17]

Risk factors for sepsis

Broadly speaking, risk factors for sepsis are those factors that weaken/breach host defenses, increasing the likelihood of bacterial invasion of otherwise sterile tissue as summarized in Figure 44.1.

PATHOGENESIS AND PATHOLOGY

Etiologic agents of sepsis

Bacteria are the commonest underlying pathogens in patients with community and hospital-acquired sepsis. Nonbacterial pathogens may also induce the pathologic responses leading to sepsis, including fungi, rickettsiae, protozoa and certain viruses. The epidemiology of community-acquired sepsis varies considerably between tropical and nontropical zones. Alterations in host immunity also influence the nature of the underlying organisms in sepsis.

Historically, Gram-negative bacteria were most commonly associated with septic shock but a shift in etiology occurred during the 1990s, with increasing infection due to Gram-positive bacteria and

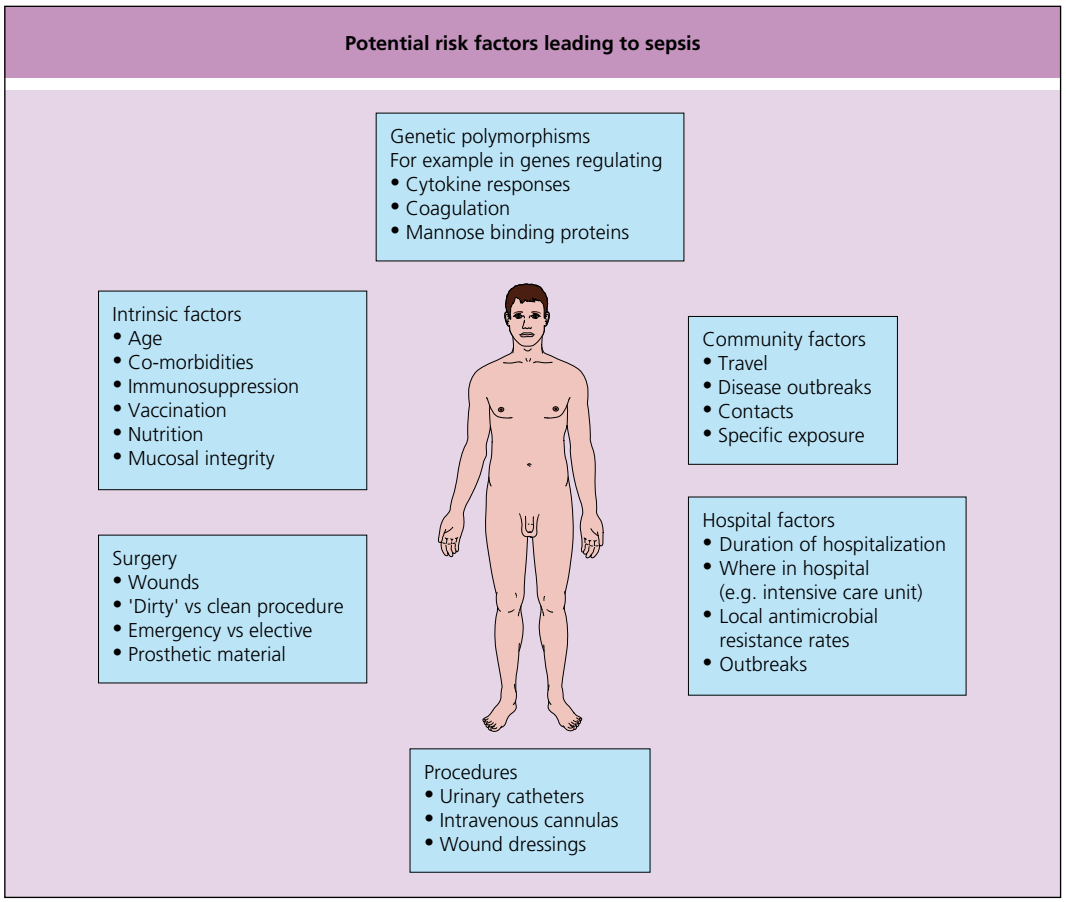


Fig. 44.1 Potential risk factors leading to sepsis. Complex interaction of factors that may influence both the susceptibility to and clinical outcome of severe sepsis.

fungal pathogens particularly within the ICU.[13,18] Vincent *et al.* surveyed over 3000 ICU patients across Europe in the SOAP study. A microbiologic cause was only identified in 60% of patients with severe sepsis which is comparable to other studies and indicates the need for better diagnostic tests. Gram-positive bacteria were responsible for 40%, Gram-negative bacteria 38% and fungi (mainly *Candida* spp.) in 17% of cases. Twenty percent of patients were bacteremic, with pulmonary, abdominal, skin and urinary tract the most common local sites of infection.[13]

The epidemiology of severe bacterial sepsis continually changes in response to the emergence of new bacterial strains and alterations in host or environmental factors. Recent years have seen the emergence of community-acquired methicillin-resistant *Staphylococcus aureus* (MRSA)[19] and more virulent strains of *Clostridium difficile*[20] as causative agents of severe sepsis in the USA with subsequent spread to other parts of the world.

Bacterial products involved in sepsis

Bacteria produce a variety of exotoxins and endogenous cell wall products that induce proinflammatory responses *in vitro* that may culminate in sepsis *in vivo*. Such products are released by bacteria during local or systemic infection and, in turn, may have local or distant proinflammatory effects.[21] The innate immune system is our first line of defense against invading micro-organisms and immune activation through this route is central to the pathogenesis of sepsis.

Innate immune recognition of microbial products

In recent years the concept of pathogen recognition systems triggering an initial, and very rapid, innate immune response to pathogens has emerged.[21] Pathogen-associated molecular patterns (PAMPs) are the microbial products that trigger these receptor recognition systems that are found in almost all higher eukaryotes.[22] The same receptor systems may be triggered by other harmful stimuli known as damage-associated molecular patterns (DAMPs). The relevance of this to severe sepsis is that innate immunity is, in most cases, the first defense mechanism against invading organisms and this activation system plays a pivotal role in setting in motion the inflammatory and anti-inflammatory processes that determine whether the infection resolves, disseminates or leads to sepsis (Fig. 44.2).

Identification of the Toll-like receptor (TLR) family and the recognition of the central role of TLR in the innate response to infection is one of the most important discoveries in understanding the molecular basis for severe sepsis.[23] Toll receptors were first identified as important in the recognition of fungi in *Drosophila* and have been recognized in widely diverse organisms including plants, animals and insects. To date, 10 functional Toll receptors have been identified in humans; they are involved in the recognition and response to PAMPs and DAMPs (Table 44.3).[24] Further elucidation of the Toll receptor pathway and signaling in sepsis may lead to a greater understanding of the molecular basis for severe sepsis in different infections and lead to new therapeutic targets.[25] Endotoxin (lipopolysaccharide) is one of the most potent PAMPs and cellular activation by endotoxin is examined in more detail below.

Gram-negative bacterial endotoxin

Endotoxin (lipopolysaccharide, LPS) is found only in Gram-negative bacteria, where it forms part of the outer leaflet of the bacterial cell wall (Fig. 44.3). It consists of a polysaccharide domain covalently bound to a unique di-glucosamine-based phospholipid, lipid A (Fig. 44.3), which is the key toxic moiety of LPS. Lipid A and LPS induce a wide range of both proinflammatory and counter-regulatory responses *in vitro* and *in vivo*. In human volunteers, administration of small amounts of endotoxin induce fever and release of cytokines including tumor necrosis factor (TNF), interleukin (IL)-1 and IL-6.[26]

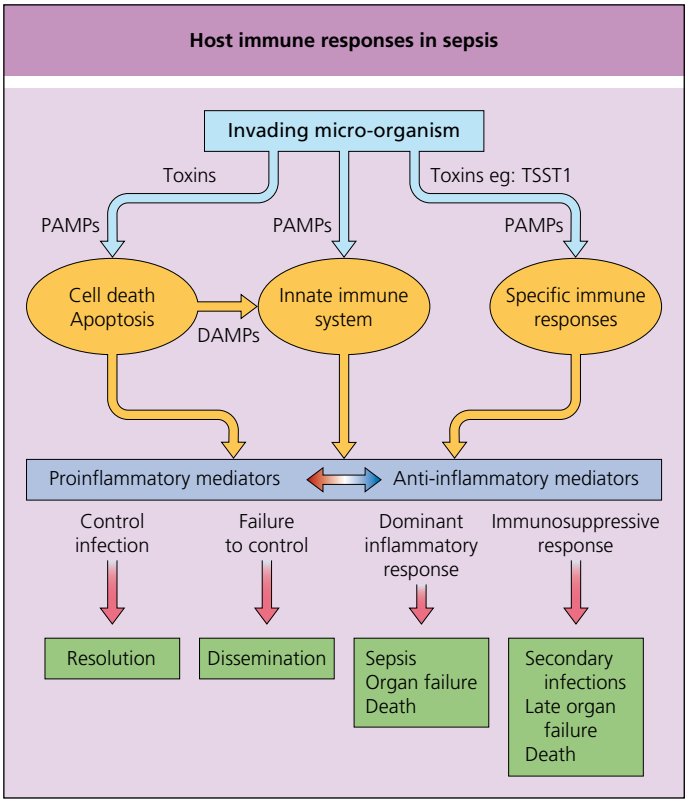


Fig. 44.2 Host immune responses in sepsis. Invading pathogens may activate the immune system by release of soluble toxins or by interaction of structural components (pathogen-associated molecular patterns, PAMPs) with the Toll-like receptor pathway. Immune activation releases a variety of inflammatory mediators that may have local or distant actions. Cell death and tissue damage may result from the direct effect of bacterial toxins or as a result of the inflammatory cascade initiated by the infection. Some host products released following cell damage are damage-associated molecular patterns (DAMPs) and may lead to further immune activation. Anti-inflammatory mediators are also released as part of a feedback loop to limit inflammatory damage. If the balance between proinflammatory and anti-inflammatory mechanisms is correct then localization and resolution of infection will occur. If there is a hyperinflammatory response then there is a risk of sepsis, organ damage and death. If an anti-inflammatory pathway predominates then there is an increased risk of secondary infection leading to further episodes of sepsis and death.

The cellular interaction of LPS is complex, involving both serum LPS-binding proteins and cell-bound and soluble LPS receptors[27] (Fig. 44.4).

Secreted toxins – exotoxins

Exotoxins are released by bacteria and other pathogens, and can cause substantial damage through direct toxic effects or by provoking inflammatory responses (Table 44.4). Type 1 toxins, such as superantigens, do not directly enter cells but bind to surface receptors, triggering specific responses. Type 2 toxins cause direct cell damage and there are many examples of these that are linked to bacterial pathogenesis (e.g. DNAse or protease enzymes). A good example discussed earlier is the emergence of a highly virulent strain of community-acquired MRSA associated with skin and soft tissue infections, necrotizing pneumonia and severe sepsis.[19] Pathogenicity is linked to the Panton–Valentine leukocidin (PVL) gene. PVL is directly toxic to neutrophils and the purified toxin can cause necrotizing pneumonia and sepsis in experimental models.

Table 44.3 Toll-like receptor binding ligands relevant to sepsis

Source	Stimulus	Released by	Receptor
Bacteria	Lipopolysaccharide, endotoxin, LPS	Gram-negative bacteria	TLR4
	Peptidoglycan	Gram-positive bacteria	TLR2
	Lipoteichoic acid	Almost all bacteria	TLR2
	Triacyl lipopeptides	Almost all bacteria	TLR1, TLR2
	Porins	*Neisseria* spp.	TLR2
	Flagellin	Flagellated bacteria	TLR5
	CpG DNA	All bacteria	TLR 9
Fungi	Zymosan	*Saccharomyces*	TLR2, TLR6
	Phospholipomannan	*Candida albicans*	TLR2
	Mannan	*Candida albicans*	TLR4
	O-linked mannosyl residues	*Candida albicans*	TLR4
	Glucans	*Candida albicans*	TLR2
Parasites	Glycosylphosphatidylinositol	*Plasmodium falciparum*	TLR2
Human cells	Heat shock proteins	Tissue damage	TLR4
	Fibronectin	Tissue damage	TLR4
	Hyaluronic acid	Tissue damage	TLR4
	Biglycans	Tissue damage	TLR4
	HMGB1	Tissue damage	TLR2, TLR4

HMGB1, high-mobility group box 1 protein; TLR; Toll-like receptor.
Adapted from van der Poll & Opal.[21]

A third type of bacterial exotoxin is formed of A and B subunits; the A subunit mediates cell entry and the B component is cytotoxic. A good example of the relevance to severe sepsis has come with the emergence of a hypervirulent strain of *C. difficile* known as NAP1/027.[20] Virulence of *C. difficile* NAP1/027 is linked to mutation of a toxin suppressor gene leading to increased production of both A and B toxins. Patients with infection due to NAP1/027 have more symptomatic disease and higher rates of toxic megacolon, severe sepsis and death.

Superantigenic toxins

Superantigenic bacterial toxins can cause profound hypotension, inflammation and organ failure.[28] Strains of *Staph. aureus* and *Streptococcus pyogenes* that are able to express these toxins are implicated in the pathogenesis of staphylococcal and streptococcal toxic shock. The principle behind immune activation by superantigens is outlined in Figure 44.5. Essentially, the bacterial toxin is capable of bypassing the normal highly antigen-specific mechanisms of T-lymphocyte activation by directly linking major histocompatibility complex (MHC) II on antigen-presenting cells to the Vβ subunit of the T-lymphocyte receptor. This triggers cell activation and massive cytokine release which, in turn, can lead to severe sepsis and organ failure.[29] Whether an individual infected with a superantigen-producing organism develops toxic shock is complex depending on the quantity of toxin produced, the presence or absence of pre-existing neutralizing antitoxin antibodies and on the composition of an individual's MHC molecules and T-lymphocyte repertoire of Vβ subunits.[30] An epidemic of menstruation-associated staphylococcal toxic shock syndrome occurred in the 1970s linked to use of a particular type of superabsorbent tampon that enhanced proliferation and increased toxin production by staphylococci in the vagina.[31]

Host genetic factors in susceptibility to sepsis

One of the most intriguing aspects of severe sepsis is why one individual will respond to a particular infection with devastating consequences of septic shock whilst another resolves a similar infection. A full discussion is beyond the scope of this chapter but it is clear that genetic variation explains aspects of the human response to infection. Animal models and some human studies have linked polymorphisms in key bacterial response genes, including cytokine, mannose-binding protein, CD14 and Toll-receptor genes, to outcome in severe sepsis.[30,32] Human leukocyte antigen (HLA) type II polymorphisms have been linked to responses to superantigens.[33] Further understanding of the genetic basis for response to infection may lead to targeted therapies for patients with sepsis.

Host responses in severe sepsis

Some of the central pathways leading to tissue damage in severe sepsis are shown in Figure 44.5 and key mediators are listed in Table 44.5. Selected pathogenetic mechanisms are discussed in more detail below.

Inflammatory and anti-inflammatory cytokines

Historically much attention was given to the search for the 'key mediator' of sepsis and the focus has at various times settled on complement, arachidonic metabolites, kinins, lipid mediators and cytokines. Rather than being due to one mediator, severe sepsis is the result of a complex interaction of inflammatory and anti-inflammatory processes as depicted in Figure 44.5.

Throughout the 1990s attention focused on the cytokines TNF, IL-1 and interferon gamma (IFN-γ). The evidence that proinflammatory cytokines, such as TNF and IL-1, play a causative role in sepsis stems from several lines of research. First, IL-1 and TNF can induce septic shock in animals and inhibitors of these cytokines can prevent the onset of sepsis in these models.[34] Secondly, mice deficient in the genes encoding receptors for TNF are resistant to septic shock induced by endotoxin. Finally, these cytokines are elevated in human sepsis, and high levels of cytokines correlate with a poor outcome in some but not all studies. TNF is released early in response to LPS challenge,[26] and the cooperative effects of TNF, IL-1 and IFN-γ in producing inflammatory responses made these three cytokines prime targets for experimental intervention in sepsis.

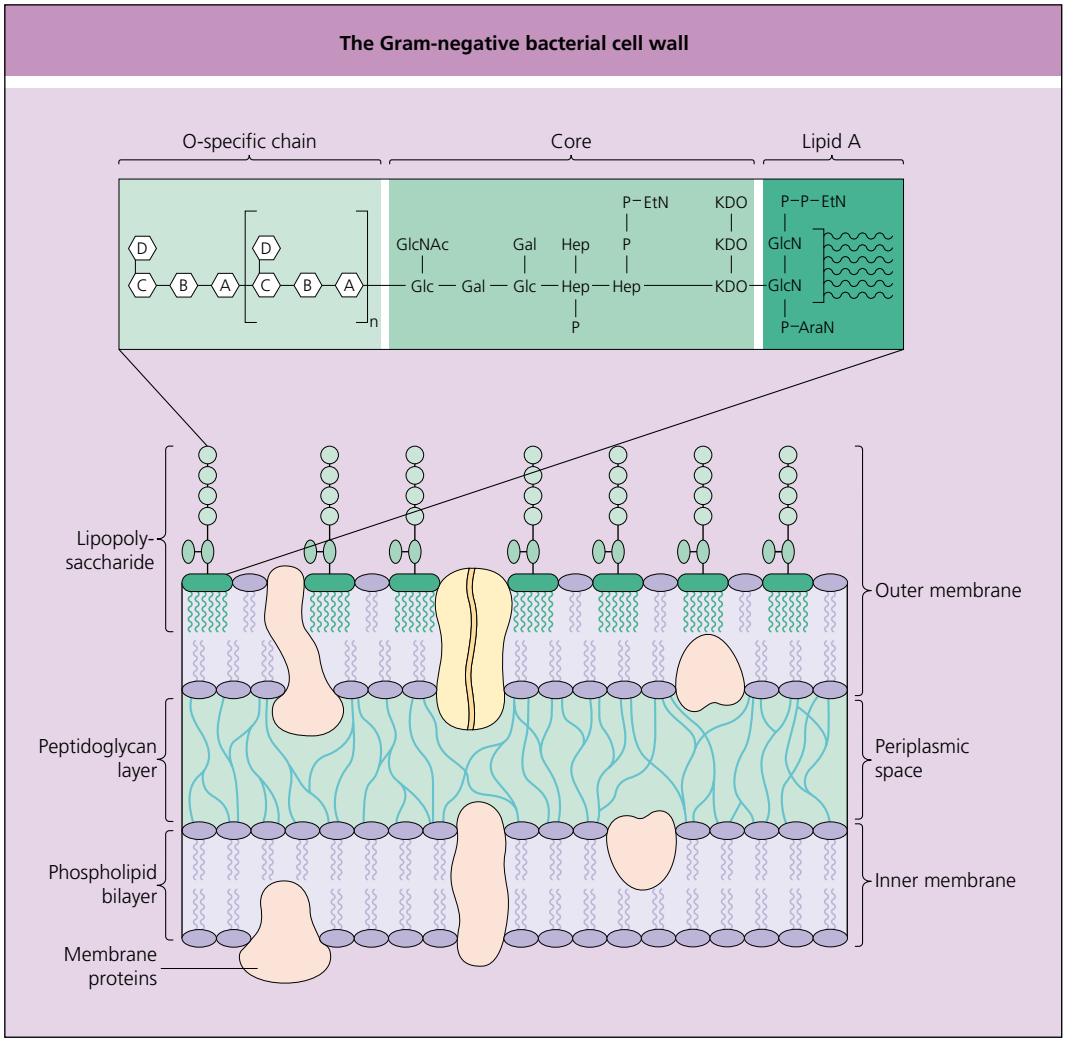


Fig. 44.3 The Gram-negative bacterial cell wall. In the cell wall of a Gram-negative bacterium such as *Escherichia coli*, the inner membrane is composed of phospholipids and membrane proteins and is separated from the outer membrane by the periplasmic space and peptidoglycan. Lipopolysaccharide (expanded box) is found only in the outermost leaflet of the outer membrane with the lipid A moiety in the membrane and the polysaccharide (O) side chain directed outwards. Lipid A is highly conserved across Gram-negative bacteria and consists of a phosphorylated diglucosamine backbone decorated with six or seven acyl side chains. Dephosphorylation or deacylation of lipid A abrogates its toxicity. Lipid A is covalently linked to an inner core of sugar residues that is relatively well conserved across species and antibodies directed against the core may protect against challenge with heterologous Gram-negative bacilli. The core is followed by an outer polysaccharide chain, of repeating sugar residues, that varies between different bacterial strains (O antigen). Antibodies directed against the O antigen will only protect against challenge with that individual bacterial strain. Gal, D-galactose; Glc, D-glucose; GlcNAc, *N*-acetyl-D-glucosamine; Hep, L-glycero-D-manno-heptose; KDO, 3-deoxy-D-manno-octuylosonic acid.

High-mobility group box 1 protein (HMGB1) has attracted attention as a late mediator of inflammatory responses in sepsis.[35] HMGB1 does not appear until more than 8 hours after the onset of sepsis. It is a nuclear protein found in all human cells and is released from macrophages and natural killer cells following infectious challenge. Many cell types following cell injury also release it. HMGB1 is released following apoptosis and anti-HMGB1 monoclonal antibodies improve outcome in animal models of sepsis.[36]

Macrophage migration inhibitory factor (MIF) is released by a variety of cells during sepsis and also in response to glucocorticoids. MIF appears to be important in sepsis pathogenesis as it enhances release of other proinflammatory cytokines, including TNF, and upregulates macrophage expression of TLR4 which increases responsiveness to LPS.[37] Furthermore, MIF prolongs macrophage life by delaying apoptosis. These actions will tend to sustain a proinflammatory profile, potentially worsening tissue damage, and blockade of MIF protects animals from lethal sepsis.

In addition to the large number of proinflammatory mediators induced during severe sepsis, there are many counter-regulatory mediators (see Table 44.5). Counter-regulatory mediators have specific actions and may neutralize or detoxify bacterial products, block the synthesis or release of cytokines, neutralize circulating inflammatory mediators or downregulate cell responses to inflammatory stimuli. Thus, it is the balance between these opposing mechanisms that will determine whether inflammation is successful in eliminating infection and then 'shutting down', or progressing so that healthy tissues become victims of 'friendly fire' (see Fig. 44.5). In individual patients with severe sepsis it may be this balance between the proinflammatory and anti-inflammatory processes that determines whether infection resolves, disseminates or leads to severe sepsis.

Coagulopathy

Widespread activation of coagulation pathways is common in sepsis and disseminated intravascular coagulation (DIC) may occur as a result. Bacterial products and inflammatory mediators can activate coagulation, anticoagulation and fibrinolytic pathways with a net procoagulant profile. Activated monocytes and endothelial cells increase secretion of tissue factor, which initiates the coagulopathy

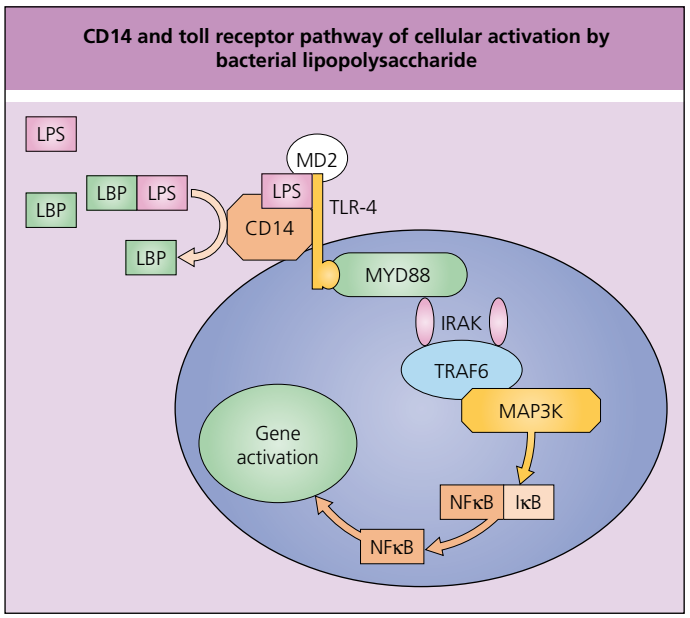


Fig. 44.4 CD14 and toll receptor pathway of cellular activation by bacterial lipopolysaccharide. Schematic representation of events at the inflammatory cell surface. Lipopolysaccharide (LPS), either free or as part of lipoprotein complexes, is bound by LPS-binding protein (LBP) in the fluid phase. The LPS–LBP complex binds to the cell surface receptor CD14 on neutrophils and macrophages. CD14 lacks an intracellular domain and acts as a co-receptor presenting LPS to toll-like receptor 4 (TLR-4), leading in turn to activation of intracellular signaling and gene activation. IκB, inhibitor of kappa B; IRAK, interleukin 1 receptor-associated kinase; MAP3K, mitogen-activated protein 3-kinase; MYD88, myeloid differentiation factor 88; NFκB, nuclear factor kappa B; TRAF6, tumor necrosis factor receptor-associated factor 6.

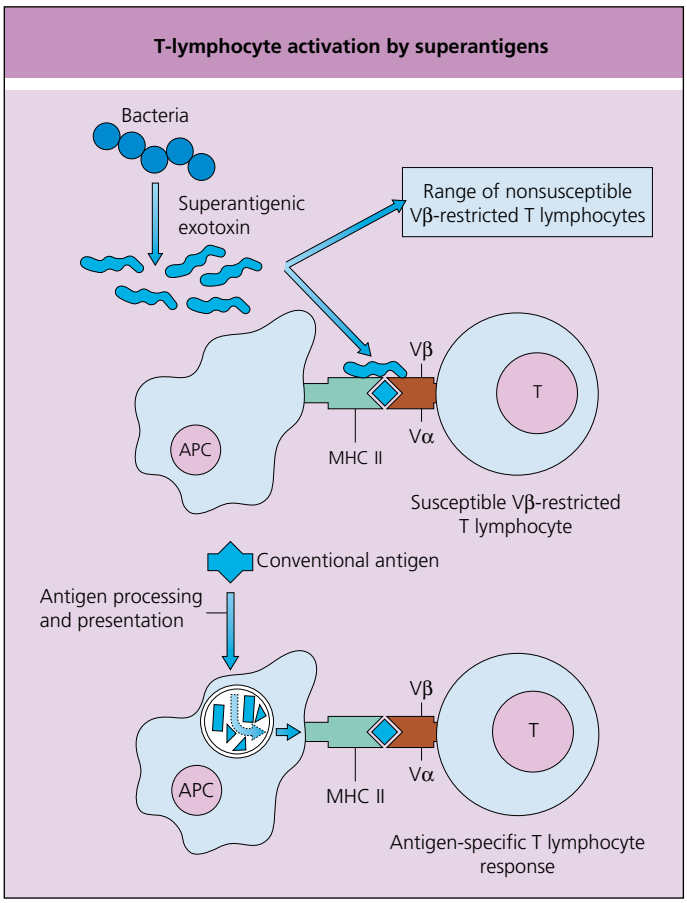


Fig. 44.5 T lymphocyte activation by superantigens. In the conventional response to bacterial antigens (bottom) the antigen is processed and presented by the antigen-presenting cell (APC) in association with MHC II. Only T lymphocytes with the correct antigen recognition site can then be activated (i.e. this is a highly antigen-specific process). Superantigens (top) are able to bypass this process by bridging between MHC II and the Vβ subunit of the T-lymphocyte receptor. Thus the entire population of T lymphocytes expressing that particular Vβ subunit can be activated; this can be up to 20% of the total T-lymphocyte population.

Table 44.4 Examples of bacterial exotoxins in sepsis

Category	Source	Examples
Pore-forming exotoxins	*Staphylococcus aureus*	α-Hemolysin Panton–Valentine leukocidin (PVL)
	Streptococcus pyogenes	Streptolysin-O
	Escherichia coli	*E. coli* hemolysin
	Aeromonas spp.	Aerolysin
Superantigens	*Staph. aureus*	Toxic shock syndrome toxin 1, enterotoxins A–F
	Strep. pyogenes	SPEA, SPEC, SMEZ
Enzymes	*Staph. aureus*	Coagulase, DNAse, proteases
	Strep. pyogenes	IL-1β convertase, proteases
	Clostridium perfringens	Phospholipase C

IL-1, interleukin-1; SPE, streptococcal pyrogenic exotoxin.

seen in sepsis.[38] Involvement of platelets and von Willebrand's factor may also stimulate coagulation.

Anticoagulant pathways involve antithrombin II, activated protein C and tissue-factor pathway inhibitor (TFPI) which are important in counteracting the procoagulant effect of inflammation.[38] Plasminogen activator inhibitor type 1 (PAI-1) is important in controlling fibrinolysis. Occlusion of small blood vessels during DIC leads to further tissue damage. There is a high risk of bleeding due to thrombocytopenia and exhaustion of clotting factors and activated protein C. This is seen in its most extreme in purpura fulminans in meningococcal bacteremia. Modulation of coagulation has been a therapeutic target in severe sepsis and recombinant human activated protein C is licensed for use in severe sepsis with a high risk of mortality.

Nitric oxide

Nitric oxide (NO) may be involved in the pathophysiology of sepsis through a number of mechanisms.[39] First, NO is a free radical and has the ability to kill phagocytosed organisms. Second, NO is a potent vasodilator and a basal level of NO is required for the maintenance of normal arteriolar tone. Synthesis of NO is increased by a number of bacterial products, including LPS and cytokines. In particular, TNF, IL-1 and IFN-γ appear to increase NO synthesis synergistically. Sepsis is characterized by widespread peripheral vasodilatation and loss of the normal regulation of tissue blood flow. There is considerable experimental evidence in animals to suggest that the vasodilatation in sepsis is mediated by increased NO levels. In humans, elevated urinary nitrite levels are found in sepsis, implying increased NO synthesis.

Table 44.5 Examples of important soluble mediators in sepsis

Target	Proinflammatory mediators	Inhibitory or counter regulatory mediators
Monocyte/ macrophage	TNF, IL-1, IL-8, IL-6, IL-12, IFN-γ, tissue factor, prostanoids, kinins, leukotrienes, PAF, NO, MIF, HMGB1	IL-1 Ra, sTNFr, TGF-β
Neutrophils	Integrin expression, superoxide production, kinins	BPI, CAP 57, defensins, acyloxyacylhydrolase
Lymphocytes	IFN-γ, IFN-β, IL-2	IL-4, IL-10, sIL-2r
Endothelia	Selectins, VCAM, ICAM, NO, tissue factor	Tissue factor pathway inhibitor
Platelets	Serotonin, prostanoids	PDGF
Coagulation pathway	Tissue factor, coagulation cascade leading to thrombin generation	TFPI, AT-III, PAI
Plasma components	Complement activation, bradykinin	Lipoprotein complexes, LBP, CRP

AT-III, antithrombin III; BPI, bacterial/permeability-increasing protein; CRP, C-reactive protein; HMGB1, high-mobility group box 1 protein; ICAM, intercellular adhesion molecule; IFN, interferon; IL, interleukin; LBP, lipopolysaccharide-binding protein; MIF, macrophage migration inhibitory factor; NO, nitric oxide; PAF, platelet-activating factor; PAI, plasminogen activator inhibitor; PDGF, platelet-derived growth factor; sIL-2r, soluble IL-2 receptor; sTNFr, soluble tumor necrosis factor receptor; TFPI, tissue factor pathway inhibitor; TGF-β transforming growth factor beta; TNF, tumor necrosis factor; VCAM, vascular cell adhesion molecule.

Complement

Activation of complement leads to a proteolytic cascade resulting in the liberation of molecules that can act as opsonins and chemoattractants, and can also mediate bacterial lysis. Lipopolysaccharide and other bacterial products activate complement by the alternative pathway. Massive local activation of complement during sepsis will fuel the inflammatory response by recruiting neutrophils, and through the activation of kinins and histamine will contribute to increased endothelial permeability and capillary leakage. C5a is increased during severe sepsis and C5a receptors are upregulated.[40] C5a recruits neutrophils and other inflammatory cells to sites of inflammation. C5a also activates neutrophils by increasing the release of neutrophil granule proteins and prolongs neutrophil lifespan by inhibiting apoptosis. Blockade of C5a improves outcome in models of severe sepsis.

Neutrophil–endothelial cell interactions

An early response to the local production of inflammatory mediators is the upregulation of adhesion molecules on endothelial cells and the release of chemokines that recruit neutrophils and other inflammatory cells to the site of infection. Exposure of neutrophils to LPS and some cytokines enhances the ability of neutrophils to release inflammatory molecules and primes neutrophils for the generation of oxygen radicals and proteolytic enzymes. These mechanisms enable neutrophils to survive longer in an inflammatory environment and to kill more bacteria, but excessive activation of endothelial adhesion may result in the migration of primed activated neutrophils into sites distant from infection. The ensuing tissue damage stimulates a vicious cycle of re-recruitment of inflammatory cells that may ultimately lead to organ damage and failure.

Immunosuppression as a result of sepsis

Sepsis is a dynamic condition and the dominant pathologic processes can change rapidly. After the initial inflammatory episode of sepsis patients may enter a phase of relative immunosuppression.[41] This can be followed by recovery but additional infectious or noninfectious insults may trigger further inflammatory responses, relapse of severe sepsis and worsening of tissue damage (see Fig. 44.5).

The mechanism underlying immunosuppression is multifactorial. The responses of immune cells may be blunted for a period of time with neutrophils, monocytes and lymphocytes releasing fewer inflammatory mediators for a given stimulus.[42] In part this is due to downregulation of cellular receptors, anti-inflammatory cytokines such as IL-10 and transforming growth factor beta. In addition, evidence has accumulated that sepsis can trigger apoptosis and cell death of a variety of cell types including lymphoid cells.[43] The contribution of sepsis-related immunosuppression to mortality is not fully understood and experimental approaches to boost immunity after the initial phase of sepsis are under evaluation.

Pathophysiologic events leading to organ failure in sepsis

How do the complicated and often confusing inflammatory responses outlined above lead to tissue damage and eventually multiorgan failure? At the cellular level, the end result of the inflammatory process is apoptosis and cell death. In part this is mediated by local hypoxia but apoptosis may also be induced by some proinflammatory mediators. In the whole organism the key to organ damage lies in the breakdown of normal vascular homeostasis, oxygen delivery and oxygen utilization by tissues. This leads to hypoxia and lactic acidosis which further impair organ function (Fig. 44.6). All organs may be affected but clinically the most important are depressed myocardial function, alterations in the peripheral vasculature and failure of oxygen exchange in the lung.

Cardiac function

One of the features of severe sepsis is peripheral vasodilatation, which should lead to an increase in cardiac output to compensate. The rise in cardiac output is often less than that predicted and it is recognized that most patients who have severe sepsis have impaired myocardial function. Cumulative evidence suggests that the impairment of myocardial contractility seen in sepsis is a consequence of circulating cytokines. Tumor necrosis factor directly reduces myocyte contractility and induction of myocardial NO synthesis by cytokines also impairs myocardial function. IL-6 has also been shown to impair myocardial function.

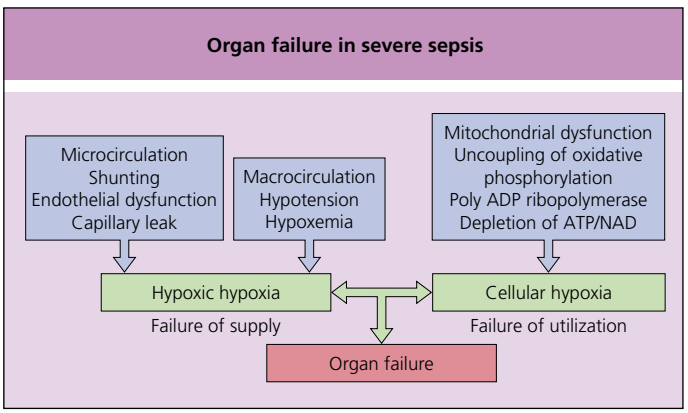


Fig. 44.6 Organ failure in severe sepsis. Organ dysfunction and finally organ failure is the result of cellular hypoxia and acidosis. Hypoxia and acidosis result both from a failure of oxygen delivery due to disturbances in circulation and limitation of the cellular ability to utilize oxygen as a result of mitochondrial dysfunction.

Peripheral vasculature

The hallmark of severe sepsis is widespread peripheral vasodilatation and, in refractory septic shock, it may resist all attempts at pharmacologic intervention. As mentioned earlier, this is largely the consequence of increased NO production in response to cytokines. The most serious consequence of the vasodilatation is the loss of homeostatic regulation of tissue blood flow. Thus, much of the circulating blood volume is shunted through capillary beds, bypassing deep tissues and reducing the opportunity for oxygen extraction. This in turn exacerbates tissue hypoxia and helps to drive the metabolic acidosis that is one of the prominent features of severe sepsis. Further local impairment of perfusion may occur as the result of aggregation of platelets and activation of coagulation in the microcirculation.

Renal failure

Renal impairment is common in severe sepsis and is related to impairment of the renal microvasculature reducing renal perfusion and glomerular filtration followed by the development of acute tubular necrosis.[44]

Respiratory failure

The lung is one of the most vulnerable organs in sepsis, and TNF, platelet-activating factor (PAF), C5a, IL-8 and thromboxane appear to play prominent roles in the development of the adult respiratory distress syndrome (ARDS). The passage of neutrophils across the endothelium and subsequent degranulation in the lung interstitium leads to impairment of endothelial integrity and the accumulation of fluid and inflammatory cells in the alveolar spaces. Impaired gas exchange inevitably produces hypoxia, which further worsens the situation. The combination of hypoxia and increased pulmonary thromboxane synthesis increases pulmonary vascular resistance, in contrast to the fall in systemic vascular resistance. Thus the end result of the pulmonary events in sepsis is the development of interstitial edema and pulmonary hypertension, the hallmarks of ARDS.[45]

PREVENTION

The morbidity, mortality and hospital costs associated with severe sepsis demand that strenuous efforts must be made to reduce the incidence of this serious disease. These can be divided as follows:

- vaccination strategies for at-risk populations;
- assiduous infection control for high-risk patients;
- use of prophylactic antibiotic therapies; and
- prompt recognition and treatment of infection and severe sepsis.

Vaccination strategies are discussed elsewhere under sections on specific pathogens but it is worth noting the success of meningococcal type C conjugate vaccine in reducing the incidence of fulminant meningococcal disease. Interestingly, a recent study from the USA detected a reduction in invasive pneumococcal disease in adults after their children had been immunized with the pneumococcal conjugate vaccine.[46] Unfortunately the majority of cases of severe sepsis are caused by organisms for which no vaccine is available.

CLINICAL FEATURES

A rigorous history, physical examination and directed investigations are essential to arrive at the correct diagnosis of any infectious disease. However, severe sepsis and shock is a medical emergency and therefore full assessment may have to be delayed until resuscitation and empiric antimicrobial therapy have been commenced. The aims of clinical evaluation are to establish the diagnosis of sepsis, estimate disease severity and prognosis, and elucidate the underlying cause. The exact presentation will depend on the site of infection, the nature of the infecting organism, the host response and coexistent illness.

History and symptomatology

Symptoms that suggest the onset of sepsis are often nonspecific and include sweats, chills or rigors, breathlessness, nausea and vomiting or diarrhea, and headache. Confusion may be found in 10–30% of patients, especially the elderly, and sepsis-related encephalopathy is associated with a poorer clinical outcome. There may be specific localizing symptoms or signs to suggest the underlying pathology, such as cough, dysuria or meningism, but in many cases there are no clues. Other important information to elicit includes recent travel, contact with animals, local infectious disease outbreaks, recent surgical procedures, indwelling prosthetic devices, hospitalization, prior antibiotics, underlying pathology and immunosuppressive illness or medication (see Fig. 44.1).

Physical signs on examination

With progression to severe sepsis and shock there is increasing evidence of organ dysfunction; the key physical signs and physiologic changes indicating this are encapsulated in Table 44.2 and Figure 44.7. Remember that sepsis is a dynamic evolving clinical picture and frequent re-evaluation of the patient is essential.

The characteristic patient is febrile, tachypneic, tachycardic with warm peripheries and a bounding arterial pulse, hypotensive, disoriented and oliguric. With impending circulatory collapse the patient

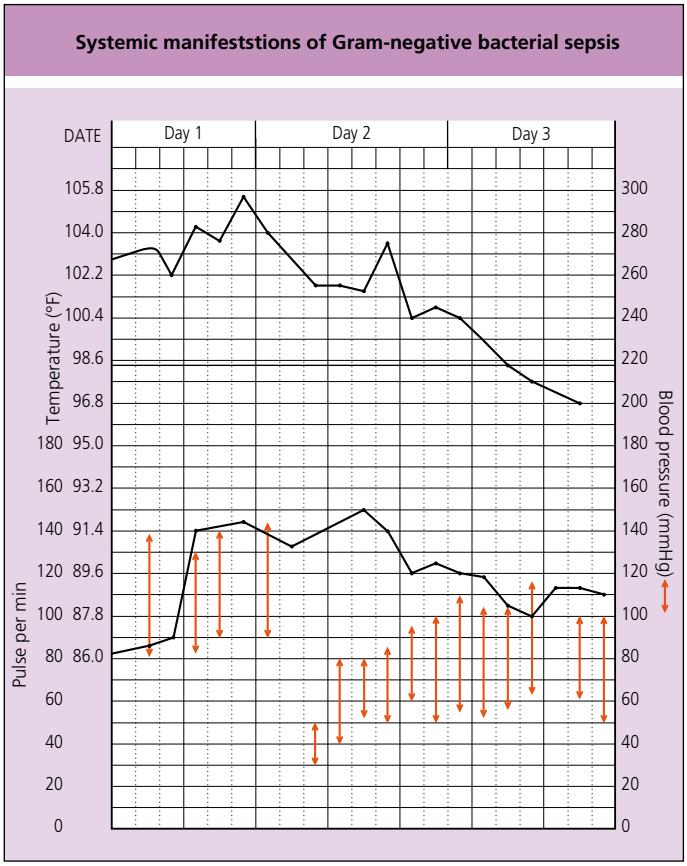


Fig. 44.7 Systemic manifestations of Gram-negative bacterial sepsis. Observation chart of a 49-year-old woman admitted to hospital with a suspected drug fever. For the first 24 hours she was observed without antimicrobial chemotherapy and demonstrated a persistent fever and tachycardia (sepsis). At this point she suddenly became confused, hypotensive and oliguric, indicating the development of severe sepsis. The underlying cause was an *Escherichia coli* bacteremia from an unsuspected urinary tract infection. She responded to fluid replacement and antibiotics and made a full recovery.

may develop peripheral vasoconstriction with cool peripheries and a prolonged capillary refill time. The observation chart of a patient with severe sepsis is shown in Figure 44.7 and the clinical course of a patient with streptococcal toxic shock in Figure 44.8. Some patients, particularly the elderly or immunocompromised, have a more subtle presentation necessitating a high index of suspicion to recognize early disease. Most patients are febrile but severe sepsis may present with hypothermia. A detailed physical examination is vital and it is important to examine the skin, all wounds and to perform full ear, nose and throat, rectal and vaginal examinations and fundoscopy as these sites are often overlooked. In the hospitalized patient pay particular attention to indwelling intravenous/intra-arterial lines, insist on exposing and examining all wounds, and carefully review the pressure areas as these are frequently neglected sites.

Occasionally, the physical examination will directly establish the diagnosis; helpful signs include the purpuric rash or peripheral gangrene of meningococcemia (Fig. 44.9), peripheral emboli in endocarditis, the erythematous rash or desquamation in staphylococcal or streptococcal toxic shock (Fig. 44.10), ecthyma gangrenosum in patients who have neutropenia and *P. aeruginosa* bacteremia, or the

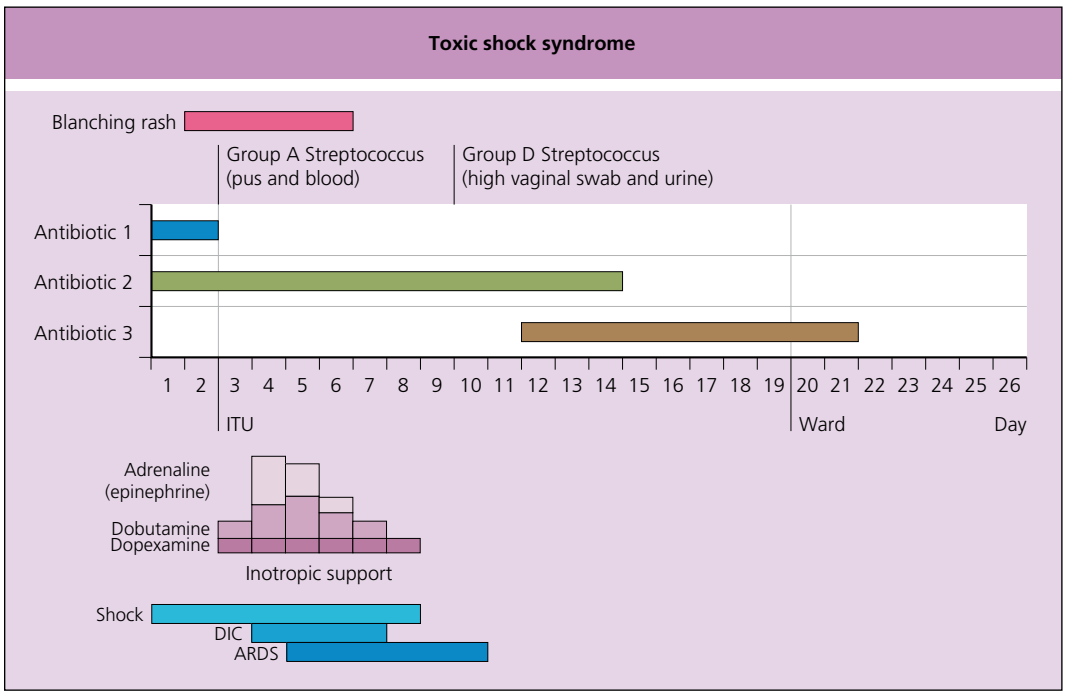


Fig. 44.8 Toxic shock syndrome. A 30-year-old woman presented with a short history of fever, breathlessness, diarrhea and abdominal pain. She was pyrexial and hypotensive with a blanching macular rash noted on day 2. Blood cultures grew a group A β-hemolytic streptococcus. Laparotomy revealed 800 ml of peritoneal pus with no intestinal perforation consistent with spontaneous bacterial peritonitis. She required ventilatory support plus inotropes to maintain blood pressure for 6 days and developed evidence of disseminated intravascular coagulation (DIC) and adult respiratory distress syndrome (ARDS). She made a gradual recovery complicated by an enterococcal urinary tract infection, finally leaving hospital 27 days after presentation. The group A streptococcal isolate from her blood was subsequently shown to release streptococcal mitogenic exotoxin Z (SMEZ), a potent superantigen. Courtesy of Dr P Gothard and Dr S Sriskanden, Hammersmith Hospital, London.

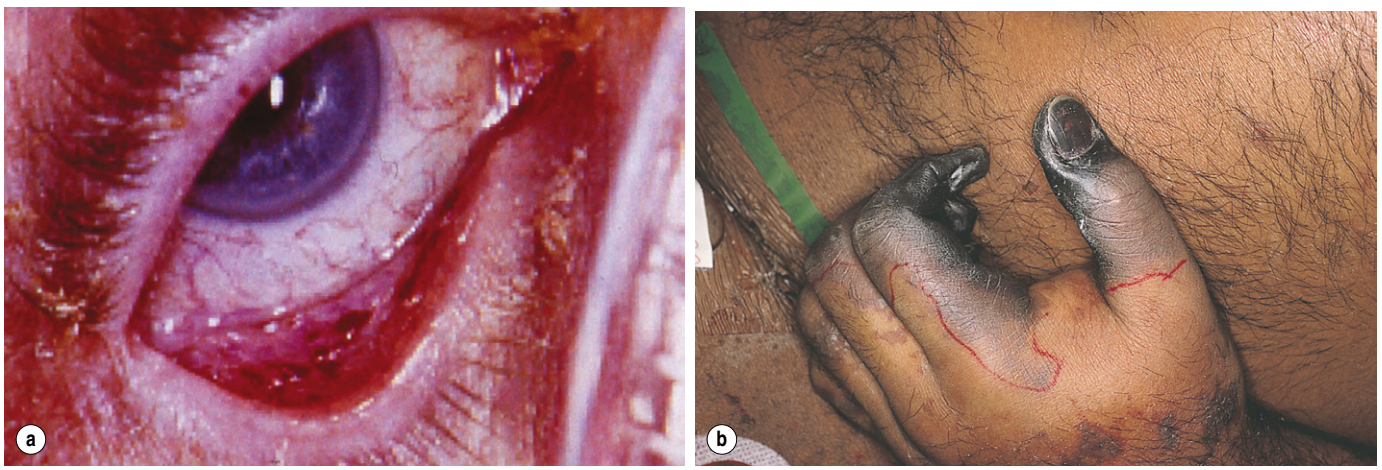


Fig. 44.9 Cutaneous changes in meningococcal infection. (a) Petechial hemorrhages are the hallmark of meningococcal infection and may be found on the periphery or, as in this case, the conjunctivae. (b) In severe disease the purpura may become confluent (purpura fulminans) and lead to severe digital gangrene. Courtesy of J Cohen, Brighton.

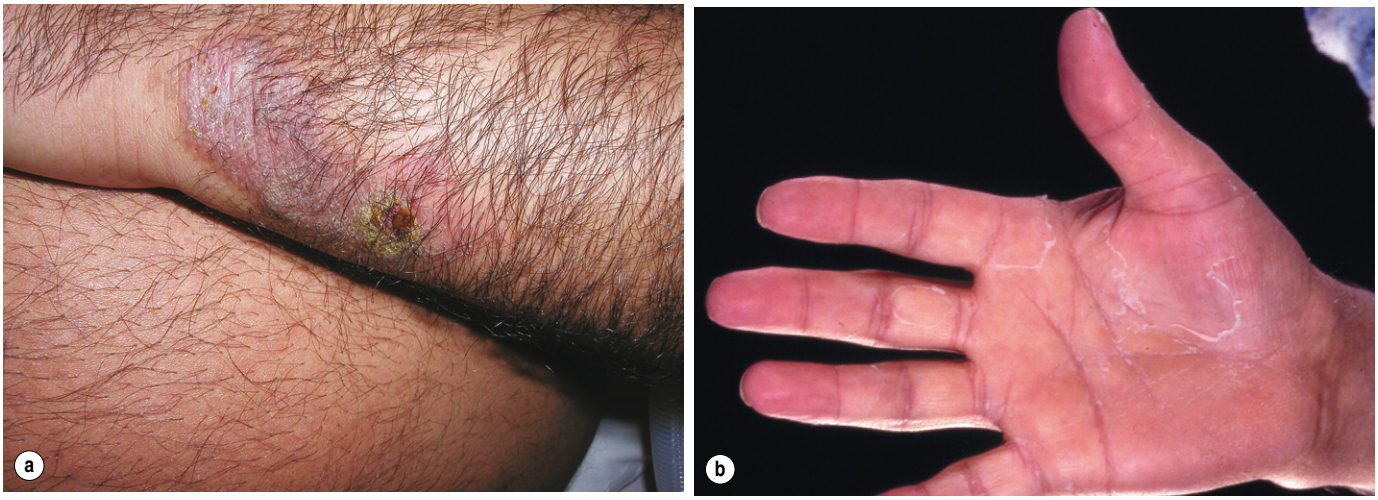


Fig. 44.10 Cutaneous changes in toxic shock. (a) Localized infection at the edge of a patch of eczema in a patient presenting with staphylococcal toxic shock syndrome. (b) Desquamation of the palm following an episode of staphylococcal toxic shock. Courtesy of Dr M Jacobs.

retinal lesions of *Candida* endophthalmitis. Focal physical signs may help to identify the site of infection, for example renal angle tenderness, pulmonary consolidation, new cardiac murmur or finding an intra-abdominal mass.

If the patient is hypotensive, other causes of shock such as cardiac dysfunction (including myocardial infarction and cardiac tamponade), hypovolemia and redistributive shock from pancreatitis and physical injuries need to be considered. Remember that hypotension in sepsis is often multifactorial and sepsis may complicate or coexist with other causes of shock.

DIAGNOSIS

Laboratory investigations

These can be broadly divided into those that help to confirm that the patient has sepsis and detect and follow complications such as organ failure, and those that establish the underlying cause.

Hematologic and biochemical evaluation in sepsis

Full blood count and blood film

Look for a neutrophil leukocytosis or leukopenia. The blood film may suggest bacterial infection with toxic granulation of neutrophils (increased band forms) even when the white cell count is normal. Leukopenia is a poor prognostic sign. Low platelet count suggests DIC and evidence of microangiopathic hemolysis may be visible. In patients who have traveled to an endemic area perform blood films for malaria parasites.

Coagulation screen

Look for evidence of DIC – prolonged prothrombin or activated partial thromboplastin time, low fibrinogen and elevated markers of fibrinolysis (fibrin degradation products or D-dimer levels).

Electrolytes and renal function

Monitor renal function closely; elevated potassium may indicate rhabdomyolysis, which may complicate severe sepsis.

Liver tests

Minor abnormalities are common in patients with sepsis and do not necessarily signify hepatic infection. Elevated bilirubin, alkaline phosphatase or transaminases of two to three times the normal level may be seen in up to 30–50% of patients. These are generally transient and not of prognostic importance. More markedly abnormal liver tests suggest underlying hepatic or biliary tract infection. Progressively deteriorating liver biochemistry in patients who have sepsis suggests hepatocellular damage or acalculous cholecystitis, which may complicate severe sepsis.

Plasma albumin

An acute fall in albumin, to as low as 1.5–2.0 g/dl over 24 hours, may occur as a result of widespread endothelial damage and capillary leakage of protein. In patients with chronic underlying illness or prolonged infection, the albumin may fall due to poor nutrition and a switch in hepatic metabolism towards acute-phase proteins.

Blood glucose

Hypoglycemia or hyperglycemia may occur in severe sepsis. In patients who are diabetic close monitoring and normalization of blood glucose are essential.

Arterial blood gases

Typically the patient has a metabolic acidosis with compensatory respiratory alkalosis. The degree of acidosis is a marker of the severity of illness. The onset of hypoxia indicates severe disease and a high risk of ARDS. Measurement of venous and arterial oxygen content allows calculation of oxygen delivery and consumption.

Plasma lactate

This is often increased three- to fivefold in severe sepsis (normal 1.0–2.5 mmol/l) and relates to the degree of tissue hypoxia. Lactate estimations are helpful in diagnosing severe sepsis, predicting mortality and monitoring the response to therapy.[5,47]

Other biochemical investigations

Amylase, creatinine phosphokinase, calcium and magnesium levels may be disturbed in patients with severe sepsis.

C-reactive protein

C-reactive protein (CRP) is an acute-phase reactant that rises within a few hours of bacterial infection. High levels are detectable in most patients who have severe sepsis although the level of CRP does not reliably distinguish infective from noninfective causes of shock or between sepsis and severe sepsis.[48] Patients with fulminant sepsis may have a normal CRP at presentation.

Procalcitonin

Procalcitonin has been suggested as a more sensitive and specific marker of bacterial infection than CRP and is preferred in some centers. Procalcitonin levels may be able to discriminate between sepsis and severe sepsis but this has not entered routine clinical practice.[48,49]

Endotoxin or cytokine levels

In some but not all studies plasma levels of endotoxin and cytokines such as TNF and IL-6 have correlated with outcome of Gram-negative sepsis. Variation in reported data along with technical difficulties in performing these assays in real time makes routine testing impractical in most settings.

Microbiologic investigations in sepsis

Establishing an accurate microbiologic diagnosis of the cause of sepsis is a crucial part of patient management. Microbiologic diagnosis is needed to confirm the diagnosis and to direct antimicrobial therapy, particularly with reports of increased antimicrobial resistance in patients with severe sepsis. Despite modern laboratory techniques microbiologic confirmation can only be achieved in around 60% of cases and there is a clear need for improved diagnostic methods.[50] Ideally, all relevant cultures should be taken before initiating antibiotic therapy, but treatment is urgent and should not be unduly delayed.

Blood cultures are the most important microbiologic investigation. Two or three separate blood cultures (total 20–30 ml of blood) should be inoculated into one of the standard commercial blood culture media.[5] Culturing an inadequate volume of blood is the most common reason for not detecting bacteremia.[51] The bottles should be incubated aerobically and anaerobically at 98°F (37°C), preferably agitated, for 7 days. The development of automated blood culture analysis allows for more rapid identification of positive cultures. Where there is a high risk of underlying fungal infection then specific fungal media may be helpful.

Sputum and urine microscopy and culture should be performed in all cases. All wounds should be swabbed and sterile body sites such as cerebrospinal fluid (CSF), joint or pleural fluid sampled as indicated. In cases in which toxic shock syndrome is suspected, wound, nose, throat and vaginal swabs should be taken. If staphylococci or streptococci are isolated in cases of toxic shock then the appropriate reference laboratories can assay the isolate for toxin production. Cultures should be repeated as directed by previous results and the condition of the patient.

Histopathologic changes in sepsis

Organ pathology in sepsis is due to the combined effects of hypoxia, impairment in tissue perfusion and severe acidosis. In the lung the changes are those of ARDS, with early findings of interstitial and alveolar edema, fibrosis developing at a later stage. The kidneys may show acute tubular necrosis that is generally reversible (Fig. 44.11). Hepatic changes are of an ischemic zonal necrosis and some cases may develop an acalculous cholecystitis. In the brain there may be areas of focal ischemia or hemorrhage. In severe cases of septic shock associated with *Neisseria meningitidis* or *Capnocytophaga canimorsus* there may be peripheral gangrene due to severe impairment of perfusion (see Fig. 44.9 and also Fig. 12.6) and hemorrhage into the adrenal glands (Fig. 44.12). The gut is also affected by ischemia and may show mucosal ulcerations and areas of infarction.

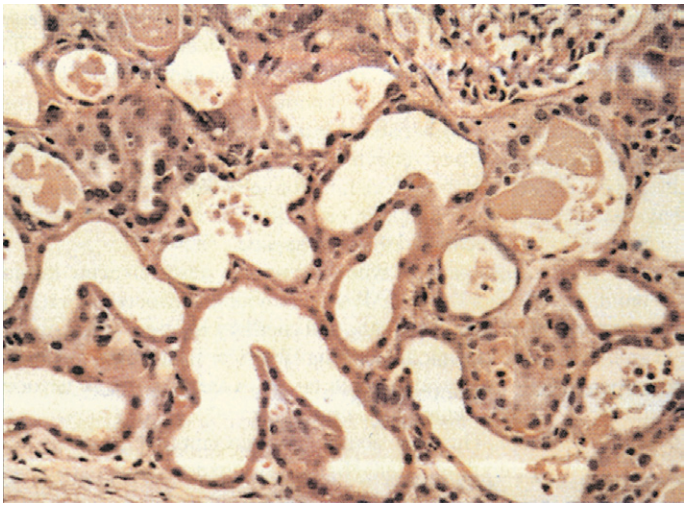


Fig. 44.11 Acute tubular necrosis. The tubules are dilated with flattened epithelial cells, and contain debris; the glomerulus is not greatly affected. Hematoxylin and eosin. With permission from Williams JD *et al*., Clinical Atlas of the Kidney. London: Mosby; 1991.



Fig. 44.12 Acute hemorrhagic necrosis of the adrenal glands (Waterhouse–Friderichsen syndrome). Both adrenal glands of a child with meningococcal septicemia show hemorrhagic necrosis leading to acute adrenal failure. With permission from Stevens A, Lowe J. Pathology. London: Mosby; 1995.

Radiologic investigations

Chest radiography is necessary in all cases and may show signs of ARDS. Other studies may also be indicated. Radiologic investigations can be of great help in identifying occult sites of infection (Fig. 44.13) and close liaison with the diagnostic radiology department is vital. Ultrasound and CT are particularly useful when trying to detect deep abscesses; in selected cases nuclear medicine techniques may pinpoint the site of infection. Access to an interventional radiologist who can perform percutaneous sampling/drainage of sites of infection is invaluable.



Fig. 44.13 Radiologic detection of occult foci of infection in severe sepsis. Plain abdominal X-ray in a diabetic with an *Escherichia coli* urinary tract infection and pyelonephritis who had developed hypotension and oliguria. X-ray reveals gas around the kidney due to a perinephric abscess requiring urgent drainage.

Table 44.6 Principles of antibiotic administration and selection of antimicrobial agents in severe sepsis

Antibiotic administration
Intravenous high dose therapy within 1 h of the recognition of severe sepsis
Broad-spectrum choice to include one or more drugs active against the likely infecting pathogen
Chosen antibiotic should penetrate the likely site of infection
Review antibiotic choice every 24 h in the light of clinical response and microbiology results
Consider combination therapy for known or suspected *Pseudomonas* infection or in neutropenic sepsis
Selection of antimicrobial agent
Clinical syndrome/likely microbiology
Site of infection
Risk of resistance based on local and national data
Host factors including immunocompromised state
Co-morbidities including organ function and drug allergy
Risk of *Clostridium difficile* and other hospital-acquired infections based on local epidemiology

MANAGEMENT

Mortality from sepsis and multiorgan failure remains high but by early recognition and prompt therapeutic intervention patients can be prevented from progressing down this path. There is no complete consensus on how best to manage severe sepsis and few randomized trial data dealing with optimal treatment. An approach to therapy is encapsulated in the Surviving Sepsis Campaign guidelines first produced in 2004 and updated in 2008.[5,52] These guidelines have not met with universal approval, mainly because severe sepsis is not a homogeneous condition and a generalized approach to treatment can be criticized. However, they do provide a logical and structured approach to the septic patient.

The key principles in the management of severe sepsis are:

- treat infection with prompt and effective antimicrobial therapy and, where indicated, source control;
- maintain tissue perfusion/oxygenation and preserve organ function; and
- prevent complications during/following the episode of severe sepsis.

Antibiotic chemotherapy

Incorrect empiric antibiotic choice is strongly associated with a higher mortality in severe sepsis.[18,53] Furthermore, delay in administration of antibiotics worsens survival. Kumar *et al*. reviewed 2154 patients with septic shock and found that only 50% had received antibiotics within 1 hour of the onset of shock.[14] In this study the mortality rate was 20.1% if antibiotics were administered within the first hour after the onset of hypotension and rose by 7.6% per hour of delay, becoming statistically significant if antibiotics were delayed more than 2 hours.

It is not possible to provide specific antimicrobial guidelines for severe sepsis as there are many local variables that must be taken into consideration such as local resistance patterns and risks of *C. difficile*. An overview of the principles underpinning antibiotic choice in severe sepsis is provided in Table 44.6. There are few outcome data on duration of antibiotic therapy in severe sepsis and a course of 7–10 days is frequently recommended, although shorter courses may be given to selected patients and helps to reduce resistance.

Antibiotic-induced endotoxin release

In experimental models, and some limited situations in humans, release of endotoxin from bacteria has been shown to increase the inflammatory response.[54] Thus, a school of thought has developed that antibiotics should be chosen on the basis of their potential to cause endotoxin release.[55] However, beyond a handful of small studies there are few clinical data to support a major role for this hypothesis in severe sepsis. Antibiotics should be selected on the basis of efficacy for the specific clinical picture, rather than on the risk of endotoxin release.

Source control – detection and removal of infected material

It is essential to drain/remove all possible infective foci as these are often the cause for treatment failure (see Fig. 44.13). Thus, abscesses should be drained, dead tissue resected and infected foreign material, such as an infected central venous catheter, removed. Guidance suggests that source control should occur as early as possible after initial resuscitation and is ideally performed within 6 hours of the onset of severe sepsis.[5] One exception to early intervention is severe necrotic pancreatitis where current practice is to defer percutaneous drainage due to the high complication rate and secondary infective risk.[56] The critically ill patient on intensive care may have repeated episodes of sepsis; locating the underlying focus often requires considerable determination, repeated radiologic investigation and sometimes persistent discussions with surgical colleagues.

Supportive therapy

The goal of supportive therapy is to try to maintain tissue oxygen delivery, a concept advanced by Shoemaker and others throughout the 1970s.[57] The potential benefit of early aggressive supportive therapy is shown in a study by Rivers *et al*.[58] in which 263 patients with severe sepsis were randomized to receive standard therapy or 'goal-directed' therapy where cardiac preload, afterload and contractility were actively managed to try to balance oxygen delivery with demand prior to admission to the ICU. In-hospital mortality was reduced from 46.5% in the standard group to 30.5% in the goal-directed group (p <0.009). Goal-directed therapy appears to improve outcome during initial resuscitation when patients first develop severe sepsis and the applicability of this approach to all comers with sepsis has been questioned. However, the Rivers study establishes the importance of paying attention to prompt and effective resuscitation in severe sepsis.

Ideally, patients who have sepsis should be closely monitored in an intensive therapy or high-dependency unit. Indeed a review of 41 patients who had septic shock revealed increased mortality when patients were managed outside of the intensive therapy unit, 70% versus 39%, even though the patients had less severe illness.[59] Minimal requirements for safe management include facilities for measurement of blood pressure, cardiac monitoring, central venous pressure recording, arterial blood gas analysis, oxygen and facilities for assisted/mechanical ventilation or dialysis when required. Invasive monitoring is useful in excluding other causes of shock and in directing therapy. However, invasive monitoring is not a substitute for repeated examination and clinical assessment of the patient. Many factors such as cardiovascular disease, hypovolemia or inotropic drugs can confound such data; results must be interpreted in the context of the clinical situation. Normal values and the typical ranges for hemodynamic parameters in severe sepsis are given in Table 44.7.

Severe sepsis is a heterogeneous condition and it is not possible to be didactic about the management of individual patients. Patients should be monitored closely during resuscitation and interventions tailored to physiologic measurements of organ function/perfusion. Improvement in acidosis and reduction in serum lactate is a reliable guide to improved tissue perfusion and oxygenation. A stepwise approach to initial resuscitation is provided by the Surviving Sepsis Campaign guidelines:[5]

- Begin resuscitation immediately in patients with hypotension or serum lactate >4.0 mmol/l.
- Do not delay pending ICU admission.
- Resuscitation goals:
 - CVP 8–12 mmHg
 - Mean arterial pressure >65 mmHg
 - Urine output >0.5 ml/kg/h
 - Central venous oxygen saturation >70%.

Table 44.7 Hemodynamic changes in severe sepsis

Parameter	Normal range	Changes in severe sepsis
Heart rate (HR)	72–88 beats/min	Sinus tachycardia
Mean arterial pressure (MAP)	70–105 mmHg	Hypotension <60 mmHg
Cardiac output (CO)	4–8 l/min	Increased but not enough to compensate for low SVR
Systemic vascular resistance (SVR)	800–1500 dyne/s/cm^2	Reduced (<600 if no pressor agents)
Oxygen delivery (Do_2)	520–720 ml/min/m^2	Decreased
Oxygen consumption (Vo_2)	100–180 ml/min/m^2	Typically increased

CO = SV × HR; SVR = (MAP – CVP)/CO × 79.92
Do_2 = CI × arterial oxygen × 10; Vo_2 = CI × (arterial – venous oxygen) × 10
CI, cardiac index (CO/m^2 surface area); CVP, central venous pressure; SV, stroke volume.

Intravenous fluid therapy

Fluid resuscitation is critical and should precede any pharmacologic agents. Crystalloid or colloids can be used as there are no clear data supporting one over the other.[5] Concerns over the safety of human albumin have been decreased by the SAFE study which showed equal efficacy and safety when albumin was used in comparison to saline in ICU patients.[60] One recent study has found an unexplained increase in adverse events in patients who were resuscitated with pentastarch compared to Ringer's lactate.[61] This finding requires further investigation before a definitive recommendation can be made about colloid solutions based on starch derivatives. In hypotensive or hypovolemic adults, an initial fluid challenge of 1000 ml crystalloid or 300–500 ml colloid should be administered over 30 minutes. Fluid filling should be continued as long as patients have evidence of hypovolemia and impaired organ perfusion. Patients must be monitored closely as cardiac filling pressures increase to avoid cardiac failure.

Vasopressors

Pharmacologic intervention is indicated if evidence of hypoperfusion – persistent hypotension or acidosis – persists despite fluid filling. Initial treatment is with noradrenaline (norepinephrine) or dopamine. There is no evidence that one agent is superior to the other. In refractory cases vasopressin or terlipressin may also be considered but large trial data on these agents are lacking.[62] The dose of pressor agents is titrated to maintain an arterial pressure of 65 mmHg or greater with improved organ perfusion. Patients receiving pressor agents require continuous arterial pressure monitoring and close attention to peripheral perfusion as ischemia of the extremities may complicate their use. There is no role for 'renal dose' dopamine in the management of severe sepsis.

Inotropes

Substantial experimental and clinical data indicate that myocardial function is impaired in severe sepsis. Inotropes increase cardiac contractility and can increase cardiac output and tissue perfusion in severe sepsis. Adrenaline (epinephrine) or dobutamine can fulfill this role and there is no evidence that either agent is superior. Inotropes are indicated where there is evidence of a persistent low cardiac output despite fluid resuscitation and treatment with pressor agents. Inotropes should not be given to patients who are still hypovolemic. Some researchers have used inotropes to try to achieve supranormal oxygen delivery in severe sepsis but there is no evidence that this strategy improves clinical outcome and it is potentially deleterious.[63]

Insulin therapy in severe sepsis

Assiduous control of the blood glucose level is essential in diabetic patients with severe sepsis. Several studies had previously indicated a benefit from tight glycemic control with intensive insulin therapy in nondiabetic patients with critical illness, including those with severe sepsis.[64] However, a large multicenter randomized study reported in 2008 has shown no overall benefit from insulin therapy in sepsis and a significant increase in hypoglycemic events.[61] Therefore, at this time, insulin should only be used in septic patients with hyperglycemia until more data are available.

Blood products in severe sepsis

There is some controversy around red cell transfusions in patients with severe sepsis. Early goal-directed therapy suggests transfusion of red cells to maintain a hematocrit >30% in patients with a superior vena cava oxygen saturation of <70% after fluid resuscitation. In contrast, the Transfusion Requirements in Critical Care trial suggests that there is no benefit in transfusing above a threshold hemoglobin of 7 g/dl in patients in ICU.[65]

Thrombocytopenia is common in severe sepsis. Platelet transfusion should be considered if the absolute platelet count is less than 5×10^9/l in the absence of bleeding or less than 30×10^9/l in the presence of bleeding. Replacement of coagulation factors with fresh frozen plasma or cryoprecipitate may be required to combat bleeding. Plasma also contains potentially proinflammatory components such as complement and replacing these may increase inflammation.

Other supportive treatments

Patients with severe sepsis should receive thromboprophylaxis unless there is a specific contraindication. Stress ulcer prophylaxis is indicated with an H_2 blocker or a proton pump inhibitor. Nutrition should be maintained, preferably by the enteral route. In renal impairment or severe acidosis early consideration should be given to hemofiltration. In the intubated and ventilated patient a reduced risk of ARDS and improved clinical outcome can be achieved using a volume and pressure limited strategy and, where necessary, permissive hypercapnia.[5]

IMMUNOMODULATION AND ADJUNCTIVE THERAPIES IN SEVERE SEPSIS

The concept that organ damage in sepsis is the result of the host inflammatory response has led to an enormous research effort to understand and intervene in this process. Many therapeutic strategies have been investigated in the laboratory setting and within large randomized clinical trials. This has highlighted the difficulties inherent in performing interventional studies in sepsis and initial results have often been conflicting and disappointing with the notable failure of antiendotoxin and anticytokine treatments. In the past decade a number of adjunctive treatments – namely low-dose corticosteroids, intravenous immunoglobulins and recombinant human activated protein C – have entered clinical practice for selected groups of patients with severe sepsis and these are discussed in more detail below.

Corticosteroids

There has been considerable controversy surrounding the use of corticosteroids in severe sepsis. In animal models pretreatment with corticosteroids protected against endotoxemia, and initial promising reports in humans led to the widespread use of high-dose corticosteroids in sepsis. Subsequently, two large multicenter trials failed to show benefit and meta-analyses of suitable trials confirmed that the use of high-dose corticosteroids in sepsis is not of benefit and may potentially be deleterious by increasing rates of secondary infection.[66] However, impairment of the hypothalamic–pituitary–adrenal axis is seen in some patients with sepsis and is associated with a poor outcome. This led to a re-evaluation of the role of lower dose corticosteroid replacement therapy.

In 2002 Annane *et al*. reported a significant increase in survival of patients with severe sepsis, who had catecholamine-refractory shock and a poor response to synacthen, indicating adrenal failure, when treated with a combination of hydrocortisone and fludrocortisone.[67] As a result of this, corticosteroid replacement has been recommended for patients with severe sepsis and a poor response to fluids and vasopressors.[5] Hydrocortisone is usually given at a dose of 200–400 mg/day in divided doses with or without fludrocortisone 50 μg/day.

In 2008 a large multicenter study, CORTICUS, was reported which failed to confirm the mortality benefit seen in earlier studies.[68] However, in this study, shock resolution was significantly faster in patients receiving hydrocortisone therapy, supporting a rationale for steroid replacement therapy in severe sepsis. Further work will be required to define the precise role of corticosteroid therapy for patients with severe sepsis.

Intravenous immunoglobulin therapy

Intravenous immunoglobulin therapy (IVIG) administration has been examined in bacterial sepsis with variable data.[69] At present it is not possible, on the basis of the available evidence, to recommend IVIG for unselected patients with severe sepsis. In severe sepsis associated with toxin-producing bacteria, for example staphylococcal or streptococcal toxic shock syndrome, there is a stronger rationale for the use of high-dose IVIG. Toxic shock is seen in persons with low levels of antitoxin antibodies and pooled IVIG contains neutralizing antibodies against a variety of superantigenic toxins. Experimental data and anecdotal reports would support the use of IVIG in this setting but there are as yet no substantial clinical trials. More recently IVIG has been suggested for use in patients with severe infections due to *Staph. aureus* strains producing the Panton–Valentine leukocidin toxin[70] and for severe *C. difficile* infection.[71]

Recombinant human activated protein C

Modulators of coagulation are protective in animal models of severe sepsis and have entered human trials. Of these, recombinant human activated protein C (rhAPC) is most promising. In a phase III multicenter trial of rhAPC versus placebo (PROWESS) in 1690 patients with severe sepsis, 28-day mortality was reduced from 30.8 to 24.7% (p <0.005), a relative reduction in risk of death of 19.4%.[72] There was a small but significant increase in serious bleeding in the active therapy group. On the basis of these results rhAPC (drotrecogin alpha) was granted a product license for the treatment of severe sepsis and organ failure in adults in 2002. rhAPC has been recommended in adult patients with sepsis-induced organ dysfunction and a high risk of death (i.e. APACHE II score >25) and who have no contraindications that would increase the risk of bleeding.

PROWESS was followed by the ADDRESS trial which looked at the use of rhAPC in patients with sepsis at a lower risk of death.[73] In this study, involving 2613 patients, no mortality benefit from rhAPC could be detected over placebo but there was a significant increase in 28-day mortality rate if they received rhAPC rather than placebo. Furthermore, in subgroup analysis of patients enrolled into ADDRESS but at high risk of death (similar criteria to the PROWESS study) no benefit from rhAPC could be detected.

So where does this leave rhAPC in the treatment of severe sepsis? On the basis of a well-randomized and performed clinical trial rhAPC has a product license and has entered treatment guidelines in many countries. On the available evidence it is reasonable to continue to prescribe rhAPC for patients with severe sepsis who fulfill the original PROWESS entry criteria and who do not have a high risk of bleeding complications. rhAPC should not be given to patients with single organ failure or a low risk of death due to the risk of bleeding. Further randomized trials of rhAPC are planned which may answer these questions.

REFERENCES

References for this chapter can be found online at http://www.expertconsult.com

Chapter 45

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Infections associated with intravascular lines, grafts and devices

Infections of Intravascular Lines and Devices

INTRODUCTION

Intravascular lines and devices are used increasingly in health care for the administration of fluids, medication, blood products and nutrition, for hemodynamic monitoring and for hemodialysis. However, catheter-related bloodstream infection (CRBSI) has become a leading cause of nosocomial bacteremia, and carries an associated morbidity, mortality, increased length of hospital stay, and cost of care.[1,2] Approximately 3 in every 1000 patients admitted to hospital in the UK will acquire bacteremia, and almost one-third of these are secondary to intravascular catheter infection.[3]

EPIDEMIOLOGY

All kinds of intravascular catheters and devices carry a risk of infection, but the risk varies considerably with the device used. Peripheral cannulae, usually only in place for up to 72 hours, are associated with a low risk of bloodstream infection, although the frequency of their use means they still cause significant morbidity. The majority of serious catheter-related infections are associated with central venous catheters (CVCs). Tunneled and cuffed or surgically implanted catheters carry much lower rates of infection than standard non-tunneled CVCs, with totally implantable ports having the lowest rates per 1000 catheter days (Table 45.1).

In addition to the type of catheter used, a number of factors during insertion, as well as patient factors, may predispose to catheter-related infection (Table 45.2).

PATHOGENESIS

Colonization of intravascular catheters may occur by either of two main routes. Extraluminal colonization, originating from the skin insertion site and migrating along the extraluminal surface, is the predominant mechanism in short-term catheters such as the non-tunneled central venous catheters used most frequently in intensive care units. Intraluminal colonization, originating from colonization of the catheter hub, or less commonly from contaminated infusate, is the predominant mechanism in longer-term lines including tunneled CVCs used for chemotherapy or parenteral nutrition. Less commonly, catheters may become colonized hematogenously, secondary to a distant site of infection (Fig. 45.1). The relative likelihood of each of the two main routes of colonization is important when considering methods for the prevention and diagnosis of catheter-related infection in a particular clinical setting.[1,2]

The commonest micro-organisms implicated in catheter-related infection include skin flora, particularly the staphylococci (most commonly coagulase-negative staphylococci but also *Staphylococcus aureus*), yeasts and aerobic Gram-negative bacilli (Fig. 45.2).

The ability of micro-organisms to form a biofilm on the surface of an intravascular catheter is important in explaining the persistence of catheter infection. A biofilm comprising host proteins, microcolonies of the infecting organisms and the extracellular polysaccharide matrix (slime) they produce, allows persistence of organisms in a relatively protected environment. Antibiotics at doses much higher than those needed to kill organisms in planktonic state may still be insufficient to kill sessile bacteria within a biofilm.[8] This explains why systemic antibiotics alone often do not succeed in eliminating infection until the line is removed.

CLINICAL FEATURES

Catheter-related infection may be local or systemic or the two may coexist.

Local infection

Localized catheter infections include exit site infection, tunnel infection and pocket infection (Fig. 45.3). They may present with signs of inflammation, purulence or frank cellulitis. Local infection may coexist with systemic infection (frank purulence may be predictive of CRBSI[9]), but may also exist independently. The vast majority of CRBSI occur without local signs[9] and as such the absence of such signs should not reassure against a diagnosis of CRBSI in a patient with unexplained fever.

Systemic infection

Catheter-related BSI may present nonspecifically with fever and chills, with or without septic shock, and should be considered when these features are present in a patient without an obvious alternative source of infection. Blood cultures growing *S. aureus*, coagulase-negative staphylococci or *Candida* species should particularly suggest the diagnosis.

Definitions of catheter colonization, local infection and CRBSI are outlined in Table 45.3.

Table 45.1 Rates of bloodstream infection (BSI) caused by the various types of devices used for vascular access

Catheter type	RATES OF DEVICE-RELATED BSIs PER 100 CATHETERS		RATES OF DEVICE-RELATED BSIs PER 1000 CATHETER DAYS	
	Pooled mean	95% CI	Pooled mean	95% CI
Peripheral venous cannulas				
Plastic catheters	0.2	0.1–0.3	0.6	0.3–1.2
Steel needles	0.4	0.0–2.3	1.6	0.0–9.1
Cutdowns	3.7	0.1–20.6	8.8	0.2–49.3
Arterial catheters used for hemodynamic monitoring	1.5	0.9–2.4	2.9	1.8–4.5
Central venous catheters				
Standard noncuffed, nonmedicated	3.3	3.3–4.0	2.3	2.0–2.4
Antibiotic coated	0.2	0.0–1.0	0.2	0.1–1.4
Control nonmedicated	4.4	3.5–5.5	—	—
Antiseptic-impregnated	3.1	2.4–3.9	2.9	2.3–3.7
Control nonmedicated	3.8	1.5–7.8	—	—
Peripherally inserted central catheters (PICCs)	1.2	0.5–2.2	0.4	0.2–0.7
Silver-impregnated cuffs	3.3	1.9–5.4	3.2	1.9–5.3
Tunneled but noncuffed	12.4	10.7–16.6	1.8	1.4–2.0
Pulmonary artery catheters	1.9	1.1–2.5	5.5	3.2–12.4
Heparin-bonded	0.4	0.2–0.9	2.6	1.1–5.2
Hemodialysis catheters				
Noncuffed	16.2	13.5–18.3	2.8	2.3–3.1
Cuffed	6.3	4.2–9.2	1.1	0.7–1.6
Tunneled and cuffed central venous catheters	20.9	18.2–21.9	1.2	1.0–1.3
Subcutaneous central venous ports	5.1	4.0–6.3	0.2	0.1–0.2
Peripherally inserted central ports	0.0	0.0–2.8	0.0	0.0–0.1
Intra-aortic balloon counter-pulsation devices	1.9	0.9–3.6	5.5	2.5–10.5
Left ventricular assist devices	22.5	17.5–28.5	4.5	3.6–5.7

Based on 206 published prospective studies in which every device was evaluated for infection. From Kluger & Maki.[4] Copyright The University of Chicago Press.

PREVENTION

Catheter-related infections are highly preventable. Considerable evidence now exists confirming the efficacy of specific interventions, and these measures have been incorporated into several national guidelines for prevention, such as those of the US Centers for Disease Control and Prevention (CDC) Healthcare Infection Control Practices Advisory Committee (HICPAC)[2] (Table 45.4), the English epic2 (evidence-based practice in infection control) guidelines[3] and the French Intensive Care Society (SRLF) consensus guidelines.[11]

As catheter-related infection may follow either extraluminal or intraluminal colonization, prevention strategies should focus on these two potential routes of acquisition.

Prevention of colonization of the external surface of the line

Measures to prevent extraluminal colonization are of primary importance with short-term lines, where the role of microbial colonization of the insertion site is so important in the pathogenesis of infection. The aim is to reduce the populations of skin micro-organisms at the time of insertion and during subsequent manipulations, dressings and care.

Maximum sterile barrier

The use of maximum sterile precautions, similar to those used in the operating theater (including sterile gown, gloves and cap, and a large sterile drape) has been shown to significantly reduce infection when compared with standard precautions (sterile gloves, small sterile drape).[2] In a randomized controlled trial, use of maximum sterile precautions led to a reduction in catheter colonization, bacteremia and cost.[12] This approach has also been found to reduce catheter-related infection associated with pulmonary artery catheters.[13]

Choice of cutaneous antiseptic

The impact of the choice of antiseptic solution is the subject of a meta-analysis of eight randomized controlled trials of chlorhexidine gluconate versus povidone-iodine.[14] The use of chlorhexidine gluconate rather than povidone-iodine was associated with a 50% reduction in the risk of catheter-related bloodstream infection. The efficacy of chlorhexidine may be concentration related, and a study comparing 0.5% chlorhexidine with 10% povidone-iodine failed to show a significant difference in catheter colonization or bacteremia,[15] whereas a study by Maki *et al.* showed a trend to less bloodstream infection with 2% aqueous chlorhexidine compared with 10% povidone-iodine or 70% alcohol.[16] Finally, povidone-iodine in ethanol is more effective than povidone-iodine alone.[17] The HICPAC guidelines strongly recommend cutaneous antisepsis with a 2% chlorhexidine-based preparation.[2]

Table 45.2 Risk factors for intravascular device-related bloodstream infection

Risk factors (number of studies)		Relative risk or odds ratio
Patient characteristics	Underlying disease:	
	AIDS (2)	4.8
	Neutropenia (2)	1.0–15.1
	Gastrointestinal disease (1)	2.4
	Surgical service (1)	4.4
	Placement in intensive care unit or coronary care unit (3)	0.4–6.7
	Extended hospitalization (3)	1.0–6.7
	Other intravascular devices (2)	1.0–3.8
	Systemic antibiotics (3)	0.1–0.5
	Active infection at another site (2)	8.7–9.2
	High APACHE III score (1)	4.2
	Mechanical ventilation (1)	2.0–2.5
	Transplant patient (1)	2.6
Features of insertion	Difficult insertion (1)	5.4
	Maximal sterile barriers (1)	0.2
	Tunneling (2)	0.3–1.0
	Insertion over a guidewire (8)	1.0–3.3
	Insertion site:	
	Internal jugular vein (6)	1.0–3.3
	Subclavian vein (5)	0.4–1.0
	Femoral vein (2)	3.3–4.8
	Defatting insertion site (1)	1.0
	Use of multilumen catheter (8)	6.5
Catheter management	Routine change of iv set (2)	1.0
	Nurse: patient ratio	
	1:2.0	61.5
	1:1.5	15.6
	1:1.2	4.0
	1:1	1.0
	Inappropriate catheter usage (1)	5.3
	Duration of catheterization >7 days (5)	1.0–8.7
	Colonization of catheter hub (3)	17.9–44.1
	Parenteral nutrition (2)	4.8

Adapted from Safdar *et al*.[5]

Site of catheter

The subclavian site is associated with less risk of infection than either the femoral or internal jugular site.[2,3] The commonly used internal jugular site is at risk of infection for several reasons, including its proximity to oropharyngeal secretions, the difficulty of applying an occlusive dressing and the presence of hair.[18] Peripherally inserted central catheters (PICC lines) are inserted in the superior vena cava via the veins of the upper arm. The HICPAC guidelines suggest that they are associated with lower rates of infection than other non-tunneled central venous catheters, and that this may be due to being distant from the oropharyngeal secretions and less heavy colonization of the antecubital site.[2] However, a prospective cohort study using data from two randomized trials found rates of infection in hospitalized patients with PICC lines to be similar to that of other non-tunneled lines, and much greater than when PICCs are used exclusively in the outpatient setting.[19]

Dressings

Appropriate adhesive dressings are crucial in the prevention of extraluminal colonization. Transparent, semipermeable dressings have gained favor, allowing regular observation of the line site, permitting showering and requiring less frequent replacement. Although initial reports described a high rate of colonization of the exit site with these dressings, a meta-analysis comparing transparent with gauze dressings found no significant difference in rates of CRBSI.[20]

Chlorhexidine-impregnated sponges, which can be placed over the line insertion site and fixed with a transparent dressing, have been the subject of a meta-analysis of eight randomized controlled trials.[21] Although their use was associated with a trend towards reduction of vascular catheter infection, the number of sponges needed to prevent one infection was high and their use has not been widely recommended. A recent large randomized controlled trial, however, confirms the preventive efficacy of this method, showing a 70% decreased rate of CRBSI.[22]

Prevention of endoluminal contamination

Number of lumens

Despite a perception that the greater the number of lumens, the higher the risk of catheter infection, this has not been shown conclusively in either randomized controlled trials or meta-analysis, with two meta-analyses reaching differing conclusions.[23,24] The English epic guidelines suggest using a single-lumen catheter unless multiple lumens are essential for patient management.[3]

Heparin

Catheter-related thrombosis may be associated with catheter-related sepsis. A meta-analysis of 14 clinical trials indicates that the risk of catheter-related thrombosis is significantly reduced by prophylactic-dose heparin.[25] The risk of microbial colonization of the CVC was also significantly reduced, but not that of CRBSI. The epic guidelines conclude that there is insufficient evidence to recommend the routine use of heparin to reduce CRBSI, but make the point that patients with intravascular catheters are often receiving prophylactic heparin for deep vein thrombosis prophylaxis, and that heparin flushes may be useful for maintaining line patency in infrequently accessed lines or in accordance with manufacturers' recommendations for hemodialysis lines or implanted ports.[3]

Antibiotic locks in prevention

Routine use of antibiotic locks for prevention of CRBSI has not been shown to be beneficial and may promote resistance. Their use may be appropriate in selected cases, for example for the prevention of recurrent infection in patients with long-term tunneled or implanted lines (e.g. patients on hemodialysis) who experience multiple episodes of confirmed infection despite optimum antiseptic practice.[2,3]

Replacement of administration sets

Studies have shown that replacement of intravenous administration sets no more frequently than every 72 hours is safe and cost-effective. Administration sets for total parenteral nutrition should be changed every 24 hours, and those for blood products should be disposed of after the infusion or after 12 hours, whichever is sooner.[3]

Other strategies for prevention

Tunneled or totally implanted devices

Long-term tunneled or totally implanted venous access devices are associated with lower rates of infection than non-tunneled long-term lines, with totally implanted systems having the lowest rates.

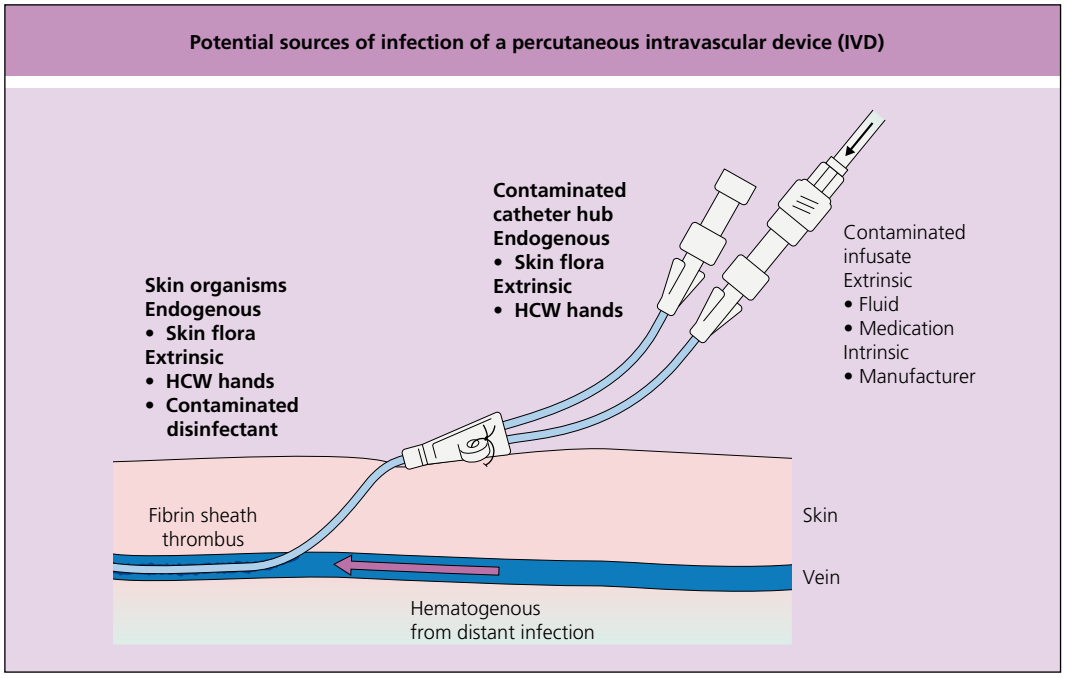


Fig. 45.1 Potential sources of infection of a percutaneous intravascular device (IVD). These include contiguous skin flora, contamination of the catheter hub and lumen, contamination of infusate and hematogenous colonization of the IVD from distant, unrelated sites of infection. HCW, health-care worker. From Crnich & Maki.[6] Copyright The University of Chicago Press.

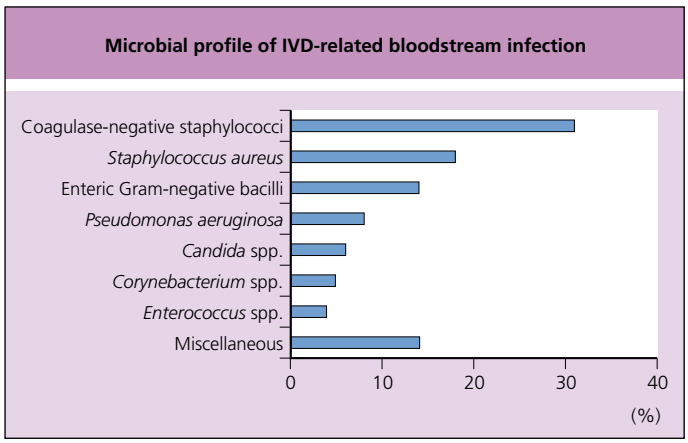


Fig. 45.2 Microbial profile of intravascular device-related bloodstream infection. Based on an analysis of 159 published prospective studies. From: Maki *et al.*[7]



Fig. 45.3 Drained infusion port pocket abscess with evidence of intravascular device-related inflammation. From Raad *et al.*[34]

For patients requiring long-term intermittent access, totally implanted devices are preferred. For situations where frequent or regular access is needed, a tunneled line is preferable.[2,3]

Tunneling of short-term internal jugular or femoral lines reduces infection rates;[26] no such difference is seen with subclavian lines.[27] However, the apparent risk reduction associated with the tunneling of nutrition catheters disappeared completely with the introduction of a specialist nutrition nurse in one study,[28] suggesting that rigorous aseptic nursing care may be the more significant factor.

Antimicrobial catheters

Antiseptic catheters

Catheters coated with chlorhexidine and sulfadiazine silver on their outer surface were associated with reduced rates of colonization and catheter-related bloodstream infection in a meta-analysis,[29] and have been shown to be cost-effective in high-incidence settings.[30] The antimicrobial activity decreases over time and benefit is likely to be realized in the first 15 days.[31] A second-generation catheter impregnated on both the external and internal surfaces, and with three times the concentration of chlorhexidine, was associated with reduced rates of colonization but without significant reduction in bacteremia.[32] However a possible explanation for this may be underpowering of the studies, given a low baseline infection rate. Rare cases of anaphylaxis have been reported to these catheters in Japan and the UK.[33]

Antibiotic-coated catheters

Catheters impregnated with minocycline and rifampicin on both the external and internal surfaces have been shown to significantly reduce rates of CRBSI in randomized controlled trials.[34,35] A prospective randomized study comparing first-generation chlorhexidine/sulfadiazine catheters with minocycline/rifampicin showed the latter to be associated with significantly lower rates of CRBSI;[36] this has subsequently been confirmed in a meta-analysis.[33] The duration of antimicrobial activity is greater than that of first-generation chlorhexidine/sulfadiazine catheters and may extend beyond 4 weeks.[33]

Table 45.3 Proposed definitions for intravascular device (IVD)-related colonization, local infection and bloodstream infection, based on microbiologic confirmation of the IVD as the source

IVD colonization	• Positive semiquantitative* (or quantitative†) culture of the implanted portion or portions of the IVD • Absence of signs of local or systemic infection
Local IVD infection	• Positive semiquantitative* (or quantitative†) culture of the removed IVD or a positive microscopic examination or culture of pus or thrombus from the cannulated vessel • Clinical evidence of infection of the insertion site (i.e. erythema, induration or purulence), *but* • Absence of systemic signs of infection and negative blood cultures, if done
IVD-related bloodstream infection (BSI)	If the IVD is removed: • Positive semiquantitative* (or quantitative†) culture of the IVD or a positive culture of the catheter hub or infusate (or positive microscopic examination or culture of pus or thrombus from the cannulated vessel) *and* one or more positive blood cultures, ideally percutaneously drawn, concordant for the same species, ideally by molecular subtyping methods • Clinical and microbiologic data disclose no other clear-cut source for the BSI If the IVD is retained: • If quantitative blood cultures are available, cultures drawn both from the IVD and a peripheral vein (or another IVD) are both positive and show a marked step-up in quantitative positivity (fivefold or greater) in the IVD-drawn culture • Clinical and microbiologic data disclose no other clear-cut source for the BSI *or* • If automated monitoring of incubating blood cultures is available, blood cultures drawn concomitantly from the IVD and a peripheral vein (or another IVD) show both are positive, but the IVD-drawn blood culture turns positive more than 2 hours before the peripherally drawn culture • Clinical and microbiologic data disclose no other clear-cut source for the BSI

*Roll plate of cannula segment(s) >15 cfu
†Sonication culture of cannula segment(s) $\geq 10^3$ cfu.
Adapted from Crnich & Maki.[10]

Table 45.4 Guideline for the prevention of intravascular device-related bloodstream infection

Recommendation		Strength of evidence*
General measures	Educate all health-care workers involved with IVD care and maintenance	IA
	Ensure adequate nursing staffing levels in intensive care units	IB
Surveillance	Monitor institutional IVD infection rates of IVD-related BSI	IA
	Express rates of CVC-related BSI per 1000 CVC-days	IB
At catheter insertion	Aseptic technique:	
	Hygienic hand care before insertion or manipulation of any IVD	IA
	Clean or sterile gloves during insertion and manipulation of noncentral IVDs	IC
	Maximal barrier precautions during insertion of CVCs: mask, cap, sterile gown, gloves, drapes	IA
	Dedicated IVD team strongly recommended	IA
	Cutaneous antisepsis: first choice, 2% chlorhexidine; however, tincture of iodine, an iodophor or 70% alcohol are acceptable (no recommendations for use of chlorhexidine in infants less than 2 months, unresolved issue)	IA
	In adults, other than hemodialysis catheters (jugular site preference), use a subclavian site rather than a jugular or femoral site for CVC access (in pediatric patients, no recommendations for preferred site, unresolved issue)	IA
	Use of sutureless securement device	NR
	Sterile gauze or a semipermeable polyurethane dressing to cover site	IA
	No systemic or topical antibiotics at insertion	IA
Maintenance	Remove IVD as soon as no longer required	IA
	Monitor IVD site daily	IB
	Change dressing of CVC insertion site at least weekly	II
	Do not use topical antibiotic ointments	IA
	Change needleless iv systems at least as frequently as the administration set; replace caps no more frequently than every 3 days or per manufacturers' recommendations	II
	Complete lipid infusions within 12 hours	IB

Table 45.4 Guideline for the prevention of intravascular device-related bloodstream infection—cont'd

Recommendation		Strength of evidence*
	Replace administration sets no more frequently than every 72 hours. When lipid-containing admixtures or blood products are given, sets should be replaced every 24 hours; with propofol, every 6–12 hours	IA
	Replace peripheral IVs every 72–96 hours	IB
	Do not routinely replace CVCs or PICCs solely for prevention of infection	IB
	Do not remove CVCs or PICCs solely because of fever unless IVD infection is suspected but replace catheter if there is purulence at the exit site, especially if the patient is hemodynamically unstable and IVD-related BSI is suspected	II
Technology	Use antimicrobial-coated or antiseptic-impregnated CVC in adult patients if institutional rate of BSI is high despite consistent application of preventive measures and catheter likely to remain in place >5 days (no data or recommendations for pediatric patients)	IB
	Use of chlorhexidine-impregnated sponge dressings (no recommendation for adults or children – unresolved issue; do not use in neonates <7 days old or of gestational age <26 weeks)	NR
	Use prophylactic antibiotic lock solution only in patients with long-term IVDs who have continued to experience IVD-related BSIs despite consistent application of infection control practices	II

BSI, bloodstream infection; CVC, central venous catheter; IVD, intravascular device; PICC, peripherally inserted central catheter.
*IA, strongly recommended for implementation and supported by well-designed experimental, clinical or epidemiologic studies; IB, strongly recommended for implementation and supported by some experimental, clinical or epidemiologic studies and a strong theoretical rationale; IC, required by state or federal regulations, rules or standards; II, suggested for implementation and supported by suggestive clinical or epidemiologic trials or a theoretical rationale – unresolved issue, an unresolved issue for which evidence is insufficient or no consensus regarding efficacy exists; NR, no recommendation for or against at this time.
Adapted from O'Grady *et al*.[2]

Clinical studies have shown conflicting results regarding emergence of resistance to either of the antibiotics concerned, although *in-vitro* studies have indicated that resistance may develop, particularly among staphylococci.[2,33] Some studies have reported an increased risk of colonization with *Candida* species in patients with these catheters.[33]

Silver-impregnated catheters

Silver has broad-spectrum antimicrobial activity; however activity is greater against Gram-negative than Gram-positive organisms. A meta-analysis failed to show any benefit in terms of catheter colonization or CRBSI with any of the available silver-impregnated vascular catheters.[33]

The CDC's Hospital Infection Control Practices Advisory Committee guidelines[2] suggest that antimicrobial catheters (chlorhexidine/sulfadiazine or minocycline/rifampicin) be considered in settings where a high rate of CRBSI persists despite the implementation of other measures (education, training, optimal skin antisepsis, maximum sterile barrier) for patients who are expected to need the catheter for more than 5 days. The English epic guidelines[3] suggest the use of antimicrobial catheters for patients who are at high risk of infection and likely to need central venous access for 1–3 weeks.

Results of a meta-analysis of antimicrobial catheters are shown in Figure 45.4.

Antiseptic cuffs

Silver-impregnated cuffs failed to reduce the incidence of CRBSI, in either short-term or long-term catheterization.[2,3]

Catheter replacement

Short-term peripheral cannulae should be replaced every 48–72 hours to prevent thrombophlebitis and infection.[2] However, routine replacement has not been shown to reduce the infection risk associated with central venous catheters, dialysis catheters or peripheral arterial lines[24] and is not recommended.[2,3] The use of guidewire exchange should not be used in cases of confirmed catheter-related infection. However, exchange over a guidewire may be used in cases of suspected line infection where there is no evidence of local infection of the line site. The tip should be sent for culture and if infection is confirmed, the new catheter should be removed and access established at an alternative site.[3]

Multifaceted approach

The very nature of the prevention measures outlined above necessitates a multifaceted approach to their implementation. A number of studies have demonstrated that the combination of several simple measures (the 'prevention bundle' approach)[37] may dramatically reduce the incidence of CRBSI. A multicenter study across 103 intensive care units in Michigan evaluated the implementation of five prevention measures (hand hygiene, maximum sterile barrier, chlorhexidine skin antisepsis, avoidance of the femoral site where possible and prompt removal of unnecessary lines).[38] A dramatic reduction in the incidence of CRBSI, with median rates lowered to zero, was seen at 3 months and maintained at 18. Several authors have demonstrated the importance of staff education programs, standardization of aseptic care and adequate staffing levels. Surveillance and feedback of infection rates can reduce the incidence of CRBSI without any additional intervention.[39] Rates of infection go up when inexperienced staff are responsible for line care, and specialist 'IV teams' have been shown unequivocally to reduce rates of CRBSI.[2]

DIAGNOSIS

Clinical diagnosis

Clinical features are unreliable for the diagnosis of catheter-related infection. Fever and chills have poor specificity, whereas local inflammation of the line site is associated with greater specificity but a

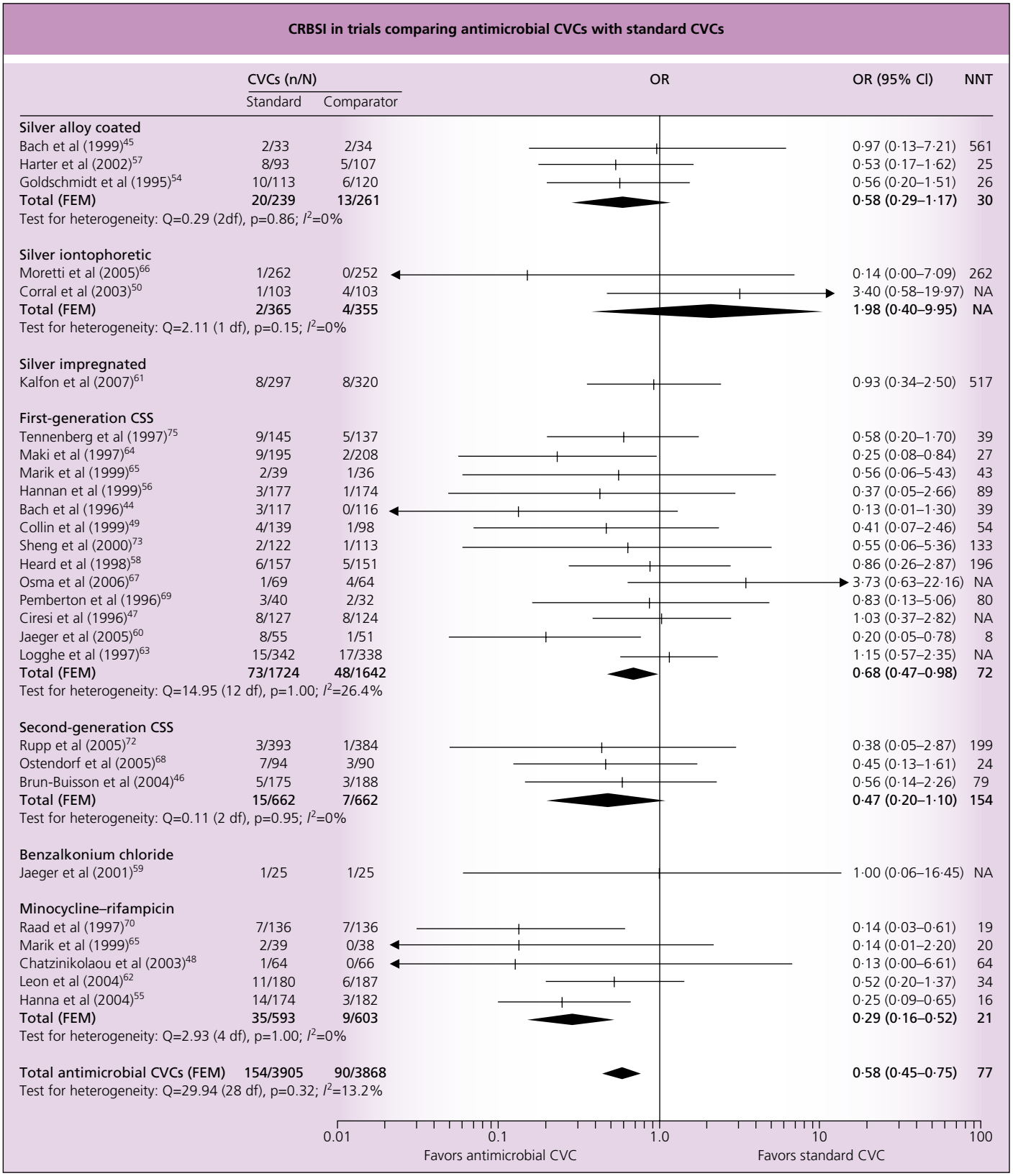


Fig. 45.4 Catheter-related bloodstream infection in trials comparing antimicrobial central venous catheters (CVCs) with standard CVCs. From Casey *et al*.[33] Reprinted from the Lancet Infectious Diseases. Copyright Elsevier 2008.

sensitivity of 3% or less.[9] Removal of a catheter in response to a clinical suspicion alone in a febrile patient results in unnecessary removal in 70–80% of cases.[26] It is therefore preferable to make a diagnosis of catheter-related infection microbiologically, ideally while the catheter remains in place.

Diagnostic methods with the catheter *in situ*

In the absence of septic shock, a 'watchful waiting' approach has been shown in a randomized trial to be a safe alternative to immediate catheter removal in patients in intensive care.[40] The notable exception to this approach might be those patients where the consequences of missing a catheter-related infection are potentially more serious, such as in the presence of an intravascular foreign body.

In order to rule out the line as the source of an unexplained fever, diagnostic techniques with a high negative predictive value are required.

Culture of the skin insertion site or hub

Several studies have evaluated the role of culturing swabs taken from the skin around the catheter insertion site, and swabs of the catheter hub.[41–44] Routine surveillance cultures of these sites have failed to predict line sepsis and therefore, in the absence of clinical suspicion, these methods are unhelpful. However, several authors have noted that catheter-related bacteremia is uncommon when these cultures are negative (negative predictive value >90%[41,42,44]), and as such these samples may be useful in eliminating the line as a source in cases of unexplained fever.

Paired quantitative blood cultures

This method involves taking paired blood cultures simultaneously from the line and peripherally, and culturing each using a quantitative technique. If the blood culture taken from the line is positive with a colony count several times higher than that of the peripheral culture, it is predictive of catheter-related bloodstream infection.[1,45] When sepsis is not related to the catheter, the colony counts are similar from the two sites. Studies have reported different cut-off points of between 4- to 10-fold as being highly predictive of CRBSI; both the Infectious Diseases Society of America (IDSA) and the French Society of Intensive Care (SRLF)[11] have chosen a ratio of 5:1 for a positive diagnosis. A meta-analysis found paired quantitative cultures to be the most accurate test for diagnosis of catheter-related bloodstream infection, with sensitivity and specificity of 75% and 97%, respectively, for short-term lines and 93% and 100%, respectively, for long-term lines.[46] However, as this technique is labor intensive and expensive, it has not been widely adopted.

A single catheter-drawn quantitative blood culture yielding more than 100 colony forming units (cfu)/ml may be diagnostic without a paired peripheral culture, particularly when taken from tunneled catheters.[1] However, this method cannot distinguish between catheter-associated bacteremia and high-grade bacteremia from another source, and is associated with low sensitivity in short-term catheters.

Differential time to positivity of paired blood cultures

This technique is derived from the same concept as paired quantitative blood cultures as described above, but takes advantage of the fact that most diagnostic laboratories use automatic blood culture machines which detect 'time to positivity' on a continuous basis. The 'time to positivity' is inversely proportional to the initial inoculum[47] and can therefore be used as a less labor-intensive method of estimating differences in bacterial load between the two samples. When a blood culture taken from the CVC becomes positive at least 2 hours earlier than that taken peripherally, it is highly predictive of CRBSI. This method has been validated in oncology, hematology and pediatrics.[47–51] Although paired culture techniques are more sensitive for long-term catheters, evidence exists to support their use in short-term lines also.[51] A meta-analysis found this technique to have a sensitivity and specificity of 89% and 87%, respectively, for short-term catheters and of 90% and 72%, respectively, for long-term catheters. Differential time to positivity is a relatively easy technique with minimal added costs. Difficulties may arise when insufficient blood can be aspirated via the line in order to carry out the culture, which may occur in up to a quarter of cases.[52]

Acridine orange leukocyte cytospin, endoluminal brush

Aspiration of a small (50–100 microliter) sample of blood from the catheter and direct examination using acridine orange leukocyte cytospin (AOLC) is a rapid diagnostic method, giving a result in 30–60 minutes. The sample is spun, stained with acridine orange and examined for the presence of bacteria using ultraviolet microscopy (Fig. 45.5). First evaluated favorably in neonates, in adults the sensitivity of this method has been very variable between studies.[53] The use of endoluminal brushing of the catheter prior to sampling has been proposed to increase sensitivity.[54] However, theoretical risks of embolization or induced bacteremia cannot be excluded. A study using AOLC followed by Gram staining reported a sensitivity of 96% and sensitivity of 92%.[55] Currently these techniques have not been widely adopted and require further investigation.

Diagnostic methods requiring line removal

Qualitative culture of catheter tip

Older methods of qualitative culture of the catheter tip in liquid broth were oversensitive and did not distinguish infection from colonization or contamination, and have been abandoned.

Semiquantitative culture of catheter tip

Semiquantitative culture (roll plate) of the catheter tip, described by Maki *et al*. in 1977,[56] is one of the most widely used methods of

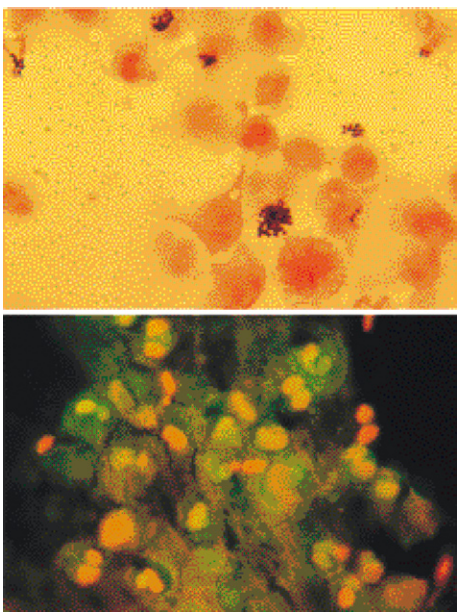


Fig. 45.5 Examples of Gram stain and acridine orange leukocyte cytospin (AOLC) tests positive for septicemia. Top: Gram-positive staphylococci (*Staphylococcus aureus*); bottom: stained with acridine orange (*Candida albicans*). Red and green cells are leukocytes. From Kite *et al*.[55] Copyright Elsevier 1999.

catheter culture. The distal catheter segment is rolled across the surface of an agar plate and incubated for 24–48 hours; the presence of more than 15 cfu was found to correlate with local signs of inflammation in the patients studied, most of whom had short-term peripheral catheters rather than CVCs. A meta-analysis examined 14 trials of this method in short-term central venous catheters and found pooled sensitivities and specificities of 84% and 85%, respectively.[46] A significant limitation of this method is that it only analyses the extraluminal portion of the catheter. The accuracy of this technique in diagnosing CRBSI in long-term CVCs, for which the intraluminal route of colonization is more important, is of concern, with sensitivity reportedly as low as 45%.[34]

Quantitative culture of catheter tip

Several quantitative methods have been described. Flushing of the catheter tip with broth examines only the internal surface. Vortexing the catheter tip in 1 ml sterile water as described by Brun-Buisson *et al.*[57] allows the release of organisms from both the internal and external surfaces of the catheter. A threshold of 10^3 cfu/ml correlated with systemic signs of infection, with or without catheter-associated bacteremia, and exhibited high specificity (88%) and sensitivity (97%) in critically ill patients. An alternative method using sonication of the catheter tip in broth gave similar results.[58] Quantitative culture of catheter segments was more accurate than roll plate and qualitative methods in a receiver operating characteristic (ROC) curve analysis.[59] Pooled sensitivity and specificity of quantitative segment culture for short-term catheters was 82% and 89%, respectively, and 83% and 97%, respectively, for long-term catheters in a meta-analysis.[46]

Direct examination of catheter tip

Gram staining or acridine orange staining of the catheter tip appears to offer good sensitivity and specificity but is labor intensive and time consuming and has not been widely adopted.

MANAGEMENT

Line removal

In the presence of septic shock, without an alternative source, the line should be removed and empiric antibiotics commenced. Other indications for early line removal include deep localized infection such as tunnel infection, frank cellulitis, pocket infection or port abscess. Catheter-related infections caused by *S. aureus* or *Candida* spp. are associated with high failure rates if treatment is attempted with the line still in place, and therefore line removal is recommended when these organisms are implicated.[1] The same is probably true of *Pseudomonas* and possibly other aerobic Gram-negative bacteria. Catheter infections complicated by septic thrombosis, endocarditis or osteomyelitis also require line removal. When dealing with tunneled or surgically implanted lines, it is clearly particularly important to make every attempt to confirm the diagnosis of catheter-related infection in order to avoid unnecessary line removal. In the case of coagulase-negative staphylococci, treatment with prolonged antibiotics plus lock therapy may be successful without line removal. Some authors have also reported the success of this approach with other organisms, provided no complications are present, in cases where line salvage is required.[1]

Empiric antibiotic choice

Antibiotics for suspected catheter-related infection are often started empirically. The choice of antibiotic will depend on clinical severity and risk factors in an individual patient. Direct staining of a catheter insertion site swab, or of the catheter itself, may help guide therapy.[60] Although evidence is lacking to support a particular choice of antibiotic combination for empiric treatment, usually a glycopeptide is chosen, due to the high prevalence of resistant Gram-positive organisms in these infections. The addition of broad spectrum Gram-negative (including pseudomonal) cover is appropriate in cases of severe sepsis or immunocompromise.[1] Antibiotics can then be rationalized when culture results become available.

Antibiotic choice and duration

Limited evidence exists regarding optimal duration of antibiotic treatment for CRBSI; suggestions below are based on consensus opinion as outlined in the guidelines of the IDSA.[1]

Coagulase-negative staphylococci

Coagulase-negative staphylococci, being skin commensals, are the commonest cause of catheter-related infection but also the commonest contaminants. In order to confirm the diagnosis of coagulase-negative staphylococcal CRBSI, at least two positive blood cultures, of which one should be from a peripheral sample, are needed.[34] If the line has been removed, and the patient lacks risk factors for complicated disease (such as a prosthetic graft), a short course of 5–7 days of antibiotic is likely to be sufficient.

If it is preferable that the line be retained, and the patient lacks risk factors for complicated disease, it is reasonable to try treatment with the line *in situ*. Eighty percent of CRBSI caused by coagulase-negative staphylococci may be successfully treated without catheter removal, with a 20% chance that bacteremia will recur.[61] The IDSA guidelines recommend a 10–14-day course of systemic antibiotic and antibiotic lock therapy for non-tunneled lines or 7 days of systemic antibiotic followed by 14 days of lock therapy for tunneled lines.

Staphylococcus aureus

Staphylococcus aureus is associated with a higher rate of complicated or metastatic infection, such as septic thrombosis or endocarditis. The duration of treatment will depend on the likelihood of such complications. Persistence of fever and/or bacteremia at 72 hours after catheter removal and antibiotic initiation is predictive of complicated disease.[34] A study examining the use of transesophageal echocardiography in patients with *S. aureus* bacteremia made a diagnosis of endocarditis by Duke Criteria in 23% of patients.[62] The use of transthoracic echocardiography was associated with a sensitivity of just 32%. The authors, and the IDSA guidelines, recommend transesophageal echocardiogram in all patients with *S. aureus* bacteremia, and the extension of treatment to 4–6 weeks when this complication is present.[1] For treatment of seemingly uncomplicated *S. aureus* bacteremia, not less than 14 days of treatment following catheter removal is recommended.[1,63] In selected cases where salvage of a tunneled line is required, in the absence of complications and with a negative transesophageal echo, the IDSA guidelines suggest 14 days of systemic antibiotics plus lock therapy.

Gram-negative bacilli

The incidence of Gram-negative intravascular catheter infections may be increasing. They are a relatively common cause of CRBSI in immunocompromised patients with long-term tunneled catheters and of infections associated with contaminated infusate.[1] Retrospective studies have shown high rates of relapse if the catheter is not removed;[64,65] however, anecdotal success has been reported with systemic antibiotics plus antibiotic lock therapy for salvage of tunneled catheters.

Candida species

All cases of catheter-related candidemia should be treated with systemic antifungals to avoid the complication of sight-threatening

candidal endophthalmitis.[1] Several prospective studies have identified catheter retention as a risk factor for persistent candidemia or increased mortality.[34] Failure to remove the catheter was associated with poorer response to antifungals in a large retrospective study of patients with cancer, provided the candidemia was catheter related.[66] However, in cases of noncatheter related candidemia, catheter removal had no effect on outcome. Clinical characteristics that suggested a noncatheter source for the candidemia included disseminated infection at diagnosis, recent chemotherapy and neutropenia; characteristics that implicated the catheter included isolation of *Candida parapsilosis*, paired blood culture techniques supportive of a catheter source of candidemia, and parenteral nutrition.

Fluconazole at a dose of 400 mg/day has been shown to be as effective as amphotericin in non-neutropenic patients with candidemia, with less associated toxicity.[67] Fluconazole may therefore be used for susceptible isolates.[1] For empiric treatment before speciation and sensitivity results are available, amphotericin B is recommended in hemodynamically unstable patients or those who have received prolonged fluconazole therapy. Otherwise, empiric treatment with fluconazole is recommended.[1] Catheter-related *Candida krusei* infections should be treated with amphotericin B.[1] Non-tunneled CVCs should be removed; in the case of a tunneled line it is important to determine if the candidemia is catheter related or secondary to another source such as the gastrointestinal tract. If the catheter is confirmed as the source, it should be removed. Duration of treatment should be for 14 days after the last positive blood culture and when signs and symptoms of infection have resolved.[1]

Treatment of a positive catheter tip culture without bacteremia

There is a lack of evidence to guide the management of positive catheter tip cultures without definite evidence of bacteremia. IDSA guidelines suggest that it is not usually necessary to treat; however, if *S. aureus* or *Candida* spp. are isolated, particularly in the context of risk factors such as valvular heart disease or prosthetic graft, patients should be watched closely for evidence of persistent infection and blood cultures should be taken. Some experts would advocate a short course (5–7 days) of antimicrobial treatment.

Complications

Persistence of fever and/or bacteremia 72 hours after catheter removal and initiation of appropriate antibiotics should prompt a thorough search for complicated or secondary infection including septic thrombosis, endocarditis and osteomyelitis. This is particularly important in the case of *S. aureus* infection. If a secondary source of infection is found, antibiotic treatment will need to be prolonged, usually to a minimum of 4–6 weeks. Superficial septic thrombosis may also necessitate excision of the vein; septic thrombosis of the great central veins and arteries requires anticoagulation in addition to antibiotic treatment.[1]

Antibiotic line locks

The technique of line locking involves instilling antibiotics at high concentration (>100 times the minimum inhibitory concentration of the organism) at sufficient volume to fill the lumen of the catheter, and leaving them in place for a period of several hours or even days while the catheter is not in use (such as overnight). *In-vitro* studies have demonstrated that these higher concentrations of antibiotics are needed to kill sessile bacteria growing in a biofilm,[8] and *in-vivo* studies have shown higher rates of successful catheter salvage using antibiotic locks in addition to systemic treatment when compared with systemic antibiotics alone.[1] Antibiotics that can be used in this way include vancomycin, teicoplanin, gentamicin or amikacin, usually for a duration of 2 weeks. Attempts to use antifungals in this way have been less successful and are not recommended. Lock therapy is unlikely to be successful if extraluminal infection is present and as such the role of this approach with short-term or recently inserted lines is uncertain.

Infections of Arterial Grafts

INTRODUCTION

Reconstructive surgery of occluded or aneurysmal arteries may involve the implantation of either an autologous vein graft or a prosthetic graft. Graft infection, more common with prosthetic grafts, is a much feared complication which may lead to limb loss or death.

EPIDEMIOLOGY

The risk of arterial graft infection varies with the site of the graft, with higher rates associated with lower limb revascularization and lower rates in aortic graft replacement (Table 45.5). Many patient or operative factors may influence the risk of infection, including diabetes, prolonged operating time, malnutrition and lower limb infection at the time of surgery (Table 45.6).

PATHOGENESIS

Early graft infection (during the first 30 days) is relatively uncommon and usually secondary to early groin or lower limb wound infection. Lower limb graft infection more commonly presents beyond 4 months, and aortic graft infection may present years later.[69] Intraoperative contamination of the graft with skin flora may occur in as many as 56% of arterial grafts,[70] implying this as the likely route of acquisition for the majority of graft infections. Contiguous spread from local wound infection or hematogenous spread from a distant site (such as an infected intravascular catheter) may also occur. Once present, micro-organisms can adhere to the graft and form biofilms similar to those seen in catheter-related infection. In this way they can evade the immune system and antibiotic activity, making treatment without graft removal extremely difficult.

Staphylococci, predominantly *S. aureus*, are the most commonly implicated pathogens, with *S. aureus* being responsible for up to 55% of all deep wound and graft infections in the UK.[71] Around 70% of *S. aureus* isolated from vascular surgical-site infections are methicillin resistant.[71] Gram-negative bacteria account for to up to one-third of such infections and anaerobes may be implicated in patients with ischemic tissue. Early infection is more likely to be caused by *S. aureus* or Gram-negative organisms, with less virulent organisms such as coagulase-negative staphylococci, enterococci and streptococci being implicated in late infection.

Table 45.5 Rates of infection by location of arterial graft

Location of arterial graft	Rates of infection (%)
Thoracic aorta	1.2–3.0
Aortoiliac	0.0–1.3
Aortofemoral	0.5–3.0
Femorofemoral	3.6–5.0
Femoropopliteal	2.0–4.6
Axillofemoral	5.3–6.0
Cervical	0.0–0.2

Adapted from Goëau-Brissonnière & Coggia.[68]

Table 45.6 Patient, procedure and environmental risk factors for surgical site infection

Patient-related risk factors	Nasal carriage of *Staphylococcus aureus* Prolonged preoperative length of stay Postoperative bacteremia End-stage renal disease Obesity Malnutrition/low serum albumin Older age Smoking, nicotine use Diabetes mellitus Prior incision site irradiation Malnutrition/low serum albumin Autoimmune disease/corticosteroid therapy Malignancy and chemotherapy
Procedure-related risk factors	Femoral/groin incision Remote infection Biomaterial implant Emergency/preoperative procedure American Society of Anesthesiology score >2 Extended operative time Hypothermia Shock Hyperglycemia
Environmental risk factors	Operating suite ventilation – environmental surface cleaning Instrument and vascular implant sterility Surgical attire and sterile operative technique

From Bandyk.[69] Copyright Elsevier 2008.

PREVENTION

Prior to elective vascular surgery, every attempt should be made to reduce a patient's individual risk factors for infection. Diabetic control and nutritional state should be optimized, and local or distant sites of infection eradicated or minimized.

Despite being classified as clean procedures, the additional risk associated with the implantation of prosthetic material justifies the use of prophylactic antibiotics in vascular surgery. A meta-analysis has examined 35 randomized controlled trials of preventive measures in arterial surgery.[72] Prophylactic systemic antibiotics reduced the risk of wound infection and early graft infection by between three-quarters and two-thirds (RR 0.25 and 0.31, respectively). Prophylaxis continued beyond 24 hours was not associated with any additional benefit. Other interventions including preoperative bathing or showering with antiseptic agents or suction groin-wound drainage lacked evidence of effectiveness. No benefit was demonstrated from the use of rifampin (rifampicin)-impregnated grafts in preventing either early or late graft infection.

First- or second-generation cephalosporins or co-amoxiclav are commonly used for prophylaxis in vascular surgery. The increasing prevalence of methicillin-resistant *S. aureus* (MRSA) and high rates of methicillin resistance among coagulase-negative staphylococci has raised the question as to whether glycopeptides should be added to prophylaxis regimens. Routine use of glycopeptides has not generally been adopted, in part due to concern that this would promote the emergence of glycopeptide resistance among enterococci and staphylococci. However, their use in high-risk patients has been advocated, for example in patients with a history of MRSA colonization or infection, or in settings with a high prevalence of methicillin-resistant staphylococci.[63,73,74] However, a threshold defining 'high prevalence' has not been well established. Wide use of MRSA screening and subsequent decontamination has been recommended in the UK, including preoperatively for all elective surgery.

CLINICAL FEATURES

Early infection will often present with local signs of cellulitis, purulent drainage or wound dehiscence in combination with systemic signs of sepsis. Early infection with virulent organisms may quickly progress to pseudoaneurysm formation and hemorrhage, whereas more indolent infection may present with cutaneous sinus tracts or graft thrombosis. Late infection of aortic grafts will more typically present with unexplained fever or bacteremia, but may present with catastrophic gastrointestinal hemorrhage from aortoenteric fistula formation.

DIAGNOSIS

Before empiric antibiotics are started, at least two blood cultures should be taken. The presence of fluid collections around the graft is highly suggestive of graft infection, although it may be a normal finding in the early postoperative period. Deep fluid collections should be aspirated for culture, preferably prior to antibiotic treatment in a hemodynamically stable patient. Fluid cultures may, however, be negative in cases of infection caused by less virulent organisms such as coagulase-negative staphylococci. Imaging with contrast CT may show failure of the graft to become incorporated into adjacent tissues, perigraft fluid or gas, pseudoaneurysm formation, focal bowel wall thickening or intragraft thrombus[75] (Fig. 45.6). Nuclear medicine scans are unhelpful in the early postoperative period when they are likely to be falsely positive, but may be useful in difficult late cases. Sinography, in which contrast is injected into a cutaneous sinus, may show tracking of dye along a non-incorporated infected graft.[75] Ultrasound may be used to identify infrainguinal pseudoaneurysm formation or graft thrombus.

MANAGEMENT

With few exceptions, the management of arterial graft infection is surgical. Intravenous antibiotic therapy is initiated preoperatively and usually continued for a minimum of 4–6 weeks postoperatively.

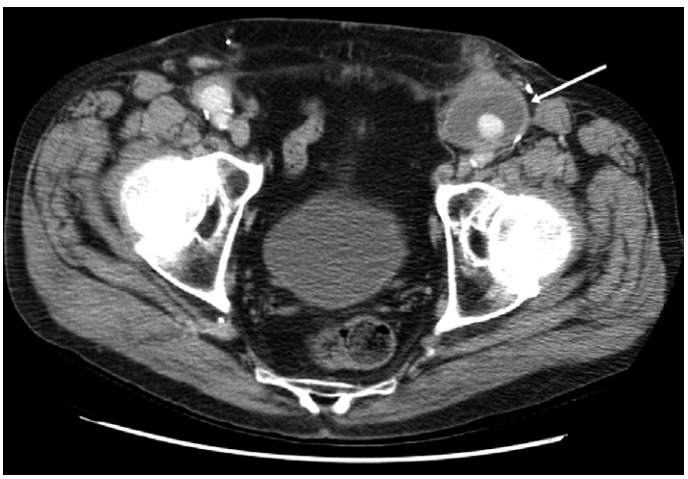


Fig. 45.6 Contrast CT scan of the pelvis demonstrating perigraft fluid in a patient with an infected left-sided aortoiliac graft (arrow).

In patients who are profoundly septic on presentation, empiric antibiotics should cover the range of nosocomial pathogens, for example combining a glycopeptide with an antipseudomonal penicillin, to be rationalized in the light of positive culture results.

Options for surgical intervention

Choice of surgical intervention must consider the location of the graft, extent of infection, virulence of the organism, options for preserving distal perfusion and a patient's likely ability to tolerate the proposed procedure.

For infrarenal aortic grafts, the gold standard approach has been total excision of the graft with bypass through a noninfected (extra-anatomic) site, most often axillobifemoral, to restore distal perfusion. Risks of this approach include stump blowout, bypass thrombosis and graft re-infection.[75] Graft excision and *in-situ* replacement with a prosthetic graft has greater patency but, as would be expected, higher rates of re-infection, and is generally only recommended as a holding procedure in low-virulence infections. Attempts at replacement with fresh or cryopreserved aortic allograft have been disappointing. *In-situ* replacement with an autogenous vein graft carries a lower risk of re-infection (although this may still occur in the context of virulent Gram-negative pathogens), but disadvantages include longer operative time, and risks of deep vein thrombosis, lower limb paresis and edema, and the need for fasciotomy. A meta-analysis of surgical options for low-virulence infections of aortic grafts[76] questioned whether extra-anatomic bypass should remain the gold standard, as *in-situ* replacements were associated with fewer adverse events. However, the authors comment that drawing such conclusions may be misleading, given the broad clinical spectrum of graft infection and individual patient subgroups that may benefit from one approach over another.

For infrainguinal graft infection, complete excision is usually required, with either *in-situ* replacement with autogenous vein graft or extra-anatomic prosthesis. Successful management without graft removal has been described in selected patients, using surgical debridement of the graft bed followed by the placement of a muscle flap.[77]

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References for this chapter can be found online at http://www.expertconsult.com

Chapter 46

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Myocarditis and pericarditis

Myocarditis

EPIDEMIOLOGY

The term myocarditis applies to a variety of disease states that produce inflammation of the myocardium. In its acute form, myocarditis ranges from an asymptomatic illness with reversible changes to fulminant myocardial necrosis and death. In its chronic form, lymphocytic infiltration of the myocardium may cause subacute deterioration of cardiac function; indeed, chronic myocarditis may predate the development of 'idiopathic' dilated cardiomyopathy (IDC). The possible association between viral myocarditis and IDC is intriguing and potentially important. Although frequently ascribed to inflammation caused by an infectious agent, myocarditis may also be seen in allergic reactions, drug reactions and in association with systemic inflammatory disease. A diagnosis of acute infectious myocarditis is suggested when unexplained heart failure or malignant arrhythmias occur in the setting of a systemic febrile illness or after symptoms of an upper respiratory tract infection.

The incidence of infectious myocarditis in the general population is unknown. In a prospective study of Finnish military recruits conducted over several years, a mean annual incidence of 0.02% was found.[1] The prevalence of clinically significant myocarditis is higher in children and young adults than in older adults, and is thought to be a major cause of sudden cardiac death in adults under the age of 40 years.[2–4] Within immunosuppressed patients, myocarditis is more prevalent, affecting approximately 50% of AIDS patients at autopsy.[5]

PATHOGENESIS AND PATHOLOGY

In myocarditis, damage to cardiac myocytes appears to involve one or more of four possible mechanisms:

- direct cytopathic effects of an infectious agent;
- cellular injury secondary to circulating exogenous or bacterial toxins;
- specific cell-mediated or humoral immunologic response to the inciting agent or induced neoantigens; and
- nonspecific cellular injury caused by generalized inflammation.

Histologically, both myocyte necrosis and infiltration by inflammatory cells in the absence of ischemia are pathognomonic of the disease. The infiltrate may be composed of a variety of cell types, including neutrophils, lymphocytes, macrophages, plasma cells, eosinophils and/or giant cells (Fig. 46.1).

The pathologic abnormalities associated with myocarditis vary considerably depending on the etiologic agent and individual host response. Coxsackie virus, for example, appears to infect cardiac myocytes directly while infection with parvovirus B19 appears to involve the vascular endothelium. The time after infection when tissue is obtained for analysis also greatly influences the histologic appearance. In acute enteroviral myocarditis, for example, few inflammatory cells may be seen, whereas cellular infiltrates are the hallmark of chronic enteroviral myocarditis. Analysis of endomyocardial biopsy specimens obtained during infection with different agents shows considerable overlap, however. Thus, a histologic diagnosis of myocarditis usually does not indicate the agent responsible.

Bacteria

Bacteria may cause myocarditis by a variety of mechanisms. Bacteremia caused by a wide variety of species may result in metastatic foci within the myocardium. Myocarditis has been noted in association with streptococcal and staphylococcal bacteremia, meningococcemia, bartonellosis, brucellosis, leptospirosis and Whipple's disease. However, the resulting myocardial dysfunction is only clinically significant in a subset of patients with overwhelming infections. In contrast, myocardial involvement in bacterial endocarditis is more common and is often clinically significant. Bacteria (especially *Staphylococcus aureus*) may directly invade the myocardium from infected valves to cause abscesses, valvular failure and conduction abnormalities, or may embolize throughout the myocardium to cause global ventricular dysfunction. Cardiac infections caused by salmonellae are particularly serious; mural involvement responds poorly to antimicrobial agents and, without surgical therapy, mortality is 100%.

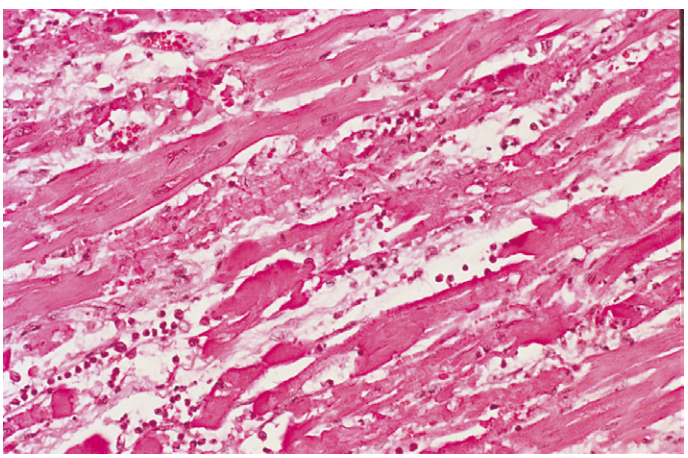


Fig. 46.1 Acute viral myocarditis, with a characteristic mononuclear infiltrate.

Bacterial toxin production can also be clinically significant. Subtle evidence of toxin mediated myocarditis can be detected in as many as two-thirds of patients who have diphtheria, occurring 1–2 weeks after the onset of illness, often when the oropharyngeal manifestations are improving.[6] Patients who have electrocardiogram (ECG) changes of myocarditis have a mortality rate three to four times that of patients who have normal tracings, with atrioventricular nodal and left bundle branch block carrying a mortality rate of 60–90%.[7]

Finally, cardiac involvement (including myocarditis) occurs in up to 50% of cases of acute rheumatic fever (ARF). Pancarditis (e.g. endocarditis, myocarditis and pericarditis) in cases of ARF is likely the result of an overexuberant host immune reaction to upper respiratory tract infection with rheumatogenic strains of group A streptococci. 'Molecular mimicry' (e.g. immunologic cross-reactivity to cardiac antigens elicited by streptococcal products) underlies the postulated pathogenesis of this disease, as investigators have failed to demonstrate the presence of streptococci within inflammatory cardiac lesions.

Spirochetes

Spirochetes such as *Borrelia burgdorferi*, the etiologic agent of Lyme disease, are important causes of myocarditis with cardiac manifestations occurring in approximately 8% of patients.[8] The cardiac manifestations of Lyme disease may occur in an isolated manner, or coincident with other features such as erythema chronicum migrans or neurologic abnormalities. The most prevalent abnormality is fluctuating atrioventricular block, but some patients have evidence of more diffuse myopericardial involvement. Indeed, organisms have occasionally been demonstrated in endomyocardial biopsy tissue both pre-mortem[9] and at autopsy,[10] providing supportive evidence for direct spirochetal invasion. Myocardial involvement is common in cases of fatal leptospirosis (Weil's disease), where arrhythmias or acute circulatory collapse may occur in conjunction with hepatorenal or central nervous system syndromes. At autopsy, myocardial inflammation, coronary arteritis and aortitis are common findings.

Gummatous involvement of the myocardium during late syphilis is an exceedingly rare cause of myocarditis. Cardiac manifestations reported in the literature include conduction abnormalities and myocardial infarction usually involving the left ventricle at the base of the interventricular septum with destruction of myocytes and replacement with fibrous tissue.[11]

Rickettsiae

Rickettsiae produce systemic vasculitis by endothelial invasion, which not infrequently involves the myocardium. Rocky Mountain spotted fever (caused by *Rickettsia rickettsii*) and scrub typhus (caused by *R. tsutsugamushi*) infections may cause transient cardiac dysfunction in severe illness, which invariably clears with disease resolution. *Coxiella burnetii* (the agent of Q fever) is a rare cause of myocarditis, but this may progress to heart failure and death.[12]

Parasites

Several parasites are known to cause chronic myocarditis and sustained myocardial dysfunction, primarily in the developing world. Chagas disease (American trypanosomiasis), widely distributed in Central and South America, is caused by the protozoan *Trypanosoma cruzi*. The organism enters the human host via the bite of the reduviid bug. Rarely, patients develop fever, myalgias, hepatosplenomegaly and myocarditis during acute infection, when myocardial parasites are abundant. Far more common is the development of biventricular failure from chronic myocarditis, which occurs in 30% of infected individuals. In chronic Chagas disease the heart is enlarged and microscopic evidence of focal mononuclear cell infiltrates is commonly found, despite the fact that parasites may be demonstrated in only a minority of patients. A history of residence in Central or South America should increase clinical suspicion for this pathogen.

Assessment of epidemiologic exposure is also essential for the diagnosis of *Trichinella spiralis*, an important parasite with worldwide distribution that has been linked to fatal myocarditis. Myocarditis generally develops in severe infections, in which the cardinal features of periorbital edema, myositis, fever and eosinophilia are present. Recent consumption of poorly cooked pork enhances the likelihood of this diagnosis. Other parasites and/or their ova, including *Ascaris*, *Schistosoma* and *Taenia solium*, may lodge in the myocardium during their systemic phase. The presence of eosinophilia in the context of acute heart failure should prompt a search for their presence, although eosinophilia is an important distinguishing factor for patients who have hypersensitivity myocarditis and hypereosinophilic syndrome as well.

Viruses

Viral infections are the most common cause of myocarditis in the Western world (Table 46.1). The enteroviruses (Coxsackie A and B, echovirus) have historically been regarded as the most common cause of viral myocarditis. However, the increasing use of molecular techniques such as polymerase chain reaction (PCR) have implicated other viral pathogens such as parvovirus B19 and human herpesvirus 6 (HHV-6). In a recent study from Germany, parvovirus B19 and HHV-6 were the most frequently isolated viruses in a cohort of 87 patients with viral myocarditis, accounting for 56% and 18% of infections, respectively.[13] In addition, the persistence of these viral genomes within the myocardium may play a role in the progression of acute viral myocarditis to dilated cardiomyopathy.[14–16] Earlier studies using molecular techniques suggest that 18–53% of patients who have myocarditis or IDC may have persistent enteroviral infection.[17]

Until recently, evidence of a causal link between enterovirus infection and myocarditis was primarily circumstantial, since many patients report an antecedent viral syndrome.[18,19] Observations of increased enteroviral antibody titers or a fall in convalescent titers have been offered as further evidence that enteroviruses are the causative agents. Unfortunately, these infections are common in the general population and it has been difficult temporally to associate serologic changes with changes in cardiac function. In one study it was found that 34% of patients who had IDC had Coxsackie virus B titers of 1:40, but the same incidence was found in control individuals.[20] However, if a cut-off antiviral titer of 1:1024 was used, 30% of patients who had IDC were abnormal as compared with 2% of control individuals.[21] Viral culture from myocardial biopsies has not been revealing.

Given the lack of confirmatory culture or serologic data, animal models of viral myocarditis have been constructed to demonstrate the pathogenicity of the enteroviruses. Murine models of acute Coxsackie B viral myocarditis demonstrate viral attachment, myocyte penetration and subsequent viral replication, with resulting scattered foci of cellular necrosis. This initial phase terminates with viral clearance by mononuclear cells and the expression of proinflammatory cytokines, including tumor necrosis factor and interferon gamma (IFN-γ).[22–24] The chronic phase of infection is characterized by the presence of macrophages and T cells, not replicating virus, within myocardial tissue.[25–27] Interestingly, depletion of T cells prevents the later stages of myocardial injury although replicating virus persists within the myocardium after T-cell depletion.[28] These experiments highlight the importance of T cells in the perpetuation of myocardial injury although the antigens to which they are directed remains unknown.

Immunocompromised patients

Immunocompromised patients are subject to the same infections as the immunocompetent; however, their risk for clinically significant myocarditis is higher than in the general population, and they are uniquely at risk for opportunistic pathogens. In end-stage AIDS, as many as 10% of patients will have clinically significant cardiomyopathy.[29] The increasing use of highly active antiretroviral therapy has led to a significant decrease in the incidence of this type of cardiac

Table 46.1 Infectious causes of myocarditis

Region	Normal host		Immunocompromised host
Developed world	**Common and/or important**	Viruses: Coxsackie viruses (A and B), human herpes virus 6, influenza echovirus, cytomegalovirus (CMV), Epstein–Barr virus (EBV), influenza viruses (A and B), adenovirus, parvovirus B19, hepatitis B virus, hepatitis C virus Bacteria: *Corynebacterium diphtheriae, Borrelia* spp., any organism associated with infective endocarditis Parasites: *Trichinella spiralis, Trypanosoma cruzi*	Viruses: HIV, CMV, EBV, varicella-zoster virus (VZV), adenovirus, parvovirus Fungi: *Candida, Aspergillus, Cryptococcus* Parasites: *Toxoplasma gondii, Trypanosoma cruzi*
	Uncommon	Viruses: adenovirus, parvovirus, respiratory syncytial virus, hepatitis B virus, ?hepatitis C virus Bacteria: staphylococci, streptococci, meningococci, *Salmonella, Listeria, Clostridium, Rickettsia, Bartonella, Ehrlichia*, *Campylobacter jejunii*	Fungi: *Histoplasma, Blastomyces, Coccidioides imitis*, zygomyces
Developing world		Viruses: poliovirus, mumps virus, rubella virus, arenaviruses, dengue virus, rabies virus, chikungunya virus, Ebola virus, yellow fever virus Bacteria: *Leptospira* spp. Parasites: *Trypanosoma cruzi, Trypanosoma gambiense*	

involvement in patients with HIV.[30] In a study of 33 patients infected with HIV who underwent cardiac biopsy, specific DNA hybridization demonstrated HIV in five out of 33 patients and cytomegalovirus (CMV) in 16, suggesting that cardiotropic viral infections may be important in pathogenesis.[31] Although HIV itself has been cultured from heart tissue[32] and shown to be present by *in-situ* hybridization,[33] it is only rarely present in cardiac myocytes in patients with demonstrable pathology. Thus, whether the virus itself causes heart failure, sets the stage for other cardiotropic pathogens, or is a correlate for the causative nutritional wasting present in late stage AIDS is unknown. In children with AIDS, myocarditis is perhaps even more common postmortem than in adults, and may be associated with different pathogens; in one study, adenoviruses were detected in over 30% of hearts with histologic evidence of myocarditis.[34] Both *Cryptococcus neoformans*[35] and *Toxoplasma gondii*[36] are also important opportunistic causes of myocarditis in the setting of advanced HIV disease.

Other disseminated fungal infections (such as disseminated candidiasis, aspergillosis and histoplasmosis) or viral infections such as herpes simplex virus or varicella-zoster virus may also present with myocarditis in the immunocompromised patient, but can usually be identified by associated findings. Cytomegalovirus is an important pathogen in solid organ and hematopoietic stem cell transplant recipients with myocarditis. In heart transplant recipients, CMV infection is a risk factor for a form of immune-mediated cardiac rejection that presents as accelerated coronary atherosclerosis; importantly, prophylactic ganciclovir significantly reduces the incidence of this complication.[37] Finally, the role of CMV in myocarditis of immunocompetent patients is also intriguing; a recent study found intramyocyte CMV DNA in 14% of patients who had myocarditis but none was found in control individuals.[38]

A wide variety of other diseases may cause myocardial inflammation. Important noninfectious causes of myocarditis are listed in Table 46.2.

Table 46.2 Noninfectious causes of myocarditis

Connective tissue disorders	Systemic lupus erythematosus, rheumatoid arthritis, systemic sclerosis, dermatomyositis, polymyositis,
Idiopathic inflammatory/ infiltrative disorders	Kawasaki disease, sarcoidosis, giant cell myocarditis
Insect and arachnid stings	Wasp, scorpion, spider stings
Medications	Cocaine, ethanol, arsenic, cyclophosphamide, daunorubicin, adriamycin, sulfonamides, tetracycline, methyldopa
Post-irradiation myocarditis	
Peripartum myocarditis	
Pheochromocytoma	
Thrombotic thrombocytopenic purpura	
Thyrotoxicosis	

CLINICAL FEATURES

The clinical expression of acute myocarditis ranges from an asymptomatic state to rapidly progressive myocardial dysfunction and death. Complaints on presentation may include fever, fatigue, malaise, chest pain, dyspnea and palpitations; arthralgias and upper respiratory tract symptoms may be associated with viral myocarditis, but are nonspecific. Chest pain may be vague, pleuritic (suggesting pericardial involvement) or angina-like. The majority of patients, however, have no precordial discomfort.

Physical examination may reveal tachycardia out of proportion to the height of fever or degree of heart failure. Cardiac auscultation may be unrevealing, or may demonstrate muffled heart sounds, extra beats, transient murmurs or loud ventricular gallops; friction rubs are uncommon and indicate pericardial involvement. In severe cases, signs of congestive heart failure (CHF) are present, and pansystolic apical pulses may be palpated.

The electrocardiographic manifestations of myocarditis are usually transient and occur far more frequently than clinical myocardial involvement. Indeed, mild asymptomatic myocardial involvement diagnosed by serial electrocardiographic changes was present in over

1% of military conscripts with acute viral syndromes. ST segment elevation and T wave inversions may be seen acutely, and reflect the focal nature of the myocardial inflammation. These changes usually return to normal within 2 months. Atrial and ventricular arrhythmias are common in severe cases. Atrioventricular nodal or intraventricular conduction defects denote involvement of the conduction system and suggest more widespread disease or specific etiologies (e.g. Lyme carditis). Although usually transient and without sequelae, complete heart block may cause sudden death in these patients. Routine radiologic examination demonstrates cardiomegaly and pulmonary vascular congestion in severe cases.

DIAGNOSIS

The clinical diagnosis of myocarditis is often difficult and requires a high index of suspicion. When unexplained heart failure or malignant arrhythmias occur in the setting of an acute febrile illness, the clinical diagnosis of infectious myocarditis is suggested. Heart failure of recent onset mandates that the physician first consider ischemic, valvular, primary pulmonary or congenital disease in the differential diagnosis. Other causes of acute myocardial dysfunction, such as rheumatologic disease, endocrinopathies, electrolyte disturbances and toxin exposure (e.g. ethanol, cocaine and heavy metals), must also be ruled out.

Electrocardiogram changes are important ancillary findings but, given the high incidence of nonspecific ST segment and T wave changes seen in acute viral syndromes, they alone are often nondiagnostic. Similarly, laboratory abnormalities such as leukocytosis and an elevated erythrocyte sedimentation rate are also nonspecific. Serum creatinine phosphokinase (CPK) elevations, on the other hand, do signify acute myocardial injury and thus are very important. Unfortunately, these elevations do not differentiate the cause of that injury (i.e. ischemic versus inflammatory). Myocardial enzyme release parallels myocyte necrosis, as in small-to-moderate myocardial infarctions. However, in contrast to ischemic necrosis, in which CPK levels return to normal within 72 hours, elevated CPK levels may persist for 6 days in myocarditis.[39] The CPK-MB is elevated in 70% of patients who have myocarditis and ST segment elevation on ECG, but is usually normal if only T wave changes are present. Although serum assays for troponin T (a cardiac contractile protein) offer greater specificity for myocardial damage and persist for 2–3 days longer than CPK-MB determinations, they also are not specific for myocarditis.

Echocardiography is a valuable tool in the initial evaluation of the patient and in follow-up. Echocardiograms in myocarditis commonly show variable degrees of cardiac dysfunction, often with striking focal wall motion abnormalities. Dyskinesia or akinesia is most often biventricular. Furthermore, the test may eliminate other anatomic causes of CHF and may demonstrate the presence of ventricular thrombi or pericardial effusions. Echocardiographic changes generally resolve within a few days in parallel with the clinical course; if progressive ventricular dysfunction is demonstrated, chronic myocarditis may be suggested.

Many other imaging modalities for diagnosing acute myocarditis have been studied. However, validation of these diagnostic tools has been challenging in the absence of a reliable gold standard. Cardiovascular magnetic resonance (CMR) is able to distinguish between the different etiologies of myocardial damage, including myocarditis, ischemic heart disease and other cardiomyopathies, as well as assessing ventricular volume and function.[40] The high spatial resolution and contrast used in CMR allows for small areas of myocardial injury to be identified, which is useful for diagnosis of myocarditis since it usually presents as focal or patchy inflammation of the ventricles. Though safe and non-invasive, CMR cannot be used in patients with pacemakers or defibrillators. In addition, cost, availability and expertise continue to limit its widespread use in the clinical setting.

Despite advances in imaging techniques, endomyocardial biopsy is still considered the reference technique for diagnosis of myocarditis. However, it remains limited by the lack of sensitivity and specificity along with its invasive nature. Even in post-mortem specimens with proven myocarditis, the probability of diagnosing myocarditis based on the Dallas criteria (see below) with one biopsy was only 17–28%, which increased to approximately 67% with more than five biopsies.[41–43] Because the inflammation involves both ventricles, a transvenous approach is generally employed in order to obtain a biopsy of the right ventricular septum. In experienced hands the procedure is relatively safe, although deaths have occurred. Biopsy studies in acute disease have generally been carried out in cases of fulminant myocarditis. Studies using serial biopsies have demonstrated findings similar to those seen in the murine model. In the majority of cases, histologic evidence of myocarditis has resolved 3–4 weeks after the onset of symptoms and late biopsy is therefore unhelpful.[44] Because of the focal nature of the disease, many practitioners have recommended obtaining four or five biopsies from different sites at initial catheterization. Repeat biopsies and/or left heart catheterization with biopsy have sometimes been necessary to establish the diagnosis firmly but increase the risk of complications such as ventricular wall rupture. In addition, the use of quantitative (qPCR) and qualitative (nested PCR) molecular techniques in conjunction with endomyocardial biopsies has increased the ability to detect viral genomes. However, due to the uncertainty regarding the number of biopsy specimens required to attain clinical sensitivity, a positive PCR result is diagnostic while a negative PCR does not exclude viral disease.[45]

Disagreement among pathologists regarding histopathologic interpretation of specimens has also been problematic. A working standard, termed the Dallas criteria, is now used by the majority of investigators to define the disease.[44]

- Active myocarditis is defined as 'an inflammatory infiltrate of the myocardium with necrosis and/or degeneration of adjacent myocytes not typical of the ischemic damage associated with coronary heart disease'.
- Borderline myocarditis is present when infiltration is sparse, or when cardiac myocytes are infiltrated with leukocytes, but associated myocyte necrosis is not present; myocarditis cannot be diagnosed in the absence of inflammation.
- The absence of myocarditis indicates normal myocardium or pathologic changes of a noninflammatory nature (fibrosis, atrophy, hypertrophy).

The adoption of the Dallas criteria has led to 90% concordance among experienced pathologists. Application of the criteria in the Multicenter Treatment Trial for Myocarditis (MTT) suggests that the prevalence of 'active myocarditis' in patients who have recent onset (<12 months) CHF is approximately 10%;[46] a recent study demonstrated identical findings.[47] However, these patients are qualitatively different from those who have acute infectious myocarditis in which fever and acute ECG changes are present. They are best characterized as having IDC with myocardial inflammation.

The rationale for the development of the Dallas criteria was to identify patients prospectively who were likely or unlikely to benefit from immunosuppressive therapy. Given the results of the MTT (see below), many physicians have called into question the utility of the endomyocardial biopsy. In selected cases, however, the biopsy can provide valuable clinical data. Rarely, a biopsy will identify specific disease processes (i.e. toxoplasmosis, CMV, giant cell myocarditis, trichinosis, sarcoidosis) for which specific therapy is available or for which a prognosis can be given (i.e. Pompe's disease, amyloidosis). Because the prognosis in most cases of acute myocarditis is favorable (and an etiology is rarely discovered in cases of cardiomyopathy of gradual onset), we believe that the endomyocardial biopsy is unnecessary for the majority of patients with suspected myocarditis. Notable exceptions include transplant recipients and patients who have AIDS, for whom the discovery of a treatable etiology is more likely. In the setting of progressive clinical deterioration (such as arrhythmias or heart failure refractory to standard therapy) in which the diagnosis is unknown, endomyocardial biopsy should also be considered.

Thus, the approach for the immunocompetent patient who has acute myocarditis should focus on the management of CHF and its

complications (see below). A thorough history (with attention to epidemiologic detail) and physical examination will unearth the majority of nonviral etiologies, in which signs and symptoms other than CHF frequently dominate the clinical picture. In the febrile patient, blood cultures should be obtained. Serologic testing for HIV should always be performed given the high prevalence of myocarditis in patients with AIDS; testing for Lyme disease, Chagas disease and autoimmune diseases should be performed in the appropriate clinical context. A complete blood count with differential should be performed to rule out eosinophilia (which may suggest parasitic infection or hypersensitivity myocarditis). Testing for CMV in blood is unlikely to be helpful because CMV reactivation in the presence of unrelated acute febrile illnesses is relatively common. Although enteroviruses are prominent pathogens, routine use of acute and convalescent enteroviral titers adds little to clinical management. Noninvasive testing for enteroviruses and adenoviruses by serum PCR is possible (although not widely available), but studies have not been performed to demonstrate that this information affects outcome. Perhaps future advances in virology and molecular biology coupled with targeted antiviral therapy will provide more options for the diagnosis and treatment of myocarditis; for now, however, the majority of cases remain classified as 'idiopathic' and are treated expectantly.

MANAGEMENT

The natural history of acute infectious myocarditis is quite variable, although the majority of cases run a benign, self-limited course. Acute cardiac dysfunction does not predict chronic impairment, as most of these individuals demonstrate normalization of laboratory, echocardiographic and histologic parameters within 1 month of symptom onset. For this reason, supportive care in lieu of aggressive, invasive procedures is of primary importance.

General measures target CHF, arrhythmias and other derangements associated with myocarditis. Animal models have demonstrated that exercise during viral myocarditis is associated with higher mortality and more extensive histologic damage; thus, bed rest may be important. Conventional therapy has included oxygen, diuretics, angiotensin-converting enzyme (ACE) inhibitors and beta-blockade, as for any patient who has CHF. Anticoagulation for patients who have intracardiac thrombi and/or severely depressed myocardial function is also important. In the murine model, early treatment with the ACE inhibitor captopril decreased left ventricular mass and myocardial necrosis, suggesting benefits above and beyond afterload reduction.[48] Randomized trials in humans have yet to be conducted, although afterload reduction appears safe and effective in other settings. Rarely, fulminant heart failure requires the use of inotropic support, intra-aortic balloon pumps or ventricular assist devices as a bridge to the resumption of cardiac function.[49] Unfortunately, there are no clinical or laboratory indicators to identify those patients who will spontaneously recover; indeed, patients who have fulminant myocarditis may have a better prognosis than those with acute, nonfulminant myocarditis.[50] For those who do not respond, cardiac transplantation is an option; however, patients who have myocarditis before transplantation have a significantly higher incidence of rejection and death in the first year.

As significant arrhythmias are probably associated with the majority of deaths in acute myocarditis, all hospitalized patients should be monitored on telemetry. Premature beats are common and do not require therapy. Supraventricular tachycardia, however, worsens heart failure and should be electrically converted. The use of anti-arrhythmic agents for high-grade ventricular ectopy has not been studied. Care should be exercised with any antiarrhythmic agent (including digoxin, which has a low threshold for toxicity in these patients) but sustained ventricular arrhythmias should be treated aggressively. Complete heart block is an indication for temporary venous pacing. The condition often resolves without a need for permanent pacemaker placement.

If a specific infection is identified (such as Lyme or staphylococcal carditis), antimicrobial therapy should be directed at the causative pathogen. As noted above, viruses are the likely cause of most cases of myocarditis, but patients who have putative viral myocarditis most commonly present after viral replication has ceased. Murine models of CMV-induced myocarditis confirm that ganciclovir or cidofovir improves outcomes in the acute (<24 hours) phase of infection, but are ineffective later.[51] Pleconaril is a novel compound that has antiviral activity against the enteroviruses (including Coxsackie viruses A and B) and thus has potential for treating the most common etiology of myocarditis. Clinical response was favorable in three-quarters of patients treated in one report,[52] but high rates of spontaneous recovery make conclusions speculative. Antiviral therapy with interferon-α for patients with enterovirus-induced myocarditis has also been studied.[53]

Contrary to expectations, most studies of immunosuppressive therapy such as prednisone, azathioprine and ciclosporin have not favorably influenced outcome. A meta-analysis evaluating 316 patients enrolled in five trials demonstrated no benefit of immunosuppressive therapy as compared to placebo or conventional therapy in terms of the pooled outcome of all-cause death and heart transplantation (odds ratio 1.03; 95% confidence interval 0.58–1.80).[54] Similarly, although multiple case reports and case series have documented successful treatment of acute myocarditis with intravenous immunoglobulin (IVIG), this difference was not observed in the only randomized controlled trial comparing IVIG to placebo for treatment of acute myocarditis in 62 patients. Treatment with IVIG showed no benefit in terms of improvement in cardiac function, survival, or need for a left ventricular assist device or transplant.[55] Trials evaluating the use of immunosuppression and other treatment strategies are needed, but are inherently difficult to perform given the low incidence of acute myocarditis, difficulty in diagnosis and the low rate of events such as death or need for cardiac transplant.

Pericarditis

EPIDEMIOLOGY

Interest in the pericardium dates to antiquity. The writings of Homer and Maximus relate the history of the 'hairy hearts of heroes' such as Aristomenes, the legendary Messinian warrior; his heart was cut out in battle and found to be 'stuffed with hair', probably the first recorded case of fibrinous pericarditis. Significant understanding of the etiology and pathophysiology followed, from Galen's first pericardial resection in the 2nd century AD to Lower's classic description of tamponade, constrictive pericarditis and pulsus paradoxus in the 17th century. Medical advances in antibiotic therapy, surgical technique, antineoplastic therapy and hemodialysis have substantially altered the spectrum and prognosis of pericardial disease in the 21st century. Imaging modalities have also made a significant impact; since the advent of echocardiography in 1955, pericardial effusions are now readily diagnosed. The etiologic determination of pericardial disease, however, remains difficult. Timely, directed therapy depends in large part on the diligence of the clinician.

The incidence of pericardial inflammation detected in several autopsy series ranges from 2% to 6%, whereas pericarditis is diagnosed clinically in only about 1 out of 1000 hospital admissions. The relative frequency of each etiologic process depends upon the clinical setting. Thus, viral or idiopathic pericarditis often presents in the outpatient clinic, whereas malignant or uremic effusions are more frequently seen in referral centers.

PATHOGENESIS AND PATHOLOGY

The human pericardium forms a strong, flask-shaped sac that encloses the heart and the origins of the great vessels. It is composed of a fibrous outer layer and an inner serous membrane formed by a single

layer of mesothelial cells. This membrane is attached to the surface of the heart to form the visceral pericardium; it reflects upon itself, lining the inside of the collagen-based fibrous layer to form the parietal pericardium. The visceral pericardium continuously produces a clear pericardial fluid, which serves as a lubricant; it is also the source of excess fluid in disease states. The human pericardium normally contains up to 50 ml of this fluid, which drains via the thoracic and right lymphatic duct into the central circulation. Pericardial effusion may develop in response to pericardial injury (as in pericarditis) or may be secondary to other processes that alter the secretion and drainage of pericardial fluid. The pathologic changes seen in acute pericarditis are those of nonspecific inflammation with cellular infiltration, fibrin deposition and the outpouring of pericardial fluid. These changes may resolve spontaneously over time, or may organize with fibrous adhesions between the epicardium and visceral pericardium, the visceral and parietal pericardium, or the pericardium and adjacent sternum and pleura (Fig. 46.2). Thus, inflammation, fluid exudation and fibrin organization account for the cardinal manifestations of pericarditis: chest pain, pericardial effusion and constriction.

The causes of this pericardial inflammation are numerous, including both infectious (Table 46.3) and noninfectious (Table 46.4) etiologies.

Noninfectious agents

The three most prevalent noninfectious causes of pericardial disease are malignancies, uremia and connective tissue disorders.

- Neoplasms may cause pericarditis or effusions by direct involvement of the pericardium (in which malignant cells are usually demonstrated within pericardial fluid) or by obstruction of the pericardial lymphatic drainage (with resulting 'benign' effusions).
- Uremic pericarditis, which affects up to 20% of patients on chronic hemodialysis, is characterized by the appearance of a shaggy, fibrinous exudate without cellular infiltration.
- Collagen vascular diseases (most commonly systemic lupus erythematosus and rheumatoid arthritis) are notable for their propensity to involve the pericardium; immune complex deposition is thought to be primary in the pathogenesis of pericardial disease.

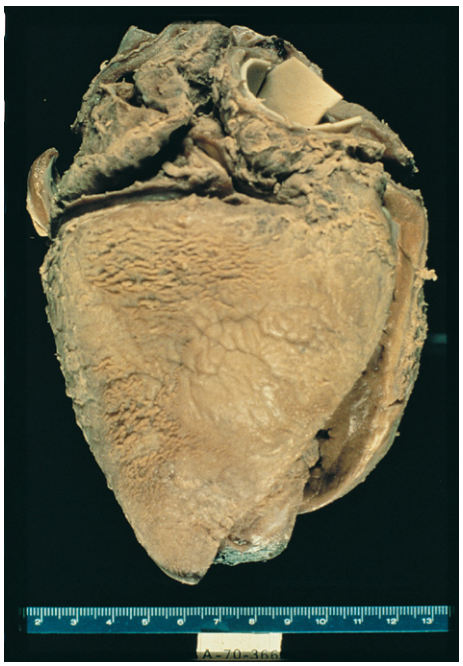


Fig. 46.2 Heart at autopsy of a patient who had acute suppurative pericarditis. The parietal pericardium has been stripped from the specimen, revealing a 'bread and butter' appearance.

Table 46.3 Infectious causes of pericarditis

Viruses	Mycobacteria
Cytomegalovirus Herpes simplex virus Coxsackie A virus Coxsackie B virus Echovirus Adenovirus Influenzavirus Mumpsvirus Varicella-zoster virus Epstein–Barr virus HIV	*Mycobacterium tuberculosis* *Mycobacterium chelonae* *Mycobacterium avium* complex
Bacteria *Streptococcus pneumoniae* *Streptococcus* spp. *Staphylococcus aureus* *Neisseria meningitidis* *Listeria monocytogenes* *Haemophilus influenzae* *Francisella tularensis* *Brucella melitensis* Enteric Gram-negative rods *Actinomyces* spp. *Nocardia asteroides* *Legionella pneumophila* *Tropheryma whipplei* *Salmonella* spp. *Campylobacter* spp. *Rickettsia/Coxiella burnetii*	Spirochetes *Borrelia burgdorferi*
	Mycoplasma *Mycoplasma pneumoniae* *Ureaplasma urealyticum* *Mycoplasma hominis*
	Fungi *Histoplasma capsulatum* *Coccidioides immitis* *Cryptococcus neoformans* *Blastomyces dermatiditis* *Candida* spp. *Aspergillus fumigatus*
	Parasites *Toxoplasma gondii* *Entamoeba histolytica* *Echinococcus granulosus* *Schistosoma* spp.

Table 46.4 Noninfectious causes of pericarditis

Idiopathic
Connective tissue disorders
Acute rheumatic fever, systemic lupus erythematosus, rheumatoid arthritis, scleroderma, mixed connective tissue disease, Wegener's granulomatosis, polyarteritis nodosa, temporal arteritis
Metabolic
Uremia, hypothyroidism
Malignancies
Lung cancer, breast cancer, leukemia, lymphoma, melanoma, others
Acute myocardial infarction
Post-myocardial infarction syndrome (Dressler syndrome)
Dissecting aortic aneurysm
Traumatic
Chest trauma, postsurgical hemopericardium, pacemaker insertion, cardiac catheterization, esophageal rupture, pancreatic–pericardial fistula
Post-irradiation
Idiopathic infiltrative/inflammatory disorders
Sarcoidosis, amyloidosis, inflammatory bowel disease, Behçet's disease, familial Mediterranean fever
Medications
Procainamide, hydralazine, isoniazid, phenylbutazone, dantrolene, doxorubicin, dilantin, methysergide, minoxidil

Infectious agents

A variety of microbes have been reported to cause pericarditis. Chief among these are viruses, which can produce a clinical syndrome of myopericarditis, but often other infectious agents are also implicated.

Viruses

The most common etiologies of viral pericarditis include Coxsackie A and B, echovirus type 8, adenovirus and HIV (see Table 46.3). As is the case for myocarditis, these viruses have only rarely been isolated from pericardial fluid or tissue; as such, evidence for viral causation of pericardial inflammation is primarily based upon isolation of virus from other sites, such as stool, and by demonstration of a fourfold rise in serum antibody titers. Most recently, PCR testing has found evidence of adenovirus, enterovirus and CMV in pericardial fluid and tissue from patients with acute pericarditis.[56]

Bacteria and other infectious agents

Bacteria may cause pericarditis by a number of different mechanisms. Hematogenous seeding of the pericardium may occur during the course of bacteremia caused by a variety of organisms. In the pre-antibiotic era, most cases of purulent pericarditis were seen as complications of bacteremia or pneumonia. Today, extension of infection from a contiguous focus within the chest is seen as a postoperative or post-traumatic complication. Subdiaphragmatic abscesses may also cause pericardial infection by direct extension. Highly invasive bacterial infections within the heart, such as acute staphylococcal endocarditis, may erode into the pericardium from a perivalvular abscess to cause purulent pericarditis. Pericardial effusions in patients who have subacute infective endocarditis provide another mechanism by which bacterial infection can lead to pericardial disease. Pathologic changes in the pericardium are caused by immune complex deposition, resulting in sterile exudates. Although common, these effusions are not correlated with prognosis, and most resolve without specific therapy.

The microbiology of bacterial pericarditis continues to evolve (see Table 46.3). Before antibiotics, uncontrolled pneumococcal, streptococcal or staphylococcal pulmonary infections were most frequently implicated. Streptococci and staphylococci remain important pathogens today (particularly in traumatic and post-thoracotomy pericarditis), with Gram-negative bacilli, atypical bacteria and *Candida* also assuming important roles. Pericardial involvement has also been documented in the course of such illnesses as tularemia, brucellosis, salmonellosis, legionellosis, meningococcal disease and Q fever.[57] In children, bacteria cause proportionately more cases of pericarditis than is the case in adults; *Staph. aureus* and *Haemophilus influenzae* are the most common etiologic agents.

Pericarditis caused by *Mycoplasma* spp. deserves special mention. Although pericarditis has been recognized in the course of *Mycoplasma* disease since 1944, culture of the organism has proved difficult. Therefore, autoimmune phenomena have been invoked to explain the association. In one report, *Mycoplasma pneumoniae, M. hominis* and *Ureaplasma urealyticum* were isolated from pericardial fluid and/or tissue cultures in five patients with large pericardial effusions.[58] Treatment with doxycycline after drainage of the effusions resulted in complete resolution in all five cases. Pericarditis caused by *Mycoplasma* spp. is thus more common than previously recognized, and fluid obtained for culture should always be analyzed for the presence of these organisms.

Mycobacteria continue to be important causes of acute pericarditis, pericardial effusion and constrictive pericarditis, particularly in developing countries. The incidence of tuberculous pericarditis among patients who have pulmonary tuberculosis ranges from 1% to 8%.[59] While the overall incidence of tuberculous pericarditis has significantly declined in the United States, countries with a high prevalence of tuberculosis and HIV such as South Africa continue to have a higher burden of disease. In a study from the Western Cape province of South Africa, tuberculous pericarditis was the most common cause of pericardial effusions and accounted for 69.5% of the 233 cases of pericardial effusion. Of note, 50% of patients in this study were HIV positive.[60]

Histoplasma capsulatum is the most common cause of fungal pericarditis; in large outbreaks, pericarditis was noted in 6% of patients who had symptomatic histoplasmosis. It most commonly develops as a noninfectious inflammatory response that resolves spontaneously without therapy. Occasionally, seeding of the pericardium occurs in the course of disseminated infection. In contrast, pericarditis has only rarely been reported in cases of severe coccidioidomycosis.

In the severely immunosuppressed or post-thoracotomy patient, infection caused by *Candida* spp., *Aspergillus fumigatus* or *Cryptococcus neoformans* has occasionally resulted from either fungemia or direct inoculation.

Pericarditis in AIDS

In contrast to other immunocompromised states, pericardial disease in patients who have AIDS is quite common. Effusions are frequently noted in end-stage AIDS (occurring in 16–40% of patients) and are associated with a poor prognosis. Etiologies include a variety of pathogens (including viruses, bacteria, fungi and mycobacteria), although in the majority of cases no causative agent can be defined. Malignant effusions secondary to lymphomatous involvement of the pericardium have also been noted. Furthermore, although extrapericardial disease suggested specific infectious or malignant etiologies in 55% of patients who had pericardial effusions in one trial, these assumptions proved incorrect for all those in whom pericardiocentesis was performed.[61]

CLINICAL FEATURES

Acute pericarditis is most often recognized by its chief presenting manifestation: chest pain. The pain is usually precordial or retrosternal, often with radiation to the trapezius ridge or neck; it is exacerbated by lying supine, coughing or deep inspiration, with relief upon sitting upright or forward. The patient's discomfort may be caused by inflammation of the adjacent pleura, accounting for the pleuritic component that often accompanies the pain. This pain is distinguished from the pain of myocardial ischemia by its quality, its duration (pain may last for days without therapy) and the absence of associated factors (i.e. pain is unchanged with exertion or rest). Patients may also report that splinting reduces the pleuritic discomfort.

A pericardial friction rub is the pathognomonic physical finding of acute pericarditis. Characterized as scratchy or grating, it is best appreciated along the left sternal border with respirations suspended and the patient leaning forward. The classic friction rub has three components, corresponding to atrial systole, ventricular systole and the rapid ventricular filling phase of early diastole, although one or more of these phases are usually absent. Of note, the friction rub frequently waxes and wanes in intensity and may disappear altogether with the accumulation of fluid within the pericardial sac. The pericardial rub may again become prominent in tamponade, in which the pericardium rubs against the adjacent pleura.

Pericardial effusions range from the asymptomatic to those causing cardiac tamponade. The rate of fluid accumulation is a major determinant in physiologic manifestations. When the effusion develops slowly, the pericardium may stretch to accumulate as much as 2 liters of fluid. The normal pericardium, however, can accommodate the rapid accumulation of only 100–200 ml of fluid before signs and symptoms of tamponade develop. Patients may then complain of dyspnea or a dull retrosternal ache, and examination will reveal jugular venous distension, the most common physical finding in acute tamponade. A fall of 10 mmHg or more in systolic blood pressure during inspiration (the pulsus paradoxus) is recognized as a hallmark of critical cardiac tamponade, although it may be absent if hypotension is already present.

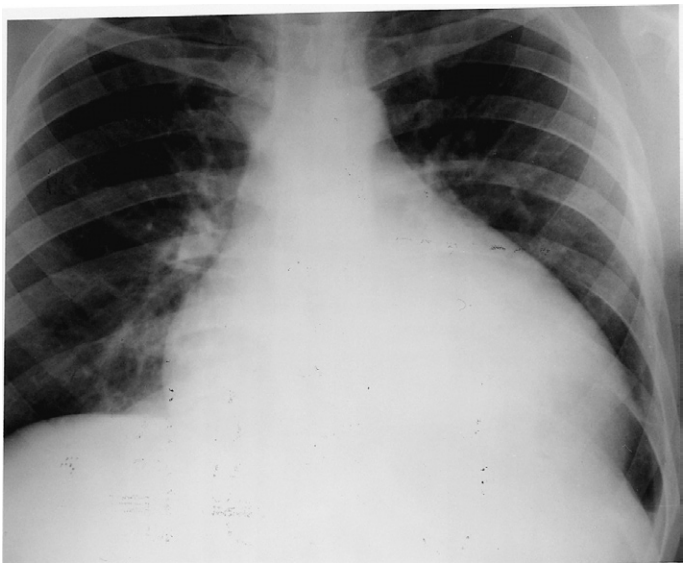


Fig. 46.3 Cardiomegaly in a patient who has pericarditis. The presence of a 'water-bottle' heart on this plain film suggests a large pericardial effusion.

Enlargement of the cardiac silhouette on routine radiography does not usually occur until at least 250 ml of fluid have accumulated in the pericardial space. Other findings on chest radiogram, such as a 'water bottle' heart (Fig. 46.3) or a prominent fat stripe sign, are found only in large pericardial effusions; their absence does not rule out the presence of a hemodynamically significant effusion. Chest radiograms may also provide etiologic clues for pericardial disease, such as pneumonic infiltrates or mediastinal adenopathy.

Electrocardiographic changes in acute pericarditis imply inflammation of the pericardium. Thus, in uremic or neoplastic pericardial effusions, characteristic ECG changes are often absent. Cardiac arrhythmias are uncharacteristic in isolated pericardial disease; their presence implies myocardial involvement. The ECG typically evolves through four stages during acute pericarditis.

- Diffuse ST-segment elevation (usually concave up) with reciprocal ST depression in aVR and V1 accompanies the onset of chest pain and is virtually diagnostic of pericarditis; these findings are present in 50% of patients who have acute pericarditis.[62] PR depression in the inferolateral leads is frequently seen in this stage (Fig. 46.4).
- ST and PR segments normalize, typically several days later.
- Diffuse T wave inversions develop, generally after ST segments become isoelectric.
- Electrocardiograph changes normalize; long-term inversion of T waves suggests 'chronic' pericarditis.

Elevated CPK and troponin I levels are common in cases of acute pericarditis. Whether this signifies clinically relevant myocardial changes has not been adequately evaluated.

DIAGNOSIS

A number of studies have established the utility of using a stepped approach.[63] One study prospectively evaluated 231 consecutive patients who had acute pericardial disease of unknown cause.[64] Pericardiocentesis was performed in patients who had tamponade, suspicion of purulent pericarditis or symptoms and/or effusion persisting for more than 1 week after initiation of nonsteroidal anti-inflammatory drug (NSAID) therapy. Pericardial biopsy was undertaken if clinical activity persisted at 3 weeks and the etiology was unknown. Despite this extensive evaluation, a specific diagnosis was confirmed in only 32 patients: neoplasia in 13, tuberculosis in nine, rheumatic disease in four, purulent pericarditis in two, toxoplasmosis in two and viral pericarditis in two. Diagnostic yield was substantial when pericardiocentesis or biopsy was performed to relieve tamponade, but poor when used solely for diagnostic purposes. Over a mean follow-up of 31

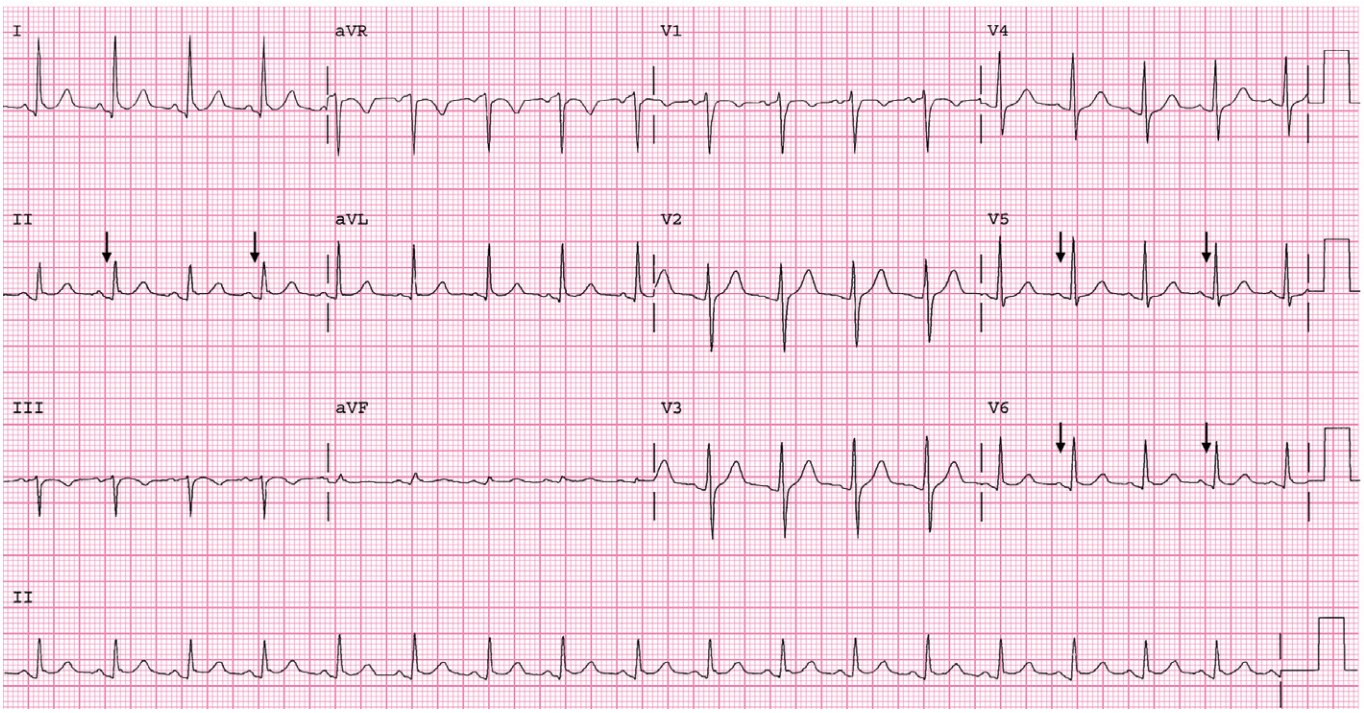


Fig. 46.4 Electrocardiogram of a patient who has early acute pericarditis. Note the presence of diffuse ST segment elevation and PR depression in the inferolateral leads (arrows). 25 mm/s; 10.0 mm/mV; F–W 0.05–100.

months, no patient diagnosed with idiopathic pericarditis and treated with NSAIDs showed signs of recurrent or chronic pericardial disease.

Because of the recent interest in subxiphoid pericardial biopsy and drainage of pericardial effusions, we recently undertook a prospective nonrandomized trial of all patients who had large pericardial effusions hospitalized at our institution. These patients underwent a similar stepped preoperative approach, with subsequent subxiphoid pericardial biopsy and drainage of their effusions.[65] Diligent handling and extensive microbiologic analysis (including cultures for aerobic and anaerobic bacteria, viruses, chlamydiae, mycoplasmas, fungi and mycobacteria) of pericardial fluid and tissue allowed specific diagnoses to be established in 53 out of 57 patients, confirming prior reports of high diagnostic yield when stepped algorithms are used for large effusions. More than one-third of the patients had malignancy or a history of irradiation to the thorax for malignancy. Infections (mostly viral), collagen–vascular disease and uremia were also frequently implicated (Table 46.5). Unexpected pathogens included CMV in three patients, herpes simplex virus 1 in one, *M. pneumoniae* in two, *Mycobacterium avium* complex in one and *Mycobacterium chelonei* (see Table 46.3) in one patient. No patient showed evidence of Coxsackie A or B viral infection. Comparison of this study with two other important series shows a significant variation in etiologies as a result of different populations, diagnostic strategies and investigator interests (i.e. infectious disease specialist versus cardiologist).

A comparison of diagnostic yield between pericardial fluid and biopsy demonstrated that fluid analysis was far more sensitive for malignancy; tissue provided additional information only in infected patients in whom fluid was not available for analysis. Previous studies demonstrated similar utility of pericardial fluid analysis; in a retrospective study of 93 cases of malignant effusion with both pericardial fluid and tissue analysis, cytology was correct in 87 cases (diagnostic accuracy 94%) with 100% specificity.[67]

In summary, acute pericarditis is most often viral or idiopathic in etiology; as such, invasive workups are usually not necessary. For the patient who has large effusions, tamponade or presentation suggestive of purulent pericarditis, early and aggressive intervention will often yield diagnostic and therapeutic rewards.

As noted previously, a wide variety of infectious and noninfectious agents can cause acute pericarditis and/or pericardial effusions (see Tables 46.1 and 46.2). For the patient presenting with acute chest pain, initial evaluation should focus on identifying conditions that may be rapidly fatal. Thus, myocardial infarction, aortic dissection, purulent pericarditis and cardiac tamponade should be systematically ruled out. An appropriate workup includes a thorough history and physical examination, ECG and chest X-ray (to rule out intrathoracic malignancy, tuberculosis or a widened mediastinum suggestive of aortic dissection) and routine laboratory studies including complete blood counts, serum chemistries, serial CPK with MB fraction determination, thyroid function tests, rheumatoid factor and antinuclear antibodies; blood cultures should be obtained for the febrile patient. Due to the serious consequences of untreated tuberculous pericarditis, a tuberculin skin test should be placed as well.

For the majority of individuals, a specific etiology will not be apparent, and a diagnosis of acute viral or idiopathic pericarditis will be made. Because either entity typically follows a brief and benign course, a full diagnostic evaluation is not appropriate. The confirmation of a particular viral agent is not necessary, as serologic titers and/or viral cultures are quite nonspecific, the workup is costly and a retrospective diagnosis is usually not helpful in management. Because significant pericardial effusion may accumulate even in idiopathic disease, however, all patients should be carefully evaluated for its presence; hemodynamic compromise on physical examination, cardiac enlargement on chest radiography or significant effusions on echocardiography necessitates rapid intervention. For patients who have large effusions, and for those in whom another diagnosis is suggested, a more thorough workup often includes pericardial drainage with or without pericardial biopsy.

As noted above, noninvasive diagnosis of pericardial disease in patients who have AIDS is extremely difficult. Because specific etiologic diagnosis often has important treatment ramifications, pericardial effusions in these patients should be managed on an individualized basis, with invasive diagnostic testing employed in those whose baseline health would benefit from aggressive therapeutic measures.

Echocardiography has largely replaced other methods for the detection of pericardial fluid. With experience, operators can detect as little as 20 ml of excess fluid posterior to the left ventricle. Echocardiography can also provide ancillary data in assessing the patient who has an effusion. Increased respiratory flow variation across the mitral valve with Doppler echocardiography is characteristic of cardiac tamponade. In addition, other etiologies of myocardial dysfunction such as left and right ventricular infarction can be ruled out. Finally, the echocardiogram can direct attempts at pericardiocentesis by identifying the location of pericardial fluid.

CT scans of the chest are primarily helpful in the diagnosis of pericardial thickening. Significant effusions are also readily demonstrated (Fig. 46.5). In addition, CT scans are more sensitive for the demonstration of small parenchymal nodules and mediastinal lymphadenopathy than is conventional radiography, and thus have clinical utility in the diagnosis of malignant pericardiditis. Similarly, MRI may be useful in the non-invasive characterization of pericardial fluid as well as pericardial thickness.

Table 46.5 Etiology of large pericardial effusions[65]

Etiology	% of 75 diagnoses
Malignancy	27
Viral	16
Collagen vascular disease	14
Radiation	11
Uremia	11
Mycobacterial	5
Mycoplasma	3
Bacterial	1
Idiopathic	5
Other	8

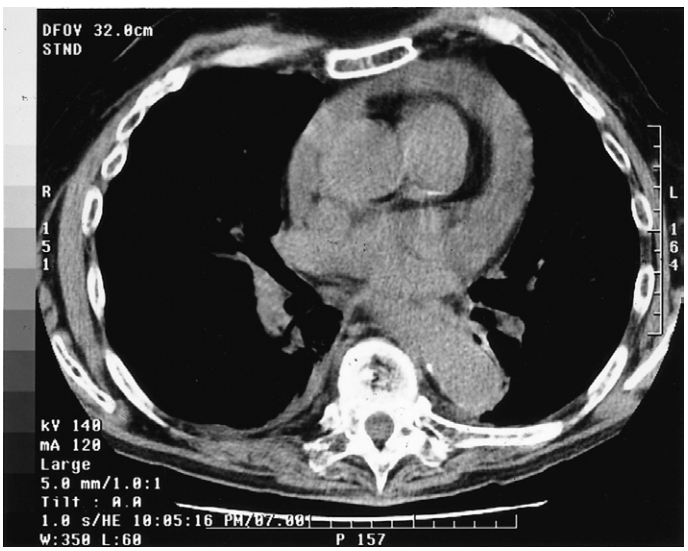


Fig. 46.5 Chest CT of a patient who has a large crescent-shaped pericardial effusion.

Cardiac catheterization is reserved for patients in whom pericardial constriction is believed to play a role in symptomatology. In those who have chest pain of undetermined etiology, catheterization is also useful for ruling out myocardial ischemia as a confounding diagnosis.

MANAGEMENT

Nearly all patients who have acute pericarditis should be hospitalized for relief of symptoms, diagnostic evaluation and observation for complications. Specific medical therapy is tailored to the cause of pericarditis. Aspirin, at doses of 2–6 g/day, or other NSAIDs are effective in reducing symptoms of pericarditis and are the agents of choice for idiopathic or viral pericarditis. Corticosteroids should be reserved for symptomatic nonresponders. Prednisone, however, is the drug of choice in pericarditis associated with connective tissue disease.

Idiopathic or viral pericarditis generally follows a benign, self-limited course but the occasional patient will present with recurrent pericardial pain. Treatment for recurrences generally begins with NSAIDs; corticosteroids have been used successfully for NSAID-resistant cases. Colchicine has shown promise as a steroid-sparing agent in a number of small trials.[70] Pericardiectomy for recurrent pericarditis should be reserved for those who fail medical therapy.[71]

Targeted intravenous antibiotics and surgical drainage of the pericardium remain the mainstays of therapy for purulent pericarditis. Pericardiocentesis urgently performed for the critically ill patient does not obviate the need for complete drainage and irrigation; fluid may reaccumulate rapidly and sequelae such as constriction may develop in hours. There is no rationale for intrapericardial antibiotic administration, as pericardial penetration of antibiotic is excellent.

Tuberculous pericarditis remains a diagnostic and therapeutic challenge. Clinical features are nonspecific, the disease course is confusing and laboratory evaluation is often nondiagnostic, particularly in low prevalence settings and in patients who have localized disease. For example, although large effusions are more likely to be tuberculous, up to 50% of tuberculous effusions resolve spontaneously despite ongoing tissue infection. The tuberculin skin test may be negative in up to 30% of patients as a result of cutaneous anergy yet may be positive in the patient who has acute idiopathic pericarditis and benign natural history. Suggestive, but not diagnostic, findings include a recent history of pulmonary tuberculosis, a positive sputum smear or culture or a high pericardial fluid adenosine deaminase level (>45 units/l).[72] Even granulomatous inflammation of the pericardium is not diagnostic, as this may be demonstrated in pericardial disease from other causes, such as histoplasmosis, sarcoidosis and rheumatoid arthritis. Additionally, a negative biopsy of the pericardium does not rule out tuberculous pericarditis, as removal of the entire pericardium may be necessary to demonstrate clear-cut evidence of tuberculosis.[73]

Definitive diagnosis rests upon the demonstration of the tubercle bacillus in pericardial fluid and/or tissue. However, the need for early therapy demands that treatment often be undertaken based upon a presumptive diagnosis. Initial treatment should consist of four drugs including isoniazid and rifampin (rifampicin) until sensitivities are known. The use of concomitant prednisone (at doses of 60 mg/day initially) to reduce pericardial inflammation is supported by two large controlled trials in Transkei, South Africa. Clinical improvement occurred more rapidly, 2-year mortality was lower (4 versus 11) and pericardiectomy was required less often (21% versus 30%) compared with those treated with four-drug therapy alone.[74] These data also highlight the incidence of constrictive complications in this disease. Complete pericardiectomy is advocated for those who have recurrent effusions or cardiac compression with constrictive physiology after 4–6 weeks of oral therapy. Such early pericardiectomy is associated with a good outcome; mortality is substantially higher in patients who undergo pericardiectomy at the late stage of calcific pericardial constriction.[75,76]

REFERENCES


References for this chapter can be found online at http://www.expertconsult.com

Chapter 47

Philippe Moreillon

Endocarditis and endarteritis

INTRODUCTION

This chapter reviews infective endocarditis (IE) and other endovascular infections in the context of their evolving epidemiology, diagnostic tools and therapeutic strategies. These diseases are invariably lethal if not aggressively treated with antibiotics associated or not to surgery. Continuous developments in diagnostic criteria, including the use of echocardiography and molecular microbiology, have led to prompter disease recognition and therapy.[1] In spite of global improvements in health care, the incidence of IE has not decreased. Rheumatic heart disease, which was a primary risk factor for IE, has almost vanished from industrialized countries, but remains the primary risk factor in developing countries, where it is associated with poverty, crowding and poor health-care conditions[2] (see Chapter 48). In industrialized countries it is being replaced with new at-risk groups, including intravenous drug users (IVDU), elderly people with degenerative valves lesions, patients with health-care-associated conditions such as chronic dialysis, and patients who have intravascular prostheses.[3–5]

Whenever appropriate, the text and the figures in the chapter indicate the levels of recommendations and the levels of evidence for treatment suggestions, as summarized in Table 47.1.

Table 47.1 Levels of recommendations and levels of evidence for treatment suggestions

Classification of recommendations
Class I: Conditions for which there is evidence and/or general agreement that a given treatment is effective *Class II*: Conditions for which there is conflicting evidence and/or divergence of opinion about the efficacy of a given treatment • *Class IIa*: Evidence in favor of efficacy • *Class IIb*: Efficacy not well established by opinion or evidence *Class III*: Conditions for which there is evidence and/or general agreement that a given treatment is not effective and in some cases may be harmful
Level of evidence
Level of evidence A: Data derived from multiple randomized clinical trials or meta-analyses *Level of evidence B*: Data derived from a single randomized trial or nonrandomized studies *Level of evidence C*: Only consensus opinion of experts, case studies or standard of care

Adapted from European Society of Cardiology[24] and American Heart Association[38] guidelines.

EPIDEMIOLOGY

The overall incidence of IE has remained between 2 and 6 per 100 000 population per year over the last 30 years.[3–5] There is a persisting predominance in males, with a 1.5:1 to 2:1 male:female ratio. Mortality remains high, and ranges from 10% to more than 30%, depending on multiple factors including the type of organism (e.g. oral streptococci are less aggressive than *Staphylococcus aureus*), underlying conditions and whether the infection occurs on a native or a prosthetic valve. In one study, mortality of *S. aureus* prosthetic valve IE was reported to be as high as 47%.[6]

In parallel, risk factors and infecting micro-organisms are evolving. Chronic rheumatic heart disease is now rare in industrialized countries[5] (see Chapter 48), and formerly classic pathogens such as pneumococci and gonococci have become uncommon. Oral streptococci tend to remain predominant in the general population,[2,4,5] but *S. aureus* and coagulase-negative staphylococci (mainly *S. epidermidis*) have become predominant in IVDU, in prosthetic valve IE and in patients with health-care-related conditions,[3,7–9] and group D streptococci (mainly *Streptococcus bovis*, recently renamed *S. gallolyticus*) are increasingly prevalent in elderly patients and often related to colon tumors.[3–5]

Intravenous drug users involve mostly young individuals (mean age 30–40 years).[10] However, the other risk factors are more frequent in the elderly, and the mean age of patients with IE has progressively increased from 30 years in the 1950s, to 50 years in the 1980s and 55 to >60 years since the 1990s.[3,4,11,12] In one review totalizing 3784 episodes of IE between 1993 and 2003, the incidence of IE varied from <5 to >15 per 100 000 patients per year in individuals younger and older than 65 years, respectively.[3] The clustering of risk factors in elderly patients correlated with a more than three times increased incidence of IE in this group. Therefore, the results of different epidemiologic studies may vary according to the population analyzed.[5,9]

Infective endocarditis is commonly classified in four categories, which are discussed below:

- native valve IE (NVE);
- prosthetic valve IE (PVE);
- IE in intravenous drug users; and
- nosocomial (or health-care-related) IE.

Native valve infective endocarditis

Risk factors for NVE include congenital heart disease and acquired abnormalities such as chronic rheumatic heart disease and degenerative heart disease. People who have cardiac abnormalities that result in high-to-low pressure gradients are at greater risk of infection. Turbulent blood flow may provoke damage or peeling of the endothelium and formation of nonbacterial thrombotic vegetations. Circulating bacteria tend to adhere on the low-pressure side of such Venturi-like systems.

These observations explain why left-sided valves and left-to-right ventricular or arterial shunts are the most common sites of IE.

Congenital heart disease

Congenital heart disease is a lifelong risk factor. It is a major risk factor in children, in whom it accounts for 30–40% of IE.[13] Although a less frequent risk factor in adults, it still represents about 5% of IE cases.[14] This includes all of the cardiac abnormalities associated with turbulent blood flow. Tetralogy of Fallot carries the highest risk for IE, followed by bicuspid aortic valve, coarctation of the aorta and ventricular septal defect. In contrast, secundum atrial septum defects rarely put the patient at risk, probably because they result in a low-pressure shunt.

Surgical or medical closure of a patent ductus arteriosus usually eliminates the risk of endovascular infection. However, surgical correction does not exclude the risk of IE in patients suffering major congenital heart disease, such as tetralogy of Fallot.[14] The type of surgical correction may influence the risk of subsequent infection in this situation. In the largest long-term survey, involving 1142 patient years of observation, IE occurred in 23% of patients treated with anastomotic operations but in only 9% of patients treated with pulmonary valvulotomy or infundibular resection or both.[15] Vascular anastomoses are likely to generate turbulent blood flow, which might explain the greater risk of IE following anastomotic rather than plastic repair.

Rheumatic heart disease

Rheumatic heart disease was the most frequent acquired cardiac anomaly leading to IE in the pre-antibiotic era. In one series, the frequency of chronic rheumatic heart disease in patients who had developed IE decreased from approximately 22% between 1933 and 1952 to less than 1% between 1963 and 1972.[16] Although the prevalence of the disease has decreased to less than 10 per 100 000 population per year in industrialized countries, it remains the predisposing factor of IE in up to 50% of cases in certain developing countries.[2] Moreover, occasional clusters of rheumatic fever were recently described in the USA, and were linked to peculiar clones of group A streptococci.[17] Thus, rheumatic heart disease remains an important risk factor for IE.

Mitral valve prolapse

Mitral valve prolapse is a relatively common condition affecting 2–3% of the population. It has been associated with at least three autosomal-dominant inherited loci, located on chromosomes 11, 13, and 16.[18] Patients with valve regurgitation have a 10- to 100-fold increased risk of IE, and the risk of IE may be especially important in children and in patients over the age or 50 years.[19] Interestingly, mitral valve prolapse is associated with body leanness, lower blood pressure and lower prevalence of diabetes in American Indians. Thus, the inherited valve alteration is ironically associated with cardiovascular protective parameters, referring to as a Darwinian paradox.

Degenerative valve lesions

Degenerative valve lesions are present in up to 25% of patients aged over 40 years and in 50% of patients aged over 60 years with IE.[20] They involve local inflammation, micro-ulcers and microthrombi of the endothelium, which are quite similar to atherosclerosis. A recent echocardiography study identified degenerative valve lesions that might increase the risk of IE in up to 50% of patients aged more than 60 years.[21] This may explain, at least in part, the increased risk of IE in elderly individuals.

Prosthetic valve infective endocarditis

PVE occurs in 1–5% of cases, or 0.3–0.6% per patient years.[22,23] The issue of whether mechanical or bioprosthetic valves are more prone to infection remains unresolved.[22] PVE is usually classified as either early infection or late infection, depending on whether the symptoms of infection occur within 12 months after surgery or later.[24,25] This stratification is based on differences in the types of pathogen responsible for PVE during the two periods:

- early PVE implicates more often surgically related and drug-resistant microbes (e.g. methicillin-resistant staphylococci);
- late PVE implicates more often oral streptococci and sometimes Gram-negative bacteria of the so-called HACEK group (including *Haemophilus* spp., *Actinobacillus actinomycetemcomitans*, *Cardiobacterium hominis*, *Eikenella corrodens* and *Kingella kingae*).

The switch between these two periods is somewhat empiric and was formerly considered to be 2 months post-surgery. However, it was recently shown that the major shift from one type of organisms to the other occurred earlier than 12 months post-surgery.[25] Patients with a prosthetic valve implanted during active IE have a greater risk (up to seven times) of subsequent PVE than patients undergoing elective valve replacement, although the infection may be due to a different organism.

Infective endocarditis in intravenous drug users

Intravenous drug users and HIV patients constitute a special risk group of relatively young people, with a median age varying between 30 and 40 years.[10] However, they may progressively increase in elderly patients in coming years. Men are more often affected than women. The tricuspid valve is infected in more that 50% of cases, followed by the aortic valve in 25% and the mitral valve in 20%, with mixed right-sided and left-sided IE in a few cases. It has been suggested that repeated injections of impure drugs and particulate material might produce microtrauma to the tricuspid leaflets, thus facilitating microbial colonization and infection. However, 20–40% of intravenous drug users suffering IE have pre-existing cardiac lesions, often caused by previous valve infection. The responsible bacteria often originate from the skin, which explains the predominance of *S. aureus* infections (Table 47.2), but streptococci and other micro-organisms are also encountered; some pathogens, such as *Pseudomonas aeruginosa* and fungi, may produce severe forms of IE.

Immune deficiency related to HIV is a risk factor, as well behaviors associated with this particular at-risk group (e.g. alcohol intake and intravenous injections).[10] In one study, the risk of IE was unaffected in patients with >500 CD4 cells/mm^3, but increased approximately fourfold in those with <200 CD4 counts. The mortality of IE was also higher in patients with AIDS, especially in advanced cases, than in other patients.[26]

Nosocomial (or health-care-related) infective endocarditis

Nosocomial IE is another growing category of patients. In a recent series it accounted for 22% of 109 cases overall, and 16% of cases excluding early PVE in cardiac surgery patients.[27] Many patients had debilitating underlying conditions, but less than 50% had obvious cardiac predisposing factors. In most circumstances a potential source of bacteremia could be identified, such as the presence of intravenous lines or invasive procedures. Pathogens usually originated from the skin or the urinary tract, staphylococci and enterococci being the most common. In one study, up to 13% of nosocomial *S. aureus* bacteremias were responsible for subsequent IE.[28] Moreover, possible right-sided IE was reported in 5% of bone marrow transplant recipients who had central venous catheters. Overall, mortality was up to 50%.

The general increase in health-care-associated IE was confirmed in recent surveys, where it represented more than 30% of all IE due to *S. aureus*, and in which chronic hemodialysis – which increased fourfold between 1993 and 1999 – was an independent risk factor.[8] As a result, it was recently proposed that a fifth category of 'hemodialysis-related IE' be added to the four categories of IE listed above.[29]

Table 47.2 Microbiology of IE in the general population and in specific at-risk groups

Pathogens	NO. OF EPISODES (% OF TOTAL)			
	Native valve	Intravenous drug abusers	Prosthetic valve	
			Early	Late
Staphylococci:	124 (44)	60 (69)	10 (66)	33 (45)
Staphylococcus aureus	106 (38)	60 (69)	3 (21)	15 (20)
Coagulase negative	18 (6)	0 (0)	7 (45)	18 (25)
Streptococci:	86 (31)	7 (8)	0 (0)	25 (35)
Oral streptococci	59 (21)	3 (3)		19 (26)
Others (non-enterococcal)	27 (10)*	4 (5)		6 (9)
Enterococcus spp†	21 (8)	2 (2)	1 (7)	5 (7)
HACEK group	12 (4)‡	0 (0)	0 (0)	1 (1.5)
Polymicrobial	6 (2)	8 (9)	0 (0)	1 (1.5)
Other bacteria	12 (4)§	4 (5)	0 (0)	2 (3)
Fungi	3 (1)	2 (2)	0 (0)	0 (0)
Negative blood culture	16 (6)	4 (5)	4 (27)	5 (7)
Total episodes	280 (100)	87 (100)	15 (100)	72 (100)

*Including 9 *S. agalactiae*, 6 *S. bovis* (renamed *S. gallolyticus*), 3 *S. pneumoniae*, 2 *S. pyogenes*, 1 group G streptococcus, and 1 *Abiotrophia* spp.
†Mostly (>80%) represented by *S. faecalis*.
‡Includes *Haemophilus* spp., *Actinobacillus actinomycetemcomitans*, *Cardiobacterium hominis*, *Eikenella corrodens* and *Kingella kingae*.
§Includes 4 *Escherichia coli*, 2 *Corynebacterium* spp., 2 *Proteus mirabilis*, 1 *Mycobacterium tuberculosis* and 1 *Bacteroides fragilis*.
Adapted from Moreillon & Que.[3]

PATHOGENESIS, PATHOLOGY AND INFECTING MICRO-ORGANISMS

The key issues in the pathogenesis of IE, discussed below, are:

- the predisposing host factors;
- the characteristics of the infecting micro-organisms;
- the role of transient bacteremia; and
- the inability of the immune system to eradicate micro-organisms once they are located on the endocardium.

Predisposing host factors

The normal valve endothelium is very resistant to colonization and infection by circulating bacteria. On the other hand, mechanical lesions of this endothelium result in exposure of the underlying extracellular matrix proteins, the production of tissue factor, and the deposition of fibrin and platelets as a normal healing process. Such nonbacterial thrombotic endocarditis is an ideal nidus for bacterial adherence and infection (Fig. 47.1) (reviewed in Moreillon *et al.*[30]).

Endothelial damage may result from mechanical aggression due to turbulent blood flow in cases of congenital cardiac abnormalities or prosthetic valves, or lesions provoked by electrodes or catheters. Alternatively, endothelial damage can result from inflammation, as rheumatic carditis or in elderly individuals with degenerative valve lesions, which involve inflammation, micro-ulcers and micro-thrombi.[20,21] It was suggested that the presence of *Chlamydia pneumoniae* or cytomegalovirus in endovascular locations could be linked to arteriosclerosis. Whether these organisms also promote degenerative valve lesions promoting IE remains to be demonstrated.

Characteristics of the micro-organisms

The organisms most frequently responsible for IE are also those that have the greatest ability to adhere to and colonize damaged valves.[30] Together, *S. aureus*, *Streptococcus* spp. and enterococci are responsible for over 80% of all cases of IE (see Table 47.2). IE pathogens possess several surface adhesins that mediate attachment to extracellular matrix proteins of the host. These adhesins are collectively referred to as MSCRAMMs, for microbial surface component reacting with adhesive matrix molecules. They include both proteins and polysaccharides.

In *S. aureus*, fibrinogen-binding protein A (also called clumping factor A or ClfA) and fibronectin-binding protein A (FnBPA) are involved in valve colonization and invasion, whereas other MSCRAMMs seem less implicated.[31] Figure 47.2 presents a likely scenario in the case of IE due to this organism. In streptococci, several surface proteins as well as platelet-activating factors and exopolysaccharides are involved in bacterial adherence to damaged valves. This abundance of surface adhesins makes IE pathogens particularly prone to colonize susceptible valve tissues.

Aside from bacterial colonization of pre-existing valve lesions, invasion of endothelial cells may also occur in certain circumstances. Direct invasion of the valve endothelium was demonstrated in experimental endocarditis with *S. aureus*.[31] During inflammation, endothelial cells express a variety of molecules, including integrins of the β_1 family (very late antigen, VLA), which bind fibronectin. Fibronectin bound to endothelia is a privileged ligand for pathogens expressing fibronectin-binding proteins, including *S. aureus*. Bridging *S. aureus* and endothelial cells via fibronectin triggers internalization of the attached microbes by the host cell. Thus, local inflammation due to degenerative lesions, e.g. arteriosclerosis, or as yet undetermined conditions may promote direct endothelial infection. These events may also be important in the pathogenesis of IE due to intracellular pathogens such as *Coxiella burnetii* (the agent of Q fever), *Chlamydia* spp., *Legionella* spp. and *Bartonella* spp.[32]

The role of transient bacteremia

The role of transient bacteremia was demonstrated in animals with catheter-induced aortic valve vegetations and dental manipulation.[33] Both the magnitude of bacteremia during dental procedures and the ability of the pathogen to attach to damaged valves were important. Out of a great variety of gingival micro-organisms circulating in the blood after the procedure, only those able to attach to damaged valves (i.e. streptococci and *S. aureus*) produced IE.

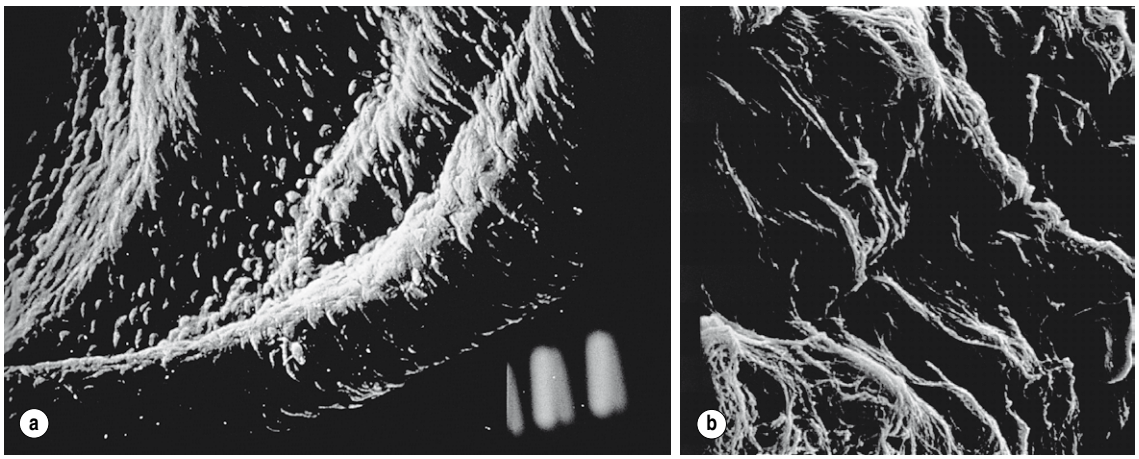


Fig. 47.1 Scanning electron microscopy of a rabbit aortic valve leaflet. (a) A normal valve, covered by a monolayer of endothelial cells. (b) The meshwork of fibrin and platelets covering a damaged valve. Mechanical lesions of the valve were created by inserting a catheter through the right aortic carotid and across the aortic valve.

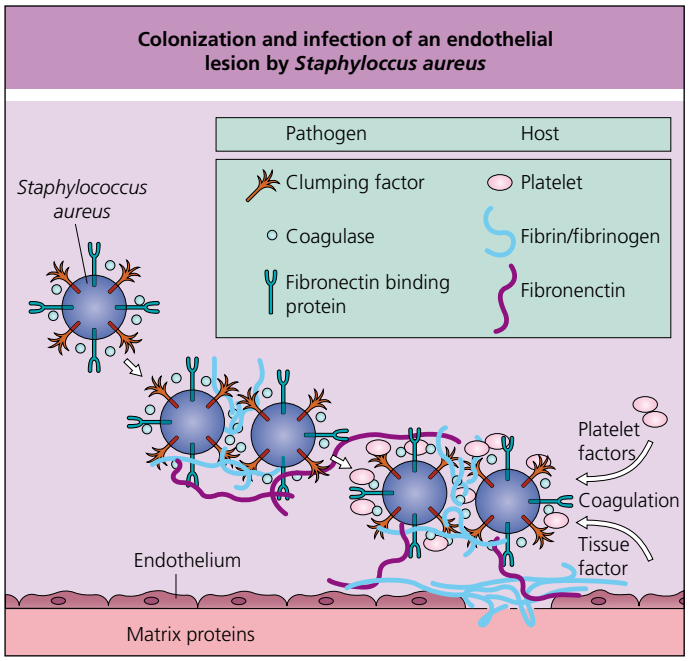


Fig. 47.2 Colonization and infection of an endothelial lesion by *Staphylococcus aureus*. Exposure of the subendothelial matrix triggers the deposition of platelet–fibrin clots and other plasma-soluble and matrix proteins, including fibrinogen, fibrin, fibronectin and thrombospondin. Triggering of the coagulation cascade is also mediated by tissue factor, which contributes to platelet activation and the constitution of a nonbacterial thrombotic vegetation. *S. aureus* is equipped with a wealth of surface determinants that may promote binding and colonization of nonbacterial thrombotic vegetations. The main players are fibrinogen-binding protein A (clumping factor A, or ClfA) and fibronectin-binding protein A (FnBPA), which synergize for valve adhesion and invasion of endothelial cells.[31] Coagulase might also help trigger platelet aggregation.

Bouts of transient bacteremia occur not only during medicosurgical procedures, but also during normal activities such as chewing and tooth-brushing. Such spontaneous bacteremias are usually of low grade and short duration (1–100 CFU/ml of blood for less than 10 minutes),[34] but their high frequency may explain why most IE cases occur outside medicosurgical procedures.[12,35] Also of increasing concern are health-care-associated bacteremias, such as in chronic hemodialysis patients who represent a quasi new at-risk group for IE.[8,29]

The role of host defenses

IE is most often due to Gram-positive organisms, and rarely to Gram-negative bacteria (see Table 47.2). The reason for this is probably multifactorial. Differences in bacterial adherence to damaged valves may be one explanation. However, differences in the susceptibility of Gram-positive and Gram-negative bacteria to serum-induced killing may also account for these variations. The C5b–C9 membrane-attack complex of complement kills Gram-negative bacteria by perforating their outer membrane. In contrast, complement does not kill Gram-positive bacteria because they lack an outer membrane, and their plasma membrane is protected from the membrane-attack complex by the thick surrounding peptidoglycan.

The importance of serum was demonstrated in experimental IE induced by serum-susceptible *Escherichia coli*. These organisms were spontaneously cleared from the valves. Yet, some Gram-negative bacteria may carry thick capsules or other modifications of their surface that help them resist complement-induced killing. Important Gram-negative bacteria that provoke IE include bacteria of the HACEK group, as well as *P. aeruginosa*.

Although Gram-positive bacteria are resistant to complement, they may be the targets of other nonspecific immune factors such as platelet microbicidal proteins (PMPs). PMPs are peptides produced by activated thrombocytes that kill bacteria by damaging their plasma membrane. The protective role of PMPs was suggested by experimental endocarditis, where thrombocytopenic rabbits demonstrated greater bacterial densities in their vegetations than rabbits with normal platelet counts. Likewise, micro-organisms recovered from patients with IE were consistently resistant to PMP-induced killing, whereas similar bacteria recovered from patients with other types of infection were susceptible to PMPs.[36] Therefore platelets, which are a major component of the vegetations, are key players in the nonspecific defense against IE.

Humoral and cellular immunity seem to play only a limited role in defense against IE. Immunization studies in experimental endocarditis gave contradictory results. Immunization of rats against the streptococcal MSCRAMM FimA conferred cross-protection against IE due to several viridans group streptococci.[37] In contrast, immunization of rabbits against the enterococcal aggregation substance failed to protect



Fig. 47.3 Microscopic appearance of a vegetation from a patient suffering mitral valve infective endocarditis due to *Streptococcus sanguis*. The purple area represents clusters of streptococci packed within a fibrin-platelet meshwork. Professional phagocytes are essentially absent from the lesion.

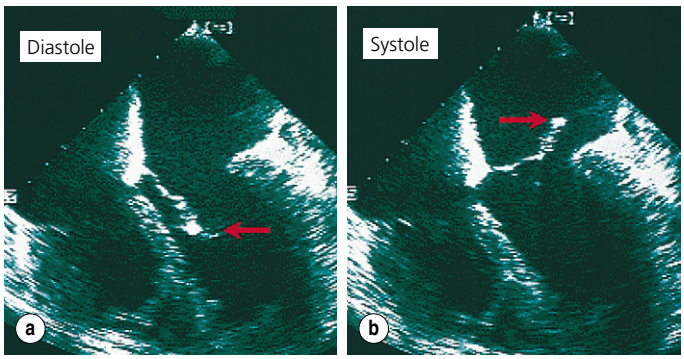


Fig. 47.4 Transesophageal echocardiography of a mitral valve infective endocarditis. The echocardiogram is from the patient described in Figure 47.3. Arrows indicate a large pediculated vegetation, which oscillates from the left ventricle to the left atrium during the systole. Courtesy of Dr X Jeanrenaud.

the animals against experimental IE. In this case, specific antibodies arising from vaccination could not appropriately penetrate inside the vegetation *in vivo*. Administration of granulocyte colony-stimulating factor did not influence the course of infection either. IE is not noticeably more frequent in immunocompromised patients than in those without immune defects, except for HIV patients who may have additional risk factors and risk components.[10] In established infection, bacteria are clustered in amorphous platelet–fibrin clots (the vegetations), permitting little access to professional phagocytes to remove the bacteria (Fig. 47.3). Figure 47.4 presents an echocardiographic picture of a large oscillating vegetation, which occurred in the context of a mitral valve streptococcal IE. Such a 'therapeutic sanctuary' explains why successful treatment of IE relies primarily on antibiotic-induced killing of bacteria rather than on host defenses.

PREVENTION

Because of its severity, it is generally agreed that IE should be prevented whenever possible. Choice of appropriate prophylactic measures requires:

- identification of patients at risk;
- determination of the procedures or circumstances that may result in bacteremia;
- choice of an appropriate antimicrobial regimen; and
- balancing of the known risks against the possible benefits of intervention.

Because prospective studies of IE prophylaxis in humans raise ethical issues and would require too many patients, the practice of IE prophylaxis is still based on its proven efficacy in experimental animal models. Recently, a working group of the American Heart Association revisited the guidelines for prophylaxis based on a risk assessment of both underlying cardiac conditions and medicosurgical practices most likely to promote IE.[38] The group reviewed the English language literature on the subject from 1950 to 2006 and concluded the following:

- Only an extremely small number of cases of IE might be prevented by antibiotic prophylaxis for dental procedures, even if such prophylactic therapy were 100% effective.
- IE prophylaxis for dental procedures is reasonable only for patients with underlying cardiac conditions associated with the highest risk of adverse outcome from IE.
- For patients with these underlying cardiac conditions, prophylaxis is reasonable for all dental procedures that involve manipulation of gingival tissue or the periapical region of teeth or perforation of the oral mucosa.
- Prophylaxis is not recommended based solely on an increased lifetime risk of acquisition of IE.
- Administration of antibiotics solely to prevent IE is not recommended for patients who are to undergo a genitourinary or gastrointestinal tract procedure.

The content of the new guidelines is summarized in Tables 47.3 and 47.4. The critical review by the working group helps settle unproven assumptions and potential medicolegal issues on the topic. Moreover, the new guidelines greatly simplify the choice of prophylactic drugs and thus may improve compliance. Due to the lack of controlled prospective studies, the level of evidence of most of these propositions is II-B, except for prophylaxis before cardiac surgery, which is I-A.

Since the working group gathered international experts, the new guidelines will most likely be adopted by most European countries, with possible minor modifications.[24] For example, the 2008 guidelines from the British National Institute for Health and Clinical Excellence (NICE) are even more restrictive, as they propose to completely abandon IE prophylaxis (www.nice.org.uk/CG064). Patients with healthy gums are at lower risk of bacteremia than those with severe gingivitis, hence the importance of primary prevention by general hygiene of the dental sphere and other body sites that might be susceptible to release pathogens into the blood.

CLINICAL FEATURES

IE may follow an acute or subacute course. The general clinical features (Table 47.5) are not usually specific. Figure 47.5 depicts the dynamics of infection-related events (critical phase) and infection-unrelated events (continuation phase) that may occur during antibiotic treatment.

Acute infective endocarditis

Acute IE can be devastating. It is most frequently caused by *S. aureus* (and sometimes by *Staphylococcus lugdunensis* and *Staphylococcus capitis*) followed by certain streptococci of the *Streptococcus milleri* group (*Streptococcus constellatus*, *Streptococcus anginosus* and *Streptococcus intermedius*) and enterococci. Bacterial production of proteases and other exoproteins contributes to rapid destruction of valve leaflets and the development of abscesses located in the valve ring and the myocardium. Myocarditis and pericardial effusions are frequent. Patients are prostrate and have a high fever. Hypotension and shock may occur, caused both by the septic state and by cardiac failure. Valve vegetations may vary from a few millimeters in diameter to more than 1 cm. Large vegetations are frequent in acute *S. aureus* and fungal IE (Fig. 47.6) and are more likely to detach and give rise to septic emboli. Complications in peripheral organs mainly result from embolic lesions; these may include skin abscesses and retinal emboli (Fig. 47.7), as well as abscesses in the brain and the spleen (Fig. 47.8).

Table 47.3 Cardiac conditions associated with the highest risk of adverse outcome from infective endocarditis (IE), and procedures for which antibiotic prophylaxis is recommended or not recommended

Underlying cardiac conditions where antibiotic prophylaxis is recommended

Prosthetic cardiac valve
Previous IE
Congenital heart disease (CHD)*
- Non-repaired cyanotic CHD, including palliative shunts and conduits
- Repaired congenital heart defect with prosthetic material or device, either by surgically or by catheter intervention, during the first 6 months after the procedure†
- Repaired CHD with residual defects at the site or adjacent to the site of a prosthetic patch or device (a situation that inhibits endothelialization)

Cardiac transplantation recipients who develop cardiac valvulopathy

Procedures where antibiotic prophylaxis is recommended for the above conditions

All dental procedures that involve manipulation of gingival tissues or periapical region of teeth, or perforation of oral mucosa
Procedures on respiratory tract or infected skin, skin structures or musculoskeletal tissues only if they imply overt incision of the skin or mucosa

Procedures where antibiotic prophylaxis is not recommended

Procedures on the genitourinary or gastrointestinal tracts, if indication for prophylaxis implicates only endocarditis prevention

*Antibiotic prophylaxis is not recommended for any CHD other than those listed above.
†Endothelialization occurs during the first 6 months after implantation.
Adapted from European Society of Cardiology[24] and American Heart Association[38] guidelines.

Major indications for urgent valve replacement include refractory cardiac failure due to valve destruction and persistent sepsis related to myocardial abscesses. A defect in atrioventricular conduction is often an early sign of septal invasion by a contiguous valve ring abscess, which usually requires urgent surgery.

Subacute infective endocarditis

Subacute IE is not usually due to *S. aureus*, but it may be due to any of the organisms listed in Table 47.2. It can mimic chronic wasting diseases. The duration between initiation and diagnosis can vary from a few days to more than 5 weeks. Fever is almost always present (see Table 47.5). Physical signs reflect the existence of cardiac or peripheral complications. These include a new or changing heart murmur, evidence of embolic events and immunologic signs of chronic inflammation. Immune complexes (or rheumatoid factor) are present in up to 50% of patients after 6 weeks of subacute infection. Their level decreases during effective treatment. Immune phenomena may be the cause of petechiae, splinter hemorrhages, Osler nodes and Roth spots, arthritis and glomerulonephritis. Osler nodes are small and painful nodular lesions on the pads of the fingers or toes or on the thenar or hypothenar eminences (Fig. 47.9). They are caused by an allergic vasculitis. Although classic, they are not pathognomonic of subacute IE. Roth spots are rounded retinal hemorrhages with a white center. Focal or diffuse glomerulonephritis is present in most cases.

Vascular complications

Peripheral septic emboli are due to fragmentation of the vegetations and often bring the patient to consultation. Therefore they peak at the time of diagnosis and decrease rapidly during the first week of effective antibiotic therapy (see Fig. 47.5).[39] They are more frequent with *S. aureus* than with oral streptococci, when the size of vegetation is >10 mm, and when the mitral valve is involved.[40] Other types of vascular manifestation are the consequence of immune-related vasculitis. Mycotic aneurysms are found in up to 15% of cases, and are especially common in *S. aureus* IE. They may arise from direct

Table 47.4 Recommended antibiotic regimens for endocarditis prophylaxis for risk factors and procedures listed in Table 47.3

		REGIMEN: SINGLE DOSE 30–60 MIN BEFORE PROCEDURE	
For dental procedures*	**Agent**	**Adults**	**Children**
Oral route	Amoxicillin	2 g	50 mg/kg
Unable to take oral antibiotics	Ampicillin	2 g iv or im	50 mg/kg iv or im
	or		
	cefazolin or ceftriaxone	1 g iv or im	50 mg/kg iv or im
Allergic to penicillin – oral route	Cephalexin†,‡	2 g	50 mg/kg
	or		
	clindamycin	600 mg	20 mg/kg
	or		
	clarithromycin	500 mg	15 mg/kg
Allergic to penicillin – unable to take oral antibiotics	Cefazolin or ceftriaxone	1 g iv or im	50 mg/kg iv or im
	or		
	clindamycin	600 mg iv or im	20 mg/kg iv or im

*Regimens for other procedures should be targeted at the most probable pathogen implicated. For streptococci, antibiotics are the same as above. For staphylococci, anti-staphylococcal β-lactams or vancomycin should be considered. For enterococci, amoxicillin or ampicillin, as above, or vancomycin should be considered. The risk of methicillin-resistant staphylococci (preferred vancomycin) and vancomycin-resistant enterococci (preferred amoxicillin or ampicillin) should be taken into account.
†Or other first- or second-generation cephalosporin.
‡Cephalosporins should not be used patients with type 1 allergy to β-lactams.
Adapted from European Society of Cardiology[24] and American Heart Association[38] guidelines.

Table 47.5 Clinical features of IE in the general population and in specific at-risk groups

Clinical features	NO. OF EPISODES (% OF TOTAL)			
	Native valve	Intravenous drug abusers	Prosthetic valve	
			Early	Late
Symptoms				
Fever	170 (75)	42 (87)	7 (77)	47 (78)
Chills	104 (46)	(73)	5 (55)	32 (53)
Arthralgias/myalgias	44 (19)	(27)	–	10 (17)
Back pain	22 (10)	5 (10)	–	2 (2)
Pleuritic chest pain	10 (4)	17 (45)	–	3 (5)
Clinical signs				
Cardiac murmur	196 (86)	45 (93)	8 (88)	58 (96)
Petechiae, emboli	80 (35)	3 (6)	3 (33)	30 (50)
Osler nodes, Janeway lesions, Roth spots	26 (11)	3 (6)	0 (0)	6 (10)
Splinter hemorrhages*	28/80 (35)	3/15 (30)	5/7 (71)	17/33 (51)
Palpable spleen*	15/80 (19)	3/15 (30)	2/7 (28)	2/33 (6)
Central nervous system manifestations†	38/148 (25)	4/33 (12)	0/2 (0)	5/27 (18)
Renal insufficiency†	45/148 (30)	5/33 (15)	1/2 (50)	8/27 (30)
Temperature (°C)				
<37.8	64 (28)	15 (31)	3 (34)	21 (35)
37.8–38.9	82 (36)	12 (25)	6 (66)	22 (37)
≥39	81 (36)	21 (44)	0 (0)	17 (28)
Total episodes	228	48	9	60

*As reported by Sandre & Shafran.[58]
†As reported by Watanakunakorn & Burkert.[57]

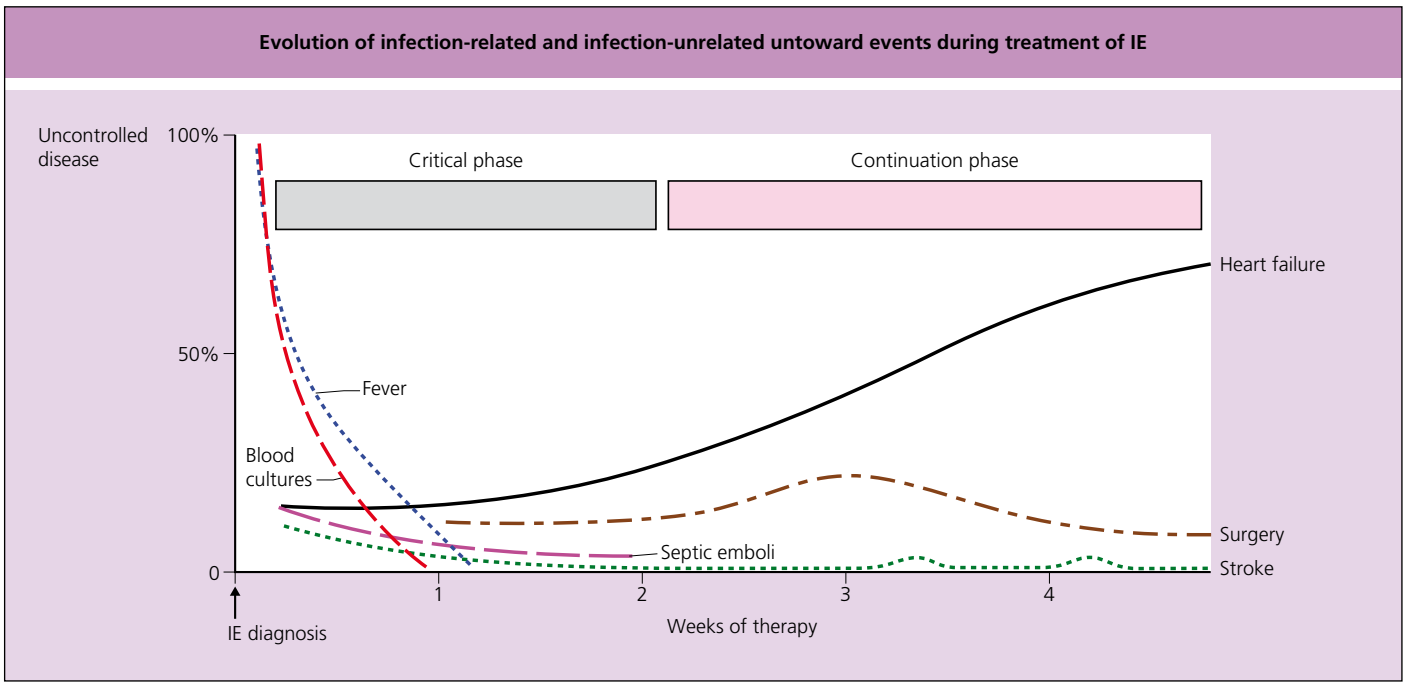


Fig. 47.5 Evolution of infection-related and infection-unrelated untoward events during treatment of IE. Infection-related events occur mostly during the first 2 weeks of therapy. These include persistent fever, positive blood cultures, acute heart failure and septic emboli, which if persistent may require therapy readjustment and/or adjunctive surgery. These first 2 weeks represent a critical phase during which initiation of outpatient parenteral antibiotic therapy (OPAT) has to be carefully weighed. OPAT should not be initiated if all these parameters are not under control. Later events (continuation phase) are not related to inappropriate antibiotic treatment and may occur irrespective of hospital stay, i.e. hospital stay does not prevent and OPAT does not provoke such complications. OPAT is feasible during this phase, provided that logistics and medical follow-up (once or twice weekly) are organized. (For details, see Andrews & von Reyn.[54])

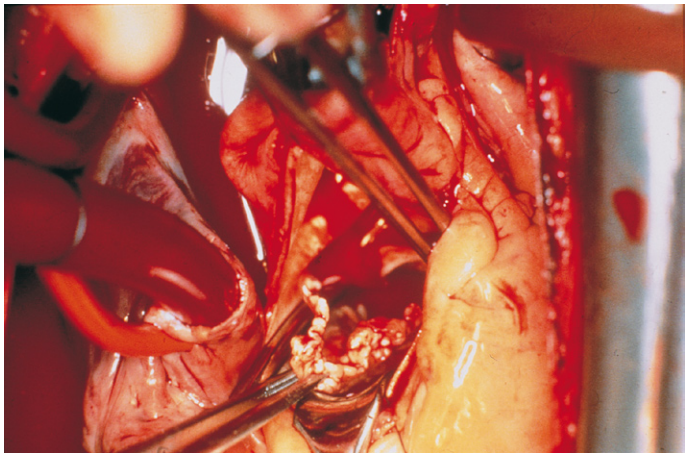


Fig. 47.6 Aortic valve of a patient undergoing emergency valve replacement for acute endocarditis caused by *Staphylococcus aureus*. In this patient, emergency valve replacement was mandatory because of multiple embolizations and acute heart failure. Here, the aorta has been opened and the valve is viewed from its upper side. The lower tweezers are holding a valve leaflet covered with vegetations. Next to the upper tweezers, a portion of a cuspid with a normal appearance can be distinguished.

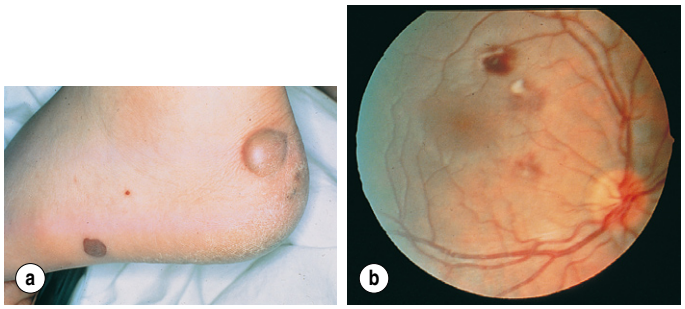


Fig. 47.7 Skin lesions (Janeway spots) on the foot (a) and septic emboli of the retina (b), the results of peripheral emboli in acute endocarditis caused by *Staphylococcus aureus*. These occurred in the patient described in Figure 47.6 and were present on admission to hospital.

invasion of the arterial wall by the infecting organisms, from septic embolization of the vasa vasorum or from the deposition of immune complexes with ensuing inflammation and weakening of the arterial wall. Mycotic aneurysms tend to be located at the bifurcation points of vessels. They may heal during antibiotic therapy or become clinically evident later, even months after the clinical cure of the disease. As such, the true incidence of mycotic aneurysms during IE is probably underestimated. In right-sided IE, embolization occurs in the pulmonary circulation and gives raise to pulmonary infiltrates and lung abscesses.

Neurologic complications

Neurologic manifestations occur in up to 40% of cases.[27] Anatomic alterations include cerebral infarction, arteritis, abscesses, mycotic aneurysms, intracerebral or subarachnoid hemorrhage, encephalomalacia, cerebritis and meningitis. Like peripheral emboli, embolic strokes peak at the time of diagnosis and decrease rapidly during efficacious antibiotic therapy.[40] The frequency of stroke is similar between NVE and PVE.

Neurologic events may be considered as a contraindication for cardiac surgery, due to the risk of anticoagulation-related cerebral hemorrhage. Nevertheless, recent studies indicate that surgery may be performed with a minimum risk of neurologic complications (3–6%), which offsets the risk of postponing valve surgery for uncontrolled infection and/or acute heart failure.[41] Such difficult situations should be handled via an interdisciplinary approach.

DIAGNOSIS

Precise clinical and microbiologic diagnosis is mandatory in order to guide therapy. In theory, IE combines persistent bacteremia and anatomic lesions of the valves. However, blood cultures may remain negative in up to 10% of cases (Tables 47.2, 47.6). Diagnosis of IE is difficult in blood culture-negative IE, or when changes in the valve status cannot be assessed owing to lack of information on pre-existing cardiac lesions. Therefore, criteria taking into account microbiology, valve status (as determined by echocardiography or pathology), serology and clinical features were recently assessed and validated to improve the diagnosis of IE in difficult cases (Table 47.7).[1]



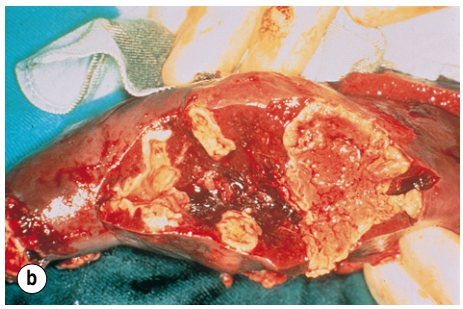


Fig. 47.8 A cerebral abscess (a) and multiple abscesses and ischemic necroses of the spleen (b), the results of peripheral emboli in acute endocarditis caused by *Staphylococcus aureus*. Again these occurred in the patient described in Figure 47.6; they developed after admission to hospital.



Fig. 47.9 Osler node on the thumb during subacute endocarditis. This was a rounded, tender, inflamed mass about 5 mm in diameter.

Table 47.6 Rare causes of IE associated with negative blood cultures

Pathogen	Diagnostic procedure	Proposed therapy and criteria for cure*
Brucella spp.	Blood cultures Serology Culture, immunohistology and PCR of surgical material	Doxycycline plus rifampin (rifampicin) or co-trimoxazole Treatment for >3 months Cure is considered when antibody titers are <1:160
Coxiella burnetii (agent of Q fever)	Serology: IgG phase I >1:800 Tissue culture, immunohistology and PCR of surgical material	Doxycycline plus hydroxychloroquine Doxycycline plus quinolone Treatment >18 months Cure is considered when IgG phase I titers are <1:800, and IgA and IgM titers are <1:50
Bartonella spp.†	Blood cultures Serology Culture, immunohistology and PCR of surgical material	β-lactams or doxycycline plus aminoglycoside Treatment >6 weeks Surgery required in up to 90% of cases
Chlamydia spp.‡,§	Serology Culture, immunohistology and PCR of surgical material	Doxycycline Newer fluoroquinolones Long-term treatment, optimal duration unknown
Mycoplasma spp.§	Serology Culture, immunohistology and PCR of surgical material	Doxycycline Newer fluoroquinolones Treatment >12 weeks
Legionella spp.§	Blood cultures Serology Culture, immunohistology and PCR of surgical material	Macrolides plus rifampin Newer fluoroquinolones Treatment >6 months
Tropheryma whipplei¶ (agent of Whipple's disease)	Histology and PCR of surgical material	Co-trimoxazole β-lactam plus aminoglycoside (long-term treatment, optimal duration unknown)

*Because of the lack of large series on IE due to these pathogens, optimal treatment duration is mostly unknown (evidence level II-B). Treatment durations in the table are indicative and are based on selected case reports.
†Several therapeutic regimens were reported, including amino-penicillins and cephalosporins combined with aminoglycosides, doxycycline, vancomycin and quinolones.
‡Beware of serologic cross-reaction with the more common IE pathogen *Bartonella* spp.
§Newer fluoroquinolones are more potent than ciprofloxacin against intracellular pathogens such as *Mycoplasma* spp., *Legionella* spp. and *Chlamydia* spp.
¶Treatment of Whipple IE remains highly empiric. Successes were reported with long-term (>1 year) co-trimoxazole therapy.
Adapted from Brouqui & Raoult (2001)[32] and (2006).[44]

Blood cultures

Blood cultures are of primary importance because they reveal the infecting organism and guide antibiotic therapy. The volume of blood cultured is critical because bacteremia is often of low grade, representing only 1–100 bacteria/ml of blood. A minimum of two (ideally three) sets of blood cultures consisting of 20 ml of blood should be taken at 60 min intervals before any antibiotic administration, and distributed into a two-bottle system for incubation. For the most frequent pathogens, two sets will reveal the organism in *c.* 90% and three sets in up to 98% of cases.[42] Some centers have developed special IE culture protocols to optimize the detection of fastidious organisms, including blood collection in lysis-centrifugation tubes and subcultures on specific media. Whenever possible, the laboratory should be made aware of the possibility of IE.

Culture-negative infective endocarditis

Blood culture-negative IE falls into two categories that are important for diagnosis and treatment. In the first category are patients who have received antibiotics prior to blood cultures. These represent *c.* 50% of culture-negative cases.[43] In the second category are patients with IE due to fastidious or as yet noncultivable organisms, represented mostly by *Coxiella burnetii* and *Bartonella* spp. (in *c.* 70% of cases), *Brucella* spp. (in endemic areas) and rarely (in *c.* 1% of cases) *Tropheryma whipplei*, maybe *Legionella* spp. and sometimes fungi (especially *Aspergillus*).[43] The laboratory must be made aware of these possibilities. Table 47.6 lists the principal organisms of this group, and proposed diagnostic procedures and therapies.[32] Since these cases of IE are rare, most therapeutic recommendations are based on limited experience including case reports and small series. Therefore, any of these conditions require a special case-by-case attention by the caregiver (evidence level II-A to II-B).

Microbiologic cultures

Bacteria of the HACEK group are usually detected after prolonged (>1 week) incubation of the blood cultures. In contrast, *Abiotrophia* spp. grow well in blood, but fail to grow when subcultured on conventional agar media. These so-called deficient bacteria do grow as satellite colonies if the plates are streaked with *S. aureus*, or if the growth medium is enriched with vitamin B6. These methods should be applied when bacteria are observed microscopically in blood cultures, but fail to grow on the subculture plates.

Brucella spp., *Bartonella* spp., *Legionella* spp. and *Mycobacteria* spp. are rare causes of IE that require special culture techniques.[44] The precise description of these techniques is beyond the scope of this chapter. Serologic tests may help define both culture strategies and other diagnostic procedures. Tissue cell cultures may be performed in specialized laboratories for the detection of strict or facultative intracellular bacteria. These include *C. burnetii*, *Chlamydia* spp., *Legionella* spp. and *Bartonella* spp.

If the patient undergoes surgery, it is of the utmost importance to obtain valve samples for cultures and other microbiologic tests. The importance of cultures cannot be emphasized enough, because it fulfills both diagnostic and therapeutic purposes. Indeed, cultures allow testing for antimicrobial susceptibility.

Table 47.7 Modified Duke criteria for diagnosis of infective endocarditis

Definition terminology used in the criteria	
Major criteria	
1. Blood culture	Positive blood cultures (≥2/2) with typical IE micro-organisms (viridans streptococci, *S. bovis* (renamed *S. gallolyticus*), HACEK* group, or community-acquired *S. aureus* or enterococci in the absence of primary focus) Persistently positive blood cultures defined as two culture sets drawn >12 h apart, or three or the majority of four culture sets with the first and last separated at least by 1 h
2. Endocardial involvement	Single positive culture for *Coxiella burnetii* or anti-phase I antibody titer >1:800 New valve regurgitation Positive echocardiogram for IE (transesophageal echo recommended in patients with prosthetic valves and patients rated as 'possible' IE by clinical criteria) defined as: (i) oscillating intracardiac mass in the valve or supporting structure, *or* in the path of regurgitant jets, *or* on implanted material, in the absence of an alternative anatomic explanation, *or* (ii) abscess, *or* (iii) new partial dehiscence of prosthetic valve
Minor criteria	
1. Predisposing cardiac condition or intravenous drug use	
2. Fever: >38°C (100.4°F)	
3. Vascular phenomena: arterial emboli, mycotic aneurysms, petechiae, Janeway lesions	
4. Immunologic phenomena: glomerulonephritis, Osler nodes. Roth spots, rheumatoid factor	
5. Microbiology: positive blood cultures, but not meeting major criteria, serologic evidence of active infection with plausible micro-organisms	

Diagnosis	
Definite	**Rejected**
• Pathology or bacteriology of vegetations, *or*	• Firm alternative diagnosis, *or*
• 2 major criteria, *or*	• Resolution of IE syndrome after <4 days of antibiotic therapy, *or*
• 1 major and 3 minor criteria, *or*	• No pathologic evidence at surgery or autopsy after <4 days of antibiotic therapy
• 5 minor criteria	• Does not meet criteria mentioned above
Possible	
• 1 major and 1 minor criterion, *or*	
• 3 minor criteria	

*Includes *Haemophilus* spp., *Actinobacillus actinomycetemcomitans*, *Cardiobacterium hominis*, *Eikenella corrodens* and *Kingella kingae*.
Adapted with modifications from Li *et al*.[1]

Histology

A number of special stains may help guide the etiologic diagnosis. Important techniques include Gram stain, periodic acid–Schiff (PAS) stain for Whipple's disease, Giemsa and Warthin–Starry stains for numerous bacteria including *Bartonella* spp., Ziehl-Neelsen stain for *Mycobacteria* spp. and Gimenez stain for *C. burnetii* and *Legionella* spp. While not strictly diagnostic, these techniques help delineate more specific etiologic procedures.

Polymerase chain reaction amplification

Polymerase chain reaction (PCR) amplification of the 16S or 23S ribosomal RNA genes (and sometimes other loci) has become a critical method for bacterial identification from culture and tissue samples. It has been successfully applied to IE surgical material,[45] including cutaneous biopsies of peripheral septic emboli. PCR is invaluable to detect poorly or noncultivable bacteria such as *Tropheryma whipplei*.[44] Figure 47.10 depicts a case of culture-negative aortic valve IE necessitating valve replacement. *T. whipplei* was identified by histology and PCR amplification of the surgical material.

Serology

Serologic tests are included in the Duke diagnostic criteria (see Table 47.7).[1] Common tests include the agglutination test for *Brucella melitensis*, indirect fluorescence for *Legionella pneumoniae*, ELISA for *Mycoplasma pneumoniae*, and complement fixation, ELISA and indi-

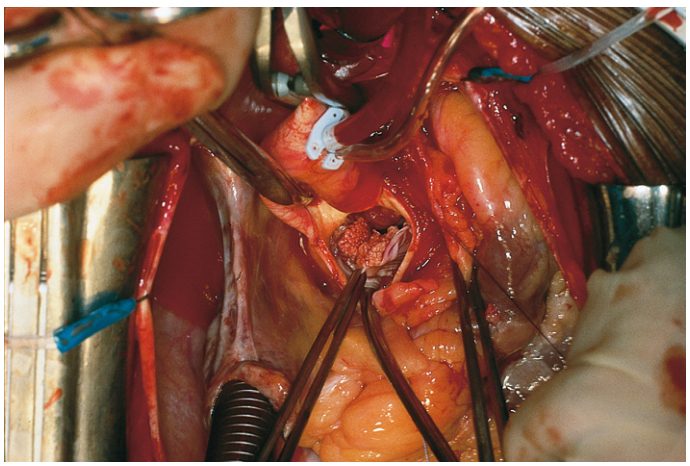


Fig. 47.10 Blood culture-negative aortic valve endocarditis caused by *Tropheryma whipplei* in a 48-year-old patient. The patient was admitted to hospital for acute abdominal pain. Abdominal surgery revealed an ischemic necrosis of the transverse colon, presumably due to arterial embolization. An echocardiogram revealed exuberant vegetations on the aortic valve. All blood cultures were negative. Emergency valve replacement was performed for cardiac insufficiency. *T. whipplei* infection was identified by histology and by using broad-spectrum polymerase chain reaction amplification of the surgical material. The patient was treated with long-term (>1 year) co-trimoxazole (see Table. 47.6). Courtesy of Dr F Bally.

rect immunofluorescence for *Chlamydia* spp. Of note, cross-reaction may occur between *Bartonella* spp. and *Chlamydia* spp. Since culture-proven IE due to *Chlamydia* spp. is notoriously rare, *Bartonella* should be suspected in the case of a positive *Chlamydia* serology. It is important because antimicrobial therapy differs (see Table 47.6). Serology is also critical in the diagnosis of *C. burnetii* IE, as an elevated IgG phase I antibody titer is pathognomonic of the disease.

Other tests

Although many laboratory findings may be abnormal, most of them are nonspecific. The erythrocyte sedimentation rate and the C-reactive protein are elevated in 90–100% of cases. Acute IE may be accompanied by high or low leukocyte counts with increased polymorphonuclear cells and immature forms, as well as other blood and chemistry abnormalities related to active infection. In subacute IE, nonspecific abnormalities accompanying chronic infection are common but not universal, including anemia with low serum iron concentration, low iron-binding capacity and thrombocytosis, leukocytosis, hypergammaglobulinemia and circulating immune complexes. Urinalysis often reveals proteinuria and microhematuria. Although these tests are not specific of IE, their return to normal values during treatment is an indirect marker of treatment efficacy.

MANAGEMENT

General principles

Successful treatment of IE relies on microbial eradication by antimicrobial drugs. Combined medical and surgical treatment is needed in more than 30% of cases, but the timing of surgery may vary along the duration of therapy (see Fig. 47.5). Both experimental and clinical studies have indicated the importance of using bactericidal drugs. For instance, 2 weeks of penicillin treatment for streptococcal IE was insufficient to prevent relapses, whereas 2 weeks of the bactericidal combination of penicillin plus an aminoglycoside successfully cured patients. Synergistic drug combinations are even more important against enterococcal IE, where combining β-lactams with aminoglycosides is mandatory to ensure treatment success.

Therapeutic schemes recommended for the most common pathogens are presented in Tables 47.8–47.11. High concentrations of antibiotics in the serum are desirable to ensure penetration into vegetations. Moreover, prolonged (4–6 weeks) treatment is mandatory to kill dormant bacteria clustered in the infected foci (see Fig. 47.3). Antibiotic choice is based on the minimal inhibitory concentration (MIC) of the drug for the pathogen. More sophisticated tests, such as inhibitory or bactericidal concentrations of the serum, have been proposed, but their utility for conducting therapy is not validated.

Drug treatment of PVE is longer (at least 6 weeks) than that of NVE (2–6 weeks), but qualitatively similar, except for staphylococcal PVE that should include rifampin (rifampicin) whenever the strain is susceptible to this agent. Treatment failure may be due to inadequate antibiotic administration, the presence of a surgically removable focus or antibiotic resistance. The three most problematic microbes with respect to antibiotic resistance are penicillin-resistant streptococci, methicillin-resistant and vancomycin-resistant staphylococci and multidrug-resistant enterococci. Culture-negative IE due to intracellular pathogens may also fail to respond to standard therapy (see Table 47.6).

Penicillin-resistant streptococci

The most common streptococci responsible for IE are oral streptococci of the viridans group, followed by enteric *S. bovis* (recently renamed *S. gallolyticus*), and more rarely group A, B, C and G streptococci, pneumococci and nutritionally variant streptococci (*Abiotrophia defectiva*, *Granulicatella adiacens* and *Gemella*). Except for *S. gallolyticus* and group A streptococci, which are still uniformly susceptible to penicillin, all streptococci may demonstrate intermediate penicillin resistance (MIC of 0.1–1 mg/l) or full penicillin resistance (MIC >1 mg/l). Treatment of IE due penicillin-susceptible and penicillin-resistant streptococci is qualitatively similar, except that short-course (2 weeks) therapy should not be used in case of resistance, and combinations of β-lactams with aminoglycosides are preferred in these cases (Table 47.8).[46] Little experience exists with highly resistant isolates (MIC >4 mg/l). Vancomycin might be preferred in such circumstances.

Methicillin-resistant and vancomycin-resistant staphylococci

Methicillin-resistant *Staphylococcus aureus* (MRSA) produces low-affinity penicillin-binding protein 2A (PBP2A), which confers cross-resistance to most β-lactams. They are usually resistant to multiple antibiotics, leaving only vancomycin to treat severe infections (Table. 47.9). Treatment for health-care-associated MRSA (HC-MRSA) and community-acquired MRSA (CA-MRSA) is similar.

However, vancomycin-intermediate *S. aureus* (VISA) (MIC 4–16 mg/l) and hetero-VISA (MIC <2 mg/l, but with subpopulations growing at higher concentrations) have emerged worldwide, and were associated with IE treatment failures.[47] Moreover, few highly vancomycin-resistant *S. aureus* (MIC >32 mg/l) carrying a vancomycin-resistance cassette acquired from enterococci were isolated from infected patients in recent years. Treating IE caused by vancomycin-resistant staphylococci requires new approaches. New lipopeptide daptomycin (6 mg/kg/day intravenously) was recently approved for *S. aureus* bacteremia and right-sided IE.[48] Compassionate studies suggest that daptomycin might also overcome methicillin-resistance and vancomycin-resistance in left-sided IE, but definitive studies are missing. Importantly, daptomycin needs to be administered in appropriated doses to avoid resistance selection.[48] Other alternatives include newer β-lactams with relatively good PBP2A affinity,[49] tigecycline, quinupristin–dalfopristin combined or not with β-lactams, β-lactams plus oxazolidinones, and β-lactams plus vancomycin. Such cases warrant consultation of infectious disease specialists.

Multidrug-resistant enterococci

Most (>80%) enterococcal IE cases are due to *Enterococcus faecalis*, followed by *Enterococcus faecium* and rarely other enterococci. *E. faecalis* and *E. faecium* are frequently resistant to gentamicin.[50] An aminoglycoside MIC >500 mg/l is synonymous with loss of bactericidal synergism with cell wall inhibitors. Streptomycin may remain active in such cases, and thus be used instead. A recently described alternative against gentamicin-resistant *E. faecalis* suggests combining ampicillin with ceftriaxone,[51] which synergize by inhibiting complementary PBPs. Otherwise, more prolonged courses of β-lactams or vancomycin must be envisioned (Table 47.10).

β-lactam resistance and vancomycin resistance are mainly observed in *E. faecium*. However, dual resistance is rare and β-lactams might be used against vancomycin-resistant strains and vice-versa. Various results were reported with quinupristin–dalfopristin, linezolid, daptomycin and tigecycline. Such situations require the expertise of an infectious disease specialist.

Gram-negative bacteria

HACEK-related species

Haemophilus, *Actinobacillus*, *Cardiobacterium*, *Eikenella* and *Kingella* species are fastidious organisms that represent less than 5% of all IE cases (Tables 47.2, 47.11). Because they grow slowly, MIC tests may be

Table 47.8 Suggested treatment for native valve endocarditis due to streptococci

Antibiotic	Dosage and route	Duration (weeks)	Level of evidence	Comments
Penicillin-susceptible (MIC <0.1 mg/l) viridans streptococci and *S. bovis* (renamed *S. gallolyticus*)*				
Standard treatment Penicillin G	6 × 2–3 million U iv q24h	4	I-A	Preferred in patients older than 65 years or with impaired renal function
Ceftriaxone†	1 × 2 g iv or im q24h *Pediatric doses:*‡ Penicillin G 200 000 U/kg q24h in four to six equally divided doses. Ceftriaxone 100 mg/kg iv or im q24h in one dose	4	I-A	
Short-term treatment Penicillin G with gentamicin§	6 × 2–3 million U iv q24h 1 × 3 mg/kg iv or im q24h in one daily dose *Pediatric doses:*‡ Penicillin and ceftriaxone as above. Gentamicin 3 mg/kg iv or im q24h in one dose or in three equally divided doses	2 2	I-B	
Ceftriaxone† with netilmicin	1 × 2 g iv or im q24h 1 × 4 mg/kg iv q24h	2 2	I-B	
In β-lactam allergic patients				
Vancomycin¶	2 × 15 mg/kg iv q24h *Pediatric doses:*‡ 40 mg/kg iv q24h in two to three equally divided doses	4	I-B	
Penicillin-resistant (MIC >1 mg/l) strains (some guidelines consider a limit at 0.5 mg/l for full resistance)				
Penicillin G with gentamicin§ Vancomycin¶	6 × 3 million U iv q24h 3 mg/kg iv or im q24h in one daily dose 2 × 15 mg/kg iv q24h *Pediatric doses:*‡ As above	4 2 4	I-B I-B	Recommended against highly resistant strains or for β-lactam allergic patients

*Notes for other streptococcal species: (i) short-term 2 weeks' therapy should not be used due to the lack of experience; (ii) IE due to *S. pneumoniae* is often accompanied by meningitis. Ceftriaxone (not penicillin) and perhaps vancomycin should be used in this case; (iii) IE due to nutritionally variant streptococci (e.g. *Abiotrophia*) should include aminoglycosides for at least 4 weeks.
†Preferred for outpatient therapy.
‡Pediatric doses should not exceed adult doses.
§Renal function and gentamicin concentrations in the serum should be monitored once a week. When given in a single daily dose, pre-dose (trough) concentrations should be <1 mg/l and post-dose (peak; 1 h after injection) concentrations should be c. 10–12 mg/l.
¶Vancomycin concentrations in the serum should achieve 10–15 mg/l at pre-dose (trough) level and 30–45 mg/l at post-dose level (peak; 1h after infusion is completed).
Adapted with modifications from Moreillon & Que,[3] and from European Society of Cardiology[24] and Infectious Diseases Society of America[59] guidelines.

difficult to interpret. Some HACEK bacilli now produce β-lactamases, thus ampicillin is no longer the first-line option. Conversely, they are susceptible to ceftriaxone, other third- and fourth-generation cephalosporins, and quinolones. β-lactam based treatments are presented in Table 47.11. Ciprofloxacin (2 × 400 mg/day intravenously or 1000 mg/day orally) is a less well-demonstrated alternative (II-C).

Non-HACEK species

The International Collaboration on Endocarditis (ICE) reported non-HACEK Gram-negative bacteria in 49/2761 (1.8%) of IE cases.[52] Most (59%) were PVE, and only 4% occurred in intravenous drug users. *Escherichia coli* (29%) and *Pseudomonas aeruginosa* (14%) were most frequently reported, followed by *Salmonella* spp., *Klebsiella* spp., *Serratia marcescens* and *Neisseria gonorrhea*. Early surgery was frequent (51%) and intrahospital mortality was 24%. The treatment paradigm of such cases is early surgery plus long-term (>6 weeks) antibiotics with bactericidal combinations of β-lactams with aminoglycosides, and sometimes with quinolones or co-trimoxazole (II-B).

Culture-negative endocarditis

Treatments of IE due to rare pathogens and outcome criteria are summarized in Table. 47.6. Treatment durations in the table are indicative, and based on selected case reports. IE due to *Brucella* spp. responds to prolonged (>3 months) treatment with doxycycline plus co-trimoxazole or rifampin combined or not with streptomycin and surgery.[32]

Table 47.9 Suggested treatment for native valve and prosthetic valve endocarditis due to staphylococci (see suggestions for right-sided IE in footnotes)

Antibiotic	Dosage and route	Duration (weeks)	Level of evidence	Comments
Native valves				
Methicillin-susceptible staphylococci				
Oxacillin or (flu)cloxacillin with gentamicin*	6 × 2 g iv q24h 3 mg/kg iv or im q24h in two to three equally divided doses *Pediatric doses*:§ Oxacillin or (flu)cloxacillin 200 mg/kg iv q24h in four to six equally divided doses. Gentamicin 3 mg/kg iv or im q24h in three equally divided doses	4–6 3–5 days	I-A	The clinical benefit of gentamicin addition has not been formally demonstrated. Its use is optional
Alternative for patients allergic to penicillins (except for immediate-type penicillin hypersensitivity)				
Cefazolin (or other first generation cephalosporins) with gentamicin*	3 × 2 g iv q24h 3 mg/kg iv or im q24h in two to three equally divided doses *Pediatric doses*:§ Cefazolin 100 mg/kg iv q24h in three equally divided doses. Gentamicin as above	4–6 3–5 days	I-B	The clinical benefit of gentamicin addition has not been formally demonstrated. Its use is optional
Vancomycin†	2 × 15 mg/kg iv q24h *Pediatric doses*:§ 40 mg/kg iv q24h in two to three equally divided doses	4–6	I-B	Recommended only for β-lactam allergic patients
Methicillin-resistant staphylococci				
Vancomycin†	2 × 15 mg/kg iv q24h *Pediatric doses*:§ As above	4–6	I-B	
Prosthetic valves				
Methicillin-susceptible staphylococci				
Oxacillin or (flu)cloxacillin, with rifampin‡ and gentamicin*	6 × 2 g iv q24h 3 × 300 mg po q24h 3 mg/kg iv or im q24h in two to three equally divided doses *Pediatric doses*:§ Oxacillin and (flu)cloxacillin as above. Rifampin 20 mg/kg iv or po q24h in three equally divided doses	≥6 ≥6 2	I-B	Rifampin increases the hepatic metabolism of warfarin and other drugs Although the clinical benefit of gentamicin has not been demonstrated, it is recommended for PVE
Vancomycin† with rifampin‡ and gentamicin*	2 × 15 mg/kg iv q24h 3 × 300 mg po q24h 3 mg/kg iv or im q24h *Pediatric doses*:§ As above	≥6 ≥6 2	I-B	Recommended only for β-lactam allergic patients Monitor renal function and serum levels of gentamicin
Methicillin-resistant staphylococci				
Vancomycin† with rifampin‡ and gentamicin*	2 × 15 mg/kg iv q24h 3 × 300 mg po q24h 3 mg/kg iv or im q24h in two to three equally divided doses *Pediatric doses*:§ As above	≥6 ≥6 2	I-B	Monitor renal function and serum levels of gentamicin

Right-sided IE: Uncomplicated staphylococcal right-sided IE (i.e. with fully susceptible organisms and no cardiac failure or peripheral complications) has been successfully treated with short-course (2 weeks) treatment of cloxacillin alone or combined with gentamicin,[60] and with 4 weeks' oral therapy combining ciprofloxacin (2 × 750 mg q24h), or preferentially a more recent quinolone with rifampin (2 × 300 mg q24h). Recently, daptomycin (6 mg/kg q24h for 4 weeks) was accepted against both methicillin-susceptible *S. aureus* and MRSA.

*Renal function and gentamicin concentrations in the serum should be monitored once a week (twice in case of renal failure). When given in three divided doses, pre-dose (trough) concentrations should be <1 mg/l and post-dose (peak; 1 h after injection) concentrations should be between 3 and 4 mg/l.

†Monitor vancomycin concentrations in the serum as indicated in Table 47.8.

‡Rifampin (rifampicin) is believed to play a special role in prosthetic device infective endocarditis (PVE), because it helps eradicate bacteria attached to foreign material. Rifampin should never be used alone, because it selects for resistance at a high frequency (*c.* 10^{-6}).

§Pediatric doses should not exceed adult doses.

Adapted with modifications from Moreillon & Que,[3] and from European Society of Cardiology[24] and Infectious Diseases Society of America[59] guidelines.

Table 47.10 Antibiotic treatment of endocarditis due to *Enterococcus* spp.

Antibiotic	Dosage and route	Duration (weeks)	Level of evidence	Comments
β-lactam and gentamicin-susceptible strains (for resistant isolates, see footnotes)				
Penicillin G with gentamicin*	6 × 3–5 million U iv q24h 3 mg/kg iv or im q24h in two to three equally divided doses *Pediatric doses*:‡ Penicillin G 200 000 U/kg iv q24h in four to six equally divided doses. Gentamicin 3 mg/kg iv or im q24h in three equally divided doses	4–6 4–6	I-A	6 weeks of therapy recommended for patients with symptoms of >3 months' duration
Ampicillin or amoxicillin with gentamicin*	6 × 2 g iv q24h 3 mg/kg iv or im q24h in two to three equally divided doses *Pediatric doses*:‡ Ampicillin or amoxicillin 300 mg/kg iv q24h in four to six equally divided doses. Gentamicin as above	4–6 4–6	I-A	
Vancomycin† with gentamicin*	2 × 15 mg/kg iv q24h 3 mg/kg iv or im q24h in two to three equally divided doses *Pediatric doses*:‡ 40 mg/kg iv q24h in two to three equally divided doses. Gentamicin as above	6 6	I-A	

In cases of high-level resistance to gentamicin (MIC >500 mg/l): if susceptible to streptomycin, replace gentamicin with streptomycin 15 mg/kg q24h in two equally divided doses (I-A); otherwise, use a more prolonged course of β-lactam therapy. Combining ampicillin with ceftriaxone was recently suggested against gentamicin-resistant *E. faecalis*[51] (II-A, II-B).
In cases of β-lactam resistance: (i) if due to β-lactamase production, replace ampicillin with ampicillin–sulbactam or amoxicillin with amoxicillin–clavulanate (I-C); (ii) if due to PBP5 alteration, use vancomycin-based regimens.
In cases of multidrug resistance to aminoglycosides, β-lactams and vancomycin: some suggested alternatives are: (i) linezolid 2 × 600 mg iv or po q24h foo 8 weeks (II-A, II-C) (control hematologic toxicity); (ii) quinupristin–dalfopristin 3 × 7.5 mg/kg q24h for ≥8 weeks (II-A, II-C); (iii) β-lactam combinations including imipenem plus ampicillin or ceftriaxone plus ampicillin for ≥8 weeks (II-B, II-C).
*Monitor renal function and gentamicin concentrations in the serum as indicated in Table 47.9.
†Monitor vancomycin concentrations in the serum as indicated in Table 47.8.
‡Pediatric doses should not exceed adult doses.
Adapted with modifications from Moreillon & Que,[3] and from European Society of Cardiology[24] and Infectious Diseases Society of America[59] guidelines.

Table 47.11 Antibiotic treatment of endocarditis due to fastidious Gram-negative bacteria of the HACEK group*

Antibiotic	Dosage and route	Duration (weeks)	Level of evidence	Comments
Ceftriaxone†	1 × 2 g iv or im q24h	4	I-B	
Ampicillin‡ with gentamicin§	6 × 2 g iv q24h 3 × 1 mg/kg iv or im q24h *Pediatric doses*:¶ Ceftriaxone 100 mg/kg iv or im q24h in one dose. Ampicillin 300 mg/kg iv q24h in four to six equally divided doses. Gentamicin 3 mg/kg iv or im q24h in one dose or in three equally divided doses	4 4	II-A, II-B	Studies suggest that gentamicin once a day might be adequate

*Includes *Haemophilus* spp., *Actinobacillus actinomycetemcomitans*, *Cardiobacterium hominis*, *Eikenella corrodens* and *Kingella kingae*.
†Preferred for outpatient treatment.
‡Ampicillin should not be used if microbes produce β-lactamases.
§Monitor renal function and gentamicin concentrations in the serum as indicated in Table 47.9.
¶Pediatric doses should not exceed adult doses.
Adapted with modifications from Moreillon & Que,[3] and from European Society of Cardiology[24] and Infectious Diseases Society of America[59] guidelines.

IE due to *C. burnetii* is often treated with a combination of doxycycline plus a fluoroquinolone given for up to 3 years, but recurrences are common. Recently, doxycycline combined with hydroxychloroquine appeared more effective. Hydroxychloroquine increases the phagolysosome pH (from 4.7 to 5.8) and improves tetracycline-induced bacterial killing.

IE due to *Bartonella* spp. should be suspected in homeless at-risk patients. Treatment includes primarily β-lactams (amoxicillin and ceftriaxone) combined with aminoglycosides (netilmicin or gentamicin) for at least 2 weeks, or β-lactams combined with other drugs (e.g. doxycycline) for a total of over 6 weeks. Up to 90% of patients may undergo surgical valve replacement.

Treatment of IE due to *Chlamydia* spp., *Mycoplasma* spp. and *Legionella* spp. is unknown. Since these organisms are highly susceptible to newer fluoroquinolones *in vitro*, these drugs should be part of the therapeutic regimen.

IE due to *T. whipplei* is very rare. A review of 35 cases proposes sequential administration of penicillin or ceftriaxone plus gentamicin for 2–6 weeks, followed by long-term (>1 year) co-trimoxazole.[53] Surgical valve replacement may be required for successful therapy (see Table 47.6).

Outpatient parenteral antibiotic therapy for infective endocarditis

Outpatient parenteral antibiotic therapy (OPAT) for IE should be envisioned to consolidate antimicrobial therapy once infection-related complications are under control. These occur during the first 2 weeks of treatment (critical phase; see Fig. 47.5) and include persistent fever or positive blood cultures, perivalvular abscesses, acute heart failure necessitating emergency surgery, septic emboli and stroke.[54] The critical phase is followed by a continuation phase, during which noninfection complications may occur (see Fig. 47.5). These are not related to antibiotic failure and may occur independently of the hospital stay. OPAT may be envisioned at this stage provided that feasibility and logistics are ensured. Critical issues are medical and social staff support, enforcing drug administration and monitoring drug efficacy. In case of problems, the patient should have direct access to the medical staff in charge. In such appropriate conditions OPAT performs equally well independently of the pathogen and NVE or PVE.[54]

Surgery

The leading indications for surgery are:

- refractory cardiac failure caused by valvular insufficiency;
- persistent sepsis caused by a surgically removable focus or a valvular ring or myocardial abscess; and
- persistent life-threatening embolization.

The decision when to operate on an unstable patient requires a multidisciplinary approach.

Endarteritis and Mycotic Aneurysms

Endarteritis is the inflammation of the arterial wall. The term mycotic aneurysm was coined by Osler to define a nonsyphilitic aneurysm resulting from infective endarteritis. Arterial infection may result from:

- microembolization of bacteria in the vasa vasorum;
- hematogenous seeding of the arterial intima or of thrombi lining atherosclerotic plaques during bacteremia;
- extension from contiguous infected foci; or
- arterial trauma with direct bacterial contamination.

The first two mechanisms are most important during IE. Other risk factors include pre-existing endarterial lesions (e.g. congenital malformations such as patent ductus arteriosus and aortic coarctation, atherosclerosis of large vessels, vascular prosthesis and sometimes depressed host immunity due to diabetes, cirrhosis or corticosteroid therapy). Infected aneurysms represent 2.6% of all aneurysms, with a male:female ratio of about 3:1.[55]

MICROBIAL PATHOGENS AND CLINICAL MANIFESTATIONS

In the context of IE, the pathogens of endarteritis follow the pattern shown in Table 47.2. In the absence of IE, on the other hand, Gram-negative bacteria are found more frequently. *Salmonella* spp., *E. coli* and other enterobacteria are isolated in more than 50% of cases of abdominal aortitis.[55] *Salmonella choleraesuis* and *Salmonella typhimurium* seem to have a particular tropism for atherosclerotic arteries. It was proposed that all patients over 50 years of age who develop *Salmonella* bacteremia should undergo abdominal imaging to detect possible aortic lesions. *S. aureus* is the organism most often associated with extra-abdominal lesions and infections of vascular prostheses, and it has been reported in about 30% of endarteritis overall. Other pathogens, including fungi, are occasionally reported.

Signs and symptoms depend on the anatomic site of the disease. Local signs include pain, erythema and manifestations due to compression of contiguous organs and distal ischemia or embolization. Fever and leukocytosis are almost always present.

DIAGNOSIS AND MANAGEMENT

Diagnosis requires a high degree of clinical suspicion. Microbiologic documentation is essential. Blood cultures and cultures of surgical specimens should be performed, if possible before starting antibiotic treatment. All laboratory techniques described for IE etiology diagnosis apply to endarteritis. Ultrasonography, CT, MRI and gallium- or indium-labeled leukocyte scans and especially fluorodeoxyglucose–positron emission tomography (FDG-PET) scanning (see Practice Point 21) are helpful to delineate the lesions. Although none of these techniques will distinguish between sterile and infected fluids, they may guide more invasive diagnostic procedures such as percutaneous needle aspiration of the affected area.

Appropriate antibiotic therapy and surgical resection of the infected vessel with extensive local debridement is mandatory. The material should be carefully examined both by the pathologist and by the microbiologist. Whenever possible, autologous vascular graft and bypass in uninfected tissue should be performed in order to decrease the risk of re-infection. Implantation of rifampin-bond prostheses was also shown to decrease re-infection.[56] Antibiotics should be given for at least 4–6 weeks. Certain situations are so complicated that therapeutic strategies must be adapted for each particular case. Sometimes patients are kept on antibiotics for much longer periods of time, even for life. Despite antibiotic treatment, the morbidity of vascular infections remains high. Infections of lower extremity arteries result in amputation in as many as 20% of cases. Moreover, the mortality rate of patients who have infected aneurysms that are diagnosed late may exceed 70%.

REFERENCES


References for this chapter can be found online at http://www.expertconsult.com

Chapter 48

Cameron Wolfe
Kumar Visvanathan

Rheumatic fever

Acute rheumatic fever (ARF) is an autoimmune condition arising from group A *Streptococcus* infection, characterized by nonsuppurative, inflammatory lesions of heart valves, brain, joints and skin. Less commonly, cardiac muscle, pericardium or lungs are involved. In its classically described form, ARF presents as a febrile and essentially self-limited illness. However, inflammation of the heart valves has the potential for chronic progressive damage, known as rheumatic heart disease (RHD). Globally RHD remains the most common cause of acquired heart disease and cardiac morbidity in childhood.

EPIDEMIOLOGY AND HISTORY

Original descriptions of the disease date to Thomas Sydenham (1624–1689), but it was not until 1812 that William Charles Wells made the association of rheumatism and carditis. Associations with sore throat became evident in the 1880s and by the turn of the 19th century histopathologic descriptions were available through the work of Ludwig Aschoff.

Rheumatic fever is a disease of poverty and social injustice. It is borne of, and indicative of, community disadvantage. During the 1900s it became progressively uncommon in industrialized countries, and was consigned to the realms of the medically esoteric. Conversely, it became increasingly recognized in resource-poor countries.

In the West, the incidence of ARF and rheumatic heart disease began to decline before the availability of antibiotics, and dropped in earnest through the early 1960s and 1970s. Human factors such as improvements in living, sanitation and medical care, as well as reduction in household crowding, are proposed to have made the greatest impact in reducing the incidence of group A streptococcal infections. Current reliable data on the incidence of ARF are rare; however, estimates of the developed world suggest an annual incidence of 1 per 100 000; this compares to 100 and 150 per 100 000 in the Sudan and China, respectively.[1] The highest reported incidence was 508 per 100 000 children aged 5–14 years, between 1987 and 1996, in the Aboriginal population of northern Australia[2] – extraordinary for a country so developed in other respects. Recent estimates put the global burden at a minimum of 18.1 million people affected, with clear regional differences as seen in Figure 48.1.[3] Yearly mortality has been put at approximately 376 000 deaths. It is not clear if the increase in developing countries represents a higher disease burden or better diagnostic efforts.

In the indigenous Australian population residing in northern Australian communities, rheumatic heart disease affects over 2% of the community, leading to hospitalization rates eight times as great in this group as in the nonindigenous.[4] Acute rheumatic fever is common in other Pacific Island nations with very high rates. Much of sub-Saharan Africa, the Indian subcontinent, poorer sections of Latin America and large parts of Asia also have prevalence rates of rheumatic heart disease of greater than 2 per 1000.

Whilst the incidence has fallen in most areas of the developed world, there have been sporadic outbreaks in the United States, for example in Salt Lake City, Utah.[5] It would seem that emergence of more virulent group A streptococci are to blame in these instances.

First episodes of ARF occur most commonly in children between the ages of 5 and 15 years. It is most uncommon to present in the first 2 years of life. (Whether this is because of a need for multiple 'primary' infections or simply reflective of a more immature immune response is not known.) Conversely, first initial episodes become rare beyond the age of 35. Incidence is equal in both males and females, although females appear more likely to suffer Sydenham's chorea and mitral stenosis. The attack rate of rheumatic fever after untreated streptococcal exudative tonsillitis in military recruitment camps has been carefully studied; it is consistently around 3%.

PATHOGENESIS

Acute rheumatic fever requires infection of a susceptible host with a 'rheumatogenic' group A streptococcus. An abnormally vigorous, tissue-specific immune response then ensues. Several theories exist as to the cause of the pathogenicity:

- that streptococcal products (e.g. streptolysins) are directly toxic;
- that a serum sickness develops; or
- that molecular mimicry occurs.

Whilst not proven beyond doubt, mimicry appears likely given the presence of epitopes in human tissue that are immunologically similar to group A streptococcal antigens. This leads to both humoral and cellular responses. Cross-reacting antibodies bind epitopes in human myosin, sarcolemmal membrane proteins, keratin, actin and proteins in synovium and articular cartilage. For example, similarities exist between the α-helical coiled region of the M protein and the rod region of the cardiac myosin.[6] In patients who suffer from Sydenham's chorea, antibodies cross-reacting with neurons of the caudate and subthalamic nuclei, as well as the streptococci, have been found.[7]

Cell-mediated immune responses are also elevated early in ARF, suggesting early T-cell activation may be critical in pathogenesis, with humoral responses simply secondary to antigens released from already damaged tissues. CD4 and CD8 lymphocytes in rheumatic fever patients are found in increased numbers in the blood, as well as in human heart valve tissue. The M protein contains both T- and B-cell epitopes. The M protein (as well as streptococcal pyogenic exotoxins) can also act as a superantigen to further augment the immune response.

Recent interest has also focused on the innate immune system. Toll-like receptors (TLRs) form a component of microbial recognition and innate response. As an indicator of host factors being capable of affecting the pathogenesis of ARF, polymorphisms in TLR-2 (in particular Arg 753 Gln) have been shown to have a strong association

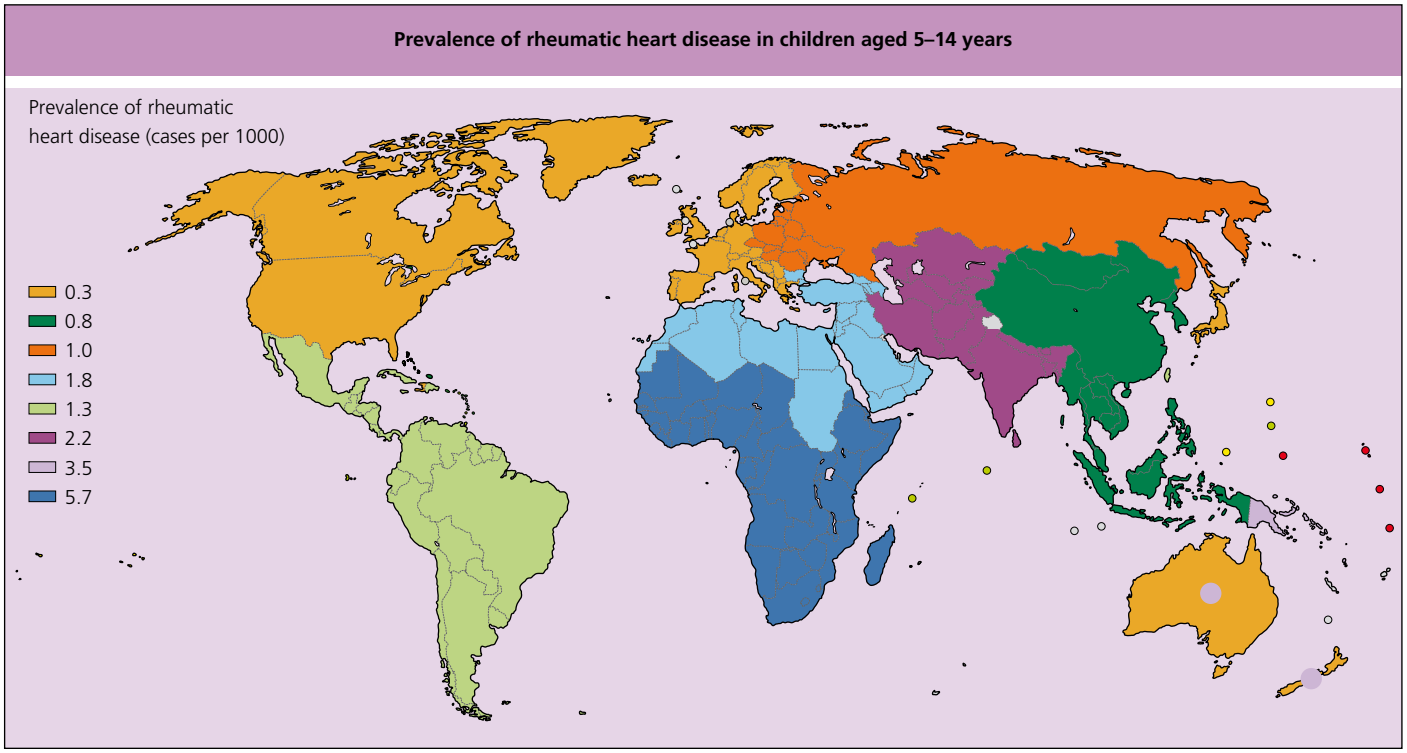


Fig. 48.1 Prevalence of rheumatic heart disease in children aged 5–14 years. The circles within Australia and New Zealand represent indigenous populations. Courtesy of JR Carapetis

with risk of ARF.[8,9] There has also been much interest in detecting an HLA association, although this has been difficult to prove and studies have given conflicting and varied reports depending on the population studied. The HLA-DR7 allele is frequently associated with the disease, especially with chronic multivalvular lesions.[10] HLA-DRB1*07 has been strongly associated in populations from Pakistan whilst HLA-DRB1*0701 and DQA1*0201 are associated in Egyptian groups. In addition, different associations have been found in Turkish, Taiwanese and Brazilian populations.[11,12]

There are variations in the organism that also affect the probability of rheumatic fever development. Variations in the M protein serotype affect 'rheumatogenicity'. Table 48.1 outlines the serotypes associated with nonsuppurative sequelae of group A streptococci. The serotypes can be divided into class I or class II M proteins. Outbreaks of ARF have almost exclusively occurred with class I M proteins. Streptococci from this class typically express more M protein and possess a larger capsule. They also lack the production of serum opacity factor. The effect of the M protein in this regard was confirmed when comparisons of the two outbreaks in Salt Lake City revealed similar M protein structure and capsular size in >80% of isolates, even though they were 12 years apart. They both possessed the *emm18* gene which encodes for M subtype 18. The waning of the outbreak corresponded to the absence of the *emm*18 strain. There have also been interesting changes in the serotypes in circulation. At least in the USA there appears to have been a virtual disappearance of types 14, 18, 19 and 29 when comparing isolates from the 1960s to those found in the early part of this decade.[13] Whether this is the cause of, or is unrelated to the decline in incidence in the USA has not been elucidated.

Previously it was assumed that pharyngeal infection with group A streptococci was required in order to precipitate ARF. This was challenged recently by an Australian group looking at strains causing pyoderma where ARF was postulated in the absence of recent pharyngitis. This theory has yet to be proved.[14]

Patients who have suffered an initial attack of rheumatic fever continue to have a significant predilection for recurrence of ARF following subsequent episodes. This risk appears greatest in the first 2 years after primary presentation and certainly wanes considerably after 5 years.

Table 48.1 M protein serotypes

Condition	Serotypes
Acute rheumatic fever	1, 3, 5, 6, 14, 18, 19, 24, 27, 29
Acute glomerulonephritis – pharyngitis associated	1, 4, 12, 25
Acute glomerulonephritis – pyoderma associated	2, 49, 55, 59–61

CLINICAL FEATURES

The onset of ARF always follows a latent period after a streptococcal sore throat. The average duration of this is 19 days, although it can last between 1 and 5 weeks. There is no difference in latency between first and recurrent episodes. Acute illness may present with a variety of signs and symptoms. The most important of these are described by the 'major manifestations' contained within the modified Jones criteria currently used for diagnosis. They include carditis, polyarthritis, chorea, subcutaneous nodules and erythema marginatum. A number of other additional features have been associated with ARF but are less specific, and are hence termed 'minor manifestations'.

Most manifestations of ARF are accompanied by fever (chorea may occur in isolation). Temperatures rarely exceed 102.2°F (39°C) and are very responsive to salicylate therapy.

Arthritis is the most common feature, occurring in 75% of first attacks, and is usually one of the first symptoms. Carditis occurs in a little under half of all cases, whereas chorea (15%), nodules and erythema marginatum (10%) are less common. The most common presentation is fever and polyarthralgia, with or without the murmur of mitral regurgitation.

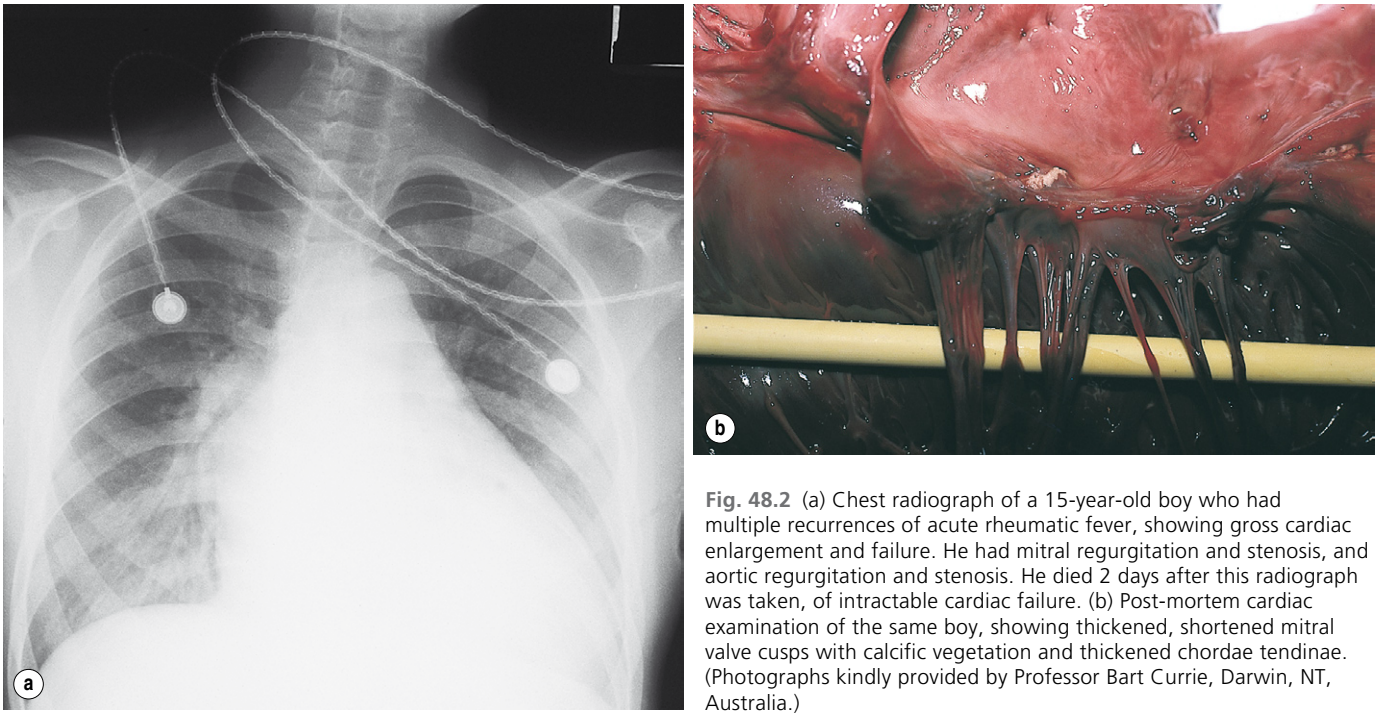


Fig. 48.2 (a) Chest radiograph of a 15-year-old boy who had multiple recurrences of acute rheumatic fever, showing gross cardiac enlargement and failure. He had mitral regurgitation and stenosis, and aortic regurgitation and stenosis. He died 2 days after this radiograph was taken, of intractable cardiac failure. (b) Post-mortem cardiac examination of the same boy, showing thickened, shortened mitral valve cusps with calcific vegetation and thickened chordae tendinae. (Photographs kindly provided by Professor Bart Currie, Darwin, NT, Australia.)

Carditis is more common in younger patients. Acute rheumatic fever can affect the pericardium (usually asymptomatic, present only with a subtle pericardial rub or effusion), the myocardium (hard to detect without the assistance of echocardiography, and rarely a cause of cardiac failure) or the endocardium (the most common and important). Initially valves become inflamed, friable and often have regurgitation. With time, and especially with repeated infections, the valves stiffen, become thickened and shortened, and eventually stenotic. Occasionally the valvular chordae can also shorten. The mitral valve is by far the most commonly affected. The aortic valve is also commonly affected, whereas disease of the pulmonary or tricuspid valve is rare (Fig. 48.2).

Clinically, a pansystolic murmur, consistent with mitral regurgitation, is the most common sign, sometimes associated with the low-pitched mid-diastolic (Carey Coombs) murmur. This is heard over the left ventricular impulse and is associated with acute valvulitis. It may be a feature of primary heart block, leading to early atrial systole and therefore increased flow at the time of the rapid filing phase.

Cardiac failure is possible in the acute phase of rheumatic fever. Rarely, it can even be life-threatening. Using echocardiography it is evident that the congestive heart failure is due to acute valvular dilatation, rather than myocarditis. Accordingly, troponin levels are usually not elevated. First-degree or greater degrees of heart block are also possible, although not specific enough to be diagnostic alone (Fig. 48.3).

Histopathology classically reveals 'Aschoff bodies' within the myocardium; these are perivascular foci of inflammation, consisting of areas of central necrosis surrounded by a rosette of large mononuclear and multinuclear giant cells (macrophage/histiocyte in origin).[15]

Examination reveals erythematous, painful, hot and swollen joints, which may contain sterile purulent effusions. The arthritis tends to be migratory, asymmetric, polyarticular and affects large joints. Each joint is most severely affected from a few days to a week, and returns to normal within 2–3 days. There are atypical cases of only monoarticular involvement, or persistent and additive disease.[16] The hallmark of rheumatic fever arthropathy is that it is exquisitely responsive to non-steroidal anti-inflammatory medication.

Perhaps the most intriguing manifestation of ARF was named by the English physician Thomas Sydenham. He described a classic chorea in 1686, also known as St Vitus' dance. It occurs in between 1% and 30% of cases of rheumatic fever, and may follow a latent period of up to 6 months after the primary infection. It commonly occurs in isolation from other symptoms, and is far more common in females. Choreiform movements most frequently involve the face, arms and hands. It is generalized in almost 80% of patients and is unilateral in the remainder. The patient's tongue may dart erratically when protruded. Classic patterns of the chorea also include the 'mild-maid's grip' (the rhythmic squeezing when grasping the examiner's fingers), the 'spooning' position (a flexion of the wrists and extension of the fingers when the hands are extended) and the 'pronator sign' (when the arms and palms are outturned when held above the head). All movements worsen upon purposeful action or with anxiety and disappear during sleep. The chorea typically lasts for 1–2 months, and is almost always gone within 6.

Recent work looking at the EEG patterns of patients with Sydenham's chorea shows a parieto-occipital localization in almost 90% with lateralization in a little over 50%. Almost a third had abnormalities seen even after clinical symptoms had resolved. MRI scanning rarely if ever reveals pathology; positron emission tomography (PET) imaging can reveal abnormalities, especially in the basal ganglia, but is rarely used in clinical settings.[17,18]

Erythema marginatum and subcutaneous nodules are both rare manifestations of acute rheumatic fever (2%). The rash is pink and serpiginous, beginning as a macule which then clears centrally (Fig. 48.4). Multiple lesions affect the trunk and limbs, but rarely the face, and can move in front of the examiner, appearing more vasomotor than truly cutaneous. Although they can be present for weeks to months, this does not correlate with ongoing rheumatic inflammation. Nodules in ARF usually appear some weeks after the onset of fever and arthralgia. They are most common over bony surfaces and tendons, for example at the elbows, knees or Achilles tendon, even the spinous processes. Typically they are painless. Their clinical relevance is tied to their strong association with severe carditis.

Less common clinical symptoms seen in acute rheumatic fever include 'rheumatic pneumonia', referring to pulmonary infiltrates seen in some patients with acute carditis. Mild elevations in liver transaminases, microscopic hematuria, pyuria and proteinuria are occasionally found. Blood C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR) are usually impressively elevated in the acute phase of disease.

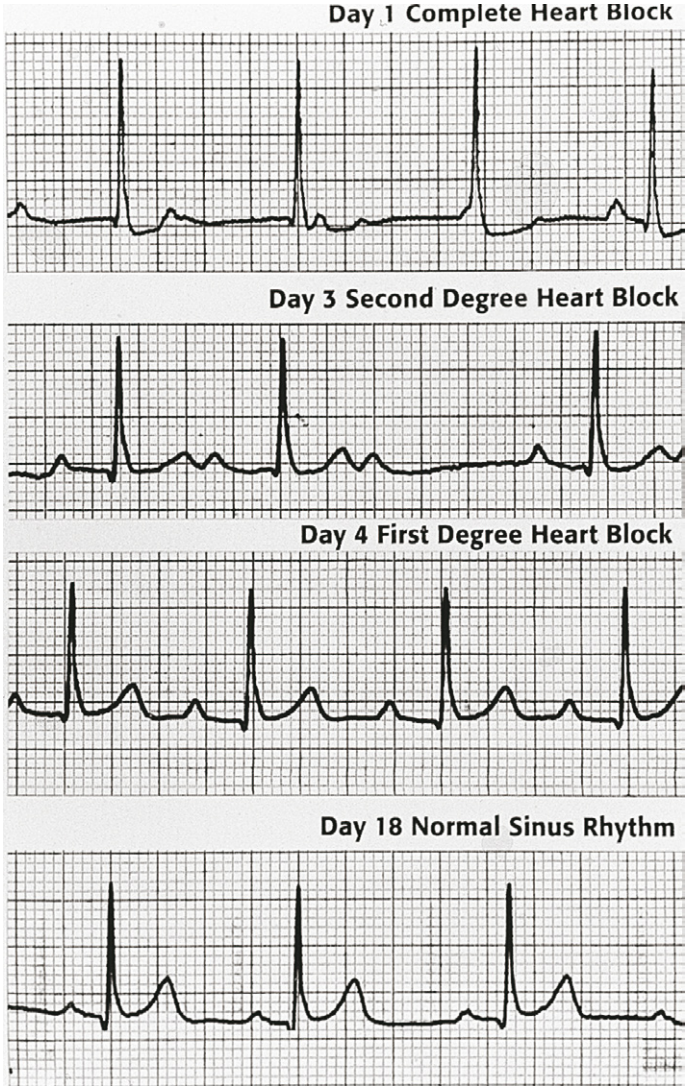


Fig. 48.3 Electrocardiographic changes in a young adult with acute rheumatic fever, showing evolution over 18 days from complete heart block to second-degree (Wenckebach) block to first-degree block and then to normal sinus rhythm. (Reproduced with permission from Bishop W, Currie B, Carapetis J, Kilburn C. A subtle presentation of acute rheumatic fever in remote northern Australia. *Aust N Z J Med* 1996;26:241–2.)

There is a separate clinical entity, known as poststreptococcal reactive arthritis. It has a shorter incubation period after bacterial infection, can follow non-group A streptococci and does not have the same dramatic responsiveness to salicylates. It carries no long-term risk of carditis, and therefore needs no long-term secondary prophylaxis. That said, only a very careful clinician would make this diagnosis in an area with highly endemic rheumatic fever. Hence some authorities recommend treating this for a year, and discontinuing if no evidence of carditis is found.

A group of disorders known as pediatric autoimmune neuropsychiatric disorders associated with streptococci (PANDAS) warrant attention; they include some of the tic disorders, some patients with Tourette's syndrome and obsessive–compulsive symptoms. They all have a temporal association with group A streptococcal infections. Some studies have associated antineuronal antibodies with the development of these conditions, but this remains inconclusive. The majority seem to have concurrent cardiac complaints. Currently there are insufficient data to support secondary prophylaxis in this group of patients.[19] In populations where acute rheumatic fever remains highly prevalent, it may be very difficult to differentiate PANDAS, hence some regional boards recommend rarely, if ever, diagnosing the syndrome.[4]



Fig. 48.4 Erythema marginatum on the trunk of an 8-year-old Caucasian boy. The pen mark shows the location of the rash approximately 60 minutes previously. (Photograph kindly provided by Professor Mike South, Royal Children's Hospital, Adelaide, Australia.)

DIAGNOSIS

There remains no definitive diagnostic test for ARF. In 1944, Jones developed the first set of diagnostic criteria, which were later revised in 1992. The 'updated' Jones criteria (Table 48.2) apply to the initial diagnosis of rheumatic fever. Their careful application is critical to arrive at the correct diagnosis, given the ongoing implications for secondary prophylaxis and cardiac follow-up. At least two major manifestations, or one major and two minor manifestations are required, in addition to evidence of recent streptococcal infection. Exceptions to the criteria exist, allowing a 'presumptive diagnosis': rheumatic chorea may be diagnosed in the absence of other manifestations or preceding streptococcal infection. Low-grade carditis may also occur as a delayed presentation, after streptococcal titers have fallen.

The modified Jones criteria are not beyond reproach. When one considers that merely fever and arthralgia, along with an elevated CRP would be diagnostic, the necessity to include recent streptococcal disease becomes evident. Because the presentation can be varied between individuals, careful exclusion of differential diagnoses is important. A number of differentials are listed in Table 48.3.

Table 48.2 Guidelines for the diagnosis of initial attack of rheumatic fever*

Major manifestations	Minor manifestations	Supporting evidence of group A streptococcal throat infection
Carditis Polyarthritis Chorea Erythema marginatum Subcutaneous nodules	Clinical findings: • Arthralgia, fever Laboratory findings: • Elevated acute phase reactants (CRP, ESR) Prolonged PR interval	Positive throat culture Rapid streptococcal Ag test Elevated or rising Ab titer: • antistreptolysin, anti-DNaseB, antihyaluronidase

CRP, C-reactive protein; ESR, erythrocyte sedimentation rate.
*Jones criteria, updated 1992.[20]

Table 48.3 Differential diagnoses of the three most common major manifestations of acute rheumatic fever

	Polyarthritis	Carditis	Chorea
Differential diagnoses	Connective tissue disease Immune complex disease Septic arthritis (including gonococcal) Viral arthropathy Henoch–Schönlein purpura Reactive arthropathy Sickle cell anemia Infective endocarditis Leukemia or lymphoma	Innocent murmur Mitral valve prolapse Congenital heart disease Infective endocarditis Hypertrophic cardiomyopathy Myocarditis (viral or idiopathic) Pericarditis (viral or idiopathic)	Systemic lupus erythematosus Drug reaction Wilson's disease Tic disorder Choreo-athetoid cerebral palsy Encephalitis Huntington's chorea Intracranial malignancy Chorea gravidarum

What constitutes evidence of recent streptococcal infection is important to define. It may be demonstrated by a positive throat swab and culture, rapid antigen test or positive antistreptococcal serology. Within 2 months of onset, approximately 80% of patients with ARF will have an ASO titer greater than 200 Todd units/ml. If one of the other serologic tests (anti-DNase B, antihyaluronidase) is also performed, the sensitivity exceeds 90%. Serology, in the absence of culture, is clearly limited in areas with high prevalence of streptococcal impetigo, as children will have continually positive serology. Testing for class I antibodies as an indicator of rheumatic fever has poor specificity and is not presently recommended given the high likelihood of having class I antibodies in any endemic population.[21]

The B cell antigen D8/17 is expressed in a high percentage of those affected by ARF, a moderate amount in close family members, and yet sparsely in the general community. It appears to be inherited in a non-HLA-associated and autosomally recessive manner.[22,23] Although its availability is not extensive, this test has potential for aiding diagnosis; however, laboratories with flow cytometry facilities are required to undertake testing.[24,25]

The increasing specificity of the Jones criteria is appropriate for Western communities where the incidence is low and overdiagnosing rheumatic fever leads to unnecessary prescribing of long-term antibiotics. However, in developing countries where the incidence remains high, a greater sensitivity is desirable, given the profound morbidity of undertreated cardiac disease.

Echocardiographic recognition of clinically silent carditis is not included in the modified Jones criteria. However, it is increasingly recognized that these lesions may progress and warrant attention. The World Health Organization therefore incorporated echocardiographically diagnosed, clinically silent rheumatic valve involvement into recent guidelines, stating they should be treated as rheumatic heart disease until proven otherwise. To support this, recent studies in the developing world screening schoolchildren with echocardiography detected 2.2–3% prevalence of rheumatic heart disease. Only 2.2 per 1000 cases were detected in corresponding groups based on clinical grounds.[26] The use of echocardiography to screen was also supported by Brazilian work showing that, despite 34% of carditis patients normalizing their clinical examination after acute phase resolution, echocardiography showed progression to chronic subclinical valvular disease in over 80%. The financial costs in providing accurate echocardiography to the Third World, as well as suitable training of technicians, remain sizable stumbling blocks.

PREVENTION

Primary prophylaxis (antibiotic therapy of group A streptococcal upper respiratory tract infection) has been the cornerstone of primary prevention since the widespread availability of antibiotics. As treatment initiated within 8 days of the onset of symptoms, oral or intramuscular penicillin is effective at preventing subsequent development of rheumatic fever (for suggested dosing of antibiotics, see Table 48.4). Whilst courses of macrolides, cephalosporins and even later generation quinolones are effective against group A steptococci, they have not been studied in the context of prevention. Their added expense and broader antimicrobial spectrum argue against their use as first-line agents. Recent New Zealand data suggest daily amoxicillin is noninferior to twice-daily penicillin V for the treatment and eradication of streptococci.[27]

Table 48.4 Recommended doses* of antibiotics to prevent rheumatic fever

Indication and antibiotic	Route	Dose in children (<27 kg)	Dose in adult	Frequency	Duration
Options for treatment of group A streptococcal infection ('primary prophylaxis')					
Penicillin V (phenoxymethyl penicillin)	po	250 mg	500 mg	q12h	10 days
Benzathine penicillin G	im	450 mg (600 000 U)	900 mg (1.2 million U)	–	Single dose
Erythromycin (allergy to pen)	po	20 mg/kg (max 250 mg)	250 mg	q12h	10 days
Options for prevention of rheumatic fever recurrences ('secondary prophylaxis')					
Benzathine penicillin G	im	900 mg (1.2 million U)	900 mg (1.2 million U)	Every 3–4 weeks	Depending on severity of carditis
Penicillin V (phenoxymethyl penicillin)	po	250 mg	250 mg	q12h	
Erythromycin (allergy to pen)	po	250 mg	250 mg	q12h	

*Doses as recommended by the American Heart Association.[28]

In regions where acute rheumatic fever remains prevalent, primary antibiotic prophylaxis is important. In settings where the disease is now rare, however, the benefits of antibiotics in the treatment of group A streptococcal pharyngitis (prevention of rheumatic fever, suppurative sequelae and attenuating symptoms) do not outweigh cost, adverse drug reactions and potential for induction of resistance in other organisms.[29] Protecting sore throat sufferers against suppurative and nonsuppurative complications in modern Western society can be achieved only by treating with antibiotics many who will derive no benefit. In emerging economies where rates of, for example, acute rheumatic fever are high, the number needed to treat may be much lower.

Unfortunately there are few data showing conclusive reduction in the incidence of ARF in developing countries. Additionally, not all cases of ARF are preceded by an obviously sore throat, making prevention difficult. Diagnosing and treating acute pharyngitis remains imperative, although countries also require aggressive measures to improve housing, control skin disease, advance education of health staff and the wider community and attempt to close social and health disparities.[30,31]

There have been no controlled studies showing tonsillectomy to be effective. It is not currently recommended for primary prevention of ARF.[1]

Use of vaccines in primary prevention

A number of different M-protein based subunit vaccines are in varying stages of development. The M protein contains both a hypervariable region and a conserved *C* terminal region. The hypervariable *N* region has been shown to be highly immunogenic and can induce strain-specific immunity. A 26-valent vaccine candidate (recombinant protein vaccine) that has been successful in both phase I and phase II human clinical trials has been shown to induce antibodies capable of opsonization of not only the specific M types represented in the vaccine, but also subtype variants. There can be very small differences in amino acid sequences of subtype variants, indicating potential for broad coverage even in highly immunized populations where variant strains may develop under selective pressure.[32]

The *C* terminal has also been incorporated into a number of vaccine studies, showing reduced colonization of pharyngeal tissues in mice. Given this is the primary route of infection with group A streptococci in humans, it raises hope for ARF prevention. Conjugating *N* terminal proteins to either tetanus or diphtheria toxoid has also been an avenue of investigation successful in mouse models. Alternatively, a number of different peptides derived from the *N* terminus have been combined into a larger 'heteropolymer' for subcutaneous injection, showing protective effects in proof-of-concept animal models. Lipopeptide molecules and even protein vesicles ('proteosomes') have also been explored.[33] A novel approach taking shape in vaccine research includes using a nonpathogenic *Lactococcus lactis* strain that expresses a protein derived from the conserved region of the M protein as an intranasal vector for immune stimulation.[34]

Unfortunately, widespread rollout of a tested, cost-effective and efficacious human vaccine remains some years away. An excellent review of current development is included.[35]

Secondary prophylaxis

After an initial attack of ARF, patients are at higher risk of recurrence after subsequent episodes of streptococcal upper respiratory infections. This risk is highest in the first few years after the initial attack and fades thereafter. With each relapse, the likelihood of more significant rheumatic heart disease increases. Secondary prophylaxis (see Table 48.4) therefore becomes crucial to reduce long-term morbidity and mortality. Best preventive responses have been achieved with 3-weekly benzathine penicillin G injections, although 4-weekly injections are often given to improve compliance and decrease cost.[36,37] Daily oral penicillin is an alternative, although compliance is problematic; the same is true for daily oral sulfadiazine. Penicillin-allergic patients should be offered erythromycin.

The duration of secondary prophylaxis is determined by risk of recurrent disease. Certainly in the first 5 years it appears significant. It is also judged on the degree of pre-existing heart disease and the likelihood of ongoing streptococcal exposure. In New Zealand experience, data collected in the 1990s suggest antibiotic cessation may be considered at 21 years of age, or at least 10 years after the last attack.[38] For those with more severe carditis (based on at least moderate clinical or echocardiographic valvular dysfunction) some groups would consider cessation of prophylaxis only after age 30 years and more than 10 years after the last clinical relapse. Others would argue that continued prophylaxis for life is warranted.[28]

On a regional and national level, the establishment of formal control programs is highly desirable.[1,4,30] Such a program requires national commitment (including budgetary), a centralized registry of patients and ideally a multidisciplinary steering committee for appropriate oversight. Priorities include education and advocacy, case finding through surveillance, coordinating secondary prophylaxis and follow-up. The more complex primary preventive strategies that address health and social inequalities must follow.

MANAGEMENT

The main initial aims of management in ARF are to confirm the diagnosis, treat any cardiac failure and quiet inflammation. Ensuring ongoing secondary prophylaxis and clinical follow-up are subsequently important. While there is no evidence that acute treatment reduces the likelihood or severity of long-term valvular damage, there is considerable potential symptomatic benefit for the patient. Hospital admission is still recommended so as to ensure relevant investigations are performed in a timely manner, medical treatment is conducted, and education for patient and family takes place.

Initial investigations should include attempts at proving recent streptococcal infection through throat cultures and at least two of the serologic tests. Additionally, an ESR, CRP, white blood count, chest X-ray and, if possible, an echocardiogram should be considered in every patient. Other investigations may be required to exclude alternate diagnoses, including blood cultures for infective endocarditis, viral serology and autoimmune markers.

Previously bed rest was recommended. This is no longer the case, provided heart failure is adequately controlled. Ongoing active streptococcal infection warrants treatment with antibiotics. Oral penicillin V or amoxicillin is reasonable, or erythromycin if allergic. Intravenous administration is not required unless the patient is unable to take oral medication.

Anti-inflammatory medications are useful for a number of reasons. They result in rapid symptomatic improvement for patients, with fevers and arthralgias resolving within 1–2 days. Occasionally they are withheld to allow characteristic clinical features (e.g. migratory polyarthritis) to appear. Pain in these cases can be controlled by codeine until the diagnosis is made. Unfortunately, meta-analysis has shown no long-term benefits in those using anti-inflammatory agents (either steroidal or nonsteroidal) but this does enable clinicians to choose to withhold them for diagnostic purposes with reassurance; for example, aspirin at a dose of 80–100 mg/kg/day (4–6 g/day in adults) in four or five divided doses for 2 weeks, then reducing to 60–70 mg/kg/day for a further 2–4 weeks.

The more potent anti-inflammatory action of corticosteroids should be brought to bear when salicylates fail or when carditis with heart failure is severe. For example, 40–60 mg/day of oral prednisolone or even intravenous methyl prednisone in fulminant cases can be considered. All anti-inflammatory agents should be tapered as there is a risk of rebound if stopped abruptly. Intravenous immunoglobulin was ineffective in a randomized, placebo-controlled trial.[39]

The management of cardiac failure includes use of diuretics, angiotensin-converting enzyme inhibitors and fluid restriction. Digoxin

may be used for atrial fibrillation, although it also has the potential to induce arrhythmias in the rare patient with concurrent active myocarditis. It is now a rare situation to require valve surgery for life-threatening cardiac failure in the acute setting.

Valve surgery is more often required for chronic disease. In recent years there has also been a trend to offer valve repair rather than replacement or to use homografts or xenograft valves rather than mechanical prostheses (to avoid the need for anticoagulation). This trend further enhances the need for regular monitoring of chronic valvular disease, as late surgical repair is technically difficult with an extensively damaged valve.

Mild cases of chorea do not usually require treatment. If chorea impairs normal daily activities, or leads to considerable embarrassment or discomfort to the patient, intervention is reasonable. Haloperidol, carbamazepine and diazepam have been traditionally used, although valproate, pimozide and chlorpromazine are equally effective. Associated anxiety can respond to behavioral therapy. Plasmapheresis and intravenous immunoglobulin have been shown to have benefit in a small trial, but cannot yet be recommended as mainstream treatment.[40] Salicylates and steroids have no role in chorea therapy.

PROGNOSIS AND FOLLOW-UP

Acute rheumatic fever resolves spontaneously, usually within 12 weeks, even if left untreated. With treatment most patients will be able to leave hospital in less than 2 weeks. The likelihood of developing rheumatic heart disease relates to the severity of the acute carditis and the number of episodes of subsequent recurrent ARF. Some 30–50% of all people with ARF will eventually develop rheumatic heart disease, increasing to 70% in patients with severe carditis on initial presentation. All patients should be added to a local registry, where available, to facilitate accurate follow-up.

Mortality in chronic rheumatic heart disease can result from progressive cardiac failure, infective endocarditis or thromboembolic stroke. Estimates of stroke-related deaths range from 100 000 per year to almost 270 000 per year in less developed countries.[3]

Depending on the population studied, 75% of recurrences occur in the first 2 years, and 90% in the first 5 years. This is likely related to immune sensitization that wanes with time. Unfortunately, a lack of carditis initially does not ensure subsequent episodes are free. As a result, regardless of the clinical features of the initial episode, the priorities of long-term management are to ensure adherence to secondary prophylaxis, treat cardiac failure and educate about the need to treat streptococcal upper respiratory or skin infections early.

Those patients who have proven rheumatic heart disease should be stratified according to the severity of disease. Mild cases should undergo echocardiography every 1–2 years where possible. Severe cases should be followed every 3–6 months, depending on progression. Patients should be given endocarditis prophylaxis during dental procedures, and should have yearly dental reviews. In those who take oral daily penicillin for ARF secondary prevention, their oral cavity may contain viridans streptococci relatively resistant to penicillin, and hence should be offered clindamycin, azithromycin or clarithromycin for endocarditis prophylaxis.[41] (See Chapter 47 for further information on endocarditis prophylaxis.) Some national guidelines also suggest vaccination with the influenza vaccine (yearly) and a locally available pneumococcal vaccine (5-yearly) could be considered.[4] This is in addition to penicillin, and is solely based on expert opinion.

REFERENCES


References for this chapter can be found online at http://www.expertconsult.com

Practice point | 21 |

Chantal P Bleeker-Rovers

Nuclear medicine scanning

INTRODUCTION

Nuclear medicine techniques have a lot to offer in visualization of infectious foci. They point to the localization of a particular metabolic process, leading to elevated uptake of a radiopharmaceutical. Furthermore, with these techniques it is possible to monitor the effect of therapy.

TECHNIQUES

A wide variety of approaches to scintigraphic imaging of infectious disorders, depicting the different stages of the inflammatory response, have been developed (Table PP21.1). Table PP21.2 shows the advantages and disadvantages of the scintigraphic techniques that are currently used in clinical practice.

PROSTHETIC JOINT INFECTION

A negative bone scintigraph effectively rules out infection. However, the majority of infections occur within 1 year after implantation, and at that time enhanced periprosthetic uptake is observed on the bone scan in most patients. Therefore, an abnormal bone scan will require additional studies to determine the cause of the abnormality.

Although not the ideal agent in these patients, ^{67}Ga-citrate can be used to delineate infected prostheses, with a moderate overall accuracy of 70–80%. Radiolabeled leukocytes can accurately delineate infections in this patient population. However, in many cases the physiologic uptake of the labeled leukocytes in the bone marrow complicates the interpretation of the images.

In combination with sulfur colloid that accumulates in active marrow but not in infection, accuracy in diagnosing painful prostheses exceeds 90%. Furthermore, infected prostheses can be diagnosed very accurately using radiolabeled antigranulocyte antibody preparations. ^{18}F-fluorodeoxyglucose positron emission tomography (FDG-PET) also has a high sensitivity in diagnosing infected joint prostheses. Specificity, however, is lower than specificity of combined leukocyte scintigraphy and bone scanning.

Table PP21.1 Approaches to scintigraphic imaging of infectious disorders

Physiologic characteristics	Targeting mechanism	Radiolabeled compound
Enhanced vascular permeability	Nonspecific uptake	^{67}Gallium-citrate
Enhanced influx of granulocytes	Granulocyte influx	^{111}Indium-leukocytes ^{99m}Technetium-leukocytes
	Antigen binding	Radiolabeled antigranulocyte monoclonal antibodies (e.g. LeuTech®, LeukoScan®)
Increased metabolic activity	Enhanced glucose uptake	^{18}F-fluorodeoxyglucose (FDG)

OSTEOMYELITIS

A three-phase bone scan in otherwise normal bone is able to diagnose osteomyelitis with high sensitivity and specificity. In patients with other osseous abnormalities, ^{67}Ga-citrate or radiolabeled leukocytes can be applied to increase specificity; however, radiolabeled leukocytes are less suitable for diagnosing chronic low-grade osteomyelitis or suspected vertebral osteomyelitis. FDG-PET proved to be superior to MRI, ^{67}Ga-citrate scintigraphy and three-phase bone scan in patients suspected of vertebral osteomyelitis (Fig. PP21.1). In addition, FDG-PET is a very sensitive tool even for chronic and low-grade osteomyelitis.

Case study (Fig. PP21.1)

A 56-year-old woman experienced persisting fever despite adequate antibiotic treatment of her *Staphylococcus aureus* sepsis and endocarditis. PET showed increased FDG uptake in both shoulders, the left sternoclavicular joint, and the thoracic and lumbar spine. Septic arthritis of both shoulders was confirmed by puncture and culture and surgical treatment was needed several times. MRI confirmed spondylodiscitis of the sixth and seventh thoracic vertebrae and the third and fourth lumbar vertebrae, for which prolonged antibiotic treatment was prescribed.

VASCULAR INFECTIONS

CT is most often used for the assessment of vascular graft infection. However, hematomas and seromas in the vicinity of a vascular graft appear anatomically similar to an abscess, thus making it difficult to distinguish between noninfected and infected prosthetic grafts on CT images. The background activity of the images obtained with ^{67}Ga and radiolabeled immunoglobulins in many cases is too high for accurate visualization of vascular lesions.

Table PP21.2 Advantages and disadvantages of currently available scintigraphic techniques

^{67}Gallium-citrate

Mechanism

- Accumulates as an iron analogue through binding to circulating transferrin
- This complex extravasates at the site of inflammation due to locally enhanced vascular permeability
- ^{67}Ga is transferred to lactoferrin that is locally excreted by leukocytes or to siderophores produced by micro-organisms

Advantages

- High sensitivity for both acute and chronic infection and noninfectious inflammation
- Inexpensive, easy labeling technique

Disadvantages

- Poor specificity (bowel excretion, accumulation in malignant tissues and areas of bone modeling)
- High-energy gamma radiation, causing high radiation absorbed doses
- Optimal imaging often requires delay of up to 72 h after the injection

^{111}Indium- or ^{99m}Technetium-leukocyte scintigraphy

Mechanism

- A blood sample (50 ml) is collected
- Leukocytes are separated *in vitro* from red blood cells
- Leukocytes are labeled with radioactive isotopes (^{111}In or ^{99m}Tc) and re-injected

Advantages

- Good diagnostic accuracy

Disadvantages

- Laborious procedure
- Handling of potentially contaminated blood, risk of transmission of infection
- Less sensitive in chronic low-grade infections and infections of the central skeleton

Antigranulocyte monoclonal antibodies and antibody fragments

Mechanism

- *In-vitro* labeling of monoclonal antibodies against surface antigens present on granulocytes
- Injection of radiolabeled antibodies and *in-vivo* binding to leukocytes

Advantages

- Easier labeling procedure
- No need for handling of potentially contaminated blood

Disadvantages

- Long time interval between injection and imaging
- Monoclonal antibodies: induction of human anti-mouse antibodies

^{18}F-fluorodeoxyglucose positron emission tomography (FDG-PET)

Mechanism

- Accumulation in tissues with high rate of glycolysis (malignant cells, activated leukocytes)

Table PP21.2 Advantages and disadvantages of currently available scintigraphic techniques—cont'd

Advantages

- High resolution resulting in superior images
- Sensitivity in chronic low-grade infections and in infections of the central skeleton
- Early imaging (1 h after injection)

Disadvantages

- No differentiation between infection and malignancy (an advantage in fever of unknown origin)
- Relatively expensive
- More limited availability

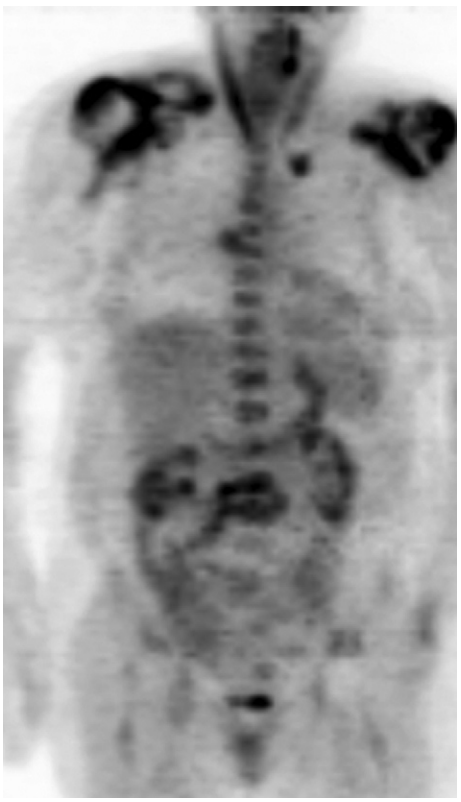


Fig. PP21.1 PET scan showing increased FDG uptake in both shoulders, the left sternoclavicular joint, and the thoracic and lumbar spine.

Vascular infections can be detected accurately with radiolabeled white blood cells; the overall sensitivity, specificity and accuracy of ^{111}In-labeled leukocytes is >90%. More recently, FDG-PET has been shown valuable in diagnosing vascular infections (Fig. PP21.2). In a systematic comparison between CT and FDG-PET in patients suspected of vascular graft infection, the sensitivity of FDG-PET (91%) was significantly higher than that of CT (64%). When the PET criterion for infection was defined as focal abnormal uptake, the specificities of FDG-PET and CT were comparable. It is to be expected that specificity and positive predictive value are even better after a certain period following surgery.

Case study (Fig. PP21.2)

A 69-year-old woman was admitted because of fever and chills. She also complained of pain in the upper abdomen radiating to her left leg. Her medical history was remarkable for severe arterial insufficiency

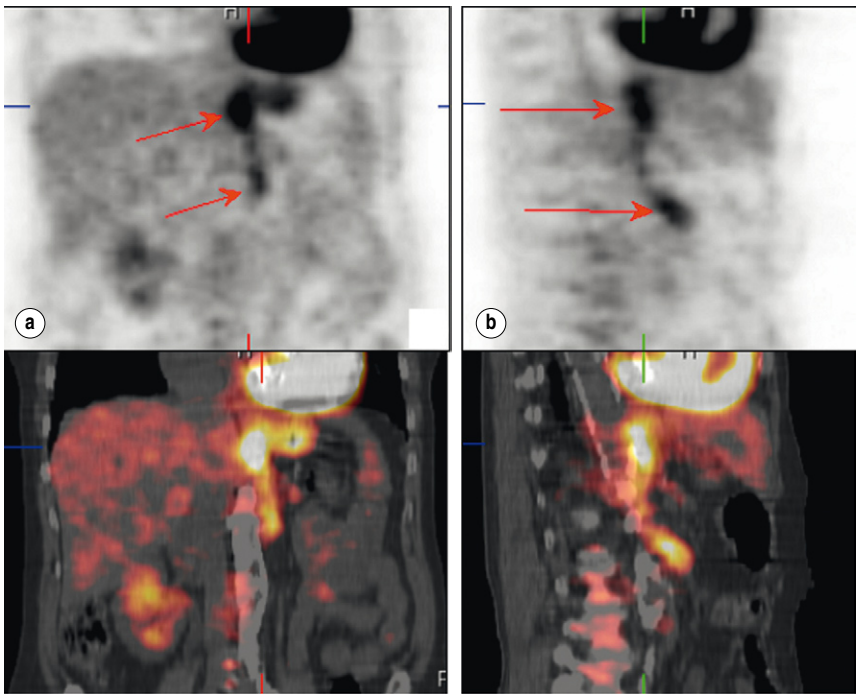


Fig. PP21.2 PET scan showing increased FDG uptake at the aortomesenteric bypass: (a) coronal view; (b) sagittal view, upper panels. Lower panels: FDG-PET-CT scan.

with mesenterial ischemia for which a bypass from the aorta to the superior mesenteric artery was performed 2 years earlier. She had also undergone an aorto-iliacal bypass some 15 years previously.

Blood cultures grew *Escherichia coli*. The first abdominal CT scan showed no signs of infection. PET, however, showed increased FDG uptake at the aortomesenteric bypass. An infection of the vascular prosthesis was diagnosed. The prosthesis could not be removed, so suppressive therapy with ciprofloxacin was started.

METASTATIC INFECTIOUS FOCI

Timely identification of metastatic complications of bloodstream infections, although critical, is often difficult. In a retrospective study in 40 patients with a high suspicion of metastatic complications after bloodstream infection, FDG-PET diagnosed a clinically relevant new focus in 45% of cases while, on average, four conventional diagnostic tests had been performed before FDG-PET. FDG-PET is very promising in detecting metastatic infectious foci in patients with a high level of clinical suspicion (see Fig. PP21.1).

ABDOMINAL INFECTIONS

For this purpose, ultrasonography, CT and MRI are currently considered the modalities of choice. However, in early stages of infection or in postoperative patients with equivocal anatomic changes, scintigraphic imaging can be very useful.

Although ^{67}Ga-citrate has a reasonable diagnostic accuracy for diagnosing abdominal infection, the physiologic gastrointestinal uptake of ^{67}Ga-citrate limits the interpretation of the images. Radiolabeled white blood cells are more suitable in these patients. Because ^{111}In-labeled leukocytes normally do not localize in the gastrointestinal tract, they have an advantage over ^{99m}Tc-labeled leukocytes. In patients with polycystic kidney disease, FDG-PET proved to be very helpful in suspected renal or hepatic cyst infection by correctly pointing to the infected cysts. In addition, FDG-PET appears to be a sensitive and specific adjunct in the diagnosis of suspected alveolar echinococcosis and can help in differentiating alveolar from cystic echinococcosis in the liver. FDG-PET also appears to be valuable in assessing the efficacy of chemotherapy in these patients.

FEVER OF UNKNOWN ORIGIN

The value of FDG-PET has been studied in 292 patients with fever of unknown origin (FUO) showing an overall helpfulness of FDG-PET of 36%. FDG-PET appears to be superior to ^{67}Ga-citrate scintigraphy because the diagnostic yield is at least similar to that of ^{67}Ga-citrate scintigraphy and the results are available within hours instead of days.

Based on the results of these studies and resulting from the favorable characteristics of FDG-PET, conventional scintigraphic techniques may in the future be replaced by FDG-PET in the investigation of patients with FUO in institutions where this technique is available.

DISCUSSION

Leukocyte scintigraphy is still considered the 'gold standard' nuclear medicine technique for the imaging of infection and inflammation by many clinicians, but a gradual shift from cumbersome and even hazardous techniques to approaches, based on small agents binding to their targets with high affinity, is ongoing. The ideal agent will be determined by the clinical situation and local availability.

FDG-PET has a lot to offer in visualization of various infectious foci in many organ systems. It is shown to be superior to other scintigraphic techniques in FUO, chronic low-grade osteomyelitis and vertebral osteomyelitis. FDG-PET has also shown promising results in vascular graft infections and the evaluation of metastatic infectious foci in patients with bloodstream infections. FDG-PET may prove to be as useful in the rapid detection and management of infectious diseases as it is in the management of malignant diseases. In addition, combined PET-CT scans are suspected to further increase the usefulness of this imaging modality.

FURTHER READING

Further reading for this chapter can be found online at http://www.expertconsult.com

Practice point 22

Aadia I Rana

Approach to the patient with persistent bacteremia

INTRODUCTION

Persistent bacteremia, generally defined as continuous bacteremia for at least 3 days, is a common clinical problem and presents a unique therapeutic challenge. The frequency of persistent bacteremia has been rising over the past 10 years, reflecting an increase in resistant organisms such as meticillin-resistant *Staphylococcus aureus* (MRSA) and the upsurge of implantable intravascular devices. This trend is particularly concerning as persistent bacteremia is associated with longer hospitalizations and higher morbidity and mortality compared with shorter duration bacteremia. Effective management entails a comprehensive approach including a diligent search for metastatic foci of infection coupled with optimal antibiotic therapy. The differential diagnosis of persistent bacteremia is usually quite straightforward:

- inappropriate or inadequate antibiotics;
- an extravascular focus of infection; or
- endocarditis or intravascular infection.

INAPPROPRIATE OR INADEQUATE ANTIBIOTICS

Persistent bacteremia is often related to inappropriate antibiotic therapy through either inadequate empiric coverage or subtherapeutic dosing. Careful reassessment of initial empiric antibiotic choices based on culture and susceptibility results is the critical element in the management of persistent bacteremia.

The choice for empiric coverage of antibiotics should be dictated by the clinical presentation. In patients with endocarditis, adequate antistaphylococcal coverage, including MRSA, and antistreptococcal coverage in conjunction with a synergistic aminoglycoside should be considered (see Chapter 47). Those patients with foreign bodies (e.g. prostheses or implantable intravascular devices) should be administered antibiotics that cover nosocomial pathogens including MRSA and resistant gram-negative bacteria such as *Klebsiella*, *Serratia*, and *Pseudomonas* species.

Suboptimal dosing represents a selective pressure that is imposed on the bacteria which in turn facilitates the emergence of resistance. MRSA is being reported with increasing frequency as a cause of persistent bacteremia and infective endocarditis.

Recommendations of maintaining higher vancomycin trough levels of 15–20 μg/ml in MRSA pneumonia have now been extrapolated to MRSA bacteremias. MRSA infections with strains expressing higher vancomycin minimum inhibitory concentrations (MICs) of 2–4 μg/ml are associated with vancomycin-treatment failure and poor outcomes. As such, recent guidelines suggest lowering the breakpoint of the MIC for susceptibility for MRSA from 4 μg/ml to 2 μg/ml and consider increasing vancomycin dosing or use alternative antibiotics when the vancomycin MIC is ≥2 μg/ml.

For infections with *Pseudomonas* spp. and other resistant gram-negative pathogens such as *Acinetobacter* spp., successful eradication necessitates higher doses of broad-spectrum β-lactams at shorter intervals to maintain levels above the MIC. Likewise, higher doses of fluoroquinolones and aminoglycosides emphasize the concentration-dependent activity of these antimicrobials while taking advantage of their sustained postantibiotic effect. Studies are underway with continuous infusions of anti-pseudomonal β-lactams to facilitate consistent levels of the antimicrobial above the MIC, especially in the critically ill patient. Such continuous infusions have been shown to be effective in animal experiments.

FOCUS OF INFECTION

The identification of a metastatic focus of infection, particularly a removable or drainable focus, is a key component in the evaluation of a patient with persistent bacteremia (Table PP22.1). Even optimal antibiotic therapy is unlikely to be curative unless source control is achieved.

The growing use of devices such as central venous catheters, automatic implantable cardioverter defibrillators (AICDs), cardiac pacemakers and central implantable spinal pumps in the medical community has resulted in increased incidence of bloodstream infections. The presence of a central vascular catheter is an independent risk factor for persistent bacteremia even if another source of infection is identified. Patients with persistently positive blood cultures should

Table PP22.1 Potential foci of infection in persistent bacteremia

- Implanted devices
 - Central venous catheters
 - Automatic implantable cardioverter defibrillator (AICD) or cardiac pacemaker
 - Implantable spinal pump
- Intra-abdominal process
- Osteomyelitis (particularly vertebral)
- Epidural abscess
- Intravascular infection
 - Endocarditis with or without valvular ring abscess
 - Mycotic aneurysm
 - Thrombophlebitis
 - Pylephlebitis

be examined carefully for evidence of local inflammation at the site of implanted devices. If present, the device should be removed and cultured. Even if there is no local inflammation, in the absence of another identifiable source, persistent bloodstream contamination necessitates the removal of the device. Empiric antibiotic regimens that cover nosocomial pathogens such as MRSA and *Pseudomonas* spp. should be initiated while awaiting definitive diagnostics and procedures.

In those patients without implanted devices, other foci of infection should be explored. Certain pathogens such as *Salmonella* spp. and *Staphylococcus aureus* are more likely to result in metastatic infectious lesions and their presence should trigger an investigation for metastatic disease. Some studies indicate that intermittent bacteremia often denotes an occult abscess whereas persistent bacteremia is more suggestive of an endovascular source. However, in a review of patients with persistent bacteremia, a non-endovascular source was implicated in 23% of cases. Patients with burns, cirrhosis or baseline intra-abdominal pathology are at particular risk of persistent bacteremia, perhaps related to mucosal barrier dysfunction and/or diminished bacterial clearance capacity. In these patients, an aggressive search for an intra-abdominal process should be undertaken.

Infections in areas with poor blood supply, such as bone or the peripheral extremities of patients with severe neuropathy, may contribute to persistent bacteremia without an apparent endovascular focus. Persistent bacteremia in a patient with bone pain, back pain and/or neurologic symptoms should trigger a workup for osteomyelitis (particularly vertebral) or an epidural abscess with MRI of the involved area.

ENDOCARDITIS OR INTRAVASCULAR INFECTION

Endovascular infections account for most cases of persistent bacteremia. Factors supporting a diagnosis of endocarditis include:

- a new or changing heart murmur;
- valvular vegetations on echocardiogram;
- peripheral stigmata of endocarditis, including septic emboli or glomerulonephritis; and
- persistent bacteremia with a typical pathogen such as *Streptococcus* spp., *Staphylococcus aureus,* enterococci or the HACEK organisms.

In patients with prosthetic heart valves, persistently positive blood cultures without another identifiable source of infection are virtually pathognomonic for prosthetic valve endocarditis and should immediately be assessed with a transesophageal echocardiogram to assess for valve function and possible leakage.

Persistently positive blood cultures while on optimal antibiotic therapy, in the absence of an intravascular device and without other identifiable foci of infection, most commonly result from intravascular suppuration. Possible intravascular infections are native valve endocarditis with or without associated valvular ring abscess, mycotic aneurysm, thrombophlebitis or pylephlebitis (Fig. PP22.1) and should be explored with appropriate diagnostic studies such as transesophageal echocardiogram or nuclear medicine studies (for more details on imaging studies, see Practice Point 21).

While diagnostic tests are pending, optimal empiric antibiotic therapy should be initiated in all patients where the clinical scenario supports this diagnosis. In addition, when the diagnosis of suppurative intravascular infection has been established, a low threshold for surgical exploration should be maintained because this often subtle and highly lethal process is unlikely to be eradicated unless the lesion is excised, even in the presence of optimal antibiotic therapy.

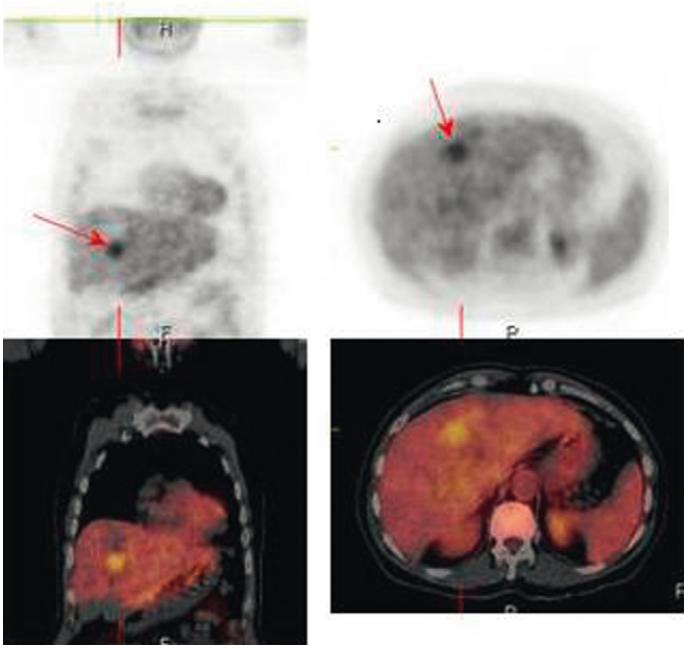


Fig. PP22.1 Pylephlibitis. This previously healthy 68-year-old man was admitted with fever that had been present for 2 weeks and diffuse abdominal pain. Physical examination was normal except for a temperature of 102.4°F (39.1°C) and a heart rate of 98 bpm. Blood cultures grew *Escherichia coli*, *Pseudomonas aeruginosa* and coagulase-negative staphylococci. Because of persisting fever despite treatment with piperacillin–tazobactam, an FDG-PET CT scan was performed, which showed increased FDG-uptake in the portal vein (arrow) and several smaller areas in the liver. CT confirmed the presence of a portal vein thrombosis and small liver abscesses, so a diagnosis of pylephlebitis was made. The patient was treated with amoxicillin–clavulanic acid and ciprofloxacin for 6 more weeks and low-molecular-weight heparin for 6 months, with a complete resolution of his symptoms. Courtesy of Chantal Bleeker-Rovers, MD.

CONCLUSION

Many hospitalized individuals with bacteremia are at risk for persistent bacteremia, particularly those with resistant organisms, intravascular infections and foreign bodies. Rates of persistent bacteremia are expected to rise as populations at risk expand with the increase of implantable devices.

Table PP22.2 outlines the approach to the patient with a persistent bacteremia. The key points in the diagnostic approach remain a careful history and physical examination, thorough consideration of likely pathogens and further diagnostic workup with imaging studies as indicated by the clinical scenario, judicious assessment of antimicrobials, and pursuit of definitive treatment through source control. Aggressive attempts to minimize the risk of complications associated with persistent bacteremia are vital in view of the higher rates of morbidity and mortality associated with this clinical entity.

Table PP22.2 Evaluation of a patient with persistent bacteremia (≥3 days)

- Obtain full history and physical, examining the patient thoroughly for any identifiable sources of infection, visible or implanted
- Assess antibiotic coverage and dosing to ensure optimal levels based on culture and susceptibility results and/or on clinical scenario
- Obtain diagnostic imaging based on organisms isolated and clinical scenario:
 - if organism isolated is consistent with a diagnosis of endocarditis, obtain transthoracic or transesophageal echocardiogram
 - if organism(s) is gram-negative, fungal pathogen or polymicrobial, or patient is at risk of bacterial translocation via the gut, obtain abdominal imaging to rule out occult abdominal process
 - if patient has back pain or neurologic symptoms, obtain MRI of head/spine to evaluate for epidural abscess or vertebral osteomyelitis
- For patients with intravascular devices and/or catheters:
 - identify and remove any temporary peripheral catheters and send for culture
 - if there is expressible purulence, culture and consider surgical intervention
 - if intravascular device such as AICD or tunneled catheter is present and no other source of infection is identified, remove and send for semiquantitative culture
- For patients who remain persistently bacteremic on optimal antibiotic therapy despite removal of all foreign bodies:
 - consider radiolabeled white blood cell scan or positron emission tomography to evaluate for evidence of intravascular suppuration
 - if suggestive of a suppurative process, obtain surgical consult for definitive evaluation and treatment

FURTHER READING


Further reading for this chapter can be found online at http://www.expertconsult.com

Chapter 49

Jack D Sobel

Vaginitis, vulvitis, cervicitis and cutaneous vulval lesions

Vaginitis

Vaginal symptoms are extremely common, and vaginal discharge is among the 25 most common reasons for consulting physicians in private office practice in the USA. Vaginitis is found in more than one-quarter of women attending sexually transmitted disease (STD) clinics. Not all women with vaginal symptoms have vaginitis; approximately 40% of women with vaginal symptoms will have some type of vaginitis (Table 49.1).

Bacterial Vaginosis

EPIDEMIOLOGY

Bacterial vaginosis is the most common cause of vaginitis in women of child-bearing age. It has been diagnosed in 17–19% of women seeking gynecologic care in family practice or student health-care settings.[1]

Table 49.1 Causes of vaginitis in adult women

Common infectious vaginitis	Bacterial vaginosis (40–50%) Vulvovaginal candidiasis (20–25%) Trichomonal vaginitis (15–20%)
Uncommon infectious vaginitis	Foreign body with secondary infection Desquamative inflammatory vaginitis (clindamycin responsive) Streptococcal vaginitis (group A) Ulcerative vaginitis associated with *Staphylococcus aureus* and toxic shock syndrome Idiopathic vulvovaginal ulceration associated with HIV
Noninfectious vaginitis	Chemical/irritant Allergic, hypersensitivity and contact dermatitis (lichen simplex) Traumatic Atrophic vaginitis Postpuerperal atrophic vaginitis Desquamative inflammatory vaginitis (corticosteroid responsive) Erosive lichen planus Collagen vascular disease, Behçet's syndrome, pemphigus syndromes Idiopathic

The worldwide prevalence ranges from 11% to 48% of women of reproductive age, with variation according to population studied.[2] The prevalence increases considerably in symptomatic women attending STD clinics, reaching 24–37%. Bacterial vaginosis has been observed in 16–29% of pregnant women. *Gardnerella vaginalis* has been found in 10–31% of virgin adolescent girls, but is found significantly more frequently among sexually active women, reaching a prevalence of 50–60% in some at-risk populations.

Evaluation of epidemiologic factors has revealed few clues as to the cause of bacterial vaginosis. Use of the intrauterine device and douching was found to be more common in women with bacterial vaginosis. Bacterial vaginosis is significantly more common among black and sexually active women, including lesbians.

PATHOGENESIS AND PATHOLOGY

Bacterial vaginosis is not due to a single organism and is the result of massive overgrowth of mixed complex flora or microbiota, including peptostreptococci, *Bacteroides* spp., *G. vaginalis*, *Mobiluncus* spp. and genital mycoplasma.[1] There is little inflammation, and the disorder represents a disturbance of the vaginal microbial ecosystem rather than a true infection of tissues. The overgrowth of mixed flora is associated with a loss of the normal *Lactobacillus* spp. dominated vaginal flora. No single bacterial species is responsible for bacterial vaginosis. Experimental studies in human volunteers and studies in animals indicate that inoculation of the vagina with individual species of bacteria associated with bacterial vaginosis (e.g. *G. vaginalis*), rarely results in bacterial vaginosis. In support of the role of sexual transmission is the higher prevalence of bacterial vaginosis among sexually active young women than among sexually inexperienced women, and the observation that bacterial vaginosis-associated micro-organisms are more frequently isolated from the urethras of male partners of females with bacterial vaginosis.[1]

The cause of the overgrowth of anaerobes, *Gardnerella*, *Mycoplasma* and *Mobiluncus* spp. is unknown. Theories include increased substrate availability, increased pH and loss of the restraining effects of the predominant *Lactobacillus* spp. flora. It has been reported that normal women are predominantly colonized by hydrogen peroxide-producing strains of lactobacilli, whereas women with bacterial vaginosis have reduced population numbers of lactobacilli, and the species present lack the ability to produce hydrogen peroxide.[3] The hydrogen peroxide produced by lactobacilli may inhibit the pathogens associated with bacterial vaginosis, either directly by the toxicity of hydrogen peroxide, or as a result of the production of a hydrogen peroxide–halide complex in the presence of natural cervical peroxidase.

Accompanying the bacterial overgrowth in bacterial vaginosis is the increased production of amines by anaerobes, facilitated by microbial decarboxylases. Volatile amines in the presence of increased vaginal pH produce the typical fishy odor, which is also produced when 10%

potassium hydroxide is added to vaginal secretions. Trimethylamine is the dominant abnormal amine in bacterial vaginosis. It is likely that bacterial polyamines together with the organic acids found in the vagina in bacterial vaginosis (acetic and succinic acid) are cytotoxic, resulting in exfoliation of vaginal epithelial cells and creating the vaginal discharge. *Gardnerella vaginalis* attaches avidly to exfoliated epithelial cells, especially at the alkaline pH found in bacterial vaginosis. The adherence of *Gardnerella* organisms results in the formation of the pathognomonic clue cells. Using broad-range DNA probes, new molecular methods have identified at least 35 new bacterial phylotypes (species) previously noncultivable in women with bacterial vaginosis, including *Atopobium vaginae* and clostridial species BVAB I, II and III, offering new insights into pathogenesis.[4]

PREVENTION

Because the pathogenesis of bacterial vaginosis is obscure, preventive measures have not been forthcoming. Although not proven to be sexually transmitted, barrier contraception may reduce occurrence and avoiding douching is recommended.

CLINICAL FEATURES

As many as 50% of women with bacterial vaginosis may be asymptomatic. An abnormal malodorous vaginal discharge, often described as fishy, that is infrequently profuse and often appears after unprotected coitus, is usually described. Pruritus, dysuria and dyspareunia are rare. Examination reveals a nonviscous, grayish-white adherent discharge.

Bacterial vaginosis has been incorrectly considered to be largely of nuisance value only. There is now considerable evidence of serious obstetric and gynecologic complications of bacterial vaginosis, including asymptomatic bacterial vaginosis diagnosed by Gram stain. Obstetric complications include chorioamnionitis, pre-term labor, prematurity and postpartum fever.[5] Gynecologic sequelae are postabortion fever, posthysterectomy fever, cuff infection and chronic mast cell endometritis. A more recent association is reported between untreated bacterial vaginosis and cervical inflammation and low-grade dysplasia.[6] Bacterial vaginosis is a risk factor for HIV acquisition and transmission.[7] Bacterial vaginosis is also a risk factor for acquisition of herpes simplex virus 2, gonorrhea and chlamydial infection.[8]

DIAGNOSIS

Signs and symptoms are unreliable in the diagnosis of bacterial vaginosis (Table 49.2). The clinical diagnosis can reliably be made in the presence of at least three of the following objective criteria:

- adherent, white, nonfloccular homogeneous discharge;
- positive amine test, with release of fishy odor on addition of 10% potassium hydroxide to vaginal secretions;
- vaginal pH >4.5; and
- presence of clue cells on light microscopy.

These features are simple and reliable, and tests for them are easy to perform. The presence of clue cells is the single most reliable predictor of bacterial vaginosis. Clue cells are exfoliated vaginal squamous epithelial cells covered with *G. vaginalis*, giving the cells a granular or stippled appearance with characteristic loss of clear cell borders. Of observed epithelial cells, diagnostic significance is indicated by 20% clue cells. Occasionally, clue cells covered exclusively by curved

Table 49.2 Diagnostic features of infectious vaginitis

		Normal	*Candida* vaginitis	Bacterial vaginosis	*Trichomonas* vaginitis
Symptoms		None or physiologic leukorrhea	Vulvar pruritus, soreness, increased discharge, dysuria, dyspareunia	Malodorous moderate discharge	Profuse purulent discharge, offensive odor, pruritus, and dyspareunia
Discharge	Amount	Variable, scant to moderate	Scant to moderate	Moderate	Profuse
	Color	Clear or white	White	White/gray	Yellow
	Consistency	Floccular nonhomogeneous	Clumped but variable	Homogeneous, uniformly coating walls	Homogeneous
	'Bubbles'	Absent	Absent	Present	Present
	Appearance of vulva and vagina	Normal	Introital and vulvar erythema, edema and occasional pustules, vaginal erythema	No inflammation	Erythema and swelling of vulvar and vaginal epithelium (strawberry cervix)
	pH of vaginal fluid	<4.5	<4.5	>4.7	5.0–6.0
	Amine test (10% potassium hydroxide)	Negative	Negative	Positive	Occasionally present
	Saline microscopy	Normal epithelial cell, lactobacilli predominate	Normal flora, blastospores (yeast) 40–50% pseudohyphae	Clue cells, coccobacillary flora predominate, absence of leukocytes, motile curved rods	PMNS +++, motile trichomonads (80–90%), no clue cells, abnormal flora
10% potassium hydroxide microscopy		Negative	Positive (60–90%)	Negative (except in mixed infections)	Negative

Gram-negative rods belonging to *Mobiluncus* spp. can be demonstrated. The offensive fishy odor may be apparent during the physical examination or may become apparent only during the amine test. Gram strain of vaginal secretions is extremely valuable in diagnosis, with a sensitivity of 93% and specificity of 70%.

Although cultures for *G. vaginalis* are positive in almost all cases of bacterial vaginosis, *G. vaginalis* may be detected in 50–60% of women who do not meet the diagnostic criteria for bacterial vaginosis. Accordingly, vaginal culture has no part in the diagnosis of bacterial vaginosis. DNA probes for *G. vaginalis* are both sensitive (95%) and specific (99%), but are not widely used by practitioners because of costs and lack of insurance reimbursement. Diagnostic cards are now available for rapid diagnosis, measuring pH, amines or sialidase.

MANAGEMENT

Poor efficacy has been observed with triple sulfa creams, ampicillin, quinolones, erythromycin, tetracycline, acetic acid gel and povidone–iodine vaginal douches.[9]

The most widely used oral therapy remains metronidazole or tinidazole. Most studies using multiple divided dose metronidazole regimens of 800–1200 mg/day for 1 week achieved clinical cure rates in excess of 90% immediately, and of approximately 80% at 4 weeks. Although single-dose therapy with 2 g metronidazole achieves comparable immediate clinical response rates, higher recurrence rates have been reported. The CDC recommended regimen is metronidazole 500 mg twice daily for 7 days.[9] The beneficial effect of metronidazole results predominantly from its anti-anaerobic activity and because *G. vaginalis* is susceptible to the hydroxymetabolites of metronidazole. Although *Mycoplasma hominis* and *Mobiluncus curtisii* are resistant to metronidazole, the organisms are usually not detected at follow-up visits of successfully treated patients. Similarly, metronidazole and tinidazole are considered therapeutic equivalents although tinidazole has fewer of the commonly experienced side-effects.

Topical therapy with 2% clindamycin cream once daily for 7 days, clindamycin ovules for 3 days or metronidazole gel 0.75% administered daily for 5 days have been shown to be as effective as oral metronidazole, without any of the side-effects of the latter.[9]

In the past, asymptomatic bacterial vaginosis was not treated, especially because patients often improve spontaneously over several months. However, the growing evidence linking asymptomatic bacterial vaginosis with numerous obstetric and gynecologic upper tract complications has caused reassessment of this policy, especially with additional convenient topical therapies.[5,10] Asymptomatic bacterial vaginosis should be treated before pregnancy, in women with cervical abnormalities and before elective gynecologic surgery. Routine screening for and treatment of asymptomatic bacterial vaginosis in pregnancy remains controversial, pending the outcome of studies proving that therapy of bacterial vaginosis reduces pre-term delivery and prematurity.[11]

Despite indirect evidence of sexual transmission, no study has documented reduced recurrent rates of bacterial vaginosis in women whose partners have been treated with a variety of regimens, including metronidazole. Accordingly, most clinicians do not routinely treat male partners.

After therapy with oral metronidazole, approximately 30% of patients initially responding experience recurrence of symptoms within 3 months.[1] Reasons for recurrence are unclear, including the possibility of re-infection, but recurrence more likely reflects vaginal relapse, with failure to eradicate the offending organisms and re-establish the normal protective *Lactobacillus* spp. dominant vaginal flora. Management of bacterial vaginosis relapse includes oral or vaginal metronidazole, or topical clindamycin, usually prescribed for 14 days. New experimental approaches including exogenous *Lactobacillus* spp. recolonization using selected bacteria-containing suppositories have been encouraging. Maintenance antimicrobial agents administered twice weekly for 4–6 months have been reasonably effective but results are less than desirable.[12]

Trichomoniasis

EPIDEMIOLOGY

Studies estimate that 3–5 million American women contract trichomoniasis annually, with a worldwide distribution of approximately 180 million annual cases.[1,13] The prevalence of trichomoniasis correlates with the overall level of sexual activity of the specific group of women under study, being diagnosed in about 5% of women in family planning clinics, in 13–25% of women attending gynecology clinics, in 50–75% of prostitutes and in 7–35% of women in STD clinics. In many industrialized countries, recent surveys indicate a decline in the incidence of trichomoniasis.[13]

PATHOGENESIS AND PATHOLOGY

Sexual transmission is the dominant method of introduction of *Trichomonas vaginalis* into the vagina.[1] *Trichomonas vaginalis* was identified in the urethra of 70% of men who had had sexual contact with infected women within the previous 48 hours. There is also a high prevalence of gonorrhea in women with trichomoniasis, and both of these are significantly associated with use of nonbarrier methods of contraception.

Recurrent trichomoniasis is common and is indicative of a lack of significant protective immunity. Nevertheless, an immune response to *Trichomonas* spp. does develop, as indicated by low titers of serum antibody, but this is insufficient for diagnostic serology. Antitrichomonal IgA has been detected in vaginal secretions, but a protective role is not defined.

Delayed hypersensitivity in natural infection can also be demonstrated. The predominant host defense response is provided by the numerous polymorphonuclear leukocytes (PMNs), which respond to chemotactic substances released by trichomonads and are capable of killing *T. vaginalis* without ingesting trichomonads. *Trichomonas vaginalis* destroys epithelial cells by direct cell contact and cytotoxicity. The urethra, paraurethral, Bartholin's and Skeene's glands are infected in the majority of patients, and organisms are occasionally isolated from urine.

PREVENTION

Sexual transmission of trichomonads is efficiently prevented by use of barrier contraception. Spermicidal agents such as nonoxynol-9 also reduce transmission. Re-infection of women is common, hence the mandatory requirement of treatment, preferably simultaneously, of all sexual partners with metronidazole. Women can acquire the disease from other women, but men do not usually transmit infection to other men.

CLINICAL FEATURES

Infection with *Trichomonas* spp. in women ranges from an asymptomatic carrier state to severe acute inflammatory disease.[14,15] Vaginal discharge is reported by 50–75% of women diagnosed with trichomoniasis; however, the discharge is not always described as malodorous. Pruritus occurs in 25–50% of patients and is often severe. Other infrequent symptoms include dyspareunia, dysuria and, rarely, frequency of micturition. Lower abdominal pain occurs in fewer than 10% of patients and should alert the physician to the possibility of concomitant salpingitis caused by other organisms. Symptoms of acute trichomoniasis often appear during or immediately after menstruation. Although controversial, the incubation period has been estimated to range from 3 to 28 days.

Physical findings represent a spectrum depending on the severity of disease. Vulvar findings may be absent, but are typically characterized in severe cases by diffuse vulvar erythema (10–33%), edema and a copious, profuse and malodorous vaginal discharge, which is often described as being yellow-green and frothy, but is frequently grayish-white.[11] Frothiness is seen in a minority of patients and is more commonly seen in bacterial vaginosis.

The vaginal walls are erythematous and in severe cases may be granular in appearance. Punctate hemorrhages (colpitis macularis) of the cervix may result in a strawberry-like appearance that, although apparent to the naked eye in only 1–2% of patients, is present in 45% of cases on colposcopy.[11]

The clinical course of trichomoniasis in pregnancy is identical to that seen in the nonpregnant state, and when untreated it is associated with premature rupture of membranes and prematurity. Trichomoniasis is reported to facilitate HIV transmission. Trichomoniasis is a risk factor for development of posthysterectomy cellulitis, tubal infertility, cervical neoplasia and pelvic inflammatory disease.[16]

DIAGNOSIS

None of the clinical features of vaginitis caused by *Trichomonas* spp. are sufficiently specific to allow a diagnosis of trichomonal infection based on signs and symptoms alone (see Table 49.2).[10] Definitive diagnosis requires the demonstration of the organism. Vaginal pH is markedly elevated, almost always above 5.0, and not infrequently 6.0. On saline microscopy, an increase in the number of PMNs is almost invariably present. The ovoid parasites are slightly larger than PMNs and are best recognized by their motility. The wet mount is positive in only 40–80% of cases (low sensitivity). Gram stain is of little value because of its inability to differentiate PMNs from nonmotile trichomonads, and use of Giemsa, acridine orange and other stains has no advantage over saline preparations. Although trichomonads are often seen on Papanicolaou smears, this method has a sensitivity of only 60–70% when compared with saline preparation microscopy, and false-positive results are not infrequently reported.

Several equivalent culture medium methods are available, and growth is usually detected within 48 hours. Culture including 'In-Pouch' is now recognized as the most sensitive method for detecting the presence of trichomonads (95% sensitivity) and should be considered in patients with vaginitis in whom an elevated pH is found together with PMN excess and absence of motile trichomonads. Several new diagnostic kits using DNA probes or rapid antigen detection are now available commercially and are replacing culture because of rapid confirmation[17] (e.g. Affirm VP, BectonDickenson; OSOM Trichomonas Rapid Test, Genzyme).

MANAGEMENT

Therapy is indicated in all nonpregnant women diagnosed with *Trichomonas* vaginitis, even if asymptomatic, and consists of administering the 5-nitroimidazole group of drugs – metronidazole, tinidazole and ornidazole – which are all of similar efficacy.[9] Oral therapy as opposed to topical vaginal therapy is preferred because of the frequency of infection of the urethra and periurethral glands, which provide sources for endogenous recurrence. Treatment of all sexual partners is mandatory.

Treatment consists of oral metronidazole, 500 mg every 12 hours for 7 days, which has a cure rate of 95%. Comparable results have been obtained with a single oral dose of 2 g metronidazole, achieving cure rates of 82–88%. The latter cure rate increases to greater than 90% when sexual partners are treated simultaneously. The advantages of single-dose therapy include better patient compliance, lower total dose, shorter period of alcohol avoidance and possibly decreased incidence of subsequent vaginitis caused by *Candida* spp. A disadvantage of single-dose therapy is the need to insist on simultaneous treatment of sexual partners.

The 5-nitroimidazoles are not in themselves trichomonacidal, but low-redox proteins reduce the nitro group, resulting in the formation of highly cytotoxic products within the organisms. Aerobic conditions interfere with this reduction process and decrease the antianaerobic activity of the 5-nitroimidazoles. Most strains of *T. vaginalis* are highly susceptible to metronidazole, with minimum inhibitory concentrations (MICs) of 1 mg/l. Similar efficacy results are reported with tinidazole but the latter has a superior tolerance profile.

Patients not responding to an initial course often respond to an additional standard course of 7-day therapy. Some patients are refractory to repeated courses of therapy, even when compliance is assured and sexual partners are known to have been treated. If re-infection is excluded, these rare patients may have strains of *T. vaginalis* that are resistant to metronidazole, which can be confirmed *in vitro*. Increased doses of metronidazole and longer duration of therapy are necessary to cure these refractory patients. The patients should be given maximal tolerated dosages of oral metronidazole of 2–4 g/day for 10–14 days. Rarely, intravenous metronidazole, in dosages as high as 2–4 g/day, may be necessary, with careful monitoring for drug toxicity. Considerable success has been observed in treating resistant infections with oral tinidazole; however, the drug is not readily available and the optimal dose to be used is unknown.[18] Most investigators use high-dose tinidazole 1–4 g/day for 14 days.[18] Rare patients not responding to nitroimidazoles can be treated with topical paramomycin.

Side-effects of metronidazole include an unpleasant or metallic taste. Other common side-effects include nausea (10%), transient neutropenia (7.5%) and a disulfiram-like effect when alcohol is ingested. Caution should be taken when 5-nitroimidazoles are used in patients taking warfarin. Long-term and high-dose therapy increase the risk of neutropenia and peripheral neuropathy. In experimental studies, metronidazole has been shown to be mutagenic for certain bacteria, indicating a carcinogenic potential, although cohort studies have not established an increase in cancer morbidity. Thus, the risk to humans of short-term low-dose metronidazole treatment is extremely small. Superinfection with *Candida* spp. is by no means uncommon.

Treatment of symptomatic trichomoniasis in pregnancy is identical.[9] Metronidazole readily crosses the placenta and, because of concern for teratogenicity, some consider it prudent to avoid its use in the first trimester of pregnancy. More recently investigators have become more comfortable with the use of metronidazole throughout pregnancy. Topical clotrimazole and povidone–iodine jelly offer minimal benefit. Some concern exists about treating asymptomatic trichomoniasis in pregnancy because one trial found a higher rate of pre-term birth (RR 1.8) compared to control women.[19]

Vulvovaginal Candidiasis

EPIDEMIOLOGY

Vulvovaginal candidiasis (VVC) accounts for approximately one-third of vaginitis cases. In the USA, *Candida* spp. are now the second commonest cause of vaginal infections.[20,21]

It is estimated that 75% of women experience at least one episode of VVC during their child-bearing years, and approximately 40–50% experience a second attack. A small subpopulation of women of undetermined magnitude, probably 5–8% of adult females, suffers from repeated, recurrent, often intractable episodes of *Candida* vaginitis.[22]

Point-prevalence studies indicate that *Candida* spp. may be isolated from the genital tract of approximately 20% of asymptomatic, healthy women of child-bearing age. The natural history of asymptomatic colonization is unknown, although animal and human studies suggest that vaginal carriage continues for several months and perhaps years. Several factors are associated with increased rates of asymptomatic vaginal colonization with *Candida* spp., including pregnancy (30–40%), use of oral contraceptives, uncontrolled diabetes mellitus and frequency of visits to STD clinics. The rarity of isolation of

Candida spp. in premenarchal girls, the lower prevalence of *Candida* vaginitis after menopause and the possible association with hormone replacement therapy emphasize the hormonal dependence of VVC.

PATHOGENESIS AND PATHOLOGY

The organism

Between 85% and 90% of yeast isolated from the vagina are *Candida albicans* strains. The remainder are other species, the commonest of which are *Candida glabrata* and *Candida tropicalis*. Non-*albicans Candida* spp., although less virulent, are capable of inducing vaginitis and are often more resistant to conventional therapy. Some, but not all, recent surveys indicate an increase in VVC caused by non-*albicans Candida* spp., particularly *C. glabrata*.[21,23]

Germination of *Candida* spp. enhances colonization and facilitates tissue invasion. Factors that enhance or facilitate germination (e.g. estrogen therapy and pregnancy) tend to precipitate symptomatic vaginitis, whereas measures that inhibit germination (e.g. bacterial flora and local mucosal cell-mediated immunity) may prevent acute vaginitis in women who are asymptomatic carriers of yeast.

Candida organisms gain access to the vaginal lumen and secretions predominantly from the adjacent perianal area. This finding is borne out by epidemiologic typing studies. *Candida* vaginitis is seen predominantly in women of child-bearing age, and only in the minority of cases can a precipitating factor be identified to explain the transformation from asymptomatic carriage to symptomatic vaginitis in individual patients.

Host factors

A number of host factors are associated with increased asymptomatic vaginal colonization by *Candida* spp. and with *Candida* vaginitis. During pregnancy, the vagina is more susceptible to vaginal infection, resulting in higher incidences of vaginal colonization, vaginitis and lower cure rates. The clinical attack rate is maximal in the third trimester, and symptomatic recurrences are also more common throughout pregnancy. The high levels of reproductive hormones result in a higher glycogen content in the vaginal environment, which provides an excellent carbon source for growth and germination of *Candida* spp. A more common mechanism is where estrogens enhance vaginal epithelial cell avidity for *Candida* spp. adherence, and a yeast cytosol receptor or binding system for female reproductive hormones has been documented. These hormones also enhance yeast mycelial formation. Several studies have shown increased VVC associated with oral contraceptive use[24] and uncontrolled diabetes mellitus. Glucose tolerance tests have been recommended for women with recurrent VVC; however, the yield is low, and testing is not justified in otherwise healthy premenopausal women.

Symptomatic VVC is frequently observed during or after courses of systemic antibiotics. Although no antimicrobial agent is free of this complication, broad-spectrum antibiotics, such as tetracycline, and β-lactams are mainly responsible, and are thought to act by eliminating the normal protective vaginal bacterial flora. The natural flora provides a colonization resistance mechanism and prevents germination of *Candida* spp. The provider of this protective function has been singled out to be *Lactobacillus* spp.[25] *Lactobacillus–Candida* interaction includes competition for nutrients, steric interference with adherence of *Candida* spp. and elaboration of bacteriocins that inhibit yeast proliferation and germination.

Other factors that contribute to an increased incidence of *Candida* vaginitis include the use of tight, poorly ventilated clothing and nylon underclothing, which increase perineal moisture and temperature.

Candida spp. may cause cell damage and resulting inflammation by direct hyphal invasion of epithelial tissue. It is possible that proteases and other hydrolytic enzymes facilitate cell penetration with resultant inflammation, mucosal swelling, erythema and exfoliation of vaginal epithelial cells. The characteristic nonhomogeneous vaginal discharge consists of a conglomerate of hyphal elements and exfoliated nonviable epithelial cells with few PMNs. *Candida* spp. may also induce symptoms by hypersensitivity or allergic reactions, particularly in women with idiopathic recurrent VVC (see Noninfectious vaginitis and vulvitis, below).[18,26]

Oral and vaginal thrush correlate well with depressed cell-mediated immunity in debilitated or immunosuppressed patients.[26] This is particularly evident in patients who have chronic mucocutaneous candidiasis and AIDS.

Pathogenesis of recurrent and chronic *Candida* vaginitis

Careful evaluation (Fig. 49.1) of women with recurrent vaginitis usually fails to reveal any precipitating or causal mechanism.[22] In the past, investigators attributed frequent episodes to repeated fungal re-inoculation of the vagina from a persistent intestinal source or to sexual transmission.[22]

The intestinal theory is based on the report of recovery of *Candida* spp. on rectal culture in almost 100% of women with VVC. Typing of simultaneously obtained vaginal and rectal isolates almost invariably reveals identical strains. This theory has been criticized in the past few years because of lower concordance between rectal and vaginal cultures in patients with recurrent VVC. Moreover, long-term therapy with oral nonabsorbable nystatin is not effective in preventing recurrences. It is likely that frequent relapses of VVC originate from a vaginal and not an intestinal reservoir. Vaginal persistence of *Candida* spp. is a consequence of use of fungistatic and not fungicidal drug therapy.

Although sexual transmission of *Candida* organisms occurs via vaginal intercourse and orogenital contact, the role of sexual re-introduction of yeast as a cause for recurrent VVC is doubtful. Recurrent VVC frequently occurs in celibate women and only a minority of male partners of women who have recurrent VVC are colonized with *Candida* spp. Although most studies aimed at treating male partners have not reduced the frequency of recurrent episodes of vaginitis, in one study reduction was achieved in recurrent VVC by treating colonized male partners.[27]

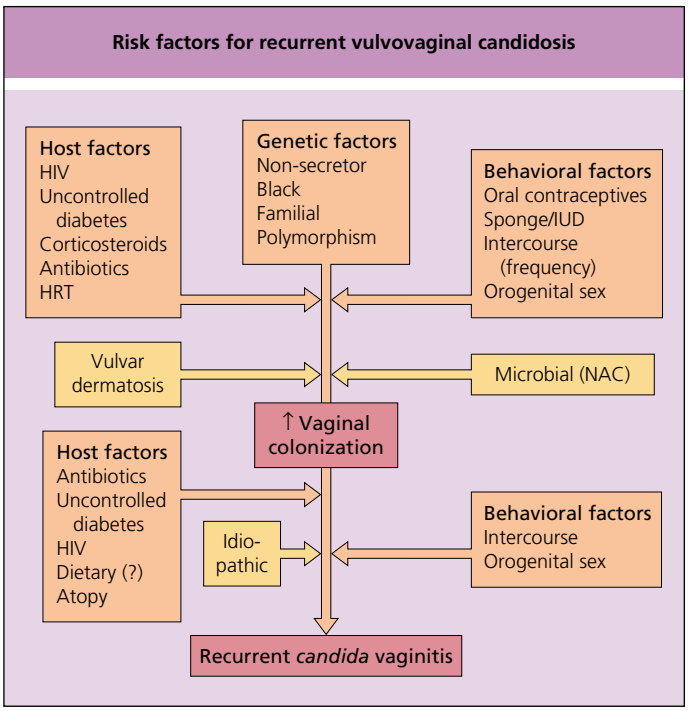


Fig. 49.1 Risk factors for recurrent vulvovaginal candidosis. HRT, hormone replacement therapy; NAC, non-*albicans Candida* spp., IUD, intrauterine device.

Vaginal relapse implies that incomplete eradication or clearance of *Candida* spp. from the vagina occurs after antimycotic therapy. Organisms persist in small numbers in the vagina and result in continued carriage of the organisms, and when host environmental conditions permit, the colonizing organisms increase in number and undergo mycelial transformation, resulting in a new clinical episode.

Whether recurrence is caused by vaginal re-infection or relapse, women with recurrent VVC differ from those with infrequent episodes in their inability to tolerate small numbers of *Candida* organisms re-introduced or persisting in the vagina. On the basis of typing of organisms, women with recurrent and infrequent infection have the same distribution frequency of *Candida* strains as women without symptoms.

Host factors responsible for frequent episodes are not clearly delineated, and more than one mechanism may be operative. There is no evidence of complement, phagocytic cells or immunoglobulin deficiency in these patients. Recurrent VVC is rarely caused by drug resistance.[28]

Current theories about the pathogenesis of recurrent VVC include qualitative and quantitative deficiency in the normal protective vaginal bacterial flora and an acquired, often transient antigen-specific deficiency in T-cell function that similarly permits unchecked yeast proliferation.[26,29] Another theory is that of an acquired acute hypersensitivity reaction to *Candida* antigen, which is accompanied by elevated vaginal titers of *Candida* antigen-specific IgE. This theory has a clinical basis in that patients with recurrent VVC often present with severe vulvar manifestations (rash, erythema, swelling and pruritus) with minimal exudative vaginal changes, little discharge and lower numbers of organisms. Allergic responses to *Candida* spp. have been reported to involve the male genitalia immediately after coitus with a woman infected with *Candida* spp. and are characterized by the acute onset of erythema, edema, severe pruritus and irritation of the penis. As yet, only a minority of women with recurrent VVC have been shown to have elevated *Candida*-specific vaginal IgE. Limited studies using *Candida* antigen desensitization have been found to be helpful in reducing the frequency of recurrent episodes of vaginitis.

Women who are HIV seropositive have higher vaginal colonization rates than seronegative women, but the attack rate of symptomatic VVC appears similar. Reports of chronic, severe recurrent VVC are largely unsubstantiated. Recurrent VVC in the absence of other risk factors for HIV is not an indication for HIV testing.[22]

Recent reports indicate an important role for host genetic factors that explain racial blood group and family predilections to VVC. Genetic factors include quantity of vaginal mannose-binding lectin (MBL) which is reduced in vaginal secretions of women with recurrent VVC. Other genetic factors influence host immune reactivity in response to *Candida* antigen.[30]

PREVENTION

In women with confirmed recurrent VVC linked to frequent courses of systemic antibiotics, prophylactic antimycotics are justified. A useful regimen is fluconazole 150 mg once weekly for the duration of antibiotic therapy. No other dietary or alternative method such as the use of probiotic preparations of lactobacilli has stood the test of time in preventing VVC.[31] In women prone to VVC, avoiding the use of oral contraceptives, intrauterine devices and the contraceptive sponge is prudent.

CLINICAL FEATURES

The most frequent symptom of VVC is vulvar pruritus because vaginal discharge is not invariably present and is frequently minimal.[21] Although described as typically cottage cheese-like in character, the discharge may vary from watery to homogeneously thick. Vaginal soreness, irritation, vulvar burning, dyspareunia and external dysuria are commonly present. Odor, if present, is minimal and nonoffensive. Examination frequently reveals erythema and swelling of the labia and vulva (Fig. 49.2), often with discrete pustulopapular peripheral lesions and linear fissures (Fig. 49.3). The cervix is normal and vaginal mucosal erythema with adherent whitish discharge is present. Characteristically, symptoms are exacerbated in the week before the onset of menses, with some relief with the onset of menstrual flow.



Fig. 49.2 Typical *Candida* vulvovaginitis with bilateral symmetric erythema and edema of vestibule and labia.



Fig. 49.3 Severe *Candida* vulvovaginitis with bilateral painful fissure formation in the vulva.

DIAGNOSIS

The relative lack of specificity of symptoms and signs precludes a diagnosis that is based only on history and physical examination. Most patients with symptomatic VVC may be readily diagnosed on the basis of simple microscopic examination of vaginal secretions and finding of a normal pH. A wet mount or saline preparation has a sensitivity of 40–60%. The 10% potassium hydroxide preparation is more sensitive in diagnosing the presence of germinated yeast. A normal vaginal pH (4.0–4.5) is found in *Candida* vaginitis, and the finding of a pH in excess of 4.5 should suggest the possibility of bacterial vaginosis, trichomoniasis or a mixed infection.[21]

Although routine fungal cultures are unnecessary, vaginal culture should be performed in the presence of negative microscopy in a symptomatic patient. The Papanicolaou smear is unreliable, being insensitive and positive in only about 25% of cases. There is no reliable serologic technique for the diagnosis of *Candida* vaginitis. Unfortunately, no reliable laboratory test based upon rapid antigen detection is available commercially. DNA probes are useful and reliable but not widely available.

MANAGEMENT

Topical agents for acute *Candida* vaginitis

Antimycotics are available for local use as creams, vaginal tablets, suppositories and coated tampons (Table 49.3). There is little to suggest that the formulation of the topical antimycotic influences clinical efficacy.[22] Extensive vulvar inflammation dictates local vulvar application of antimycotic cream and, if severe, in combination with a topical steroid.[32]

The average mycologic cure rate of 7- and 14-day courses of nystatin is 75–80%. Azoles appear to achieve slightly higher clinical mycologic cure rates than the polyenes (nystatin): 85–90%. Although many studies have compared the clinical efficacies of the various azoles, there is little evidence that any one azole agent is superior to others.[33] Topical azoles are remarkably free of local and systemic side-effects; nevertheless, the initial application of topical agents is not infrequently accompanied by local burning and discomfort.

Systemic agents for acute *Candida* vaginitis

There has been a major trend toward shorter treatment courses with progressively higher antifungal doses, culminating in highly effective single-dose topical regimens. Although short-course regimens are effective for mild and moderate vaginitis, cure rates for severe and complicated vaginitis are lower.

Oral systemic azoles available for the treatment of VVC include ketoconazole 400 mg every 12 hours for 5 days, itraconazole 200 mg/day for 3 days (or every 12 hours single-day regimen) and, finally, fluconazole 150 mg single dose.[34] All the oral regimens achieve clinical cure rates in excess of 80%. Oral regimens are generally preferred by women because of convenience and lack of local side-effects. None of the systemic regimens is approved for use during pregnancy. Hepatotoxicity with ketoconazole precludes its widespread use in VVC.[33]

Table 49.3 Therapy for vaginal candidiasis – topical agents

TOPICAL AGENTS		
Drug	**Formulation**	**Dosage regimen**
*Butoconazole	2% cream	5 g q24h for 3 days
*Clotrimazole	1% cream 100 mg vaginal tablets 100 mg vaginal tablets 500 mg vaginal tablets	5 g q24h for 7–14 days 1 tablet q24h for 7 days 2 tablets q24h for 3 days 1 tablet, single dose
*Miconazole	2% cream 100 mg vaginal suppository 200 mg vaginal suppository 1200 mg vaginal suppository	5 g q24h for 7 days 1 suppository q24h for 7 days 1 suppository q24h for 3 days 1 suppository, single dose
Econazole	150 mg vaginal tablet	1 tablet q24h for 3 days
Fenticonazole	2% cream	5 g q24h for 7 days
*Tioconazole	2% cream 6.5% cream	5 g q24h for 3 days 5 g, single dose
Terconazole	0.4% cream 0.8% cream 80 mg vaginal suppository	5 g q24h for 7 days 5 g q24h for 3 days 80 mg q24h for 3 days
Nystatin	100 000 U vaginal tablets	1 tablet q24h for 14 days

*Drugs available over the counter, without prescription.

Table 49.4 Classification of vulvovaginal candidiasis

Uncomplicated	Complicated
Candida albicans + Infrequent episodes + Mild-to-moderate vaginitis + Normal host	Non-*albicans Candida* spp. Resistant *Candida albicans* (rare) *or* History of recurrent vulvovaginal candidiasis *or* Severe vulvovaginal candidiasis *or* Abnormal host, for example, uncontrolled diabetes, pregnancy, immunocompromised

Vulvovaginal candidiasis is classified as uncomplicated or complicated on the basis of the likelihood of achieving clinical and mycologic cure with short-course therapy (Table 49.4). Uncomplicated VVC represents by far the most common form of vaginitis seen, is caused by highly sensitive *C. albicans* and, provided that the severity is mild to moderate, patients respond well to all topical or oral antimycotics, including single-dose therapy. In contrast, patients who have complicated VVC have a relatively resistant organism, a host factor or a severity of infection that dictates more intensive and prolonged therapy lasting 7–14 days. Most non-*albicans Candida* infections respond to conventional topical or oral antifungals provided they are administered for sufficient duration. However, vaginitis caused by *C. glabrata* often fails to respond to azoles and may require treatment with vaginal capsules of boric acid 600 mg/day for 14 days.[35] Patients with severe vulvovaginitis require more prolonged systemic or topical therapy. The former can be provided by a second dose of fluconazole 150 mg, 72 hours after the first dose.[36]

Treatment of recurrent vulvovaginal candidiasis

The management of women who have recurrent VVC aims at control rather than cure. The clinician should first confirm the diagnosis of recurrent VVC. Uncontrolled diabetes mellitus must be controlled and use of corticosteroids or other immunosuppressive agents should be discontinued where possible. Unfortunately, in the majority of women with recurrent VVC, no underlying or predisposing factor can be identified. Recurrent VVC requires long-term maintenance with a suppressive prophylactic regimen. Because of the chronicity of therapy, the convenience of oral treatment is apparent, and the best suppressive prophylaxis has been achieved with weekly oral fluconazole at a dosage of 150 mg.[37] An effective topical prophylactic regimen consists of weekly vaginal suppositories of clotrimazole 500 mg.[21,22]

Atrophic Vaginitis

Clinically significant atrophic vaginitis is quite rare, and the majority of women with mild-to-moderate atrophy are asymptomatic. Because of reduced endogenous estrogen, the epithelium becomes thin and lacking in glycogen, which contributes to a reduction in lactic acid production and an increase in vaginal pH. This change in the environment encourages the overgrowth of nonacidophilic coliform organisms and the disappearance of *Lactobacillus* spp. Despite these major but usually gradual changes, symptoms are mostly absent, especially in the absence of coitus.

With advanced atrophy, symptoms include vaginal soreness, dyspareunia and occasional spotting or discharge. Burning is a frequent complaint and is often precipitated by intercourse. The vaginal mucosa is thin, with diffuse redness, occasional petechiae or ecchymoses with few or no vaginal folds. Vestibular atrophy may also be apparent and discharge may be serosanguinous, thick or watery, and the pH of the vaginal secretions ranges from 5.5 to 7.0. The wet smear frequently shows small, round epithelial or parabasal cells which represent immature squamous cells that have not been exposed to sufficient estrogen. The *Lactobacillus* spp. dominated flora is replaced by mixed flora of Gram-negative rods. Bacteriologic cultures in these patients are unnecessary, and can be misleading.

The treatment of atrophic vaginitis consists primarily of topical vaginal estrogen. Nightly use of half or all the contents of an applicator for 1–2 weeks is usually sufficient to alleviate the atrophic vaginitis.

Noninfectious Vaginitis and Vulvitis

Women frequently present with acute or chronic vulvovaginal symptoms caused by noninfectious etiologies. Symptoms are indistinguishable from those of infectious syndromes, but are most commonly confused with those of acute *Candida* vaginitis, including pruritus, irritation, burning, soreness and variable discharge.

Noninfectious causes include irritants [physical (e.g. minipads) or chemical (e.g. spermicides, povidone–iodine, topical antimycotics, soaps and perfumes, topical 5-fluorouracil)] and allergens, which are responsible for immunologic acute and chronic hypersensitivity reactions, including contact dermatitis (e.g. latex condoms, antimycotic creams). An enormous list of topical factors responsible for local inflammatory reactions and symptoms exists and many more have yet to be defined. Depending on the site of contact, symptoms may be vaginal or vulvar. Included in this category are systemic dermatoses that may present in the vulva (e.g. psoriasis, Fig. 49.4) or vulva-specific dermatosis (e.g. lichen sclerosus).

A noninfectious mechanism may coexist with or follow an infectious process, and should be considered when the three common infectious causes and hormone deficiency are excluded and in the presence of a normal vaginal pH, normal saline and potassium hydroxide microscopy, and, ultimately, a negative yeast culture. Unfortunately, given the anticipated 20% colonization rates in normal asymptomatic women, occasionally a positive yeast culture in a symptomatic patient reflects the presence of an innocent bystander and not the cause of the vulvovaginal symptoms. The only logical way of establishing the role of *Candida* spp. in this context is to treat with an oral antifungal agent and assess the clinical response.



Fig. 49.4 Vulvar psoriasis resulting in pruritus vulva and misdiagnosed as *Candida* vulvovaginitis.

Once a local chemical irritant or allergic reaction is suspected, a detailed inquiry into possible causal factors is essential. Offending agents or behaviors should be eliminated wherever possible, including avoiding chemical irritants and allergens (e.g. soaps, detergents). The immediate management of severe vulvovaginal symptoms of noninfectious etiology should not rely on topical corticosteroids, which are rarely the solution and frequently high-potency corticosteroid creams cause intense burning. Local relief measures include sodium bicarbonate sitz baths and oral antihistamines.

A syndrome of hyperacidity of the vagina causing overgrowth of lactobacilli has been described but not confirmed. A rebound increase in population numbers of lactobacilli is thought to occur after completion of topical antimycotics and is alleged to suppress population numbers of healthy resident flora. The proposed syndrome of cytolytic vaginosis is characterized by vulvovaginal burning, irritation, soreness and dyspareunia, and is usually incorrectly diagnosed as VVC. The finding of large numbers of lactobacilli on wet count and a low pH, together with extensive squamous epithelial cell cytolysis is said to confirm the diagnosis. Recommended therapy for cytolytic vaginosis is daily alkaline douching using sodium bicarbonate to elevate the low vaginal pH and suppress growth of lactobacilli.

Vulvitis

Most of the important infectious and noninfectious causes of vulvitis have been described in the section on vaginitis. Human papillomavirus and genital herpes are described in Chapters 59 and 58, respectively. Bacterial vulvitis due to streptococci, anaerobes and Gram-negative rods occurs infrequently and should be diagnosed by clinical features and bacterial culture. Specific antimicrobial treatment is indicated for patients with the diagnosis of bacterial vulvitis. Occasionally a Bartholin's abscess creates a painful swelling in the vulva. This can be due to *N. gonorrhoeae* or to a variety of pathogens, particularly Gram-positive organisms. A Bartholin's cyst infection should be treated with appropriate antibiotics and occasionally may require drainage. Other causes of vestibulitis or vulvitis include lichen sclerosus, erosive lichen planus and theoretically any cause of dermatosis or dermatitis such as psoriasis (see Fig. 49.4), eczema and pemphigus.

Cervicitis

EPIDEMIOLOGY

The presence of a purulent exudate in the cervical os has been highly associated with cervical infection with *Chlamydia trachomatis*, *N. gonorrhoeae*, herpes simplex virus and cytomegalovirus. Infection with *T. vaginalis* correlates with colpitis macularis and inflammatory changes of the ectocervix.[38,39] Not infrequently, mucopurulent endocervicitis or ectocervicitis is seen in the absence of these pathogens, indicating that additional, as yet unrecognized causes exist. A role for disruption of vaginal flora, specifically overgrowth of anaerobes as typified in bacterial vaginosis in causing cervical inflammation has been proposed. Rare causes of cervicitis include *Mycobacterium tuberculosis* and *Actinomyces israelii*, the latter almost invariably in the presence of intrauterine devices. Although the most important and prevalent infection of the cervix is undoubtedly human papillomavirus, this virus does not cause cervicitis and is discussed in Chapter 156. To the growing number of etiologic agents implicated in cervicitis, must be added *Mycoplasma genitalium*.[40]

The prevalence of genital chlamydial infection ranges from 8 to 40%.[41] Risk factors include young age, unmarried status, lower socioeconomic conditions, number and recent change of sexual partner, ectopy, oral contraceptive use and concurrent gonococcal infection; the last may reactivate latent chlamydial infection and increases shedding of chlamydia from the endocervix.[42] Risk factors for gonococcal mucopurulent cervicitis are

identical to those of *Chlamydia* spp. but also include urban dwelling, prostitution, illicit drug use and minority racial status. Up to 60% of women with *N. gonorrhoeae* have co-infection with chlamydia.[41] Herpetic cervicitis is rare in the absence of genital lesions and is most commonly associated with first episode, primary disease with an 80% viral isolation rate.[39] Cytomegalovirus is thought to be responsible for approximately 5% of cases of cervicitis and is usually asymptomatic. When it is isolated from cervical secretions, the detection may not imply a causal relationship with present pathology.

CLINICAL FEATURES

Cervicitis is frequently asymptomatic and is detected on routine pelvic examination. Alternatively, cervical inflammation is recognized because of signs and symptoms of concomitant infection (e.g. vaginal trichomoniasis, genital herpes or salpingitis). Mucopurulent cervicitis may result in a purulent vaginal discharge in its own right. Accordingly, cervical speculum evaluation should be an essential part of vaginal examination in women with an abnormal discharge. Mucopurulent endocervicitis results in swelling and erythema of the zone of ectopy, associated with friability, contact bleeding, spotting and a yellow or green endocervical exudate. The purulent discharge is best appreciated by obtaining an endocervical swab specimen and observing the latter against a white background.

Trichomoniasis is associated with ectocervical squamous epithelial mucosal inflammation, giving the cervix a 'strawberry' appearance due to microscopic focal patchy petechiae (colpitis macularis) in 5–20% of patients.[15] Primary herpes cervicitis may be associated with severe necrosis that is reminiscent of cervical cancer. Most commonly, primary herpetic cervicitis is characterized by increased surface vascularity, and micro- and macro-ulcerations with and without necrotic areas. Asymptomatic shedding of herpesvirus occurs in the absence of cervical lesions.

DIAGNOSIS

Mucopurulent cervicitis is confirmed when a Gram-stained specimen of green or yellow endocervical exudate reveals more than 30 PMNs per high power field.[1] Microscopic examination of cervical mucus from a patient with mucopurulent endocervicitis reveals an overabundance of inflammatory cells, obliterating the background ferning pattern. A similar excess of inflammatory cells can be found in a Papanicolaou smear. These two microscopic studies are not reliable in identifying the underlying cause of mucopurulent cervicitis. For a diagnosis of *C. trachomatis* cervicitis, culture techniques to identify the obligate intraparasites served as the gold standard in the past. Now the development of the more widely available enzyme-linked immunosorbent assay antigen detection tests has been replaced by highly sensitive DNA amplification techniques, particularly ligase chain reaction, allowing diagnosis not only from cervical specimens, but also by screening urine specimens. Gram stain of cervical mucus may reveal intracellular Gram-negative diplococci, but has low sensitivity and specificity in the diagnosis of gonococcal cervicitis. Diagnosis relies mainly upon culture of the endocervix using a modified Thayer–Martin medium; however, diagnostic methodologies now include use of DNA probes, especially for screening purposes, given the high sensitivity of these newer techniques.

Although Papanicolaou smears in herpetic cervicitis are useful in revealing multinucleated giant cells, viral culture and fluorescein-conjugated monoclonal antibodies are the mainstay of clinical diagnosis; polymerase chain reaction is used for monitoring asymptomatic viral shedding in a research context.

The clinical differentiation of the various causes of cervicitis is not possible, but requires the aforementioned diagnostic tests, recognizing that frequently more than one etiologic agent may be present simultaneously because many of the pathogens share risk factors and behavior. The most important diagnostic problem is that of overdiagnosis of cervicitis. All too frequently physiologic changes in the appearance of the cervix, in spite of the use of colposcopy, are interpreted as reflecting pathologic cervicitis. Regrettably, after failed attempts to identify pathogenic micro-organisms, patients are needlessly treated with cervical ablative techniques. Cervical ectopy is often mistaken for cervicitis with eversion of endocervical columnar cells, and is commonly seen in women on oral contraceptives.[42] Other physiologic changes related to childbirth and dilatation of the cervical canal are mistakenly diagnosed as cervicitis.

Equally important is the failure to recognize that a friable, abnormal cervix may reflect dysplasia and neoplasia. If the Papanicolaou smear reports inflammatory cells with or without atypia, the presence of atypical squamous cells of undetermined significance (ASCUS) should be considered in the differential diagnosis between a benign change in reaction to a stimulus and a low-grade squamous intraepithelial lesion. Accordingly, women with a Papanicolaou smear showing ASCUS should have their smears repeated. Persistence of an ASCUS smear should prompt colposcopy.

MANAGEMENT

Antimicrobial regimens for infectious cervicitis are provided in the chapters on pelvic inflammatory disease, gonorrhea and chlamydial infections (see Chapters 50 and 177; and Practice Point 29).

REFERENCES


References for this chapter can be found online at http://www.expertconsult.com

Chapter 50

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Infections of the female pelvis including septic abortion

Infections of the female pelvis constitute a diverse group. This chapter considers three groups of infections: pelvic inflammatory disease (PID), postpartum and postabortal infections (including postpartum endometritis and Caesarian section, episiotomy infections and postabortion sepsis) and postsurgical gynecologic infections.

Pelvic Inflammatory Disease

EPIDEMIOLOGY

Pelvic inflammatory disease (PID) refers to an acute clinical syndrome that results when vaginal or cervical organisms ascend into the upper structures of the female reproductive tract unrelated to pregnancy or surgery.[1] Depending on which areas are infected, PID may manifest itself as endometritis, parametritis, salpingitis, oophoritis, pelvic peritonitis, tubo-ovarian abscess, periappendicitis, perihepatitis (Fitz-Hugh–Curtis syndrome) and perisplenitis.

Pelvic inflammatory disease and sexually transmitted diseases (STDs) share many of the same risk factors and, in the USA, 40–80% of PID is attributed to STDs. Bacterial vaginosis (BV) is commonly found in women with PID.[2] Identified risk factors for PID include younger age, unmarried status, lower socioeconomic status, sexual behavior (number of sexual partners, age of sexual debut, rate of acquiring new partners), substance abuse, poor health-care behavior (treatment-seeking and compliance with treatment instructions), douching and intrauterine device insertion.[1,3] In studies from the USA and Europe, about three-quarters of women who had PID were under 25 years of age, and about one-half had never been pregnant.[1] Oral contraceptive users tend to have clinically and laparoscopically milder infection than do nonusers, and oral contraceptives appear to protect against chlamydial PID.[4]

PATHOGENESIS AND PATHOLOGY

The vast majority of PID cases result from a direct canalicular spread of organisms from the endocervix to the mucosa of the endometrium and fallopian tubes, although the precise mechanisms are poorly understood. Postinfectious scarring (e.g. intratubal adhesions, tubal occlusion, peritubal scarring and damaged fimbrial ostia) results in the long-term sequelae of PID.

Occurrence of PID is described only among sexually active women, and its risk is associated with the number of sexual partners and the frequency of sexual acts among women with only one sexual partner. Multiple organisms have been implicated as etiologic agents of PID (Table 50.1). The rates of isolation of these organisms are variable. They may vary with geographic region, duration of infection and the site of sampling (i.e. cervix, fallopian tubes or endometrium). The most commonly recovered organisms are *Neisseria gonorrhoeae* and *Chlamydia trachomatis*, followed by other aerobic and anaerobic bacteria associated with BV (e.g. *Gardnerella vaginalis*, *Mycoplasma hominis*, *Prevotella bivia* and *Porphyromonas*, *Prevotella* and *Peptostreptococcus* spp.). *Mycoplasma genitalium* may also be an etiologic agent of PID. Rates of isolation of 10–20% in the upper genital tract, and as high as 85% (5–27% in studies from Europe versus 44–70% in those from North America) in the lower genital tract, have been reported for *N. gonorrhoeae*. For *C. trachomatis*, rates of 1.2–31% in the upper genital tract and 31% in the lower genital tract have been reported.[1,2,5] It is estimated that between 10% and 40% of women who have untreated gonococcal or chlamydial cervical infection will develop acute upper tract infection. Finally, *M. genitalium* can be found in up to 14% of women with PID.[6] However, PID is a polymicrobial infection, even in the setting of gonococcal or chlamydial cervicitis. It has been suggested that, in many cases of PID, STD organisms initiate the inflammation of the tubal mucosa and this process facilitates the invasion of the mucosa by organisms endogenous to the lower genital tract.[1]

Table 50.1 Common etiologic agents of pelvic inflammatory disease

Aerobic bacteria	*Neisseria gonorrhoeae* *Chlamydia trachomatis* *Gardnerella vaginalis* *Escherichia coli* *Streptococcus* spp. *Haemophilus influenzae*
Anaerobic bacteria	*Bacteroides* spp. *Peptostreptococcus* spp. *Peptococcus* spp. *Prevotella* spp. *Porphyromonas* spp.
Mycoplasmas	*Mycoplasma hominis* *Mycoplasma genitalium*

Data from Weström & Eschenbach.[1]

PREVENTION

Prevention is directed at reducing a woman's risk of acquiring an STD, and the detection and treatment of lower genital tract infections. Women screened and treated for asymptomatic chlamydial infection

were nearly 60% less likely than unscreened women to develop PID.[7] Prompt and correct treatment of upper tract infections will ameliorate some of the long-term sequelae.

CLINICAL FEATURES

The most common clinical complaint in a woman who has PID is bilateral lower abdominal or pelvic pain. Other complaints include an abnormal vaginal discharge, dyspareunia, dysmenorrhea and abnormal uterine bleeding. In more severe cases, nausea, vomiting, fever and diffuse abdominal pain may occur. The hallmark findings of PID are cervical motion tenderness and uterine or adnexal tenderness in the absence of other potential cause of the illness. Whereas gonococcal PID tends to have an abrupt, fulminant presentation within 1 week of the onset of menses, chlamydial PID is characterized by a subacute course with mild symptoms, often described as just a dull pain, and mild findings. It is not unusual for a woman who has chlamydial PID to be unaware of her infection and to present as a relatively asymptomatic contact of a male with urethritis. The clinician should be alert to adnexal masses or fullness as the major signs of chlamydial infection. Women who have HIV infection and PID may present with more clinically severe disease.[8]

The common complications and sequelae of PID include ectopic pregnancy, tubal infertility, recurrent PID, chronic abdominal pain, tubo-ovarian abscesses and pelvic adhesions. A woman's risk of ectopic pregnancy increases seven- to 10-fold after an episode of PID. The number of episodes of PID, the woman's age and the severity of tubal inflammation determined at laparoscopy influence the fertility prognosis of PID.[1] It appears that PID caused by chlamydial infection may result in more infertility than PID caused by gonococcal infection. Recurrent pelvic infections will develop in up to one-third of women who have had PID. Chronic abdominal pain lasting more than 6 months occurs in 15–18% of women after PID. Pyosalpinx, tubo-ovarian abscesses and pelvic adhesions occur in 15–20% of women who have had PID and often require surgical intervention. Mortality from acute PID is rare. The most common cause of death from PID is a ruptured tubo-ovarian abscess with subsequent peritonitis. The mortality rate from this complication of PID is 6–8%.

DIAGNOSIS

The clinical diagnosis of PID is imprecise. The traditional clinical signs and symptoms of PID are neither sensitive nor specific for the syndrome. Laparoscopy confirms salpingitis in 45–89% of women who have clinically diagnosed PID. Of women who have acute clinical PID, 6–45% have normal fallopian tubes and 5–33% have other conditions, including ectopic pregnancy, appendicitis, hemorrhagic ovarian cysts, endometritis, pelvic adhesions and torsion of an adnexal structure.[5,9] False-negative clinical diagnoses for PID range from 16% to 47%.[9] The diagnoses prior to laparoscopy in women who have laparoscopically confirmed PID included ectopic pregnancy, ovarian cyst, hemorrhagic ovarian cyst, endometriosis, fibroids, ovarian tumor, appendicitis, pyelonephritis and uncertain diagnosis. Although laparoscopy can be used to obtain a more accurate diagnosis, it is neither readily available in most cases nor justifiable in clinically mild disease.

Women who have lower abdominal tenderness, adnexal tenderness or cervical motion tenderness – the Centers for Disease Control minimum criteria – should be treated for PID if there is no other diagnosis that should be considered.[10] One analysis on a large number of women enrolled in a clinical trial showed that lower abdominal tenderness alone was the most sensitive of the physical findings.[11] However, many clinicians may be uncomfortable treating for PID based on history and physical examination alone.

Tests to document an inflammatory and infectious process in the lower genital tract can be used to increase the sensitivity of the clinical signs. A pregnancy test should be done to make sure that the pain is not caused by an ectopic pregnancy. Within the setting of a patient with lower abdominal or pelvic tenderness, the presence of an increased number of leukocytes (a ratio of more than 1:1 white blood cells:vaginal epithelial cells) or findings consistent with BV on a wet mount are considered supportive of a diagnosis of PID. Similarly, an endocervical Gram stain, obtained after cleaning the ectocervix, showing Gram-negative intracellular diplococci, 30 or more polymorphonuclear leukocytes per high power field, or a positive test (culture or PCR) for either *N. gonorrhoeae* or *C. trachomatis* are also useful in establishing the diagnosis. Erythrocyte sedimentation rate, C-reactive protein and complete white blood cell count may also be useful. Pelvic and endovaginal ultrasound can detect findings consistent with severe PID, including tubo-ovarian abscesses, dilated fallopian tubes and cul-de-sac fluid. Ultrasound is less useful in mild or atypical clinical presentations. CT scanning has the same limitations as ultrasound. However, in severe cases of PID with atypical ultrasound findings CT can be useful.[12] For example, spiral CT scanning optimizes identification of small air bubbles that are specific for abscess.[13]

More invasive tests may also be considered in a woman where PID is suspected. Endometrial biopsy documenting endometrial neutrophils or plasma cells confirms the diagnosis but requires at least 24 hours for processing. Purulent material from the peritoneal cavity obtained by culdocentesis, a painful procedure, may support the diagnosis of PID but may also occur with other intra-abdominal infections such as appendicitis. Laparoscopy is the gold standard for the diagnosis and staging of acute PID. The minimum criteria for visual confirmation of PID include hyperemia of the tubal surface, edema of the tubal wall and a sticky exudate on the tubal surface and from the fimbriated end when patent.[14]

MANAGEMENT

Management of a patient who has PID includes therapy, education, careful follow-up and partner management. The goal is to cure the patient, prevent recurrences and, ultimately, to preserve fertility. Empiric treatment should be instituted as soon as the diagnosis is suspected. Based on the polymicrobial nature of PID, therapy must provide broad-spectrum coverage. Several antimicrobial regimens have proved to be highly effective in achieving clinical cure of PID.[10] Studies from the pre-antibiotic era documented infertility rates after PID of 60–70%, indicating that prompt institution of antimicrobial therapy does influence fertility outcome.[1]

Pelvic inflammatory disease should always be treated with a minimum of two antibiotics for at least 10–14 days (Table 50.2). The combination regimen of an extended-spectrum parenteral cephalosporin plus doxycycline provides good coverage for most potential pathogens, including β-lactamase-producing strains. An alternative parenteral inpatient regimen is the combination of clindamycin plus an aminoglycoside. Oral regimens listed in Table 50.2 also provide excellent coverage. However, with recent data suggesting that quinolone-resistant *N. gonorrheae* (QRNG) is becoming more common, quinolones are no longer recommended for first-line therapy unless QRNG is not suspected as being involved, based on either negative testing or low local prevalence of this organism.[15]

Recent data from a large randomized clinical trial comparing inpatient and outpatient therapy indicate that outpatient therapy is associated with similar short- and long-term treatment outcomes in women with mild to moderate PID.[16] Hospitalization is recommended for women whose tolerance or compliance with outpatient regimens is uncertain, or who have factors that complicate treatment, severe illness or an uncertain diagnosis (Table 50.3).

Supportive therapy includes hydration, bed rest in the semi-Fowler position to localize the infection to the pelvis, pelvic rest and pain relief. The patient should abstain from sexual intercourse until test-of-cure studies and resolution of signs and symptoms. All sexual partners in the previous 30 days should be evaluated and presumptively treated for gonococcal and chlamydial infection. Explicit and clear patient education cannot be overemphasized in the treatment of PID, especially in the context of outpatient management.

Table 50.2 Recommended treatment of pelvic inflammatory disease

Oral treatment		
Regimen A	With or without	Ofloxacin 400 mg po q24h for 14 days, or Levofloxacin 500 mg po q24h for 14 days Metronidazole 500 mg po q12h for 14 days
Regimen B	Either	Ceftriaxone 250 mg im, or Cefoxitin 2 g im plus probenecid 1 g po in a single dose concurrently, or Other parenteral third-generation cephalosporin (e.g. ceftizoxime or cefotaxime)
	Plus	Doxycycline 100 mg po q12h for 14 days
	With or without	Metronidazole 500 mg po q12h for 7 days
Parenteral treatment		
Regimen A*	Either	Cefotetan 2 g iv q12h, or Cefoxitin 2 g iv q6h
	Plus	Doxycycline 100 mg iv or po q12h
Regimen B	Plus	Clindamycin 900 mg iv q8h Gentamicin loading dose iv or im (2 mg/kg body weight) followed by a maintenance dose of 1.5 mg/kg; single daily dosing may be substituted

Parenteral regimens should be continued for 48 hours after substantial clinical improvement. Doxycycline 100 mg po q12h or clindamycin 450 mg po q6h should then be administered for a total of 14 days.
*This regimen should not be used if quinolone-resistant *N. gonorrheae* is suspected.
Data from Centers for Disease Control and Prevention.[10]

Table 50.3 Recommendations for hospitalizing patients who have pelvic inflammatory disease

Uncertain tolerance or compliance with outpatient regimen	Substance abusers Nausea and vomiting Follow-up at 72 hours after starting antibiotic treatment is problematic
Complicating factors	Pregnancy Suspected pelvic or tubo-ovarian abscess
Severe illness	Temperature over 101°F (38.3°C) White blood cell count >15 000/ml Peritoneal signs Septic
Uncertain diagnosis	Failure to respond clinically to outpatient treatment Inability to exclude surgical emergencies (ectopic pregnancy, appendicitis)

The incidence of ascending infection among pregnant women who have intact membranes is unknown but is believed to be rare.[17] However, given the high-risk of miscarriage and pre-term delivery associated with PID, pregnant women who have PID must be hospitalized and given parenteral antibiotic therapy.[10]

Women who have tubo-ovarian abscesses should be hospitalized and begun on broad-spectrum antibiotics (aminoglycoside plus clindamycin or metronidazole). The vast majority of abscesses with a diameter of 4–6 cm respond to antibiotics alone, whereas only 40% of those that are 10 cm or larger respond to medical therapy alone.[18] Increasing abscess size or failure to defervesce 72 hours after administration of antibiotics suggests medical failure and requires surgical intervention (e.g. percutaneous or transvaginal drainage under sonographic guidance, laparoscopic drainage or laparotomy). Leaking or ruptured abscesses require immediate laparotomy after stabilization of the patient. Extensive surgery such as complete hysterectomy is rarely indicated except in life-threatening complications such as extensive necrotic myometrium.

Postpartum Endometritis and Caesarian Section

EPIDEMIOLOGY

Postpartum endometritis, an infection of the uterus, is the most common cause of maternal postpartum fever and includes the inflammatory conditions of endometritis, endomyometritis and endoparametritis. It can be categorized into early infection (i.e. onset within 48 hours of delivery) and late infection (i.e. onset 2 days to 2 weeks after delivery). The most significant risk factor for postpartum endometritis is Caesarian section; the incidence of postpartum endometritis after vaginal delivery is 2–5% whereas the rate after Caesarian section ranges from 20% to 55%.[19] Postpartum upper tract infections following vaginal delivery are about 10 times more common in developing countries than in developed countries as a result of unclean delivery practice, traditional birth practices and the high prevalence of STDs in some populations.[20]

PATHOGENESIS AND PATHOLOGY

Early postpartum endometritis usually is associated with nonelective Caesarian section and is probably the result of direct uterine contamination by organisms in the amniotic cavity. This is in direct contrast to women who develop late postpartum endometritis, which usually develops after vaginal delivery. The timing of these late infections suggests an ascending infection similar to the mechanisms for PID. Wound infections after Caesarian section appear to be the result of a direct contamination of the wound by organisms in the endometrium at the time of surgery.

Postpartum endometritis is a mixed aerobic–anaerobic infection (Table 50.4). *Ureaplasma urealyticum* and *Mycoplasma hominis* have also been isolated from the endometrium and blood of infected women, but their clinical significance is not clear.[21] *Chlamydia trachomatis* is associated with the late form of postpartum endometritis.[22] Group A β-hemolytic streptococcal endometritis is rare and clustered cases are probably related to a common source, often a caregiver. Herpes simplex endometritis has also been reported.[23] Bacteremia occurs in 10–20% of patients and most common blood isolates are group B streptococci, *Gardnerella vaginalis* and *Peptostreptococcus* spp.

PREVENTION

The timely diagnosis and treatment of lower tract syndromes during pregnancy, especially bacterial vaginosis, may prevent some postpartum endometritis. However, the only intervention which clearly reduces the risk of endometritis is the use of prophylactic antibiotics for patients

Table 50.4 Common endogenous micro-organisms identified as potential etiologic agents in postoperative pelvic infections

Aerobic bacteria	*Streptococcus* spp. *Enterococcus* spp. *Staphylococcus aureus* *Staphylococcus epidermidis* *Escherichia coli* *Klebsiella pneumoniae* *Gardnerella vaginalis*
Anaerobic bacteria	*Bacteroides* spp. *Peptostreptococcus* spp. *Prevotella bivia* *Prevotella disiens* *Fusobacterium* spp.
Mycoplasmas	*Mycoplasma hominis* *Ureaplasma urealyticum*

requiring a nonelective Caesarian section after labor or rupture of membranes, with a two-thirds to three-quarters reduction in infection.[19,24,25]

CLINICAL FEATURES

Risk factors for postpartum endometritis include duration of labor, length of time membranes remain ruptured, presence of STDs, presence of BV, the number of vaginal examinations, the use of internal fetal monitoring and socioeconomic status.[21,26,27]

Postpartum endometritis should be suspected in any woman who develops significant fever (oral temperature 101.3°F (38.5°C) or higher in the first 24 hours after delivery or 100.4°F (38°C) or higher for at least 4 consecutive hours, 24 hours or more after delivery). The diagnosis of postpartum endometritis can be made on the basis of clinical features of fever and when signs which suggest an endometrial inflammatory process, such as abdominal pain, uterine tenderness, foul lochia, increased uterine bleeding and uterine subinvolution, are present. Late-onset endometritis tends to have a mild, subacute clinical presentation.

Acute complications of postpartum endometritis include pelvic abscess and puerperal ovarian vein thrombophlebitis. Puerperal ovarian vein thrombophlebitis is an acute thrombosis of one or both ovarian veins postpartum, is usually associated with postpartum endometritis with an onset of 2–4 days after delivery, and can be associated with septic pulmonary emboli. The reported incidence is 1 in 2000 deliveries.[28] Chronic complications and sequelae result from postinfectious scarring.

DIAGNOSIS

Blood cultures should be obtained from all patients before starting therapy. Tests for the detection of cervical infection with *N. gonorrhoeae* and *C. trachomatis* should be obtained from all women at risk of STDs. Quantitative endometrial cultures obtained using a triple lumen catheter, which minimizes contamination, may provide useful information but is rarely used in clinical practice. A complete white blood cell count should be done. If an adnexal mass is felt on examination, then ultrasound or CT can be used to confirm the diagnosis.

MANAGEMENT

Postpartum endometritis is commonly treated parenterally with a broad-spectrum antibiotic regimen with activity against *Bacteroides fragilis* and other penicillin-resistant anaerobic bacteria, such as second-generation cephalosporins (cefoxitin or cefotetan) or the extended-spectrum penicillins (ticarcillin–clavulanate or sulbactam–ampicillin).[27] The combination of aminoglycoside plus clindamycin remains an appropriate regimen.[29] Once-daily administration of aminoglycosides appears to be effective and safe in the treatment of endometritis.[29]

Women should continue to receive parenteral therapy until fever has resolved, uterine tenderness and abdominal pain are gone, and white blood cell count has normalized. Subsequent oral antibiotic therapy with erythromycin or doxycycline is only indicated in women who require it for a documented chlamydial infection for a total of 10–14 days.

Reasons for failure to respond to antimicrobial therapy include inappropriate antibiotics (enterococcal infection or resistant anaerobic infection), pelvic or wound abscess, or ovarian vein thrombophlebitis. When postpartum septic pelvic thrombophlebitis is suspected, heparin should be given.[28] Late postpartum endometritis can be managed in the same way as PID (see Table 50.2).

Episiotomy Infections

EPIDEMIOLOGY

Episiotomy infections are rare. The rate of infection of episiotomies is 0.1% overall but increases to 1–2% of episiotomies complicated by third- or fourth-degree extensions. Episiotomy infections, however, can have severe and even fatal consequences.

PATHOGENESIS AND PATHOLOGY

Episiotomy infections have been classified into four categories based on the depth of infection in the soft tissue: simple infection, superficial fascial infection, superficial fascial necrosis and myonecrosis.[30] Bacteria implicated in episiotomy infections include skin pathogens, streptococci and staphylococci, and bacteria associated with vaginal flora, Enterobacteriaceae and anaerobic bacteria, including *B. fragilis*. *Clostridium perfringens* and *C. sordellii* are likely if myonecrosis is present. Methicillin-resistant *Staphylococcus aureus* has also been reported as a cause of episiotomy infection.[31]

PREVENTION

Treatment standards should be introduced that reduce the liberal or routine use of episiotomies, as they appear to increase the risk of third- and fourth-degree tears. In the USA from 1980 to 2005 there has been an overall decline in the use of episiotomy from 64% to 19%.[32]

CLINICAL FEATURES

The simple wound infection is a local infection limited to incision site in the skin and the superficial fascia. Clinically there is edema and erythema only along the incision. A superficial fascial infection involves two layers of the superficial fascia and resembles a cellulitis with erythema, edema and pain. Often, infection of the episiotomy site may be associated with dehiscence. The superficial infection may be indistinguishable on the basis of skin appearance from an early superficial fascial infection with necrosis (necrotizing fasciitis). Necrotizing fasciitis involves all layers of the superficial fascia (and may involve the deep fascia). Skin anesthesia may precede the skin breakdown, because of nerve involvement. As nutrient vessels are occluded, the skin may turn dusky and develop bullae and then frank necrosis. Subcutaneous gas may be present with the mixed infection. These patients often have evidence of marked toxicity out of proportion to the clinical findings. In myonecrosis, pain is often the dominant feature. Patients are extremely toxic, restless and confused or disoriented.

DIAGNOSIS

Prompt diagnosis is of paramount importance because necrotizing fasciitis and myonecrosis are rapidly progressive. Frozen section examination of full-depth biopsy specimens has been helpful in the diagnosis of necrotizing fasciitis.[33] However, surgical exploration is warranted if necrotizing fasciitis or myonecrosis is suspected.

MANAGEMENT

Management of a simple episiotomy wound infection includes opening of the incision and exploration to ensure that there is no accumulated blood or a rectovaginal opening. Any superficial fascial infection should be managed with broad-spectrum antibiotic coverage (e.g. ampicillin–gentamicin–metronidazole or ampicillin–gentamicin–clindamycin) and observed closely. After initial management and control of the infection, early repair in the immediate postpartum period can be considered in a patient with dehiscence secondary to infection.[34]

More extensive surgical exploration should be undertaken if erythema and edema extend beyond the incision site, there is no improvement in 24–48 hours after the start of antibiotics, if the patient deteriorates, or if the patient has severe systemic manifestations. In the case of necrotizing fasciitis, the superficial fascia will separate easily from the deep fascia with a probe or finger (this does not occur in healthy tissue), the incisions will be bloodless and the exudate will be serosanguinous rather than purulent. Surgical debridement of all necrotic and pale tissue should be performed promptly and broad-spectrum antibiotic therapy should be instituted. The wound should be left open after debridement. A second-look procedure is often necessary after 24 hours.

Myonecrosis is an extremely rare event that is usually caused by *C. perfringens* or *C. sordellii* but may result from an extension of necrotizing fasciitis through the deep fascia to the muscle. Therapy includes high-dose penicillin and urgent surgical debridement. Hyperbaric oxygen therapy remains controversial and should be considered only as adjunctive therapy.

Postabortion Sepsis

EPIDEMIOLOGY

Postabortion sepsis is an ascending infection of the female pelvis after spontaneous or induced abortion. Inflammatory conditions associated with infectious complications of abortion are similar to those for PID but can be complicated by retained, poorly perfused tissue and uterine or bowel trauma. The mortality from abortion in developed countries is low (an estimated 0.6 per 100 000 cases), and abortion accounts for only 5% of all maternal mortality in the USA. Infection is the major cause when mortality does result from abortion complications. In a review of 107 deaths caused by abortion in the USA between 1975 and 1977, 33% were caused by sepsis.[35] In developing countries the World Health Organization estimates that illegal abortion accounts for 25–50% of the 500 000 maternal deaths that occur each year.

PATHOGENESIS AND PATHOLOGY

The bacteria associated with postabortion sepsis are similar to those associated with PID. However, the potential of direct uterine or bowel injury and retention of the products of conception after an abortion may result in injured and poorly vascularized tissue and an enlarged spectrum of enteric organisms, including *C. perfringens*. In developing countries, tetanus is a cause of mortality after abortion. More recently, *C. sordellii* has been associated with fulminant infections, secondary to lethal toxic shock-like syndrome, in women undergoing medical abortion with mifepristone.[36]

PREVENTION

Prevention measures include providing effective and acceptable contraception and appropriate medical management of abortion. Prophylaxis for cervical and vaginal infections, particularly for BV, before voluntary termination of pregnancy seems to decrease the risk of postabortal infection.[10] Prompt diagnosis and effective treatment of endometritis after the procedure is extremely important as delayed treatment is a common feature in cases of death from septic abortion.

CLINICAL FEATURES

The diagnosis of septic abortion should be considered in any woman who has a temperature of 100.4 °F (38 °C) or higher on two occasions more than 24 hours after an abortion and in any woman of reproductive age who presents with fever, abdominal pain and bleeding. Additionally, details of the procedure, including microbiologic studies and pathology of the aborted tissues, should be obtained. Physical examination findings typically include uterine tenderness, foul or purulent cervical discharge and products of conception at the cervical os. In more severe infections the patient may be hypotensive or in shock. The presence of cervical or vaginal lacerations should be assessed. Women who have clostridial infection may have severe disseminated intravascular hemolysis.

Fortunately rare, infection with *C. sordellii* may have atypical features, in that classic indicators of infection, such as fever, a purulent discharge, uterine tenderness and an elevated white blood cell count, are notably absent until the patient is in shock, and the only early warning may be excessive uterine bleeding.[36]

Acute complications of septic abortion are seen in advanced stages of the disease process and include the respiratory distress syndrome, septic shock, renal failure, abscess formation, septic pelvic vein thrombophlebitis and septic emboli, and disseminated intravascular coagulopathy; death may also occur. The chronic complications are similar to those of PID and include infertility, chronic pelvic pain and ectopic pregnancy.

DIAGNOSIS

Except for women who have mild, early, uncomplicated postabortion endometritis, all women should have blood and cervical cultures as well as a complete white blood cell count and urinalysis. Upright and flat abdominal and pelvic radiographs should be done to assess the presence of air in the abdominal cavity, dilated bowel and gas in the uterus. Pelvic ultrasound can determine the presence of retained tissue and other fluid collections, and the disruption of the myometrium by fluid or gas. CT is useful in assessing the entire abdomen. Laparoscopy can be used to examine the uterus for perforation but is suboptimal for a detailed examination of the bowel.[35]

MANAGEMENT

Any woman who has an incomplete or failed abortion or retained clotted or liquid blood (hematometra) should undergo immediate re-evacuation.[37] This tissue serves as a nidus for infection. The recommended treatment of PID (see Table 50.2) is appropriate for a woman who has early, uncomplicated postabortion infection limited to the endometrial cavity. The patient should be evaluated 48 hours after institution of therapy. If fever or pain persists, then the patient should be hospitalized and evaluated as above.

Women who have more severe illness should be hospitalized and begun on broad-spectrum antibiotics such as ampicillin, gentamicin and clindamycin. If clostridial infection is suspected, high-dose penicillin therapy should be used in place of ampicillin. These patients should be monitored closely and aggressively evaluated for uterine perforation and bowel injury. In the case of suspected *C. sordellii* infection, some experts recommend the combination of clindamycin and imipenem–cilastatin.[38] Laparotomy with possible hysterectomy should be performed if there is failure to respond to uterine evacuation and medical therapy, uterine perforation with necrotic myometrium or suspected bowel injury, pelvic and adnexal abscesses, or clostridial myometritis. Indications for a total hysterectomy with removal of adnexae include a discolored, woody appearance of the uterus and adnexae, clostridial sepsis, pelvic tissue crepitation and gas in the uterine wall on radiographs.

Postoperative Gynecologic Infections

EPIDEMIOLOGY

Hysterectomy is the most frequently performed elective surgical procedure among women of reproductive age in the USA.[32] The spectrum of postoperative infections after hysterectomy includes vaginal cuff cellulitis, pelvic cellulitis, vaginal cuff abscess, phlegmon, pelvic abscess and wound infections.[24] Rates of infection after abdominal hysterectomy ranged from 11% to 38% without antibiotic prophylaxis and from 4% to 8% with antibiotic prophylaxis. For vaginal hysterectomies, infection rates varied between 12% and 64% without antibiotic prophylaxis and between 0% and 10% with antibiotic prophylaxis.[39] Risk factors for postoperative infection include duration of surgery, younger age, lower socioeconomic status and the presence of BV.

PATHOGENESIS AND PATHOLOGY

Bacterial contamination of the operative site with flora of the lower reproductive tract occurs at the vaginal incision. The exposure in a vaginal hysterectomy occurs from the initial vaginal incision throughout the procedure and the exposure in an abdominal hysterectomy occurs near the end of the procedure. Hospitalization itself, regardless of whether antimicrobial prophylaxis is given, changes the vaginal flora, resulting in an increase in colony counts of *Escherichia coli*, *Enterococcus faecalis* and *Bacteroides* spp. and a decline in *Staphylococcus epidermidis* and *Streptococcus* and *Peptostreptococcus* spp.

Postoperative infections involve a mix of aerobic and anaerobic bacteria from the lower reproductive tract (see Table 50.4). *Bacteroides fragilis* and *Fusobacterium* spp. are more common in infections than when they are found in normal vaginal flora.

PREVENTION

Patients undergoing elective hysterectomy should be screened and, if necessary, treated for bacterial vaginosis several weeks before surgery. Preoperative single-dose antibiotic prophylaxis substantially reduces the rate of postoperative febrile morbidity in these procedures.[24,39]

CLINICAL FEATURES

Postoperative fever itself does not indicate infection but should prompt the clinician to evaluate the patient for one.

In pelvic cellulitis symptoms usually occur 2–3 days after surgery and include fever (temperature over 100.4°F (38°C)) and complaints of increasing abdominal and pelvic pain that may not be symmetric.[40] Parametrial tenderness without palpable mass is found on bimanual examination.

Histologic vaginal cuff cellulitis will develop in all women postoperatively as part of the normal healing process and most cases resolve without antibiotic therapy. Women who have more severe cellulitis will complain of increasing central or lower abdominal pain, increasing vaginal discharge or low-grade fever, usually within the first 2 weeks postoperatively. Bimanual pelvic examination may show only mild suprapubic tenderness to deep palpation without masses. Speculum examination will show a tender, indurated, hyperemic vaginal surgical margin.

In a vaginal cuff abscess patients typically have fever 2–3 days postoperatively and may report vaginal fullness. On examination a tender, palpable collection will be found above the vaginal surgical margin. A phlegmon would be diagnosed if a tender mass were felt in one or both parametrial areas and if no abscess could be identified on radiographic studies.

Pelvic abscesses are a late postoperative complication that present many weeks after surgery and most have a palpable mass in the pelvis. Wound infections are characterized by pain, marginal cellulitis and purulent exudate. Septic pelvic vein thrombophlebitis and osteomyelitis pubis are both rare complications of gynecologic surgery.

DIAGNOSIS

Microbiologic cultures obtained from drained abscesses are useful in guiding therapy. Cultures of the vaginal cuff are likely to be contaminated with vaginal flora. Abdominal and pelvic ultrasound and CT scans are useful to confirm the presence of a fluid collection when pelvic abscesses are suspected.

MANAGEMENT

Patients who have pelvic cellulitis should be treated with a broad-spectrum parenteral antibiotic regimen, such as a second-generation cephalosporin (cefoxitin or cefotetan), extended-spectrum penicillin (ticarcillin–clavulanate or sulbactam–ampicillin), or an aminoglycoside plus clindamycin, for 24–36 hours after the patient becomes afebrile. Cuff abscesses should be managed similarly, and the abscess should be drained. Vaginal cuff cellulitis can be managed on an outpatient basis with oral antibiotics such as amoxicillin–clavulanic acid, but patients should have a follow-up evaluation 72 hours after starting therapy. Medical therapy that covers Gram-negative aerobes and Gram-negative anaerobes (aminoglycoside–clindamycin or aminoglycoside–metronidazole) is often successful in treating postoperative pelvic abscesses that are inaccessible to drainage.

Patients who fail to respond to medical therapy alone (no defervescence in 72 hours or enlarging abscess) will require laparotomy and drainage, or excision. Abscesses accessible from a cutaneous surface should be drained. Patients should be treated with parenteral antibiotics until all signs and symptoms have resolved.

Some clinicians give postdischarge outpatient treatment with metronidazole and amoxicillin for 1 week after discharge in those patients who responded to medical management. All patients who have pelvic abscesses should be re-evaluated 2 weeks after discharge to ensure no recurrence of the abscess has occurred.

REFERENCES

References for this chapter can be found online at http://www.expertconsult.com

Chapter 51

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Complications of pregnancy: maternal perspectives

EPIDEMIOLOGY

Medical progress, such as effective antibiotics and vaccines, in combination with improved living conditions has modified the sequelae of infections, yet infectious morbidity in pregnancy remains a serious problem.

Any acute or chronic infection may occur before conception, during pregnancy or during the puerperium and may have serious consequences for the mother, the fetus and the neonate. Some micro-organisms are known to cause congenital infections and are discussed in Chapter 52. Others primarily influence the health of pregnant women and are described below.

The problem of maternal infections during pregnancy is addressed with emphasis on organ systems, including genitourinary tract infections, respiratory tract infections, gastrointestinal infections, puerperal sepsis, wound infection, mastitis, thrombophlebitis, endocarditis and meningitis. The infectious etiology of pre-term birth, premature pre-term rupture of membranes (pPROM) and chorioamnionitis deserves special attention. In addition, the implications of specific infections, including malaria, listeriosis, Lyme disease, varicella-zoster, HIV and other sexually transmitted diseases (STDs), are summarized in Tables 51.1 and 51.2.

The topic of infections in pregnancy is too wide to summarize in a single chapter. The interested reader will find excellent reviews by Sweet and Gibbs, Ledger, Hurley and Lamont.[1–4]

Incidence and prevalence

The primary infectious risk factor to poor pregnancy outcome, including pre-term birth, pPROM, spontaneous abortion, perinatal morbidity and mortality and maternal infections, does not relate to a specific micro-organism, but rather to a perturbation of the vaginal ecosystem and to bacterial vaginosis in particular. Bacterial vaginosis is a particularly common, albeit often asymptomatic condition, characterized by a loss of the resident lactobacilli and the concomitant overgrowth with a wide variety of mostly anaerobic species associated with ascending genital tract infection.[5–10]

Group B streptococci (GBS) continue to be a leading cause of perinatal infections.[11] The reported rates of GBS colonization in the genital tract range from 5% to 40%, with an average transmission rate to the neonate of 60%. The rates of early-onset GBS infection in the neonate, especially in pre-term and low-birth-weight babies, can be as high as 3 in 1000; however, the role of GBS in the occurrence of pre-term birth remains unclear.

Sexually transmitted infections, including trichomoniasis, gonorrhea, *Chlamydia* infection and syphilis, have also been associated with adverse pregnancy outcome, including pre-term birth, pPROM, low birth weight and postpartum infection.[12,13]

Prevalence rates of HIV in pregnant women are increasing all over the world but have reached endemic proportions in developing countries. The impact of maternal HIV infection on pregnancy outcome is still debated but most data from large studies of pregnant women who do not use drugs show an increased risk of adverse obstetric outcome, including abortion, prematurity, low birth weight and stillbirth.[13,14] However, the main risk remains mother-to-child transmission, with perinatal HIV transmission rates reported to occur with 15–40% of births among HIV-infected women.

Urinary tract infections (UTIs) are the most common infections in pregnancy, with or without clinical signs or symptoms. Asymptomatic bacteriuria is found in 4–7% of pregnant women, of whom 25–30% will develop pyelonephritis later in pregnancy.

Upper respiratory tract infections are common but of limited consequence for mother and child. In contrast, pneumonia is a serious illness for a pregnant woman.

Gastrointestinal infections caused by viruses are usually mild with no harm to the pregnancy and no need for specific medication. Meningitis is rare except for areas in which HIV and cryptococcal meningitis are endemic. Bacterial endocarditis is also uncommon and incidence rates vary from 1 in 4000 to 1 in 16 000 deliveries.

Febrile illness at delivery is uncommon in uncomplicated term pregnancies. Common underlying causes are chorioamnionitis, pyelonephritis, influenza and listeriosis.

The overall incidence of postpartum infections varies between 1% and 10%, depending on the definitions used, particularly for mastitis and postpartum endometritis. Postpartum infections consist of genital tract infections, puerperal mastitis, pelvic thrombophlebitis, UTIs, wound infections, complications of anesthesia and other infectious complications.

Genital tract infections of the uterus are the most common cause of puerperal infection and are categorized as endometritis, endomyometritis or endoparametritis depending on the extent of the infection. Wound and episiotomy infections occur frequently. After Caesarian section, wound infection defined as erythema, positive discharge and/or positive wound cultures varies between 5% and 10%, with emergency cases at higher risk of infection.

Septic thrombophlebitis is a rare complication of pregnancy with reported incidence rates of 1 in 2000 deliveries. The incidence of puerperal mastitis is estimated at around 1% in lactating women. Most have a mild disease.

Burden of disease, morbidity and mortality

In the general population the attributable risk of infections for adverse pregnancy outcome depends on the prevalence rates of infections in the population as well as on the socioeconomic and cultural factors that influence health, health behavior and health-seeking behavior. All infections that manifest with fever increase the risk of pre-term birth because of the release of pyrogens that increase myometrial activity.

Table 51.1 Implications of specific infections on pregnancy

	Impact on mother and child	Prevention	Management
Malaria	More frequent, more severe in pregnancy, especially in nonimmune women; increased risk of hypoglycemia in the mother LBW, IUGR, pre-term birth, abortion and stillbirth increased Congenital malaria (fever, hepatosplenomegaly, jaundice, anemia)	Chemoprophylaxis in travelers to endemic areas If no *Plasmodium falciparum* resistance, chloroquine phosphate 500 mg/week po In case of resistance proguanil 200 mg po q24h plus chloroquine 500 mg po q24h Avoid exposure	Prompt treatment with chloroquine or quinine according to resistance patterns Similar treatment regimens to those in nonpregnant women
Listeriosis	Mild maternal infection, but increased susceptibility Serious impact on the fetus: amnionitis, pre-term birth, septic abortion, stillbirth, fatality rate 3–50%	Early diagnosis in any febrile illness in pregnancy, cervical and blood cultures for *Listeria monocytogenes* Avoid implicated foods (e.g. unpasteurized cheese)	Ampicillin 2 g iv q6h plus gentamicin 2 mg/kg q8h for 1 week
Lyme disease	Erythema migrans in the mother, risk of transmission unknown, probably low Pre-term birth, stillbirth, syndactyly, cortical blindness, rash	Protective clothes in tick-infected areas (rural forest); remove ticks	Early treatment with amoxicillin 500 mg po q6h for 10–30 days or ceftriaxone 2 g iv for 14 days
Varicella-zoster	Rare in adults, fever, malaise followed by rash, 20% risk of varicella pneumonia Risk of abortion, stillbirth Congenital varicella (limb hypoplasia, cortical atrophy, retardation, IUGR, cutaneous scars, microphthalmia)	IgG testing if exposed If no IgG: varicella-zoster immunoglobulin 125 units/10 kg, max 625 units im, <96h after exposure	In cases of pneumonia: admission, respiratory support, aciclovir 10–15 mg/kg for 7 days Ultrasound assessment of the fetus
Measles	Increased maternal mortality secondary to pneumonia Risk of prematurity Developmental abnormalities (e.g. congenital heart disease, cleft lip, cerebral leukodystrophy and cyclopia have been reported)	Passive immunization in susceptible exposed women with pooled immunoglobulins 0.25 ml/kg within 6 days of exposure Avoid measles vaccine in pregnancy	Symptomatic
Group B streptococci	Sepsis in 1–3/1000 neonates, high mortality rates	Non consensus Antenatal case detection and treatment, or intrapartum treatment of women at risk?	Penicillin G 5 million units iv followed by 2.5 million units q4h until delivery or ampicillin 2 g followed by 1 g q4h until delivery

LBW, low birth weight; IUGR, intrauterine growth retardation.

Intra-amniotic infection diagnosed on clinical criteria occurs in 1–5% of pregnancies, with or without ruptured membranes. Consequences are pre-term birth, pPROM and postpartum and neonatal infections. The mother and the fetus are put at risk with pPROM as it is associated with pre-term birth and frequent infectious morbidity. Ascending infections, either the cause or the result of pPROM, may lead to intra-amniotic infection, chorioamnionitis, placentitis and fetal infections, including pneumonia and bacteremia.

The impact of asymptomatic UTI on pregnancy complications such as hypertension, anemia and poor obstetric outcome remains controversial. In contrast, ascending UTIs clearly play a role in the etiology of pre-term delivery and neonatal death.

Lower respiratory tract infections, meningitis and bacterial endocarditis are all life-threatening conditions for the mother and should be treated without delay.

Although postpartum infections are seldom life-threatening, sepsis remains an important cause of maternal death worldwide. Maternal mortality rates of 1–5 per 100 000 live births are registered in the Western world, whereas 100–600 per 100 000 pregnant or child-bearing women in developing countries die as a consequence of reproduction. In addition, for every woman who dies in childbirth another 30 women suffer from injuries, infections and disabilities. Overall, 25% of maternal mortality is considered to be caused by infections and this number can be lowered substantially by better health services and prompt treatment.

A number of reports estimating the role of infections in maternal death are summarized in Table 51.3. Few etiologic studies have been carried out in developing countries but the role of infections is likely to be more important. Personal observations (MT) from Nairobi, Kenya indicate that infections play a role in up to 40% of mothers dying in childbirth. The silent tragedy of maternal death should receive more attention from the international community, and also from the research world, because a substantial proportion of maternal deaths as a result of infections, bleeding and eclampsia are avoidable and interventions have to be tested to lower this unacceptable consequence of giving birth.

Postpartum genital infections may lead to chronic pain and discomfort, bleeding irregularities and infertility caused by ascending infections. Wound infections may increase pain and discomfort and prolong hospital stay. Pelvic vein thrombophlebitis may lead to serious complications such as septic pulmonary emboli.

Table 51.2 Implications of some sexually transmitted diseases on pregnancy

	Impact on mother and child	Prevention	Management
Neisseria gonorrhoeae	Ophthalmia neonatorum, pre-term delivery, puerperal infections	Silver nitrate 1% or tetracycline eye ointment	Spectinomycin 2 g im, ceftriaxone 250 mg im, or standard antimicrobial treatment
Chlamydia trachomatis	Ophthalmia neonatorum, puerperal infections, pre-term delivery	Tetracycline eye ointment	Erythromycin 500 mg for 4–7 days
Bacterial vaginosis	Risk of pre-term birth	Case detection and treatment is still under study	Metronidazole 250 mg q8h for 3–7 days or erythromycin base 333 mg q8h for 3–14 days
Trichomonas vaginalis	Risk of pre-term birth	Case detection and treatment no proven effect	Metronidazole 2 g single dose
Condylomata acuminatum	Risk of respiratory papillomatoses 1/80–1/1500	Case detection and treatment	Topical trichloroacetic acid (85%)/ surgery
Herpex simplex	Neonatal herpes 50% in mother with primary herpes at delivery	History from pregnant woman, careful inspection of the genital tract on the day of delivery	In case of active lesions, Caesarian section or vaginal delivery under aciclovir 200–400 mg q8h (under study)
HIV	Transmission in 25–45% Risk of abortion, pre-term delivery, puerperal infections	Antiretroviral therapy according to the most recent guidelines Elective Caesarian section No breast-feeding	Avoid long labor, rupture of membranes <4 h Treat with antiretroviral therapy, according to the most recent guidelines

Table 51.3 Cases of maternal death from infections

	Date of study	%
Michigan	1950–1971	23
Iowa	1926–1980 1950–1980	56 16
South Carolina	1970–1984	14
Oklahoma	1950–1979	7

Data from Sweet & Gibbs.[1]

Risk factors

Poverty is the most important risk factor for maternal infections during pregnancy. Poor women are more susceptible to malnutrition, infections, including STDs, less adequate sanitary conditions and lower access to preventive and curative health care than those who are financially better off.

Risk factors for UTI in pregnancy include sexual activity, older age, history of UTIs, lower socioeconomic status, diabetes mellitus, sickle-cell disease and specific bacterial factors such as the serotype and the virulence determinants of the micro-organisms.

Risk factors for lower respiratory disease include low socioeconomic status and HIV infection, particularly for infections with *Streptococcus pneumoniae*. Bacterial endocarditis has been reported more often in urban settings and among drug users.

Risk factors for puerperal genital infections include socioeconomic variables, anemia, STD, obstetric factors such as length of rupture of membranes, pre-term delivery, Caesarian section and number of vaginal examinations. Puerperal fever caused by group A β-hemolytic streptococci, once one of the most striking examples of iatrogenic infections in the 19th century, is a rare event in modern obstetrics, although sporadic outbreaks have been reported. A toxic-shock-like syndrome can occur caused by the release of pyrogenic exotoxins from streptococcal isolates (see Chapter 44).

Factors known to increase the risk for wound infections are age, obesity, bacterial contamination, operating time and duration of pre-operative hospitalization, emergency procedures, number of vaginal examinations, duration of internal fetal monitoring, length of labor and underlying maternal disease. Puerperal mastitis can be related to poor nursing techniques and lack of strict hygiene measures.

PATHOGENESIS AND PATHOLOGY

Pathogenesis

The pathogenesis of most infections is similar in pregnant and non-pregnant women except for possible alterations in the immune system as noted below. Of specific interest is the role of infectious agents in the onset of labor or, more importantly, of pre-term labor. Although the exact mechanism of the onset of labor is still part of the human parturition puzzle, there is convincing evidence for the role of prostaglandins in the initiation of parturition. Arachidonic acid, one of the precursors of prostaglandins, is made available for prostaglandin synthesis by the enzyme phospholipase A_2. This enzyme, produced by many micro-organisms but especially by anaerobes, might be one of the mechanisms of pre-term initiation of labor. Micro-organisms can stimulate the release of cytokines, such as interleukins and tumor necrosis factor, that stimulate prostaglandin precursors, thus leading to uterine contractions.

In theory, the amniotic cavity is sterile, protected by the placental membranes, with the cervical mucus serving as an effective barrier preventing micro-organisms from entering the uterine cavity. With the onset of labor and the rupture of membranes, bacteria may ascend and result in an amniotic infection. Pathogens may also gain access to the amniotic cavity through intact membranes or after invasive procedures such as amniocentesis, chorion villus sampling, umbilical blood sampling and cervical cerclage.

Urinary tract infections are caused by organisms that are part of the normal fecal flora, with *Escherichia coli* responsible for 80–90% of infections. Others are facultative Gram-negative bacteria, including *Klebsiella, Proteus, Enterobacter* and *Pseudomonas* spp., and Gram-positive bacteria such as staphylococci and GBS. Symptomatic UTIs are more frequent in pregnancy for several reasons, including decreased ureteric muscle tone and activity, dilatation of the ureter and renal pelvis because of the progesterone effect, and mechanical obstruction caused by an enlarging uterus, changes of the bladder and alterations in the properties of urine during pregnancy. Bacteriuria is of concern because of the increased risk of pyelonephritis associated with preterm labor caused by pyrogens, ureteric contractions leading to reflex myometrial contractions, the release of bacterial enzymes that may weaken the membranes and bacterial products that stimulate prostaglandin synthesis.

The most common organisms causing pneumonia in pregnant women are *S. pneumoniae, Haemophilus influenzae*, group A β-hemolytic streptococci and coagulase-positive staphylococci.

Postpartum endometritis seems to be a mixed infection with aerobic and anaerobic bacteria from the genital tract. Sexually transmitted diseases, including *Chlamydia trachomatis* and *Neisseria gonorrhoeae*, are important risk factors for ascending infections. Caesarian section is the single most important predisposing factor for pelvic infection. Wound infections are determined by the surgical techniques used, the amount of bacterial contamination and the resistance of the patient. An adequate blood supply is necessary to avoid acidosis in the wound. Organisms involved are *Enterococcus faecalis, E. coli, Staphylococcus aureus, Strep. pyogenes, Proteus* spp. and anaerobes. Predisposing factors for septic pelvic thrombophlebitis include changes in coagulation factors, alterations in the vein wall and stasis of blood flow.

In most cases of mastitis, *Staph. aureus* is the responsible organism, although *Staph. epidermidis* and viridans streptococci may also be isolated and are of questionable significance. Sporadic mastitis, usually the result of poor nursing technique, manifests as a cellulitis of the breast, primarily involving the interlobular connective tissue, to which the pathogens gain entry via a cracked or fissured nipple. In epidemic mastitis, however, infection occurs via the ductal system and spreads throughout the entire breast, resulting in mammary adenitis. The role of *Candida albicans* in causing mastitis is controversial.

Immunity

The normal course of pregnancy is associated with a variety of changes in humoral and cellular immunity, such as a loss in $CD4^+$ cells and other alterations in T-cell subsets.[15–19] Reports on T-cell subsets during pregnancy have been conflicting. Some studies have found a progressive fall in the $CD4^+$ count throughout pregnancy, from a mean of 950 cells/ml before 18 weeks to 720 cells/ml at term. Others have either reported a U-shaped $CD4^+$ cell count profile during pregnancy, with a minimum at approximately 32 weeks of gestation (CD4 of 30% and a $CD4^+$ count of 876 cells/ml), or have found stable CD4 levels and CD4:CD8 ratios during pregnancy with a rise (rebound) afterwards. Such differences may be attributable to different methodology (manual fluorescence microscopy versus automated flow cytometry), to differences in study populations or to the fact that blood was taken at different times during pregnancy. The altered immune status of pregnant women may possibly alter the response of the host to infectious agents.

Despite conflicting laboratory data, most studies agree that the humoral immune response (polymorphonuclear cells, PMNs) in pregnancy is similar to that in nonpregnant women but that the cellular immune response is diminished. Mortality rates of, for example, pneumococcal pneumonia, malaria or influenza have been found to be higher in pregnant than in nonpregnant women.

PREVENTION

Elimination of poverty and improvement of antenatal and obstetric care in deprived groups of society are primary strategies to prevent adverse pregnancy outcome. The challenge of identifying women at risk of adverse obstetric outcome is still a subject of intensive research. Early markers of infection-related pre-term birth are needed to identify a subset of women at risk for pre-term delivery who could benefit from antimicrobial therapy. A vaginal pH ≥5.0 and the presence of vaginal neutrophils at a rate of more than 5 per oil-field on Gram stain were found to be strongly associated with early pre-term birth.[20] A modified scoring system applied to Gram-stained vaginal smears in early pregnancy differentiating normal smears from bacterial vaginosis-like smears, smears dominated by atypical Gram-positive rods and lactobacilli-dominated smears showing heavy leukorrhea of unknown cause yielded a sensitivity of 70% in predicting spontaneous pre-term birth.[21] A number of molecular markers have been scrutinized for their putative predictive value with regard to the occurrence of pre-term birth. Overall, at present the most potent factors found to be associated with early spontaneous pre-term birth are a positive cervical–vaginal fetal fibronectin test, and elevated alpha-fetoprotein, alkaline phosphatase and granulocyte colony-stimulating factor serum levels, along with a shortened cervical length.[21,22]

Case detection and treatment of genitourinary infections is recommended in pregnant women who note an abnormal discharge. Screening for genitourinary pathogens with a potential negative impact on pregnancy outcome, such as bacterial vaginosis, trichomoniasis, GBS, gonorrhea, *Chlamydia* and others, depends on the prevalence of infectious agents in the population, the expected outcome of the intervention and the available resources. Recent intervention studies have provided convincing evidence of the benefit of identifying and treating bacterial vaginosis in pregnancy but only in women at high risk for pre-term birth.[10,11] As a result, the practice of routine screening for bacterial vaginosis in asymptomatic women at low risk for pre-term delivery cannot be justified according to published studies.

Screening for GBS in pregnant women is still a subject of debate. Between 10% and 30% of pregnant women are colonized with GBS, an important source of perinatal morbidity and mortality. Recently, the Centers for Disease Control and Prevention issued recommendations for the active prevention of GBS, which have been adopted by the American College of Obstetricians and Gynecologists.[23] A strategy based on late prenatal cultures, followed by intrapartum treatment with penicillin or other broad-spectrum antibiotics is recommended. Others argue that routine antimicrobial treatment with a broad-spectrum antibiotic is optimal for pregnant women at high risk of adverse pregnancy outcome in order to reduce the incidence of infectious complications such as pre-term birth, neonatal infections and maternal infectious morbidity.[24] Risk may be based solely on laboratory findings or on clinical and obstetric factors (bad obstetric history, clinical signs and symptoms). However, many questions remain unanswered, especially the benefit of screening and treating low-risk groups and the ideal antimicrobial regimen. The existing evidence is hampered by methodologic weaknesses such as small numbers, use of combinations of antibiotics and differences in populations studied. Although practice guidelines have been established it is essential to prove that the costs and the benefits associated with screening and treating genital infections in pregnant women outweigh the potential risks and effects. Only prospective, randomized and blinded clinical trials with large study populations can determine the effect of antimicrobial therapy on infectious morbidity and mortality in pregnancy.

Intercourse during pregnancy has been implicated as a risk factor for pre-term birth. This could be because of the effect of STDs or because of increased myometrial activity and cervical ripening caused by the prostaglandins in sperm. After correcting for STDs, there is little evidence of a causal relation between sexual intercourse and pre-term birth.

Randomized trials of condom use versus unprotected sex in high-risk groups for poor pregnancy outcome have not been carried out to date.

Detection and treatment of maternal bacteriuria in early pregnancy, preferably around 16 weeks, can reduce the risk of pyelonephritis, preterm delivery and neonatal mortality.

Routine examination of stools for pathogens is not useful in pregnant women, except in populations that have high rates of anemia and malnutrition.

Prevention of puerperal infections is a major concern in obstetrics. Antenatal detection and treatment of STDs and hygienic standards during delivery decrease the risk for puerperal infection. The indications for cervical cerclage have to be carefully weighed against the risks, and antibiotic prophylaxis should be given. Active management of labor – including shorter labor, fewer vaginal examinations and reduced Caesarian section rates – help to prevent genital infections. Antibiotic prophylaxis in cases of long, complicated or operative deliveries is effective, although resistance is a limiting factor.

Mastitis can be prevented through good nursing technique, including strict hygiene measures.

CLINICAL FEATURES

Infections in pregnancy can be asymptomatic but usually manifest with symptoms similar to those in nonpregnant individuals.[1–4] The course of the infection may be worse because of alterations in immune response and because of the potential hazards for the outcome of pregnancy and the well-being of the fetus.

The clinical diagnosis of intra-amniotic infection is based upon fever, uterine tenderness, fetal tachycardia, leukocytosis and elevated C-reactive protein, with or without ruptured membranes.

Urinary tract infections are the most common infectious complications of pregnancy and can be asymptomatic or manifest with signs of cystitis such as frequency, urgency and dysuria. Fever, flank pain and chills occur with ascending UTIs.

Lower respiratory tract infections manifest with cough, fever and chest pain.

Gastrointestinal infections appear as diarrhea and are usually self-limiting and without complications. If the diarrhea persists beyond 24 hours, a stool specimen should be obtained for culture. Acute appendicitis can be a diagnostic dilemma as the clinical presentations differ from those seen in nonpregnant women because of the large uterus and the altered immune response. Appendicitis may manifest as upper right quadrant pain with nausea and vomiting, without leukocytosis or fever, and should be differentiated from acute cholecystitis and amebic liver diseases.

Meningitis should be considered in every patient with headache, malaise, nausea, vomiting and fever. The diagnosis of bacterial endocarditis in pregnant women should be considered in any febrile, lethargic patient with no signs of localizing infection. Cutaneous lesions and heart murmurs should be sought. Blood cultures are required for diagnosis.

Diagnostic criteria for postpartum endometritis include fever, uterine and/or adnexal tenderness, purulent or foul lochia and leukocytosis in the absence of other signs of infection within the first 5 days after delivery. Late postpartum endometritis may occur weeks after delivery. Retention of placental products has to be excluded.

Early wound infection starts usually within 48 hours postpartum and manifests with fever and cellulitis or edema of the wound. Early wound infection is often caused by group A or group B streptococci, or *Clostridium perfringens*. In clostridial infection, wound cellulitis is associated with a watery discharge and a bronze appearance of the skin. Late-onset wound infections occur about 4–8 days after surgery, and manifest with fever and an erythematous, draining wound.

Early recognition of life-threatening complications such as necrotizing fasciitis is crucial. Cutaneous findings can be minimal and include cellulitis, edema and sometimes crepitations. However, the patient may be critically ill and require prompt treatment. A surgical exploration of the wound may be necessary to make the diagnosis.

Puerperal mastitis occurs with breast engorgement and milk stasis, often in the second or third week after delivery. The onset of sporadic mastitis is rather sudden, with breast tenderness, chills, fever, malaise and headache mimicking a flu-like syndrome. The breast may show foci of local infection characterized by erythema, tenderness and warmth. The development of a breast abscess is rare in lactating women.

Patients with ovarian vein thrombophlebitis, which is more frequently present on the right, usually have distinct clinical findings. They present with fever and lower abdominal pain, and on examination are acutely ill with tachycardia and tachypnea and may be in respiratory distress. Abdominal examination usually shows direct tenderness, guarding and a tender abdominal mass. Pelvic pain thrombophlebitis without pulmonary emboli manifests less dramatically and has a more rapid response to therapy. These patients are more often not as critically ill, and just have fever and tachycardia.

DIAGNOSIS

Most infections that manifest with clinical signs and symptoms do not give rise to diagnostic difficulties, as they are related to specific infections of the urinary or reproductive tract, or to common infections in the community. Outlining a complete scheme of investigations is beyond the scope of this chapter, but a summary of the most important diagnostic leads and laboratory tests is presented in Table 51.4 (see also Chapter 98).

Rapid and inexpensive tests for the early detection of intra-amniotic infection in patients in pre-term labor, including amniotic fluid Gram stain, leukocyte esterase, amniotic fluid glucose concentration and the Limulus amebocyte lysate assay for endotoxin, have been tested in women admitted with pPROM or pre-term birth. The greatest sensitivity for predicting infection was demonstrated by a low glucose level in amniotic fluid but none of the tests had sufficient accuracy to allow clinical decisions.[24,25] For women with cervical dilatation in the mid-trimester of pregnancy, amniocentesis to determine the microbiologic characteristics of the amniotic cavity should be considered before placing a cerclage because of the poor prognosis in women with microbial invasion of the amniotic cavity.

The diagnosis of UTI is based on quantitative cultures with more than 100 000 cfu/ml clear-voided urine (midstream) in asymptomatic patients, or more than 100 cfu/ml in symptomatic patients. Direct suprapubic bladder aspiration is a better technique for obtaining

Table 51.4 Clinical and laboratory criteria in the diagnosis of infection in pregnancy

History	Signs, symptoms, onset, specific localization, additional signs such as pain, rash, uterine tenderness, leakage of amniotic fluid, exposure to infections, pets, occupation, hobbies, travel, place of residence, history of infections
Clinical examination	Auscultation of heart and lungs, assessment of the uterus and cervix, examination of the breasts, detection of masses, enlargement of spleen, liver, lymph nodes, signs of thrombophlebitis
Laboratory tests	White blood cell count and differential, C-reactive protein, serum enzymes, blood smears, blood cultures, throat and vaginal swabs, specific antibodies, urine culture

uncontaminated urine but is less readily accepted by patients and/or physicians. To avoid screening all pregnant women with expensive and time-consuming urine cultures, rapid screening tests such as leukocyte esterase dipstick, microscopy for pyuria, nitrite tests and enzymatic screening tests have been developed. The rapid enzymatic test seems to be a reliable alternative to culture screening, with a sensitivity of 100%, a specificity of 81% and a negative predictive value of 100%.[26]

The key to the care of lower respiratory tract infections is an early diagnosis, with careful clinical evaluation and examination, if possible, of a sputum sample and a blood culture. One should not hesitate to take a chest radiograph, as well as an arterial P_{O_2} in pregnant women if a serious lower respiratory tract infection is suspected. In patients with meningitis, a spinal tap with Gram stain, culture and chemical analysis of the cerebrospinal fluid is indicated.

Postpartum endometritis is a clinical diagnosis supplemented by a cervical swab for aerobic culture to identify pathogens that may require additional measures besides the antibacterial therapy. Isolation of group A streptococci should lead to isolation of the patient whereas that of GBS should prompt further action in relation to the neonate. Culturing techniques of the endometrium with double- and triple-lumen devices have been hampered by vaginal and cervical contamination and are not used routinely.

The diagnosis of early wound infection is made clinically and confirmed by a Gram stain. Gram-positive rods are highly suggestive of clostridia, and Gram-positive cocci indicate the presence of group A streptococci or *Staph. aureus*.

The diagnosis of mastitis is a clinical diagnosis. Mammography and ultrasound can be useful in the early diagnosis of an abscess and to differentiate infection from a breast malignancy. However, this technique is rarely used because of pain and discomfort to the patient.

Pelvic vein thrombophlebitis is a difficult clinical diagnosis often confused with acute appendicitis, torsion of an adnexa, urolithiasis, pyelonephritis, leiomyoma and pelvic abscess. In case of clinical suspicion of a pelvic vein thrombophlebitis, additional examinations such as venography, CT scan and sonography have to be performed.

MANAGEMENT

A number of interventions with proven value in the management of morbidity related to infection during pregnancy are discussed. The serologic screening and subsequent management of viral infections, including rubella, toxoplasmosis, cytomegalovirus, HIV and others, is discussed in Chapter 52.

Although the initiating mechanism of pre-term labor is unknown, the potential role of ascending infection and intrauterine inflammation is clear. Consequently, attempts to prolong gestation and improve pregnancy outcome using antimicrobials have been made. A number of prospective, randomized clinical trials with antibiotics have been reviewed. Among women in pre-term labor with intact membranes the use of prophylactic antibiotics is associated with a reduction in maternal infection, though not with a clear benefit for length of gestation and neonatal outcome, and hence this treatment is not recommended for routine practice.[27] In contrast, among women with pre-term rupture of membranes, antibiotic administration is associated with a significant reduction in intrauterine infection, with delay in delivery and a reduction in major markers of neonatal morbidity. These data support the routine use of antibiotics in pPROM, such as erythromycin, while co-amoxiclav should be avoided.[28] In addition, routine antibiotic prophylaxis given during the second or third trimester of pregnancy reduces the risk of pre-labor rupture of membranes when given to unselected pregnant women and is associated with beneficial effects on birth weight and the risk of postpartum endometritis among women with a previous pre-term birth. However, as infection is the underlying cause of pre-term birth in only a fraction of women, improved diagnostic methods are needed to identify those patients who will most gain from routine antibiotic prophylaxis.[29]

As bacteriuria in pregnancy, if untreated, may lead to pyelonephritis in up to a third of women, with potential hazards for mother and fetus, screening and management in pregnancy is justified. Antibiotic treatment is effective in clearing asymptomatic bacteriuria and in reducing the risk of pyelonephritis in pregnancy.[30] There are, however, insufficient data to recommend any specific regimen. The choice of antibiotic should be guided by antimicrobial susceptibility testing, but this decision is becoming more difficult because of increasing rates of antimicrobial resistance to commonly prescribed antibiotics.[31] Also, the optimal duration of antimicrobial therapy for treatment of bacteriuria in pregnant women has not been determined.[32]

Similarly, antibiotic treatment has been shown to be highly effective for the cure of symptomatic cystourethritis; however, there are insufficient data to recommend any specific treatment regimen for symptomatic urinary tract infections during pregnancy. Accordingly, susceptibility tests should be obtained. Most antibiotics studied were shown to be very effective, while complications are rare.[33] Common antimicrobial treatment options include 3-day courses of trimethoprim–sulfamethoxazole (TMP–SMZ) 160/800 mg q12h or a 7-day course of nitrofurantoin 100 mg q6h, or cephalexin 500 mg q6h. TMP–SMZ should be avoided in the third trimester because sulfonamides cross the placenta and compete with bilirubin in the fetus. Trimethoprim is a folate antagonist and should be combined with folic acid if given in high doses. In view of the high rate of recurrence of bacteriuria in pregnancy, suppressive therapy is recommended until 2 weeks postpartum in women with recurrent bacteriuria. Pregnant women with acute pyelonephritis require admission for parenteral administration of antibiotics and careful monitoring.

Penicillin is the drug of choice in women with lower respiratory tract infections caused by pneumococci. The fever may cause premature contractions. Because of possible cardiopulmonary complications, β-mimetic tocolytic drugs should be avoided or, if necessary, administered with caution.

Treatment of gastrointestinal infections is usually not necessary during pregnancy except when the problems persist and interfere with the mother's health. Metronidazole should be prescribed for *Entamoeba histolytica* infection. Pregnant women with acute appendicitis should undergo surgical exploration by the obstetrician together with the surgeon, and antibiotics should be prescribed.

Treatment of postpartum genital infection includes appropriate antibiotics with good anaerobic coverage. After Caesarian section the desired results have been obtained with a combination of clindamycin and gentamicin.[34] Newer antibiotics such as the monobactams may replace gentamicin in combination with clindamycin, and the newer cephalosporins with a wide spectrum of activity are increasingly used. Intravenous antibiotics should be continued for 24–48 hours after the patient has become afebrile, and can be stopped without changing to oral antibiotics unless a staphylococcal infection is present. The reduction of endometritis by some two-thirds and the decrease in wound infections justifies a policy of routine prophylactic antibiotics to women undergoing elective or nonelective Caesarian section.[35]

The treatment of early wound infections may require excision of the necrotic tissue and aggressive antibiotic treatment with a cephalosporin or clindamycin. In late-onset (after 5 days) wound infection incision of the wound and drainage is required. If the patient does not become afebrile after 24 hours, antibiotics should be prescribed. Open wounds can be allowed to close spontaneously by granulation after wound debridement and packing. Surgical closure of Caesarian section has been shown to be successful and requires less healing time. The procedure may be carried out under general or local anesthesia, and antibiotic prophylaxis is generally used. Episiotomy incisions should not be re-sutured but given time to heal by granulation, unless the sphincter muscle or the rectal mucosa is involved. In rare complications such as necrotizing fasciitis or clostridial gas gangrene, treatment must be aggressive, including high doses of broad-spectrum antibiotics and extensive drainage and debridement.

Therapy of mastitis includes continuation of lactation and treatment with a penicillinase-resistant penicillin or a cephalosporin, given orally except in the case of a severely sick patient. Ice packs, breast support, analgesics and regular emptying of the infected breast may help to prevent abscesses. In case of abscess formation, surgical incision and drainage should be performed.

Table 51.5 Risk factor assignments for antibiotics in pregnancy[36]

Agent		Category	Potential toxicity
1	Penicillin	B	
2	Cephalosporin	B_M	
3	Monobactam	B_M	
4.1	Aminoglycoside/group 1	C*	Fetal ototoxicity, maternal oto- and nephrotoxicity
4.2	Aminoglycoside/group 2 (spectinomycin)	B	
5	Chloramphenicol	C	Gray syndrome (cardiovascular collapse in babies) when given near term
6	Tetracycline	D	Adverse effects on fetal teeth and bones, congenital defects, maternal liver toxicity
7.1	Macrolide: erythromycin	B	! Estolate salt form can induce maternal hepatotoxicity
7.2	Other macrolides	$C–C_M$	
8	Clindamycin	B	
9	Fluoroquinolone	C_M	
10.1	Sulfonamides	B*	No teratogenic effect, to be avoided near term because of potential toxicity to the newborn (kernicterus)
10.2	Trimethoprim–sulfamethoxazole	C_M	Megaloblastic anemia, folate activity

*Adapted from ref.[36]

Treatment of pelvic vein thrombophlebitis includes broad-spectrum antibiotics, heparin for 7–10 days intravenously, followed by long-term anticoagulation with oral anticoagulants. Surgery, including bilateral ovarian vein and inferior vena cava ligation, may be required for patients who do not respond to treatment. Hysterectomy is seldom required.

Antibiotics in pregnancy

Antibiotics are frequently used during pregnancy. Several studies have shown that 25–40% of pregnant women take antibiotics, mainly in the second trimester, while the incidence of antibiotic intake is around 5% in the first trimester. Administration of a drug to a pregnant woman presents a unique problem. The pharmacologic mechanisms must be well considered, and the fetus must always be kept in mind. Most drugs or chemical substances taken during pregnancy can cross the placenta to some extent throughout pregnancy, but the fetus is at highest risk during the first 3 months of gestation.

Risk factors have been assigned to all level of risk that a drug poses to the fetus (A, B, C, D and X). The definitions used for the risk factors, described by Briggs *et al.*,[36] are summarized below.

- Category A: Controlled studies in women fail to demonstrate a risk to the fetus in the first trimester (and there is no evidence of a risk in later trimesters), and the possibility of fetal harm appears remote.
- Category B: Either animal reproduction studies have failed to demonstrate a risk to the fetus and there are no adequate and well-controlled studies in pregnant women, or animal reproduction studies have shown an adverse effect, but adequate and well-controlled studies in pregnant women have failed to demonstrate a risk to the fetus in any trimester.
- Category C: Animal reproduction studies have shown an adverse effect on the fetus and there are no adequate and well-controlled studies in humans. However, potential benefits may warrant use of the drug in pregnant women despite potential risks.
- Category D: There is positive evidence of human fetal risk based on adverse reaction data from investigational or marketing experience or studies in humans, but potential benefits may warrant use of the drug in pregnant women despite potential risks.
- Category X: Studies in animals or humans have demonstrated fetal abnormalities or there is evidence of fetal risk based on human experience or both, and the risk of the use of the drug in pregnant women clearly outweighs any possible benefit. The drug is contraindicated in women who are or may become pregnant.

Many older drugs have not been given a letter by their manufacturers, and the risk factor assignments were made by Briggs *et al.*[36] In cases where the manufacturer has rated the product in its professional literature, the risk factor is shown with a subscript (e.g. C_M). Risk markers with an asterisk indicate that risks to the fetus depend on when or for how long the antibiotic is used. The classification of the most commonly used antibiotics is summarized in Table 51.5.[36]

CONCLUSIONS

Infectious diseases are important risk factors for maternal and neonatal morbidity and mortality and can be detected early with improved outcomes or prevented entirely. Maternal mortality due to infections is an unbearable tragedy and must be addressed by improved access to modern obstetric care for all pregnant women. This care must be affordable, even in resource-limited countries, and should be a priority for governments and international agencies.

Preventing pre-term birth is a global issue, including industrialized countries. A substantial portion of pre-term births can be prevented with improvements in the detection and management of infections during pregnancy. Further research is required to identify women and babies at risk, and to develop preventive, diagnostic and management strategies to enhance care with maximal benefits at minimal costs and adverse effects.

REFERENCES

References for this chapter can be found online at http://www.expertconsult.com

Chapter 52

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Implications for the fetus of maternal infections in pregnancy

The number of pathogens capable of infecting the fetus, and the spectrum of disease in the fetus and newborn infant caused by various pathogens has expanded substantially. Advances in molecular technology (e.g. polymerase chain reaction, PCR) and wider availability of intrauterine diagnostic testing (e.g. maternal serum alpha-fetoprotein screening, ultrasound, amniocentesis, fetal blood sampling) have provided new opportunities to link pathogens to untoward events. Congenital infection in pregnancy may come to clinical attention through:

- a history of maternal risk factors;
- known exposure;
- documented acute maternal infection;
- laboratory screening;
- detection of fetal abnormalities on clinical examination or ultrasonography;
- suggestive findings in the neonate; and
- suggestive findings in the child.

Depending on the scenario, one or a range of infections must be considered in the differential diagnosis.

Fortunately, the vast majority of maternal infections have no deleterious effect on the fetus, either because there is no transmission to the intrauterine site or because the fetal infection is asymptomatic. Fear and poor understanding of risk on the part of the parents or physician can lead to unnecessary termination of pregnancy.

EPIDEMIOLOGY

Although many micro-organisms are known to cause congenital (intrauterine) infection (Table 52.1), only more common agents are discussed (see below). A brief summary of infections transmitted primarily at the time of delivery is given at the end of the chapter.

Because the use of appropriate diagnostic testing is highly variable and because many of these diseases are not reportable to public health departments, there are few data on incidence.

Geography

There is considerable geographic variation in the risk of exposure to pathogens associated with congenital and perinatal infections. This variation may be related directly to the biology of the pathogen (e.g. *Plasmodium* spp., *Trypanosoma cruzi*), culinary practices, including handling and ingestion of fresh raw meat (e.g. toxoplasmosis) or regional variation in breast-feeding rates. The geographic distribution of certain pathogens may change over time, particularly arthropod-borne or zoonotic infections; an example of this is the recent introduction of West Nile virus to North America.

Other factors associated with infection

Epidemiologic factors associated with maternal–fetal infection are summarized in Table 52.2. The risk of congenital infection often reflects maternal immunity rates as well as the risk of exposure to the pathogen. Cytomegalovirus (CMV) and *Treponema pallidum* are excellent examples of this. Annual seroconversion rates to CMV for healthcare workers, usually in the range of 2–4%, are generally lower than rates in day-care workers or susceptible parents of children in day care (12–45%).[3] Syphilis in pregnancy generally affects women who are young, unmarried, of low socioeconomic status and who receive inadequate prenatal care.

PATHOGENESIS AND PATHOLOGY

Infections occurring in the neonate may be acquired in the following ways:

- transplacentally in utero (congenital or intrauterine) by direct blood flow to the amniotic fluid or from the genital tract via the cervical amniotic route, during pregnancy or just before delivery. The placenta can be infected and even act as a repository for pathogen growth;
- at the time of birth (perinatal) through vaginal secretions and blood; and
- after birth but during the neonatal period (postnatal), from the mother, her breast milk or other sources.

Adverse outcomes from intrauterine infection include abortion, stillbirth, premature delivery, physical defects, intrauterine growth restriction (e.g. rubella, enterovirus, herpes simplex virus (HSV)) and postnatal persistence of infection (e.g. rubella, CMV, HSV, toxoplasmosis).

A general association between certain outcomes and timing and site of infection exists:

- embryo: malformations, spontaneous abortion;
- fetus: stillbirth, neurologic sequelae; and
- placenta: preterm birth, stillbirth and neonatal death.

Factors affecting fetal disease

The risk of transplacental transmission and disease manifestations are influenced by a multitude of factors, including the pathogen's propensity for causing fetal infection, the pregnant woman's immunologic status to the pathogen, placenta function and the timing of infection in regard to fetal development.

Table 52.1 Infectious agents known to cause congenital infection

Viruses	Herpesviruses Cytomegalovirus Herpes simplex virus Varicella zoster virus Human herpesvirus 6[2] Human herpesvirus 7[2] Parvovirus B19 Rubella virus Measles virus Enteroviruses Coxsackie B Echoviruses Polioviruses Parechoviruses Human immunodeficiency viruses HIV-1 HIV-2 Lymphocytic choriomeningitis virus Hepatitis B virus Vaccinia Smallpox Adenovirus Western equine encephalitis virus Venezuelan encephalitis virus West Nile virus
Bacteria	*Treponema pallidum* *Mycobacterium tuberculosis* *Listeria monocytogenes* *Brucella* spp. *Campylobacter fetus* *Salmonella typhi* *Borrelia burgdorferi* *Coxiella burnetti*
Protozoa	*Toxoplasma gondii* *Plasmodium* spp. *Trypanosoma cruzi*
Fungi	*Coccidioides immitis*

Adapted with permission from Guerina.[1]

Table 52.2 Factors associated with maternal–fetal infection

Association	Pathogen
Seasonality (in North America)	Parvovirus B19 (winter, spring) Rubella (winter, spring) Enterovirus (summer, autumn) West Nile virus (summer, autumn)
Handling/ingestion of uncooked, previously unfrozen meat	*Toxoplasma gondii*
Children: Day care School Household	 CMV, parvovirus Parvovirus CMV, parvovirus
Exposure in travel to certain geographic regions	*Toxoplasma gondii, Mycobacterium tuberculosis, Plasmodium* spp., *Trypanosoma cruzi, Borrelia burgdorferi*, hepatitis B virus, *Brucella* spp., West Nile virus
Kitten/cat feces within 21 days of primary infection (handling animals, kitty litter, gardening)	*Toxoplasma gondii*
Number of sexual partners, sex industry worker/ partner, illicit drug use	*Treponema pallidum*, herpes simplex virus, hepatitis B virus, hepatitis C virus, HIV
Sexually active adolescents	CMV, herpes simplex virus, hepatitis B virus, hepatitis C virus, HIV
Unimmunized (e.g. immigrant from developing world; World Health Organization Expanded Program of Immunization does not include rubella)	Rubella
Other incompletely immunized	Measles, mumps, tetanus, diphtheria, poliomyelitis, hepatitis B, varicella-zoster virus

Pathogen

The explanation as to why certain pathogens have a propensity for causing congenital infections is incompletely understood. In part, this may reflect the tendency of certain infecting organisms to invade the mother's bloodstream, thereby coming in contact with the placenta and fetus. Inoculum size, as measured by quantity of pathogen in the blood, may also be an important factor (e.g. viral load in HIV).

The specific manifestations of different pathogens reflect their individual propensity for certain stages of organogenesis as well as specific organs and tissues. Rubella virus and varicella zoster virus (VZV) cause developmental anomalies through cell death, inhibition of cell growth or chromosomal damage. Inflammation, and the resulting tissue destruction, is thought to be responsible for the structural abnormalities characteristic of HSV, congenital syphilis and toxoplasmosis. Parvovirus B19, by binding to the P antigen on erythrocyte precursors and thereby inhibiting the required rapid increase in erythrocyte numbers during the second trimester, can cause profound anemia and nonimmune hydrops.

Postnatal persistence of CMV, HSV, *Toxoplasma gondii* and rubella can result in progressive tissue damage and onset or worsening of clinical disease later in life. Thus, hearing loss associated with congenital CMV can become manifest after 2 years of age, and the chorioretinitis of congenital toxoplasmosis can recur in later years despite prior therapy.[4,5] Progressive encephalitis may occur with congenital rubella, with onset typically in the teenage years.[6]

Maternal immunity

Before conception

Preconception immunity in the immunocompetent mother is usually protective to the fetus. Exceptions include viruses with known latency such as CMV and occasionally HSV, rubella when maternal immunity has waned, untreated *Treponema pallidum* and HIV. Immunosuppressive disorders and immunosuppressive therapy will also alter the risk of fetal disease (e.g. HIV-1 infection facilitates the transmission of *T. gondii*).

The length of time before conception during which infection may occur without causing later fetal damage is not known. In the immunocompetent mother, it is advisable to have an interval of 7–9 months between *T. gondii* acquisition and conception. Over 50% of infants born to mothers with primary or secondary syphilis will have congenital infection, decreasing to 40% with early latent syphilis and to 10% or less with late latent infection.[7]

During pregnancy and before delivery

The risk of transplacental transmission and severity of disease are increased with primary maternal infection during pregnancy. Thus, the rate of intrauterine transmission of CMV is about 40% with primary maternal infection, but only 1% among previously seropositive mothers. Neurologic sequelae are approximately three times higher in the context of primary infection.

In late gestational infections, the fetus is at high risk of severe disease when delivered before transplacental transfer of specific antibody has taken place. For example, the mortality from varicella-zoster virus infection acquired transplacentally from a mother who develops skin lesions from 5 days prior to delivery to 2 days after delivery is 30% in the absence of treatment.

Placenta

In early gestation, the small developing placenta effectively excludes most pathogens. As the placenta matures, with expansion of the maternal–fetal interface, this barrier becomes more porous, rendering transplacental transmission more likely. For a variety of pathogens, including CMV, rubella virus, *Treponema pallidum*, *Plasmodium* spp. and *Mycobacterium tuberculosis*, placental infection may occur without fetal infection. Thus, the placenta can prevent infections from reaching the fetus; in part, this is due to it serving as a physical barrier to the pathogen. In addition, placental macrophages and local production of antibodies and cytokines may be important.

Fetus

Fetal disease may result from many pathogens at any time during gestation. In general, transplacental transmission (via umbilical blood flow or direct spread to the amniotic fluid) is less likely early in gestation, but the results of infection, if it occurs, are more likely to be severe. The deficiencies in immune function of the young fetus in both humoral and in cellular function contribute to the increased risk of severe disease through unchecked tissue damage, organ dysfunction and teratogenicity. Detectable IgM is rarely produced before 20–24 weeks of gestation.

Certain pathogens are associated with particular effects at certain stages of cell development. For example, maternal rubella infection in pregnancy prior to 16 weeks, but not thereafter, is associated with the development of congenital defects.[8] Congenital varicella syndrome occurs almost exclusively before 20 weeks of gestation; the highest risk occurs if the mother had varicella infection between 13 and 20 weeks.[9] While transmission of *T. gondii* occurs in only about 15% of first trimester infections, severe disease occurs in about 40% of infected infants.[10] In the third trimester the reverse is true with 60% transmission, but the disease is generally milder or asymptomatic.

PREVENTION

Prevention of maternal infection in pregnancy should include education, immunization, laboratory screening, monitoring for evidence of infection and, where appropriate, intervention (Table 52.3).

Education and exposure avoidance

While exposure to potential pathogens during pregnancy cannot be completely eliminated, relatively simple interventions can offer significant protection (Table 52.3). All women who are planning pregnancy should be given advice pertinent to infection prevention:

- Wash hands thoroughly before eating and before and after food preparation (e.g. enteric pathogens, *Listeria* spp., *T. gondii*).
- Wash hands after coming in contact with saliva, stool, urine or other body secretions (e.g. enteric pathogens, *Listeria* spp., CMV, rubella).
- Avoid eating unpasteurized milk or cheese and undercooked meat (e.g. *Listeria* spp., *Brucella* spp., *T. gondii*).
- For those with high-risk behaviors such as intravenous drug use or multiple sexual partners, provide information on the risk posed by HIV, hepatitis B, hepatitis C, syphilis and other sexually transmitted infections.
- Inform physician following exposure to erythema infectiosum (fifth disease, parvovirus B19), chickenpox/shingles, pertussis (whooping cough) or other infection.
- For women with recurrent genital herpes, inform physician if they have recurrent lesions late in gestation or when in labor.
- Inform women that testing for rubella immunity and evidence of hepatitis B virus, HIV and syphilis infection is routinely sought in pregnancy.

Active and passive immunization

All women should be immune to measles, mumps, rubella, tetanus, diphtheria, poliomyelitis, hepatitis B and varicella, either by natural infection or by vaccination. Influenza and pneumococcal immunization are recommended for women who are at high risk of infection. Immune globulin products may be indicated on exposure to measles, hepatitis A or B virus, tetanus, varicella or rabies and, in the case of certain travel, to poliomyelitis, yellow fever, typhoid and hepatitis B virus. Although inactivated vaccines are generally considered safe, some physicians wait until after the first trimester to administer them. Live viral or bacterial vaccines should be avoided during pregnancy. However, the risk of congenital rubella syndrome after administration of vaccine to a pregnant woman is very low.[11]

Screening for infection during pregnancy

Rubella screening alerts the physician to which mothers require postpartum immunization. Also, if the susceptible woman has known exposure or disease, the physician can confirm acute infection through study of a second serum sample. Antenatal screening for syphilis allows for treatment of the mother as well as prevention of infection in the infant. Hepatitis B virus screening allows for preventive management of the infant of the hepatitis B surface antigen (HBsAg)-positive mother.

The interpretation of other serology in pregnancy in the absence of seroconversion is fraught with difficulty as the acuity of the infection often cannot be determined. A woman at high risk of CMV infection (e.g. a day-care worker) would therefore be advised to establish her immune status before pregnancy. However, the general value of routine antenatal screening for CMV, as is practiced in some European countries, is debatable, in part because of the difficulty in interpreting serology results. Thus, a positive IgM for CMV in the absence of suggestive symptoms may represent a false-positive test result. If confronted with this scenario, antibody avidity assays may be helpful in determining the timing of infection.[12]

Screening for toxoplasmosis is not universally recommended, but some have argued for its implementation.[13] *Toxoplasma gondii*-specific IgM antibody can persist for more than a year in about one-third of infections, making it difficult to establish the timing of maternal infection. If *T. gondii*-specific IgM is detected during pregnancy, additional serologic testing that includes the Sabin–Feldman dye test, IgM ELISA and avidity assays should be undertaken through a reference laboratory (e.g. Toxoplasma Serology Laboratory, Palo Alto Medical Foundation, Palo Alto, CA).[14] If serology is suggestive of acute infection, amniotic fluid sampling and testing by PCR can be used to confirm fetal infection.[14] Practitioner-based testing for acute toxoplasmosis in the absence of a community-based program should be discouraged in countries where screening and treatment are not routine.

Table 52.3 Summary of preventive antenatal strategies

Pathogen	Exposure avoidance	Infection screening	Potential maternal–fetal medical interventions
CMV	Hand washing after handling respiratory secretion and urine	Some jurisdictions support routine screening of day-care workers pre-pregnancy	CMV hyperimmune globulin and oral valganciclovir for pregnant women with confirmed acute infection may be of benefit
HIV	Avoid high-risk behaviors such as unprotected sex with multiple partners, intravenous drug use Condom use	Recommended for all women; testing strategies vary with jurisdiction (opt in vs. opt out) Repeat testing late in pregnancy for those at 'high risk' should be considered Exclude associated infections such as hepatitis B, hepatitis C, syphilis and other STIs	Antiretroviral therapy to mother and baby Elective Caesarian section if maternal viral load >1000 copies/ml
Parvovirus B19	Hand washing	Following exposure or during epidemic	Intrauterine transfusion may be of benefit in selected cases
Rubella	Hand washing	Routine and following exposure	If susceptible, immunize well before conception or postpartum
VZV	Avoid exposure to children with chickenpox if nonimmune	Following exposure or during outbreak	Immunization prior to or after pregnancy if nonimmune If susceptible, some experts recommend varicella zoster immune globulin within 96 hours of exposure Oral aciclovir in mother if develops chickenpox
Treponema pallidum	Avoid high-risk behaviors such as unprotected sex with multiple partners Condom use	Routine; if high risk, third trimester intrapartum testing Exclude associated infections such as HIV, hepatitis B, hepatitis C, gonorrhea and other STIs	Penicillin Monthly serologic follow-up
Toxoplasma gondii	Avoid eating undercooked meat and wash hands thoroughly after handling Wash fruits and vegetables before eating and after handling Avoid contact with materials potentially contaminated with cat excreta such as kitty litter boxes Wear gloves while gardening	Some jurisdictions recommend routine screening; if not, screen following suspected exposure	Spiramycin or pyrimethamine–sulfadiazine with folinic acid for documented maternal infection

Monitoring for evidence of infection (clinical manifestations and ultrasound findings)

Maternal illness

Rash in pregnancy requires exclusion of syphilis, rubella, parvovirus B19 and enterovirus infection. Arthritis occurs with parvovirus B19 infection as well as with rubella. In the presence of acute mononucleosis-like symptoms of fatigue and lymphadenopathy, Epstein–Barr virus, CMV, *T. gondii* and HIV infection must be considered.

Fetal ultrasonography

Ultrasound findings of in-utero infection are provided in the Clinical features section below and in Table 52.4.

Screening for congenital infections in newborns

Routine neonatal screening for congenital toxoplasmosis is practiced in several countries;[15,16] detection of *T. gondii*-specific IgM in newborn dried blood spots has been used for this purpose. Some have also advocated for routine neonatal CMV screening.[17] Potential strategies for detection of congenital CMV infection include shell vial culture of urine or saliva or PCR for CMV DNA in neonatal dried blood spots. The cost-effectiveness of screening neonates for congenital infections has not been established.[13]

Intervention

A variety of interventions are available to prevent congenital infections. These are detailed in the management section below and in Table 52.3.

Table 52.4 Clinical features of in-utero infection

General	Intrauterine growth retardation: all etiologies Hydrops fetalis: parvovirus B19, CMV, syphilis, toxoplasmosis, HSV, Coxsackie B3 Placentamegaly: CMV, syphilis
Head and neck	Hydrocephalus: toxoplasmosis, CMV, enterovirus Microcephaly: CMV, rubella, HSV, varicella, toxoplasmosis Intracranial calcification: CMV, toxoplasmosis, HSV, rubella, HIV, parvovirus B19, West Nile virus, lymphocytic choriomeningitis virus
Heart	Congestive heart failure: parvovirus B19, syphilis, CMV, toxoplasmosis Pericardial effusion: parvovirus B19, syphilis, CMV, toxoplasmosis Cardiac defects: rubella, parvovirus B19, mumps (not proven) Myocarditis: enterovirus
Lungs	Pleural effusion: parvovirus B19, syphilis, toxoplasmosis Pulmonary hypoplasia: CMV
Abdomen	Hepatosplenomegaly: CMV, rubella, toxoplasmosis, HSV, syphilis, enterovirus, parvovirus B19 Echogenic bowel: CMV, toxoplasmosis Hepatic calcifications: CMV, toxoplasmosis Meconium peritonitis: CMV, toxoplasmosis Ascites: parvovirus B19, CMV, toxoplasmosis, syphilis
Extremities	Limb reduction, limb restriction: varicella

CLINICAL FEATURES

Fetal

The features of in-utero infections are summarized in Table 52.4. Some fetal abnormalities detected on antenatal ultrasonography may be caused by infection. However, no ultrasonographic findings are pathognomonic for a particular agent.[18] Postnatal follow-up of infants, including ophthalmologic examination, cranial neuroimaging (i.e. CT and MRI), head growth and developmental progress over at least the first 2 years of life, is important in identifying affected infants.

Spontaneous abortion and stillbirth

Pregnancy loss has been associated with infection with CMV, enterovirus, HSV, HIV, parvovirus B19, rubella, *Treponema pallidum* and *T. gondii*. Fetal loss occurring with any maternal viral infection requires comprehensive pathologic and microbiologic evaluation to determine the role of the infection in pathogenesis.

Syndromes of congenital infection

General

The majority of infected infants have no symptoms at birth although some will develop sequelae later in childhood (Table 52.5). Routine investigation of premature or low birth weight infants for possible congenital infection only rarely yields positive results. Prematurity is typical of congenital syphilis and common in perinatal HSV infection. Intrauterine growth retardation occurs with rubella, CMV, toxoplasmosis and, occasionally, enteroviruses. The clinical findings in congenital infection are summarized in Table 52.6.

Table 52.5 Early onset and late onset manifestations of selected congenital infections

Micro-organism	Proportion infected with symptoms at birth	Selected early onset manifestations	Proportion with late onset manifestations	Late onset manifestations
CMV	10%	Low birth weight, petechial/purpuric rash, hepatosplenomegaly, thrombocytopenia, chorioretinitis, microcephaly, cerebral calcifications	5–15%	Sensorineural hearing loss, microcephaly, mental retardation, motor deficits, chorioretinitis
Rubella	First 16 weeks, decreasing from 90% to 24%	Low birth weight, blueberry muffin rash, hemolytic anemia, thrombocytopenia, hepatosplenomegaly, cardiac defects, cataract, bony lucencies	>20%	Sensorineural hearing loss, visual defects, multiple endocrinopathies, panencephalitis
Treponema pallidum	30% (30–40% stillborn)	Low birth weight, snuffles, diffuse rash, pseudoparalysis (perichondritis, osteitis), chorioretinitis, aseptic meningitis, hemolytic anemia	Unknown	Sensorineural hearing loss, visual defects, abnormal dentition, bone and joint abnormalities, CNS abnormalities
Toxoplasma gondii	Severe organ damage in 20% of infants of untreated mothers and 2% in treated mothers	Low birth weight, hepatosplenomegaly, macrocephaly with hydrocephalus, or microcephaly, intracranial calcifications	40% of infants of untreated mothers; 17% of infants of treated mothers	Chorioretinitis most common, sensorineural hearing loss

Table 52.6 Clinical findings in congenital infection[19,20]

Prematurity	Syphilis, HSV
Intrauterine growth retardation	All etiologies including tuberculosis
Anemia with hydrops	Parvovirus B19, syphilis, CMV, toxoplasmosis
Bone lesions	Syphilis, rubella
Cerebral calcification	Toxoplasmosis (widely distributed) CMV and HSV (usually periventricular) Parvovirus B19, rubella, HIV, West Nile virus, lymphocytic choriomeningitis virus
Congenital heart disease	Rubella, parvovirus B19, mumps (not proved)
Hepatosplenomegaly	CMV, rubella, toxoplasmosis, HSV, syphilis, enterovirus, parvovirus B19
Hydrocephalus	Toxoplasmosis, CMV, syphilis, possibly enterovirus
Hydrops, ascites, pleural effusions	Parvovirus B19, CMV, toxoplasmosis, syphilis
Jaundice	CMV, toxoplasmosis, rubella, HSV, syphilis, enterovirus
Limb paralysis with atrophy and cicatrices	Varicella
Maculopapular exanthem	Syphilis, measles, rubella, enterovirus
Microcephaly	CMV, toxoplasmosis, rubella, varicella, HSV
Ocular findings (see below)	CMV, toxoplasmosis, rubella, HSV, syphilis, enterovirus, parvovirus B19
Progressive hepatic failure and clotting abnormalities	Echovirus, Coxsackie B virus, enterovirus, HSV, toxoplasmosis
Pseudoparalysis	Syphilis
Purpura (usually appears on first day)	CMV, toxoplasmosis, syphilis, rubella, HSV, enterovirus, parvovirus B19
Vesicles	HSV, syphilis, varicella, CMV, parvovirus B19, enterovirus
Cataracts	Rubella, HSV, VZV, parvovirus B19, toxoplasmosis, syphilis
Chorioretinitis	HSV, VZV, rubella, CMV, toxoplasmosis, West Nile virus
Optic atrophy	HSV, VZV, rubella, CMV, toxoplasmosis, syphilis
Microphthalmia	Rubella, HSV, parvovirus B19, toxoplasmosis, CMV, varicella
Coloboma	CMV
Keratoconjunctivitis	HSV
Pigment retinopathy	Rubella
Glaucoma	Rubella, toxoplasmosis, syphilis
Iritis	HSV, rubella, syphilis
Anophthalmia	CMV
Peter's anomaly	CMV
Horner syndrome	VZV

Sepsis-like illness

HSV acquired just before delivery or intrapartum may present as a perinatal syndrome, often without skin lesions, and resemble neonatal sepsis or pneumonitis. An early laboratory clue is abnormal liver enzymes. Shock, coagulopathy, fulminant hepatitis and, often, skin lesions follow. Congenital tuberculosis and enteroviral infections can also present in this way.

Hepatitis

Enteroviruses, HSV and *T. gondii* can cause overwhelming acute neonatal liver failure in the first week of life. Other infections, including CMV and parvovirus B19, can also present with hepatic findings.

Central nervous system

Central nervous system (CNS) involvement may occur with all of the congenital infections (as well as with perinatally acquired HSV and enteroviral infections), although initial findings may be very subtle. Occult findings in congenital neurosyphilis have led to general recommendations that neurosyphilis be assumed with any cerebrospinal fluid (CSF) abnormality.[21] With newer molecular techniques, CNS involvement may be better recognized (i.e. PCR detection of CMV DNA in the CSF). Clinical symptoms and signs may be preceded by ophthalmologic and neuroimaging findings. Central nervous system calcifications can be observed in congenital CMV, toxoplasmosis, HSV, HIV, rubella, parvovirus B19 and lymphocytic chorioretinitis virus (LCM).

Cardiac

Rubella infection causes structural defects including patent ductus arteriosus; parvovirus B19 may cause intrauterine congestive heart failure with resulting prenatal closure of the foramen ovale and Epstein's anomaly, and occasionally acute and chronic lymphocytic myocarditis.[22] Viral myocarditis is characteristically caused by Coxsackie B virus or other enteroviruses.

Ophthalmologic

Ophthalmologic abnormalities are seen in a variety of congenital infections (see Table 52.9).[19]

Deafness

Sensorineural deafness is a common sequela of congenital CMV and rubella, as well as of untreated *T. gondii* and *Treponema pallidum* infections. One-third of sensorineural hearing loss in childhood is caused by congenital CMV infection. Of rubella-infected infants, 80% or more may have deafness, often as the only significant consequence. Given the progressive nature of impairment, serial hearing evaluations to age 6–9 years are recommended for CMV-infected infants.

Specific infections

Cytomegalovirus

Between 85% and 90% of children with congenital CMV infection are asymptomatic at birth. Common clinical manifestations in symptomatic newborns include small for gestational age growth parameters, petechiae, thrombocytopenia, jaundice and hepatosplenomegaly. More severely affected infants may also have microcephaly, CNS calcifications, chorioretinitis or sensorineural hearing loss.

Symptomatic infants are at high risk of significant neurologic and developmental dysfunction, particularly if abnormalities are noted on cranial CT or if chorioretinitis exists. Cerebral calcifications tend to be periventricular. Most children with an abnormal newborn CT scan (90%) develop at least one neurologic sequela as compared with 29% of those with normal study, making cranial CT scan a good predictor of an adverse neurodevelopmental outcome. Normal development at 12 months of age makes subsequent neurodevelopmental or intellectual impairment unlikely. Infants with asymptomatic infection have a 5–15% risk of sensorineural hearing loss, mental retardation, motor spasticity or microcephaly evolving in the early years. Hearing loss may be late in onset, fluctuating and progressive, necessitating long-term follow-up (see Deafness above).

Enteroviruses (coxsackieviruses, echovirus)

Although high rates of maternal infection in pregnancy (up to 25%) have been reported, disease is limited to case reports or series of infected infants with a variety of entities including growth retardation, CNS malformations, blueberry muffin rash, hepatic necrosis, myocarditis or pericarditis.

Herpes simplex virus

Intrauterine HSV infection is characterized by the triad of skin vesicles or scarring, eye lesions and microcephaly or hydranencephaly. It may follow primary or recurrent, symptomatic or asymptomatic, HSV-1 or HSV-2 maternal infection at any stage of gestation. Acquisition just before delivery can lead to disease identical to that acquired at delivery, except that it occurs within the first 48 hours of life. The presence of even a single vesicle should prompt ophthalmologic examination, lumbar puncture with CSF analysis and cranial imaging.

Parvovirus B19

Seroprevalence studies indicate that approximately half of women of childbearing age are susceptible to parvovirus B19 and that the annual seroconversion rate among such women is about 1.5%. The risk of transplacental transmission of the infection is approximately 30%.[23] The vast majority of such infections, both symptomatic and asymptomatic, are followed by delivery of a healthy term infant.

The most common clinical manifestations of congenital infection with parvovirus B19 are pregnancy loss and nonimmune hydrops fetalis. The rate of fetal loss has been estimated at 5–9%, though it remains unclear what proportion of these are specifically due to parvovirus B19 infection.[23] Nonimmune hydrops fetalis is uncommon, occurring in 0–1.6% of parvovirus B19 infections; on the other hand, this virus is responsible for 10–20% of all nonimmune hydrops fetalis cases.

Rubella

The clinical features of congenital rubella syndrome (CRS) can be divided into the categories of transient, in newborns and infants; permanent, at birth or during the first year of life; and delayed, occurring in 10–20% of patients, usually in the second decade of life.[11] In rare cases, maternal reinfection has resulted in CRS.

Varicella-zoster virus

The congenital varicella syndrome (or fetal varicella-zoster syndrome, better reflecting the pathogenesis), including cicatricial skin scars, eye abnormalities including microphthalmia, hypoplastic limbs, and autonomic nervous system damage causing gastroesophageal reflux with or without CNS abnormalities, occurs almost exclusively with maternal varicella infection acquired before 20 weeks of gestation. Generally, after 20 weeks, manifestations include skin scars, as with postnatal VZV infection, and childhood shingles. Only rarely has this syndrome been reported after gestational zoster.

Subclinical maternal VZV infection is now recognized as a cause of neurologic symptoms and signs in children without other manifestations of congenital varicella syndrome, including dermatologic findings, suggesting the damage done by intrauterine varicella is underestimated. If the CNS is relatively spared, a good long-term outcome can occur.

Treponema pallidum

Most commonly, physicians are required to investigate the asymptomatic infant whose mother had positive serologic testing for syphilis. Among such infants, bone lesions are the most frequently encountered abnormality, occurring in 20% of infected cases. Cerebrospinal fluid examination is required for all infants with possible congenital syphilis and some experts believe that minimal abnormalities in the CSF should be considered to indicate neurosyphilis. These include leukocyte counts of 5/mm^3 or more and protein concentration of 100 mg/dl or greater.

Toxoplasma gondii

The diversity of findings may be classified according to timing of symptomatology as:

- symptomatic neonatal disease;
- disease in the first months of life, usually with neurologic and ophthalmologic findings;
- sequela of previously undiagnosed infection (i.e. chorioretinitis later in childhood); and
- subclinical disease.

Severely affected children may present with the classic triad of hydrocephalus, intracranial calcifications and chorioretinitis. Of asymptomatic infected infants, half will have abnormalities on cranial imaging or on ophthalmologic examination. Among 23 000 mothers and infants in the Collaborative Perinatal Project, infants of IgG *T. gondii* antibody-positive mothers had a twofold increase in hearing loss, a 60% increase in the incidence of microcephaly and a 30% increase in the occurrence of low intelligence quotient (IQ <70).

Other

Intrauterine adenovirus infection has been associated with fetal myocarditis, pneumonia and encephalitis. Measles infection in pregnancy increases the risk of prematurity in the first 2 weeks after rash. Congenital West Nile virus infection has been associated with chorioretinitis and significant brain injury.[24]

DIAGNOSIS

Diagnosis of fetal infection

Attribution of adverse outcome to a particular micro-organism in early fetal life is problematic and frequently requires molecular diagnostic testing (e.g. DNA detection by PCR). Detection of micro-organism-specific IgM is hampered not only by the testing method but also by the failure of the fetus to reliably produce IgM-specific antibody before 22–24 weeks. Fetal infection may follow maternal infection by at least 4–6 weeks, providing false reassurance if fetal diagnosis, through direct detection or antibody production, is attempted too soon after maternal infection.

Antenatal diagnosis of fetal infection requires a multidisciplinary approach to exclude noninfectious causes and to obtain maternal–fetal studies. In the absence of fetal disease, prenatal diagnosis of fetal infection is not warranted because the predictive values of positive and negative results cannot be used as a basis for management decisions.[25] For example, in contrast to genetic diseases for which the outcome is reasonably certain, most infants with congenital CMV infection are asymptomatic and do not suffer sequelae. Follow-up of infants with suspected congenital infections over the ensuing months is needed to determine the presence, extent and damage, if any, resulting from the infection.[26]

Maternal testing

Many maternal infections are asymptomatic and all infections, symptomatic or otherwise, can pose a risk to the fetus. Laboratory tests are listed in Table 52.7. Routine broad screening of a single serum is unlikely to be helpful and is rarely indicated. The diagnosis of a primary CMV infection can be made by demonstration of seroconversion of CMV-specific IgG antibodies from negative to positive, but not by boosting of a titer, as this may occur with recurrent infection. Differentiation of the serofast state from inadequately treated syphilis can be difficult. A serofast patient usually has a titer of ≤1:4; although titers can be as high as 1:8,[27] caution in interpretation is essential.

Maternal symptoms and their likelihood

Although fetal disease follows both symptomatic and asymptomatic maternal infection and maternal infections are frequently asymptomatic, the following symptoms should suggest a search for micro-organisms: a flu- or acute mononucleosis syndrome-like illness (CMV, *T. gondii*, HIV) and arthritis or rash (parvovirus B19, rubella, *Treponema pallidum*). Maternal history relevant to congenital infections is summarized in Table 52.8.

Fetal testing

Detection of the micro-organism by culture or its DNA by PCR in amniotic fluid or fetal blood is promising, but should generally not be undertaken in the absence of fetal abnormalities because of the uncertain sensitivity and specificity. After 18 weeks of gestation, and at least 4 weeks after maternal infection, PCR testing of the amniotic fluid will detect 97% of *T. gondii*-infected fetuses.[28] Fetuses infected with CMV are likely to have positive amniotic fluid cultures after 20–22 weeks of gestation because fetal kidney infection is common.

Neonatal testing

In evaluating the neonate with suspected congenital infection at birth, it is necessary to:

- review the maternal history including serologic screening (Tables 52.7 and 52.8);
- review ultrasonography undertaken in pregnancy (Table 52.4);

Table 52.7 Laboratory evidence of clinically significant maternal infection

Micro-organism	Detection by culture, PCR (site)	Serology
CMV	Infrequently (blood/serum)	• Seroconversion • Possibly CMV IgM capture ELISA • Possibly IgG avidity testing to determine timing of infection
Enterovirus	Yes (stool, throat, other)	• Seroconversion
HSV	Yes (vesicle)	• Seroconversion • Research lab required to differentiate between HSV-1 and HSV-2
Parvovirus B19	Yes (blood)	• Seroconversion, • Parvovirus B19-specific IgM
Rubella	Infrequent (nasopharynx, urine)	• Seroconversion • Rubella-specific IgM
VZV	Yes (vesicle)	• Seroconversion
Treponema pallidum	Yes (lesion, wet field)	• VDRL/RPR ≥1:8 and positive treponemal test
Toxoplasma gondii		• Seroconversion • Toxoplasmosis-specific IgM with confirmatory testing in reference laboratory to determine timing of infection

Table 52.8 Maternal history relevant to congenital infections

Woman's history

Underlying illness and medications

Previous history of sexually transmitted disease (HSV, syphilis, chlamydia, gonorrhea, HIV)

Drug or alcohol use, current and previous

Travel during pregnancy (consider culinary practices and other factors in region traveled)

Occupation
- Working with children wearing diapers or who have disabilities (CMV)
- Working with elementary school children (parvovirus, rubella)
- Working with animals or raw meat products (*Toxoplasma gondii*)
- Working in the sex trade industry (HIV, syphilis, tuberculosis, hepatitis B virus, hepatitis C virus)

Household exposure to young children (CMV)

During her pregnancy
- Has she eaten raw meat or tasted it while cooking? (toxoplasmosis)
- Has she consumed unwashed vegetables? (food-borne pathogens, toxoplasmosis)
- Has she changed kitty litter without wearing gloves? (toxoplasmosis)
- Has she worked in soil/garden without wearing gloves? (toxoplasmosis)
- Has she had any illness she might not have mentioned until now?
- Cold sore? (HSV)
- Dysuria, burning, itching? (HSV)
- Profound fatigue? (CMV, toxoplasmosis, HIV)
- Swollen glands? (CMV, toxoplasmosis, HIV)
- Arthritis? (rubella, parvovirus B19)
- Rash? (rubella, parvovirus B19, syphilis, enterovirus)

Has her husband/partner had any particular illness?
- Skin sores anywhere? (HSV, syphilis, HIV)

Routine serologic testing

HBsAg, rubella-specific antibody, syphilis serology (treponemal and/or non-treponemal tests)
- First trimester
- If high risk, repeat third trimester, delivery

Antenatal care
- If absent, see Table 52.9

Table 52.9 Evaluation of neonate with suspected congenital infection

Clinical	Physical examination (gestational age, height, weight, head circumference) to identify prematurity, intrauterine growth retardation, microcephaly Measure liver/spleen size Ophthalmologic examination (by pediatric expert)
Laboratory	Complete blood count and smear Platelet count Liver transaminase levels Bilirubin level, direct and indirect CSF examination (cells, protein, with pertinent antibody detection; see Table 52.10) Laboratory testing (detection of agent, maternal and infant (not cord) serology) Immunoglobulin determinations Hold pretransfusion blood for possible additional tests
Other	Cranial CT scan with enhancement Long-bone radiographs (if syphilis or rubella likely) Placental pathology Audiology assessment Multidisciplinary follow-up

- evaluate the infant clinically (Tables 52.6 and 52.9);
- attempt detection of the pathogen in the neonate; and
- undertake judicious maternal and infant serologic testing pertinent to the most likely diagnoses.

Detection of the micro-organism in the neonate

The best evidence for infection with CMV, enterovirus, HSV, parvovirus B19, rubella, syphilis, and *T. gondii* comes from detection of the agent in culture or by molecular detection techniques in the neonate (see below).

Maternal and infant testing pertinent to the most likely diagnoses

Diagnostic tests are summarized in Table 52.10. Over- and underdiagnosis of congenital infection has arisen as a result of failure to:

- submit maternal serology for documentation of a source of infection;
- detect the organism in the infant through culture (or molecular detection techniques);
- sequentially test the infant serologically; and
- appreciate the enormous rate of false-positive and false-negative test results obtained through IgM-specific testing and use of cord blood.

False-negative IgM tests in infants are very common (e.g. only 25% of infants with clinical manifestations of intrauterine infection had VZV IgM).[29] An exception is the rubella-specific IgM test, which is highly sensitive and specific. Parvovirus-specific IgM is frequently negative when virus is detected by PCR.

Serologic documentation of maternal infection and acute and follow-up serology of the infant over the first months or year of life can be diagnostic, albeit not sufficiently quickly to facilitate decisions about therapy. Passively transferred antibody will disappear during the first 6–12 months, whereas antibody persists or rises in the infected infant. Follow-up serology is more complicated in the case of congenital CMV as infection may also be acquired at birth or postnatally through breast milk or other contact.

Cord blood is not acceptable for specific antibody testing for syphilis, *T. gondii* or CMV because of false-positive and false-negative results. Total cord IgM levels may be falsely elevated through contamination by maternal blood. Cases of congenital syphilis have been reported in which both mothers' and neonates' titers were negative at birth but the infants subsequently developed clinical syphilis at 3–14 weeks of age with strongly positive titers.

The futility of a single serologic (syphilis, toxoplasmosis, other agents, rubella, CMV, herpesvirus (STORCH)) screen is well known and a more directed approach is required. Every effort should be made to recover the organism from the neonate and follow-up maternal and infant blood (Table 52.11).

Definitions of selected congenital infections

Because of delayed onset and/or recognition of signs of infection as well as the varied availability of diagnostic tests, definitions have been developed that take into consideration the likelihood of infection (Table 52.12).

Table 52.10 Laboratory investigation and follow-up of neonate with suspected congenital infection

Infection	Mother at birth	Neonatal (not cord blood)	Follow-up of infant
CMV	Antibody	Virus detection in urine, saliva, blood leukocytes, CSF IgM capture enzyme-linked immunosorbent assay (ELISA) and radioimmunoassay antibody	Repeated antibody testing to 6–12 months; passive maternal antibody disappears at 4–9 months; negative infant and maternal antibody rules out infection, although intrapartum cervical and postpartum breast milk transmission is common
Enterovirus	Virus detection in stool, throat, blood, of mother of infant with suspected congenital enteroviral infection Bank serum	Virus detection in stool, throat, CSF, nose, blood, other Bank birth and 2nd serum at 2–4 weeks	Selective testing of paired infant and maternal sera appropriate to infant, maternal or community isolates
HSV	Antibody	Virus detection in skin vesicles, throat, CSF, urine, nose, conjunctiva, rectal swab Antibody	Repeated antibody testing to 6–12 months (cannot differentiate between type 1 and type 2 viruses) Negative infant and maternal antibody rules out infection
Parvovirus B19	IgM and IgG antibody Detection of DNA (e.g. by PCR) in blood	Detection of DNA (e.g. by PCR) in blood, bone marrow Parvovirus-specific IgM	Repeated antibody testing to 6–12 months Infected infants and their mothers may lack IgM antibody
Rubella	Rubella-specific IgG and IgM antibody	Rubella-specific IgM antibody Virus detection in urine, nasopharynx, CSF, blood CSF rubella-specific IgM antibody	Repeated antibody testing to 6–12 months Negative infant antibody at 6–12 months usually rules out infection
VZV	Antibody	Virus detection in skin lesions Antibody	Antibody testing 6–12 months postnatally Intrauterine infection commonly manifest only as persistent antibody and childhood zoster
Toxoplasma gondii	Toxoplasma-specific IgM and IgG antibody To determine acuity of infection in IgM-positive mother, need reference laboratory testing Seroconversion or fourfold rise in *Toxoplasma*-specific IgG in pregnancy	Reference laboratory testing of *Toxoplasma gondii* specific: IgM-ISAGA, DS-IgM-ELISA. IgE-ELISA, IgA-ELISA and virus detection by PCR in CSF (screening *Toxoplasma gondii*-IgM can be falsely positive and negative) Culture, PCR and histopathology of the placenta	Repeated antibody testing to 6–12 months Negative infant antibody at 6–12 months usually rules out congenital infection
Treponema pallidum	Nontreponemal antibody tests (quantitative): VDRL, RPR Treponemal antibody tests: EIA, TPPA, FTA, ABS If positive, maternal HIV status	Detection of treponemes in nasal secretions, skin lesions, etc. by darkfield examination Quantitative VDRL, RPR Serum treponemal antibody (e.g. EIA, TPPA, FTA, ABS)	Repeated quantitative VDRL/RPR and treponemal antibody testing to 12–15 months No test at birth can differentiate between the asymptomatic infected and uninfected neonate Passively transferred antibody disappears at 6 months (VDRL, RPR) and 12–15 months (treponemal tests)

Table 52.11 Appropriate specimens to diagnose congenital infection[20]

Specimen	Tests	Interpretation
Urine	Viral culture/detection (CMV, HSV, rubella)	Urine for CMV must be obtained at ≤2 weeks of age Positive is diagnostic
Throat swab	Viral culture (CMV, HSV, rubella, enteroviruses)	Positive is diagnostic
Blood	Agent detection (CMV, parvovirus B19)	Positive is diagnostic
Neonatal serum (single specimen)	Rubella-specific IgM	Positive is diagnostic although determination of status at 10–12 months is confirmatory

(Continued)

Table 52.11 Appropriate specimens to diagnose congenital infection[20]—cont'd

Specimen	Tests	Interpretation
Sequential neonatal, infant sera over 6–12 months	All	Passive maternal antibody in uninfected infant disappears at 4–9 months for CMV (unless peri-, postnatal transmission); 8 months for *Toxoplasma. gondii*; 6 months VDRL, rapid plasma reagin; 12–15 months treponemal Positive specific antibody at 8–12 months suggests congenital toxoplasmosis, parvovirus B19, rubella, VZV infection
Single maternal serum at delivery	*Toxoplasma gondii*-specific IgM	If IgM -specific antibody positive, reference laboratory testing of maternal, infant sera and placenta
Serology of both mother and infant	All	Negative maternal serology rules out source of infection Serial infant serology identifies passive maternal antibody (titers fall) and active infection (titers remain the same, rise over months)
CSF culture, detection	Detection CMV, enteroviruses, HSV, *Toxoplasma gondii* (reference laboratory), parvovirus B19 Rubella-specific IgM antibody VDRL/RPR and/or treponemal tests (non-bloody tap)	Positive is diagnostic
Skin lesions culture, detection	If active/vesiculated at birth: herpes, enteroviruses VZV Syphilis darkfield microscopy	Positive is diagnostic
Nasopharyngeal secretions	Syphilis darkfield microscopy	Positive is diagnostic
Stool cultures	Enteroviruses	Positive is diagnostic
Placenta	Variable	May be supportive of specific pathogen

Table 52.12 Definitions of selected congenital infections

CMV	Confirmed	Detection of CMV in the first 2–3 weeks of life in urine, throat or other sources in newborn with one or more of: • Small for gestational age • Hematologic findings (petechiae, purpura, splenomegaly, jaundice at birth) • Neurologic findings (microcephaly, chorioretinitis, neurologic abnormality, intracranial calcifications, hearing impairment) • Laboratory findings: direct hyperbilirubinemia >3 mg/dl, thrombocytopenia (platelet count <75 000/ml), liver function abnormality (alanine aminotransferase >100 mg/dl)
	Possible	As for Confirmed, except viral detection only after 3 weeks of age and other diseases ruled out
HSV	Congenital	Detection of HSV within 24 hours of birth or stable positive titer over 3 months in infants with one or more of skin, eye and brain lesions
	Perinatal	Detection of HSV after 24 hours and in first 6 weeks of life further characterized by clinical and laboratory findings as one of: • Disseminated • CNS • Skin, eye, mouth disease (with negative CSF PCR)
Rubella	Confirmed	Defects of congenital rubella syndrome present and one more of: • Virus detected • Positive rubella-specific IgM antibody • Positive infant serology after disappearance of passively transferred maternal antibodies at 3–12 months
	Compatible	Insufficient laboratory data for confirmation of diagnosis but any two complications from (a) or one from (a) and one from (b): (a) Cataracts or congenital glaucoma, congenital heart disease, hearing loss, pigmentary retinopathy (b) Purpura, splenomegaly, jaundice, radiolucent bone disease, meningoencephalitis, microcephaly, mental retardation
	Possible	Presence of some compatible clinical findings, but insufficient criteria for either the Confirmed or Compatible categories

Table 52.12 Definitions of selected congenital infections—cont'd

Syphilis	Confirmed Presumptive	Identification of *Treponema pallidum* in nasal or skin lesions One or more of: • Infant born to mother with untreated or inadequately treated syphilis • Treated with drug other than penicillin and/or • <30 days to 3 months before delivery • Infant has reactive treponemal test with findings of one or more of abnormal physical examination, long bone radiographs, CSF (including reactive CSF-VDRL and/or a leukocyte count of 20 ml or greater or a protein concentration of 100 mg/dl or greater), fourfold higher VDRL than mother has • Infant has documented fourfold rise in titers and positive treponemal test • Infant has reactive treponemal test that does not revert by 12–15 months
Toxoplasma gondii	Confirmed Compatible	• *Toxoplasma gondii*-specific IgM (or if available, Sabin–Feldman dye test >300 IU) in maternal sera with infant findings of chorioretinitis, cerebral calcifications or hydrocephalus in the absence of CMV infection • Positive antibody in infant at 8–10 months (after disappearance of passively transferred maternal antibody) with or without clinical findings • Chorioretinitis with positive *Toxoplasma gondii*-specific antibody after 8–10 months of age • Cerebral calcifications and/or hydrocephalus with positive *Toxoplasma gondii*-specific antibody after 8–10 months of age in the absence of CMV infection
Parvovirus B19		Detection of parvovirus B19 by: • Direct electron microscopy or nucleic acid in blood and/or tissue obtained within the first 3 weeks of life in the presence of fetal or neonatal findings of hydrops and anemia • Parvovirus B19-specific IgM in the first 3 weeks or persistent IgG beyond 3–12 months with either documented maternal infection or fetal/neonatal findings of hydrops/anemia or cranial calcification
Varicella		One or more of: • Anomalies of congenital varicella syndrome (skin, eye, limb, neurologic) • Acute varicella at birth with viral detection • Herpes zoster in the first year of life with viral detection • VZV-specific IgM at birth, persistent IgG to 12 months or detection of specific lymphocyte transformation in response to VZV virus antigen

MANAGEMENT

General

A thorough understanding of the consequences of infection in pregnancy is required to counsel parents regarding risks to the fetus and possible courses of action. Selected maternal infections should be followed by ultrasonography to identify adverse effects. Antenatal diagnosis is generally attempted if fetal abnormalities are present. After delivery, additional information about contagiousness, breast-feeding and risk of transmission in subsequent pregnancies should be conveyed. Comprehensive long-term pediatric follow-up is required to identify and manage the spectrum of cognitive, motor, visual, hearing and other impairments that may be imparted by some of these infections.

Specific infections

Cytomegalovirus

The management of a pregnant woman with serologic evidence of acute CMV infection is difficult, in part because it is not possible to predict the outcome of the fetus; most infants born to such women are normal. Termination of pregnancy is certainly an option when significant abnormalities are detected on fetal ultrasound. The potential role of antenatal treatment of the mother with CMV-immune globulin[30] or valganciclovir[31] in preventing congenital CMV infection or disease is intriguing, but requires further study.[32]

Neurologic outcome cannot be reliably predicted until the infant is 2 or 3 years of age, during which head growth is monitored and achievement of developmental milestones is documented. Infants with abnormal cranial imaging are more likely to have cognitive and other deficits on follow-up.[33] Progression of existing retinal lesions or delayed development of chorioretinitis during childhood has been reported in about 20% of children, whereas hearing loss will evolve in about 30% of children, as late as school age. Children with asymptomatic congenital CMV infection show no differences in IQ score or other neuropsychologic test performance as compared with uninfected children.[34]

The benefit of and indications for antiviral therapy remain controversial. In a phase III randomly controlled trial, ganciclovir therapy (6 mg/kg/dose twice daily for 6 weeks), given to infants with evidence of CNS disease, chorioretinitis or hearing loss at baseline, protected infants from further hearing deterioration.[35] Transient effects on growth and resolution of transaminase elevation were also seen. Approximately two-thirds developed neutropenia, with half requiring dose reduction. The study has been criticized because less than 50% of subjects had evaluable data at baseline and at 6 months. The potential role of oral valganciclovir,[36] and prolonged therapy with ganciclovir or valganciclovir remain to be defined.[37]

Several studies have suggested a link between the development of sequelae, such as hearing loss, and viral burden, as measured by CMV antigenemia, quantitative PCR in blood and quantitative urine culture.[38,39] It is plausible that these measures of viral burden could be useful in selecting subjects for whom antiviral therapy is likely to be beneficial. Prevention through vaccination is urgently needed.

Herpes simplex virus

As intrauterine infection is rare, infected women should be assured that maternal infection is common but that fetal infection is unusual.

Parvovirus B19

After maternal exposure and documented infection, serial ultrasonography for 1–14 weeks will identify the hydropic fetus. For the fetus with nonimmune hydrops fetalis and a low hematocrit and reticulocyte count, one or more intravascular fetal transfusions of packed erythrocytes, until marrow aplasia resolves, have been used with good results. Hydropic fetuses with an intermediate hemoglobin and a high reticulocyte count, in whom the process is resolving spontaneously, need not be transfused and show no sequelae. There is no medical indication for termination of the pregnancy complicated by parvovirus B19 infection.

Rubella

Infected infants should be considered infectious for the first year of life unless repeated nasopharyngeal and urine cultures are negative. Long-term follow-up will identify progressive hearing loss and visual deterioration, endocrinopathies and subacute sclerosing panencephalitis.

Treponema pallidum

All infected women should receive penicillin therapy appropriate to their stage of disease to minimize or eliminate the risk of transmission to the fetus. If serology has been positive for less than 1 year, the patient may be treated with intramuscular benzathine penicillin G 2.4 million units as a single dose (some experts recommend two doses 1 week apart). If the patient has been seropositive for 1 year or more, benzathine penicillin G 2.4 million units in a single dose weekly for 3 successive weeks is required. Maternal CSF examination (protein, cell count, CSF-VDRL) is required only if neurosyphilis is suspected; it is not routinely required in patients with infectious syphilis if the neurologic examination is negative. Retreatment during pregnancy is unnecessary if titers followed monthly continue to fall. In the presence of penicillin allergy, desensitization is the preferred management; any other therapy will necessitate treatment of the infant after birth. All women with positive serology require HIV testing and testing of their recent sexual contacts, as well as repeat testing in the third trimester and at birth.

All infants born to seropositive mothers should be evaluated for possible congenital syphilis. Quantitative nontreponemal serology (rapid plasma reagin (RPR)/VDRL) should be done for all cases. Cord blood should not be used for RPR/VDRL testing as it may be falsely negative or positive.[40] The extent of investigation of the infant is dependent on the adequacy of and response to therapy in the mother, placental pathology and initial evaluation of the infant.[41] A full evaluation including complete blood count, treponemal and nontreponemal serology, long bone X-rays and CSF analysis (including nontreponemal titers in the CSF) is indicated for those deemed at risk.

The decision to treat an infant as possible congenital syphilis should follow a thorough evaluation of the maternal history, as well as the infant's clinical manifestations, laboratory results and radiographic findings.[41] Aqueous crystalline penicillin G 100 000–150 000 IU/kg (given every 8–12 hours) intravenously for 10–14 days is the recommended standard regimen.[41] After the second week of life, or with confirmed neurosyphilis or severe disease, aqueous penicillin 200 000–250 000 IU/kg per day may be used.[21] A preliminary study suggests that selected asymptomatic infants may be treated with intramuscular benzathine penicillin 50 000 IU/kg once, provided that there are no signs of congenital syphilis on physical examination, CSF cell count is normal and CSF-VDRL is negative, and radiographic studies of the long bones of the lower extremities, platelet counts and liver function tests are normal. Follow-up of all infants is required up to 1 year or beyond to ensure that the appropriate falls in titers have occurred and that treatment or retreatment is not required.

Toxoplasma gondii

If acutely infected women can be identified and treated in pregnancy through organization of a regional system of screening, there is evidence that the outcome is improved. Maternal spiramycin therapy may reduce the rate of transmission to the fetus and is recommended prior to 18 weeks' gestation. Once congenital infection is established, the combination of pyrimethamine and sulfadiazine is required to improve fetal outcome. The regimen currently recommended for treatment in North America is given in Table 52.13 (see also Practice Point 25).

The outcome of congenitally infected infants with treatment started at birth has been shown to be substantially better than that of historic controls not treated or treated for 1 month only.[42] Normal cognitive, neurologic and auditory outcome was observed in all children who did not have neurologic disease at birth. Furthermore, more than 70% of those with moderate or severe neurologic disease at birth had normal cognitive and/or neurologic outcome and none had sensorineural hearing loss. In contrast, when infants are not treated, late sequelae after subclinical disease include active chorioretinitis in 85%, including some cases of blindness or impaired vision, developmental delay in 20–75% and moderate hearing loss in 10–30%.

Perinatal infections

These occur as a result of ascending infection and premature rupture of the membranes or through intrapartum transmission. Their management and prevention are summarized in Table 52.14.

Specific infections

Enterovirus

Enteroviruses are among the commonest pathogens encountered by the newborn in the first months of life. The most common symptoms are undifferentiated fever and aseptic meningitis. A sepsis-like syndrome with or without myocarditis and hepatitis is occasionally seen; this is commonly associated with a nonspecific late-gestational febrile illness in the mother. Pleconaril is a potential therapeutic agent,[43] but is not currently available outside of the research setting. A randomized, double-blind, placebo-controlled clinical trial sponsored by the US National Institutes of Health evaluating pleconaril in enteroviral-associated neonatal sepsis syndrome is currently underway. Given the likelihood that an infected infant has received no passive maternal antibody, high-dose intravenous immune globulin may be reasonable in the acutely ill infant, although efficacy has not been demonstrated.

Herpes simplex virus

Because it is the primary infection, rather than recurrent infection in pregnancy, that is a risk to the fetus, transmission cannot be eliminated despite the best obstetric care. Primary infection is characterized by more prolonged shedding (mean of 11 days) and a 33% risk of transmission to the newborn if shedding occurs at delivery. Conversely, in recurrent infection, shedding lasts 2–4 days, and if present at birth, is associated with a 2% risk of transmission.

Neither a positive nor a negative clinical history will predict the neonatal risk for HSV infection. Of women with no previous history of genital HSV, 0.2% will have positive genital cultures at delivery.[44] Because viral shedding at the time of delivery cannot be predicted by third trimester cultures, screening is not recommended.

Women with recurrent disease should be reassured; the risk of neonatal infection in this situation is at most 1 in 2000 births. For women with first episode genital herpes during pregnancy or those with multiple recurrences, oral aciclovir or valaciclovir has been utilized to reduce the risk of active lesions at delivery and the need for Caesarian section.[45,46] Caesarian section is generally recommended for women with active lesions at birth or a clinical primary infection during pregnancy, particularly in the last half of gestation; infection may occur in spite of Caesarian section. Fetal scalp monitors should be

Table 52.13 Treatment of toxoplasmosis in the fetus and infant

Manifestation of disease	Medication	Dosage	Duration of therapy
In pregnant women with acute toxoplasmosis (first 21 weeks of gestation or until term if fetus not infected)	Spiramycin	1 g q8h without food	Until fetal infection documented or excluded at 21 weeks; if documented, in alternate months with pyrimethamine, leukovorin, and sulfadiazine until term (France)
If fetal infection confirmed after week 17 of gestation or if maternal infection acquired in last few weeks of gestation	Pyrimethamine and Sulfadiazine Leukovorin (folinic acid)	Loading dose: 100 mg/day in two divided doses for 2 days then 50 mg/day Loading dose: 75 mg/kg per day in two divided doses (maximum 4 g/day) for 2 days then 100 mg/kg per day in two divided doses (maximum 4 g/day) 10–20 mg/day	Until term (leukovorin is continued 1 week after pyrimethamine is discontinued)
Congenital *Toxoplasma gondii* infection in the infant	Pyrimethamine and Sulfadiazine and Leukovorin Corticosteroids (prednisolone) have been used when CSF protein is ≥1 g/dl and when active chorioretinitis threatens vision	Loading dose: 2 mg/kg per day for 2 days, then 1 mg/kg per day for 2–6 months, then this dose every Monday, Wednesday and Friday 100 mg/kg per day in two divided doses 10 mg 3 times a week 1 mg/kg per day in two divided doses	1 year 1 year 1 year Until resolution of elevated (≥1 g/dl) CSF protein level or active chorioretinitis that threatens vision

Adapted with permission from Hohlfeld *et al*[28] and Remington *et al*.[10]

Table 52.14 Preventive strategies and intrapartum management for selected perinatally acquired infections

Micro-organism	Clinical situation	Preventive management	Comment
Enterovirus	Active maternal enteroviral infection (i.e. fever, abdominal pain) at delivery	Attempt to defer delivery	May allow transmission of antibody
Hepatitis B virus	Maternal HBsAg status unknown	Neonatal passive plus active immunization	Many experts recommend universal neonatal immunization
HSV	Maternal lesions at delivery	Active observation of neonate for signs of infection	See detailed guidelines Caesarian section for active lesions or primary infection in (late) pregnancy
HIV-1	HIV-positive mother	Maternal antiretroviral therapy* Single or combination antiviral therapy of infant*	Antenatal delivery screening and therapy is recommended
Rubella	High risk, no antenatal care, no serology at delivery	Postpartum immunization of susceptible mother before hospital discharge	Failure to immunize prior to discharge has resulted in subsequent infected neonates
VZV	Maternal lesion within 1 week before or after delivery	Varicella-zoster immune globulin ± aciclovir therapy of neonate	Postpartum immunization of mothers known to be susceptible
Treponema pallidum	High risk, no antenatal care, no serology at delivery	Evaluation and possible therapy with follow-up of neonate	In high-risk women repeat screen in third trimester, delivery

*Potential interventions vary depending on the timing of diagnosis; intrapartum iv zidovudine with or without other antiretroviral agents should be given to the mother; the infant should receive zidovudine; the addition of other antiretroviral agents for the infant may be considered in selected cases.

avoided. Viral cultures should be obtained from the maternal cervix at birth. Infant cultures (i.e. urine, mouth, nasopharynx, stool/rectum) should be obtained at 24 hours and the child carefully observed for symptoms of neonatal HSV requiring therapy. Initial evaluation of all infants with suspected or confirmed HSV infection must include CSF PCR to detect subclinical CNS disease.

Neonatal HSV disease may manifest as disseminated disease, involving multiple organs, most notably the lungs and liver, localized CNS disease and isolated skin/mucosal disease. Skin lesions may be absent in disseminated and CNS disease. Aciclovir therapy for presumed or confirmed HSV infection is prescribed at a dose of 60 mg/kg per day intravenously q8h for 14–21 days.[47] For isolated skin and mucosal surface involvement, 14 days is sufficient, whereas 21 days of therapy is required with disseminated disease, encephalitis, or in the presence of an abnormal CSF profile. While mortality is certainly reduced with therapy, morbidity remains significant. Contact isolation of the infant with lesions is required.

Relapses of neonatal HSV infection with cutaneous and neurologic involvement have been reported, in some cases with the development of neurologic damage.[48] Long-term suppressive oral aciclovir has been suggested as a strategy to prevent such recurrences. In one small prospective study, 81% of infants on long-term suppressive aciclovir therapy had no recurrences, though 46% developed neutropenia.[49] Recurrent HSV encephalitis with aciclovir-resistant virus, while on suppressive acyclovir therapy, has recently been reported.[50]

Varicella-zoster virus

A neonate whose mother has developed varicella 5 days before delivery to 48 hours after delivery should receive a dose of 125 U (1.25 ml or 1 vial) of zoster immune globulin as soon as possible. These infants carry a 30% risk of infection without the protection of passive maternal antibody and a 30% mortality rate without antiviral therapy. Aciclovir at a dose of 1500 mg/m^2 per day is used to treat symptomatic neonates,[51] with dose adjustments for liver and renal failure or prematurity. Isolation of the exposed hospitalized infant is required.

HIV

In the absence of intervention the risk of mother-to-child transmission of HIV is approximately 40% (5% in utero, 20% intrapartum and 15% breast-feeding). Both early and late in-utero transmission can occur, though the latter is much more common. Fetal diagnostic testing may result in iatrogenic infection of the fetus and is contraindicated.

Prevention of mother-to-child transmission of HIV is achievable through antiretroviral therapy of the mother and infant and, where appropriate, elective Caesarian section (viral load >1000 copies/ml). Optimal preventive therapy consists of highly active antiretroviral therapy to the mother throughout the pregnancy or beginning in the second trimester, intrapartum intravenous zidovudine, and 6 weeks of oral zidovudine in the babies (see Chapter 98). In resource-poor settings, single dose nevirapine to mother and child and short course dual antiretroviral therapy have led to reductions in transmission of 50% or more. The potential for resistance development in women receiving single dose nevirapine is of concern. Elective Caesarian section can reduce the risk of transmission by half among women who received no antiretroviral therapy or zidovudine monotherapy.

Hepatitis B virus

In four studies of over 33 000 pregnant women in North America, a prevalence of HBsAg carriage of 0.8% was documented; 52% of women acknowledged no known risk factors. The risk of perinatal infection is greater if the maternal infection was acquired in the third trimester (80–90% vs <10% in the first trimester). If HBsAg positivity is accompanied by e-antigen positivity, the neonate is both more likely to become infected (70–90% vs 20–25%) and more likely to become a chronic carrier (85% vs 5%). The strongest predictor of transmission may be maternal hepatitis B virus DNA load.

Infants of carrier mothers should receive 0.5 ml hepatitis B immune globulin within 12 hours of birth and either 5 µg of the Merck hepatitis B vaccine (Recombivax HB) or 10 µg of the Smith Klein Beecham hepatitis B vaccine (Engerix-B). The second dose of vaccine is given at 1–2 months of age and the third dose at 6 months of age. Infants born to mothers of unknown status should receive the first dose of hepatitis B vaccine within 12 hours of birth and complete the vaccination series as indicated above. The need for and timing of hepatitis B immune globulin depends on availability of maternal HBsAg results and the infant's gestational age.[52]

Hepatitis C virus

The vertical transmission rate of hepatitis C virus (HCV) is between 2.4% and 11.9%. The risk of transmission is almost three times higher from women who are co-infected with HIV. Transmission from mothers who are HCV RNA negative is uncommon.[53] Confirmation of HCV infection in infancy requires the detection of HCV RNA after 1 month of age or the presence of HCV antibodies after 18 months of age.[54] Among vertically infected infants followed for 10–15 years, 20% clear the infection, 50% have chronic asymptomatic infection and 30% have chronic active infection.[55]

Group B streptococcus

Of the 10–35% of women asymptomatically carrying group B streptococcus (GBS), 50% of their infants will be colonized on their skin and mucous membranes. Of those colonized, 1–2% will develop early-onset disease. Two alternative approaches (i.e. screening, risk factor identification) to the prevention of group B streptococcal disease have been developed in the USA with a 70% reduction in the incidence of early-onset disease.[56] According to the more effective screening-based approach, all pregnant women should be screened at 35–37 weeks' gestation for anogenital group B streptococcus colonization. Intrapartum antibiotics are offered to all culture-positive women, regardless of risk factors. If the results of cultures are not known at the time of labor, intrapartum antibiotics are used in the presence of prematurity (<37 weeks' gestation), prolonged rupture of membranes (≥18 hours) or intrapartum fever (≥100.4 °F/38 °C). Also, women with a previously infected infant and women with group B streptococcus bacteriuria during the current pregnancy or a history of postpartum GBS sepsis are treated with intrapartum antibiotics (see Practice Point 26). In geographic regions with differing epidemiologic data, this approach may not be appropriate.

Absence of antenatal care

For women in whom there has been inadequate antenatal care, detection of infection is required at delivery as summarized in Table 52.15.

Table 52.15 Evaluation of women presenting in labor with limited or no antenatal care

Mother	• Genital examination for findings suggestive of sexually transmitted disease • Cultures for *Chlamydia trachomatis, Neisseria gonorrhoeae* • Serologic testing for HBsAg,* hepatitis C virus, HIV,* syphilis (treponemal and nontreponemal testing) • Adequate follow-up
Infant	• Prophylactic eye care • Serologic testing for HBsAg, hepatitis C virus, HIV, syphilis (treponemal and nontreponemal testing) • First dose of hepatitis B vaccine • Adequate follow-up

*HBsAg and HIV serology should be done stat in order to allow for prompt initiation of preventive interventions if the mother tests positive.

Breast-feeding

Contraindications and precautions with breast-feeding

In North America and other developed countries breast-feeding is contraindicated if the mother is infected with HIV, human T-cell lymphotropic virus 1 (HTLV-1) or HTLV-2.[57] In resource-poor settings, the potential benefits and harms associated with breast-feeding versus formula feeding need to be carefully weighed. Breast-feeding is not contraindicated with hepatitis B or hepatitis C infection.

Viral contamination of milk with rubella, HSV and CMV has also been reported, but serious sequelae have not generally occurred. CMV acquired through breast milk can pose a risk to extremely premature infants. In this setting, the potential benefits and risk of breast-feeding need to be considered. Mothers with herpetic lesions on their breasts should refrain from breast-feeding from the affected side(s). They should cover active lesions and wash their hands before breast-feeding.

Contaminated milk has been implicated in neonatal infection with *Staphylococcus aureus,* group B streptococcus, *Mycobacterium* spp. and possibly *Salmonella* spp. In mothers who have active pulmonary tuberculosis, contact with the infant must be avoided until the mother has received adequate antituberculous therapy and is considered noninfectious. In the setting of mastitis, breast feeding can usually be continued; in the presence of a breast abscess or frank pus, temporary discontinuation of breast-feeding on the affected side is warranted.[57]

Breast-feeding and drug therapy

The American Academy of Pediatrics recommends that breast-feeding be discontinued while a nursing mother is being treated with metronidazole, and warns about the use of nitrofurantoin and sulfa drugs, which can cause hemolysis in glucose-6-phosphatase-deficient infants.[58] Although it would be unusual for an effective maternal medication to be contraindicated because of risks to the infant through breast milk, physicians should be aware of information specific to agents being used. Frequent feeding exposes the infant to more drug than feeding at 4-hour intervals. Mothers can be encouraged to avoid frequent feedings to reduce drug exposure and the consequent changes in the infant's gastrointestinal flora and risk of oropharyngeal candidiasis.

REFERENCES

References for this chapter can be found online at http://www.expertconsult.com

Practice point | 23 |

Juan Carlos Salazar

Management of an HIV-positive pregnant woman who has a positive VDRL test from an area endemic for *Treponema* infection

INTRODUCTION

The dual epidemics of venereal syphilis (*Treponema pallidum* subsp. *pallidum*) and HIV are responsible for increasing tolls of morbidity and mortality among pregnant women and their offspring. Syphilis and HIV infection are closely interrelated. As sexually transmitted diseases, each poses a risk for the other, and there is now substantial evidence that genital ulcerative diseases, including syphilitic chancres, facilitate the bidirectional transmission of HIV-1. Although a simple screening test for syphilis and effective antibiotics are available, the disease remains uncontrolled in resource-scarce regions where antenatal testing and treatment are not always accessible. Moreover, in many of these countries, the endemic (i.e. nonvenereal) treponematoses yaws, endemic syphilis and pinta, caused by *T. pallidum* subsp. *pertenue*, *T. pallidum* subsp. *endemicum* and *T. carateum*, respectively, have not only not been eliminated but are also resurging in some places because of the breakdown of once successful control programs.

In the pregnant female, regardless of HIV-infection status, untreated syphilis poses a grave risk to the fetus or neonate on top of whatever morbidity, acute or chronic, it may inflict upon the mother. The possibility of infection with a nonvenereal treponematosis in such individuals poses a further diagnostic challenge inasmuch as it is impossible to distinguish these infections from venereal syphilis using currently available serologic tests. Health-care professionals managing patients residing in or emigrating from areas endemic for the nonvenereal treponematoses need to be aware of the clinical and epidemiologic features that distinguish these diseases from venereal syphilis. They also need to know how HIV co-infection may influence the management and outcome of syphilis in pregnant women and their offspring (Table PP23.1).

CLINICAL FEATURES

A detailed discussion of the clinical features of the treponematoses is beyond the scope of this commentary (see Chapters 57, 104 and 171); the salient clinical features of these diseases, however, are summarized in Table PP23.1.

Treponema pallidum infections are divided into early (i.e. primary, secondary and early latent) and late (i.e. late latent and tertiary) stages. Early lesions, as well as blood and exudative body fluids, are infectious. Primary lesions of venereal syphilis (chancres) occur usually in the anogenital area of sexually active individuals; a substantially greater proportion of heterosexual men present with genital ulcers than women and gay men. By contrast, acquisition of nonvenereal treponematoses occurs earlier in life, usually in childhood, with primary lesions often occurring on an extremity. Although anecdotal reports indicate that syphilis manifestations in HIV-infected patients may be florid and/or predispose to central nervous system complications, the manifestations of syphilis in most HIV-infected patients appear to be essentially the same as in HIV-uninfected patients.

No studies are available on the impact of HIV infection on the course or severity of the nonvenereal treponematoses. With both venereal and nonvenereal treponematoses, mucocutaneous lesions are the most common form of secondary disease; extracutaneous manifestations, occasionally severe, can occur in secondary syphilis (nonvenereal as well as venereal) and yaws. The gummatous lesions of late yaws can be extremely destructive. Venereal syphilis is the only treponematosis that involves the central nervous system or that causes congenital infection.

SERODIAGNOSTIC TESTS

Visualization of spirochetes in lesion exudate by darkfield examination is the definitive method for diagnosing primary treponemal infection. A presumptive diagnosis is possible with the use of nontreponemal and treponemal serologic tests. The nontreponemal tests (NTTs), represented by the VDRL test and the rapid plasma reagin (RPR) test, detect antibodies to a defined mixture of cardiolipin, lecithin and cholesterol. These tests have traditionally been designated as 'nontreponemal' based upon the belief that lipoidal antigens of host origin induce the antibodies being detected. Nontreponemal tests are used to screen for syphilis because they are easy to perform and have reasonably high sensitivity. Because they are titratable and usually decline in parallel with disease activity, they are also used to monitor the response to therapy. Falsely reactive NTTs are usually of low titer (<1:8) and may occur in a variety of conditions (see Chapter 57). Although pregnancy is a recognized cause of a falsely reactive NTT, one must exercise great caution in labeling a reactive NTT in a pregnant patient as a false-positive result.

The second type of serodiagnostic test, represented by the fluorescent treponemal antibody-absorption test (FTA-ABS), the *T. pallidum* hemagglutination assay (TPHA) and the *T. pallidum* particle agglutination assay (TPPA), detects antibodies directed against *T. pallidum* antigens and hence is designated as 'treponemal'. Treponemal tests are used to confirm that reactivity in a NTT is indeed due to syphilitic infection. Because they are more difficult and expensive to perform than NTTs, treponemal tests may not always be available in underdeveloped countries. In resource-scarce settings, traditional treponemal tests have been replaced by rapid treponemal tests which detect by immunochromatography serum antibodies specific for a variety of recombinant treponemal antigens (e.g. Determine Syphilis Tp test). These tests require only a drop of blood obtained by finger stick, can

Table PP23.1 Overview of treponemal infections

Disease	Organism	Endemic areas	Primary lesion	Secondary lesions	Tertiary lesions	Congenital infection
Venereal syphilis	*Treponema pallidum* subsp. *pallidum*	Worldwide	Chancre, usually in anogenital region	Mucocutaneous lesions (condyloma lata, papules, macules or maculopapules) Visceral involvement Central nervous system (CNS) involvement (usually aseptic meningitis)	Gummas, including CNS Carditis/aortitis Neurosyphilis (meningovascular, tabes, paresis)	Yes
Yaws	*Treponema pallidum* subsp. *pertenue*	Rural areas of Africa, Central and South America, the Caribbean, equatorial islands of South East Asia and remote parts of India and Thailand	Papule Papilloma Ulcer, usually on an extremity	Diffuse papules, papillomas and ulcers Osteitis Dactylitis	Destructive gummas of skin and bone	No
Pinta	*Treponema carateum*	Underdeveloped rural areas of Mexico and northern South America	Erythematous papule, usually on an extremity	Scaly papules Areas of altered skin pigmentation	Areas of altered skin pigmentation Hyperkeratosis	No
Endemic bejel	*Treponema pallidum* subsp. *endemicum*	West Africa, small foci in Zimbabwe, Botswana, Arabian peninsula and central Australia	Oral mucosal ulcer	Oral and pharyngeal ulcers Mucous patches Condyloma lata Periostitis	Gummas of skin, bone and joints	No

be performed with minimal training and do not require expensive equipment or refrigeration. Rapid treponemal tests have been shown to have a high sensitivity (85–90%) and specificity (92–98%) when compared to the traditional treponemal tests. However, rapid tests have not been studied in the context of nonvenereal treponematoses. Molecularly based efforts to design serodiagnostic tests capable of distinguishing venereal syphilis from the nonvenereal treponematoses, principally yaws, have thus far been unsuccessful. It is hoped that the availability of the genomic sequence for *T. pallidum* and, eventually, those of other pathogenic treponemes may enable investigators to identify polymorphisms that can be exploited for developing disease-specific serodiagnostic or polymerase chain reaction (PCR)-based tests.

Both treponemal and nontreponemal serologic tests are generally reliable for the diagnosis and management of syphilis in patients co-infected with HIV. However, HIV-infected syphilis patients may on occasion show higher than expected NTT titers, false-negative treponemal or nontreponemal tests, or delayed seroreactivity. When serologic tests and clinical syndromes suggestive of early syphilis do not correspond, alternative tests such as biopsy of suspicious lesions should be performed. When available, lesions can also be analyzed by PCR for the presence of *T. pallidum* DNA. As with serologic tests, the PCR tests in widespread usage cannot distinguish between the pathogenic treponemes.

SYPHILIS TESTING DURING PREGNANCY

It is estimated that, of all pregnant women who have untreated syphilis, only 20% will both carry the fetus to term and deliver a normal child. Complications include stillbirth (30%), neonatal death (10%) and mental handicap (40%). Because of the seriousness of these complications, pregnant women should be screened serologically for syphilis early in pregnancy. For communities and populations in which the prevalence of syphilis is high or for patients at high risk, serologic testing should be performed twice during the first trimester, at 28 weeks gestation and at delivery. Rapid tests are less useful in areas with high prevalence, since a high proportion of pregnant women could have antibodies as a result of past, treated infection. Nevertheless, pregnant women who are seropositive but who lack clinical manifestations should be considered to be infected unless an adequate treatment history is clearly documented and sequential serologic, nontreponemal (e.g. RPR) antibody titers have declined appropriately. Given the inability of serodiagnostic tests to distinguish between venereal and nonvenereal treponematoses, syphilis should be the presumed diagnosis in asymptomatic, seropositive patients unless a diagnosis of nonvenereal treponematosis can be established unequivocally (see Table PP23.1).

TREATMENT

Penicillin is the antimicrobial of choice in the treatment of syphilis during pregnancy (Table PP23.2). Treatment during pregnancy should consist of the penicillin regimen appropriate for the stage of syphilis. Some specialists recommend a second dose of benzathine penicillin 2.4 million units intramuscularly, 1 week after the initial dose. The present consensus is that alternative regimens are potentially too harmful to the fetus (e.g. tetracycline), lack efficacy because of the inability of the drug to cross the placenta (e.g. erythromycin), have documented evidence of antimicrobial resistance (e.g. azithromycin) or are insufficiently studied (e.g. ceftriaxone). The current Centers for Disease Control and Prevention guidelines remind the practitioner of the importance of evaluating the HIV-infected patient both clinically and serologically for treatment

Table PP23.2 Treatment of syphilis during pregnancy in HIV-infected women

Primary or secondary syphilis	Benzathine penicillin G 2.4 million units im (single dose)
Early latent syphilis	Benzathine penicillin G 2.4 million units im (single dose)
Late latent syphilis or latent syphilis of unknown duration	Benzathine penicillin G 7.2 million units in three doses each of 2.4 million units im at 1-week intervals
Tertiary syphilis	Benzathine penicillin G 7.2 million units in three doses each of 2.4 million units im at 1-week intervals
Neurosyphilis	Aqueous crystalline penicillin G 18–25 million units q24h administered as 3–4 million units iv q4h for 10–14 days

failure. This is accomplished by blood draws at 3, 6, 9, 12 and 24 months after the course of therapy has been completed. The guidelines go on to suggest considering cerebrospinal fluid examination approximately 6 months after treatment, emphasizing that this is of unproven benefit. The treatment regimens for nonvenereal treponematoses are the same as those used for the comparable stage of venereal syphilis.

Because penicillin is clearly the preferred treatment, penicillin skin testing is recommended for reportedly penicillin-allergic pregnant women who have syphilis. If the penicillin allergy is confirmed, desensitization can be accomplished using incremental doses of oral penicillin V over 4–6 hours. A Jarisch–Herxheimer reaction, which presents with fever, headache and myalgia, can occur within hours of initiation of penicillin therapy in early syphilis. Women treated for syphilis during the second half of pregnancy may also experience self-limiting uterine contractions, decreased fetal activity and fetal heart rate abnormalities after penicillin treatment, but premature labor or fetal distress is rare. Women should be warned of the symptoms of the Jarisch–Herxheimer reaction and be instructed to use acetaminophen (paracetamol) to control these symptoms and to self-monitor uterine and fetal activity during the first 48 hours after penicillin therapy. In the second half of pregnancy, management and counseling may be facilitated by a fetal ultrasound but this should not delay therapy. Ultrasound signs of fetal syphilis (hepatomegaly, hydrops fetalis) indicate a greater risk to fetal health; such cases should be managed in consultation with specialists in high-risk obstetrics.

FURTHER READING


Further reading for this chapter can be found online at http://www.expertconsult.com

Practice point | 24 |

Theodore Jones
William R Bowie

Treatment of a positive *Toxoplasma* titer in pregnancy

INTRODUCTION

Clinically recognized toxoplasmosis is infrequent, but serologic evidence of toxoplasmosis is common. In immunologically competent people, even symptomatic toxoplasmosis is usually of minimal clinical significance. This is not the case in pregnancy. When symptomatic or asymptomatic infection is acquired just before or during pregnancy, *Toxoplasma gondii* readily crosses the placenta to infect the fetus, often with immense clinical and financial implications. The number of individuals in this country infected with *Toxoplasma gondii* is nearly one in four. In fact, nearly 85% of women of child-bearing age have not been exposed to the organism in the past. It is estimated that in North America, approximately one pregnancy in 1000 is affected, with higher rates reported in Europe. Although definitive proof is lacking, because early treatment of the mother has been thought to decrease the risk of infection of the fetus and diminish the sequelae in infected fetuses, appropriate management of the mother is essential.

Since the majority of infected women are either asymptomatic or have nonspecific and transient symptoms, the diagnosis is rarely made clinically. Rather, the health-care provider and the woman are typically faced with a positive serologic test without clear information on when the infection was acquired. 'Positive' serology with routine tests does not reliably determine the acuteness of infection, which is what determines management. An inappropriate response may result in unwarranted psychologic distress, unnecessary evaluations and treatments, and even unnecessary termination of pregnancy. In the USA, a recent study of obstetricians and gynecologists revealed that few were aware that 'positive' IgM antibody did not necessarily indicate acute infection, or that the US Food and Drug Administration had sent out an advisory about this problem with serologic tests for toxoplasmosis.

PATHOGENESIS

The definitive hosts of *T. gondii* are felines, and people are infected by direct or indirect contact with oocysts excreted by cats. Oocysts, spread for example in cat litter or soil or sand contaminated by cat feces, are highly infective. When ingested by animals or humans, they ultimately result in cysts in tissues. These also infect humans when uncooked or inadequately cooked meat containing viable cysts are ingested. Rarely, humans can also be infected by blood transfusions (see Chapters 52 and 183).

During acute infection of humans, toxoplasmosis is disseminated widely. Infection acquired immediately before conception or during pregnancy carries with it the risk of spread to the fetus. Though the risk of transmission in the first trimester (10–15%) is the lowest, its impact on the fetus is greatest, resulting in severe sequelae or death. Infection in the second trimester has been associated with a 25–40% transmission and has been found to have nonfatal effects in most circumstances. The highest proportion of fetuses infected occurs in the third trimester (>60% transmission). However, nearly all affected fetuses have mild or asymptomatic manifestations.

The classic triad of clinical features in severely infected infants who survive includes hydrocephalus, intracranial calcifications and chorioretinitis. Asymptomatically infected infants are typically not recognized at birth, but many, if not most, are thought to be at risk of subsequently developing chorioretinitis, hearing loss or subtle neurologic manifestations.

The economic impact of congenital toxoplasmosis is substantial, and screening programs to detect infection in pregnancy or at birth have been considered. In the USA, routine screening for toxoplasmosis in pregnancy is rarely recommended, but in some other countries screening is repeated serially in pregnancy, particularly in France.

INVESTIGATION

The focus of this Practice Point is on immunocompetent pregnant women who have had a 'positive' test for antibody to *T. gondii*. This will usually have been performed as a screening test rather than a diagnostic one. Depending on the laboratory, this will be either a positive IgG titer, or possibly a positive titer for both IgG and IgM. Evaluation for falling or rising titers is standard, but unless timing is fortuitous, stable titers are usually found rather than a definitive change.

Difficulties in interpretation of serologic results

There are several substantial problems in the interpretation of results.

- First, there are problems with sensitivity and, more frequently, specificity of many of the commercially available tests.
- Second, potential markers for acute infection, such as IgM and IgA, may persist long after the risk of transmission to the fetus ends. This may diminish the ability of IgM and IgA results that may be true positives to establish acuteness of infection. For this reason, when the IgM is reported as being 'positive', the possibilities are that it can be a false-positive (with the rate of false-positives dependent on the test and the laboratory), a true-positive indicative of recent infection or a true positive but simply reflecting persistence of IgM antibody.
- Third, the history and physical examination rarely adds to the clinician's ability to determine the acuteness of infection.

Negative anti-*Toxoplasma gondii* IgG and IgM

Unless it is very early in infection, negative anti-*Toxoplasma gondii* IgG and IgM indicate absence of previous infection. Women with such results are susceptible to infection. Primary prevention messages should be strongly reinforced. Subsequent repeat testing is required if one wishes to exclude infection later on in the pregnancy.

IgG titer only available, and is positive

An IgM test should be requested on the same or a new serum specimen because a single positive IgG titer provides no information about acuteness of infection. The presence of IgG antibody with a negative IgM antibody excludes recent infection. If performed early in pregnancy, no further investigation is required for toxoplasmosis. If performed late in pregnancy, there is an outside possibility that acute infection may have arisen earlier in pregnancy, with subsequent disappearance of IgM. Because of long-term persistence of IgM this is unlikely, but if there is any clinical concern of toxoplasmosis then the infant should be screened at birth.

Initial negative IgG titer with positive or equivocal IgM titer

When the IgG titer is negative, it is highly likely that the IgM titer is falsely positive. However, because the woman could be in the process of seroconverting, testing should be repeated in parallel on a second sample that is collected approximately 3 weeks after the first. If the IgG titer remains negative, then the result is probably falsely positive. Unless there are other features suggesting active infection, no further investigation is needed. Recent acquisition of infection is strongly suggested if the IgG titer becomes positive or there is a significant increase in the IgM titer.

Initial or subsequent positive IgG titer, and IgM titer is positive

Unless there is seroconversion or a significant increase in titers, interpretation at this stage is much more difficult because this information by itself does not establish acuteness of infection. Though IgM antibody levels rise within days of the infection and remain present – in general, for 2–3 weeks – it has been reported to persist for as long as 2–3 years in as many as 27% of women when immunosorbent agglutination assays are used. Often, this will confound additional efforts at clinical assessment with serology and other studies in the mother and potentially in the fetus.

History and examination of the mother

Although symptoms are usually absent or so nonspecific in over 90% of women with primary toxoplasmosis infection in pregnancy, a careful history and examination might detect symptoms that provide a clue to the onset of the disease. Most helpful would be development of lymphadenopathy, typically involving one node or a few nodes. A physical examination should be performed, looking in particular for abnormal lymphadenopathy and ocular disease.

Serologic studies in the mother

Pregnant women are often screened for a variety of processes (e.g. rubella, HIV), and this serum might have been stored. If available, prior stored serum should be tested for antibody to *T. gondii*. The results may be very helpful in determining the chronicity of infection.

Additional testing is required on available or newly acquired serum. If repeat testing on specimens run in parallel (i.e. testing is performed on all specimens at the same time) shows or has shown seroconversion or a fourfold or greater rise, then infection is acute. Usually titers are stable, and further testing is required in specialized or reference laboratories. A battery of tests is usually performed to assess acuteness of infection. Results should be interpreted in consultation with the reference laboratory. The two tests most often employed to assess acuteness of infection are IgG avidity and differential agglutination testing, but numerous other tests may be used by reference laboratories. If these additional results are consistent with acute infection or if they do not exclude acute infection, the woman should be managed as below.

MANAGEMENT

Studies diagnostic of or consistent with acute infection in pregnancy or immediately before conception

Management is in part determined by the time when infection was acquired. If results are consistent with infection immediately before or just after conception, the risk of delivering an infected baby is low. Either the fetus is not infected or if the fetus is infected it is likely to abort. When infection occurs later, the risk of having a viable but affected infant is much greater.

The mother should immediately be started on treatment (see below) for the duration of pregnancy and the fetus should be assessed by ultrasound. Amniocentesis for polymerase chain reaction (PCR) should be strongly considered.

Fetal ultrasound may show features consistent with infection, especially increased size of the ventricles. Negative studies do not exclude fetal involvement and studies may need to be performed serially.

With or without fetal abnormalities, amniocentesis should be considered at 18 weeks or later. If performed, as a minimum the amniotic fluid should be tested by PCR. Reference laboratories may do additional testing on amniotic fluid, such as mouse or cell culture inoculation. Although PCR can be falsely positive or falsely negative, if it is positive treatment of the mother should be switched to pyrimethamine and sulfadiazine.

Some authorities also sample fetal blood to detect the parasite or a fetal immunologic response, but PCR on amniotic fluid is probably safer.

Treatment of the mother

Initial treatment is with spiramycin 1 g orally every 8 hours. Spiramycin reduces the incidence and severity of fetal infection; however, when the fetus is known to be infected, pyrimethamine and sulfonamides are more active than spiramycin. Hence, ultrasound or amniotic fluid findings suggestive of fetal involvement should prompt a change of treatment to pyrimethamine and a sulfonamide. A variety of dosage regimens have been used, but currently suggested is pyrimethamine 25 mg/day and sulfadiazine 2 g orally every 12 hours, with supplemental folinic acid 5 mg/day. When treatment of the mother is initiated, it should be continued for the duration of the pregnancy.

The reader is cautioned to consider the fact that, while treatment of the woman with primary toxoplasmosis is suggested by most references, the effectiveness of prenatal treatment is not clear. Conflicting results have been noted in controlled and uncontrolled cohort studies: controlled studies failed to demonstrate benefit in treated women compared to untreated women, while several uncontrolled studies found a higher risk for congenital toxoplasmosis in untreated neonates. A recent systematic review further demonstrated the problem, with five studies demonstrating benefit while four failed in this regard.

Spiramycin is a macrolide with possible adverse reactions including nausea, vomiting, anorexia and diarrhea. Sulfadiazine has typical adverse reactions associated with sulfonamides, including concerns

of kernicterus. The major adverse reaction to pyrimethamine is dose-related bone marrow suppression. To assess hematologic abnormalities, testing is recommended at least once weekly to detect anemia, leukopenia and thrombocytopenia.

Although other drugs have been used to treat toxoplasmosis, there is minimal experience with them in the context of pregnancy and fetal infection.

Termination of pregnancy

Many women with 'positive' serology for toxoplasmosis have had inappropriate termination of pregnancy. Positive IgG and IgM serology is not of itself an indication for termination. Most of these women will not have infected fetuses, but even if the fetus is infected, with appropriate long-term treatment the infants often do well. Termination should only be considered when there is documented evidence of fetal involvement, especially if infection was acquired early in the first trimester. However, even most of these infants who survive have done well with treatment of the mother followed by treatment of the infant.

Initial management of the infant at birth

If there is any question about the possibility of fetal acquisition of toxoplasmosis, the infant should be evaluated, including by one or a series of serologic tests. If clinical, serologic or other testing suggests congenital toxoplasmosis, the infant should be treated for 1 year. On this treatment, even infants who have clinically apparent manifestations such as intracranial calcifications, meningitis or chorioretinitis are highly likely to have a favorable outcome.

HIV infection and toxoplasmosis in pregnancy

In contrast to immunocompetent women, women who have HIV infection whose antibody to *T. gondii* is IgG positive and IgM negative are at risk of transmitting HIV to the fetus. Appropriate management is unclear. Important variables probably include the degree of immunosuppression, concurrent *Pneumocystis jirovecii* prophylaxis with trimethoprim–sulfamethoxazole (co-trimoxazole), *Mycobacterium avium-intracellulare* treatment or prophylaxis with macrolides, *T. gondii* treatment or suppression, and infection acquired in pregnancy. Acute infection with *T. gondii* in pregnancy should be managed aggressively, probably with pyrimethamine and sulfadiazine, whether or not there is objective evidence of fetal involvement (see Chapter 91).

Other considerations

Pregnant women who have possible or proven toxoplasmosis are likely to feel guilty that they did something that put their fetus at risk. It is prudent for women who are or might be pregnant to avoid direct or indirect contact with cats or raw or inadequately cooked meat. However, most people with toxoplasmosis are totally unaware of when or how they became infected. Care should be taken to avoid adding guilt to the stress that women who have 'positive' serology undergo.

FURTHER READING


Further reading for this chapter can be found online at http://www.expertconsult.com

Practice point | 25 |

Upton D Allen

A pregnant patient with a previous pregnancy complicated by group B streptococcal disease in the infant

INTRODUCTION

The group B streptococcus (GBS, *Streptococcus agalactiae*) is recognized as an important cause of invasive disease in neonates and pregnant women. Among neonates, premature infants are at the greatest risk of adverse outcomes from GBS infection. These premature infants account for 25% of cases of GBS disease among neonates. The disease manifests itself as an early-onset form (<7 days after birth), a late-onset form (7 days to 3 months after birth) and a very late form (>3 months after birth). Disease among infants usually presents as bacteremia, pneumonia and meningitis. However, they may experience other syndromes, including soft tissue and bone infection. Initial reports of GBS disease in the 1970s indicated mortality rates as high as 50%. Current mortality rates are less than 5%, largely due to advances that have evolved in neonatal care since the 1990s.

EPIDEMIOLOGY

The organism colonizes the gastrointestinal tract of humans, with the genitourinary tract being the most common site for secondary spread. Colonization rates vary widely among different ethnic groups, geographic areas and age groups. These rates generally indicate that 10–30% of pregnant women have vaginal or rectal colonization with GBS. Data from the USA have suggested that prior to the implementation of recommendations for the prevention of early-onset GBS using maternal intrapartum antimicrobial prophylaxis (IAP), the incidence of GBS neonatal disease was 1–4 cases per 1000 live births. Among these cases, early-onset disease occurred in approximately 1 infant per 100–200 colonized women and was responsible for 75% of cases among infants. Since the widespread use of IAP, the incidence of early-onset GBS disease has decreased significantly (by approximately 80%), resulting in an incidence rate of less than 1 case per 1000 live births.

The incidence of early-onset disease is higher in babies born to women less than 20 years of age and in those who are of black race in the USA. Intrapartum risk factors include premature onset of labor (<37 weeks gestation), prolonged rupture of membranes (>18 hours) and intrapartum fever (>100.4°F/>38°C). Additional risk factors include heavy vaginal colonization with GBS, previous delivery of an infant who had GBS disease and the presence of low maternal levels of anti-GBS capsular antibody. Women who have GBS bacteriuria are at an increased risk of delivering an infected baby with early-onset disease. This is related in part to the fact that women who have GBS bacteriuria are usually heavily colonized with GBS. Bacteriuria caused by GBS is associated with an increased risk of preterm labor.

MICROBIOLOGY

Group B streptococci are represented by several serotypes, including Ia, Ib and II–VIII. While all serotypes may cause disease in humans, serotype III is the main cause of early-onset meningitis as well as of late-onset GBS disease among neonates.

In the determination of the GBS carrier status of a pregnant woman, culture techniques that maximize the recovery of GBS are essential. The optimal method for GBS screening involves collection of a single vaginal–anorectal swab or two separate swabs from the vagina and rectum. These swabs may be taken by the health-care provider of the patient with appropriate instructions. Swabs should be placed in a transport medium if the bacteriology laboratory is off-site and subcultured onto selective broth medium. After overnight incubation, the specimen is subcultured onto solid blood agar. Slide agglutination tests or other tests used to detect GBS antigen may be used to enable specific identification of GBS.

PREVENTION

Vaccination as a GBS disease-preventive strategy is currently the subject of research. Chemoprophylaxis has become established as a useful preventive strategy. Given that the majority of newborns who have GBS disease acquire infection in utero, the administration of antibiotics to neonates (postnatal prophylaxis) will not prevent the majority of GBS disease. Intrapartum chemoprophylaxis (administration of antibiotic during labor) has the potential of preventing neonatal as well as maternal GBS disease.

Guidelines on intrapartum chemoprophylaxis have been established by various countries. However, it should be noted that there are differences in the approach to antenatal screening for GBS and chemoprophylaxis between countries. Some countries do not have specific recommendations for routine antenatal screening based on the perceived risks versus benefits in their regions. Guidelines are based on collective evidence showing a beneficial effect of intrapartum prophylaxis in preventing early-onset GBS sepsis in specific jurisdictions. The most widely accepted recommendations are those proposed by the US Centers for Disease Control and Prevention (Fig. PP25.1). Based on these recommendations, the following summary points can be made.

Screening

All pregnant women (exceptions noted) should be screened at 35–37 weeks gestation for vaginorectal colonization. The exceptions are women who had previous infants with invasive GBS disease and women with GBS bacteriuria during the current pregnancy.

Culture techniques that maximize the likelihood of GBS recovery should be used. Because lower vaginal and rectal cultures are recommended, cultures should not be collected by speculum examination. Laboratories should report results to both the anticipated site of delivery and the health-care provider who ordered the test. Ideally, laboratories that perform GBS cultures will ensure that clinicians have continuous access to culture results.

IAP indicated

- *Women identified as GBS carriers during the current pregnancy.* At the time of labor or rupture of membranes, IAP should be offered to these women.
- *Women who have previously given birth to an infant who had GBS disease.* Prenatal screening is not necessary for these women.
- *Women with GBS bacteriuria.* These women are usually heavily colonized with GBS. Women who have symptomatic or asymptomatic GBS urinary tract infections in pregnancy should be managed according to current standards of care for urinary tract infections in pregnancy.
- *If the results of cultures are not known at the time of labor or rupture of membranes* and *any of the following risk factors is present.* Gestation less than 37 weeks, duration of membrane rupture of 18 hours or longer, or intrapartum temperature >100.4°F (>38°C). If these women are febrile due to chorioamnionitis, appropriate broad-spectrum antimicrobial therapy that includes an agent that is active against GBS should replace intrapartum chemoprophylaxis.

IAP not indicated

- *Women who are colonized and have a planned Caesarian section prior to rupture of membranes.* These women are felt to be at an extremely low risk of delivering an infant who has early-onset GBS.
- *Previous pregnancy with positive GBS screening culture* (unless a culture is also positive in the current pregnancy or GBS bacteriuria is present).
- *Negative vaginal and rectal GBS screening culture in late gestation* (regardless of risk factors).

INFECTION CONTROL IN THE NEONATAL NURSERY

Adequate hand hygiene is regarded as the most effective way to prevent the spread of GBS within a nursery. The routine culturing of babies to determine colonization status is not recommended. The treatment of asymptomatic carriers is not recommended. Cultures may be considered as part of an epidemiologic investigation of an outbreak of late-onset or very late-onset cases. In the event of an outbreak, cohorting of ill and colonized infants and the use of contact precautions should occur.

Newborn management following IAP

The recommendations for the prevention of GBS disease are accompanied by a suggested approach to the management of neonates born to mothers who have received intrapartum antimicrobial prophylaxis (Fig. PP25.1). This approach is based on the gestational age of the neonate, the presence or absence of symptoms and signs of sepsis and whether sufficient time has elapsed between IAP and delivery. Antibiotics must be administered at least 4 hours prior to delivery to allow for adequate antibiotic levels in the amniotic fluid.

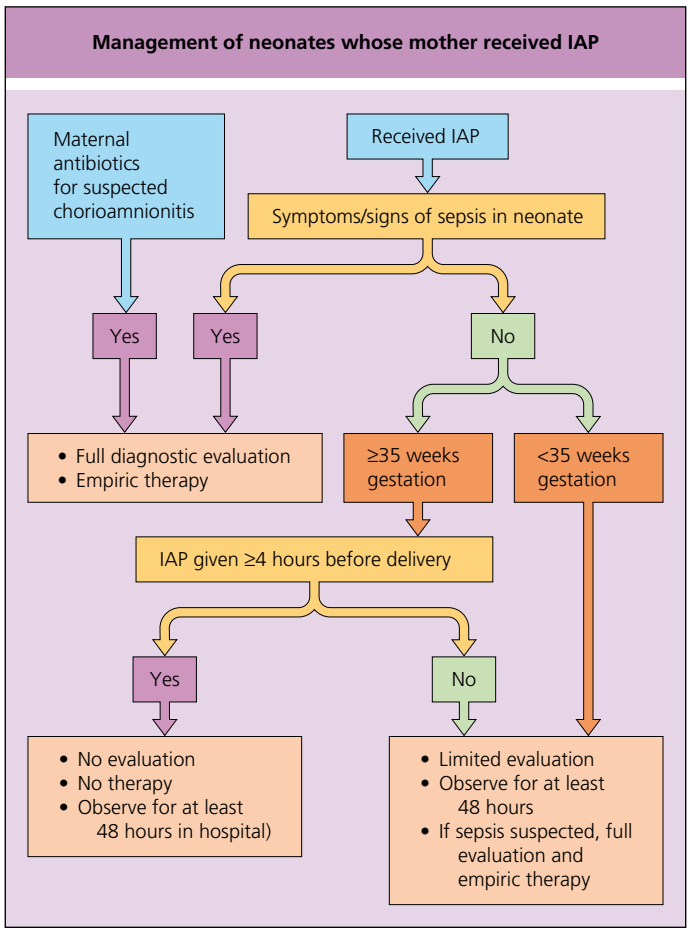


Fig. PP25.1 Management of neonates whose mothers received intrapartum antimicrobial prophylaxis (IAP).

MANAGEMENT OF A PREGNANT WOMAN WITH A PREVIOUS AFFECTED NEWBORN

Any woman who has lost an infant as a result of GBS disease needs the usual understanding and support given to any woman who has lost an infant during the neonatal period. Intrapartum prophylaxis is recommended regardless of screening cultures because of the previous delivery of a baby who had GBS disease. Routine vaginal–rectal screening for GBS is not necessary in this setting. However, it would be appropriate to obtain urine cultures at different antenatal visits to determine whether GBS bacteriuria is present, given that these women are at an increased risk of having GBS-affected infants.

Penicillin G (5 megaunits intravenously initially followed by 2.5 megaunits intravenously q4h) should be given intrapartum until delivery (Table PP25.1). Ampicillin (2 g intravenously initially and then 1 g intravenously q4h until delivery) is an acceptable alternative. Penicillin G is preferred because it has a narrow spectrum and is thus potentially less likely to select out resistant bacteria.

The use of IAP in women who are allergic to penicillin takes into account the risk of anaphylaxis and the background prevalence of GBS resistance to erythromycin and clindamycin. Given the relatively high rates of GBS resistance to these two latter agents, women who are allergic to penicillin but who are at low risk of anaphylaxis may receive cefazolin 2 g initially, then 1 g q8h. If the isolate has been shown to be susceptible to clindamycin, this agent may be used. Vancomycin should be reserved for women who are at high-risk of anaphylaxis in the setting where susceptibility testing of the GBS isolate has not been performed.

Table PP25.1 Alternative antibiotic regimens for intrapartum antimicrobial prophylaxis

Drugs	Doses	Comments
Penicillin	5 megaunits iv load, then 2.5 megaunits iv q4h	Preferred agent
Ampicillin	2 g iv load, then 1 g iv q4 h	Alternative agent
Cefazolin	2 g iv load, then 1 g iv q8 h	Use if risk of anaphylaxis is low in penicillin-allergic patient
Clindamycin	900 mg iv q8 h	Use if risk of anaphylaxis is high in penicillin allergic patient *and* the GBS isolate is known to be susceptible to clindamycin
Vancomycin	2 g iv load, then 1 g iv q12 h	Use if risk of anaphylaxis is high in penicillin allergic patient *and* the susceptibility of the GBS isolate is unknown or the isolate is known to be resistant to clindamycin

Group B streptococci are associated with various complications during pregnancy. These include septic abortion, urinary tract infections, chorioamnionitis, wound infection and endometritis. Although IAP may have a beneficial effect on endometritis, an assessment is necessary in the immediate postpartum period in order to guide further antibiotic therapy directed at the mother.

The newborn infant of a mother who has received intrapartum prophylaxis requires a special management approach. The approach outlined in Figure PP25.2 may be used as a guide to the management of the neonate. However, if the infant is believed to have invasive GBS disease, the following apply.

- Ampicillin plus an aminoglycoside is the initial treatment of presumed GBS disease.
- Penicillin G may be given alone when GBS is proved to be the etiologic agent and clinical and microbiologic responses have been documented.
- In cases of meningitis, a second lumbar puncture at 24–48 hours after the start of treatment is recommended by some experts; this may have prognostic significance.
- Bacteremia without a defined focus should be treated for a minimum of 10 days. Uncomplicated meningitis should be treated for 14–21 days (longer periods of treatment are needed in infants who have complicated courses).
- Osteomyelitis should be treated for a minimum of 4 weeks.
- There is a high incidence of co-infection among twins, and the twin of an index case should be observed or evaluated for sepsis as clinically indicated.

FURTHER READING

Further reading for this chapter can be found online at http://www.expertconsult.com

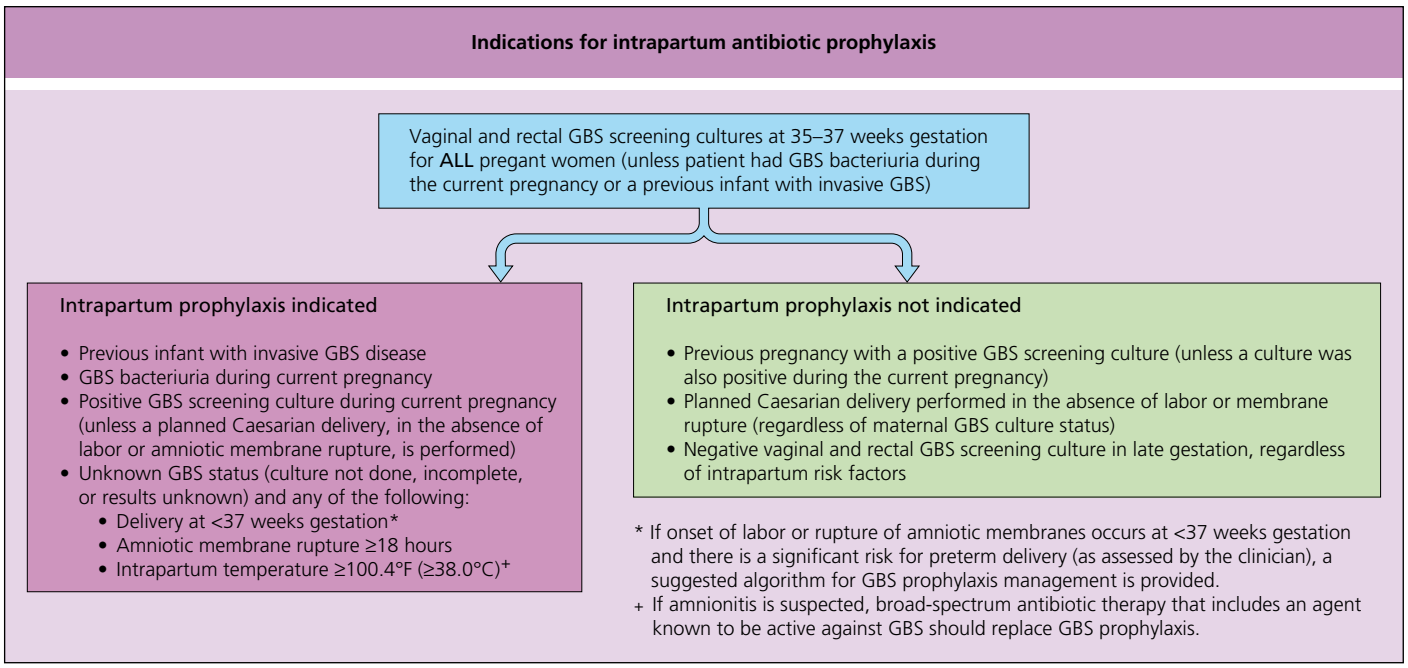


Fig. PP25.2 Indications for intrapartum antibiotic prophylaxis to prevent perinatal group B streptococcus disease under a universal prenatal screening strategy based on combined vaginal and rectal cultures collected at 35–37 weeks gestation from all pregnant women.

Chapter 53

Stephen T Chambers

Cystitis and urethral syndromes

Urinary tract infections (UTIs) are the second most common infectious cause for consultation and prescription of antibiotics among family physicians and are a common cause of morbidity in institutional care. Most infections are limited to the lower urinary tract but may cause pyelonephritis and bacteremia. The global incidence is estimated to be 2–3%, or at least 150 million cases per annum, costing billions of dollars annually.[1] Most cases of cystitis are uncomplicated and respond readily to antimicrobial treatment and typically occur in otherwise healthy nonpregnant women. Complicated infections, associated with anatomic or functional abnormalities, have an increased risk of therapeutic failure.

Acute Cystitis

EPIDEMIOLOGY

In the first 3 months of life UTIs are about three times more common in males than females, but thereafter infections occur more frequently in females. The prevalence of bacteriuria in preschool- and school-aged girls is 30 times higher than that in boys; 5–6% of girls will have had at least one episode of bacteriuria during their school-age years.[2] Thereafter the prevalence increases with onset of sexual activity before falling during the thirties and then rising at about 1% per decade thereafter (Table 53.1). About 50% of adult women report at least one UTI during their life.[1]

Table 53.1 Prevalence of bacteriuria in different age groups

	Group	Prevalence (%)
Females	Schoolgirls	1.2
	Sexually active young women	2–4
	Women >60 years 70 years 80 years	 6–8 5–10 20
	Institutionalized elderly	30–50
Males	Childhood to middle age	<1
	Men 60–65 >80 years	 1–3 >10
	Institutionalized elderly	20–30

Among college women more that two-thirds of acute episodes may be attributable to intercourse.[3] Risk factors for symptomatic UTI include asymptomatic bacteriuria during childhood, previous UTI, and use of a diaphragm and spermicide. Sexual activity is not a risk factor for UTI in postmenopausal women

In pregnancy asymptomatic bacteriuria occurs in 4–7% of women, predisposing them to developing acute pyelonephritis (15–40% of cases) during the third trimester or in the puerperium. Up to 20% of such patients have significant abnormalities of the urinary tract. Bacteriuria of pregnancy has also been associated with increased risk of pre-eclampsia, lowered fetal birth weight, prematurity and increased perinatal mortality rates.[4]

Men have low rates of bacteriuria until advanced age when rates rise dramatically and UTI is associated with abnormalities of the urinary tract.[2] A lowered CD4 lymphocyte count from HIV infection is a risk factor for UTI.[5]

In the elderly, both concurrent disease of the urinary tract and other medical conditions contribute to the high prevalence of UTI (Table 53.2). Instrumentation is associated with an increased risk of infection of 1% of ambulatory patients and 5–10% of hospitalized patients.

PATHOGENESIS

Infecting organisms

In uncomplicated cystitis more than 95% of infections are caused by a single organism. The most common pathogen is *Escherichia coli* (80–90% of cases), and these usually possess fimbriae or pili that adhere to host receptors. *Staphylococcus saprophyticus* accounts for 10–20% of cases in young women during late summer and autumn (Table 53.3).[6] A small number of serotypes of *E. coli* account for most UTIs and some clones have become widespread.[7]

Complicated infections and infections among the institutionalized elderly may be polymicrobial (30% of cases), particularly when stones are present. There is an increased incidence of resistant Gram-negative, *Pseudomonas aeruginosa* and yeast infections in this group. Urease-producing organisms (*Proteus*, *Providencia* and *Morganella* spp.) are of concern because urease leads to the conversion of urea to ammonia, an increase in the pH of urine, precipitation of struvite crystals ($MgNH_4PO_4.6H_2O$), and stone formation.

Mechanisms

Cystitis is almost always caused by ascending infection, although *S. aureus* may infect the urine from the bloodstream. The increased susceptibility of women to UTI is probably related to the shorter distance between the anus and urethral orifice, shorter length of urethra and

Table 53.2 Risk factors for lower urinary tract infection

Young adults	Women	Past history of UTI Sexual intercourse Diaphragm use Spermicide	Parity Diabetes (women) Primary biliary cirrhosis Sickle cell anemia (pregnancy) Instrumentation
	Men	Lack of circumcision AIDS	Homosexual activity
Elderly people	Women	Loss of estrogen effect Incomplete emptying of bladder	Abnormalities of urinary tract Rectoceles Urethroceles Bladder diverticula
	Men	Strictures Instrumentation	Prostatic disease Benign enlargement Calculi Loss of bactericidal secretions
	Both sexes	Neurologic disease Alzheimer's disease Parkinson's disease	Cerebrovascular disease

Table 53.3 Organisms associated with urinary tract infections

Common organisms	*Escherichia coli* *Staphylococcus saprophyticus*	
Less common organisms	*Klebsiella* spp. *Enterobacter* spp. *Proteus* spp. *Morganella* spp. *Citrobacter* spp. Group B streptococcus Group D streptococcus Enterococci	*Pseudomonas aeruginosa* *Acinetobacter* spp. *Serratia* spp. Yeasts *Corynebacterium urealyticum*
Rare indications	*Haemophilus influenzae* *Mycobacterium tuberculosis* Anaerobes	*Salmonella* spp. *Shigella* spp. Adenovirus (type 11)
Unproven causes	*Gardnerella vaginalis* *Ureaplasma urealyticum* *Mycoplasma hominis*	

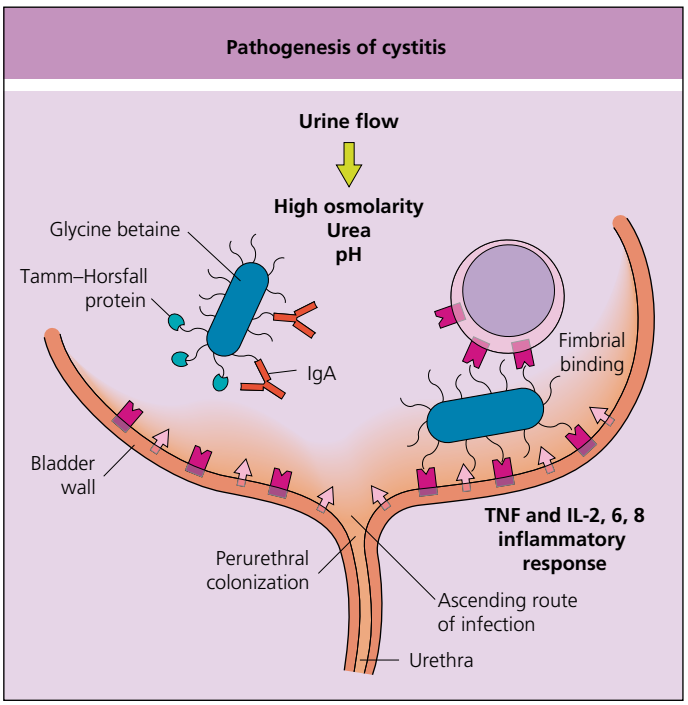


Fig. 53.1 Pathogenesis of cystitis. Factors favoring bacterial persistence and infection include bacterial binding to bladder mucosa (fimbriae), and high bacterial growth rates despite high osmolarity and urea concentrations and low pH. Factors favoring bacterial elimination include high urine flow rate, high voiding frequency, bactericidal effects of bladder mucosa, secreted proteins that bind to fimbrial adhesins and the inflammatory response. IL, interleukin; TNF, tumor necrosis factor.

the absence of the antibacterial barrier provided by prostatic fluid in males. In other respects the pathogenesis is similar in men and women and depends on a series of complex, interdependent host–parasite interactions that enable colonization of the periurethral area from the bowel, ascent of organisms into the bladder, growth in urine, tissue invasion and evasion of immune response (Fig. 53.1) to occur.

Colonization

The region between the anus and urethra is normally colonized by specialized flora including lactobacilli that inhibit colonization with enteric organisms. Spermicides (nonoxynol-9), diaphragms, estrogen deficiency and antibiotics (particularly β-lactams) may cause a reduction in these organisms and increase colonization by enteric organisms including uropathogens.[8]

Some women with recurrent UTI have an increased susceptibility to persistent colonization of the periurethral zone and *E. coli* adheres more strongly to vaginal epithelial cells from these patients.[9] Nonsecretors of histo-blood group antigens (Lewis blood group [Le(a+b–)] and recessive [Le(a–b–)] phenotypes) are at greater

risk of recurrent UTI.[10] Likewise, bowel carriage of *E. coli* possessing DNA sequences for P fimbriae, is more common among patients with the P blood group.[11]

Ascent

Bacteria normally enter the bladder by ascending along the mucosal sheath. This may result from mechanical instrumentation, sexual activity and motility of the organisms.

Fimbriae: role in mucosal adherence and inflammation

Symptomatic bacteriuria is highly correlated with the presence of bacteria that mediate attachment to uroepithelial cells. Type I (mannose-sensitive) fimbriae are important in initiating colonization of the bladder, but are not expressed subsequently following phase variation of the organisms. This may prevent binding to Tamm-Horsfall protein and IgA, and decrease recognition by phagocytic cells, which possess type I receptors.[12] Type II fimbrial attachment (mannose-resistant) is mediated by P fimbriae (Gal-Gal), which are associated with pyelonephritis, and attach to a variety of receptors associated with the globose series of glycolipids.[13] Attachment of bacteria to the mucosa and cell wall components, such as lipid A, activate an inflammatory response, including production of tumor necrosis factor (TNF) and interleukin (IL)-2, IL-6 and IL-8, and attraction of inflammatory cells.[14]

In contrast, asymptomatic bacteriuria is usually caused by organisms that neither possess fimbriae nor excite an inflammatory response unless tissue invasion occurs. The pathogenesis of these infections is not known.

Biofilm formation

Uropathogenic *E. coli* readily form biofilms on the mucosal surfaces, epithelial cells and catheter surfaces. Bacteria within biofilms are characterized by a slow metabolic rate and localization within an exopolysaccharide coating, and are protected from opsonization and phagocytosis by host cells.[15] Sessile communities are maintained by quorum sensing molecules that may interact with the host's signaling systems. Recently intracellular bacterial communities have been found within facet cells which line the transitional epithelium in animal models and up to 20% of sexually active women with *E. coli* cystitis.[16] A larger number have filamentous organisms in the urine which is the hallmark of recent residence in sessile colonies (Fig. 53.2).

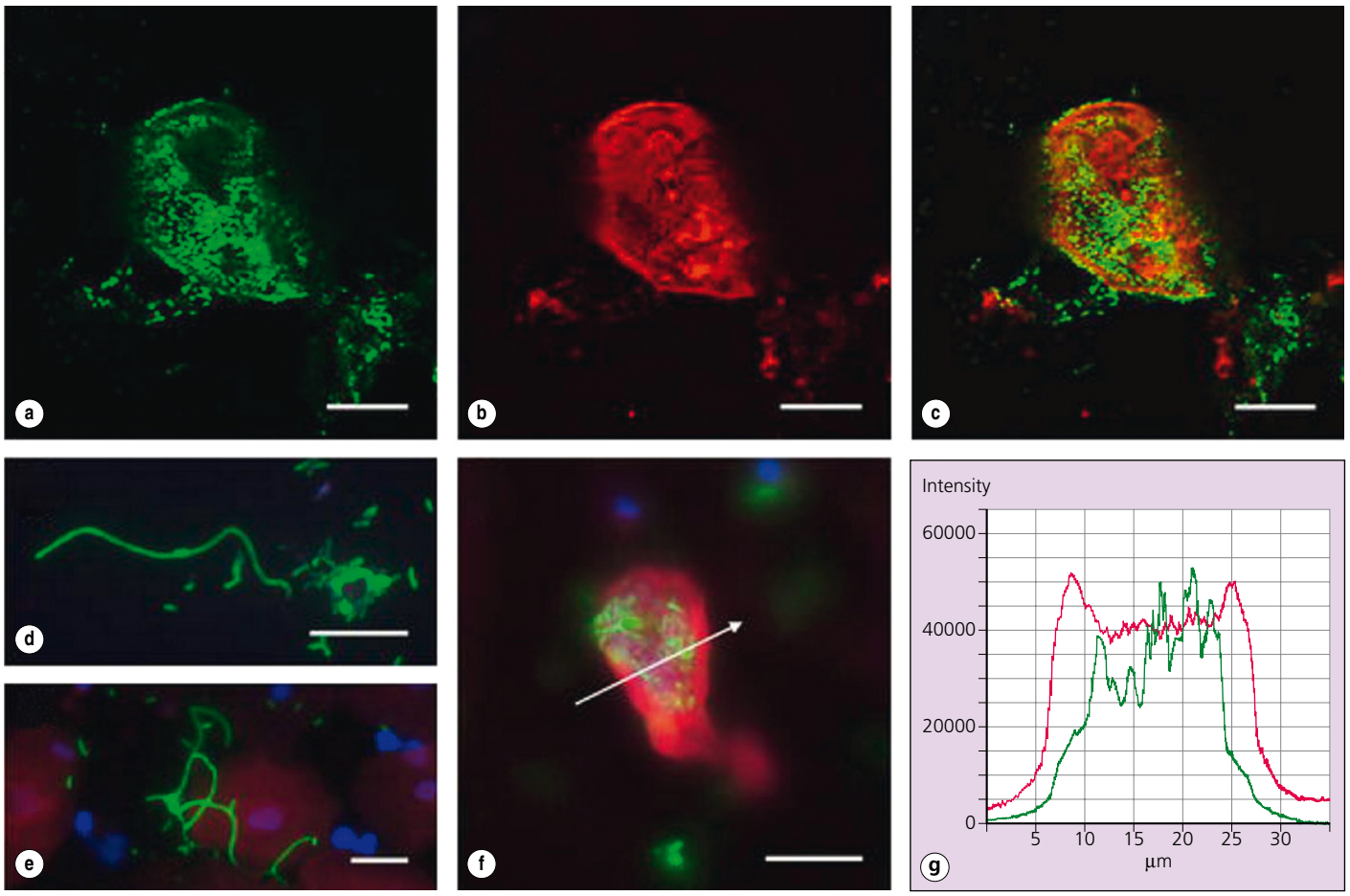


Fig. 53.2 Electron microscopy findings in urine from women with cystitis. TEM analysis of human cystitis urine specimens (a) revealed large collections of bacteria associated with nuclei and other cellular debris. These collections of bacteria from human urines (b) have similar morphology and organization as those recovered from intact murine intracellular bacterial communities (c). Bacteria and filaments were also observed intracellularly within exfoliated epithelial cells in a urine sample quickly fixed and analyzed from an *E. coli* cystitis patient (d). SEM analysis of cystitis urines deemed positive for intracellular bacterial communities and filaments captured large bacterial biofilm-like collections (e and f) composed of bacteria with a smaller, more coccoid morphology than typical *E. coli*. Long filaments were also captured by SEM (g). Scale bars: 2 µm (a and d); 1 µm (b and c); 5 µm (e–g). Courtesy of Rosen *et al*.[16]

Urodynamics

Normal structure and function of the urinary tract promotes elimination bacteria from the urinary tract because voiding eliminates free organisms and the bladder mucosa is bactericidal to many organisms on contact. Similarly, adequate urine flow promotes removal of bacteria from the upper urinary tracts. These defense mechanisms are potentially compromised by low urine flow rate, infrequent voiding, residual bladder urine and reflux of urine.[17] Neurologic disease, diabetes, debility and anatomic changes impair bacterial clearance by these mechanisms, particularly in the elderly. In postmenopausal women urinary incontinence, cystocele and postvoiding residual urine are the commonest risk factors for UTI.[18]

Growth in bladder urine

Growth in urine is essential for invasion of the urinary tract. Gonococci, anaerobes and urethral commensals are inhibited whereas uropathogens grow well in urine. Bacteria derive protection from the inhibitory effects of high osmotic forces, extremes of pH and high urea concentrations by the intracellular accumulation of osmolytes normally present in urine (e.g. glycine betaine) that stabilize macromolecular structures.[19]

Immune response

Locally produced urinary antibodies produced in response to febrile UTI (monomeric and dimeric IgA and IgG) decrease adherence by interference with adhesion receptors and agglutination of bacteria. Hyperimmunization can protect animals against experimental UTI.[20]

PREVENTION

Normal urinary tract

Recurrent or closely spaced symptomatic UTIs cause considerable morbidity and anxiety when no cause is found. Patients are often told to empty their bladder completely, maintain a high fluid intake and to void after intercourse, but there is little evidence to support these measures. Application of antiseptic cream (e.g. 0.5% cetrimide) to the urethral orifice may prevent infections. Women who use a diaphragm and spermicide should consider alternative methods of contraception. Postmenopausal women with recurrent UTI have been shown to benefit from the application of estriol vaginal cream (0.5 mg/day for 2 weeks then twice weekly).[21]

If these measures fail, chemoprophylaxis should be considered if there are two or more symptomatic UTIs within 6 months, or three or more over 12 months. A useful strategy is to give a 6-month trial of prophylaxis; if there is a reversion to the previous pattern, consider prophylaxis for 2 years. Postcoital prophylaxis is preferable, where applicable, as it results in administration of smaller amounts of antimicrobials than continuous prophylaxis and is equally effective. Continuous prophylaxis is highly effective but vaginal and oral candidiasis and gastrointestinal symptoms can be troublesome[22] (Table 53.4). Nitrofurantoin is an excellent agent because it does not alter the fecal flora, and resistance during treatment is rare. Use of trimethoprim–sulfamethoxazole (TMP–SMX) for as long as 5 years is effective and well tolerated.

Cranberry juice

Cranberry juice may decrease the number of symptomatic UTIs over a 12-month period but there are a large number of dropouts/withdrawals, indicating that cranberry juice may not be well tolerated and the optimum dosage and method of administration (e.g. juice, tablets or capsules) is uncertain.[23] Cranberry juice inhibits adherence of both type I fimbriated *E. coli* and P-fimbriated *E. coli* to uroepithelial cells.

Table 53.4 Drug regimens for prophylactic therapy administered as a single dose at night

Drug	Dose
Nitrofurantoin	50 mg
Trimethoprim	100 mg
Trimethoprim-sulfamethoxazole (co-trimoxazole)	480 mg
Norfloxacin	200 mg
Cephalexin	125 mg
Hexamine hippurate	1.0 g

Other prevention strategies

Competitive exclusion of uropathogens using probiotics (i.e. lactobacilli) or receptor analogues has been touted as a possible approach to reduce the risk of UTI by reducing colonization by uropathogens. While this approach holds promise, more research is needed.

Abnormal urinary tract

Any lesions in the urinary tract should be corrected if possible. Urologic referral is essential when abnormalities are found. Large residual volumes and high pressure may require intermittent self-catheterization. Low-dose chemoprophylaxis is often effective.

Vaccine

There is no vaccine currently available, although type I and type II pili and multiple heat-killed uropathogenic bacteria have been trialed. Anatomic and functional abnormalities may limit vaccine applicability.[24]

CLINICAL FEATURES

Symptomatic infection

The dominant complaint in cystitis is usually painful micturition (dysuria), which may be associated with frequency, urgency, strangury, initial and terminal hematuria, suprapubic discomfort and voiding small amounts of turbid urine. Low-grade fever may occur, but is usually absent. Pyuria or hematuria is almost always present. Elderly patients usually have similar symptoms but occasionally present with incontinence or smelly urine, or in men, epididymo-orchitis.[25]

Dysuria caused by bacterial cystitis should be distinguished from that caused by vaginitis, vulvitis and urethritis (see Chapter 49). Vaginal discharge, pruritus or dyspareunia points to vaginitis. Labial discomfort as the stream of urine is passed suggests vulvitis which is often caused by genital herpes and candidiasis. In urethritis there may be a history of a new sex partner, urethral discharge, mucopurulent cervicitis or bartholinitis. *Chlamydia trachomatis* and *Neisseria gonorrhoeae* are the usual causes.

Dysuria caused by chronic conditions such as interstitial cystitis and *Mycobacterium tuberculosis* infection may cause initial confusion but usually persists following attempts at treatment.

Asymptomatic bacteriuria

Asymptomatic bacteriuria is very common in elderly patients. It is poorly correlated with fatigue, poor appetite and urinary incontinence. Similarly, symptoms such as frequency, dysuria and hesitancy, which may be caused by infection, are common and nonspecific and often

fail to respond to treatment of coexisting bacteriuria.[26] Asymptomatic UTIs have been shown to increase morbidity in pregnancy but not in the elderly.

DIAGNOSIS

The diagnosis of UTI can only be proven by culture of an adequately collected urine sample. This is essential in all suspected cases in males, infants and children. A presumptive diagnosis can be made in sexually active women in the presence of typical clinical features together with the presence of pyuria if sexually transmitted infections are unlikely.[27] A culture should be performed if a complicated infection is suspected or symptoms have relaped within a month of treatment.

Pyuria

The preferred method for assessment of pyuria is microscopic examination of uncentrifuged fresh urine using a hemocytometer, although counts per microscopic field are reasonably reliable in the clinical laboratory. Urine from adult patients who have symptomatic UTI almost always (>96%) contains more than 10 leukocytes/ml. Pyuria occurs less frequently in asymptomatic bacteriuria of pregnancy (50% positive) and the elderly (90% positive).

Pyuria alone is not a reliable predictor of infection. Specimens from women with vaginitis often contain white cells and there are many other causes of inflammation within the urinary tract (Table 53.5).

Urine dipsticks using esterase provide a simple inexpensive method for detecting pyuria. A positive test indicates a minimum of eight white blood cells per high power field and has a sensitivity of 88–95% and specificity of 94–98% compared with the counting chamber method.[28] The presence of blood, rifampin (rifampicin), nitrofurantoin, bilirubin and ascorbic acid may result in a false-negative test, whereas trichomonads, imipenem and amoxicillin-clavulanate may give a false-positive test.[29]

Table 53.5 Conditions associated with pyuria but without culturable bacteria using standard bacterial isolation techniques

Recent treatment of UTI	
Organism not culturable on usual bacterial media	Adenovirus Anaerobes *Chlamydia trachomatis* Fungal infections Herpes simplex Leptospirosis *Mycobacterium tuberculosis* *Neisseria gonorrhoeae*
Noninfectious causes	Cyclophosphamide therapy Foreign bodies Glomerulonephritis Interstitial cystitis Neoplasms Stones Transplant rejection Trauma Tubulointerstitial disease Vaginal contamination

Detection of bacteria

The presence of bacteria on microscopy of urine correlates well with culture results. Experienced laboratories can reliably detect 10^8 organisms per liter in unstained specimens. The nitrite test is reliable for detecting Gram-negative bacilli in first morning urines but not other specimens because of inadequate time for reduction of nitrate to nitrite.

Midstream urine specimens

The simplest method for obtaining urine for quantitative culture is to collect a clean midstream specimen. This minimizes the confounding effects of contamination from the first few milliliters of urine.

The sensitivity and specificity of quantitative culture for diagnosis of UTI is dependent on the presence of symptoms and pyuria:

- in asymptomatic women, a single count of 10^4–10^5/ml has a 95% chance of representing contamination;[30]
- in symptomatic women with pyuria (>10 white blood cells/ml) a single count of more than 10^5/ml has a very high specificity (>99%) but a low sensitivity (51%) for UTI.[31]

Routine application of this criterion would fail to diagnose one-third of women with a UTI (Table 53.6).

Specimens from males are less likely to be contaminated and lower counts (>10^3/ml) are highly predictive of infection. Gram-positive bacteria and yeasts in urine tend to be associated with lower counts than Gram-negative bacilli.

In practice specimens are often poorly collected and delays in processing without refrigeration allow bacterial multiplication. In the presence of symptoms and pyuria, a bacterial count greater than 10^3/ml is a reasonable criterion for significant bacteriuria in routine laboratories, bearing in mind that this represents 10 organisms on a plate if a 0.01 ml loop is used. Others argue that over 10^4/ml is more realistic. Obstruction, antimicrobial agents and, possibly, diuresis can cause false-negative results.

Suprapubic aspiration

Suprapubic aspiration of urine from a distended bladder is an efficient means of diagnosis. Any bacteria identified can be regarded as significant because the technique avoids contamination. In most infected specimens bacteria can be seen microscopically and treatment started promptly. Provided the patient has a full bladder it is safe and acceptable.

Catheter specimens

Catheterization specifically for a urine culture may be justifiable if the patient is unable to co-operate to obtain an uncontaminated sample or to hold urine in the bladder for a suprapubic aspiration.

Table 53.6 Value of quantitative urine culture in diagnosis of urinary tract infection with Gram-negative bacilli in women

	Number of specimens	**Organisms/ml of urine**	**Sensitivity (%)**	**Specificity (%)**
Asymptomatic women	Two	>10^5	>95	>80
Symptomatic women with pyuria	One One One	>10^5 >10^3 >10^2	51 80 95	99 90 85

Catheterization rarely leads to false-positive results, but may introduce bacteria into the bladder. Straight plastic catheter or Alexa bag techniques are satisfactory.

Imaging of the urinary tract

All men, children and infants need investigations of the urinary tract if they have a UTI regardless of the clinical features at presentation. This is not cost-effective in women unless there is some evidence of an unusual clinical pattern, such as urinary infection in childhood, treatment failure, persistent microscopic hematuria or pyuria at follow-up. An ultrasound examination including postmicturition bladder volumes plus a plain abdominal radiograph, including the kidneys, ureters and bladder, are adequate in most instances. Cystoscopy rarely yields useful information in women with acute cystitis.

MANAGEMENT

The cornerstone of management is effective antimicrobial therapy (Table 53.7). Drinking large amounts of fluids and alkalinizing agents may decrease bacterial counts and improve symptoms, but add little to effective antimicrobial therapy.

Follow-up visits at 7–14 days after completion of therapy give the opportunity to obtain urine cultures and discuss the importance of the diagnosis. It is essential to relieve anxiety about sexual activity and perceived long-term consequences, and to discuss advice offered by the family and the media.

Acute uncomplicated bacterial cystitis

Short-course therapy has now become standard treatment because of improved compliance, lower cost, fewer side-effects and decreased likelihood of the emergence of resistant strains.[32] A treatment algorithm is given in Figure 53.3. Short-course therapy is contraindicated in complicated infections.

Three-day therapy

A 3-day regimen is recommended because it provides the advantages of short-course therapy and has a slightly higher success rate than single-dose therapy, particularly in older women. It is appreciated by patients because it may take several days for symptoms to abate. Three days of trimethoprim-sulfamethoxazole (TMP-SMX; co-trimoxazole), trimethoprim alone, the quinolones or pivmecillinam are as effective as longer courses with fewer side-effects. β-Lactams as a group are less effective than trimethoprim, TMP-SMX or the quinolones.[33] There is insufficient evidence to recommend nitrofurantoin for 3 days rather than 5 days.

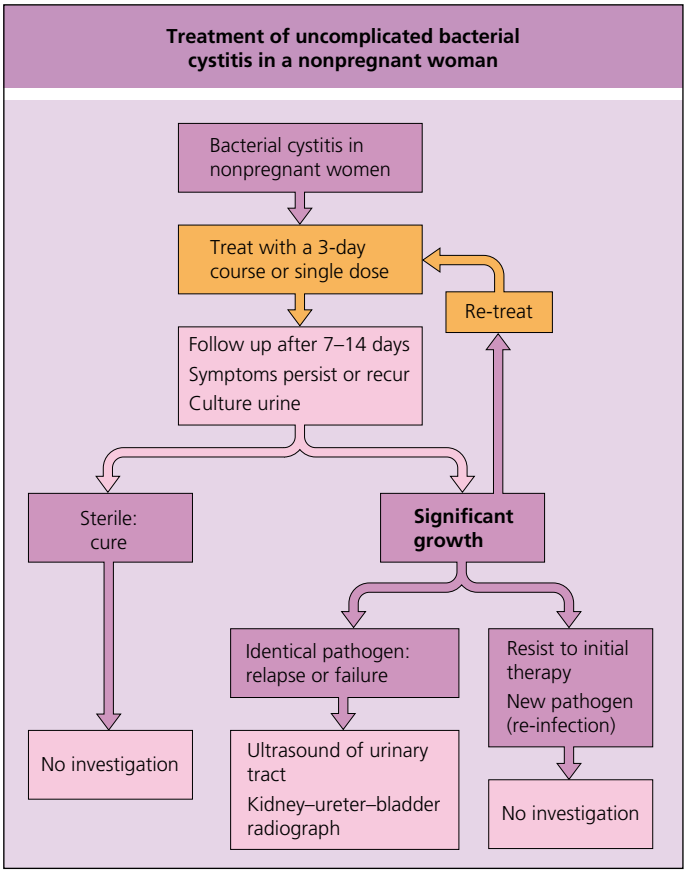


Fig. 53.3 Treatment of uncomplicated cystitis in a nonpregnant woman.

Single-dose therapy

Single-dose therapy is essentially 1 day of treatment given in a single dose that produces inhibitory concentrations of antibiotic over a 12–24-hour period. It is most suitable for cystitis in sexually active women and for younger patients who have normal urinary tracts and a short history (<7 days) (Table 53.8).

Amoxicillin is less effective than either TMP–SMX or trimethoprim alone and is no longer recommended in single-dose regimens.

Table 53.7 Drug treatment regimens for a 3-day course of oral therapy for bacterial cystitis

Drug	Dose
Trimethoprim	300 mg q24h
Trimethoprim-sulfamethoxazole (co-trimoxazole)	960 mg q12h
Ciprofloxacin	250 mg q12h
Fleroxacin	400 mg q24h
Levofloxacin	500 mg q24h
Lomefloxacin	400 mg q24h
Nalidixic acid	500 mg q8h
Norfloxacin	400 mg q12h
Amoxicillin	250 mg q8h
Amoxicillin-clavulanate	500/125 mg q12h
Cephalexin	250 mg q8h
Cephradine	250 mg q8h
Cefpodoxime proxetil	100 mg q12h
Pivmecillinam	200 mg q8h

Table 53.8 Suggested drug treatment regimens for single-dose treatments

Drug	Dose
Trimethoprim	600 mg
Trimethoprim-sulfamethoxazole (co-trimoxazole)	1.92 g
Ciprofloxacin	500 mg
Fleroxacin	400 mg
Norfloxacin	800 mg
Fosfomycin trometamol	3 g

Fluoroquinolones are very effective, although they may be less effective against *S. saprophyticus* than Gram-negative bacilli. Fosfomycin trometamol is marketed specifically as single-dose therapy and is highly effective but is relatively expensive.

Those who fail to respond to single-dose therapy have an increased risk of abnormalities within the urinary tract.[34]

Therapeutic agents

Both TMP-SMX and trimethoprim (TMP) alone are highly effective, but as sulfonamides have occasional severe side-effects, especially in the elderly, TMP is preferred. There is no evidence that the combination with sulfamethoxazole prevents the emergence of resistance.

The fluoroquinolones have essentially superseded nalidixic acid and oxolinic acid. All these agents are extremely active and effective against most pathogens, including many hospital pathogens. Norfloxacin is usually the most inexpensive fluoroquinolone for the treatment of cystitis in some countries.

Nitrofurantoin is ineffective against *Proteus mirabilis*. The side-effects of nausea and vomiting can be minimized by treating with 50 mg q8h or 100 mg q18h without loss of efficacy, rather than 100 mg q6h.

Mecillinam has been widely used in Scandinavia without apparent resistance problems developing in Gram-negative organisms. *In-vitro* studies show resistance in Gram-positive organisms, but clinical studies show that there is a high cure rate for *S. saprophyticus*, presumably because of the very high concentrations achieved in the urine.

Amoxicillin is the treatment of choice for treating *Streptococcus faecalis*, but increasing resistance has limited its usefulness against other uropathogens. Combinations of β-lactams with β-lactamase inhibitors may be effective, but there is a high rate of diarrhea. Cephalosporins such as cephalexin, cephradine and cefaclor are useful, particularly in renal failure.

Fluoroquinolones have been shown to produce arthropathies in immature animals and are therefore contraindicated in pregnant women. Antibiotics, such as penicillins, may interfere with the effectiveness of oral contraceptives by altering the enterohepatic recycling of estrogen and reducing the contraceptive effect. Women who use oral contraceptives may need to use barrier methods of contraception while on treatment.

Choice of initial therapeutic agent

Trimethoprim and TMP-SMX have been regarded as the agents of first choice but there is pressure to use quinolones as resistance rates of *E. coli* and other Gram-negative organisms rise and exceed 10–20%. *In-vitro* resistance reduces the clinical success of TMP-SMX from 95% to 60% and bacterial eradication from 93% to 50%. Thus the expected clinical and bacterial success rates for a 10% resistance rate in Gram-negative infections will be 92% and 89%, and for a 20% resistance rate 88% and 84%, respectively.[35] The net effect on success on intention to treat will be smaller, because Gram-positive uropathogens remain susceptible to TMP or TMP-SMX.

The decision to change policy is made more difficult if reliable estimates of resistance rates in the local setting are lacking. Resistance rates reported by clinical laboratories are often misleading because isolates from patients who have failed therapy or are at a high risk of resistance are over-represented.[36] Using quinolones as a first choice may be a short-term solution because resistance to these agents is steadily rising worldwide and is reported to be more than 20% in several countries. There are now efforts to reverse quinolone use in some centers.[37]

Where resistance to TMP-SMX is a potential problem a reasonable approach is to use it unless the patient has risk factors for TMP-SMX resistance. These include previous infection with TMP-SMX-resistant organisms, recent use of TMP-SMX or another antimicrobial agent, recent hospitalization or recurrent UTI in the past year. Alternatives include fluoroquinolones (3 days), nitrofurantoin (5–7 days), pivampicillin (3 days) and fosfomycin (single dose).

Recurrent infections

The major problem with uncomplicated acute cystitis is recurrence. About one-half of adult women will have another infection within 1 year, many within 3 months. Recurrence rates vary (0.3–7.6 episodes per year) and may occur in clusters. With treatment of each episode, 20–30% will cease having recurrences. If episodes are closely spaced, self-administered therapy, preferably after obtaining a urine specimen, postcoital therapy or prophylaxis can be considered. Self-initiated therapy should be reserved for those who are not at risk of sexually transmitted infections or pregnancy, and who do not have significant co-morbid conditions.[38] A treatment algorithm is given in Figure 53.4.

Complicated infections

Complicated infections are more likely to be caused by unusual organisms and should be treated for 7–14 days. Quinolones are an appropriate initial choice but treatment should be altered in response to results from culture and sensitivity testing. Patients who fail short-course treatment often have abnormalities of the urinary tract (e.g. stones, diverticula, strictures, chronic bacterial prostatitis). These should be corrected where possible and infection treated with more prolonged courses of therapy of 7–14 days.

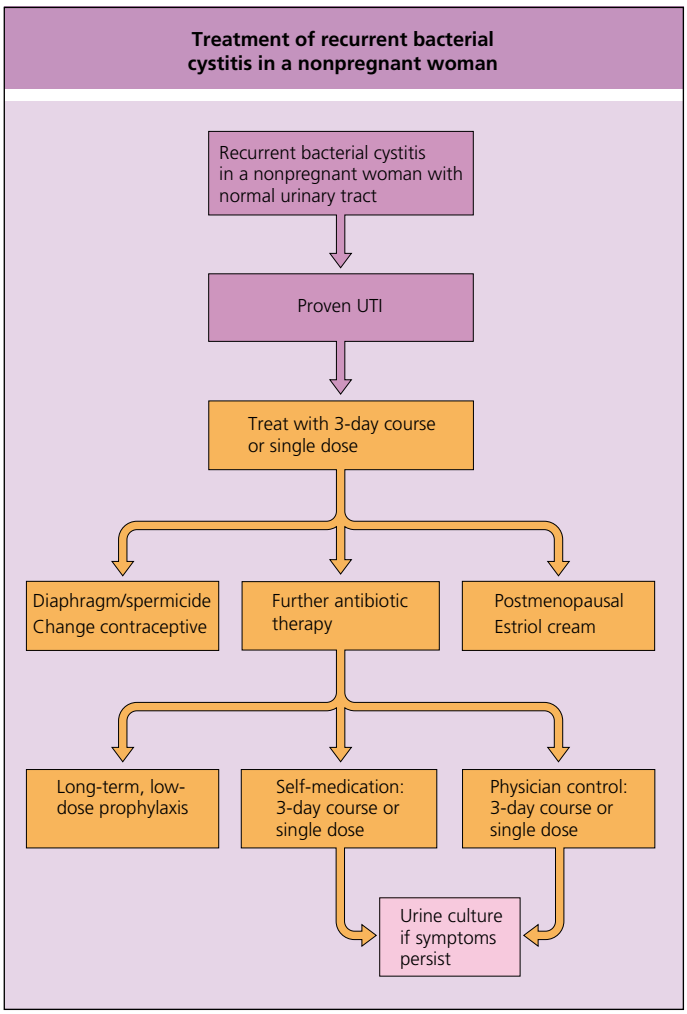


Fig. 53.4 Treatment of recurrent bacterial cystitis in a nonpregnant woman.

Urinary tract infections in pregnancy

The risks of symptomatic infection and pyelonephritis during the third trimester of pregnancy in mothers and prematurity in infants can be prevented by early detection and eradication of asymptomatic bacteriuria. In the first instance single-dose or a 3-day course of therapy is appropriate (Fig. 53.5). If relapse or re-infection occurs, then the patients should be re-treated. At that stage the simplest strategy is to institute prophylaxis (e.g. nitrofurantoin 50 mg at night), although some prefer close surveillance with repeated cultures and prompt treatment of each episode. All such patients need evaluation of the urinary tract with ultrasonography and a plain abdominal radiograph after delivery.

There is probably no absolute contraindication to any antimicrobial agent during pregnancy, but caution is urged with some agents. The β-lactams (e.g. amoxicillin, amoxicillin-clavulanate, cephalexin) are considered safe and the antifolate activity of trimethoprim and TMP-SMX is minimal in short courses and probably safe between 16 and 30 weeks of gestation (Table 53.9).

Males

Lower UTI in males may be complicated by infection of prostatic fluid, even if there is no clinical prostatitis. For this reason there is reluctance to treat men with short regimens and 7-day courses are recommended even for apparently uncomplicated cystitis. Long-term suppressive therapy may be helpful for frequent recurrences.

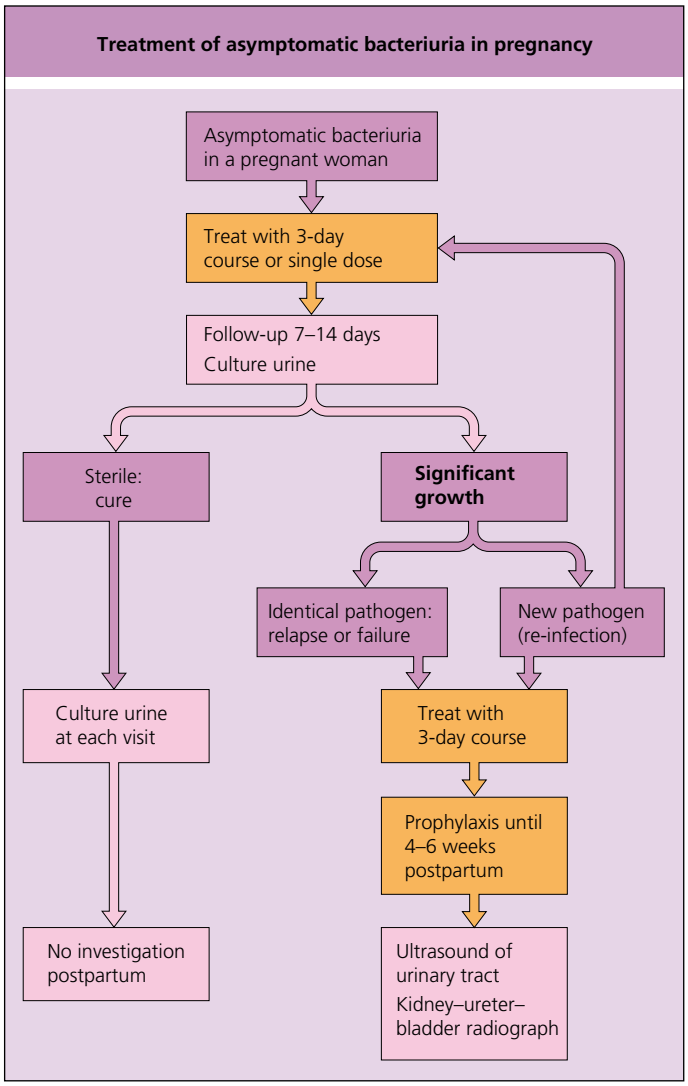


Fig. 53.5 Treatment of asymptomatic bacteriuria in pregnancy.

Asymptomatic bacteriuria

Asymptomatic bacteriuria is often best left untreated except in pregnancy. In elderly patients it is extremely common and there is no convincing evidence that treatment benefits the patient either in terms of recurrent symptomatic episodes or mortality rate. Antimicrobial therapy is associated with adverse effects, the potential for development of resistant strains and financial cost.

Invasive procedures

Asymptomatic bacteriuria should be treated if a patient is to undergo an invasive procedure of the genitourinary tract. Mucosal trauma may cause postprocedural bacteremia, and occasionally septic shock and death. The antimicrobial agent should be selected on the basis of the sensitivity of the infecting organism. Likewise, it is prudent to treat any urinary infections before the insertion of permanent indwelling devices, particularly prosthetic joints.

Treatment in the presence of renal failure

If treatment is truly indicated, drugs that achieve adequate urine concentrations in the presence of renal failure should be used. The best levels may be achieved with penicillins and cephalosporins. Trimethoprim and fluoroquinolones will probably achieve adequate concentrations, whereas nitrofurantoin, sulfamethoxazole and doxycycline are present in very low concentrations when creatinine clearance falls below about 0.16 ml/s.

Infections with *Candida* spp.

Infections with *Candida* spp. may occur either from hematogenous spread or via the ascending route. There is an increased risk in those with diabetes, prolonged antimicrobial therapy and instrumentation of the urinary tract. The natural history has not been well defined, but most infections are asymptomatic, limited to the lower urinary tract and may resolve in otherwise normal patients. Most trials in asymptomatic candiduria have failed to show a clinical benefit but candiduria in the presence of urinary manipulation should be treated. Symptomatic candiduria should always be treated. Oral fluconazole (200 mg/day, 14 days) is the treatment of choice, although some species such as *Candida krusei* may be resistant.[39] Intravenous amphotericin B (0.3 mg/kg/day, 1–7 days) is usually effective, presumably because of prolonged renal excretion. Oral flucytosine often fails with development of resistance. If a catheter is present, bladder washouts with amphotericin B may be used, but are of questionable efficacy. Casofungin may be effective despite minimal urinary excretion.

Table 53.9 Possible toxicities of antimicrobial agents in pregnancy

Agent	Toxicity
Trimethoprim sulfonamides	Antifolate activity and megaloblastic anemia
Sulfonamides (protein bound)	Kernicterus of newborn
Sulfonamides/nitrofurantoin	Hemolytic anemia in glucose-6-phosphate dehydrogenase deficiency
Tetracycline	Fatty liver/hepatic necrosis in mother, stained teeth in baby
Fluoroquinolones	Not approved

Urethral Syndromes

PATHOGENESIS AND CLINICAL FEATURES

Following the introduction of quantitative bacterial counts, about half of women with acute symptoms of cystitis did not have significant – $>10^5$ colony forming units (cfu)/ml – bacteriuria. These women were said to have acute urethral syndrome or dysuria-pyuria syndrome. Many women with acute urethral syndrome have periurethral colonization with uropathogenic organisms and low numbers of *E. coli*, *S. saprophyticus* and enteric Gram-negative organisms present in their urine. Furthermore, many such patients responded to antibiotic therapy, suggesting that this was essentially a UTI. These patients probably have bacterial urethritis with little infection of the bladder and the diagnosis may be missed because of the low numbers of organisms present.

There remains a group with similar symptoms who do not have a low-count bacteriuria. It is possible that symptoms are caused by infection confined to the proximal urethra, especially if there is pyuria. A recent randomized controlled trial found that trimethoprim (300 mg daily) was superior to placebo in women with dipstick-negative acute dysuria, suggesting that infection is one cause of this syndrome.[40] *Ureaplasma urealyticum* and *Chlamydia trachomatis* are possible pathogens but trials with tetracycline have not been helpful. Multiple other causes have been suggested, including chemicals (e.g. bubble baths and deodorants), trauma, atrophic vaginitis in postmenopausal women and psychologic factors, but are mostly unproven.

MANAGEMENT

Management of the urethral syndrome is difficult. A pelvic examination should be carried out to exclude herpes simplex, gonorrhea and vaginitis. The symptoms usually settle in a few days, although some patients appear to benefit from a high fluid intake. Antimicrobial therapy may be helpful, particularly if pyuria is present, presumably reflecting bacterial urethritis or chlamydial infection. Antibiotics useful for treatment of UTI are often prescribed in the first instance. Tricyclic antidepressants are sometimes used in chronic dysuria but the benefit is unproven.

REFERENCES

References for this chapter can be found online at http://www.expertconsult.com

Chapter 54

Florian ME Wagenlehner
Kurt G Naber
Wolfgang Weidner

Prostatitis, epididymitis and orchitis

Prostatitis

The diagnosis of prostatitis syndrome refers to a variety of inflammatory and noninflammatory conditions probably not always affecting the prostatic gland itself. In 1978 a classification system[1] was developed to differentiate inflammatory from noninflammatory entities and was much used in the past. However, many aspects of chronic prostatic symptoms remained enigmatic. In 1999 a consensus conference at the National Institute of Diabetes, Digestive and Kidney Diseases (NIDDK) of the National Institutes of Health (NIH) developed a new classification system[2,3] based on the clinical presentation of the patients, the presence or absence of leukocytes in the expressed prostatic secretion (EPS) or voided bladder urine after prostatic massage (VB_3), and the presence or absence of bacteria in the EPS or VB_3. In addition, the inflammatory conditions are categorized into symptomatic and asymptomatic presentations, identifying patients who are incidentally diagnosed from biopsies, evaluations for fertility disorders or routine examinations. The term chronic pelvic pain syndrome (CPPS) was chosen, because it has not been scientifically demonstrated either that CPPS is primarily a disease of the prostate or that it is an inflammatory process.[2,3] Table 54.1 illustrates the NIH consensus.

EPIDEMIOLOGY

Definition and nomenclature

Acute bacterial prostatitis (ABP, NIH I) is an acute febrile illness that may be characterized by intense pain in the perineum and rectum, fever, voiding difficulties, systemic symptoms of sepsis and a tender, swollen prostate on rectal examination. The chronic prostatitis syndromes – chronic bacterial prostatitis (CBP) or chronic prostatitis/chronic pelvic pain syndrome (CP/CPPS) – cause symptoms that in the majority of cases cannot be differentiated from each other (Table 54.2). Patients with CBP (NIH II), however, often present with recurrent urinary tract infections. Pathogens must be present in EPS or VB_3 for a conclusive diagnosis of CBP.[4] In patients who have signs of inflammation (CP/CPPS, NIH IIIA), leukocytes (neutrophils, macrophages) are present in EPS or VB_3 or in ejaculate. In noninflammatory CP/CPPS (NIH IIIB) no signs of inflammation are detectable. In asymptomatic inflammatory prostatitis (NIH IV), detected either by prostatic histology or by the presence of leukocytes in seminal fluid or in prostate secretion during evaluation for other disorders, the patients have no subjective symptoms (see Table 54.1).

Incidence and prevalence

Because of classification difficulties, few data are available to determine the incidences of all the prostatic entities. Acute prostatitis is infrequent, with a probable incidence of fewer than 1 in 1000 adult men per year. However, prostatic symptoms are common. In the USA approximately 30% of men between 20 and 50 years of age experience 'prostatitis-like' symptoms[5] and these symptoms are responsible for about 25% of physician office visits by men for genitourinary complaints.[5–7] Prostatitis is the most common urologic diagnosis in men under 50 years of age and the third most common urologic diagnosis in men over 50 years of age.[8] It has been demonstrated that a diagnosis of chronic prostatitis can have a quality of life impact similar to a diagnosis of angina or Crohn's disease.[9] Thus, prostatitis is a major health-care issue, perhaps as important as the other two major prostatic diseases, namely benign hyperplasia and carcinoma.[10]

The term prostatitis implies inflammation, but only 5–10% of patients who have this diagnosis actually have a proven bacterial infection;[5,11] the remainder do not have 'significant' prostatic fluid bacterial counts. About half of these men have inflammatory CP/CPPS (NIH IIIA) with an elevated leukocyte count in prostatic fluid;[4,5,11–16] the remainder are categorized as having noninflammatory CP/CPPS (NIH IIIB). This is a diagnosis of exclusion and, in most cases, it cannot be proven that the symptoms arise from the prostate itself.

The most frequent form of prostatitis is asymptomatic inflammatory prostatitis (NIH IV). Significant scientific interest in this entity has evolved because of probable involvement in the initiation of prostate cancer.[17,18]

Risk factors

Urinary tract infections (UTIs) are the major underlying determinant of both ABP and CBP. Strains of *Escherichia coli* responsible for both ABP and CBP appear to have at least similar or even more urovirulence determinants to the *E. coli* strains that cause pyelonephritis.[19,20] Prostatic calculi can account for recurrences of CBP.[4] Bacterial microcolonies enclosed within biofilms inside prostatic acini and ducts can be a focus for bacterial persistence.[14] Inflammatory CP/CPPS may be due to intraprostatic reflux of urine causing inflammation.[21] Other presumed and unproven causes of inflammatory CP/CPPS are immunologic reactions to spermatozoa and migration of sexually transmitted organisms from the urethra.

CLINICAL FEATURES

Diagnosis

ABP is diagnosed by its clinical presentation.[6,16,22] It presents as an acute febrile illness with irritative and obstructive voiding symptoms. Prostatic massage is contraindicated and the diagnosis depends upon:

- urine and blood cultures;
- a gentle examination of the prostate that demonstrates acute inflammation; and
- urinalysis, which usually demonstrates pyuria.

Table 54.1 The National Institutes of Health consensus classification of prostatitis syndromes[2]

Category	Characteristic clinical features	Bacteriuria*	Inflammation†
I. Acute bacterial	Acute urinary tract infection (UTI)	+	+
II. Chronic bacterial	Recurrent UTI caused by the same organism	+	+
III. Chronic prostatitis/chronic pelvic pain syndrome (CP/CPPS) A. Inflammatory subtype‡ B. Noninflammatory subtype§	Primarily pain complaints, but also voiding complaints and sexual dysfunction	 – –	 + –
IV. Asymptomatic	Diagnosed during evaluation of other genitourinary diseases	–	+

*In chronic bacterial prostatitis VB_2 can be sterile and bacteriuria can only be detected in expressed prostatic secretion (EPS) or VB_3.
†Objective evidence of an inflammatory response in EPS, postprostate massage urine or semen or by histology.
‡Formerly termed 'nonbacterial prostatitis'.
§Formerly termed 'prostatodynia'.

Table 54.2 Symptoms in patients with the chronic prostatitis syndromes

Urethral symptoms	• Burning in the urethra during voiding • Discharge • Difficult urination • Stranguria • Frequency • Nocturia
Prostatic symptoms	• Pressure behind pubic bone • Perineal pressure tension in testes and epididymes • Inguinal pain • Anorectal dysesthesia • Diffuse anogenital syndromes • Lower abdominal discomfort
Sexual dysfunction	• Loss of libido • Erectile dysfunction • Ejaculatory dysfunction • Pain during or after orgasm
Other symptoms	• Myalgia • Headache • Fatigue

Depending on the patient's history, two different types of acute bacterial prostatitis – spontaneous and manipulated – have been described.[23] Approximately 10% of acute prostatitis cases might be elicited by previous manipulation of the lower urinary tract (e.g. prostatic biopsies, transurethral manipulation). Patients with acute bacterial prostatitis secondary to manipulation are older and appear to have a higher risk of prostate abscess formation.

Prostatic abscesses may occur in patients who have acute prostatitis. This diagnosis is made by clinical examination and transrectal ultrasonography. Focal hypoechoic zones with irregular internal echoes, septations and indirect borders with the surrounding parenchyma are typical patterns. The abscess may be distinct or more diffuse. Prostatic abscesses are usually due to the same uropathogens that are responsible for ABP, although a variety of anaerobes and fungi are implicated sporadically. Systemic mycoses, particularly *Cryptococcus neoformans, Blastomyces dermatitidis, Coccidioides immitis* or *Histoplasma capsulatum*, can rarely involve the prostate gland and produce prostatic abscesses. *Candida albicans* can also cause prostatic abscesses.

Chronic bacterial prostatitis is a rather precise diagnosis. It often presents as the source of acute recurrent UTI and/or recurrent ABP. Patients presenting with prostatic complaints should have a prostatic massage to localize the infection. The method of choice is the Meares and Stamey localization technique (Fig. 54.1).[24] Increased numbers of neutrophils and fat-laden macrophages are typical cytologic signs in the EPS. Although increased numbers of leukocytes may be found in the EPS, it is generally accepted that over 10 neutrophils per high-power field indicates prostatitis.[16,20] In patients for whom an EPS cannot be obtained, increased numbers of neutrophils in the urine after prostatic massage (VB_3) is an indication of prostatitis if first voided urine (VB_1) and midstream urine (VB_2) do not contain these cells. In patients who have CBP, bacterial pathogens will be present in the EPS or VB_3 in larger numbers, usually a 10-fold higher concentration than in the VB_1 and VB_2.[16,20] The exact technique for localizing infection with the Meares and Stamey technique is outlined in Figure 54.1 and should be followed carefully.[24]

A simpler screening test to assess inflammation/infection is the two-glass pre- and post-massage test (PPMT). Compared with the four-glass test, the PPMT has a rather good concordance with the four-glass test for the initial evaluation.[25] The PPMT is therefore a reasonable alternative when EPS cannot be obtained or when microbiologic assistance is not available (EPS, due to its usually small volume, has to be processed and plated immediately).

The role of *Chlamydia trachomatis* and *Ureaplasma urealyticum* in bacterial prostatitis is uncertain and there are no widely accepted criteria for defining prostatitis due to these or other infrequently isolated pathogens (Table 54.3).[26–28]

Ejaculate analysis is sometimes recommended in men who have CBP to obtain further information but studies of seminal fluid are mostly unhelpful. A proportion of men who have CBP have bacteriospermia (>10^3 cfu/ml) and the organisms present are usually identical to those in the EPS.[29] As semen cultures identify significant bacteriospermia in only about 50% of semen specimens from men with CBP,[30] culture of the ejaculate alone is not sufficient to diagnose CBP.[22,30]

Biochemical analysis of EPS has been used as an additional diagnostic criterion for CBP but these observations have not been shown to be sufficiently sensitive or specific to add to the diagnosis (Fig. 54.2).[16,20] The pH is usually increased (>7.8) in the EPS from patients who have CBP.

Biopsy under ultrasonographic guidance, particularly if nodules are present, is used for histology and culture.[4,12,31] Inflammatory findings in the prostate are usually nonspecific and the primary indication for biopsy is to exclude prostatic cancer.

Evaluation of bladder emptying by flow rate measurements and ultrasonography can be useful in patients who have voiding disturbances.[31] On occasion this diagnostic workup should include a voiding

Meares and Stamey localization technique

1. Approximately 30 minutes before taking the specimen, the patient should drink 400 ml of liquid (two glasses). The test starts when the patient wants to void
2. The lids of four sterile specimen containers, which are marked VB_1, VB_2, EPS and VB_3, should be removed. Place the uncovered specimen containers on a flat surface and maintain sterility
3. Hands are washed
4. Expose the penis and retract the foreskin so that the glans is exposed. The foreskin should be retracted throughout
5. Cleanse the glans with a soap solution, remove the soap with sterile gauze or cotton and dry the glans
6. Urinate 10–15 ml into the first container marked VB_1
7. Urinate 100–200 ml into the toilet bowl or vessel and without interrupting the urine stream, urinate 10–15 ml into the second container marked VB_2
8. The patient bends forward and holds the sterile specimen container (EPS) to catch the prostate secretion
9. The physician massages the prostate until several drops of prostate secretion (EPS) are obtained
10. If no EPS can be collected during massage, a drop may be present at the orifice of the urethra and this drop should be taken with a 10 ml calibrated loop and cultured
11. Immediately after prostatic massage, the patient urinates 10–15 ml of urine into the container marked VB_3.

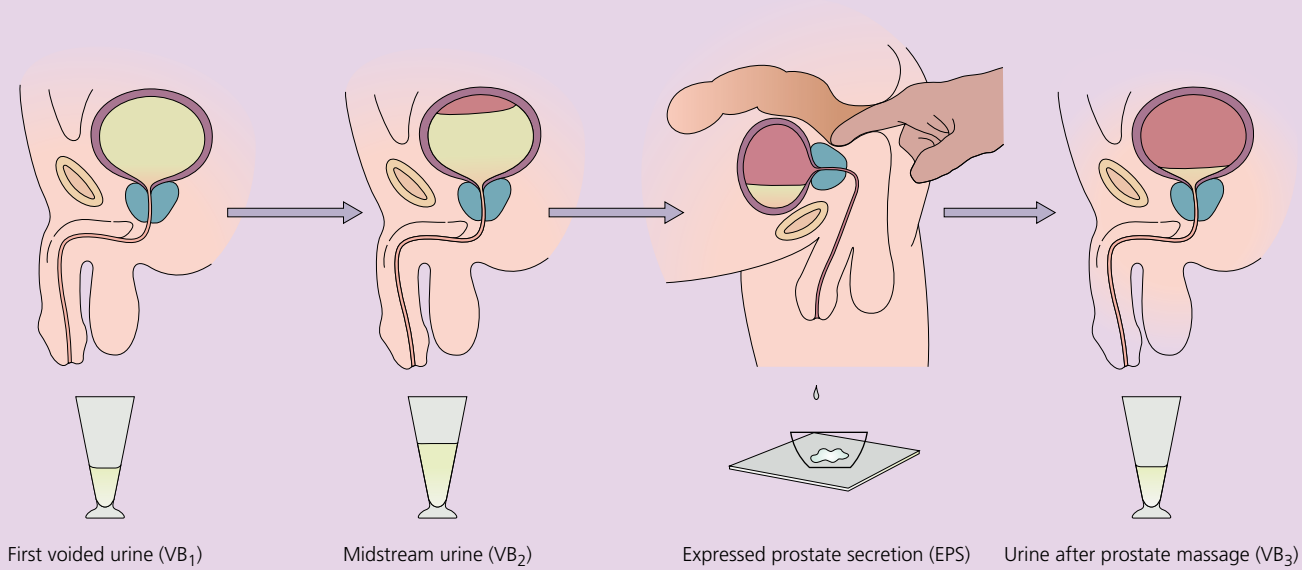


Fig. 54.1 Meares and Stamey[24] localization technique to diagnose chronic bacterial prostatitis. Prostate secretion can be more readily obtained if the patient has not ejaculated for approximately 3–5 days before the examination.

cystourethrogram. Urodynamic changes are present in about one-third of patients who have CBP. In the presence of abnormal flow rate measurements, further studies should be performed to differentiate between functional and anatomic changes.

Urethrocystoscopy may reveal visible inflammatory changes in the posterior urethra. Prostatic sonography may demonstrate prostatic calculi (Fig. 54.3). Prostatic calculi may serve as nidi for pathogens and lead to CBP; however, as they are common and increase with age, their role remains controversial. Figure 54.4 outlines the diagnostic investigation of patients who present with possible CBP.[22]

Chronic prostatitis/chronic pelvic pain syndrome is a less specific diagnosis. In CP/CPPS (NIH IIIA) inflammatory cells in the EPS with negative cultures from both the EPS and VB_3 are found. Although numerous investigators have attempted to demonstrate that CP/CPPS (NIH IIIA) is due to difficult-to-culture pathogens such as *C. trachomatis* or genital mycoplasmas, there is no consensus that these organisms cause CP/CPPS (NIH IIIA).[26,32,33] As a result, this diagnosis is currently poorly defined and is presumed to be caused by largely unknown etiologic and pathogenetic processes.

In CP/CPPS (NIH IIIB) neither inflammatory cells nor positive cultures are found in EPS and VB_3. For a better discrimination of NIH IIIA from NIH IIIB, novel parameters, such as interleukin-8, have been investigated.[34,35] The exact limits of these parameters, however, are currently under discussion.

MANAGEMENT

Treatment varies according to the severity of the patient's presenting symptoms and the probable etiologic agent. Antimicrobial treatment should be initiated immediately in patients who have acute bacterial prostatitis after blood and urine cultures have been obtained. Prostatic massage is contraindicated. Parenteral treatment with a fluoroquinolone or a β-lactam with an aminoglycoside are appropriate initial regimens. After initial improvement, a switch to an oral regimen is appropriate and should be prescribed for about 2–4 weeks. Optimal antibiotics would be fluoroquinolones, if tested susceptible.

Patients who have possible CBP require investigation for evidence of inflammation and an etiologic agent. Selection of an appropriate antimicrobial agent that has optimal pharmacokinetics for prostatic secretion and tissue is important.[36] Antibacterial diffusion into prostate secretion depends upon the lipid solubility, molecular size and pKa of the agent.[36] For example, trimethoprim, a weak base with a pKa of 7.4, penetrates well into the acid prostatic secretion. However, because the pH of prostatic fluid in patients who have CBP is often alkaline, concentrations in prostatic secretion may be inadequate.[37,38] In contrast, the fluoroquinolones exist as zwitterions with a pKa in acid and alkaline milieus[39] (Table 54.4). Due to the relatively large

Table 54.3 Prostatitis infections by unconventional fastidious pathogens

Species	Clinical features	Comment
Haemophilus influenzae		Single case reports
Neisseria gonorrhoeae	Associated with history of gonococcal urethritis	Decreasing due to effective antibiotic treatment
Mycobacterium tuberculosis	Urogenital manifestation	Associated with HIV infection
Anaerobes	Prostatic abscesses	
Brucella spp.	Disseminated disease	Consumption of unpasteurized diary products and occupational contact
Candida spp.	In immuno-compromised patients with indwelling urinary catheters	
Coccidioides immitis, Blastomyces dermatitidis, Histoplasma capsulatum	Disseminated disease	Associated with HIV infection
Trichomonas vaginalis	Chronic inflammation	May be associated with urethritis

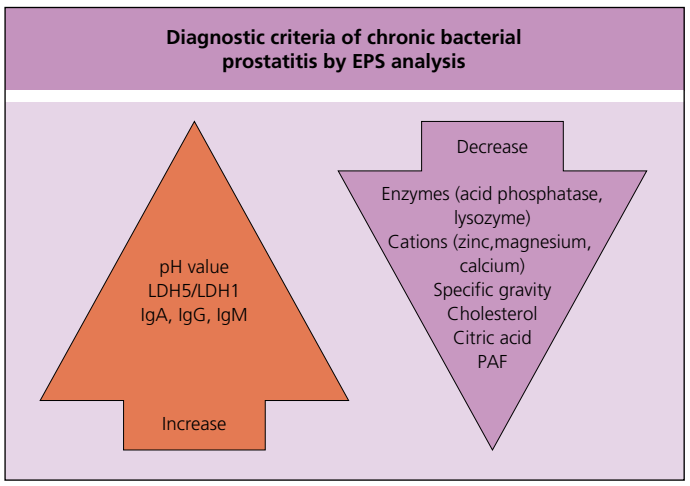


Fig. 54.2 Diagnostic criteria of chronic bacterial prostatitis by expressed prostatic secretion analysis. LDH, lactate dehydrogenase; PAF, prostatic antibacterial factor.

differences in pKa values of the different fluoroquinolones (Table 54.4), the prostatic fluid:plasma concentration ratios range from 0.10 to 1.57 (Table 54.5).[40–46] The concentration of some fluoroquinolones in the alkaline seminal fluid may even exceed that in plasma (Table 54.5).[42,44] There exists a positive correlation of the isoelectric points of the fluoroquinolones and the prostatic secretion to plasma concentration ratios, investigated in volunteers (Fig. 54.5).

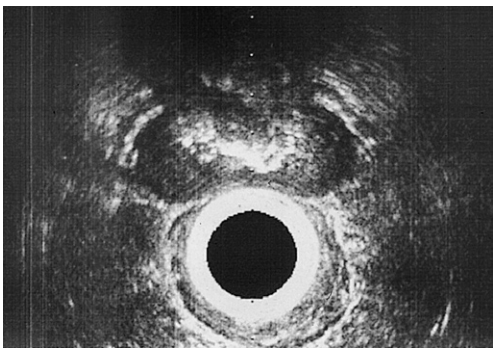


Fig. 54.3 Transrectal ultrasonography of the prostate with diffuse calcifications (prostatitis calcarea).

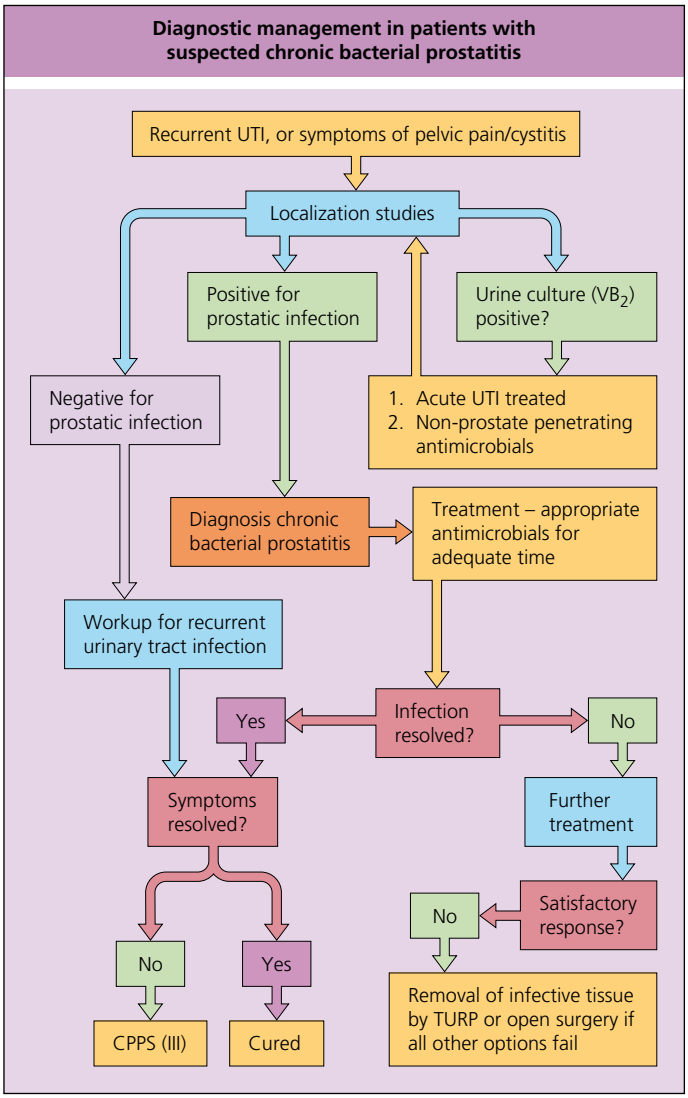


Fig. 54.4 Diagnostic management in patients with suspected chronic bacterial prostatitis. TURP, transurethral resection of prostate; UTI, urinary tract infection. Modified from Schaeffer *et al.*[22]

Other studies have examined fluoroquinolone concentrations in prostatic tissue obtained at transurethral resection and they appear to be consistently at or above corresponding plasma concentrations.[47] These investigations, however, resemble a mixture of different pharmacokinetic compartments (e.g. intracellular, extracellular, interstitial

Table 54.4 Dissociation constants of fluoroquinolones[39]

Quinolone	Pk_{a1}	Pk_{a2}
Ciprofloxacin	6.1	8.7
Enoxacin	6.3	8.7
Fleroxacin	5.5	8.1
Gatifloxacin	6.0	9.2
Levofloxacin	6.1	8.2
Lomefloxacin	5.8	9.3
Moxifloxacin	6.4	9.5
Norfloxacin	6.3	8.4
Ofloxacin	6.0	8.2
Pefloxacin	6.3	7.6
Sparfloxacin	6.3	8.8

compartment). Therefore assessment of concentrations in the prostatic fluid is the more precise method. Macrolides also penetrate into prostatic and seminal fluids very well; however, due to their restricted Gram-positive activity, they play only a limited role in the monotherapy treatment of CBP.[36,48]

Although it remains unproven, considerable evidence suggests that bacteria in prostatic tissue survive in a milieu protected by biofilms. Antimicrobial agents (particularly the fluoroquinolones and the macrolides) that are active in biofilm infection may be preferred drugs. Theoretically a combination therapy of fluoroquinolones and macrolides might be beneficial because of the anti-biofilm activity of the macrolides; however, no such data from studies in CBP patients are available. Most studies in patients who have CBP have not been well controlled and have been variably designed.[47,49] As a result, comparison is difficult. Duration of therapy has ranged from 14 to 150 days and follow-up investigation has not been standardized. An EPS should

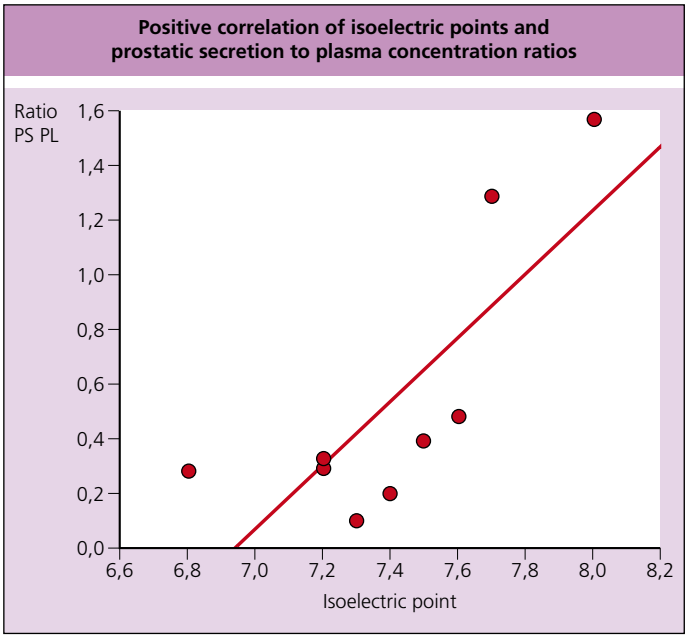


Fig. 54.5 Positive correlation of the isoelectric points and the prostatic secretion to plasma concentration ratios (PS/PL) of nine quinolones (see Table 54.5) (Pearson correlation, $P=0.013$). Modified from Wagenlehner *et al.*[42]

be obtained from all patients at 4–8 weeks and at 6 months after treatment to ensure that the pathogens have been eradicated.[49]

Overall, it appears that 60–80% of patients who have *Escherichia coli* and other Enterobacteriaceae can be cured with a 4- to 6-week course of therapy (Table 54.6).[50–56] However, prostatitis due to *Pseudomonas aeruginosa* or enterococci often fails to respond to treatment and the increasing fluoroquinolone resistance among Enterobacteriaceae leads to increasing treatment failures in those species as well. In the case of CBP due to fluoroquinolone-resistant strains, prolonged treatment with co-trimoxazole for 2–3 months is recommended.

Table 54.5 Median concentrations of fluoroquinolones in prostatic and seminal fluid and fluid to plasma concentration ratios (normalized to a dose of 400 mg)

Quinolone	Dose (400 mg)	Time (h)	Subjects (n)	Prostatic fluid (mg/l)	Ratio of prostatic:plasma concentration*	Seminal fluid (mg/l)	Ratio of seminal:plasma concentration	Reference
Norfloxacin	po	1–4	7	0.08	0.10	n.d.	–	Naber & Sorgel[41]
Ciprofloxacin	iv	4	8	0.18	0.20	5.06	7.1	Naber *et al.*[45]
Fleroxacin	po	2–4	8	1.00	0.28	5.80	1.7	Naber & Sorgel[41]
Levofloxacin	po	3	8	1.42	0.29	5.14	1.0	Bulitta *et al.*[40]
Ofloxacin	iv	4	5	0.66	0.33	2.05	4.0	Naber *et al.*[46]
Enoxacin	po	2–4	10	0.29	0.39	2.19	2.2	Naber & Sorgel[41]
Lomefloxacin	po	4	7	1.38	0.48	2.03	1.2	Naber & Sorgel[41]
Gatifloxacin	po	4	7	1.03	1.29	1.75	1.0	Farker *et al.*[43] Naber *et al.*[44]
Moxifloxacin	po	3–4	8	3.99	1.57	2.43	1.0	Wagenlehner *et al.*[42]

n.d., no data.
*Ordered according to prostatic fluid to plasma ratios.

Table 54.6 Eradication of pathogens (bacteriologic cure) in patients with chronic bacterial prostatitis treated with fluoroquinolones

Quinolone	Daily dosage (mg)	Duration of therapy (days)	Number of evaluable patients	Bacteriologic cure (%)	Duration of follow-up (months)	Year of study	References*
Norfloxacin	800	28	14	64	6	1990	Schaeffer & Darras[50]
Ofloxacin	400	14	21	67	12	1989	Pust *et al.*[51]
Ciprofloxacin	1000	14	15	60	12	1987	Weidner *et al.*[52]
Ciprofloxacin	1000	28	16	63	21–36	1991	Weidner *et al.*[53]
Ciprofloxacin	1000	60–150	7	86	12	1991	Pfau[54]
Ciprofloxacin	1000	28	34	76	6	2000	Naber *et al.*[55]
Ciprofloxacin	1000	28	78	72	6	2001	Naber[56]
Lomefloxacin	400	28	75	63	6	2001	Naber[56]

Only studies are listed in which the diagnosis was derived from application of the Meares and Stamey technique and a follow-up of at least 6 months was available.

Chronic bacterial prostatitis can be a relapsing illness and recurrent episodes are best managed by continuous low-dose suppressive therapy with an effective regimen such as a fluoroquinolone, intermittent treatment whenever symptoms recur, or efforts to resect infected prostatic tissue, particularly prostatic calculi, in order to effect a surgical cure.[5] The last is rarely successful and should only be carried out with very specific indications.

A prostatic abscess may require drainage in addition to antimicrobial treatment. Occasionally, anaerobes or mixed infections may be responsible for the abscess. Cultures should always be obtained and, if fungal infection is suspected, the laboratory should be informed. Most treatment regimens should include an agent effective against anaerobes. Prostatic abscesses can be drained through the urethra, the perineum or the rectum.

Inflammatory CP/CPPS (NIH IIIA) is managed with α-blockers for 3–6 months, although a recent study has not shown any improved effect compared to placebo.[56A] A pollen extract (Cernilton) has recently been shown to significantly improve pain and total symptoms, specifically in patients with CP/CPPS (NIH IIIA).[56B] Occasionally, patients appear to have a very specific response to antimicrobial therapy and, whenever this occurs, a prolongation of therapy is indicated. However, most patients who have inflammatory CP/CPPS do not experience any change in symptoms with antibacterial therapy. Other treatment regimens include anti-inflammatory agents, phytotherapeutics and pain medications. However, all regimens are empiric and treatment is often unsatisfactory.

Noninflammatory CP/CPPS (NIH IIIB) is an imprecise diagnosis for which therapy is controversial. Although the symptoms can mimic those of CBP, the absence of inflammation or any signs of infection are presumed to mean that no microbial agent is involved.[2,3] Noninflammatory CP/CPPS is probably the most frequent entity when patients are presenting with symptoms of prostatitis. Treatment regimens are similar to those used for inflammatory CP/CPPS, with the exception that antibiotics do not have any role in this entity.

Epididymitis and orchitis

Epididymitis is an acute painful swelling in the scrotum, which is usually unilateral.[57] The testes may be involved in the inflammatory process as 'epididymo-orchitis'. Inflammatory processes of the testes, especially viral orchitis, less often involve the epididymis.

EPIDEMIOLOGY

Orchitis and epididymitis are classified as acute or chronic processes according to their cause (Table 54.7). Chronic inflammation with induration develops in about 15% of patients following an episode of acute epididymitis. Viral and bacterial inflammation of the testes can lead to testicular atrophy and destruction of spermatogenesis.[58]

Epididymitis is common among individuals who have high-risk sexual behaviors (frequent change of sexual partners) and is one of the leading causes of acute admission to hospital among military personnel. It occurs in 1–2% of patients who have gonococcal and chlamydial urethritis, with an equal risk from each. It is usually unilateral and is due to an extension of the urethral infection via the vas deferens to the epididymis.

In middle-aged and older men, epididymitis is usually due to the same organisms as those that cause UTI and is presumably a direct extension from the urinary tract. Epididymitis is more common in patients who have indwelling catheters. Bladder outlet obstruction and urogenital abnormalities are also risk factors for acute and chronic epididymo-orchitis.

Mumps orchitis was common before widespread vaccination but became rare in the 1990s. However, due to decreasing vaccination coverage in western populations, mumps notifications and orchitis cases

Table 54.7 Classification of epididymitis and orchitis

Acute epididymitis or epididymo-orchitis	Granulomatous epididymitis or orchitis	Viral orchitis
Neisseria gonorrhoeae	*Mycobacterium tuberculosis*	Mumps
Chlamydia trachomatis	*Treponema pallidum*	Enteroviruses
Escherichia coli	*Brucella* spp.	
Streptococcus pneumoniae	Sarcoid	
Klebsiella spp.	Fungal	
Salmonella spp.	Parasitic	
Other urinary tract pathogens	Idiopathic	
Idiopathic		

have increased rapidly in the past few years. Mumps orchitis occurs in 20–30% of postpubertal men who have mumps. Other viral infections can also cause orchitis, particularly enteroviruses. The testes can also be involved as a continuation of epididymitis, particularly when suppurative UTI pathogens are involved. Granulomatous orchitis is a rare condition of uncertain etiology.[59] With regard to chronic inflammatory conditions, a so-called 'low-grade autoimmune orchitis'[60] has been described.

Epididymo-orchitis can lead to abscess formation, testicular infarction, testicular atrophy, chronic epididymitis and infertility.[57] In men who have azoospermia, postinflammatory epididymal obstruction can sometimes be cured by reconstructive surgery.[61]

CLINICAL FEATURES

Inflammation, pain and scrotal swelling characterize acute epididymitis.[57] Frequently the tail of the epididymis is involved first. The spermatic cord is usually tender and enlarged. The testes may be spared or may be involved to produce a contiguous large painful mass. Acute epididymitis always requires immediate evaluation by Doppler duplex scanning to differentiate between acute epididymitis and spermatic cord torsion. The latter requires urgent surgical intervention to prevent testicular infarction.[62]

The microbiologic diagnosis of acute epididymitis must be made as specifically as possible. A urethral Gram stain, urine culture and other studies (e.g. amplification techniques) for identification of *Neisseria gonorrhoeae* and *C. trachomatis* should be obtained for all patients. Blood cultures are valuable if the patient is febrile or has systemic signs of toxicity. Ejaculate analysis according to World Health Organization (WHO) criteria, including leukocyte analysis, may be of value. A transiently decreased sperm count or azoospermia is common. Infertility is a rare complication unless there is bilateral involvement.

Chronic epididymitis is characterized by thickening and induration of the epididymis. Especially in patients who are infertile, ejaculate analysis concerning semen quality is a necessary investigation to exclude azoospermia[60] and changes of sperm maturation.[63]

Orchitis, an isolated inflammation of the testis, is a rare event. Most frequently it occurs in association with epididymitis, as epididymo-orchitis (see Table 54.7). Testicular swelling, frequently accompanied by fever, is typical. Antibody and other specific serum investigations should be carried out to identify mumps, enteroviruses and other potential viral pathogens. In chronic infections, ejaculate analysis may demonstrate structural sperm defects providing reduced sperm motility and number.[60] Testicular biopsy in these cases may demonstrate focal inflammation, mixed atrophy and complete Sertoli-cell-only syndrome in the follow-up.[58]

MANAGEMENT

In acute epididymitis (epididymo-orchitis), antimicrobial agents should be chosen for initial empiric treatment based on the probability of the etiologic agent. In sexually active men who are at risk of *C. trachomatis* or *N. gonorrhoeae*, a therapeutic regimen that covers both these pathogens is mandatory.

Antibiotic resistance in *N. gonorrhoeae* has increased dramatically over the past few years. The WHO surveillance of antibiotic resistance in *N. gonorrhoeae* in the Western Pacific Region, carried out in 2005, revealed resistance rates of penicillins and quinolones up to 100%, and of tetracyclines up to 80% in some countries.[64] Penicillin- and fluoroquinolone-resistant strains have already reached western countries. The Centers for Disease Control and Prevention (CDC) therefore adapted to this resistance trend and now recommend ceftriaxone as first-line agent for the treatment of acute epididymitis caused by *N. gonorrhoeae* in a rather low single dose of 250 mg intramuscularly.[65] Additional treatment of nongonococcal agents with a tetracycline is also recommended.[65]

Additional therapy includes scrotal support. Abscesses may require surgical drainage. If urinary tract pathogens are considered to be the probable etiologic agent, fluoroquinolones or trimethoprim–sulfamethoxazole are appropriate choices. Experimental[66] and clinical[67] studies suggest that the fluoroquinolones are very effective, if the pathogens test susceptible.

Interferon alpha (IFN-α) has been prescribed for the treatment of acute mumps orchitis.[60,68] However, further studies in which testicular biopsies were performed after treatment have shown that systemic treatment with IFN-α is not completely effective in preventing testicular atrophy after mumps orchitis.[69] Therapy with nonsteroidal anti-inflammatory agents has also been recommended,[70] as has treatment with long-acting gonadotrophin-releasing hormone agonists.[71] Use of steroids should be avoided, because concentrations of testosterone could be decreased and concentrations of follicle-stimulating and luteinizing hormones could be increased, facilitating testicular atrophy.[72]

REFERENCES

References for this chapter can be found online at http://www.expertconsult.com



Chapter 55

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Pyelonephritis and abscesses of the kidney

INTRODUCTION

Acute pyelonephritis, an acute infection (usually bacterial) of the kidney and renal pelvis, is one of the most common serious infectious diseases of otherwise healthy individuals, and is an even greater problem for compromised hosts. New approaches to the diagnosis and management of this disorder and its sequelae, including intrarenal and perinephric abscess, have resulted in improved outcomes for patients.

EPIDEMIOLOGY

Annually in the USA, approximately 200 000 adults are admitted to hospital for renal infection;[1,2] many others are managed as outpatients. In Seattle, Washington, the annual risk of pyelonephritis is approximately 12–13/10 000 for women and 3–4/10 000 for men.[3]

Complicated versus uncomplicated pyelonephritis

Pyelonephritis can be stratified as 'complicated' or 'uncomplicated', depending on the presence of underlying urologic or medical conditions that predispose to kidney infection or that aggravate the severity or intransigence of such infections once they occur.[1] Uncomplicated and complicated pyelonephritis have distinctive host substrates, microbial flora, pathogenetic mechanisms, clinical presentations and requirements for and response to therapy.

Risk factors

Although little is known about the specific risk factors for uncomplicated pyelonephritis, recognized risk factors for uncomplicated cystitis would be predicted to predispose to pyelonephritis also. Such associations include female sex and, among adolescent or adult women, sexual intercourse, a history of previous urinary tract infections (UTIs), use of spermicide-diaphragm contraception and being a nonsecretor of blood group substances (see Chapter 42). The postmenopausal state has also been associated with increased rates of UTI.[4,5] Among children, the P_1 blood group phenotype is associated with an increased pyelonephritis risk.[6] Further evidence for an inherited cause of pyelonephritis comes from a study involving a cohort of children predisposed to acute pyelonephritis. Investigators found increased rates of pyelonephritis in relatives of these children when compared to relatives of matched controls, despite similar rates of cystitis in the two groups. Expression of CXCR1 on peripheral blood neutrophils was significantly decreased in the families with a history of pyelonephritis, suggesting this receptor may play a role in the pathogenesis of the disease.[7]

Pyelonephritis in compromised hosts, which by definition is 'complicated', is promoted by almost any anatomic or functional abnormality of the urinary tract, urinary tract instrumentation, diabetes mellitus, pregnancy (during which the risk of pyelonephritis is 1–2%) and conditions associated with sensory impairment (such as diabetic or alcoholic neuropathies and spinal cord injury).[1] Among the commonly implicated urologic conditions are posterior urethral valves (in infant boys), congenital vesicoureteral reflux (in girls), indwelling or intermittent urinary catheterization, other instrumentation of the urinary tract, neurogenic bladder, urolithiasis, ureteral diversions, any obstruction to normal urinary flow and kidney transplantation. Pyelonephritis in an immunosuppressed host does not imply that the infection is complicated per se, as the spectrum of expected infectious agents is similar to that seen in immune competent hosts, as is the response to and duration of therapy.

Renal abscess, which can be intrarenal, intrarenal with perirenal extension or entirely perirenal, typically develops as a consequence of acute pyelonephritis and is among the most serious local complications of this illness. It occurs predominantly in compromised hosts, notably patients who have diabetes mellitus or have undergone recent surgery or instrumentation of the urinary tract. Urinary reflux and obstruction are prominent risk factors for renal abscess. Rarely, a renal abscess may develop during a severe episode of otherwise uncomplicated pyelonephritis in an intact host.

PATHOGENESIS

Route of infection

Irrespective of the presence of predisposing host conditions, in almost all patients acute pyelonephritis arises via an ascending route of infection.[1] The causative micro-organisms enter the urethra, colonize the bladder, then ascend the ureters to the renal pelvis and subsequently invade the renal parenchyma. In most cases, the pathogens arise from the host's own intestinal (and, in women, vaginal) flora,[8] although in patients who have indwelling catheters or nephrostomy tubes organisms may be transferred on the hands of health-care workers and thus bypass the intestinal, vaginal and/or bladder colonization steps.

Microbial flora

Organisms must have substantial intrinsic virulence to overcome the many defense mechanisms of a healthy urinary tract and cause pyelonephritis in an intact host. In contrast, organisms of lesser intrinsic

virulence can infect the kidney in patients who have impaired urinary tract defenses. Paradoxically, the less virulent organisms associated with complicated pyelonephritis are more often resistant to antimicrobial agents than are the more virulent ones that cause uncomplicated pyelonephritis. This, together with the impaired defense mechanisms of compromised hosts, makes such infections more difficult to treat and cure than uncomplicated pyelonephritis.

In uncomplicated pyelonephritis the distribution of microorganisms is similar to that in uncomplicated cystitis, with approximately 80% of isolates being *Escherichia coli* and the remainder other Gram-negative bacilli (predominantly *Klebsiella* and *Proteus* spp.), *Staphylococcus saprophyticus* (especially in young women), *Enterococcus* spp. (especially in older men) and occasionally group B or other streptococci.[1] The *E. coli* strains that cause uncomplicated pyelonephritis exhibit multiple virulence properties that contribute to their ability to invade the urinary tract and stimulate inflammation and tissue damage (Fig. 55.1).[9]

Among the various adhesins expressed by these strains, the most prevalent and pathogenetically important are type 1 fimbriae and P fimbriae. P fimbriae are strongly epidemiologically associated with pyelonephritis[9] and contribute to kidney infection in a monkey model.[10] They recognize Galα(1–4)Gal-containing receptors on host epithelial surfaces, including the mucosal lining of the colon, vagina and urinary tract. The P fimbrial adhesin molecule PapG is situated at the tip of the fimbrial stalk and mediates attachment to receptors on the host cell. PapG occurs in three known variants, of which the class II variant is the most common among strains that cause uncomplicated pyelonephritis and bacteremic UTIs, whereas the class III variant is associated with cystitis and with complicated UTIs.

Type 1 fimbriae are structurally similar to P fimbriae but have a binding specificity for mannose-containing receptors on host cells. Since type 1 fimbriae are produced by almost all *E. coli*, epidemiologic associations of type 1 fimbriae with UTI or pyelonephritis are difficult to demonstrate. However, in the mouse model of UTI, deletion of the type 1 fimbrial adhesin gene or immunization against the corresponding FimH adhesin molecule reduces both bladder and kidney infection. Other important virulence factors of pyelonephritogenic *E. coli* include cytotoxins such as α-hemolysin (which destroys or impairs the function of host epithelial cells, phagocytes and lymphocytes), iron sequestration systems such as the aerobactin system, polysaccharide capsules, lipopolysaccharide and serum resistance proteins (which protect the organism against phagocytosis and/or complement-mediated lysis).[9,11]

In complicated pyelonephritis, although *E. coli* still is the single most common pathogen, it is less prevalent than in uncomplicated pyelonephritis and is represented by less virulent strains. Other Gram-negative bacilli are more commonly encountered, including *Pseudomonas aeruginosa*, *Enterobacter* spp. and other Enterobacteriaciae.[1]

Factors promoting ascending infection

Vaginal colonization with urovirulent organisms is promoted by sexual intercourse, particularly with the use of a spermicide, which kills normal lactobacillus-based vaginal flora and permits overgrowth with *E. coli* and other coliform bacteria.[8] Similar changes in the vaginal flora occur after the menopause as a result of estrogen depletion, and are induced by the use of certain antimicrobial agents, notably β-lactams.

In women, sexual intercourse promotes the entry of periurethral bacteria into the bladder on a mechanical basis. In catheterized patients, bacteria can be introduced into the bladder at the time of catheter insertion, or can migrate into the bladder along the external or luminal surfaces of catheters.[12] With improper catheter care, infected urine from the collecting bag and drainage tubing can reflux into the bladder. Antireflux valves are standard on most indwelling catheters; however, there is little evidence that they prevent bacteriuria or catheter-associated UTI. Catheter-associated organisms persist within the urinary tract in part by cementing themselves to the catheter within glycocalyx matrices that protect them against natural host defense mechanisms and antimicrobial agents. Uropathogenic *E. coli* have been demonstrated to invade the bladder epithelium in a mouse UTI model, suggesting a possible method for evading host defenses and establishing a persisting intracellular reservoir from which organisms could re-emerge to cause recurrent UTI. Whether this phenomenon

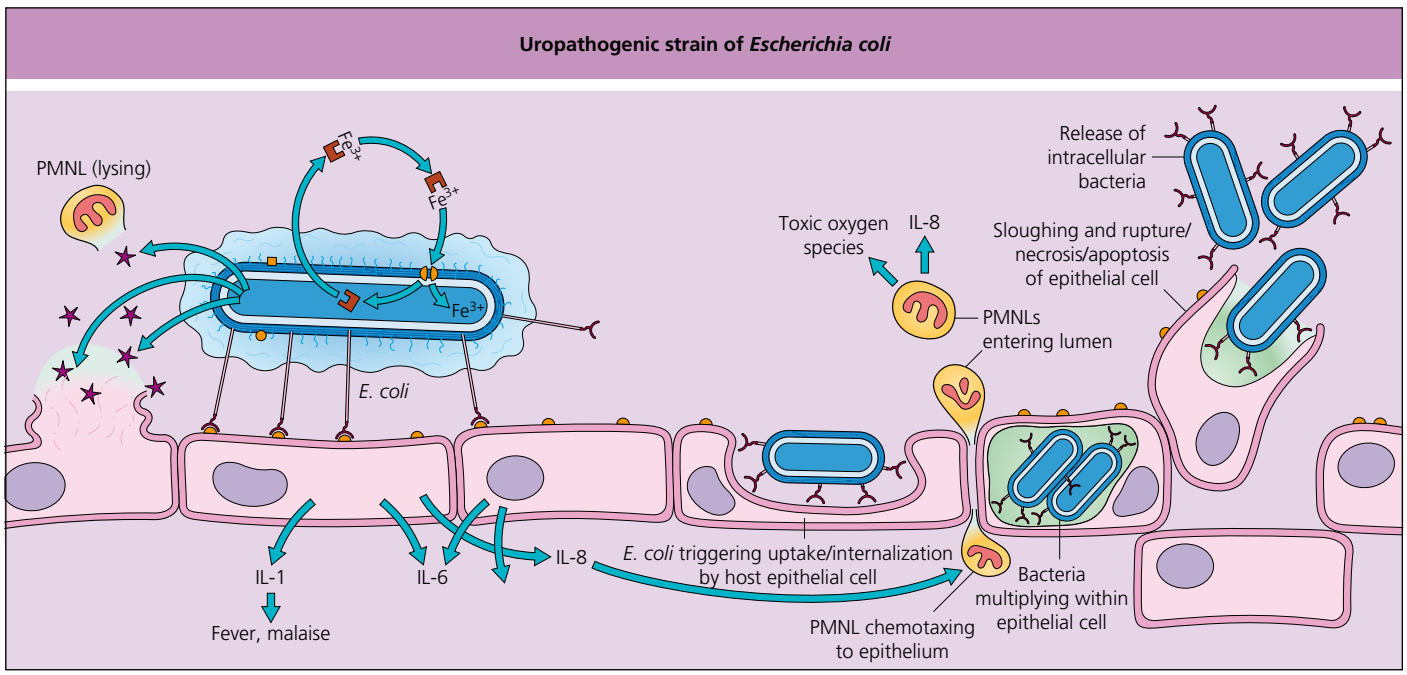


Fig. 55.1 Uropathogenic strain of *Escherichia coli*. Note the typical virulence properties, including adhesive fimbriae, cytotoxins, lipopolysaccharide (LPS), capsular polysaccharide, the aerobactin iron sequestration system and outer membrane proteins important in serum resistance. Bacterial interactions with host cells trigger cytokine production, inflammatory cell infiltration and bacterial internalization within epithelial cells. Internalized bacteria can multiply intracellularly and stimulate sloughing, rupture, necrosis or apoptosis of host cells. IL, interleukin; PMNLs, polymorphonuclear leukocytes.

contributes to the pathogenesis of UTI, and specifically pyelonephritis, in humans is undefined.

Ascent of pathogens from the bladder up the ureters is facilitated by vesicoureteral reflux, which may be pre-existing or which in the intact host can result from a reversible ureteral aperistalsis induced by exposure of the ureteral wall to lipopolysaccharide from adherent bacteria.[13] Bacterial flagellae also likely contribute to ureteral ascent against urine flow.[14] Among several UTI-promoting physiologic alterations of pregnancy, ureteral hypotonia and some degree of ureteral obstruction may contribute to bacterial entry into the upper urinary tract in pregnant women and its persistence once there.[10] Once within the renal pelvis, micro-organisms migrate up the collecting ducts into the tubules, a process promoted by intrarenal reflux (if present in the particular host) and by bacterial adhesins that recognize receptors along this epithelial surface or in subjacent tissues.[9,13]

PATHOLOGY

Within the urinary tract, pathogenic bacteria adhere to the mucosa and trigger a local cytokine/chemokine network, with production of interleukin (IL)-1, IL-6, IL-8 and tumor necrosis factor, and recruitment of polymorphonuclear leukocytes (PMNLs) and lymphocytes. Triggers include the interaction of bacterial lipopolysaccharide with host cell Toll-like receptor 4 (TLR4), P fimbrial binding to host membrane glycolipids, which activates an intracellular ceramide signaling pathway, and type-1-fimbria-mediated bacterial internalization. The influx of inflammatory cells leads to the generation of reactive oxygen species, leukotrienes, prostaglandins and other mediators of inflammation, which together with bacterial cytotoxins produce tissue damage, edema and, in the kidney, intense local vasoconstriction (see Fig. 55.1).[1,13] These phenomena presumably are responsible for the characteristic signs and symptoms of pyelonephritis, including dysuria and suprapubic pain from bladder involvement, flank pain and costovertebral angle tenderness from kidney involvement, and fever and malaise from inflammatory cytokines that enter the systemic circulation.

Histologically, in acute pyelonephritis the mucosa and submucosa of the collecting system, the tubules and the interstitium are edematous and infiltrated with PMNLs (Fig. 55.2). Tubules may necrose. Microabscesses form within the mucosa and interstitium, and can coalesce to form macroscopic abscesses.[1]

Grossly, the kidneys are diffusely or focally swollen and edematous. On sectioning, streaks of yellowish inflammatory infiltrate extend from the papillae and medulla toward the cortex, sometimes reaching the capsule and rupturing it.[1] When macroscopic abscesses do form, typically they localize at the corticomedullary junction but can be subcapsular or extend into the perirenal space.[7] In hyperglycemic diabetic patients, rapid fermentation of glucose by Gram-negative bacilli (or, rarely, yeasts) can produce gas within the renal parenchyma (emphysematous pyelonephritis; Fig. 55.3), within an abscess (gas abscess) or within the renal pelvis and collecting system (emphysematous pyelitis).[15] Papillary necrosis, which occasionally complicates acute pyelonephritis among diabetic patients, may make the infection worse because of the obstruction caused by sloughed tissue (Fig. 55.4).[1]

Functionally, the intense interstitial inflammatory process leads to a reduction in urinary concentrating capacity. Decreased renal blood flow, functional tubular obstruction from inflammatory cells and necrotic debris, and inflammation-induced tubular dysfunction result in delayed excretion of radiographic contrast dye[1] but only rarely manifest as clinically apparent renal dysfunction.[16]

Bacteremia develops in between 10% and 65% of patients who have acute pyelonephritis, depending on the severity of infection and increasing in proportion to the age of the host. Bacterial entry into the bloodstream may be promoted by P fimbriae and by tissue destruction mediated by microbial cytotoxins. Systemic complications of pyelonephritis, which are more common among patients who have Gram-negative bacteremia, include septic shock, disseminated intravascular coagulation and

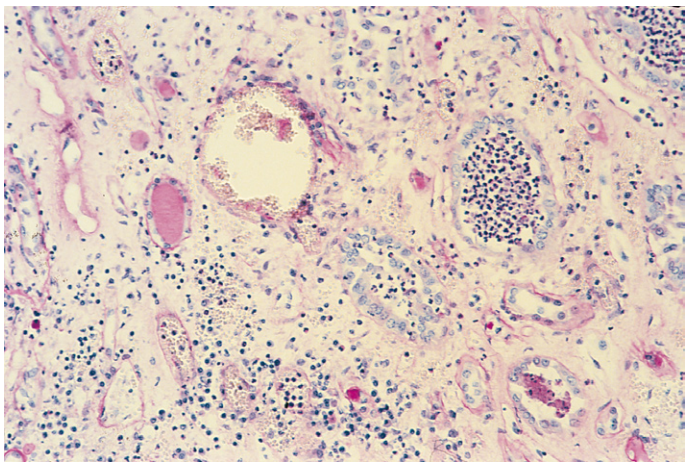


Fig. 55.2 Acute pyelonephritis. Note interstitial edema, tubules packed with PMNLs and a diffuse interstitial acute inflammatory infiltrate in this autopsy specimen from a diabetic patient who had refractory *Escherichia coli* urosepsis.

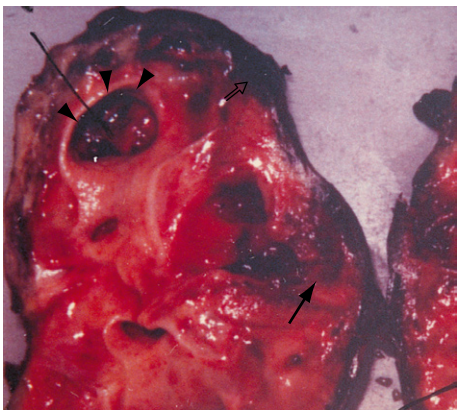


Fig. 55.3 Emphysematous pyelonephritis. Cortical necrosis (solid arrow), diffuse cortical hemorrhage (open arrow) and dilatation of the collecting system (arrowheads) in a nephrectomy specimen from a diabetic patient who received combined medical/surgical therapy and survived emphysematous pyelonephritis due to an unusual pathogen, namely *Candida albicans*.



Fig. 55.4 Acute papillary necrosis (arrows) in an autopsy specimen from a diabetic patient who died from refractory *Escherichia coli* urosepsis. Necrotic papillae (arrows) failed to take up formalin, so appear pink, in contrast to the grayish-tan formalinized tissue.

the acute respiratory distress syndrome (ARDS). Pregnant women who have pyelonephritis are particularly prone to these complications, and also may develop premature labor as a result of the irritative effect of lipopolysaccharide on the uterus.[17]

Hematogenous renal abscesses

Intrarenal abscesses can also be caused by certain hematogenously borne pathogens, most commonly *Staphylococcus aureus, Candida* spp. and *Mycobacterium tuberculosis*. In contrast to abscesses that form during acute ascending pyelonephritis, hematogenously derived abscesses are usually cortical in location, are not prone to rupture into the perinephric space and are not associated with the characteristic clinical syndrome of pyelonephritis. Conversely, the typical pathogens of acute ascending pyelonephritis almost never cause renal abscesses in patients who have bacteremia arising from an extraurinary-tract focus.[1]

PREVENTION

Little is known about the prevention of pyelonephritis or renal abscess. Presumably, the same measures that can be recommended to noncompromised women who wish to reduce their risk of uncomplicated recurrent cystitis (e.g. avoiding spermicide-diaphragm contraception, use of chronic antimicrobial prophylaxis or early patient-initiated therapy for UTI symptoms) should decrease the risk of uncomplicated pyelonephritis.[18] Postmenopausal women can reduce their risk of bacteriuria with vaginal estrogen treatment; this might also prevent pyelonephritis. Complicated pyelonephritis may be prevented by removing the precipitating factor. Urinary catheters should be avoided whenever possible, used according to current guidelines when unavoidable, and removed as soon as no longer essential.[12]

Indwelling catheters coated with antimicrobial substances have been shown to prevent bacteriuria/funguria in select patients; however, their ability to prevent clinically apparent UTI or other end points of clinical relevance in a wider population is unproven.[19] Similarly, in a small study, an avirulent strain of *E. coli* was used successfully to prevent overgrowth with more pathogenic organisms in catheterized patients.[20] Further studies involving larger, more representative populations and clinically relevant end points would be useful.

Correction of urologic abnormalities (whether surgically or medically) may reduce the associated infection risk but treatment decisions must be carefully individualized and based on the expected risks and benefits of the planned intervention(s). It is not known whether improved glycemic control among patients who have diabetes reduces their increased risk of pyelonephritis but the other documented benefits of this therapy provide ample rationale for its use.

A recent Cochrane Review concluded that cranberry juice (or cranberry extract tablets) did reduce the incidence of symptomatic UTI. However, dropout rates in the reviewed studies were high, suggesting that although effective, these interventions may not be tolerated by all patients.[21] Patients desiring nonantimicrobial therapy can be advised that daily intake of cranberry juice is an option that may provide some benefit with regard to recurrent UTI, with minimal risk. Theoretically this may also decrease the risk of pyelonephritis, although this has not been studied.

Prophylactic antimicrobial therapy is useful in women who have recurrent uncomplicated cystitis[22] and in certain compromised hosts, for example renal transplant recipients in the early post-transplant period.[23] However, in many other compromised hosts antibiotic prophylaxis is without clear benefit and often selects for resistant organisms and causes drug-related adverse effects. Whether the experimental anti-UTI vaccines that are currently being evaluated will prevent cystitis or pyelonephritis is unknown.[24] At present there is no medically defined role for vaccines, receptor analogue therapy, *Lactobacillus* preparations or yoghurt in the prevention of UTI or pyelonephritis.[18]

CLINICAL FEATURES

The clinical manifestations of acute pyelonephritis vary considerably depending on the characteristics of the host and pathogen. A typical history for the classic pyelonephritis syndrome, which is most commonly observed with kidney infections in otherwise healthy young women, includes several days of progressive flank pain, malaise, fever and chills, prostration and possibly nausea and vomiting, often preceded and/or accompanied by symptoms of acute cystitis.[1]

The physical examination characteristically shows an ill-appearing, febrile, tachycardic patient, often with evidence of volume contraction. The pathognomonic physical finding of acute pyelonephritis is tenderness to palpation or percussion over one or both costovertebral angles. Mild to moderate abdominal and suprapubic tenderness are often also present.

Atypical presentations are common. Even otherwise healthy young women who have pyelonephritis may not have all of the classic symptoms or examination findings, and infants or young children, elderly or debilitated patients and patients who have underlying systemic illnesses or neurologic impairment often have an even less characteristic clinical picture.[1] Abdominal pain, headache, nonspecific constitutional symptoms, diffuse back pain, pelvic pain or respiratory complaints may predominate, obscuring the diagnosis and suggesting other processes. A deceptively benign presentation, including sometimes even the complete absence of suggestive symptoms, can mask the presence of severe renal infections in immunocompromised or sensory-impaired hosts.[1] On the other hand, even in patients who have a classic presentation for acute pyelonephritis, other entities must be considered in the differential diagnosis, including (in the appropriate setting) pelvic inflammatory disease, acute appendicitis, urolithiasis, basal pneumonia and acute pancreatitis or biliary tract disease. The decision whether to perform a pelvic examination in a woman suspected of having pyelonephritis must be individualized, taking into consideration the patient's demographic characteristics, the specifics of the history (including the sexual history) and the findings on general physical examination.

In addition to the varied combinations of symptoms and physical findings encountered in patients who have acute pyelonephritis, a wide range of severity of illness is seen. At one extreme, patients who seem healthy and have what otherwise appears clinically to be acute cystitis may demonstrate a slight elevation of body temperature or report mild malaise, suggesting early renal involvement. At the other extreme, patients may present in full-blown septic shock, with multisystem organ failure. The severity of illness has a significant influence on subsequent management, as described below.

Abscess

The initial history and physical examination usually provide few clues as to the presence of an intrarenal or perinephric abscess, although these entities should be kept in mind in high-risk patients. The presence of a palpable mass is suggestive of renal abscess but is neither a sensitive nor a specific finding. Failure of a patient who is thought to have ordinary pyelonephritis to improve substantially after treatment for 48 hours increases the likelihood of abscess sufficiently to warrant further diagnostic studies.[1]

DIAGNOSIS

Urinalysis and urine culture

Acute pyelonephritis is a clinical diagnosis based on a combination of characteristic symptoms and signs together with supporting laboratory tests.[1] The minimal laboratory evaluation needed

to make this diagnosis in the appropriate clinical setting is microscopic examination (whether by urinalysis or Gram stain) of a voided urine specimen to evaluate for the presence of pyuria, followed by quantitative urine culture. The Gram stain is also helpful by confirming the presence of bacteria in the urine (which are seen in unconcentrated urine specimens when the urine bacterial concentration is $>10^5$ cfu/ml) and by suggesting the likely bacterial type, although effective empiric treatment often can be selected without this information.[25]

In the absence of prior antimicrobial therapy, the urine culture almost always shows high concentrations ($>10^5$ cfu/ml) of one or more bacterial species. Pure growth of a single uropathogenic organism is typical of infections in noncompromised hosts, whereas polymicrobial infections are more common in compromised hosts. Lesser bacterial concentrations are occasionally encountered and in the appropriate clinical context (e.g. a patient who has typical symptoms and examination findings, plus pyuria) do not exclude the diagnosis of pyelonephritis. Antimicrobial susceptibility testing of urine isolates is essential, both to confirm that the empirically selected treatment regimen is appropriate and for guiding selection of an effective oral agent for patients treated initially with a parenteral antimicrobial regimen.[25]

Ancillary tests

Other tests may be indicated depending on the severity of illness, the range of alternative diagnoses being considered and the presence of co-morbid conditions. Pre-therapy blood cultures are commonly collected although, interestingly, bacteremia (if present) predictably clears with appropriate therapy directed toward the urinary infection, and clinical outcomes are similar regardless of the presence or absence of bacteremia. A pregnancy test is useful if the patient might be pregnant and treatment is being considered with an agent (such as an aminoglycoside or a fluoroquinolone) that might be toxic to the fetus.[17]

Imaging studies

Imaging studies are not routinely indicated for the diagnosis or management of acute pyelonephritis.[1] For patients in whom the initial diagnosis is unclear, those who fail to respond appropriately to therapy and those in whom abscess or obstruction is suspected for other reasons, CT scan can be used to clarify the anatomy and guide a mechanical intervention, in addition to helping evaluate for an alternative diagnosis.[1] Of all urinary tract imaging modalities, contrast-enhanced CT provides the best anatomic definition of inflammatory processes in the urinary tract, including sensitive detection of abscesses and differentiation of abscesses (water density) from simple inflamed tissue (tissue density; Figs 55.5–55.8). Inflamed regions of the pyelonephritic kidney appear on enhanced CT as streaky or wedge-shaped hypodense areas that fail to concentrate contrast material normally in comparison with surrounding renal tissue. Focal bulges or diffuse swelling of the entire kidney are common, as is inflammatory stranding in the perinephric fat. Terms coined by radiologists in the 1980s for these changes, such as 'focal' (or 'lobar') nephronia and 'focal' (or 'diffuse') bacterial nephritis, were often confusing to the clinician and were applied inconsistently by different radiologists. The Society of Uroradiology has defined a uniform terminology according to which all such changes are reported under the umbrella term 'acute pyelonephritis', with modifiers that describe the observed anatomic abnormalities. The extent and severity of such CT findings at the time of presentation are predictive of the clinical course, including the likelihood of bacteremia, progression to abscess formation and death.

Ultrasonography, although commonly used as an initial imaging test for patients who have a suspected focal infectious complication during pyelonephritis, is comparatively insensitive[1] and is often followed by a CT scan irrespective of the ultrasound results. Consequently, it may be best to omit this test and proceed directly to CT. (However, serial directed sonographic examinations can be used subsequently to follow the response of abscesses or hydronephrosis to therapy, without the higher cost and exposure to radiation and contrast material of repeated CT scans.) Single photon emission CT (SPECT) using technetium-99 dimercaptosuccinate (^{99}Tc-DMSA), the newest imaging modality for use in pyelonephritis, is slightly more sensitive than CT for identifying areas of inflammation within the kidney, which can be advantageous if the initial diagnosis is in question. However, it cannot distinguish between frank abscesses and inflamed but viable tissue, and so is of little help in evaluating the patient who fails to respond to therapy. Excretory urography and MRI have little role in the management even of complicated pyelonephritis.[1] Nonenhanced spiral CT is more sensitive than excretory urography in detecting urinary calculi and avoids exposing the patient to contrast material, and so may be the modality of choice (when available) if urolithiasis is a concern. Occasionally, antegrade or retrograde ureterography may be indicated, usually when stent placement or calculus removal is needed to relieve obstruction.

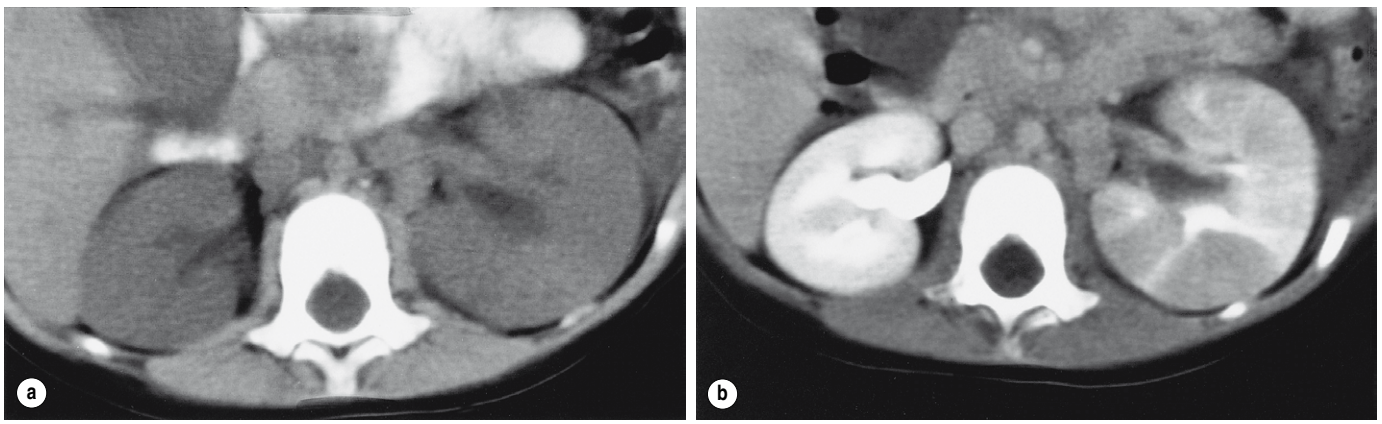


Fig. 55.5 Febrile urinary tract infection with white blood cell count of 36 000/ml (girl, 3 years). (a) Precontrast CT scan: left kidney is diffusely swollen; parenchymal attenuation is the same as that of the right kidney. (b) Postcontrast CT scan: wedge-shaped regions of hypoenhancing parenchyma in the left kidney are most pronounced in the posterior portion. Inflamed parenchyma enhances from 32 to 93 Hounsfield units (HU), whereas normal kidney enhances from 33 to 140 HU. The right kidney shows normal cortical enhancement and pronounced medullary blush. With permission from Talner *et al*.[24]

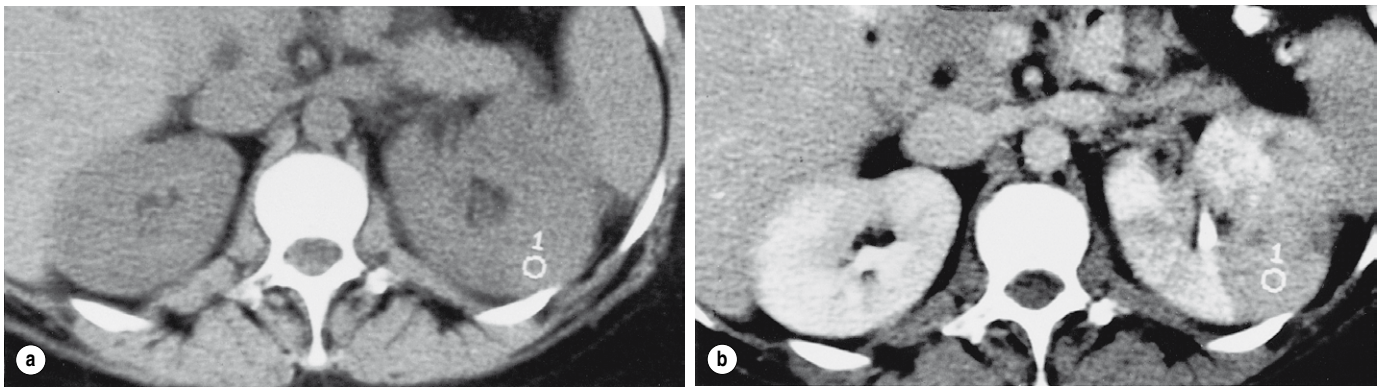


Fig. 55.6 Woman with clinical signs of acute pyelonephritis. (a) Precontrast CT scan: focal bulge present in anterolateral aspect of left kidney. Attenuation is the same as that of normal kidney parenchyma. (b) Postcontrast CT scan: rounded and streaky regions of hypoenhancing parenchyma in the left kidney are most pronounced anterolaterally. Attenuation in the region of interest (cursor) was 22 HU on precontrast scans and increased to 93 HU on postcontrast scans. Normal parenchyma increased from 25 to 130 HU. With permission from Talner *et al*.[24]

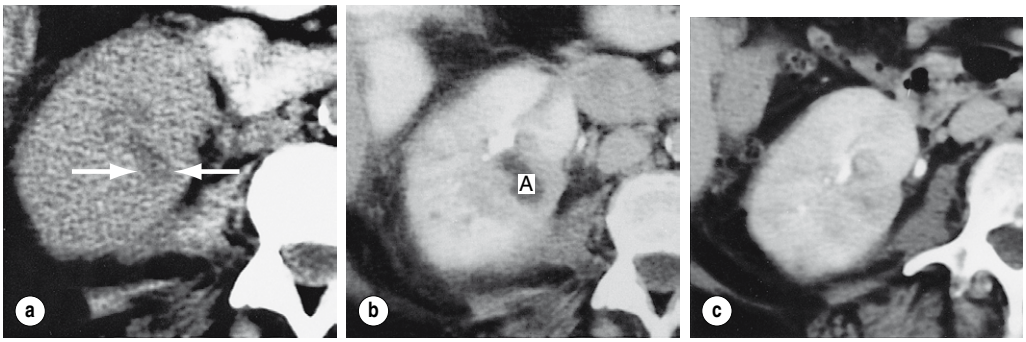


Fig. 55.7 Acute pyelonephritis with small intrarenal abscess. (a) Precontrast CT scan shows small region of low attenuation (arrows). (b) On the postcontrast CT scan, the abscess (A) fails to enhance at all. Surrounding inflamed parenchyma bulges and enhances less than adjacent normal parenchyma. (c) Follow-up CT scan obtained after prolonged antibiotic therapy. The abscess has resolved without drainage. The focal swelling is gone but the parenchyma still shows hypoenhancement. With permission from Talner *et al*.[24]

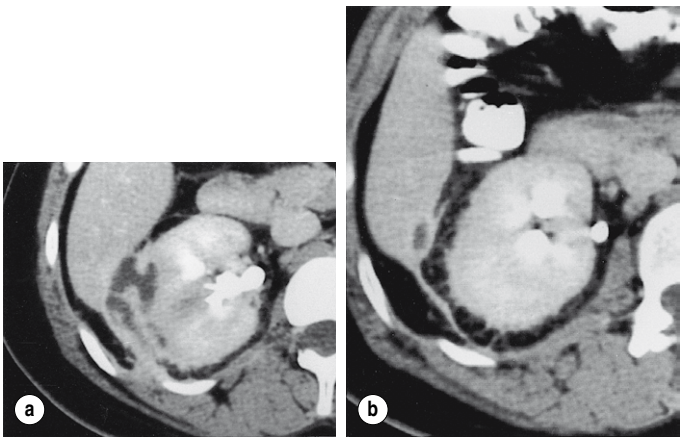


Fig. 55.8 Renal abscess perforating into subcapsular and perinephric spaces (woman, 29 years). (a) Postcontrast CT scan. Dumbbell-shaped nonenhancing region laterally in right kidney represents parenchymal abscess breaking through subcapsular and perinephric spaces. Note marked thickening of perinephric fascia posterolaterally. (b) CT section obtained caudal to (a). Note thickening of perinephric inflammation. At this level there is a small pararenal abscess pocket adjacent to the liver. With permission from Talner *et al*.[24]

MANAGEMENT

In comparison with the treatment of acute cystitis, which has been extensively studied, there have been relatively few large, high-quality treatment trials for acute pyelonephritis on which to base therapeutic recommendations.[25,26] Much of the prevailing wisdom regarding the treatment of pyelonephritis comes from tradition, anecdotal experience, extrapolation from animal models or pharmacokinetic studies, small clinical trials involving heterogeneous patient populations and *in-vitro* susceptibility test results. Nonetheless, some guidelines can be suggested for key management issues.

Inpatient versus outpatient and parenteral versus oral therapy

Traditionally, most patients who have pyelonephritis have been hospitalized and given intravenous antimicrobial therapy, at least initially. However, evidence is accumulating that oral therapy on an ambulatory basis (with or without initial parenteral treatment and observation in the emergency department or short-stay unit) is acceptable for the majority of patients who have acute pyelonephritis.[27] Outcomes with oral therapy for otherwise healthy ambulatory patients who are

clinically stable and can take medications by mouth have been similar to those obtained with sicker patients given traditional in-hospital parenteral therapy, at a considerable cost saving. Oral therapy has even been used successfully with pediatric patients and with pregnant women in whom pyelonephritis has traditionally been considered to require in-hospital management.

Thus, there is no single right answer to the question of the optimal setting for treatment. The management plan must be individualized to the patient, taking into consideration the severity of illness (including the presence of nausea or vomiting), the patient's underlying host status and reliability level, and the availability of a support system at home and a mechanism for medical follow-up (Table 55.1). Women who have uncomplicated pyelonephritis and who are only mildly ill can sometimes be treated successfully from the outset with oral therapy alone (Table 55.2). Moderately ill patients can be can be rehydrated with intravenous fluids (if needed) in the clinic or emergency department, given an initial parenteral dose of antibiotic and observed. If after several hours their condition has failed to improve sufficiently they can be admitted to the hospital for continued parenteral therapy, whereas if they are feeling better and are able to take fluids by mouth they can be discharged to home with an appropriate oral antibiotic regimen (Table 55.2), with close follow-up arranged.

Men with pyelonephritis, or women who have complicating factors other than pregnancy, have previously been thought to require admission and treatment with parenteral antibiotics. However, even within these populations there may be a subset of patients (with mild symptoms, ability to take oral medications and otherwise without signs of severe disease) who may be treated as outpatients with oral medications. Little literature specifically addressing this issue exists; therefore, most such patients probably should be admitted to the hospital initially for parenteral therapy, particularly if they are more than minimally ill.

Table 55.1 Indications for hospital admission in patients with acute pyelonephritis

Indication	Rationale
Severly ill, unstable (1)	Needs close monitoring and aggressive resuscitation
Moderate or severe host compromise	At risk of poor response to therapy, progression to 1
Suspected abscess, obstruction, stone	Needs diagnostic evaluation and intervention; at risk of progression to 1
Pregnant women*	At risk of progression to 1
Children*, men*	At risk of poor response to therapy, progression to 1
Persistent vomiting (despite antipyretic therapy and intravenous hydration)	Needs iv or im therapy†
No suitable oral therapy available	Needs iv or im therapy†
Unsuitable home situation, unreliable follow-up, or unreliable/noncompliant patient	At risk of progression to 1

*Selected patients with mild illness and suitable home situations may be treated orally as outpatients, with or without a first iv dose in the emergency department or clinic.
†Home parental therapy acceptable (where available) for mildly or moderately ill patients.

Antimicrobial regimen

Because urine culture and susceptibility testing takes several days to complete, the initial antimicrobial regimen for acute pyelonephritis is usually selected empirically (from among those agents that have suitable pharmacokinetic characteristics and a good 'track record' in pyelonephritis treatment trials) on the basis of the predicted susceptibility patterns of the expected organism(s) (Table 55.2).[1,25,26] For all patients, activity against 'ordinary' Gram-negative bacilli is essential in the empiric regimen. For patients who have complicated UTI or recent antimicrobial therapy, Gram-positive organisms and drug-resistant Gram-negative organisms must also be anticipated.

Suitable initial regimens are shown in Table 55.2, which emphasizes aminoglycosides, fluoroquinolones, third-generation cephalosporins and β-lactam–β-lactamase inhibitor combination agents for parenteral use, and trimethoprim–sulfamethoxazole (TMP–SMX), fluoroquinolones and amoxicillin–clavulanate (co-amoxiclav) for oral use. Because of the high prevalence among uropathogens of resistance to ampicillin, other penicillins and first- or second-generation cephalosporins – as well as these agents' adverse pharmacokinetic properties and inconsistent performance in clinical trials[25,26] – these drugs should be avoided as empiric monotherapy for even mild or uncomplicated pyelonephritis. Emerging resistance to TMP–SMX among uropathogens has diminished this drug's utility in the USA for empiric oral therapy of UTIs, particularly pyelonephritis, since *in-vitro* resistance is associated with clinical failure rates of more than 50%.[28] Unfortunately, resistance to fluoroquinolones, which have traditionally been regarded as the 'fall-back' option for UTIs due to TMP–SMX-resistant Gram-negative uropathogens, is already quite prevalent in some places, and resistance to extended-spectrum cephalosporins among community-source *E. coli* also is emerging globally. How this will affect future recommendations for empiric therapy of pyelonephritis remains to be seen. Whether oral third-generation cephalosporins should have a role in the empiric therapy of pyelonephritis in outpatients has not been adequately studied.

Antimicrobial regimens can be simplified by using a single agent (there being little rationale for combination therapy except in patients who are thought to have both Gram-positive and Gram-negative pathogens)[29] and by using twice-daily dosing with ciprofloxacin, TMP–MX and co-amoxiclav, or once-daily dosing with ceftriaxone, levofloxacin, ertapenem and the aminoglycosides.

Conversion to oral therapy

Patients initially admitted to the hospital for intravenous therapy have traditionally been continued on parenteral therapy until susceptibility results are known. They are then placed on an oral agent selected on the basis of the susceptibility pattern of the urine organism, and are observed in the hospital for an additional 1–2 days to evaluate the success of oral therapy (Table 55.3). This approach leads to unnecessarily prolonged hospital stays in many patients. Conversion to oral therapy can be done safely as soon as the initial indications for parenteral therapy have resolved, as evidenced by the success of oral therapy for mildly ill ambulatory patients who have pyelonephritis. When the hospitalized patient is clinically ready for oral therapy before susceptibility results are available, an oral regimen can be selected empirically, much as is done in the emergency department for patients treated with an oral agent from the outset.[25,27] In most locales in the USA, the fluoroquinolones are predictably active against community-acquired *E. coli*. Thus, despite being slightly more expensive than TMP–SMX (which might be the preferred agent for a known susceptible organism), fluoroquinolones can yield a tremendous cost saving if they permit patients to be discharged sooner.

The practice of observing patients who have pyelonephritis in the hospital for 24 hours or longer on oral therapy before discharge is without empiric support. When examined retrospectively, this approach was found to detect relapse in only 1% of patients and intolerance of the new oral agent in only 4%.[30] Thus, patients can usually be safely

Table 55.2 Suggested empiric initial treatment regimens for acute pyelonephritis*

Modifying circumstances	Treatment setting	Empiric treatment options
Uncomplicated pyelonephritis		
Mild to moderate illness, no nausea or vomiting	Outpatient therapy acceptable	Oral† fluoroquinolone (not in children), TMP–SMX caution§ or amoxicillin–clavulanate (co-amoxiclav) caution§ for 7–14 days (co-amoxiclav preferred if Gram-positive cocci present)
Severe illness or possible urosepsis	Hospitalization required	Parenteral‡ fluoroquinolone (not in children), third-generation cephalosporin, gentamicin (± ampicillin or piperacillin), piperacillin–tazobactam, aztreonam or carbapenem, TMP–SMX caution§ until patient is better; then oral† agent (see above) to complete 14 days of therapy (Initial regimen should include ampicillin or piperacillin if Gram-positive cocci are present)
Complicated pyelonephritis		
Pregnancy, mild illness	Outpatient therapy acceptable	Oral† co-amoxiclav, cephalosporin or TMP–SMX caution§ for 10–14 days (co-amoxiclav preferred if Gram-positive cocci present)
Pregnancy with moderate to severe illness	Hospitalization required	Parenteral‡ third-generation cephalosporin, gentamicin (± ampicillin or piperacillin) caution,§ piperacillin–tazobactam, carbapenem caution§ or TMP–SMX caution§ until patient is better; then oral† amoxicillin, co-amoxiclav, a cephalosporin or TMP–SMX caution§ for 14 days (Initial regimen should include ampicillin or piperacillin if pre-therapy Gram stain shows Gram-positive cocci or no organisms, or is not done)
Not pregnant, mild illness, no nausea or vomiting	Outpatient therapy acceptable	Oral† fluoroquinolone (not in children) for 10–14 days
Not pregnant with moderate to severe illness or possible urosepsis	Hospitalization required; imaging studies and urologic consultation often needed	Parenteral‡ gentamicin (± ampicillin or piperacillin), fluoroquinolone, third-generation cephalosporin, aztreonam, ticarcillin–clavulanate, piperacillin–tazobactam or carbapenem until patient is better; then oral† agent (see above) for 14–21 days (Initial regimen should include ampicillin, piperacillin or a carbapenem if pre-therapy Gram stain shows Gram-positive cocci or no organisms, or is not done)

*'Uncomplicated' is usually limited to noncompromised, nonpregnant adult women but can include carefully selected men and children who lack compromising conditions and are only mildly ill.
†**Oral regimens**: TMP–SMX, 160 mg + 800 mg q12h; norfloxacin, 400 mg q12h; ciprofloxacin, 500 mg q12h; ofloxacin 200–300 mg q12h; lomefloxacin, 400 mg q24h; levofloxacin, 500 mg q24h; amoxicillin, 500 mg q8h; amoxicillin–clavulanate (co-amoxiclav), 850 mg q12h or 500 mg q8h; cefpodoxime proxetil, 200 mg q12h.
‡**Parenteral regimens**: TMP–SMX, 160 mg + 800 mg q12h; ciprofloxacin, 200–400 mg q12h; ofloxacin, 200–400 mg q12h; levofloxacin, 500 mg q24h; gentamicin, 5 mg/kg q24h; ceftriaxone, 1–2 g q24h; ampicillin, mezlocillin or piperacillin, 1–2 g q6h; imipenem–cilastatin, 250–500 mg q8h–q6h; meropenem, 1 g q8h; ertapenem, 1 g q24h; doripenem, 500 mg q8h; ampicillin–sulbactam, 1.5–3 g q6h; ticarcillin–clavulanate, 3.2 g q8h–q6h; piperacillin–tazobactam, 3.375 g q8h–q6h; aztreonam, 1 g q12h–q8h.
§**Cautions**: fluoroquinolones (norfloxacin, ciprofloxacin, ofloxacin, levofloxacin and lomefloxacin) should not be used in pregnancy or in young children. TMP–SMX, although not approved for use in pregnancy, has been widely used (but should be avoided in the first trimester and near term). TMP–SMX and co-amoxiclav should be used only if susceptibility of urine organism is known or is highly (>95%) likely. Gentamicin should be used with caution in pregnancy because of its possible toxicity to eighth-nerve development in the fetus. The fluoroquinolones norfloxacin and lomefloxacin can only be administered po; ciprofloxacin, levofloxacin and ofloxacin can be administered iv or po. Imipenem/cilastatin is FDA class C in pregnancy, all other carbapenems (meropenem, ertapenem and doripenem) are FDA class B.
Adapted with permission from Stamm & Hooton.[26]

Table 55.3 Criteria for conversion to oral therapy

Patient no longer severely ill or unstable
Patient taking fluids by mouth; no vomiting; adequate gut function
Suitable oral agent available:
- documented or predicted activity against causative organism(s)
- highly bioavailable
- good 'track record' in UTI therapy
- no contraindication to use (i.e. no history of previous adverse reaction), no drug–drug interactions, no fetal toxicity (pregnant women), no age-related toxicities (e.g. fluoroquinolones in children)

discharged once they have demonstrated tolerance of the first dose of an appropriate oral agent, whether the drug is selected empirically or on the basis of known susceptibility results.

Expected clinical course

Nearly all patients who have pyelonephritis and who will ultimately be cured by antimicrobial therapy alone experience substantial clinical improvement within the first 2 days of therapy, sometimes even after the first liter of intravenous rehydration fluid and before receiving any antimicrobial agent. Patients commonly continue to have fever and flank pain for several days on effective therapy, but these manifestations should begin to wane and there should be improvement in the patient's energy level, appetite and sense of well-being.

If after 48 hours there is no improvement in any of these parameters, aggressive re-evaluation is required.[1] Possibilities to be considered include a mistaken diagnosis, a mismatch between the urine organism and the selected antimicrobial regimen and an anatomic complication such as obstruction or abscess. A directed history and physical examination are indicated, as is repeated laboratory testing (including blood cultures and chemistries, urinalysis and urine culture plus Gram stain) and urinary tract imaging studies, beginning with enhanced abdominal CT. In some patients this evaluation will reveal a focal process in need of an invasive procedure, such as drainage of an abscess (see Fig. 55.8) or an obstructed collecting system; in some patients, continued medical therapy (with or without adjustment) will suffice (see Fig. 55.7). Consultation with an infectious diseases specialist and/or a urologic surgeon or interventional radiologist can be extremely helpful in problematic cases to ensure that all relevant options are considered and the appropriate procedures performed.

Complications

Supportive care for patients who develop septic shock, ARDS and multisystem organ failure during pyelonephritis, which is not specific to pyelonephritis, is discussed in Chapter 44. When infection is present, obstruction to urine flow (e.g. by a stone or tumor) must be relieved, either by removal of the obstruction or by provision of alternative drainage. When possible, removal of urinary calculi from patients who have pyelonephritis is probably best delayed until the bacterial load can be reduced and the patient stabilized with medical therapy. Gas-forming UTIs have traditionally been managed surgically in most instances, often with nephrectomy in cases of emphysematous pyelonephritis (see Fig. 55.3). However, reports of successful medical therapy of gas abscesses[15] and emphysematous pyelonephritis indicate that even in these extreme situations therapy can be individualized.

Intrarenal (see Fig. 55.7) and perinephric (see Fig. 55.8) abscesses have also traditionally been managed with combined medical and surgical therapy. Recent experience with closed (catheter-assisted) drainage or medical therapy alone suggests the possibility of alternative approaches in this setting as well.[31] Small abscesses, especially those occurring in otherwise intact hosts, are most likely to respond to medical therapy, whereas large collections, particularly in compromised hosts or in patients who have severe illness, are likely to require drainage. The cost and morbidity of a drainage procedure must be weighed against the cost and morbidity of the protracted antibiotic therapy that is usually required when abscesses are treated with antibiotics alone. If an abscess is to be drained, the optimal method (open versus closed) depends in part on the anatomy, the host and local expertise. Perinephric abscesses (see Fig. 55.8) have been described as requiring a more aggressive interventional approach than intrarenal abscesses but published experience suggests that drainage is not always needed even here.

Duration of therapy

The optimal duration of therapy for acute pyelonephritis, unlike that for acute cystitis, is largely undefined and remains a source of controversy.[29] As with other aspects of the management of pyelonephritis, because of the highly variable nature of the illness and the host substrate, it is probably best to tailor duration of therapy to the individual patient.

Clinical trial data demonstrate that 14 days of a traditional sequential regimen that includes an intravenous aminoglycoside initially, followed by oral TMP–SMX or ampicillin, eliminates the initial infection in 100% of women who have moderate or severe uncomplicated pyelonephritis, with no relapses at the 6-week follow-up visit.[32] Thus, courses of therapy longer than 14 days should be unnecessary when similarly potent regimens are used in comparable hosts. In other trials, approximately 90% of patients who had uncomplicated pyelonephritis and were treated for only 5 days with aminoglycosides, third-generation cephalosporins or fluoroquinolones were cured,[29] although some of the 10% failure rate was attributable to relapses with the initial pathogen.

Whether there is a real or clinically meaningful difference in success rates between 5–7 days and 10–14 days of therapy for uncomplicated pyelonephritis is unknown. Of note, a multicenter randomized clinical trial performed in the mid-1990s demonstrated that 7 days of oral ciprofloxacin (with or without an initial intravenous dose) was 96–99% effective for uncomplicated pyelonephritis of mild to moderate severity in ambulatory women. Similarly, 14 days of oral TMP–SMX (with or without an initial intravenous dose of ceftriaxone) was 92–96% effective if the urine organism was susceptible to TMP–SMX. However, these favorable findings are not necessarily applicable to women who have more severe uncomplicated infections or to patients who have complicating factors, for whom longer treatment duration may be preferable. Duration of therapy for abscesses must be individualized, taking into consideration underlying host status, the nature of the abscess, adequacy of drainage (if undertaken) and response to therapy (both clinical and as revealed by serial imaging studies).

Follow-up

Routine repeat urine cultures are commonly performed during therapy for pyelonephritis to confirm sterilization of the urine but may add little beyond what is apparent from clinical evaluation and possibly from inspection of the urine for pyuria.[33] It is prudent to confirm at least by telephone that patients who are sent out from the emergency department with oral therapy are improving as expected. Whether routine post-therapy clinic visits, urine cultures and urinalyses contribute to favorable outcomes has not been studied. However, as it has been argued that in the setting of uncomplicated acute cystitis these measures are unnecessary,[34] it is possible that the same may be true with pyelonephritis, at least for uncomplicated cases in seemingly reliable and responsible patients. Post-therapy evaluations still are advisable in children, pregnant women[17] and probably also in other compromised hosts.

Urologic evaluation for predisposing conditions

In addition to the management of the acute pyelonephritis episode, in selected patients it is worth searching for an underlying urologic abnormality, as the surgical correction of such an abnormality might prevent future infections. The cost and morbidity of such a search must be weighed against the likelihood of finding a correctable abnormality, the morbidity of the possible corrective procedure itself and the infectious morbidity that can be averted by a successful procedure. In the absence of firm data, opinions differ as to the indications for imaging studies and corrective surgery after pyelonephritis.[34] One approach is to investigate all children and men who develop pyelonephritis, as they are the most likely to have an important correctable abnormality. Women probably should be studied if they have a second (same-strain) relapse of pyelonephritis despite an extended course of appropriate antimicrobial therapy for a first relapse. Whether women who have multiple episodes of pyelonephritis caused by diverse organisms will benefit from urologic investigation is unknown.

SUMMARY

Acute pyelonephritis is a diverse entity that challenges the clinician to intervene sufficiently but not excessively, and for which the management approach must be tailored to the individual patient. New developments in the field – such as the use of at-home oral therapy, shorter treatment courses, single-daily-dose intravenous aminoglycoside,

ertapenem or ceftriaxone therapy and early hospital discharge – provide opportunities for cost savings and enhanced patient convenience. Alertness is required to anticipate and detect complications in high-risk patients or in those who fail to respond to treatment as expected. Intrarenal and perinephric abscesses, gas-forming renal infections and infections superimposed on urinary obstruction are potentially lethal processes that require aggressive therapy, often including mechanical intervention.

REFERENCES



References for this chapter can be found online at http://www.expertconsult.com

Chapter 56

Lindsay E Nicolle

Complicated urinary infection, including postsurgical and catheter-related infections

INTRODUCTION

This chapter discusses urinary tract infections (UTIs) occurring in individuals with abnormalities of the genitourinary tract, designated 'complicated UTI'. This includes UTIs following urologic surgery and infections associated with urinary catheterization, i.e. intermittent catheterization and both short-term (<30 days) and long-term (>30 days) indwelling catheters. Urinary infection in pregnant women is not addressed.

EPIDEMIOLOGY

Urinary tract infection is the most common bacterial infection in adults. In the setting of structural or functional abnormalities of the genitourinary tract or after urologic interventions, the frequency of UTI may be exceptionally high (Table 56.1).

Infection incidence on a population basis has not been reported. In a review of hospitalization for acute pyelonephritis in Manitoba for 1989–92,[7] the rate of admissions was 11 per 10 000 population for women and 3.3 per 10 000 for men. Of these, 34% of patients with pyelonephritis admitted to two tertiary care hospitals had complicating genitourinary factors. For patients admitted to hospital with a diagnosis of UTI other than pyelonephritis, 84% of subjects at one institution and 36% at a second had complicating factors.

Table 56.1 Infection rates after genitourinary surgery, extracorporeal shock wave lithotripsy or catheterization in the absence of antimicrobial prophylaxis

Procedure	Proportion infected postprocedure
Genitourinary surgery	
Transurethral prostatectomy[1]	6–64%
Transurethral procedure with instrumentation for stone extraction[2]	25%
Extracorporeal shock wave lithotripsy (ESWL)[3]	
Negative urine culture before ESWL	1.5%
Positive urine culture before ESWL	21%
Sepsis	4.5%
Catheterization	
Urodynamic studies[4]	1.5–36%
Indwelling catheter[5]	3–7%/day
Intermittent catheterization[6]	4.06/100 patient days

The urinary tract is also the most common source of infection in elderly individuals presenting to the emergency department with bacteremia; it accounts for about one-third of all bacteremic episodes in this population.[8] Most of these bacteremic individuals have abnormalities of the urinary tract, primarily obstructing lesions and indwelling catheters.

Urinary tract infection is the most frequent hospital-acquired infection. Urinary infection accounts for 40% of all nosocomial infections, occurring at a rate of approximately 2 per 100 patient discharges.[9] Hospital-acquired UTI is associated with indwelling catheters in at least 80% of episodes, with acquisition of infection by 3–5% of exposed subjects per day of catheterization. Most catheter-associated infections are asymptomatic, but symptomatic infection, including bacteremia, sepsis syndrome and death, may occur. The catheterized urinary tract is the most frequent source of nosocomial Gram-negative rod bacteremia.[5] While bacteremia occurs in only 2–4% of patients who have catheter-acquired UTI, the high frequency of indwelling catheter use means that the absolute number of episodes of bacteremia secondary to catheter-acquired UTI is high.

Approximately 5% of individuals resident in long-term care facilities in North America have a chronic indwelling catheter. The prevalence of bacteriuria in these subjects is 100%. The urinary tract of residents with chronic indwelling catheters is the source of over half of all bacteremic episodes in these facilities. For individuals who have neurogenic bladders managed by intermittent catheterization, the reported rates of infection are 4.06 per 100 patient days[6] or 17.2 per patient year.[10]

PATHOGENESIS

The normal genitourinary tract, apart from the distal urethra, is sterile. The usual colonizing flora of the distal urethra – *Staphylococcus epidermidis*, diphtheroids, streptococci and certain anaerobes – are rarely uropathogens. The sterility of the urine and genitourinary tract is primarily maintained through the flushing action of normal urine voiding. Other variables that may contribute include the concentration and chemical composition of the urine, the bladder mucous layer, Tamm–Horsfall protein excreted from the kidneys, and the innate immune system. These additional factors are probably less important in the development of complicated UTI, where obstruction to normal urine flow overwhelms all other factors in promoting infection.

In complicated UTI, including postsurgical infection and catheter-related infections, the major factor contributing to the initiation and persistence of bacteriuria is an impaired ability to clear organisms from the urinary tract. This may be attributable to:

- obstruction of urine flow with a pool of urine remaining in the urinary tract after voiding;
- the presence of a protected environment, such as an infection stone or a bacterial biofilm on a catheter, from which organisms cannot be eradicated by usual antimicrobial therapy; or

- increased introduction of organisms into the bladder through interventions such as intermittent catheterization.

A wide variety of genitourinary abnormalities are associated with complicated UTI (Table 56.2). These are congenital or acquired functional, structural or metabolic abnormalities. The abnormality may be transient – for instance, the presence of a noninfected stone, a cystoscopy procedure or short-term catheterization. In this situation, once the abnormality is corrected there is no longer an increased risk of UTI. If the abnormality cannot be corrected, as in a patient who has an ileal conduit or with a neurogenic bladder maintained on intermittent catheterization, there is a continued risk of recurrent UTI.

Where UTI occurs in a patient who has an indwelling urethral catheter, the organisms may have gained access to the bladder by several routes:

- carried into the bladder from the periurethral flora or on contaminated equipment with initial introduction of the catheter;
- ascending the mucous sheath from the periurethral area on the outside of the catheter; or
- intraluminally by ascension up the catheter.

The intraluminal route appears to be more important in men than in women, in whom a shorter urethra probably facilitates extraluminal ascent.

When infection occurs in the presence of a foreign body in the genitourinary tract, such as a ureteral stent, nephrostomy tube or indwelling catheter, a bacterial biofilm forms on the inert material.[11] A biofilm is an adherent colony of organisms, with individual organisms encased in copious extracellular matrix which also incorporates urine constituents such as calcium, magnesium and Tamm–Horsfall protein. This biofilm provides a relatively protected environment for the bacteria by interfering with the diffusion of both antibiotics and host defenses, thus contributing to persistent and relapsing infection.

Urinary tract infection may also be acquired in urologic practice when organisms are transmitted between patients on inappropriately cleaned diagnostic or therapeutic equipment.[12] In particular, contamination is a risk where instruments are not appropriately changed or cleaned between patients and where fluid is left standing for prolonged periods at room temperature. Shared use of urinometers or urine collecting devices among patients with indwelling catheters has also been repeatedly identified as a cause of nosocomial outbreaks of infection.[13]

MICROBIOLOGY

The spectrum of micro-organisms isolated from individuals who have complicated UTI is more varied than observed in patients who have uncomplicated UTI.[14] Table 56.3 summarizes the organisms isolated in several studies of varied populations with complicated UTI. Although *Escherichia coli* remains an important infecting organism, the frequency with which it is isolated is substantially lower than that reported for acute uncomplicated UTI. *Escherichia coli* has unique virulence characteristics which promote symptomatic infection in the person with a normal genitourinary tract (see Chapter 53). Where complete urine voiding is impaired, organisms without unique urovirulence properties may also become important uropathogens. Therefore, there is a lower prevalence of genotypic or phenotypic expression of virulence factors by *E. coli* isolated from individuals who have complicated genitourinary infection than by *E. coli* isolated from acute uncomplicated UTI.[19]

The distribution of other infecting organisms is determined by factors such as:

- whether organisms are isolated from initial or recurrent infection;
- whether acquisition is nosocomial or community-acquired;
- whether there is an indwelling urinary device; and
- previous antimicrobial exposure.

Common organisms isolated include Enterobacteriaceae such as *Klebsiella*, *Citrobacter*, *Serratia*, *Proteus* and *Providencia* spp., other Gram-negative organisms such as *Pseudomonas aeruginosa* and other nonfermenters, and Gram-positive organisms such as *Enterococcus faecalis* and group B streptococci. Coagulase-negative staphylococci are frequently isolated, although rarely in symptomatic infection, and their pathogenicity is seldom clear. Yeasts, primarily *Candida* spp., may be isolated, usually in individuals who have prolonged or repeated courses of antimicrobial drugs.[20] Anaerobic organisms are isolated rarely, and then in the setting of highly complex urologic abnormalities and abscess formation in the urinary tract.

The urease-producing organisms, principally *Proteus mirabilis*, *Providencia stuartii* and *Morganella morganii*, are important pathogens. Rarely, more unusual urease-producing organisms such as *Ureaplasma urealyticum* or *Corynebacterium urealyticum* D2 may be isolated. These organisms maintain an alkaline environment leading to the formation of struvite stones or catheter encrustation, and promoting persistence of infection.

In addition to the much wider variety of infecting species in complicated UTI compared with uncomplicated UTI, there is also often increased antimicrobial resistance among the infecting bacteria. Increased resistance is promoted by:

Table 56.2 Genitourinary abnormalities associated with an increased frequency of urinary tract infection

Type of lesion	Examples
Obstructing lesion	Tumor, stricture, urolithiasis, prostatic hypertrophy, diverticulum, pelvicalyceal junction obstruction, congenital abnormality, renal cysts
Foreign body	Indwelling catheter, ureteric stent, nephrostomy tube
Functional abnormality	Neurogenic bladder, vesicoureteral reflux, cystocele
Metabolic illness	Diabetes mellitus, medullary sponge kidney, post renal transplantation
Urinary instrumentation and urologic surgery	Prostatectomy, cystoscopy
Urinary diversion	Ileal conduit

Table 56.3 Bacteria isolated in complicated urinary tract infection

Organism	Proportion of total organisms isolated* (%)
Gram-negative organisms	
Escherichia coli	21–54
Klebsiella pneumoniae	1.9–17
Citrobacter spp.	4.7–6.1
Enterobacter spp.	1.9–10
Proteus mirabilis	0.9–10
Providencia spp.	1.9
Pseudomonas aeruginosa	2.0–19
Other Gram-negative organisms	6.1–23
Gram-positive organisms	
Enterococci	6.1–23
Coagulase-negative staphylococci	1.3–3.7
Staphylococci aureus	0.9–2.0
Group B streptococci	1.2–3.5
Other Gram-positive organisms	1.9
Yeast	0–7

* Data on frequency of isolation of different bacterial species derived from references[15–18].

- repeated antimicrobial courses for recurrent UTI; and
- the high frequency of nosocomial acquisition.

Some of the infecting organisms, such as *P. aeruginosa*, are intrinsically more resistant to antimicrobials, while others such as *E. coli* or *K. pneumoniae* have acquired genetic elements for resistance.

PREVENTION

General measures

Urinary tract infection in the abnormal genitourinary tract occurs because of the presence of an underlying abnormality or intervention that breaches normal defenses and allows the introduction and persistence of micro-organisms. Therefore, the most important interventions to prevent UTI are:

- to identify and, wherever possible, correct underlying abnormalities; and
- to avoid nonessential interventional procedures.

It is important to follow appropriate aseptic technique for interventional procedures such as cystoscopy or urodynamic studies, as well as for operative procedures. All fluids used in urologic procedures must be handled in a manner that ensures sterility. In particular, equipment must be disassembled after a procedure and reassembled using sterile components before the next procedure, and aseptic technique must be maintained.[12] Institutions should establish and maintain appropriate infection surveillance programs to ensure that endemic infection rates are known and to facilitate early identification of potential outbreaks.[5]

Catheter-acquired infection

The single most important practice in preventing catheter-acquired UTI is not to use an indwelling catheter unless clearly indicated and, if a catheter must be used, to limit the duration of catheterization to as short a period as possible.[5] When a catheter is necessary, a closed urinary drainage system must be maintained. In addition, practicing aseptic technique at insertion is important (Table 56.4).

Patients who have indwelling catheters and are receiving antimicrobial therapy have a decreased incidence of infection acquisition during the initial 4 days of catheterization compared with patients who do not receive antimicrobials. After the first 4 days, the infection rates are similar, but patients receiving antimicrobials develop infection with more resistant organisms. Therefore, antimicrobial therapy to prevent infection when an indwelling catheter is *in situ* is currently not recommended.

Table 56.4 Interventions to prevent catheter-acquired infection

Proven effective	Avoid use of catheter Limit duration of catheterization Maintain closed drainage system Antibiotics first 4 days (not recommended)
Possibly effective	Aseptic insertion Antibiotics last 48 h of catheterization Antimicrobial decontamination of gut
Proven not effective	Daily meatal care with soap or antiseptic Disinfectant (formaldehyde, chlorhexidine, hydrogen peroxide) in drainage bag Antimicrobial-coated catheters Continuous antibiotic or antiseptic irrigation

Data from Pratt *et al*.[5]

Repeated evaluations of interventions using topical or local anti-infectives to prevent infection associated with indwelling catheters have consistently documented no benefit.[5] For instance, daily perineal cleansing with either soap or disinfectant does not decrease and may increase the rate of infection. Other measures that do not decrease the frequency of infection are the addition of disinfectants such as povidone–iodine or chlorhexidine to the drainage bag and routine irrigation with normal saline or antiseptics. The use of catheters impregnated with antimicrobial agents such as silver has been controversial. Some of these devices may decrease the occurrence of bacteriuria, but they have not been shown to decrease symptomatic UTI or other morbidity.[21] It is, in fact, remarkable how consistently local anti-infective measures have failed to modify the occurrence of catheter-acquired infection.

The use of antimicrobials for preventing infection in patients who have spinal cord injury and who are maintained on intermittent catheterization has also been controversial. Clinical trials report antimicrobial prophylaxis may prevent infection in the early postinjury months, but at the cost of increased antimicrobial resistance when infection occurs. Therefore, prophylactic antimicrobials are currently not recommended for these patients.[22] For spinal cord injured patients, cranberry juice has not been shown to prevent infection.[23] However, an educational program which included written educational material, self-assessment, telephone follow-up, review of catheter technique by an experienced nurse, and physician access for urinary infection care was associated with a sustained decreased frequency of infection.[24]

Indications for long-term catheterization include chronic obstruction, to assist with healing of decubitus ulcers and, occasionally, patient preference. Individuals with chronic indwelling catheters are consistently bacteriuric, usually with between two and five organisms. The incidence rate of acquisition of infection with a new organism is similar to that of short-term indwelling catheters, i.e. 3–5% per day. While it is recommended that procedures for catheter management should be similar for both long-term and short-term catheters, there are no studies reported for long-term catheters which document any specific practices that are effective in decreasing the risk of infection. In particular, systemic antimicrobial therapy does not decrease the risk of symptomatic infection, but does increase the likelihood of infection with resistant organisms.[25] It is, however, likely that avoiding trauma to the catheter and early identification of catheter obstruction will decrease the incidence of symptomatic infection for patients with long-term catheters.

Postoperative infection

The perioperative use of antimicrobials encompasses two issues:

- treatment of pre-existing bacteriuria to prevent the complications of invasive infection; and
- prophylaxis to prevent postoperative infection in individuals without positive pre-intervention urine cultures

Treatment of bacteriuria preoperatively in individuals undergoing genitourinary interventions is indicated to prevent postoperative bacteremia and sepsis. Postoperative sepsis was reduced from 6.2% to zero when appropriate antimicrobials were given before operation to patients whose urine was infected preoperatively.[26] Antimicrobial therapy should be selected on the basis of the infecting organism and antimicrobial susceptibilities, and initiated at least 1 hour before surgery.

There are some indications for the use of true prophylactic therapy in urologic surgery[27] (Table 56.5) Most authorities now suggest that antimicrobial prophylaxis is appropriate for transurethral prostatectomy even if the pre-procedure urine culture is negative,[27,28] although this recommendation was controversial in the past.[30] Renal transplant patients receive 6 months' postoperative prophylaxis with trimethoprim–sulfamethoxazole to prevent pneumocystis pneumonia and other infections.[29] This regimen also effectively prevents both asymptomatic and symptomatic UTI.

There is no generally accepted 'standard' antimicrobial regimen for prophylaxis.[27] Many different antimicrobials have been used. Generally, an aminoglycoside, with or without a cephalosporin, or a fluoroquinolone is used. Studies have documented the efficacy

Table 56.5 Prophylactic antimicrobial therapy in genitourinary surgery to prevent postoperative urinary tract infection

Procedure	Regimen	Infection rate with prophylaxis (%)	Infection rate without prophylaxis (%)
Transurethral instrumentation			
UTI with stone extraction[2]	Cefotaxime 1 g iv, one dose	8.5	25
Sepsis[26]	Cefotaxime 1 g iv, one dose	0	6.2
Prostatectomy			
Sterile urine preoperatively[28]	Various	3–22	6–70
Preoperative bacteriuria[28]	Various	35–41	65–92
Transplantation			
Renal transplantation[29]	Trimethoprim–sulfamethoxazole 160–800 mg q24h for 4 months	8	35

of second- and third-generation cephalosporins, including cefotaxime, ceftriaxone, cefotetan, cefoxitin and ceftazidime, as well as fluoroquinolones. It is not clear, however, that these agents are superior to less costly alternatives such as aminoglycosides and trimethoprim–sulfamethoxazole.

The recommended regimen is one dose 1–2 hours preoperatively.[27] The appropriate duration of antimicrobial therapy has not been defined. If an indwelling catheter remains *in situ* postoperatively some authors recommend continuation of antibiotics until the catheter is removed. However, recent studies suggest that, at least for some agents, a single dose is as effective as multidose therapy.[31,32] The shortest effective duration of therapy is preferred to limit cost and adverse effects, and the emergence of antimicrobial-resistant organisms.

CLINICAL FEATURES

The clinical presentations of complicated UTI are diverse, and vary along a spectrum from asymptomatic bacteriuria without a measurable host response to septic shock and death. Infection may be localized to the bladder or may involve the kidney and, in men, the prostate. In many patients with persistent abnormalities and recurrent infection, asymptomatic bacteriuria is the most common presentation. When symptomatic infection occurs, the clinical features are usually consistent with lower UTI including frequency, suprapubic discomfort, dysuria and urgency.

With renal infection the characteristic presentation of pyelonephritis, including fever and costovertebral angle tenderness, may occur. Urinary obstruction or trauma to the genitourinary mucosa may predispose to bacteremia and more severe infection.

Presenting symptoms may not, however, be straightforward. In males, recurrent bladder infection may be secondary to chronic bacterial prostatitis. Infection may also manifest as lower UTI irritative symptoms alone despite the presence of upper tract or renal infection. In individuals with uncomplicated UTI, fever is a reliable localizing symptom for upper UTI. This is not the case for complicated and postsurgical infection, or infection in the presence of an indwelling catheter. In these cases trauma to the bladder mucosa may lead to invasive infection and fever associated with lower UTI alone. In most cases, however, treatment decisions will not depend upon knowledge of the site of infection within the urinary tract.

Presentations in unique patient groups

Certain patient groups may demonstrate some unique clinical presentations of infection. For patients who have a spinal cord injury with a neurogenic bladder, the usual irritative lower tract symptoms may be absent because of altered sensation associated with the neurologic injury.[22] Signs and symptoms suggestive of UTI, in addition to fever, kidney pain or tenderness and bladder discomfort, may include a new onset or increase in urinary incontinence, increased sweating, increased spasticity and a general sense of being unwell. Autonomic dysreflexia may occur in patients with spinal cord injury at high thoracic or cervical levels. Patients with multiple sclerosis may present with increased fatigue and deterioration in neurologic function.

Infection may occasionally present as fever without any localizing genitourinary findings, usually in individuals who have indwelling catheters or obstruction. For patients without indwelling catheters, a diagnosis of UTI in the febrile patient who has a positive urine culture and no localizing findings must be assessed critically. In populations with a high prevalence of asymptomatic bacteriuria, the large majority of such episodes are not attributable to UTI.[33]

In patients who have undergone renal transplantation, symptoms and signs may be absent or mild in the early post-transplant period, despite the presence of bacteremia. This lack of symptoms may be due to immunosuppressive therapy or uremia.

Occasionally, symptoms of the underlying genitourinary abnormality may be prominent. For instance, if a UTI occurs in the setting of a ureteral stone, symptoms of renal colic may predominate. The bacteriuric patient with diabetes mellitus who develops papillary necrosis may also present with symptoms of renal colic. Infections with Enterobacteriaceae (usually *E. coli* and *K. pneumoniae*) in patients with diabetes mellitus and hyperglycemia with glycosuria may present as emphysematous cystitis or pyelonephritis. Acute bacterial prostatitis usually presents with prominent symptoms of urethral obstruction including retention.

Presentations with unique infecting organisms

Infection by selected organisms may also produce a unique clinical presentation. Catheter obstruction is associated with urease-producing organisms, the most common of which is *P. mirabilis*. *Corynebacterium urealyticum* infection is associated with the clinical syndrome of encrusted cystitis. This is encrustation of the bladder wall by struvite crystals precipitated by the alkaline urine generated by the urease of the organism. If a persistent fungal infection is identified, there may be a fungus ball in the bladder or kidney associated with obstruction.

Recurrent infection after antimicrobial therapy

Early recurrent infection after antimicrobial therapy is characteristic of individuals with persistent genitourinary abnormalities. Infection may be symptomatic or asymptomatic and may represent:

- relapse with recurrence of the pre-therapy infecting organism after therapy; or
- re-infection with a new organism.

Selected reports that document this high frequency of recurrent infection are summarized in Table 56.6. Bacteriologic cure rates at 4–6 weeks (long-term follow-up) are consistently less than 50% (i.e. recurrent infection is the expected outcome) if the underlying abnormality persists. If the underlying abnormality is transient or reversible, such as a single obstructing stone that is passed, permanent or long-term cure may be achieved. When the underlying abnormality promoting infection cannot be corrected, recurrent infection with organisms of increasing antimicrobial resistance is a common outcome. Some patients may ultimately have infections for years with acquisition of highly resistant organisms.

DIAGNOSIS

Clinical symptoms alone are not sufficient for a diagnosis of complicated UTI. For definitive diagnosis an appropriately collected urine specimen must be obtained for bacterial culture. The large variety of potential infecting organisms, together with the increased likelihood of antimicrobial resistance, means that obtaining a pre-therapy urine culture is essential for appropriate antimicrobial management of patients who experience complicated UTI. Blood cultures should also be obtained from patients who have evidence of sepsis, including symptoms such as high fever, rigors, hypothermia or acute confusion.

Urine specimen

The urine specimen must be collected before initiating antimicrobial therapy, using a urine collection method that limits contamination. Urine specimens should be forwarded promptly to the laboratory for semiquantitative culture and appropriate susceptibility testing. A clean-catch voided specimen or, if a voided specimen cannot be obtained, a specimen obtained through in and out catheterization, is usually appropriate. For individuals who have indwelling catheters, urine is collected by aseptic aspiration from the catheter port. Patients with obstruction for whom a urologic procedure for decompression is undertaken may have specimens obtained by ureteric catheterization or percutaneous aspiration of the renal pelvis. There is no completely satisfactory way of collecting specimens for culture from people who have an ileal conduit. Specimens collected through the conduit will always be contaminated with organisms colonizing the conduit, and clinical judgment is necessary to evaluate the significance of the organisms isolated.

Foreign material in the urinary tract, including indwelling urethral catheters, ureteric stents and nephrostomy tubes, are rapidly coated with a bacterial biofilm after insertion. Organisms isolated from urine specimens obtained through such devices reflect the microbiology of the biofilm on the inner surface of the catheter rather than bladder urine. Therefore, it is suggested that indwelling catheters should be changed before specimen collection and initiation of antimicrobial therapy.[36] The urine specimen is collected through the newly inserted catheter which is free of biofilm and samples bladder urine, leading to a more reliable identification of the infecting bacterial species.

Quantitative bacteriology

Current recommendations for interpretation of quantitative bacteriology in the diagnosis of complicated UTI are provided in Table 56.7. In the symptomatic patient, UTI may be diagnosed if the quantitative count of organisms in urine culture is 10^4 cfu/ml or more and there are symptoms consistent with genitourinary infection. For individuals who are asymptomatic, two specimens with a quantitative count of 10^5 cfu/ml or more with the same organism(s) isolated on two consecutive occasions are recommended for diagnosis.[25,37] In practice, however, a single specimen achieving counts of 10^5 cfu/ml or more is usually interpreted as bacteriuria.

Infections usually involve a single infecting organism, but there may be more than one bacterial species in the urine of patients with frequent recurrent infections. The patient who has a long-term indwelling catheter or other chronic device will usually have two to five organisms isolated.[5]

Rarely, less common organisms, such as yeast species, may be isolated in lower quantitative counts. Unusual uropathogens such as *U. urealyticum* or *Haemophilus influenzae*, which are uncommon causes of infection, will not be isolated by routine laboratory methods for urine culture. If there is complete ureteric obstruction with infection localized proximal to the obstruction, the culture may also be negative. Of course, urine cultures will also be negative with symptomatic infection if the patient has received effective antimicrobial therapy prior to collecting the urine specimens.

Pyuria

Pyuria is evidence for inflammation within the urinary tract, and may be attributed to many causes other than infection. Underlying abnormalities associated with complicated UTI, inflammation following a surgical procedure or a chronic indwelling catheter may all be associated with pyuria in the absence of infection. Thus, pyuria is not sufficient to confirm a diagnosis of a UTI in the absence of a positive urine culture. In addition, both symptomatic and asymptomatic UTI are usually associated with pyuria, so pyuria does not discriminate between symptomatic and asymptomatic infection. Therefore, a dipstick test or urinalysis that shows evidence of pyuria is consistent with but not diagnostic of UTI. A negative test for pyuria is, however, helpful to exclude a diagnosis of urinary infection.

Table 56.6 Bacteriologic outcome after antimicrobial therapy of complicated urinary tract infection

Regimen	Follow-up after therapy	Cure (%)	Re-infection (%)
Complicated urinary infection			
Lomefloxacin[16]	5–9 days	59	5.9
	4–6 weeks	43	19
Trimethoprim–sulfamethoxazole[16]	5–9 days	33	1.5
	4–6 weeks	28	9.2
UTI secondary to spinal cord injury			
Norfloxacin 14 days[34]	5–7 days	53	14
	8–12 weeks	16	NS
Various antimicrobials: 7–14 days[35]	1 week	47	NS
Various antimicrobials: ≥28 days[35]	1 week	41	NS

NS, not stated.

Table 56.7 Quantitative bacteriology in the diagnosis of complicated urinary tract infection

Clinical presentation	Bacteriologic count
Asymptomatic bacteriuria	$\geq 10^5$ cfu/ml in two consecutive urine specimens
Symptomatic urinary infection	$\geq 10^4$ cfu/ml in one specimen or $\geq 10^5$ if collected by external catheter
Percutaneous aspiration in hydronephrosis	Any quantitative count
Diuresis, diuretic therapy, renal failure, selected infecting organisms (e.g. *Candida albicans*)	Lower quantitative counts ($<10^5$/ml) may occur in these situations

MANAGEMENT

Essential elements in approaching the management of UTI in the setting of an abnormal genitourinary tract include:

- initial clinical evaluation;
- appropriate pre-therapy diagnostic specimen collection;
- selection of initial antimicrobial therapy;
- appropriate supportive care;
- an assessment of the need for genitourinary investigation or urologic interventions to correct any abnormality; and
- review of urine culture results, once available, to ensure the optimal antimicrobial regimen is being given.

Supportive therapy should be given as appropriate depending on the severity of the clinical presentation. This may include hemodynamic monitoring, parenteral fluids, measurement of urine output, or antiemetic medication. If a chronic indwelling catheter is present, catheter replacement prior to initiating antimicrobial therapy is associated with more rapid defervescence and a decreased frequency of symptomatic relapse, as well as providing a more accurate urine specimen for culture.[36]

Asymptomatic bacteriuria

Asymptomatic bacteriuria should only be treated in patient groups where clinical studies have demonstrated a benefit. Table 56.8 summarizes current recommendations for treatment of asymptomatic bacteriuria for different patient populations. These recommendations are developed based on results of published clinical trials.[25]

Where treatment of asymptomatic bacteriuria is not indicated, antimicrobial therapy does not decrease morbidity of infection but is associated with a greater frequency of negative outcomes, including the emergence of resistant organisms and adverse drug effects. Studies in children, in fact, suggest that the treatment of asymptomatic bacteriuria may increase the frequency of symptomatic infection. Screening for bacteriuria is also not indicated except in populations for whom treatment of asymptomatic bacteriuria is recommended.

Table 56.8 Indications for the treatment of asymptomatic bacteriuria

Definite	Before an invasive genitourinary procedure Pregnancy
Not indicated	In the elderly For schoolgirls or healthy women Intermittent catheterization Indwelling urinary catheter Diabetic women

Data from Nicolle *et al.*[25]

Antimicrobial therapy

Whenever possible, empiric therapy should be avoided and antimicrobial therapy specific for the infecting organism(s) isolated in urine culture should be prescribed. This is usually possible if the patient has mild symptoms. When the patient's symptoms are severe enough to warrant immediate empiric therapy, it is essential that a urine culture be obtained before initiating therapy. The empiric antimicrobial regimen should be re-evaluated once culture results are available, usually 48–72 hours after the specimen was collected.

Initial parenteral therapy is preferred for individuals who have:

- hemodynamic instability;
- nausea and vomiting;
- questionable absorption of oral antimicrobials; or
- an infection by suspected resistant organisms for which oral therapy is not available.

The majority of patients can be managed without hospitalization or with a limited (24–72 hours) admission to a short-stay unit for initial parenteral therapy. Oral therapy is then started as soon as the patient is stable, often when pre-therapy urine culture results are available to assist in selecting the appropriate medication.

The antimicrobial agents appropriate for therapy are similar to those recommended in the treatment of acute uncomplicated UTI or acute nonobstructive pyelonephritis (Tables 56.9, 56.10). The selection of a specific antimicrobial agent is based upon clinical presentation, the known or suspected infecting organism and its susceptibilities, patient tolerance, documented efficacy and, in some cases, cost.

There are many clinical trials addressing the treatment of complicated UTI, but few of these are helpful in identifying a preferred regimen in a specific clinical situation.[14] One problem is the lack of an accepted 'standard' antimicrobial therapy with respect to agent, dose or duration to serve as a comparator for new agents. In addition, studies have generally enrolled diverse patient populations for whom different outcomes with therapy would be anticipated on the basis of underlying abnormalities. The severity of illness also varies, and subjects may have clinical presentations ranging from an increase in incontinence or bladder spasms to a life-threatening illness with bacteremia.

In view of the very high frequency of relapse, therapeutic trials should provide both short-term (1 week post-therapy) and long-term (4–6 weeks post-therapy) outcomes.[37] Frequently, studies of therapy for complicated UTI have provided only short-term therapeutic

Table 56.9 Oral therapeutic regimens for the treatment of complicated urinary tract infection

Agent	Dose
Amoxicillin	500 mg q8h
Amoxicillin–clavulanate	500 mg q8h or 875 mg bid
Cephalexin	500 mg q6h
Cefuroxime axetil	500 mg q12h
Cefpodoxime proxetil	100 mg q12h
Ceftibuten	400 mg q24h
Cefixime	400 mg q24h
Nitrofurantoin	50–100 mg q6h
Nitrofurantoin monohydrate/ macrocrystals	100 mg q12h
Norfloxacin	400 mg q12h
Ciprofloxacin	250–500 mg q12h
Ciprofloxacin extended release	1 g q24h
Ofloxacin	400 mg q24h or 200 mg q12h
Levofloxacin	200–500 mg q24h
Trimethoprim	100 mg q12h
Trimethoprim–sulfamethoxazole	160 mg trimethoprim–800 mg sulfamethoxazole q12h

Table 56.10 Parenteral regimens for the treatment of complicated urinary tract infection in patients with normal renal function

Agent	Dose
Ampicillin	1 g q6h
Piperacillin	3 g q6h
Ticarcillin–clavulanate	3.1 g q6h
Piperacillin–tazobactam	4 g piperacillin–500 mg tazobactam q8h
Cefazolin	1–2 g q8h
Cefotaxime	1 g q8h
Ceftriaxone	1 g q24h
Ceftazidime	1 g q8–12h
Cefepime	1–2 g q12h
Imipenem	500 mg q6h
Meropenem	500 mg q6h or q8h
Ertapenem	1 g q24h
Gentamicin	5 mg/kg q12h or q24h
Tobramycin	5 mg/kg q12h or q24h
Amikacin	15 mg/kg q12h or q24h
Trimethoprim–sulfamethoxazole	160 mg trimethoprim–800 mg sulfamethoxazole q12h
Ciprofloxacin	400 mg q12h
Levofloxacin	500 mg q24h
Ofloxacin	400 mg q12h

outcomes. The many published studies, therefore, provide limited insight into the comparative efficacy of different antimicrobials. These studies do, however, document the effectiveness of a wide variety of antimicrobial agents.

Oral therapy

For oral therapy, the fluoroquinolone antimicrobials as a class have been widely studied and promoted.[14,27] Benefits of fluoroquinolones include a wide antimicrobial spectrum and good patient tolerance. The main concern is increasing antimicrobial resistance with widespread use of this class of antimicrobials. The isolation of a fluoroquinolone-resistant extended-spectrum β-lactamase producing *E. coli* or *K. pneumoniae* is an increasing problem and may be a therapeutic challenge. Amoxicillin–clavulanic acid is effective for the treatment of some of these strains and nitrofurantoin may be effective for *E. coli* bladder infections. Many of these strains remain susceptible to fosfomycin or pivmecillinam, but the clinical efficacy of these agents for complicated UTI has not yet been determined.

In studies of oral therapy for pyelonephritis, cell-wall-active agents such as amoxicillin or first-generation cephalosporins appear less effective than non-cell-wall-active agents such as trimethoprim–sulfamethoxazole or quinolones.[38] Appropriate comparative clinical trials have not shown whether these cell-wall-active agents have a lower efficacy for complicated UTI. For Gram-positive organisms such as group B streptococci and *Enterococcus* spp., amoxicillin orally or ampicillin parenterally would be the treatment of choice.

Nitrofurantoin may be used for patients who have lower tract symptoms. This agent should be avoided in subjects with pyelonephritis or renal impairment, and is not effective for *P. mirabilis*, *K. pneumoniae* or *P. aeruginosa* infection. It is effective for most strains of vancomycin-resistant *Enterococcus*.

Parenteral therapy

For parenteral therapy, an aminoglycoside antimicrobial remains the treatment of choice because of the documented efficacy of this group of drugs over many years of use, and the continued susceptibility of most uropathogens. The nephrotoxicity and ototoxicity of aminoglycosides are seldom a problem if the duration of therapy is limited, with an early switch to oral therapy following initial clinical improvement and review of the urine culture.

Many other antimicrobial agents are effective as parenteral therapy including penicillins (piperacillin and piperacillin–tazobactam), cephalosporins (cefazolin, cefuroxime, cefotaxime, ceftriaxone and ceftazidime), carbapenems (imipenem, ertapenem, meropenem) and fluoroquinolones (levofloxacin, ciprofloxacin). If *Enterococcus* spp. may be present, ampicillin should be added. The increasing prevalence of ampicillin resistance in *E. faecium* and *E. faecalis* in many institutions means that vancomycin may be necessary to treat nosocomial enterococcal infection.

Duration of therapy

Few reported studies have directly addressed the question of the appropriate duration of therapy. The usual recommended duration is 7–14 days.[14,37] A prospective, randomized, clinical trial of spinal cord injured patients showed that 14 days of ciprofloxacin therapy resulted in fewer symptomatic relapses post-therapy than a 3-day course.[39] However, whether an alternate treatment duration between 3 and 14 days would be as effective is not yet known. In another prospective randomized study, 2 weeks of ciprofloxacin therapy was as effective as 4 weeks for men presenting with febrile urinary infection.[40]

There are selected clinical situations where alternative durations of therapy are appropriate:

- where relapsing infection is due to a prostate source, 6 or 12 weeks of antimicrobial therapy is recommended;
- if symptomatic infection associated with an indwelling catheter is treated while the catheter remains *in situ*, it is recommended that the duration of therapy is as short as possible, usually 5–7 days, to limit the emergence of resistant organisms; and
- after successful extracorporeal shock wave lithotripsy for an infected struvite stone, continuation of antimicrobial therapy is recommended for at least 4 weeks to prevent relapse and sterilize residual stone fragments.[41]

Treatment of patients with renal failure

Patients who have abnormalities of the genitourinary tract are more likely to have impaired renal function. Renal function should be evaluated, if not already known, for patients who present with possible complicated UTI. If there is renal impairment appropriate modifications in antimicrobial dose are necessary. Nitrofurantoin and tetracyclines other than doxycycline should be avoided.

In patients with renal failure, renal perfusion is decreased and antimicrobials may not reach infected renal tissue or achieve high urine levels. The aminoglycosides, in particular, may be less effective.[14] The fluoroquinolone antimicrobials, trimethoprim–sulfamethoxazole, trimethoprim and extended-spectrum β-lactam antimicrobials appear to be effective in the treatment of UTI in the presence of significant renal failure. A more prolonged duration of antimicrobial therapy may be necessary to cure UTI in these patients.

An additional potential therapeutic problem is the patient who has normal measured renal function and satisfactory urinary antimicrobial levels but disparate kidney function. If the function of one kidney is severely impaired relative to the other, blood flow is preferentially increased in the functioning kidney and, despite adequate bladder urine antibiotic levels, little antibiotic will be filtered by the poorly functioning kidney. Antimicrobial treatment may then not eradicate infection localized to the poorly functioning kidney. This is one potential explanation for relapsing infection.

Treatment of fungal infection

Treatment of asymptomatic funguria is not beneficial and is not recommended.[42] The current treatment options and reported cure rates for symptomatic infection are shown in Table 56.11.

Table 56.11 Treatment regimens for fungal urinary tract infection

Agent	Dose	Cure rate (%)
Amphotericin B: parenteral	0.3–1 mg/kg single dose or 6 mg/kg total dose	75
Amphotericin B: bladder irrigation	Continuous, 50 mg/1 for 5 days	72–88
Fluconazole	50–400 mg q24h for 7 days	70–80
5-Flucytosine	50–150 mg/kg q6h	70

Data from Malani & Kauffman.[43]

The treatment of choice is fluconazole, which is the only azole antifungal excreted in the urine. The usual dose is 200 mg/day for 7–14 days.[43] Some non-*albicans Candida* spp., particularly *C. krusei* and *C. glabrata*, have decreased susceptibility or are resistant to fluconazole. The efficacy of voriconazole or posaconazole, which are not excreted in urine, is not known. Flucytosine may be effective for some resistant strains, but flucytosine resistance may emerge rapidly when this agent is used alone. Previously, bladder irrigation with amphotericin B was a recommended therapy. This approach, however, is costly and time-consuming and is no longer considered optimal therapy.

For many infections with *Candida*, a single dose of amphotericin B may be effective, given its prolonged excretion.[44] Echinocandin antifungals, including caspofungin, anidulafungin and micafungin, are not excreted into the urinary tract. However, preliminary case reports suggest caspofungin may be effective for treatment of some fluconazole-resistant *Candida* strains causing UTI.[45]

Suppressive therapy

Suppressive therapy is long-term antimicrobial therapy given to individuals with underlying abnormalities to prevent morbidity from urinary infection when the infection cannot be definitively cured (Fig. 56.1).

When a patient who has an underlying abnormality that cannot be corrected experiences recurrent morbidity, suppressive therapy may prevent symptomatic episodes.[46] Renal transplant patients with persistent infection of a native kidney leading to symptomatic recurrences is one example where this approach might be considered. Suppressive therapy may also be considered for men who have frequent recurrent episodes of symptomatic urinary infection from a prostate source and prolonged antimicrobial therapy has failed to cure the infection. Continuous suppressive therapy is recommended for the few individuals with a struvite (infection) stone that cannot be completely removed to prevent further stone enlargement and preserve renal function.

Long-term suppressive therapy is, however, infrequently indicated and should be used selectively because of cost and the potential for adverse effects including development of infection with resistant organisms.



Figure 56.1 Nephrocalcinosis complicated by relapsing *Pseudomonas aeruginosa* infection. Plain film of the abdomen showing multiple bilateral renal stones. This woman has had recurrent stone formation since the age of 18 despite dietary manipulation and repeated lithotripsy. Urinary infection with *P. aeruginosa* was identified at 24 years of age with subsequent recurrent episodes of symptomatic upper tract infection. She has been maintained on suppressive ciprofloxacin therapy for over 10 years with control of symptomatic infection.

INDICATIONS FOR INVESTIGATION

An important aspect of the management of UTIs is determining when to undertake urologic or imaging investigations to characterize abnormalities in the genitourinary tract (see also Chapter 55). Single infections that respond promptly to antimicrobials are unlikely to be associated with significant underlying abnormalities. On other occasions the abnormality may be obvious, such as the presence of an indwelling catheter. In selected other patients, genitourinary investigation should be considered. These clinical scenarios include:

- delayed or incomplete response to appropriate antimicrobial therapy;
- early recurrence of infection after therapy; and
- symptomatic fungal infection, where a fungus ball in the bladder or kidneys should be excluded.

Some patients may have previously well-characterized abnormalities but present with a change in frequency or severity of infection. In these cases, repeat evaluation to identify any changes (e.g. development of new bladder or renal stones) should be considered. Patients presenting with sepsis and hemodynamic instability may require urgent evaluation to identify an abscess or obstruction requiring immediate source control.

REFERENCES

References for this chapter can be found online at http://www.expertconsult.com

Practice point | 26 | John N Krieger

Management of persistent symptoms of prostatitis

INTRODUCTION

'Prostatitis' is the diagnosis given to a large group of men presenting with varied complaints referable to the lower urogenital tract and perineum. By one estimate, 50% of adult men experience symptoms of prostatitis at some time in their lives. National Health Center for Health Statistics data indicate that there were 76 office visits per 1000 men per year for genitourinary problems, with prostatitis accounting for approximately 25% of these visits. Recent epidemiologic studies suggest that 2–10% of adult men in varied populations worldwide suffer from prostatitis symptoms at any time. Patients may experience symptoms for prolonged periods. Data also suggest that men with a history of prostatitis may prove substantially more likely to develop prostate cancer and lower urinary tract symptoms associated with benign prostate hyperplasia. Management of patients who experience persistent symptoms following repeated courses of treatment represents a clinical challenge.

PATHOGENESIS

Genitourinary tract infection and inflammation are important in the pathogenesis of prostatitis syndromes. However, the proportion of cases attributable to these causes remains subject to active debate. Most prostate infections occur by ascending through the urethra. Mechanical factors such as urethral length, micturition and ejaculation provide some protection, although the relative importance of such defenses is unclear. The oblique courses of the ejaculatory ducts and some prostatic ducts have also been proposed as mechanical defenses. Other host defenses include the antimicrobial activity in the prostatic secretions, particularly a zinc-containing polypeptide known as prostatic antibacterial factor. The prostate has higher concentrations of zinc than any other organ and prostatic secretions from normal men contain high zinc levels. Men with chronic bacterial prostatitis have low prostatic fluid zinc concentrations, but their serum zinc levels are normal and oral zinc supplements do not increase prostatic secretion zinc levels. Local immunoglobulin production by the prostate also appears to be an important host defense. Many patients with prostatitis have increased leukocyte numbers in their prostatic secretions or semen, but the role of cellular immunity in resolving chronic prostatitis remains uncertain.

Hematogenous dissemination of systemic infections may result in prostatic infection in patients with tuberculosis or other granulomatous infections. This route is especially common in patients who are immunosuppressed or who have HIV infection. Rare patients develop prostatic infection or abscesses due to involvement by infection of adjacent organs, for example with perforated appendicitis or diverticulitis.

MICROBIOLOGY

Uropathogenic bacteria

Bacteriuria is a hallmark of acute and chronic bacterial prostatitis. The agents are standard uropathogens associated with bacterial urinary tract infection (e.g. Enterobacteriaceae, pseudomonads, enterococci, etc.). Recurrent infections caused by the same organism are the sine qua non of chronic bacterial prostatitis. Between episodes of bacteriuria these organisms may be 'localized' to the prostate as described below. Unfortunately, patients with well-documented acute and chronic bacterial prostatitis constitute a minority of patients presenting with prostatitis (less than 10% in our clinic).

Other genitourinary pathogens

Other pathogens have been implicated as causes of prostatitis. The best evidence supports a role for sexually transmitted pathogens. In the preantibiotic era, *Neisseria gonorrhoeae* was a frequent cause of prostatitis and the most common cause of prostatic abscess. However, in current practice *N. gonorrhoeae* is seldom implicated. Some studies suggest a role for other sexually transmitted diseases (STDs), particularly *Chlamydia trachomatis*, *Ureaplasma urealyticum* and *Trichomonas vaginalis*. The proportion of cases attributable to these pathogens remains undefined.

Granulomatous infections

A few patients develop granulomatous prostatitis, an uncommon syndrome representing a characteristic histologic reaction of the prostate to a variety of insults. Granulomatous prostatitis is classified as 'specific' when associated with particular granulomatous infections, or as 'nonspecific' in other cases. Specific causes of granulomatous prostatitis include *Mycobacterium tuberculosis*, atypical mycobacteria, bacille Calmette–Guérin (BCG) (after topical therapy for transitional cell carcinoma) and the deep mycoses (especially blastomycosis, coccidioidomycosis and cryptococcosis). Causes of nonspecific granulomatous prostatitis include acute bacterial prostatitis, prostatic surgery and rheumatoid diseases.

The clinical point is that specific diagnostic studies are required to document STDs or granulomatous infections in patients with symptoms or signs of prostatitis. Accurate diagnosis is the prerequisite for successful treatment, which may require antimicrobials seldom prescribed for standard uropathogens.

CLINICAL FEATURES

Patients with prostatitis can be classified into four syndromes: acute bacterial prostatitis, chronic bacterial prostatitis, chronic prostatitis/chronic pelvic pain syndrome and asymptomatic prostatitis (Table PP26.1).

Acute bacterial prostatitis

The clinical features of acute bacterial prostatitis are readily apparent. Characteristic complaints include acute symptoms of urinary tract infection, such as urgency, frequency, dysuria and occasionally gross hematuria or acute urinary retention. Patients may also have systemic symptoms of a 'flu-like' syndrome, with fever, chills or other symptoms associated with bacteremia. Occasional patients experience bladder outflow obstruction due to acute edema of the prostate.

Physical examination may show a high temperature, lower abdominal or suprapubic discomfort due to bladder infection, or urinary retention. The rectal examination is often impressive, with an exquisitely tender, 'tense' prostate. Urinalysis reveals pyuria, and cultures will be positive for uropathogenic bacteria. Systemic leukocytosis is common, with increased numbers of segmented cells. Bacteremia may occur spontaneously or result from vigorous rectal examinations.

Chronic bacterial prostatitis

The characteristic clinical feature of chronic bacterial prostatitis is recurrent episodes of bacteriuria caused by the same organism. Between bouts of bacteriuria, the patient may be asymptomatic or have only minimal symptoms. The infected prostate remains a focus of organisms causing relapsing infection in such patients. The prostate is usually normal on either rectal or endoscopic evaluation.

On occasion, chronic bacterial prostatitis presents as a systemic illness. Small numbers of bacteria in the prostate do not cause systemic illness but represent the source of such systemic infection. With acute exacerbations, bladder bacteriuria and secondary sepsis may occur. This is especially true among older men, who may have the combination of prostatic obstruction and infection.

Chronic prostatitis

Chronic prostatitis/chronic pelvic pain syndrome is the National Institutes of Health consensus term for the largest group of patients with prostatitis symptoms. Chronic pelvic pain symptoms are the characteristic common presentation, especially perineal, lower abdominal, testicular, penile and ejaculatory pain. Other genitourinary tract complaints include sexual dysfunction and voiding complaints. Some patients have inflammation in their prostatic secretions, post-prostate massage urine or semen (inflammatory subtype of chronic prostatitis/chronic pelvic pain syndrome, formerly termed 'nonbacterial prostatitis'), while others have no evidence of inflammation (noninflammatory subtype of chronic prostatitis/chronic pelvic pain syndrome, formerly termed 'prostatodynia').

Asymptomatic prostatitis

Asymptomatic prostatitis is the term used for men with inflammation diagnosed during evaluation for other genitourinary tract problems. For example, some patients undergoing evaluation for infertility have increased concentrations of leukocytes in their seminal fluid. Chronic prostatitis, characterized by inflammatory infiltrates in prostate specimens, represents the most common 'benign' diagnosis among men who undergo prostate biopsy for evaluation of elevated prostate-specific antigen levels or a digital rectal examination suggesting the possibility of prostate cancer.

INVESTIGATIONS

The critical practice point is to distinguish patients with lower urinary tract complaints associated with bacteriuria from the larger number of men without bacteriuria. Careful review of the history, physical examination and previous laboratory studies is helpful in making this distinction. Patients with bacteriuria may have acute or chronic bacterial prostatitis, while these conditions are rare in patients with no history of bacteriuria.

Document uropathogens

Urine culture and sensitivity testing is essential for men with acute lower urinary tract symptoms. Men with documented bacteriuria should have lower urinary tract localization studies, ideally the 'four glass' test described in Table PP26.2. A less sensitive alternative is to obtain pre- and post-prostate massage urine specimens. The purpose of these investigations is to document a prostatic focus of infection when the patient does not have bacteriuria. Documenting persistent prostatic infection supports the need for continued antimicrobial therapy.

Unequivocal diagnosis of bacterial prostatitis requires that the colony count of a recognized uropathogen in postmassage (VB_3) urine exceed the colony count in the first-void urine (VB_1) by at least

Table PP26.1 NIH consensus classification of prostatitis syndromes

Category	Characteristic clinical features	Bacteriuria	Inflammation*
Acute bacterial prostatitis	Acute urinary tract infection	+	+
Chronic bacterial prostatitis	Recurrent urinary tract infection caused by the same organism	+	+
Chronic prostatitis/chronic pelvic pain syndrome	Primarily pain complaints, plus voiding complaints and sexual dysfunction		
Inflammatory subtype†		–	+
Noninflammatory subtype‡		–	–
Asymptomatic prostatitis	Diagnosed during evaluation of other genitourinary complaints	–	+

*Objective evidence of an inflammatory response in expressed prostatic secretions, post-prostate massage urine, semen, or by histology.
†Formerly termed 'nonbacterial prostatitis'.
‡Formerly termed 'prostatodynia'.

Table PP26.2 Lower urinary tract localization study

Specimen	Abbreviation	Procedure
Voided bladder 1	VB_1	Initial 5–10 ml of urinary stream
Voided bladder 2	VB_2	Midstream specimen
Expressed prostatic secretions	EPS	Secretions expressed from prostate by digital massage after midstream specimen
Voided bladder 3	VB_3	First 5–10 ml of urinary stream immediately after prostate massage

Unequivocal diagnosis of chronic bacterial prostatitis requires a 10-fold higher concentration of a uropathogen in the VB_3 of EPS specimen when compared to the VB_1 specimen. The organism is identical to organisms causing repeated episodes of bacteriuria.

10-fold. However, many men with chronic bacterial prostatitis harbor only small numbers of pathogenic bacteria in their prostates. Direct culture of the expressed prostatic secretions (EPS) is useful in this situation because colony counts in EPS are often 1 or 2 logs higher than comparable counts in the VB_3. The hallmark of chronic bacterial prostatitis is that the uropathogen present in VB_3 or EPS may be isolated on multiple occasions and is identical to the organism causing episodes of bacteriuria.

Document other infectious agents

Patients with risk factors for STD pathogens should have appropriate testing for *N. gonorrhoeae*, *C. trachomatis* and other agents. Serologic testing should be recommended for both syphilis and HIV infection. Patients who have clinical findings suggesting granulomatous prostatitis should have appropriate studies for specific agents associated with this condition (which often entails histologic studies and cultures of prostate tissue).

Document inflammation

Traditionally, microscopic evaluation has been recommended to identify EPS inflammation because this provides objective support for the diagnosis of chronic prostatitis. Although many authorities diagnose inflammation based on finding >10 or >20 leukocytes per high power field in the prostatic secretions, we prefer to define inflammation based on chamber counts with >1000 leukocytes/mm^3 because this method is substantially more accurate than the traditional 'wet mount'. There appears to be little value in counting EPS leukocytes in patients with urethral inflammation, especially among men at risk for STDs. Therefore, we examine a urethral smear before proceeding with the lower urinary tract localization study. Diagnosis of urethral inflammation (or 'urethritis') by either VB_1 or urethral smear supports appropriate testing for STD pathogens. In contrast, the clinical utility of distinguishing between the inflammatory and noninflammatory subtypes of chronic prostatitis/chronic pelvic pain syndrome is subject to active debate because these two categories of chronic prostatitis have not been demonstrated to differ in their clinical presentation or response to therapy.

Other investigations

Our standard approach is to recommend a noninvasive uroflow study and postvoid residual testing (by ultrasound) to evaluate possible structural and functional obstructions that may contribute to the etiology or persistence of prostatitis symptoms. Patients with abnormal flow rates or significant postvoid residual urine have additional evaluation with a retrograde urethrogram to evaluate the possibility of urethral stricture. Video urodynamics is reserved for patients with abnormal uroflow findings and negative urethrograms.

Cystoscopy is not recommended for routine evaluation of men with prostatitis symptoms. We reserve cystoscopy for cases where bladder cancer or interstitial cystitis is considered likely (e.g. in older patients, those with hematuria or a history of chemical exposure, or patients in whom painful voiding complaints are prominent). Urinary cytology is obtained if transitional cell carcinoma *in situ* is considered prominently in the differential diagnosis. For patients undergoing cystoscopy, we prefer general or regional anesthesia and recommend hydrodistention with appropriate bladder biopsies. Prostate-specific antigen testing should be considered because occasional patients with carcinoma of the prostate present with persistent symptoms of prostatitis. However, such testing is not recommended for patients with more acute symptoms, since temporary elevation of prostate-specific antigen (and prostatic acid phosphatase) is common following acute episodes. Transrectal ultrasound evaluation may also be considered in selected patients to evaluate possible ejaculatory duct obstruction or complications such as prostatic abscess.

MANAGEMENT

Acute bacterial prostatitis

Appropriate therapy results in dramatic improvement. Many antimicrobials that do not penetrate the uninflamed prostate have proved effective. Thus, drugs appropriate for Enterobacteriaceae, pseudomonads or enterococci should be started once cultures are obtained. For men who require hospitalization, conventional therapy is the combination of an aminoglycoside plus a β-lactam. The fluoroquinolones or third-generation cephalosporins are attractive alternatives for monotherapy.

For less severe infections the conventional choice for outpatient therapy is the combination of trimethoprim and sulfamethoxazole. Because of increasing antimicrobial resistance, fluoroquinolones represent the preferred oral therapy for patients who do not require hospitalization. We usually prescribe one of the newer quinolones for outpatient management.

Patients with acute urinary retention require bladder drainage. In this situation we prefer a suprapubic cystostomy tube, placed using a percutaneous approach. Traditionally, it has been taught that an indwelling transurethral catheter would pass through and obstruct drainage of the acutely infected prostate, increasing the risk for bacteremia and prostatic abscess. However, this has never been evaluated in a controlled study. General measures are also indicated, including hydration, analgesics and bed rest.

Chronic bacterial prostatitis

Trimethoprim–sulfamethoxazole is the traditional 'gold standard'. Long-term therapy with trimethoprim (80 mg) plus sulfamethoxazole (400 mg) taken orally twice daily for 4–16 weeks was superior to shorter courses. Such therapy results in symptomatic and bacteriologic cure in approximately one-third of patients, symptomatic improvement during therapy in approximately one-third (who relapse after stopping treatment) and no improvement in the remaining patients. However, increasing rates of antimicrobial resistance in many areas have tarnished this gold standard.

During the last decade the fluoroquinolones have become the preferred treatment for chronic bacterial prostatitis in most settings. In contrast to the β-lactams, concentrations of many fluoroquinolones are relatively high in prostatic fluid, prostatic tissue and seminal fluid compared to plasma levels. Good results were reported for men with bacterial prostatitis, including patients who failed therapy with trimethoprim–sulfamethoxazole. Useful fluoroquinolones include

ciprofloxacin, ofloxacin and levofloxacin. Currently our first choice for curative therapy for chronic bacterial prostatitis is usually an appropriate fluoroquinolone, at full-dose for at least 3 months.

Patients who are not cured benefit from long-term suppressive treatment using low-dosage antimicrobial agents. Since most patients are asymptomatic between episodes of bacteriuria, the goal of suppressive therapy is to prevent symptoms of bacteriuria. Very low doses of drugs are remarkably effective. Available agents include penicillin G, tetracycline, nitrofurantoin, nalidixic acid, cephalexin and trimethoprim–sulfamethoxazole. Although effective, we seldom recommend fluoroquinolones for chronic suppression, because of cost and the potential for development of antimicrobial-resistant organisms.

Chronic prostatitis/chronic pelvic pain syndrome

Therapy is often unsatisfactory because the etiology of chronic prostatitis/chronic pelvic pain syndrome remains unclear. As outlined above, an etiologic role has been suggested for many infectious agents. Prostaglandins, autoimmunity, psychologic abnormalities, neuromuscular dysfunction of the bladder neck or urogenital diaphragm, allergy to environmental agents, stress and other psychologic factors have all been suggested as causes.

Antimicrobial drugs are often considered the first-line treatment. Patients with recognized uropathogens may respond to such empiric therapy. For men without evidence of infection by recognized pathogens, antimicrobial treatment often results in temporary, if any, relief. Because long-term antimicrobial therapy offers limited benefit for patients with no evidence of urogenital infection, we recommend a thorough evaluation for infectious agents. We prescribe antimicrobials only for patients with documented infections rather than recommending repeated courses of empiric therapy.

Alpha-blocker therapy has been evaluated in case series and randomized clinical trials. These agents represent our second-line therapy, after antibiotics. A number of other medications have been supported by case series or nonrandomized studies, including anti-inflammatory drugs, anticholinergic drugs, allopurinol, muscle relaxants and chronic pain medications.

Other measures that have been reported to provide relief include acupuncture, prostate massage, sitz baths, diathermy, exercises, physiotherapy and psychotherapy. Anecdotal reports suggest that transurethral resection of the prostate, microwave or thermal therapy might provide relief for highly selected patients. Some clinicians recommend increased frequency of ejaculation to relieve 'congestion'. Others recommend abstinence from ejaculation, alcohol, coffee, tea, spicy foods, etc. There is little objective evidence that any of these measures changes the natural history of chronic prostatitis/chronic pelvic pain syndrome.

Asymptomatic inflammatory prostatitis

Some practitioners prescribe antimicrobial and/or anti-inflammatory treatment for patients with asymptomatic prostatitis. There are limited data that patients benefit from such treatment. Thus, most authorities do not recommend treatment of asymptomatic prostatitis because the risk of side-effects does not justify the theoretical benefit from such treatment.

CONCLUSION

Patients with documented bacteriuria may have acute or chronic bacterial prostatitis, while these conditions are rare in patients with no history of bacteriuria. Specific diagnostic studies are necessary to document uropathogens, STD agents or granulomatous infections. Other diagnoses are possible in certain patients. Accurate diagnosis is the prerequisite for successful treatment.

FURTHER READING


Further reading for this chapter can be found online at http://www.expertconsult.com

Practice point | 27 |

George J Alangaden
John David Hinze
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Tuberculosis of the urogenital tract

DEFINITION OF THE PROBLEM

A 47-year-old man who was born in India and emigrated to the United States 5 years ago presents with painless swelling of the scrotum. He has no systemic symptoms. Examination reveals a nontender 3 cm firm mass involving the left epididymis. Urine examination demonstrates persistent pyuria with negative cultures.

INTRODUCTION

Genitourinary tuberculosis (TB) is a severe form of extrapulmonary TB that is uncommon in countries with a low prevalence of TB. In 2003 in the United States, genitourinary TB accounted for about 1% of the 14 874 cases of TB and 5% of the 3029 cases of extrapulmonary TB. However, genitourinary TB can account for up to 20% all TB cases in high TB-prevalence countries. It is uncommon in children and most cases present in adults older than 20 years, with a male:female predominance of 2:1.

PATHOGENESIS

Genitourinary TB is almost always caused by *Mycobacterium tuberculosis* (MTB) and results from hematogenous dissemination from the lungs to the kidneys during primary infection, with subsequent reactivation later in life. Infection initially involves the renal cortex, with granuloma formation and caseation necrosis. It progressively involves the medulla and papilla with development of macroscopic cavitary lesions. Subsequent MTB bacilluria results in infection of the ureters and bladder. Progressive disease can result in fibrosis affecting the ureters and bladder, and may lead to total destruction of the kidney, termed 'autonephrectomy'.

Descending infection from the kidneys in males can affect the epididymis, seminal vesicles, testes and prostate. TB of the penis is rare and can result from sexual contact. In women, hematogenous infection of the fallopian tubes occurs initially, followed by spread of infection to the endometrium, ovaries and cervix.

Rarely genitourinary TB can result from infection following intravesical immunotherapy with bacille Calmette–Guérin (BCG) for the treatment of transitional cell carcinoma of the bladder. Transmission of TB via donor kidney during renal transplantation has been reported.

CLINICAL FEATURES

Genitourinary TB is a slowly progressive infection that is clinically inapparent for decades. Many patients with pulmonary TB may have coexistent asymptomatic genitourinary TB. The symptoms of genitourinary TB are often localized to the organs involved and constitutional symptoms are uncommon. The commonest symptoms of genitourinary TB reported in large series of patients have included dysuria, frequency or urgency (31–65%), hematuria (30–43%) and flank pain (21–57%), with fever being infrequent (12–33%).

Genital TB in men generally presents with a scrotal mass due to infection of the epididymis and less frequently with prostatitis, orchitis or scrotal fistulas. In women with genital TB the most common presentation is infertility, followed by pelvic pain or dysmenorrhea.

DIAGNOSIS

The early diagnosis of genitourinary TB requires a high degree of clinical suspicion. A history of prior TB infection in the context of chronic urinary symptoms, scrotal mass in men or infertility in women should prompt consideration of genitourinary TB.

A tuberculin skin test should be performed in suspected cases, and will be positive in approximately 90% of patients with genitourinary TB.

Radiologic findings can help support the diagnosis of genitourinary TB. Chest radiographs to assess the presence of pulmonary TB may demonstrate abnormalities in about a third of cases. Simple abdominal radiography might show renal calcification. The most commonly used radiologic technique for evaluating genitourinary TB has been the intravenous pyelogram (IVP). Renal TB is suggested by the presence of abnormalities of both the upper and lower urinary tracts. An IVP may demonstrate calyceal distortion, delayed excretion, cavitation, 'moth-eaten' calyces or strictures with caliectasis. The characteristic 'phantom calyx' occurs when the IVP reveals an obstructed nonfunctioning calyx proximal to an infundibular stricture. A 'hiked-up' renal pelvis observed when the renal pelvis makes an acute angulation with the ureter is highly suggestive of renal TB. Another highly suggestive lesion is the 'putty kidney' consisting of a heavily calcified caseous mass surrounded by a thin parenchymal shell. Ureteral TB may manifest as strictures and obstructions, giving the appearance of 'beading', 'corkscrewing', straight 'pipe-stems', focal calcification or hydronephrosis. Commonly, the bladder appears thickened and fibrotic with small capacity.

Ultrasound, CT and MRI are increasingly being utilized in the evaluation of genitourinary TB and help to provide a guide for fine-needle aspiration biopsies.

- Ultrasound may demonstrate small focal kidney lesions in patients with renal TB. In women with TB of the fallopian tubes, ultrasonography demonstrates adnexal masses containing small scattered calcifications. Testicular and transrectal ultrasonography can be used to evaluate a scrotal mass and the prostate but the findings are nonspecific.
- The CT scan can provide details of the renal parenchyma and associated abnormalities in the genital tract, abdomen and pelvis. Typical CT findings are caliectasis and focal cortical scarring (>80%).
- On MRI scans tuberculous foci appear hypointense on T2-weighted images. In women with genital TB the hysterosalpingogram demonstrates characteristic abnormalities, displaying a contracted deformed uterine cavity with intrauterine adhesions. The fallopian tubes may have ragged outlines and appear beaded or rigid.

The finding of 'sterile' pyuria has been considered an important clue to the laboratory diagnosis of genitourinary TB. However, common bacterial uropathogens are often isolated from the urine of patients with genitourinary TB. This can obscure the diagnosis and result in unnecessary antibiotic therapy. The initial microbiologic testing should include at least three first-morning urine specimens for acid-fast bacillus (AFB) stain and culture. It is important to ensure that patients are not receiving antibiotics since commonly used agents such as fluoroquinolones can inhibit the growth of MTB. Mycobacterial cultures of the urine may be positive in about 77–92% cases of genitourinary TB. If urine, menstrual blood or seminal fluid cultures are negative, then ultrasound- or CT-guided fine-needle aspiration, uterine curettage or diagnostic laparoscopy should be used to obtain appropriate tissue for microbiologic and histopathologic testing.

The average time to isolation of MTB using liquid and solid media is about 2–4 weeks and susceptibility testing requires an additional 2–4 weeks. Polymerase chain reaction (PCR) assays targeting MTB DNA have been used for the rapid diagnosis of genitourinary TB on urine and tissue specimens. Reports suggest higher sensitivity and specificity compared to AFB smear and MTB cultures. However, cultures should be performed to obtain MTB isolates for susceptibility testing which is essential to guide therapy.

MANAGEMENT (SEE ALSO CHAPTERS 30 AND 143)

The primary management of genitourinary TB is medical. Short-course anti-TB therapy regimens have been shown to be effective for all forms of extrapulmonary therapy including genitourinary TB. The recommendations of the World Health Organization and the Centers for Disease Control and Prevention include initial intensive 2-month therapy with a three- or four-drug regimen using isoniazid, rifampin and pyrazinamide. Ethambutol may be utilized as the fourth agent in areas with a high prevalence of isoniazid resistance. The intensive phase is followed by 4 months of continuation therapy with isoniazid and rifampin (rifampicin). Therapy in the continuation phase should be guided by the results of susceptibility testing. The management of patients unresponsive to treatment or with drug-resistant TB should be carried out in close conjunction with an expert. Urine cultures should become negative after 2–3 months of therapy. Follow-up radiologic imaging to monitor for development of obstructive uropathy should be considered.

A common problem in the management of genitourinary TB is the progression of nephroureteric disease despite appropriate antimicrobial therapy. Fibrosis causing obstruction can occur anywhere along the urinary tract. Endoscopic balloon dilatation, ureteric stents and reconstructive urologic surgery have been utilized in the management of these sequelae. Nephrectomy is seldom necessary and may be indicated in cases of a nonfunctioning kidney, recalcitrant infection, life-threatening hematuria and uncontrollable hypertension secondary to renal TB.

FURTHER READING



Further reading for this chapter can be found online at http://www.expertconsult.com

Practice point | 28 |

George J Alangaden

Urinary tract infections in kidney transplant recipients

DEFINITION OF THE PROBLEM

A 42-year-old woman with end-stage renal disease secondary to hypertension and diabetes mellitus underwent a cadaveric renal transplant. Five weeks after her transplant, while on antibiotic prophylaxis with trimethoprim–sulfamethoxazole she develops low-grade fever and dysuria. Urine examination reveals pyuria and bacteriuria, and *Escherichia coli* resistant to trimethoprim-sulfamethoxazole is isolated from urine culture.

INTRODUCTION

In the USA, 16 646 kidney transplants were performed in 2006. The 5-year patient and kidney graft survival rates are about 80%. Mortality related to infection in the first year following kidney transplant is less than 5%. Urinary tract infections (UTIs) are the most common infectious complication after kidney transplantation; they account for 47–61% of infections and a third of bacteremias. The cumulative incidence of UTI is about 17% for both men and women within 6 months and 60% for women and 47% for men at 3 years after transplantation.

The wide range of frequencies of UTI reported is the result of varying definitions of UTI, use of antibiotic prophylaxis and the duration of follow-up. If the presence of bacteriuria (10^5 organisms/ml) alone is used to define UTIs it overestimates the incidence. The inclusion of concomitant pyuria to bacteriuria may identify patients at risk for subsequent symptomatic UTI which occurs in about 25–47% of recipients. Use of trimethoprim–sulfamethoxazole prophylaxis results in slightly lower rates of UTIs. The incidence of UTI is greatest during the first 3 months following transplantation and may be related to surgical trauma, presence of urinary catheters and ureteric stents.

PATHOGENESIS AND RISK FACTORS FOR UTI

Host immune system deficiencies that predispose to infections including UTI among renal transplant recipients are termed as the 'net state of immune suppression' and include antirejection immune suppressive therapy, surgical trauma, presence of catheters and stents, metabolic factors such as hyperglycemia and immunomodulating viral infections such as cytomegalovirus. Women and diabetic transplant recipients are at greatest risk for UTIs and are more likely to develop bacteremias resulting from UTIs. Other specific risk factors include older age, prolonged pre-transplant dialysis, polycystic kidney disease, pre-transplant UTIs, allograft trauma, microbial contamination of cadaveric kidneys, complications related to ureteral anastomosis, presence of urinary catheters and ureteric stents, re-implantation and vesicoureteral reflux.

CLINICAL FEATURES OF UTI

Most UTIs in renal transplant recipients presents as cystitis with common symptoms such as frequency, urgency, dysuria and minimal fever. Pyelonephritis and bacteremias can occur and are more likely in the early period after transplantation. In the immediate post-transplant period, the time to development of UTIs in the presence of a urinary catheter is about 1 week. Recurrent UTIs occur in 5–27% of cases.

MICROBIOLOGY FOR UTI

The pathogens isolated from kidney transplant recipients with UTI are similar to those in the general population. Enterobacteriaceae account for 40–90% of UTIs, with *Escherichia coli* being the most common pathogen. Other Gram-negative uropathogens include *Klebsiella* spp., *Enterobacter* spp., *Pseudomonas aeruginosa*, *Serratia marcescens* and *Acinetobacter* spp. Gram-positive bacteria such as *Enterococcus* spp., coagulase-negative staphylococci and *Corynebacterium urealyticum* cause 7–22% of UTIs. *Candida* spp. are uncommon uropathogens in renal transplant recipients, accounting for 1–5% of UTIs.

Enterococcus spp. and antibiotic-resistant Enterobacteriaceae are being increasingly reported worldwide in association with UTIs in renal transplant recipients. Enterococcal UTIs appear to occur early after transplantation. Resistance to trimethoprim–sulfamethoxazole, ciprofloxacin and the production of extended-spectrum β-lactamases has been noted in *E. coli* and *E. cloacae*.

PREVENTION OF UTI

Good surgical technique and early removal of urinary catheters decrease the risk of UTI.

Trimethoprim–sulfamethoxazole has been shown to be cost-effective in preventing UTIs after renal transplantation. Moreover, trimethoprim–sulfamethoxazole prevents opportunistic infections caused by *Pneumocystis jirovecii*, *Listeria monocytogenes*, *Nocardia asteroides* and *Toxoplasma gondii*. Hence prophylaxis with trimethoprim–sulfamethoxazole has been recommended for 6–12 months after renal transplantation. The doses range from 80 mg/400 mg to 320 mg/1600 mg of trimethoprim–sulfamethoxazole administered daily. Ciprofloxacin in doses of 250–500 mg daily is as effective as trimethoprim–sulfamethoxazole. Antibiotic stewardship and infection control practices are necessary to forestall the emergence and spread of multidrug-resistant pathogens.

DIAGNOSIS AND THERAPY OF UTIs

The diagnosis of UTI should be based upon the presence of symptoms, pyuria and bacteriuria. Given the emergence of drug resistance among uropathogens, Gram stain, cultures and antimicrobial susceptibilities should be performed on clean-voided midstream urine specimens. Blood cultures should be obtained if systemic symptoms such as high fevers or clinical features of pyelonephritis are present.

Initial empiric therapy of symptomatic UTI should include antibiotics that are active against Enterobacteriaceae such as broad-spectrum penicillins, cephalosporins or fluoroquinolones. Nephrotoxic agents such as aminoglycosides should be avoided, especially since these patients are often receiving other potentially nephrotoxic agents (e.g. tacrolimus). Subsequent therapy should be adjusted based upon results of urine cultures and susceptibilities. A course of therapy of about 2 weeks is recommended except in cases of relapse or prostatitis when prolonged therapy for 4–6 weeks is appropriate. Hospitalization may be necessary based upon severity of illness.

Imaging studies such as renal ultrasound or CT are indicated if there is a poor response to appropriate antimicrobial therapy, or when a surgical complication such as a ureteral anastomosis leak or obstruction is suspected. Recurrent and relapsing UTIs or breakthrough UTI on prophylaxis may warrant further urologic investigations such as voiding cystourethrography to identify any correctable structural or functional defects of the urinary tract.

ASYMPTOMATIC BACTERIURIA AND CANDIDURIA

The natural history of asymptomatic bacteriuria in renal transplant recipients is poorly defined. In the absence of controlled studies to examine effectiveness of antibiotic treatment, the optimal management of asymptomatic bacteriuria remains controversial. Routine antibiotic treatment is not recommended as it appears to have no impact on renal function and does not appear to decrease the rate of symptomatic UTIs.

Likewise the management of asymptomatic candiduria is controversial. Routine treatment of candiduria has been recommended in the past. However, in a recent study of 1738 kidney transplant recipients, of whom 11% had candiduria, no difference in outcomes was noted among treated or untreated patients with asymptomatic candiduria.

UTI IN KIDNEY–PANCREAS TRANSPLANT RECIPIENTS

Transplantation of the pancreas is increasingly used in the management of patients with type 1 diabetes mellitus with end-stage renal disease. The main types of pancreas transplantation are simultaneous kidney–pancreas transplantation (the most common), pancreas after kidney transplantation, or pancreas alone. In pancreatic transplantation, the drainage from the pancreas is diverted into the duodenum (enteric drainage) or into the urinary bladder.

Advances in surgical techniques and antirejection therapies have resulted in patient survival rates of greater than 95% and kidney and pancreas graft survival rates of 92–94% and 78–84%, respectively. Infections (especially UTIs) are more common in these patients compared to renal transplantation alone, due to the complexity of surgery and the structural and metabolic changes resulting from pancreatic secretions draining into the urinary bladder. Hence UTIs and urologic complications are more frequent in recipients with bladder drainage compared to those with enteric drainage. In contrast, recipients with enteric drainage have a higher rate of intra-abdominal infections.

Common uropathogens isolated from UTI in these patients are *E. coli*, *Klebsiella* spp., *Proteus* spp., *Pseudomonas* spp., coagulase-negative staphylococci and *Enterococcus* spp. The principles of management of UTI in these patients are the same as described in renal transplant recipients. UTIs are often recurrent and may result in epididymitis, prostatitis and prostatic abscesses. Such complications may necessitate conversion of urinary bladder drainage to enteric drainage.

CONCLUSION

UTIs are the most common infectious complication after renal transplantation and can occur despite trimethoprim–sulfamethoxazole prophylaxis. The epidemiology of UTIs varies based upon the time after transplantation. *Enterococcus* spp. and antibiotic-resistant Enterobacteriaceae have emerged as important uropathogens. Hence the antibiotic treatment of symptomatic UTIs in these patients should be guided by culture and susceptibility data. Until the natural history and optimal management of asymptomatic bacteriuria and candiduria are better defined, routine therapy of asymptomatic bacteriuria and candiduria may be unnecessary.

FURTHER READING


Further reading for this chapter can be found online at http://www.expertconsult.com

Chapter 57

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Syphilis

Venereal syphilis (from now on, referred to as 'syphilis') is a disease caused by the spirochete *Treponema pallidum* subspecies *pallidum* and is transmitted during sexual intercourse and other intimate contact; it may also be vertically transmitted by a pregnant woman to her fetus in utero or during birth.

Two other subspecies of *T. pallidum* – *pertenue* and *endemicum* – are responsible for yaws and endemic syphilis, respectively. The name 'syphilis' was drawn from a poem, 'Syphilis sive morbus gallicus', written by Fracastoro of Verona in 1530, in which the mythical swineherd Syphilis refused to make sacrifices to Apollo and was smitten as a result. In 1905, the association between *T. pallidum* and syphilis was first described by Schaudinn and Hoffman.[1]

EPIDEMIOLOGY

An epidemic of sexually transmitted syphilis spread across Europe at the end of the 15th century. There are conflicting views about its origin. Recent phylogenetic studies show that strains of *T. pallidum* subspecies *pallidum* are most closely related to yaws-causing strains found in South America rather than Old World strains.[2] This finding suggests that syphilis was most likely introduced to the Old World by Christopher Columbus and his men upon their return from the New World. The impact of syphilis in 16th century Europe was similar to the global impact of AIDS in the late 20th century. Syphilis had certainly become endemic in Europe by the 17th century, since then there have been several epidemic waves, notably during the Napoleonic wars and the period of industrialization in the 19th century, and during and after the two world wars of the 20th century.[3]

In North America and northern Europe, by the 1970s, syphilis had become predominately a disease of homosexual men. During the late 1980s, there were renewed outbreaks of heterosexual and congenital syphilis in North America in the wake of the HIV epidemic.[4] Syphilis rates reached a nadir in 2000, but began increasing shortly thereafter despite the introduction of a National Plan to Eliminate Syphilis. In 2006, 9756 primary and secondary syphilis cases (3.3 cases/100 000 population) were reported to the US Centers for Disease Control and Prevention (CDC), a 14% increase from the previous year.[5] From 2000 to 2005, these increases were predominantly among men (5.7 cases/100 000 men in 2006), with 60% occurring among men who have sex with men. In 2006, increases in women (1 case/100 000 women in 2006) and in cases of congenital syphilis (8.5 cases/100 000 live births in 2006) were noted after a steady decline.

In the UK there has been a substantial increase in syphilis cases: from 301 cases in 1997 to 3702 in 2006. The majority of cases are among men (8.2 cases/100 000 men in 2006). Almost a quarter of those with syphilis in the UK in 2006 were also infected with HIV. Early on, cases were predominantly reported from London and the North West of England,[6] but recent increases have been documented throughout the UK.

The annual incidence of congenital syphilis in infants aged less than 1 year in the US increased from 3.0 per 100 000 live births in 1980 to a peak of 107.3 per 100 000 live births in 1990. The very large increase in reported cases was artificially elevated by the introduction of a new reporting system. From 1996 to 2005, a steady 14% yearly decrease in the number of congenital syphilis cases was observed in the US. However, in 2006, a 3.7% increase from the previous year was noted in conjunction with the increased rates seen in women.

The World Health Organization (WHO) estimates that the annual global incidence of syphilis is approximately 12.2 million cases, most of which occur in developing countries, where the disease has remained a prominent cause of genital ulcer disease in heterosexual men and women, of stillbirth, and of neonatal morbidity and mortality. The prevalence of pregnant seropositive women is 0.1–0.6% in developed countries, but it may exceed 10% in many developing countries.

Transmission

The organism is transmitted from the early mucocutaneous lesions, primarily via sexual contact, and enters the body through small breaches in epithelial surfaces of genital, anorectal, oropharyngeal and other cutaneous sites (Fig. 57.1). The probability of sexual transmission to an uninfected partner in early syphilis has been estimated to be ~60% (range 9–80%).[7] Syphilis is rarely transmitted during transfusion of blood or blood products or through needle sharing by intravenous drug users.

Prenatal transmission is greatest in cases of maternal infection of short duration, but it may also occur during the latent stages of syphilis. Disease manifestations are unusual before 18 weeks in utero. Stillbirth caused by congenital syphilis has a maximum incidence at 6–8 months of gestation. Even when a previous pregnancy has resulted in an uninfected child, congenital syphilis may occur in subsequent offspring, and it may affect only one of twins. It is preventable with maternal treatment during pregnancy.[8]

PATHOGENESIS AND PATHOLOGY

Treponema pallidum subsp. *pallidum* is microaerophilic with a unique wave-like cell body that is 6–15 µm long and 0.15–0.20 µm wide. It contains a periplasmic flagellum and can be identified in darkfield microscopy by its characteristic morphology and movements, which typically include angling. The organism has evolved to become a highly invasive and persistent pathogen with little toxigenic activity and an

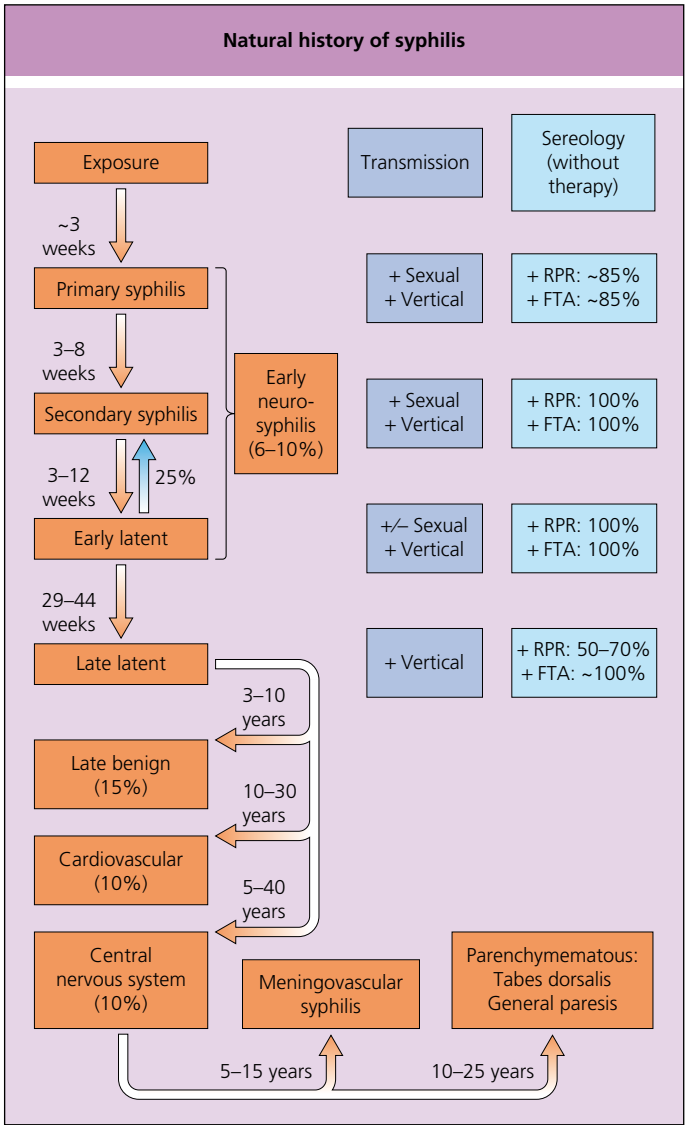


Fig. 57.1 The natural history of syphilis. FTA, fluorescent treponemal antibody absorbed test; RPR, rapid plasma reagin test.

inability to survive outside the mammalian host. It has extreme nutritional requirements, owing to deficiencies in biosynthetic pathways, and it has a narrow equilibrium between oxygen dependence and toxicity. It cannot be cultured on artificial media but it can be propagated in organ culture, such as rabbit testis. It has a slow growth rate, optimal at 91–95°F (33–35°C), with a doubling time of 30–36 hours.

Analysis of the genome, which is contained on a single circular chromosome with 1 138 006 base pairs, shows that the organism lacks lipopolysaccharide and lipid biosynthesis mechanisms, as well as many metabolic pathways, including pathways for the tricarboxylic acid cycle, for components of oxidative phosphorylation, and for most amino acids and vitamins. It requires D-glucose, maltose and mannose but cannot utilize other sugars. It is able to use exogenously supplied amino acids and is dependent on serum components such as fatty acids.

Treponema pallidum initiates an inflammatory response at the site of inoculation and is disseminated during the primary infection (reviewed by LaFond & Lukehart[9]). Animal studies have shown that treponemes appear within minutes in lymph nodes and blood,[10] and disseminate widely within hours. Eighteen hours following inoculation, treponemes are detectable in the cerebrospinal fluid (CSF) of rabbits. Following inoculation, *T. pallidum* gains access to deeper tissues by inducing the production of matrix metalloproteinase-1 in dermal cells. The organism activates endothelial cells which then signal inflammatory and immune cells to migrate into infected tissues. Polymorphonuclear lymphocytes are the first cells that respond to pathogen invasion, but their presence is limited and transient. Toll-like receptor (TLR)-2 has been shown to recognize *T. pallidum* microbial patterns and is involved in mediating the innate response.[11] Dendritic cells are stimulated by the TLR-2 pathway. They phagocytize the organisms and migrate to lymph nodes where they activate T cells.

T. pallidum components also induce the production of tumor necrosis factor (TNF) by macrophages (and other proinflammatory cytokines), which further activates the immune response. Humoral responses consisting of IgM and IgG antibodies are detected within 6 days after infection and are specific for a broad range of *T. pallidum* surface molecules. These antibodies have been shown to have opsonizing activity as well as the ability (in the presence of complement) to immobilize the organisms and neutralize their ability to form lesions. These antibodies have not been shown to be treponemicidal. The number of organisms within a lesion begins to decline significantly 14–16 days following infection.

Evasion of the host immune response is thought to occur via several mechanisms:[9]

- the antigenic inertness of the *T. pallidum* surface;
- limited number of organisms causing disease below the 'critical antigenic mass' necessary to stimulate a robust immune response;
- the invasion of immune-privileged sites;
- the slow generation time of the organism; and
- the ability of the organism to bypass the host's iron-sequestration mechanisms.

Histologic appearances and pathology

Some manifestations of syphilis (e.g. neuropathy) are immune-complex mediated. In early lesions, perivascular infiltration by lymphocytes and plasma cells is accompanied by intimal proliferation in arteries and veins. This leads to ischemia and ulceration. Organisms are most numerous in the walls of capillaries and lymphatic vessels.

In late lesions, the characteristic lesion of mucocutaneous surfaces is the syphilitic gumma. Granulation tissue forms with histiocytes, fibroblasts and epithelioid cells. Endarteritis obliterans and necrotic areas are pronounced. Gummas most often originate in subcutaneous tissues and spread in all directions. Spirochetes are not readily demonstrable in these lesions.

Heubner's arteritis occurs in cardiovascular and meningovascular syphilis. It is characterized by lymphocytic and plasma cell infiltration of the vasa vasorum and adventitia of large and medium-sized vessels. Occlusion of the vasa vasorum results in medial necrosis and fibroblast proliferation. There is associated subintimal proliferation, which leads to luminal occlusion and thrombosis.

PREVENTION

The risk factors for acquisition of syphilis mirror those of other sexually transmitted diseases (STDs). Primary prevention depends on similar methods, such as reducing the number of sexual partners and consistent use of condoms. Single-dose intramuscular benzathine penicillin G and oral azithromycin have been used as treatment for incubating syphilis.[12] Administration of treponemicidal antibiotics to treat other STDs, such as gonorrhea and chancroid, may also abort incubating concurrent syphilis. The development of a vaccine against syphilis has long been inhibited by the inability to grow the organism on artificial media.

Secondary prevention by early diagnosis, treatment, partner notification, education and counseling remain the mainstay of prevention efforts. Access to prompt and appropriate services for infected persons

is essential. In many developed countries, clinic-based specialist services have long been established. In developing countries, the WHO has recommended that STD management be integrated into basic health care and reproductive health services.

Serologic screening for syphilis remains a cost-effective measure for control. Congenital syphilis may be prevented by maternal screening and treatment during early pregnancy. In the US, screening is advocated at the first prenatal visit; among high risk women, it should be repeated twice during the third trimester (28–32 weeks' gestation and at delivery).[13] In developing countries, where congenital syphilis is more common, the disease occurs when there has been no maternal screening, when no treatment has been administered in response to positive tests, or when primary infection occurs later in pregnancy.

CLINICAL FEATURES

Studies conducted in the US and Europe in the early 20th century helped to define the natural history of syphilis,[14–16] summarized in Figure 57.1.

Incubation period

The time from transmission to the appearance of primary lesions averages 21 days (range 10–90 days); the incubation period varies inversely with the size of the spirochete inoculum.

Primary syphilis

The primary chancre appears at the site of initial treponemal invasion of the dermis. It may occur on any skin or mucous membrane surface and is usually situated on the external genitalia (Fig. 57.2). Initial lesions are papular but rapidly ulcerate. They are usually single, but 'kissing' lesions may occur on opposing mucocutaneous surfaces. Typically, the ulcers are nontender (unless there is coexisting infection) and indurated, and have a clean base and raised edges (Fig. 57.3). There is often surrounding edema, especially with vulval lesions. Chancres of the cervix, anorectum or oropharynx are commonly silent. Nontender, nonsuppurative, rubbery inguinal lymphadenopathy appears 1 week later and usually becomes bilateral after 2 weeks. The chancre usually heals spontaneously within 3–6 weeks but leaves a scar.

The differential diagnosis includes other sexually transmitted causes of genital ulcer disease (which may coexist) such as chancroid, lymphogranuloma venereum, donovanosis, genital herpes and HIV, as well as traumatic ulceration, pyogenic lesions, aphthous ulceration and malignancy.



Fig. 57.2 Primary chancre in coronal sulcus in primary syphilis. A typical solitary lesion with raised everted edges, central ulceration and undermined base. From Kinghorn GR. Syphilis. Medicine 1995;24:64–8, with permission of The Medicine Group (Journals) Ltd.



Fig. 57.3 Chancre of upper lip in primary syphilis. The chancre shows the characteristic features of a raised, rolled and everted edge, central ulceration and a granular base. From Kinghorn GR. Syphilis. Medicine 1995;24:64–8, with permission of The Medicine Group (Journals) Ltd.

Secondary syphilis

The manifestations of generalized treponemal dissemination first appear about 8 weeks after infection. Constitutional symptoms consist of fever, headache, and bone and joint pains. There is wide diversity in physical features.

Skin rashes are the most common feature. They are initially macular and become papular by 3 months. Lesions appear initially on the upper trunk (Fig. 57.4), the palms and soles (Fig. 57.5), and flexural surfaces of the extremities. The later papulosquamous eruptions typically have a coppery color and often follow skin cleavage lines, as in pityriasis rosea. Facial lesions follow the hairline of the temporal and frontal scalp (the so-called corona veneris) and cause split papules at the angles of the mouth (Fig. 57.6). There may be hypopigmented lesions on the lateral neck (collaris veneris). Lesions in hairy areas cause moth-eaten alopecia of the scalp, beard, eyebrows and eyelashes.

Condylomata lata are moist flat-topped papules that appear about 6 months after infection in the moist intertriginous areas around the genitalia, anus and axillae, and beneath the breasts. On mucous membranes, especially of the mouth, erythematous macules evolve into asymptomatic, slightly elevated, flat-topped lesions covered by a



Fig. 57.4 Maculopapular rash on trunk in secondary syphilis. From Kinghorn GR. Syphilis. Medicine 1995;24:64–8, with permission of The Medicine Group (Journals) Ltd.

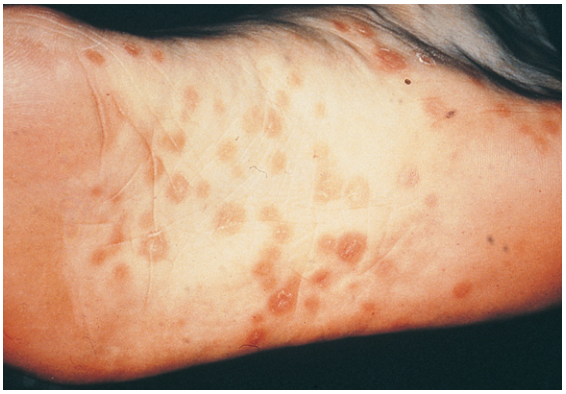


Fig. 57.5 Plantar syphilid in secondary syphilis. From Kinghorn GR. Syphilis. Medicine 1995;24:64–8, with permission of The Medicine Group (Journals) Ltd.



Fig. 57.6 Split papules at angle of mouth and mucous patch on lower lip in secondary syphilis. From Kinghorn GR. Syphilis. Medicine 1995;24:64–8, with permission of The Medicine Group (Journals) Ltd.

hyperkeratotic grayish membrane. These mucous patches may coalesce to form 'snail-track' ulcers.

Generalized lymphadenopathy occurs in 50% of cases of secondary syphilis. It has similar characteristics to the localized lymphadenopathy of primary infection. Other systemic features of secondary syphilis include panuveitis, periostitis and joint effusions, glomerulonephritis, hepatitis, gastritis, myocarditis and aseptic meningitis.

The lesions of secondary syphilis resolve spontaneously in a variable time period and most patients enter the latency stage within the first year of infection. In some, especially the immunocompromised, primary or secondary lesions may recur.

The differential diagnosis is broad and includes exanthema associated with many infectious diseases, including primary HIV infection, dermatoses (e.g. pityriasis rosea and guttate psoriasis) and connective tissue disorders such as systemic lupus erythematosus. Condylomata lata must be differentiated from viral warts, scabies and lichen planus.

Latent syphilis

In latent syphilis there are no clinical stigmata of active disease, although disease remains detectable by positive serologic tests. In early latency, within 1–2 years of infection, up to 25% of asymptomatic patients will experience secondary relapses, hence the distinction between early and late latency. Vertical transmission of infection may still occur, but sexual transmission is less likely in the absence of mucocutaneous lesions. The late manifestations of syphilis arise, often decades later, in about 30% of those who have latent syphilis.

Tertiary gummatous syphilis

The characteristic lesions of tertiary syphilis appear 3–10 years after infection and consist of granulomas or gummas. The granulomas appear as cutaneous plaques or nodules of irregular shape and outline (Fig. 57.7) and are often single lesions on the arms, back and face. Gummas are areas of granulomatous tissue, often arising from subcutaneous structures. They have a tendency for central necrosis and ulceration and for peripheral healing with tissue-paper scarring. Punched-out lesions appear most commonly on the scalp, face and sternoclavicular areas of the chest and on the lateral calf (Fig. 57.8). Gummas can also cause palatal perforation; destruction of nasal cartilage, producing saddle-nose deformity; painless testicular swelling, which may mimic a tumor; portal hypertension and portosystemic anastomoses; and diffuse interstitial glossitis and subsequent malignant neoplasms of the tongue.

Cardiovascular syphilis

The typical lesion of cardiovascular syphilis is aortitis affecting the ascending aorta and appearing 10–30 years after infection. The aortitis may be asymptomatic and detected as dilatation of the ascending aorta on chest radiography, often accompanied by linear calcification of the aortic wall, or it may lead to stretching and incompetence of the aortic valve, to left ventricular failure or to aneurysm formation.



Fig. 57.7 Cutaneous nodular gummas of upper arm in tertiary gummatous syphilis. The lesions have a serpiginous outline. From Kinghorn GR. Syphilis. Medicine 1995;24:64–8, with permission of The Medicine Group (Journals) Ltd.

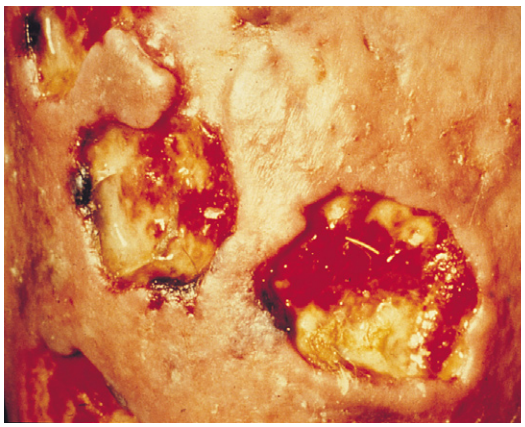


Fig. 57.8 Multiple gummatous ulcers of lower leg in tertiary gummatous syphilis. The lesions have a punched-out appearance with 'wash-leather' slough overlying a base of granulation tissue. They show a tendency for peripheral healing with thin tissue-paper scars. From Kinghorn GR. Syphilis. Medicine 1995;24:64–8, with permission of The Medicine Group (Journals) Ltd.

Aneurysms may be associated with a variety of syndromes caused by pressure on adjacent structures in the mediastinum, and they may cause sudden death from rupture. Other symptoms include angina pectoris from associated coronary ostial stenosis. Cardiovascular syphilis is more commonly associated with neurosyphilis than with gummatous disease.

The differential diagnosis of thoracic aortic aneurysm includes Behçet's disease, Takayasu's arteritis, ankylosing spondylitis and atheromatous disease. Similar histologic lesions may appear in other large arteries.

Neurosyphilis

Treponemes invade the central nervous system very early after infection[17,18] but the majority of patients are asymptomatic. Neurosyphilis is characterized by a number of heterogeneous syndromes. The onset can occur weeks or decades after treponemal dissemination.

Asymptomatic neurosyphilis precedes the development of clinically apparent disease and accounts for one-third of all neurosyphilis. It occurs in 10% of those with latent disease and has a peak incidence at 12–18 months after infection. It reverts spontaneously in 70% of patients.

Meningeal neurosyphilis usually has its onset during the early stages of disease and is characterized by symptoms of headache, confusion, nausea and vomiting, neck stiffness and photophobia. There may be focal seizures, aphasia, delirium and papilledema. Cranial nerve palsies cause unilateral or bilateral facial weakness and sensorineural deafness. Other manifestations include hydrocephalus, spinal pachymeningitis and spinal meningomyelitis.

Meningovascular syphilis occurs most frequently between 4 and 7 years after infection. Symptoms usually begin abruptly, and focal neurologic signs are most common in the territory of the middle cerebral artery. Less commonly, focal ischemia affects the basilar or spinal arteries. The clinical features of hemiparesis, seizures and aphasia reflect multiple areas of infarction from diffuse arteritis.

Gummatous neurosyphilis is the least common syndrome. Lesions arise from the pia mater and subsequently invade the brain or spinal cord, resulting in features typical of a space-occupying lesion of these structures.

Parenchymatous syphilis appears later and has become rare in its classic forms in the antibiotic era. The peak incidence of general paresis from parenchymatous disease of the brain used to be 10–20 years after infection. It was more common in males. The onset is insidious with subtle deterioration in cognitive function and psychiatric symptoms that mimic those of other mental disorders. As the disease progresses, neurologic signs develop, including pupillary abnormalities, hypotonia of the face and limbs, intention tremors and hyperreflexia. In late disease, there is progressive dementia, onset of seizures and increasing weakness leading to an incontinent bedfast state.

Tabes dorsalis, the second form of parenchymatous neurosyphilis, was the most common form of neurosyphilis before antibiotics were available. It has an onset 15–25 years after primary infection. The most characteristic symptom is of lightning pains – sudden paroxysms of lancinating pain affecting the lower limbs. Other early symptoms include paresthesia, progressive ataxia, and bowel and bladder dysfunction. The clinical signs result from leptomeningeal infiltration of the preganglionic portion of the dorsal root ganglia, with subsequent atrophic change in the posterior columns of the spinal cord. The signs are hypotonia, areflexia, and impaired joint-position and vibration sense. Pupillary abnormalities are usual, and 50% of patients have the classic Argyll Robertson pupil (small, irregular pupils that are unreactive to light but constrict normally to accommodation–convergence). Optic atrophy is also common. Later features of tabes dorsalis include visceral crises in 10–15% of patients, characterized by recurrent episodes of epigastric pain and vomiting, mimicking an acute abdomen; acute urinary retention; progressive ataxia with a wide-based slapping gait; the appearance of neuropathic joints and perforating ulcers; and coexisting palsies of the third, sixth and seventh cranial nerves.

The differential diagnosis of neurosyphilis covers the whole spectrum of neurologic and psychiatric conditions. Routine serologic screening tests for syphilis are indicated in patients who exhibit any of these features.

Congenital syphilis

Many infants with congenital syphilis are asymptomatic at birth. The placenta may show proliferative vascular changes and there may be acute inflammation of the umbilical cord (funisitis). Early congenital syphilis manifests itself as rhinitis with serosanguinous nasal discharge, vesiculobulbous eruptions of the skin and oral mucous patches. Skin lesions on the lips, nostrils and anus heal with radiating scars (rhagades). In addition, there are often bone abnormalities, characterized by diaphyseal periostitis, osteochondritis and a positive Wimberger's sign, which may present with limb pseudoparesis. Other features include chorioretinitis, visceral lesions causing pneumonia alba, hepatosplenomegaly associated with jaundice and the nephrotic syndrome.

In late congenital syphilis (presenting after 2 years of age) there may be a variety of skeletal developmental defects, including a high-arched palate, a protruding mandible, frontal bossing of the skull and saddle-nose deformity resulting in a characteristic facies (Fig. 57.9); additionally, there can be bilateral hydroarthoses of the knees (Clutton's joints), sabre tibiae from osteoperiostitis, perforation of the palate and nasal septum, and sternoclavicular thickening. Dental abnormalities occur with peg-shaped permanent incisors (Hutchinson's teeth) and mulberry multicusped molars; together with interstitial keratitis and eighth nerve deafness, these dental abnormalities form Hutchinson's triad. Other features include hydrocephalus and mental retardation, as well as other typical lesions of gummatous syphilis and neurosyphilis.

Syphilis and HIV infection

Some 16–25% of patients infected with syphilis in the US and the UK are estimated to be co-infected with HIV (reviewed by Zetola & Klausner[19]). The diagnosis of one condition requires immediate testing for the other. The presence of syphilitic ulcers increases the risk of HIV transmission and acquisition.[20] Several studies have reported differences in clinical manifestations among HIV-infected and uninfected patients.[21,22] The major finding consistently reported by these studies is the collapse of the usual timeline of the natural history of syphilis – it is not uncommon to find patients presenting with classic manifestations of primary and secondary syphilis concomitantly. In addition,



Fig. 57.9 Typical facies in late congenital syphilis. There is frontal bossing, an underdeveloped maxilla, a prominent jaw and a depressed nasal bridge, with multiple gummatous ulcers of scalp. From Kinghorn GR. Syphilis. Medicine 1995;24:64–8, with permission of The Medicine Group (Journals) Ltd.

several studies highlighted the increased frequency of early symptomatic (meningeal) neurosyphilis among dually infected patients.[23]

Diagnosis of syphilis in HIV-infected patients is similar to HIV-uninfected patients, but there is an increased frequency of false-positive reaginic tests[24] (see below). Very rarely, false-negative reaginic and treponemal tests have been reported.[25]

DIAGNOSIS

Microscopic identification

Treponema pallidum can be identified from genital lesions of primary syphilis and lesions from secondary or early congenital syphilis by darkfield microscopy. Darkfield microscopy is the gold-standard test to diagnose the genital chancres of primary syphilis as serologic tests may be negative in up to 30% of patients. The specificity of darkfield microscopy in diagnosing syphilis from oral or rectal mucosal lesions is much lower given the presence of nonpathogenic treponemes at those sites. Lesions are thoroughly cleansed with saline and scarified if necessary. Lymph node aspirates are also useful for microscopic examination in secondary syphilis.

The organism can also be identified by direct immunofluorescent antibody testing where no facilities for darkfield microscopy exist.

In biopsy specimens from late syphilis or in atypical early lesions, it may be possible to identify the organism by silver stains such as Warthin–Starry preparations or by direct immunofluorescent antibody testing.

Diagnosis using the polymerase chain reaction (PCR) has been based on primers and probes prepared from the 47 kDa gene. This technique is not routinely available.

Serologic testing

The serologic tests for syphilis can be subdivided into two types: standard nontreponemal (reaginic) tests and the specific treponemal antibody tests (Table 57.1). The former tests are sensitive but not specific and they provide titers that can be followed after therapy. The latter are sensitive and specific but do not provide titers to monitor therapeutic response (reviewed by Larsen *et al.*[26]). As the relationship between microbiologic status (i.e. presence of treponemes) and serologic status is not well defined, serologic failure may not reflect microbiologic failure; similarly, serologic response to therapy may not reflect microbiologic eradication of the organism.[27]

Standard nontreponemal tests

The nontreponemal tests detect IgM and IgG antibodies to lipoidal material released from damaged host cells and to lipoidal-like antigens of *T. pallidum*. There are several tests available that use the VDRL antigen (consisting of cardiolipin, cholesterol and lecithin) as the principal component. These tests are quantitative and are useful in assessing response to treatment. Reactivity to these tests does not develop until 1–4 weeks after the chancre appears in primary syphilis. Titers are highest in secondary syphilis. The prozone phenomenon occurs in 2% of sera; undiluted sera give negative results because of antibody excess or the presence of blocking antibodies, or both. The titer slowly declines after the 1- to 4-week period following the appearance of the chancre, and it may spontaneously become negative in some cases of late latent syphilis and neurosyphilis (see Fig. 57.1).

The VDRL slide test is widely used and requires the microscopic demonstration of antigen–antibody flocculations in heat-inactivated serum.

The rapid plasma reagin (RPR) test uses charcoal added to the VDRL reagent to enhance visualization of the antigen–antibody flocculations. The flocculations are visible macroscopically.

Biologic false-positive reactions to these tests occur in a wide variety of conditions. They can be subdivided into acute reactions, lasting for 6 months or less, and chronic reactions (Table 57.2).

Specific treponemal antibody tests

Specific treponemal antibody tests are used for confirmatory testing. They detect IgG antibodies to antigenic determinants of treponemes. They are qualitative procedures and are not helpful in assessing treatment responses; once positive, they tend to remain positive for life, irrespective of treatment. They are used to differentiate true-positives from false-positives in the standard nontreponemal antibody tests.

The fluorescent treponemal antibody absorption (FTA-ABS) test and the FTA-ABS double-staining (FTA-ABS DS) test are both indirect immunofluorescent tests. The double stain test employs a fluorochrome-labeled counterstain for *T. pallidum* and an antihuman IgG conjugate labeled with tetramethyl rhodamine isothiocyanate to detect antibody in patient serum. False-positive results may occur in about 1% of sera; possible causes include technical error, Lyme borreliosis, pregnancy, genital herpes, alcoholic cirrhosis and connective tissue diseases such as systemic lupus erythematosus and scleroderma.

The microhemagglutination assay for antibodies to *T. pallidum* (MHA-TP) detects passive hemagglutination of erythrocytes sensitized with ultrasonicated Nichol's strain *T. pallidum*. In many laboratories, the TPHA has been replaced by the similar *T. pallidum* particle

Table 57.1 Serologic tests for syphilis

	SENSITIVITY*				
	Primary	Secondary	Latest	Late	Specificity*
Nonspecific reaginic tests					
VDRL	80	100	95	70	98
RPR	86	100	98	75	98
USR	80	100	94		98
TRUST	85	100	97		97
Specific treponemal antibody tests					
MHA-TP	76	100	96	95	99
FTA-ABS	84	100	100	96	97
FTA-ABS double-staining	80	100	100		98

*The values for sensitivity are the mean values in CDC (Centers for Disease Control and Prevention) tests. The values for specificity are mean values in nonsyphilis populations in CDC tests.

Table 57.2 Causes of biologic false-positive cardiolipin tests

	Acute	Chronic
Physiologic	Pregnancy	Old age
Spirochete infections	Leptospirosis Relapsing fever Rat-bite fever Lyme disease	
Other infections	Varicella zoster Herpes simplex Infectious mononucleosis Cytomegalovirus Toxoplasmosis Viral hepatitis HIV seroconversion illness *Mycoplasma* infection Malaria Other acute viral or bacterial sepsis	Lepromatous leprosy Tuberculosis Lymphogranuloma venereum Malaria Kala-azar Trypanosomiasis Tropical spastic paraparesis HIV or AIDS
Vaccinations	Smallpox Typhoid Yellow fever	
Autoimmune diseases		Systemic lupus erythematosus Polyarteritis nodosa Rheumatoid arthritis Sjögren's syndrome Primary biliary cirrhosis Hashimoto's thyroiditis Autoimmune hemolytic anemia Idiopathic thrombocytopenic purpura
Other		Malignancy Malnutrition Injecting drug use

agglutination (TPPA) test but uses gelatin particles rather than erythrocytes as the carrier. It is more sensitive than the FTA-ABS.

Treponemal enzyme immunoassay (EIA) commercial tests were initially designed as confirmatory tests for syphilis. Serum is added to microwells coated with a treponemal antigen. After incubation, an enzyme-labeled antihuman immunoglobulin conjugate and enzyme substrate are added to detect antigen–antibody reaction. The test also has advantages of automated or semiautomated processing and objective reading of results, and it can be interfaced with laboratory computer systems to allow electronic laboratory report generation. The test can be modified to detect specific IgM antibody.

There are many commercial tests in any given format whose performance characteristics vary.

Indications for examination of cerebrospinal fluid

Examination of cerebrospinal fluid (CSF) in syphilis should be considered if:[28]

- neurologic, ophthalmic or auditory symptoms and signs are present;
- other clinical evidence of active infection (e.g. aortitis, gumma, iritis) is present;
- serologic failure occurs despite adequate therapy (and no evidence of re-infection);
- HIV infection is present (especially if RPR = 1:32 and CD4 <350 cells/ml);
- serum nontreponemal titer >1:32 if duration of syphilis is more than 1 year; and
- nonpenicillin-based treatment regimen is planned.

The typical CSF findings of neurosyphilis consist of:

- moderate mononuclear pleocytosis (10–400 cells/ml);
- elevated total protein (0.46–2.0 g/l); and
- positive CSF VDRL.

The CSF VDRL is highly specific, and false-positive results are rare in the absence of blood contamination.[29] A negative CSF VDRL does not exclude neurosyphilis. Treponemal-specific tests performed on CSF may have higher sensitivity than CSF VDRL, but false-positives have been reported.[30]

Typical neuroimaging findings have been described for neurosyphilis.[31]

Evaluation of neonates for congenital syphilis

The definitions of congenital syphilis are listed in Table 57.3. It is recommended that the following investigations be carried out in a child born to a seropositive mother if there has been no documented completion of treatment at least 4 weeks before delivery, if a nonpenicillin regimen was administered, or if relapse or re-infection is suspected:[28]

- physical examination for stigmata of congenital syphilis;
- radiography of long bones for evidence of periostitis;
- examination of the CSF; and
- darkfield microscopic examination (or DFA staining) of suspicious lesions or body fluids

Infection of the neonate is also suggested if the serum (not cord blood) nontreponemal antibody titer is four or more times greater than the mother's, or if specific IgM treponemal antibody tests are positive. Passively transferred maternal IgG antibody can persist in the infant's serum for up to 12 months.

MANAGEMENT

Current treatment regimens are based on over 50 years of clinical experience with penicillin,[32] expert opinion, and open clinical studies rather than on randomized clinical trials. Thus, significant differences

Table 57.3 Definitions of congenital syphilis

Confirmed diagnosis	An infant in whom *T. pallidum* is identified by darkfield microscopy, fluorescent antibody or other specific stains in specimens from lesions, placenta, umbilical cord or autopsy material
Presumptive diagnosis	1. Any infant whose mother had untreated or inadequately treated syphilis at delivery, regardless of symptoms or signs in the infant 2. Any infant or child who has a reactive specific treponemal test for syphilis and one of the following: evidence of congenital syphilis on physical examination evidence of congenital syphilis on long-bone X-ray reactive CSF VDRL elevated CSF cell count or protein (without other cause) reactive test for FTA-ABS-IgM using fractionated serum

Table 57.4 Treatment of acquired syphilis comparing the US and UK recommendations

Stage	US CDC-recommended regimens	US alternative regimens	UK recommended regimens
Early syphilis (primary, secondary, early latent of <1–2 years' duration)	Benzathine penicillin G 2.4 million units im single dose	Tetracycline 500 mg po q6h for 14 days Doxycycline 100 mg po q12h for 14 days Azithromycin 2 g po single dose Ceftriaxone 1g im q24h for 10 days	Procaine penicillin G 600 000 units im q24h for 10 days Benzathine penicillin G 2.4 million units im once
Late syphilis (latent syphilis of >1–2 years' duration, cardiovascular, gummatous syphilis)	Benzathine penicillin G 2.4 million units weekly for 3 weeks	Tetracycline 500 mg po q6h for 28 days Doxycycline 100 mg po q12h for 28 days	Procaine penicillin 600 000 units im q48h for 17 days Benzathine penicillin G 2.4 million units weekly for 3 doses
Neurosyphilis	Aqueous crystalline penicillin G 3–4 million units iv q4h for 10–14 days	Ceftriaxone 2 g im or iv q24h for 10–14 days	Procaine penicillin 1.8–2.4 million units im q48h plus probenecid 500 mg po q6h for 17 days Benzyl penicillin 3–4 million units iv q4h for 17 days

exist when comparing treatment guidelines from different countries (Table 57.4). Many antibiotics, with the notable exceptions of the aminoglycosides and sulfonamides, have some treponemicidal activity, and their administration for other conditions may abort or modify the natural history of syphilis.[27]

Parenteral penicillin G is the preferred drug at all stages of syphilis.[33] The preparations used, the dosage and the duration of treatment depend on the clinical stage and disease manifestations (see Table 57.4).[28] Adequate treatment requires the maintenance of serum concentrations in excess of 0.03 units/ml for at least 10 days.[34] A single intramuscular dose of 600 000 units of aqueous procaine penicillin gives an effective serum concentration for at least 24 hours; in comparison, a single intramuscular dose of 2 400 000 units of long-acting benzathine penicillin G maintains effective levels for about 2 weeks. Of note, the latter dose does not provide consistent treponemicidal levels in the CSF.

In patients who are hypersensitive to penicillin, regimens based on tetracycline, doxycycline,[35] erythromycin, ceftriaxone[36] and chloramphenicol have all been successfully used to treat syphilis; however, success is less assured than with penicillin. Azithromycin has been shown to be effective at treating early syphilis,[37] but emerging resistance may limit widespread use.[38]

Ceftriaxone, a third-generation cephalosporin, may be used as an alternate agent for the treatment of neurosyphilis,[39] but efficacy data are limited.

Desensitization of penicillin-allergic patients is recommended for the treatment of pregnant women and (may be considered for) neurosyphilis because parenteral penicillin G is the only treatment with well-documented efficacy in these situations.

Jarisch–Herxheimer reaction

The Jarisch–Herxheimer reaction is an acute febrile reaction that occurs in many patients within 24 hours of commencing treatment. It is mediated by cytokines. The fever may be accompanied by headache, myalgia, bone pains and an exacerbation of skin lesions. It must be differentiated from penicillin allergy. Patients should be advised that it may occur. Symptoms may be controlled by antipyretics.

In pregnant women the reaction may induce early labor or cause fetal distress. In late neurosyphilis and cardiovascular syphilis, the Jarisch–Herxheimer reaction can be more serious and may be associated with life-threatening sequelae. Many clinicians advocate a short course of corticosteroids to lessen its effects in these patients.

Treatment of congenital syphilis

The optimal treatment of congenital syphilis is unknown. Regimens that have been recommended for early congenital syphilis are intravenous aqueous crystalline penicillin G 50 000 units/kg every 8–12 hours for 10–14 days, or intramuscular procaine penicillin G 50 000 units/kg once daily for 10–14 days.[28] For infants with normal CSF findings, intramuscular benzathine penicillin G 50 000 units/kg in a single dose has also been successful. In children with late congenital syphilis presenting after 2 years of age, regimens should be the same as those recommended for late acquired disease.

Follow-up

The nontreponemal serologic test titers are followed after therapy at bi-yearly intervals to document response. Response is defined as a fourfold decline in titers at a pre-specified time (depending on stage) following therapy.[40] Failure is defined as a lack of fourfold decline during that interval, a fourfold increase in titers after appropriate therapy, or any clinical signs or symptoms consistent with syphilis.

For early syphilis, a serologic response is expected 6–12 months after therapy. For late stage disease, a decline is expected 12–24 months after therapy. An inappropriate serologic response may reflect treatment failure or re-infection. If treatment failure cannot be reliably ruled out, patients should undergo a CSF examination prior to retreatment. For patients diagnosed with neurosyphilis who had abnormalities in their CSF, repeat CSF examinations should be conducted every 6 months until resolution of all abnormalities. If there is worsening of CSF parameters or persistent abnormalities after 2 years, retreatment should be considered.

Treatment of syphilis in HIV-seropositive patients

The current recommendations are to treat HIV-infected patients with the standard therapeutic regimens used in HIV-uninfected patients; enhanced therapy was not shown to be beneficial.[41] Response to standard therapy in HIV-infected patients is a controversial topic, with several studies documenting increased serologic and clinical failure rates compared to HIV-uninfected patients.[42,43] Whether this reflects microbiologic failure or merely a delay in the response of the reaginic serologic titers to standard therapy is unclear. Currently, the main recommendation is to obtain more frequent serologic follow-up testing to ensure appropriate response to therapy. Thus, dually infected patients

should have follow-up reaginic tests performed at 3, 6, 9, 12 and 24 months after therapy. In HIV-infected patients treated for neurosyphilis, a follow-up lumbar puncture 6 months after therapy is recommended and at 6-month intervals until abnormalities have resolved. Several studies have suggested that CSF and neurologic abnormalities may persist for a prolonged period despite adequate therapy.[44,45] Decisions on retreatment should be individualized.

Recent data suggest that reversal of immunosuppression by the use of highly active antiretroviral therapy may decrease the risk for syphilis serologic failure among HIV-infected patients.[46,47]

Management of sexual contacts

It is recommended that attempts be made to identify, trace and offer further investigation to at-risk sexual contacts. In early syphilis, these are those contacts occurring within 3 months plus the duration of symptoms for primary syphilis, within 6 months plus the duration of symptoms for secondary syphilis and within 1 year for early latent disease. All long-term partners of patients with late syphilis should be offered investigation.

Many clinicians recommend presumptive treatment of all sexual contacts within the 90-day period preceding patient presentation of early syphilis if serologic test results are not immediately available and if follow-up cannot be assured.

Prognosis

In the pre-HIV era, cure rates with initial treatment of early syphilis were better than 95%. The long-term outcome of adequately treated cases is excellent. In late syphilis, infection can usually be arrested, although some treponemes may persist in less accessible sites (e.g. the eye and nervous system). As long as immune function is normal, this persistence of treponemes rarely has clinical sequelae. The outlook for HIV-positive and other immunocompromised patients appears to be less assured.

REFERENCES


References for this chapter can be found online at http://www.expertconsult.com

Chapter 58

Diane Hanfelt-Goade

Genital herpes

Genital herpes is one of the most common sexually transmitted diseases. Of these, the agents of genital herpes – herpes simplex virus (HSV) type 2 and, less commonly, HSV type 1 – are among the most frequently encountered human pathogens.[1,2]

EPIDEMIOLOGY

Genital herpes infections are common, with estimates of 500 000–700 000 cases of symptomatic first episodes/year in the USA.[3–7] Humans are the natural reservoir for HSV and virtually all cases of genital herpes are sporadic, acquired via person-to-person transmission. There are no reported epidemics of genital herpes.[2]

Herpes simplex virus infections have a worldwide distribution, with seroprevalence to either HSV-1 or HSV-2 approaching 90% in some age and sex groups.[3–7] Herpes simplex virus-1 infection is common early in life, typically as orolabial 'cold sores' with antibodies appearing in childhood and prevalence increasing with age; HSV-2 antibodies increase in prevalence with increasing age after the onset of sexual activity. Approximately one in every four adults in the USA is seropositive for HSV-2.[3–7] Overall, however, HSV-2 seroprevalence decreased approximately 19% in the USA by 2004, compared to surveys done 10 years previously (overall seroprevalence to HSV-2 of 17.0%, compared a previous overall seroprevalence of 21.0%).[8] Herpes simplex virus-2 seroprevalence rates in the USA differ for some racial, sex and ethnic groups. Women acquire HSV-2 infections more readily than men and overall have a higher seroprevalence rate.[6,9] Other risk factors for genital herpes include lower socioeconomic status, increased number of sexual partners, African-Caribbean race and Hispanic ethnicity.[4–7] The highest prevalence of HSV-2 antibodies is among commercial sex workers and up to 70% of prostitutes in the USA have the infection. The lowest HSV-2 prevalence is in sexually abstinent groups, including nuns, where seroprevalence is 3% or lower.[4–7]

The incidence of seroconversion is approximately 5–10%/year when discordant couples are followed longitudinally. Among HSV-naive females with male partners who have the infection, seroconversion is as high as 15–30%/year. However, when the female partner has the infection first, less than 5% of male partners seroconvert/year.[9]

After infection with an HSV, antibodies to HSV-1 and HSV-2 type common antigens provide partial protection to infection with the counterpart virus. In prospective studies, women who have HSV-1 infection have a 5–20%/year lower rate of seroconversion to HSV-2 than women who do not have HSV infection.[9] Seroconversion to HSV-1 during childhood has decreased, particularly in upper and middle class socioeconomic groups in developed countries, whereas symptomatic infection with HSV-2 during adulthood has increased, and first visit for medical care of genital herpes remains on the rise.[4,5]

PATHOGENESIS

Herpes simplex viruses are large enveloped DNA viruses with a diameter of approximately 150 nm, a dsDNA core, an icosahedral capsid composed of 162 capsomers, an amorphous tegument layer and a lipid envelope. There are 11 different glycoproteins projecting from the envelope that are crucial for virion-cell surface attachment and cell-to-cell spread (Fig. 58.1; see also Chapter 155).[1,10]

Herpes simplex viruses are spread by direct contact, including contact with infected secretions:

- HSV-1 is typically spread through close contact with infected oral secretions, and genital herpes due to HSV-1 is usually due to oral–genital contact; and
- HSV-2 is primarily spread through intimate contact with infected genital secretions and tissues.

Intact skin is fairly resistant to virus infection, but abraded skin or mucous membranes are more susceptible.

The virus attaches to the cell surface and the viral envelope fuses with the cell membrane using a specific cellular receptor. Virion–cell surface attachment and virus intracellular penetration are mediated by viral surface glycoproteins. The viral nucleocapsid is released into the cytoplasm where it is transported to the nuclear pores of the cell. Following viral DNA replication and gene expression in the cell nucleus, the replicated nucleocapsid is assembled and buds through the nuclear membrane, acquiring an envelope. The enveloped nucleocapsid is translocated across the cytoplasm and cell membrane, acquiring surface glycoproteins at both the nuclear membrane and cell surface.[1,10]

Herpes simplex virus then infects and replicates in parabasal and intermediate skin cells. Replication results in lysis of the infected cell. Infection may spread locally by direct cell-to-cell invasion or to more distant sites via sensory nerve pathways. As virus replication spreads to involve the local autonomic and sensory nerve endings, retrograde transmission of virions (or possibly nucleocapsids) occurs with transport of virus particles to the regional sensory ganglia. Transient virus replication may occur in the ganglia at this point. From the sensory ganglia, antegrade virion migration along sensory nerves allows viral spread to other sites. By this method of spread, crops of herpetic lesions may arise at nonadjacent sites such as the thighs or buttocks. Virus replication is associated with cell lysis, cell destruction and local inflammation of all tissues except the sensory ganglia.[2,11,12]

Central nervous system disease may occur as an aseptic meningitis with primary HSV-2 or as a necrotic focal encephalitis with HSV-1 via spread through the cribriform plate to the temporal horns. Recurrent aseptic meningitis has been described, but is rare. Rarely, hematogenous dissemination may occur with visceral organ involvement, most commonly in immune compromised patients.[2]

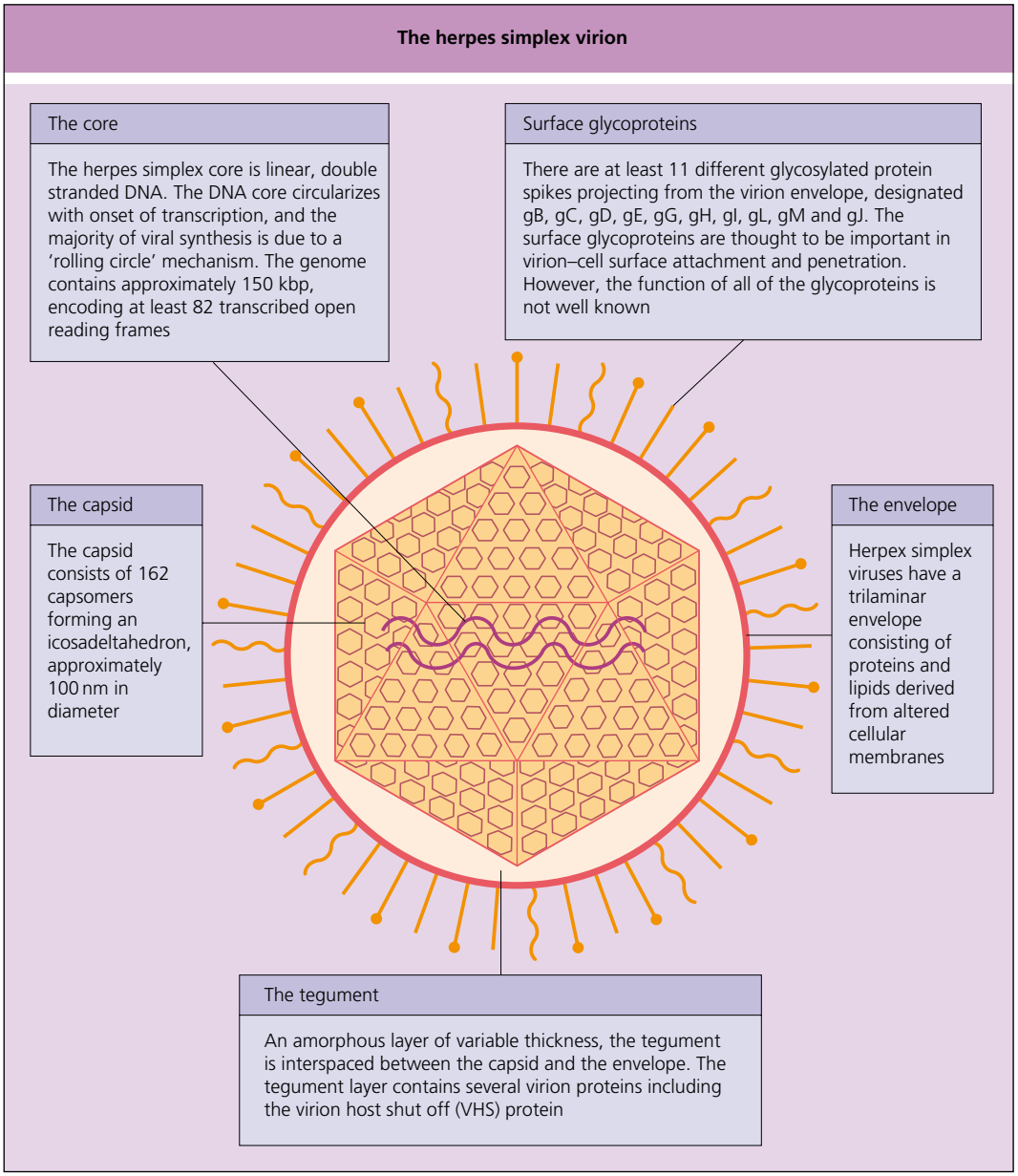


Fig. 58.1 The herpes simplex virion. This consists of a dsDNA core surrounded by a capsid, an amorphous tegument layer and a lipid envelope with numerous glycoprotein spikes. The overall diameter is 150–200 nm. Virus replication takes place within the nucleus of the infected cell. The envelope is gained as the virion passes through the nuclear membrane. Replication of virus within the host cell results in cell lysis and destruction. Latent virus does not cause neural cell lysis within ganglia.

Immune response

Both cellular and humoral host immune responses appear to be elicited by genital herpes infection.[10–17] The lysis of infected epithelial cells results in local inflammation, macrophage recruitment and T-cell activation. In a murine model, there is induction of natural killer lymphocytes. Lymphocyte activation results in the appearance of antibodies, including virus-neutralizing antibodies and antibodies that provide passive protection against infection when given to animals who are then challenged with virus.[1,10] T-cell responses, including cytotoxic T-cell responses, appear to be crucial in limiting and clearing disease.[14] Murine models also indicate that the degree of macrophage function at the site of local invasion may contribute to limiting the spread of infection.[1]

Cytokine induction is less well described. In cell culture models, HSV-infected cells express the cytokines interleukin-2, tumor necrosis factor and interferon-γ.[14] Peripheral blood mononuclear cells have been shown to produce interferon-α within hours of exposure to HSV virions.[12] Individuals with limited cellular immunity, including those at the extremes of life, patients with AIDS and bone marrow transplant recipients, tend to have prolonged severe disease with extensive tissue involvement and a higher rate of dissemination.[15]

Human humoral immune responses to HSV include the rapid generation of IgM antibodies following infection, with the appearance of detectable IgG and IgA antibodies approximately 10–14 days later. Antibodies appear first to certain structural proteins, and then sequentially to the various surface glycoproteins. Seroconversion to all virus antigenic determinants after infection may require months

as measured by Western blot.[5,13,16] Lifelong virus neutralizing antibodies and antibody-dependent cellular cytotoxic antibodies are detectable approximately 1 month after infection. Lack of antibody-dependent cellular cytotoxic antibodies has been correlated with a poor clinical outcome and may be a factor in severe disease in the newborn.[1,17,18]

Histology

The characteristic lesion of HSV infection is an erythematous macular or papular lesion, which progresses to a thin-walled vesicle on an erythematous base. Histologically, HSV infection is characterized by edema, multinucleate giant cells and Cowdry type A intranuclear inclusions. The inflammatory reaction present within HSV lesions consists of a mononuclear cell infiltrate of predominantly $CD4^+$ T cells (Fig. 58.2).[19]

Latency

Lifelong latency is a unique property of the herpesviruses and is characterized by persistence of virus or viral DNA in aquiescent state in sensory and autonomic ganglia.[1] Viral transcription is limited to three latency-associated transcripts[1] and antiviral medications are unable to eradicate latent virus.[1,2,10]

Periodic recurrences of symptomatic disease are due to reactivation of latent virus. Latent virus begins to replicate and form active virus particles. The virus particles are transported by antegrade axonal flow from the ganglion to the epithelium in the genital tract. Following reactivation, fully infectious virus may be detected in cutaneous lesions at the site of recurrence.

Reactivation of latent virus tends to be site and virus specific. HSV-2 reactivates much more readily from the sacral ganglia than HSV-1 and HSV-1 reactivates more readily from the trigeminal ganglia.[2,10,20] How the virus remains latent within the ganglia and how reactivation occurs are not fully understood. In the epithelial cell, actively replicating virus DNA exists in a circular form. During latency the viral DNA assumes a linear configuration and latent viral DNA is episomal. Latency is not a wholly quiescent period, however, as some herpes simplex genes thought to be regulatory transcripts are expressed during this time.[1,2,10] Sequences within the herpes simplex genome specify 'latency-associated transcripts', which may be important in reactivation of virus replication. These sequences are highly divergent between HSV-1 and HSV-2 and may account for the preferential reactivation of virus type with its corresponding ganglion following latency.[2,10,20]

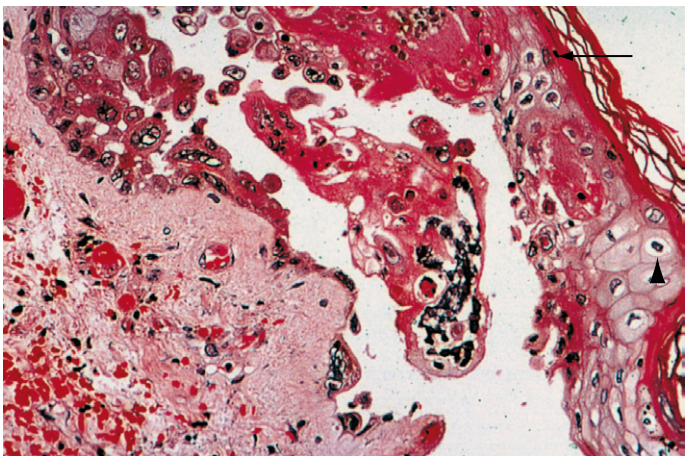


Fig. 58.2 Histology. Section of human skin showing typical herpes simplex virus effects: multinucleated giant cells (arrowhead) and intranuclear inclusion (arrow).

Virtually all recurrent genital herpes is due to reactivation of latent infection rather than to re-infection.[21] Several events may contribute to reactivation, including trauma to the ganglia, immunosuppression, UV light, fever and possibly sexual activity.[2,20]

PREVENTION

The only proven prevention strategy is avoidance of skin-to-skin contact when HSV is present in the genital tract. This includes avoidance of close physical contact during clinically symptomatic outbreaks when virus is present in high concentrations. Most patients can recognize symptomatic clinical outbreaks even when their symptoms are 'atypical' or mild and can avoid intimate contact during this time.

Frequent, intermittent asymptomatic or subclinical shedding of low titers of virus from the genital tract occurs in both men and women,[22] and accounts for most cases of transmission of genital herpes.[22–24] There is no current method to predict when asymptomatic virus shedding is occurring. It has been demonstrated that use of suppressive aciclovir, valaciclovir or famciclovir decreases both the number of days of virus shedding and the quantity of virus shed in an immunocompromised host.[25–27] In a landmark study, decreased shedding due to chronic suppressive antiviral therapy has been shown to lead to decreased transmission in a randomized, placebo-controlled trial among immunocompetent heterosexual couples where one person had genital HSV-2 and the sexual partner did not.[28] Valaciclovir was given at a dose of 500 mg daily in the treatment group, which resulted in 48% reduction in transmission in the susceptible partner. There are no data on risk of transmission between homosexual couples; however, the safety of the antiviral therapy and the data on decreased viral shedding regardless of sex would tend to favor routine use of suppressive therapy among HSV-2 discordant couples.

Vaccines

Protective vaccines may become important preventive options. The increasing incidence of genital herpes, the large number of HSV-2-seropositive individuals who are unaware of their infection status and the risk of disease transmission from asymptomatic shedding underscore the need for an effective vaccine.[23,24]

In vaccine studies, various animal models, including a guinea-pig vaginal HSV-2 model and a mouse footpad inoculation or scarification model, indicate that certain vaccine candidates provide protection from acquiring infection or ameliorate disease.[29,30] When studied in humans, these vaccine candidates have not been as effective as in the animal models. In a previous controlled trial in discordant couples, an inactivated HSV-2 glycoprotein vaccine failed to provide protection.[31] Recently, human trials have been completed or phase III trials are underway to evaluate recombinant surface glycoprotein vaccines.[32,33] No vaccine for HSV-2 is currently licensed and none has currently been shown to be effective.

Barrier contraception

Barrier contraception such as latex condoms can reduce the risk of disease transmission by decreasing contact with the partner's infected skin or mucous membranes or infected secretions. Frequent condom use decreased the risk of HSV-2 acquisition in persons with high-risk behavior, such as four or more partners per year or those with a history of a sexually transmitted disease.[34] Because herpesvirus may be present outside the portion of genital tract protected by barrier contraception, condom use is not infallible, and many couples may not place a condom until sexual activity is already underway. However, condom use should be encouraged as one arm of a multifactorial approach to decrease risk of transmission.

CLINICAL FEATURES

Genital herpes is a lifelong infection characterized by an initial infection followed by latency, and frequent recurrences. Infection may range from severe and symptomatic to asymptomatic. Herpes simplex virus disease is defined as:

- primary disease when a person lacking any antibodies to HSV acquires an infection with HSV-1 or HSV-2; and
- nonprimary first-episode disease when an individual with pre-existing antibodies to one serotype, typically HSV-1, acquires disease with the second type.

As the presence of pre-existing type common antibodies and cell-mediated immune responses modifies the course of disease, first-episode nonprimary disease is typically less severe than primary disease.[2,10]

Genital herpes may recur from none to six or more times/year. Recurrent disease tends to be milder than symptomatic first-episode disease, with fewer vesicles, less discomfort and a shortened duration of symptoms.[2,10]

Transmission of HSV-2 to a sexual partner or a neonate may be the first indication of the presence of genital herpes infection in an asymptomatic source partner.[24,35] The time and source of disease acquisition are often difficult to prove conclusively because:

- genital herpes can have a prolonged asymptomatic phase after acquisition and may be transmitted via asymptomatic shedding; and
- clinically silent or unrecognized disease is common.

Primary first-episode genital herpes

Primary genital herpes is most often seen as a disease of sexually active teenagers and young adults. Following exposure, a clinically silent incubation period lasts for 2–7 days. Onset of clinically apparent disease may be heralded by fever, headache and local genital pain and burning. Patients may appear to be systemically ill. In general, females tend to have more severe disease than males, with estimates of urinary retention occurring in approximately 10% of females. Up to 25% of females manifest symptoms of aseptic meningitis.[2,10]

The characteristic painful lesions in the genital area initially present as erythematous macules, which then progress to vesicles on an erythematous base, pustules, ulcers and finally to crusts (Fig. 58.3). Each crop of lesions takes an average of 8 days to heal completely, and successive crops of lesions may arise during the course of the disease. Untreated genital herpes may require weeks to resolve, averaging 3 weeks to cessation of lesions. Healing is usually complete, although particularly severe or large ulcers may result in scarring.[2]

In the male, lesions typically appear on the penis and glans penis (Fig. 58.3). In females, lesions may be present throughout the genital tract including the perineum, vulva, labia, perianal regions and buttocks. The vesicles are typically distributed bilaterally in primary disease. Cervical involvement is usually present and may escape detection if limited external disease is present and a complete pelvic examination is not performed. A vaginal discharge may accompany cervical and, less commonly, vaginal herpes. In either sex, the perianal and rectal mucosa may be involved, especially if exposure was due to rectal intercourse. Tender bilateral inguinal adenopathy is generally present. Vesicles may also be present on the thighs.

If herpetic involvement of the urethra occurs, severe dysuria may result. Sacral radiculopathy may occur during the course of primary genital herpes, with urinary retention, neuralgias, dysesthesia and diminished rectal tone. Tenesmus and rectal pain may be present with rectal herpes.[2,10]

In HSV-naive individuals, primary genital disease due to either HSV-1 or HSV-2 is clinically indistinguishable, making identification of infection by genital cultures and virus typing important for diagnosis. Atypical symptoms are quite common, with up to 30% of genital herpes presenting as paresthesias or urinary retention rather than the classically described vesicular genital lesions.[2,9,10,36]

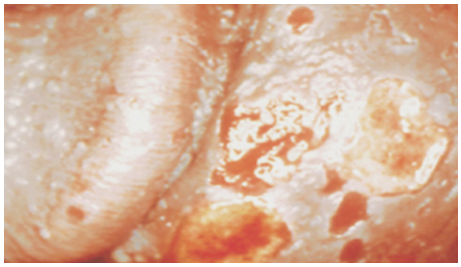


Fig. 58.3 Primary HSV. Multiple painful, erythematous, ulcerating lesions on shaft and head of penis. Exudative crust is visible over one lesion.

Nonprimary first-episode disease

Nonprimary first-episode genital HSV-2 infection is typically intermediate in severity when compared with that of primary and recurrent genital herpes.[2] Untreated disease lasts for approximately 10–14 days, with fewer lesions and fewer crops of lesions than are typical for primary disease. Systemic symptoms, including fever, are less common than in primary genital herpes, and virus can be cultured less frequently from the cervix and genital tract.[2,10]

Recurrent genital herpes

In approximately 50% of patients, a prodrome of symptoms heralds the onset of recurrent disease. Commonly reported prodromal symptoms include local burning and itching, tingling and dysesthesia. Patients may have their own recognizable cluster of symptoms. The prodromal symptoms may occur without the development of noticeable lesions or may be followed by the typical vesicular eruption lasting 6–10 days. In contrast to the widely distributed bilateral lesions of primary genital herpes, the crop of vesicular lesions in recurrent disease tends to be localized and unilateral. Lesions are typically present on the vulva in women and the glans penis and penile shaft in men, although lesions may also present on the thigh or buttocks, in the rectum and at other sites. Paresthesia, dysesthesia, local edema, pain, regional adenopathy and local swelling may accompany recurrent cutaneous herpes.[2,10]

Recurrence rates vary widely from none to six or more episodes/year, with some individuals having one or more recurrences each month. Recurrences may be triggered by sexual activity, with high rates of recurrent disease in commercial sex workers. Immunocompromised patients may experience frequent, prolonged and severe recurrences.[37] Genital herpes due to infection with HSV-2 reactivates much more readily than genital herpes due to HSV-1. Recurrent genital herpes has been reported in over 80% of individuals following primary genital HSV-2, whereas recurrence in patients who had HSV-1 primary genital herpes was less than 50%.[2] Recurrence of genital herpes averages 0.33/month with HSV-2 disease, but only 0.02/month for HSV-1 genital disease.[20]

Asymptomatic shedding

Landmark studies have demonstrated both by culture and by polymerase chain reaction (PCR) that herpesvirus is frequently present in the genital tract when lesions are absent and skin is intact, or when small or unrecognized lesions are present (Fig. 58.4).[38,39] During periods of asymptomatic virus shedding, virus titers are typically lower than during a symptomatic acute outbreak. When detected by virus culture, asymptomatic shedding occurs on an average of 4% of days, whereas women with primary genital herpes may shed virus on up to 17% of days in the first few months following their primary outbreak.[22,23]

Uncommon sites of infection

Although the majority of herpes virus infections occur in the genital and orolabial regions, cutaneous disease may occur in virtually any site, causing localized to widespread disease. Genital herpes may present as:

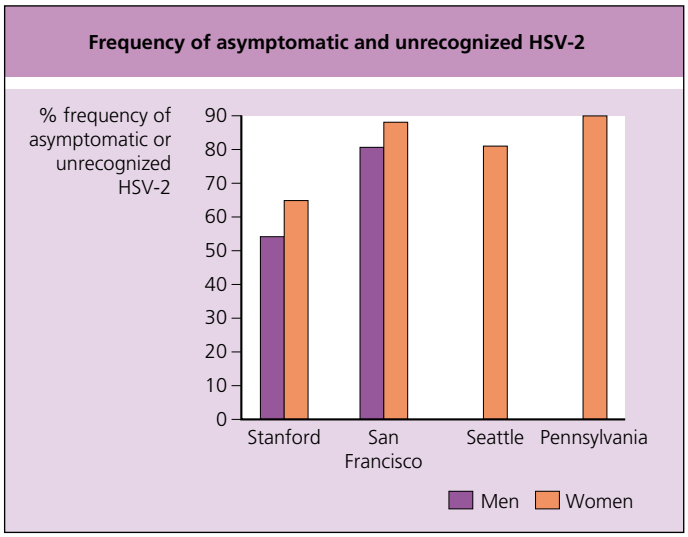


Fig. 58.4 Frequency of asymptomatic and unrecognized HSV-2 in four major US cities.[4,36,40–42]

- rectal herpes, with symptoms of proctitis, including rectal discharge, anal pain and pain on defecation;
- sacral paresthesia with urine retention; and
- painful anal fissures.

Males with rectal herpes may also present with impotence as a manifestation of neurologic involvement with HSV.[2,10]

Neonatal herpes is a rare complication of genital herpes resulting from exposure of a newborn to HSV-2 from the maternal genital tract (see Chapter 52). Acquisition may occur during maternal primary genital disease via exposure to infectious virus in the birth canal during labor and delivery. Less commonly, the fetus acquires the infection during gestation. The incidence of neonatal herpes is approximately 1/3500 live births. An increased incidence is noted in pre-term infants. Maternal acquisition of genital herpes during the third trimester of pregnancy increases the risk of neonatal transmission more than 10-fold compared with the risk of transmission from longstanding maternal HSV infection.[35,43]

Disease in the immunocompromised host

Herpes simplex viruses may be significant pathogens in immunocompromised hosts. Patients who have HIV disease may have both frequent and increased duration of recurrences of HSV mucocutaneous disease. Delayed healing and continued virus replication lead to local spread and large, painful ulcers extending through the cutaneous and subcutaneous layers. In patients with $CD4^+$ lymphocyte counts <100 cells/mm^3, treatment of genital herpes may be complicated by emergence of aciclovir-resistant HSV strains.[2,10,37,44]

DIAGNOSIS

Despite an overall increasing awareness of sexually transmitted diseases, recognition of genital herpes by both the patient and the health-care provider is often limited, and the majority of infections are undiagnosed or unrecognized (Table 58.1).[4,5,36] Diagnosis of herpes simplex disease is important:

- to establish presence or absence of infection;
- to guide treatment considerations; and
- to allow the health-care practitioner an opportunity to intervene in disease transmission.

Diagnosis may be accomplished by serologic and microbiologic methods.

Table 58.1 Differential diagnosis of genital herpes

Disease	Diagnostic clues
Syphilis	Lesions usually single and painless; positive darkfield microscopy
Chancroid	Nonindurated ulcers, positive bacterial cultures for *Haemophilus ducreyi*
Lymphogranuloma venereum	Constitutional symptoms follow onset of lesions; responds to doxycycline
Genital warts	Chronicity of lesions: no vesiculation; minimal pain
Fixed drug eruption	History; viral culture negative
Contact dermatitis	History; lack of systemic symptoms; no adenopathy
Trauma	History; lack of systemic symptoms; no adenopathy
Psoriasis	Chronicity of lesions; lesions elsewhere on body

Viral culture

Viral culture and typing is the preferred method for identifying infection, as it is the most accurate and most widely available diagnostic modality. It is recommended for all patients who have not had previous virologic confirmation of disease, including those with first-episode genital disease. Culture provides confirmation of the clinical diagnosis, and typing of virus predicts subsequent recurrence patterns. Culture of body fluids, vesicles and tissues from patients with symptomatic disease yields positive results in most cases when obtained early in the disease. Cervical cultures are positive in up to 90% of women with primary or first-episode genital herpes during the first week of symptoms, with decreasing rates of virus recovery during disease resolution. Virus may be recovered from mucocutaneous ulcers, cutaneous vesicles, cerebrospinal fluid (CSF), rectum, urethra, urine and elsewhere in the genital tract.[2,45]

In recurrent disease, virus may not be present in readily detectable quantities. Culture of multiple sites such as urethra, rectum and genital tract improves the chance of virus recovery. Growth and typing of virus requires less than 5 days, and modified culture techniques that detect herpes antigens (the shell-vial assays) yield preliminary results in 1–2 days.[38,45]

Serologic assays

Type-specific serologic diagnosis is important:

- for serosurveys to gauge prevalence rates;
- for identification of asymptomatic patients;
- in situations where culture is not feasible or available; and
- for patient counseling in scenarios of sexually transmitted disease and other public health clinics.

Herpes simplex antibodies are readily detectable following infection, but it may take several months for complete antibody development to all antigenic determinants. Differentiating HSV-1 from HSV-2 antibodies is more problematic due to the antigenic similarity of the viruses.[1,10,16] Antibody responses in general are broadly cross-reactive between HSV-1 and HSV-2.[45] Antibodies to glycoprotein G are type specific, as are antibodies to portions of glycoprotein B.[46] Recently developed tests using glycoprotein G-based serologic assays are now commercially available and include both ELISA and immunoblot formats. Sensitivities for HSV-2 using IgG antibody development range from 80% to 98%, and perform worst in low-risk groups where false-positives are more common. One recent study of a low-risk group comparing the Focus HSV-1

and -2 ELISA to type-specific Western blot showed a specificity of 94%, a sensitivity of close to 100%, but a positive predictive value of only 37.5%, indicating that commercially available tests must be interpreted with caution in low-risk populations.[47] Overall, serologic assays that rely on virus-specific antibodies to IgG have a specificity approaching 96%. Older assays do not discriminate between HSV type 1 and 2 and have little to no practical value.[46,48]

Other diagnostic techniques

The advent of the PCR allows amplification and detection of HSV gene segments even when present in virus copy numbers too low for detection by culture. Assays based on PCR are rapid and sensitive, but strict control measures are necessary to avoid false-positive results. Detection of HSV by PCR is most useful for herpes meningitis, encephalitis and other central nervous system infections that are not readily diagnosed by CSF culture or would otherwise require brain biopsy for diagnosis. The sensitivity of HSV PCR approaches that of biopsy for the diagnosis of herpes encephalitis. HSV-2 can be detected by PCR in a high proportion of people with recurrent aseptic meningitis.[1,10,49,50]

Use of PCR to detect and quantify virus sequences on the skin and in the genital tract in the absence of lesions has provided important information about the duration and amount of asymptomatic virus shedding as the virus copy number is often below the threshold of detection by culture.[22] Neonatal herpes infection may also be diagnosed by PCR amplification of HSV gene segments in the serum and CSF.

MANAGEMENT

Currently, the practitioner has a choice of effective, safe and well-tolerated medications (Table 58.2). Antiviral therapy is effective:

- in the treatment of primary and first-episode genital herpes;
- for episodic treatment of recurrences; and
- for suppression of frequent recurrences.

Continuous antiviral therapy has also been shown to decrease viral shedding. Antiviral therapy has not yet been shown to affect latency or 'cure' genital herpes. Recent data are encouraging that continuous antiviral therapy may decrease, but not eliminate, the risk of transmission. The combination of education, counseling, barrier contraception and antiviral therapy has the potential to greatly decrease the risk of partner-to-partner transmission and potentially the anxiety and decreased quality of interpersonal interactions that may accompany a diagnosis of genital herpes.

Pharmacologic agents (see also Chapter 146)

Aciclovir is the acyclic analogue of the nucleoside guanosine. In order to be active, it must first be phosphorylated by the virus-encoded enzyme, thymidine kinase, to aciclovir monophosphate. The monophosphorylated form is then further phosphorylated by cellular enzymes to the di- and triphosphate form. The active form, aciclovir triphosphate, inhibits the HSV-specific DNA polymerase and terminates the replicating DNA chain by competing with its analogue deoxyguanosine triphosphate. Aciclovir lacks the 3′-hydroxyl group necessary for subsequent phosphodiester linkages, so that extension of the replicating DNA chain is no longer possible.[39]

Aciclovir has proved to be a well-tolerated and effective medication with a wide margin of safety. Its safety and specificity result from the relative inability of cellular kinases to phosphorylate aciclovir to its active form and from the more potent inhibition of HSV DNA polymerase than human DNA polymerase by aciclovir triphosphate. Resistance to aciclovir occurs when HSV strains develop that lack thymidine kinase or, less commonly, by strains with an altered thymidine kinase or DNA polymerase.[39]

Aciclovir is currently available as an oral, topical and intravenous agent. Clearance is via the kidney, with approximately 90% of the drug excreted unchanged in the urine.[39] Side-effects for the oral and topical forms are minimal. Topical aciclovir may cause burning and is not approved for use on mucous membranes.[51] Oral aciclovir may

Table 58.2 Antiviral therapy

Medication	Indication	Dose, route	Side-effects
Aciclovir	Primary genital herpes	200 mg po five times daily or 400 mg po q8h for 7–10 days	Nausea
	Recurrent genital herpes	400 mg q8h for 5 days or 800 mg q12h for 5 days or 800 mg q8h for 2 days	Nausea
	Suppression of frequent recurrences	400 mg po q12h	Nausea
	Severe or disseminated disease	5 mg/kg slow iv q8h for 14 days	Reversible crystalline nephropathy, tremors
	Encephalitis	10 mg/kg slow iv q8h for 14–21 days	Reversible crystalline nephropathy, tremors
Valaciclovir	Primary genital herpes Recurrent genital herpes Suppression of frequent recurrences	1 g q12h for 7–10 days 500 mg q12h for 3 days or 1 g q24h for 5 days or 1 g q12h for 1 day 500 mg q24h or 1 g q24h	Nausea
Famciclovir	Primary genital herpes Recurrent genital herpes Suppression of frequent recurrences	250 mg po q8h for 7–10 days 125 mg po q12h for 5 days or 1 g q12h for 1 day 250 mg po q12h	Nausea
Foscarnet	Aciclovir-resistant herpes simplex virus	60 mg/kg q8h, infuse over 2h for 10 days	Azotemia, seizures, hypo- or hyperkalemia and hypo- or hyperphosphatemia

Note: The topical aciclovir 5% ointment available in the USA is of little value in the treatment of genital herpes and is not recommended as effective therapy. Valaciclovir 1 g q24h and 500 mg q24h are both approved for chronic suppressive therapy, but the authors recommend 500 mg q12h.

cause nausea, especially with high doses. These symptoms are generally mild and resolve over time with continued use of the drug.[52] Intravenous aciclovir may cause local pain and phlebitis if drug extravasates during administration.[39] The most common significant side-effect of intravenous aciclovir is a reversible nephropathy secondary to crystallization of aciclovir in the renal tubules. Administration of intravenous doses slowly over 1 hour decreases the incidence of nephropathy. Aciclovir nephropathy is rarely seen in normal adults, but is more common in the elderly and in those with underlying renal dysfunction. Intravenous aciclovir may rarely cause neurologic complications, including lethargy, delirium and tremors.[39]

Other therapeutic agents

Antiviral medications that have recently become available include valaciclovir, penciclovir and famciclovir.

Valaciclovir

Oral bioavailability of aciclovir is limited, with only 15–20% of the oral dose absorbed. Valaciclovir, a prodrug of aciclovir, was developed to increase the oral bioavailability and is absorbed at levels three to five times greater than those for aciclovir. Following absorption, valaciclovir is then hydrolyzed to aciclovir. Oral administration of valaciclovir can lead to serum levels of aciclovir approaching those following intravenous administration of aciclovir.[53]

Penciclovir

Penciclovir is also an acyclic nucleoside analogue and is available in oral and topical forms. Topical penciclovir is a treatment option for orolabial HSV-1 but has not been approved for use in genital herpes. Like aciclovir, the drug must first be phosphorylated by the HSV-encoded thymidine kinase to penciclovir monophosphate. Cellular kinases then phosphorylate the compound to the di- and triphosphate forms. Like aciclovir triphosphate, penciclovir triphosphate is an inhibitor of HSV DNA polymerase. Although penciclovir is phosphorylated by thymidine kinase much more readily than aciclovir, this benefit is offset by reduced activity against the viral polymerase compared with that of aciclovir. DNA chain termination is not a significant property of penciclovir triphosphate. The intracellular half-life of penciclovir triphosphate is much longer than that of aciclovir triphosphate. It is still unclear whether these differences have an effect on the effectiveness or safety of penciclovir compared with those of aciclovir.[54,55]

Famciclovir

Famciclovir is an oral prodrug of penciclovir. Similar to valaciclovir, famciclovir was developed in an effort to increase oral bioavailability. Following ingestion, famciclovir is rapidly converted by deacetylation to penciclovir. Approximately 60–70% of the dose is excreted in the urine as penciclovir.[56]

Foscarnet

Foscarnet is a pyrophosphate analogue that inhibits viral DNA polymerase.[57] Because it does not require phosphorylation to become active, its efficacy is not dependent on the presence of HSV-specific thymidine kinases. As such, foscarnet is one of the few drugs available for the treatment of aciclovir-resistant herpes simplex due to thymidine kinase or thymidine kinase-altered strains. Foscarnet is only available as an intravenous preparation. The most common side-effects include nephrotoxicity, electrolyte abnormalities, seizures and penile ulcers. Saline hydration decreases the renal toxicity and is recommended to diminish the risk of azotemia. Foscarnet-resistant HSV strains have occasionally developed in immunosuppressed hosts.[58]

Cidofovir or (S)-9-(3-hydroxy-2-phosphonylmethoxypropyl) cytosine

Cidofovir is an acyclic nucleoside phosphonate antiviral agent currently used in the treatment of cytomegalovirus retinitis. Cidofovir is active *in vitro* against HSV and has activity against thymidine kinase-negative and thymidine kinase-altered HSV strains. Trials of cidofovir in humans for the treatment of resistant genital herpes have not been completed. The primary toxicity of cidofovir at doses used to treat cytomegalovirus is nephrotoxicity. The doses of cidofovir necessary to treat aciclovir-resistant genital herpes have not been established.[59,60]

Ineffective therapies

Ineffective therapies include bacille Calmette–Guérin (BCG) vaccination, topical povidone–iodine (Betadine), topical vidarabine, topical idoxuridine and gammaglobulin. Topical therapy with foscarnet has been disappointing, with recent studies of treatment of recurrent genital herpes showing little to no benefit.[61] Lysine is a popular over-the-counter supplement purported to decrease the symptoms of genital herpes. Well-controlled clinical trials of lysine, including a double-blind cross-over study, have failed to demonstrate efficacy.[62,63]

General treatment guidelines

Antiviral therapy decreases both the duration of symptoms and viral shedding in genital herpes. Maximum benefit occurs when antiviral therapy is initiated promptly, ideally with the first prodromal symptoms with recurrent genital herpes. With the wide margin of safety of the oral antiviral medications and the need to initiate therapy promptly, it is appropriate to begin medication before receiving culture results or other confirmatory test results.

Aciclovir, famciclovir and valaciclovir are all appropriate agents for the treatment of genital herpes. For some indications, valaciclovir and famciclovir offer more convenient dosing regimens. However, for many health-care providers, generic aciclovir remains the drug of choice for the treatment of genital herpes due to the extensive clinical experience with the drug, its excellent safety and efficacy profiles, wide availability and lower cost (Table 58.3).

Treatment of primary genital herpes

Antiviral therapy is clearly of benefit in the treatment of primary genital herpes. When compared with placebo it has been shown to reduce median:

- duration of viral shedding by 7 days;
- duration of pain by 4 days; and
- time to healing by 7 days.[64]

Antiviral therapy does not prevent the establishment of latency or affect the likelihood and frequency of recurrences when compared with placebo.[71] Higher than recommended doses of antiviral medications are no more effective in the treatment of genital herpes and may be associated with an increased risk of nausea.[52] Treatment for longer than 10 days in the normal host does not improve outcome when compared with the standard regimen.

Primary or first-episode genital herpes may be adequately treated with a variety of oral regimens, including aciclovir at doses of 200 mg five times daily or 400 mg q8h for 10 days. Alternatively, valaciclovir 1 g q12h for 7 days or famciclovir 250 mg q8h for 7–10 days is also effective treatment for primary genital herpes.[63,65]

Individuals with severe disease or who are unable to take oral medications should be treated with intravenous aciclovir at doses of 5 mg/kg administered over 1 hour q8h. Topical aciclovir is of little value in the treatment of primary disease.[64] The combination of oral and topical aciclovir offers no therapeutic advantage.[63,72]

Oral famciclovir and oral valaciclovir have been compared with oral aciclovir for the treatment of first-episode genital herpes in recent clinical studies. Oral famciclovir in doses of 125, 250 or 500 mg q8h for 10 days has been shown to be as effective as aciclovir 200 mg five times daily for 10 days.[63,73] Valaciclovir 1 g q12h was also as effective as aciclovir 200 mg five times daily in the treatment of first-episode genital herpes.[63,74] Neither famciclovir nor valaciclovir appears any more effective than aciclovir.

Table 58.3 Drug comparison trials

Medications	Indication	Authors	Results	Conclusions
Aciclovir 200 mg po five times daily for 10 days versus placebo	First-episode genital herpes	Corey *et al*.[64]	With po or iv aciclovir, pain decreased by 4 days, viral shedding decreased by 7 days, time to healing reduced by 7 days, 60% fewer new lesions; topical aciclovir of limited value	Oral aciclovir significantly effective in treatment of first-episode genital herpes
Famciclovir various doses po q8h versus aciclovir 200 mg po five times daily	First-episode genital herpes	Loveless *et al*.[65]	Famciclovir 125, 250 or 500 mg q8h equal to aciclovir 200 mg five times daily, each given for 10 days; famciclovir 250, 500 or 750 mg q8h equal to aciclovir 200 mg five times daily, each given for 5 days	Famciclovir equal in efficacy to standard dose aciclovir and required less frequent dosing interval
Valaciclovir 1 g po q12h po versus aciclovir 200 mg po five times daily	First-episode genital herpes	Fife *et al*.[66]	Valaciclovir q12h doses as effective as aciclovir five times daily	Valaciclovir as effective as aciclovir, but required less frequent dosing interval
Valciclovir 1 g po q12h versus aciclovir 200 mg po five times daily versus placebo	Recurrent genital herpes	Smiley *et al*.[67]	Time to healing equivalent for valaciclovir and aciclovir (115–116 hours) and shorter than with placebo (144 hours); duration of viral shedding also decreased in treated group	Treatment with valaciclovir or aciclovir significantly more effective than with placebo, and no difference between the two drug treatments (valaciclovir 500 mg q12h now shown to be as effective as 1 g q12h in a separate study)
Famciclovir 125, 250 or 500 mg po q12h compared with placebo	Recurrent genital herpes	Sacks *et al*.[68]	Time to healing reduced by 1.1 days in treated group; significant reduction in shedding	Famciclovir effective in treatment of recurrent genital herpes
Aciclovir 400 mg po q12h versus placebo	Suppression of frequent recurrences	Mertz *et al*.[69]	Mean number of recurrences 11.4/year for placebo group, 1.8/year for treated group; number free from recurrences for 1 year: 2% for placebo group, 44% for treated group	Aciclovir highly effective at suppressing recurrences; minimal side-effects, frequent high patient compliance and satisfaction with treatment
Famciclovir various doses po versus placebo	Suppression of frequent recurrences	Mertz *et al*.[70]	Time to first recurrence 82 days for placebo, greater than 120 days for treatment; 250 mg q12h most effective and 78% of patients with no recurrences at 120 days versus 42% of those treated with placebo	Famciclovir 250 mg q12h effective for suppressing frequent recurrences
Aciclovir 400 mg po q12h versus placebo	Suppression of asymptomatic viral shedding	Wald *et al*.[25]	Five of 34 treated shed virus during therapy compared with 25 of 34 in placebo group; days shed were 6.9% with placebo, 0.3% with aciclovir	Aciclovir dramatically decreases asymptomatic viral shedding

Episodic treatment of recurrent genital herpes

Recurrent genital herpes is generally milder and of shorter duration than primary disease, so the expected benefits of treating individual episodes with antiviral therapy are not as profound as those seen in primary disease. Episodic treatment of recurrent genital herpes with antiviral therapy results in only a modest decrease in disease severity, with a mean decrease in the duration of symptoms of 1 day or less.[73]

Aciclovir at a dose of 200 mg five times daily or 400 mg q8h for 5 days remains the recommended therapy.[75] Patient-initiated therapy at the time of onset of prodromal symptoms has been shown to be of significantly greater benefit than therapy delayed to initial lesion onset.[73]

Both valaciclovir and famciclovir have been evaluated for effectiveness in the treatment of recurrent genital herpes. Recent clinical trials comparing valaciclovir (1 g q12h) with either aciclovir (200 mg five times daily) or placebo showed the following in both treatment arms when compared with placebo:

- decreased time to lesion healing;
- decreased duration of pain; and
- reduced duration of viral shedding.[67]

Oral famciclovir at doses of 125, 250 or 500 mg q12h reduced viral shedding and duration of symptoms and reduced time to healing when compared with placebo.[76] The lowest effective dose, 125 mg q12h for 5 days, is the currently approved treatment dose. Recently, a 1-day course of famciclovir 100 mg twice over 24 hours was found to be more effective than the standard 3-day course of valaciclovir.[77] A convenient 1-day course of valaciclovir at a dose of 2 g twice daily, for a total of 4 g over 24 hours, was found to be safe and effective, but has not been evaluated in comparison trials.[67] Neither famciclovir nor valaciclovir appears to offer any therapeutic advantage over aciclovir in the immunocompetent host.

The 5% topical aciclovir ointment available in the USA is not effective at reducing viral shedding, symptoms or time to healing, and is not licensed or recommended for treatment of recurrent genital herpes.[78] A different preparation available in Europe, a 5% topical aciclovir cream, has been shown to be effective and may be an appropriate alternative to oral therapy if available to the patient.[79]

Suppression of frequently recurring genital herpes

Chronic antiviral therapy has proven to be a safe and well-tolerated mechanism for suppressing symptomatic recurrences in patients with moderate to frequently (more than five episodes per year) recurring genital herpes.

Aciclovir

Aciclovir was the first antiviral agent evaluated for effectiveness in suppressing recurrent disease. In one study, patients with frequent recurrences had a reduction of recurrences from more than 12/year to an average of 1/year when treated with aciclovir 400 mg q12h.[69]

Suppressive therapy is safe and well tolerated, with no long-term effects on sperm motility, no noted laboratory abnormalities and minimal side-effects.[69,80]

Aciclovir has now been used for suppressive therapy for up to 10 years and has not been associated with development of tolerance or with any significant development of resistant strains in the normal host.[69,81,82] However, interruption of therapy at 1- or 2-year intervals is suggested to assess the frequency of episodes and the need for suppressive therapy. It has recently been shown that suppressive therapy with aciclovir decreases the frequency of asymptomatic viral shedding in women.[25] Aciclovir is now available as a generic prescription in most places, which may provide significant cost-saving considerations when deciding on pharmacotherapy.

Famciclovir

Famciclovir is now licensed in the USA for suppressive therapy at a dose of 250 mg q12h, the most effective dose tested in a dose-ranging study of famciclovir compared with placebo for suppression of frequently recurring genital herpes in women.[70] In this trial, once-daily dosing regimens were not as effective as the q12h dosing regimens at equal or higher cumulative daily doses. A subsequent 1-year trial in men and women also found that the 250 mg q12h dosage was effective and well tolerated. At present, there are no results available of trials comparing the efficacy of suppressive therapy with famciclovir to therapy with valaciclovir or aciclovir.

Valaciclovir

Valaciclovir has also been used for suppression of genital herpes and has recently been licensed in the USA at a dose of 500 mg or 1 g orally daily for suppression of genital herpes in the normal host. The 500 mg daily dose appears to be effective in persons with a history of 6–10 episodes/year. This regimen appears to be less effective than aciclovir 400 mg orally q12h or valaciclovir 1 g daily or 250 mg q12h in patients with very frequent recurrences (10 or more episodes/year). Once-daily dosing with valaciclovir 1 g appears to have acceptable efficacy in the normal host, but should not be used in the immunocompromised host because there is evidence of reduced efficacy in people who have HIV infection. Valaciclovir 1 g daily or 250 mg q12h and aciclovir 400 mg q12h appeared equivalent in efficacy, even among people with frequent recurrences. Valaciclovir was found to decrease viral shedding by 71% at a dose of 1 g daily in immunocompetent persons.[83] Valaciclovir 500 mg q12h was found to be safe and was also significantly more effective than valaciclovir 1 g daily in suppressing genital herpes in people who have HIV infection and a median $CD4^+$ lymphocyte count of 320 cells/mm.[3,84]

Summary

In summary, there are no data at present suggesting that suppressive therapy with either famciclovir or valaciclovir at the licensed doses is more effective than suppressive therapy with aciclovir 400 mg q12h. In addition, cost comparisons favor the use of generic aciclovir rather than the newer agents.

CONCLUSION

Genital herpes is one of the most commonly encountered sexually transmitted diseases. Its management requires:

- recognition of disease;
- patient education about the unique features of the disease, including latency and virus shedding; and
- judicious use of antiviral medications.

REFERENCES


References for this chapter can be found online at http://www.expertconsult.com

Chapter 59

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Papillomavirus infections

INTRODUCTION

Human papillomavirus (HPV) is the most common viral sexually transmitted infection (STI) in humans.[1] Mild disease includes genital warts (condyloma acuminata), verruga or cytologically evident dysplasia of the cervix or anus. Persistent anogenital infection with certain 'high-risk' HPV types is associated with advanced cervical neoplastic disease and invasive carcinoma of the anogenital region, most notably the cervix.[2] Oncogenic 'high-risk' HPV genotypes (notably types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 73 and 82) are likely to be responsible for a high proportion of carcinomas of the cervix, vagina, vulva, anus and penis worldwide.[3] Other HPV genotypes termed 'low-risk' and nongenital types of HPV cause benign epithelial warts, as well as a number of other dermatologic conditions[4] not reviewed in this chapter (see Chapter 156).

HPV-associated anogenital disease

Human papillomavirus represents a family of DNA viruses with more than 120 related genetic types, more than 40 of which infect the anogenital tract.[3] The genome consists of a circular double-stranded DNA about 8000 base pairs long within an icosahedral protein coat with no membrane envelope. Most HPV genotypes have three regions:

- a transcription and replication control region;
- an early region encoding proteins for replication, regulation and modification of the host cytoplasm and nucleus; and
- a late region encoding capsid proteins.[5]

HPV is a strictly epitheliotropic virus with various HPV genotypes targeting specific epithelial targets (e.g. soles of feet, nongenital skin, anogenital skin, anogenital/oropharyngeal mucosa).

Strong epidemiologic and molecular evidence identifies HPV as a necessary but insufficient cause of cervical cancer.[2,3] Cervical cancer is the second most common cancer among women worldwide. Globally, an estimated 500 000 new cases and 275 000 deaths occurred in 2002, with developing countries accounting for more than 80% of cases (Fig. 59.1). In the absence of screening programs, 85% of women with cervical cancer will die given the advanced stage of the disease at the time of diagnosis. Despite widespread screening, it is estimated that there are still more than 12 000 new cases of cervical cancer and 4000 deaths due to the disease annually in the USA. For every case of invasive cervical cancer found by Papanicolaou-type cervical cell examination testing (Pap smear), an estimated 50–100 cases of squamous intraepithelial lesions are detected. In the USA about 35% of invasive cervical cancers and 57% of deaths occur in women older than 55 years of age. The lifetime risk for acquiring invasive cervical cancer in the USA is 0.85% and the risk of dying from it is 0.30%. However, black women are 61% more likely to develop invasive disease and have about twice the risk (0.56% vs 0.27%) of dying from invasive cervical cancer than white women in the USA.[6] The racial disparity in the incidence of cervical cancer becomes more prominent among women older than 40 years.

Cervical cancer development involves four major steps: HPV infection, viral persistence, progression of persistently infected cells to precancer cancer, and invasion. Therefore, HPV infection is the single most important and necessary risk factor for cervical squamous intraepithelial lesions (SILs) and cancer. Although most women with cervical cancer are well into their forties when diagnosed, HPV infection and disease pathogenesis are known to begin at the onset of sexual activity.[7] Early signs of cervical intraepithelial neoplasia (CIN) are detectable through cervical cytology screening programs that provide periodic Pap smear or other screening modalities. An overwhelming majority of cervical cancer cases occur in women receiving suboptimal cervical screening and treatment regimens. Older women, women from ethnic or racial minorities, and women from poor and disadvantaged backgrounds are more likely to be diagnosed with advanced-stage disease as a direct consequence of fewer or absent early detection opportunities.

EPIDEMIOLOGY, PATHOGENESIS AND PATHOLOGY

Epidemiology

Genital HPV is a common infection among sexually active adults and adolescents.[1] The estimates of HPV prevalence vary greatly depending on the methods used for HPV detection and the populations sampled. Diagnostic limitations, subclinical cases, repeated and multiple infections, and the phenomenon of inconsistent HPV types noted at separate clinical encounters make the incidence and prevalence estimates for HPV infection imprecise. In the latter circumstance, one cannot be sure whether a new viral type has been acquired or whether it was missed at a prior diagnosis.

Estimates based on visible warts grossly underreport prevalence, while estimates based on molecular evidence of infection (presence of HPV DNA in exfoliated genital tract cells) show the highest rates (20% in a general or 75% in a very high-risk population of women or adolescents). The estimates of HPV prevalence, based on the polymerase chain reaction (PCR) test for HPV DNA among asymptomatic women in the general population, have ranged from 2% to 44% in various populations.[8] A meta-analysis of studies from around the world estimates the global HPV prevale nce to be 10.4%, with an estimated 291 million HPV-infected women.[9] In the USA, approximately 20 million women are estimated to be infected with HPV at any one time. A 2007 survey using PCR for HPV DNA detection found an overall HPV

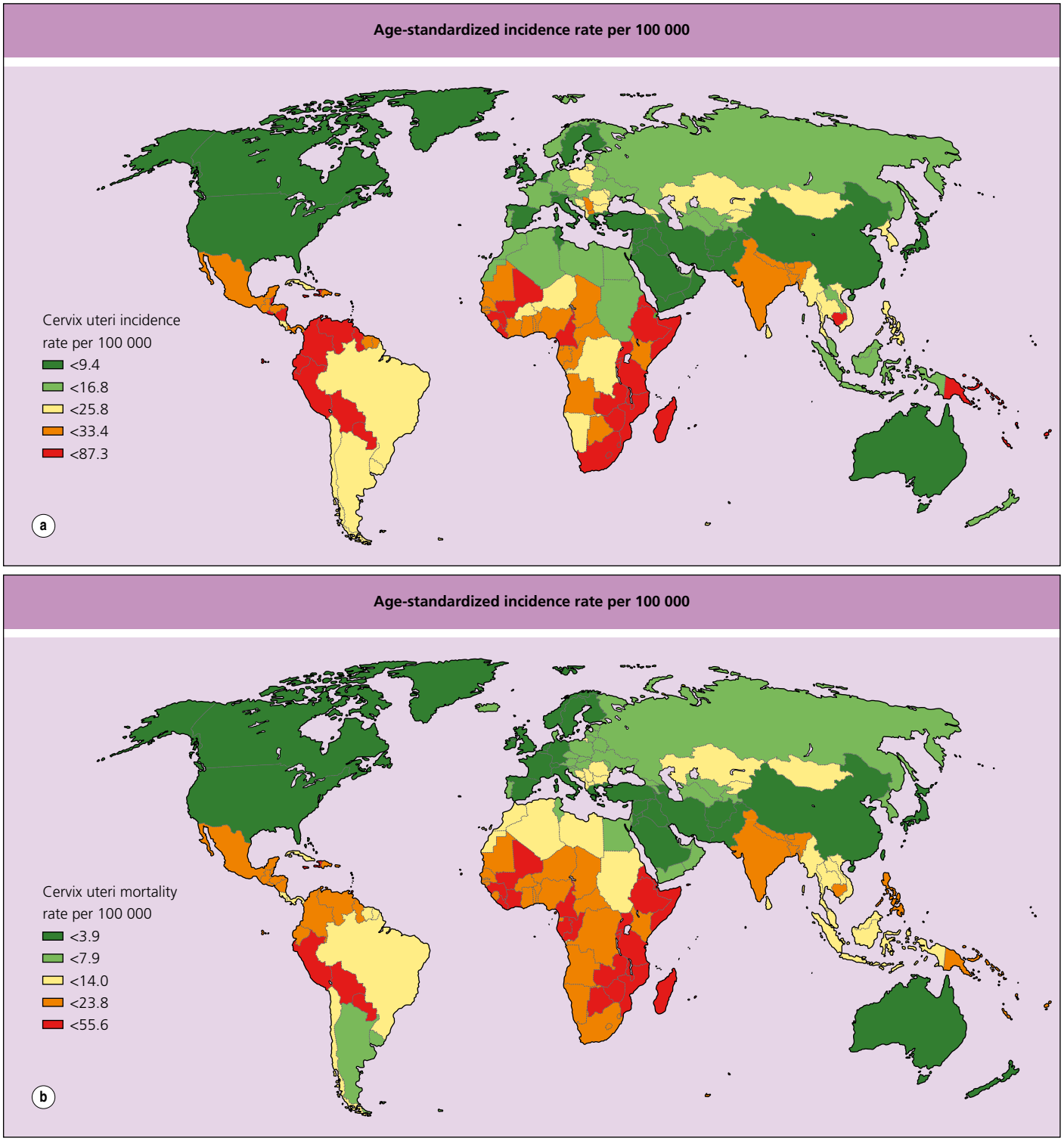


Fig. 59.1 Age-standardized incidence (a) and mortality (b) rates due to cervical cancer worldwide. Adapted from GLOBOCAN 2002, International Agency for Research on Cancer; Parkin DM, Bray F, Ferlay J, Pisani P. Global cancer statistics, 2002. CA Cancer J Clin 2002;55:74–108.

prevalence of 26.8% among US females aged 14–59 years.[10] The prevalence showed an increasing trend, starting at age 14 years and peaking at 44.8% among women aged 20–24 years. The prevalence gradually declined thereafter, through age 59. A longitudinal study of HPV infection in a cohort of college women in the USA found an average annual incidence of 14% and that about 60% of the women were infected with HPV at some time during the 3-year follow-up period.[11] Similar results were also observed in a slightly older cohort (mean age 32 years) of women with a cumulative incidence of 54% after 3 years.[12]

There is considerable regional variation in both overall prevalence and type-specific distribution of HPV among women in the general population. For example, a study of worldwide distribution of HPV

types based on the PCR test for HPV DNA found an age standardized HPV prevalence of 1.4% in Spain versus 25.6% in Nigeria.[13] HPV prevalence rates as high as 97% have been reported in some studies among specific populations, such as HIV-infected women. The rate of active HPV infection decreases with age; sexually active women under age 25 consistently show the highest rates. Both acquired genital tract immunity and diminishing behavioral risk with age may be responsible for this strong inverse prevalence–age relationship. Other risk factors associated with HPV infections include a higher number of recent and lifetime sexual partners, higher frequency of sexual intercourse, compromised immune status, high-risk characteristics of the sex partner including partner genital warts, and a high number of lifetime sex partners of the partner.

Genital HPV infections are primarily transmitted by skin-to-skin contact; penetrative sexual intercourse is not always necessary for transmission. The rate of HPV transmission per sexual contact is not known but is likely to be high, with similar transmission rates for various HPV types. A high proportion (20–30%) of concurrent infections with multiple HPV types has been observed when women in general populations are sampled. Most sexually active women are probably infected with at least one if not several HPV types during their lifetime.[1] HPV infection in women is typically short lived, with a 70% clearance rate in 12 months and greater than 90% clearance rate in 2 years. Whether the virus is totally cleared or simply suppressed by cell-mediated immunity to a latent state without full viral expression is unclear.

The degree and duration of immunity conferred by a natural HPV infection is not known. It is estimated that only 50–60% of women develop serum antibodies to HPV after natural infection.[14] The transient nature of HPV DNA detection, lack of all infections producing humoral response and inaccurate serology make it difficult to measure total exposure to HPV infection. Estimates based on HPV DNA detection methods suggest that the prevalence of HPV and concurrent HPV infections in men may be similar to that found in women.

Persistence of type-specific HPV infection is an important risk factor for cervical cancer development and progression. Though methodologically challenging to assess, screening studies show that the median time of clearance of HPV infections ranges from 6 to 18 months.[15] The longer an HPV type persists, the less likely the virus will subsequently clear up over a fixed interval, and the risk of precancer diagnosis increases.[15] While both prevalence and incidence of HPV are higher among younger women, it is older women who seem to be at higher risk of persistent HPV infection. There is not yet an accepted definition of persistence, particularly one that is clinically relevant. Of all the carcinogenic HPV infections, only a small portion (about 10%) is associated with a high absolute risk of precancer diagnosis. HPV type risk factors for persistence of infection and progression to precancer are not well understood. Other factors associated with persistence of infection may include older age, immunosuppression, higher HPV virus load and infection with multiple HPV types.

Human papillomavirus transmission can also occur intrapartum due to vaginal delivery.[16] Viral load in cervical and vaginal cells may be an important determinant for the risk of perinatal HPV transmission. Whether acquisition of HPV during the perinatal period predisposes to an increased risk of CIN among female infants in later life is unclear, as the evidence for high-risk HPV transmission and persistent infection throughout childhood is uncertain. Juvenile laryngeal papillomatosis may result from vertical transmission resulting in obstructive warts years later. Genital warts in children, in contrast, do not seem to occur from perinatal transmission and are typically the consequence of child sexual abuse when seen in immunocompetent children.

Oncogenesis and co-factors for oncogenesis

Molecular studies demonstrate that HPV has a direct mechanistic role in oncogenesis.[17] The integration of viral DNA is critical for malignant transformation, while continued expression of HPV proteins is necessary for maintenance of the transformed state. In benign warts and in preneoplastic lesions, the HPV genome is maintained in a circular non-integrated form, whereas in cervical cancer cells the previously circular viral DNA is often found integrated into the linear host cell genome. Proteins from both HPV and endogenous origins can deregulate cell cycles and enable carcinogenesis to occur. The expression of some of the 'early' genes (E6/E7) of high-risk HPV types (16, 18 and others) seems to be an essential factor for malignant conversion of the cervical epithelium. The viral DNA will integrate into the host DNA within the E1/E2 open reading frame of the viral genome.[5] Because the E2 region of the viral DNA normally represses the transcription of the E6 and E7 early viral genes, HPV integration causes overexpression of the E6 and E7 proteins of high-risk HPV types. Transformation can occur when these expressed E6 and E7 proteins bind the products of tumor suppressor genes *p53* and retinoblastoma (*pRB*), respectively. This modifies or inactivates their normal cell-regulating functions, namely the transcription of genes involved in cell cycle control. The E6/*p53* and E7/*pRB* interactions cause genomic instability, resulting in the accumulation of chromosomal abnormalities followed by clonal expansion of malignant cells.

Human papillomavirus DNA from oncogenic viral types is found in nearly all cervical cancer cells. Infection with a high-risk HPV type is a necessary cause for both squamous cell carcinoma and adenocarcinoma of the cervix. HPV-16 and HPV-18 are the two most common high-risk types, accounting for 70% of cervical cancers and about 50% of CIN grade 3.[18] Since high-risk HPV type infection is common in women with normal cervical cytology and only a fraction of HPV-infected women develop cervical cancer, HPV infection is a necessary but not sufficient cause of cervical and many other anogenital cancers. However, carcinogenic risk may be enhanced by a number of additional factors.

Women infected with more than one HPV type are at a higher risk of precancer than women with a single infection. Co-infections with *C. trachomatis* and herpes simplex virus type-2 may increase carcinoma risk in women with HPV infection. Local inflammation from infectious or other causes may stimulate cytokine responses that impact HPV expression. Retinoids such as β-carotene, antioxidants such as vitamin C and methylation agents such as folic acid have been implicated in cervical disease, but the role of nutritional factors is still unclear. There is evidence of higher folate levels in blood having a protective effect against precancer. Smoking has been shown to increase the risk of squamous cell carcinoma twofold among HPV-infected women. Long-term use of oral contraceptive hormones is associated with an almost threefold increase in the risk of cervical cancer in HPV-infected women. Women with seven or more full-term pregnancies are four times more likely than nulliparous women, and two times more likely than women with one to two full-term pregnancies to develop squamous cell carcinoma.

A 'male factor' is apparent in increased cervical cancer risk to women. Women married to men whose first wives died of cervical cancer, monogamous women married to men who are frequently away from home, and female sexual partners of men with penile cancer all have a higher than expected cervical cancer risk.[19] Lack of male circumcision is associated with higher penile HPV infection rates and increased risk of cervical cancer in their female sex partners. Unlike other STIs, condom use is neither strongly nor consistently associated with HPV prevention, perhaps indicating higher infectiousness per contact of HPV or the possibility of genital transmission from perineal or other noncoital sexual contact.[20] Sexual couples can be discordant in HPV infection status or type, demonstrating the transient nature of infection in many persons, its low level in at least one partner and/or resistance to HPV by the uninfected partner through local immune or genetic factors.[21] HPV-related genital cancers can cluster in families due, perhaps, to shared risk factors and/or host immunogenetics.

Host genetics and immunosuppression

A current research challenge is to investigate the role of host genetics in HPV acquisition, retention and disease pathogenesis. Theoretically,

HLA type or other host cell receptor genetic profiles may affect the degree of host cell susceptibility, efficiency of viral replication, nature of host immune responses and likelihood that infection will persist. Molecular techniques have improved the precision and affordability of immunogenetic assessments within epidemiologic and clinical studies. There may be a protective effect of HLA DRB1*1301 on HPV clearance and precancer development.[22]

Impaired immunity increases susceptibility to many infections and malignancies. Immunosuppressed persons, including those with low $CD4^+$ T-cell counts due to HIV infection, cancer chemotherapy, renal transplant or rheumatology on immunosuppressive drugs, may have an impaired ability to clear up HPV, permitting longer duration of infection and increased likelihood of oncogenic integration of HPV into the host genome. Immunosuppression can exacerbate the severity of epithelial warts and facilitate the persistence, pathogenicity and progression of HPV-induced neoplasia.[23,24] HIV-infected women have higher prevalence, incidence, regression and recurrence of HPV infection, cervical neoplasia and cervical cancer than HIV-uninfected women with comparable risks. Invasive cervical cancer was added to the list of acquired immunodeficiency syndrome (AIDS)-defining conditions by the US Centers for Disease Control and Prevention (CDC) in 1993. Women with lower $CD4^+$ T-cell level are more likely to manifest HPV infection and cervical neoplasia.

The magnitude of HPV prevalence is proportional to the severity of immunosuppression, although HPV-16 is more weakly associated with immune status than other high-risk HPV types. A meta-analysis of HPV types among HIV-infected women found an overall prevalence of 56.6% in African women compared to 31.4% in women in North America.[25] Additionally, studies from different populations suggest that HIV-infected women may also be infected with a broader range of HPV types than HIV-uninfected women. The proportion of HPV prevalence attributable to HPV-16 is lower in HIV-infected women, including those with high-grade SIL, than in women in the general population.[25]

Cervical cytology is now deemed an adequate screening tool for CIN in HIV-positive women, but the high recurrence rate and multifocal nature of this disease reinforces the need for annual screening. Highly active antiretroviral therapy (HAART) does not seem to impact the natural history of HPV infection or reduce the risk of cancer or precancer in HIV-infected women. However, prolonged exposure of the cervix to high-risk HPV in a relatively immunosuppressed state in HIV-infected women may confound this relationship.

Other genitourinary cancers

Anal cancers in women and men are strongly associated with HPV, with 80–95% reported HPV prevalence in anal carcinomas.[26] A high level of HPV infection may be important for the development of anal intraepithelial neoplasia. Men and women who practice receptive anal intercourse have higher anal cancer rates, but anal disease in women can occur even without anal intercourse, presumably through perineal HPV spread. Prior to the era of highly active antiretroviral combination chemotherapies, the HIV epidemic was associated with a huge rise in anal cancer rates affecting both men and women. Cytologic screening for anal disease with a Pap smear-type anal swab is advocated for high-risk persons, especially for sexually active homosexual men; the utility of HPV for anal disease screening is not established.

Invasive squamous carcinoma is not common in the vulva or vagina. Using PCR methods, the prevalence of HPV infection in vaginal cancer is about 60–65% and in vulvar cancers 20–50%. Both HPV types 16 and 18 are associated with vulvar squamous cell carcinoma, although the prevalence of HPV-16 is less than in cervical carcinoma. Primary squamous cell carcinoma of the ovary is rare; most cases represent malignant transformation of ovarian teratomas and are not HPV related.

Oncogenic HPV types can be detected in 40–50% of carcinoma of the penis and urethra. Other malignancies may be HPV related.[27] Other urologic malignancies (e.g. prostate and bladder) may not have an HPV etiology, although this continues to be studied. It has been suggested, but not clearly demonstrated, that HPV can act as an oncogenic agent in persons who are already predisposed to bladder cancer for other reasons.

Additionally, infection with HPV is most likely a risk factor for a subset of squamous cell carcinoma of the head and neck.[28] However, further research is needed to elucidate the full role of HPV in the development of nongenital tract cancers. 'Low-risk types' such as HPV-11 can nonetheless cause severe non-neoplastic disease including juvenile laryngeal papillomatosis. The non-anogenital disease spectrum of HPV is discussed in Chapter 156.

PREVENTION

Primary HPV prevention is promoted through vaccination as well as through measures to prevent or reduce exposure. Measures for preventing exposure are not absolute, but reduce risk of transmission, nonetheless. These measures include encouraging sexual abstinence or delaying sexual debut among sexually inexperienced youth, mutual monogamy in couples, correct and consistent condom use, and other sexual risk reduction strategies. Measurable successes in reducing the incidence of cervical cancer have been achieved in industrialized nations through screening by cervical cytology, followed by clinical treatment when indicated. In countries where screening has been extensive, the mortality rate from cervical cancer has fallen markedly since the 1950s. However, lower-income women may have higher sexual risk profiles and the lowest rates of access to preventive health services, thus resulting in cervical cancer death rates greater than those seen among socioeconomically higher women.

As a medical screening procedure, Pap smears have numerous operational challenges in developing country settings.[29] A proper Pap smear requires a pelvic examination, an adequate sample of cells, including those from the transformation zone (the squamocolumnar junction), proper fixing of the cells, excellence in cytopathologic processing and assessment, adequacy of the clinical response to the results, and appropriate follow-up by patients. This operational complexity, combined with the Pap smear's only moderate sensitivity, has motivated a search for improved cervical cancer screening approaches.[29] In western industrialized settings, liquid-based cytology (Thin Prep™ Pap smear) is now the test of choice among many clinicians and laboratories for cervical cytology due to its relative ease of standardization and increased sensitivity (over conventional Pap smear); however, the high costs of infrastructure and maintenance, as well as the need for multiple visits, make it difficult to use in resource-limited settings.[30]

Visual inspection with acetic acid (VIA) stands out as one of the most promising low-cost alternatives to conventional Pap smears, especially for use in low-resource settings.[29] VIA involves the application of 3–5% dilute acetic acid (common household vinegar) to the cervix after examination through a vaginal speculum, and observing the color changes on the cervix after 1 minute. Due to reversible protein coagulation and increased reflectivity of precancerous cells having a high nuclear:cytoplasmic ratio, precancerous areas of the squamocolumnar junction appear white in color (Fig. 59.2). Trained nonphysician health-care workers can perform the screening. VIA gives immediate results, which can help in linking screening and treatment in the same visit, through the use of cryosurgery, an ablative treatment method for cervical precancerous lesions that has been proven to be safe, acceptable and feasible for large-scale implementation in developing countries. Other modifications to VIA have been proposed, including use of Lugol's iodine instead of acetic acid (visual inspection with Lugol's iodine, VILI) or visual inspection with magnification (VIAM). VILI seems to have comparable or better accuracy measures than VIA, while VIAM does not seem to offer any additional benefits. Digital camera imaging of the cervix has been proposed as another adaptation to enhance the accuracy of VIA. This method provides visual documentation for medical records, a measure of quality control, patient and nursing education, and also allows distance consultation (telecervicography).

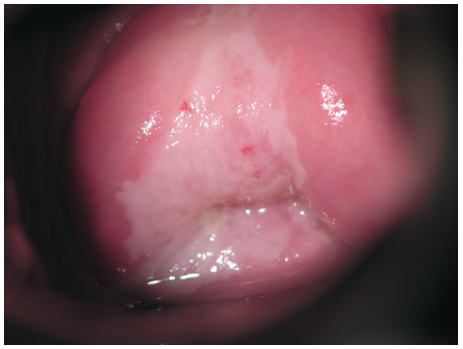


Fig. 59.2 Appearance of the cervix after application of 3–5% acetic acid. Note the well-defined, opaque acetowhite area, with regular margins, involving a large part of the visible squamocolumnar junction. Courtesy of Dr Mulindi Mwanahamuntu.

Current screening guidelines

Molecular HPV screening of cervicovaginal samples is most sensitive and objective. Many HPV DNA tests are used and others are being developed, but the only Food and Drug Administration (FDA)-approved, commercially available HPV detection kit as of 2008 is Digene/Qiagen Corporation's Hybrid Capture-2® (HC-2). This assay utilizes a nonradioactive chemiluminescence method that stratifies HPV genotypes into the following risk strata:

- high/intermediate-risk oncogenic HPV types (types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68); and
- low-risk non-oncogenic HPV genotypes (types 6, 11, 42, 43, and 44).

Multiple studies across the world have used HPV HC-2 for categorizing patients into low- and high-risk groups. Whether HPV HC-2 has value as a stand-alone screening tool, can complement cytology screening or is too cost-ineffective for use has been the subject of intense investigation; it has been shown to be a useful triage tool for patients with minor cytologic abnormalities on Pap smears in clinical practice. Results from the Atypical Squamous Cells of Undetermined Significance (ASCUS) Low-grade Squamous Intraepithelial Lesion (LSIL) Triage Study (ALTS), a consensus report crafting revised guidelines for reporting cervical cytologies (2001 Bethesda System), another consensus report for management of women with abnormal cervical cytology, many meta-analyses, reports and cost-effectiveness/decision analysis models have now suggested logical roles for HPV testing in clinical practice and cervical cancer screening programs.[31–33] Based on the ALTS results, the 2001 Bethesda System subdivides the previous ASCUS cytopathologic category into two: 'atypical squamous cells of undermined significance' (ASCUS) and 'atypical squamous cells, cannot exclude high-grade SIL' (ASC-H).

Liquid-based cytologic assessment makes possible retention of the Pap test specimen for subsequent HPV screening. Thus, a valuable role for HPV testing is use of the Pap test using the liquid cytology method and testing the remaining sample should the cytology result demand further HPV testing analyses. Note that a second patient visit and examination are not needed with this approach. A large expert panel developed the 2006 consensus management guidelines, recommending HPV testing as the preferred method of follow-up evaluation for women aged 20–30 years with ASCUS cytology. HPV DNA testing as a primary screening test for women above age 30 is also approved and the test is being marketed in selected countries as a primary cervical cancer screen, with or without cytology. The sensitivity of a single lifetime HC-2 test for detection of high-grade SIL is around 80–90% (higher than for cytology) and specificity has ranged from 57% to 89%. Human papillomavirus testing holds promise as a cost-effective tool in resource-limited settings, especially since it needs much less quality control and assurance than cytology.

Cost-effectiveness of both primary and secondary prevention through cervical cancer screening has been used to evaluate public policy.[34,35] Analyses in countries with existing screening programs have focused on identifying the optimal screening interval, the ages for starting and stopping screening, and consideration of the various recommended roles for HPV testing. Analyses for resource-poor settings with infrequent or no screening have focused on strategies that enhance the linkage between screening and treatment, consider non-cytologic alternatives such as VIA, VILI and HPV DNA testing, and target women between the ages of 35 and 45 years for screening one, two or three times per lifetime. Cervical cancer screening strategies incorporating visual inspection of the cervix with acetic acid or HPV DNA testing for HPV in one or two clinical visits are cost-effective alternatives to conventional three-visit cytology-based screening programs in resource-poor settings.[35,36] Single lifetime screening is as cost-effective as hepatitis B vaccination, second-line treatment regimens for tuberculosis and malaria prevention with bed nets.[35,36]

Vaccination against HPV

Immunization with the L1 protein of the HPV virus induces a strong and long-lasting immune response and, as of 2008, two prophylactic vaccines are commercially available for public health application.[37–39] Both are composed of HPV type-specific L1 proteins that self-assemble into noninfectious, recombinant virus-like particles (VLPs).[39] Gardasil® (Merck) is a quadrivalent HPV-6/11/16/18 L1 VLP vaccine manufactured in a yeast system. Each injection includes 20 µg of HPV-6, 40 µg of HPV-11, 40 µg of HPV-16 and 20 µg of HPV-18 in an adjuvant of 255 µg of aluminum hydroxyphosphate sulfate.[39] Cervarix® (GlaxoSmithKline) is an HPV-16/18 L1 VLP vaccine manufactured in an insect-cell system and includes 20 µg of HPV-16 and 20 µg of HPV-18 VLP in an adjuvant of 500 µg of aluminum hydroxide with 50 µg of 3-deacylated monophosphoryl lipid A (ASO4).[39] For both vaccines, administration of three doses induces high levels of serum antibodies in virtually all vaccinated individuals.

In women who have no evidence of past or current infection with the HPV genotypes in the vaccine, both vaccines show >90% protection against persistent HPV infection for up to 5 years after vaccination, which is the longest reported follow-up so far.[39] The CDC Advisory Council for Immunization Practices (ACIP) recommends the use of Gardasil® to girls as young as 9 years old, before the initiation of sexual activity, and women up to 26 years of age (for 'catch-up' vaccination, in case they were not vaccinated when they were younger).[40]

As yet, the duration of antibody protection induced by vaccination is unknown, as is the need for future booster doses. Other biologic issues include unknown vaccine efficacy in pre-adolescents/adolescents, for whom the vaccine is recommended but no efficacy end points were assessed in the original trials. 'Bridging studies' have shown high immunogenicity and favorable safety profiles in this age group (9–15 years), but it is not known whether this higher immune response in younger children translates into detectable differences in clinical efficacy or duration of protection. The vaccine performance in immunocompromised individuals is unclear, an important issue for Africa and elsewhere given the frequency of immunosuppression due to HIV/AIDS. The 'cross-protection' against other, nonvaccine HPV types is now reported as only partial, and limits the initially projected claims of wider applicability of the vaccine.[41] In particular, the impact of HPV-16/18 eradication on the predominance of other HPV types in cervical cancer will be known only after decades, unless a broadly neutralizing antibody response against several high-risk types can be generated by second-generation prophylactic vaccines. Clinical trials are already ongoing to evaluate a nine-valent vaccine (types 6, 11, 16, 18, 31, 35, 45, 52, 58).

Finally, real-world implementation suggests that vaccine 'roll-out' will be difficult. Overcoming patient, parent and provider level attitudes that do not favor vaccination, especially against a sexually transmitted infection, is a formidable challenge. It is also critical to study optimal policies on male vaccination (assuming future approval of the vaccine for male patients) and determining whether HPV vaccines should be available for adolescents without requiring parental consent or if HPV vaccines should be mandated for school entry. Tiered pricing for HPV vaccines, innovative financing mechanisms and multidisciplinary partnerships will be essential in order for the vaccines to

reach populations in greatest need, especially in developing countries. Prices in 2008 are among the highest of any commonly used vaccine. Since HPV vaccines do not eliminate the risk of cervical cancer, cervical screening will still be required to minimize cancer incidence.

Prophylactic (VLP-based) antibody-generating vaccines do not eliminate pre-existing persistent infections.[42] Vaccines proposed as immunomodulators or therapeutic adjuncts to radiation therapy, termed therapeutic vaccines, are thus being investigated for impacting established HPV infection and its consequences. Based on successes in several animal models, phase I trials of the safety and immunogenicity of multiple therapeutic vaccine candidates have been undertaken. Various approaches are being investigated.[43]

CLINICAL FEATURES

Human papillomavirus infection may be asymptomatic or manifested in various benign or malignant lesions on cutaneous and mucosal surfaces. Genital warts can be flat (condyloma lata), as is common in the cervix, or more papillary (condyloma acuminata), as is typical in the vulva, vagina or anus. Human papillomavirus is present in a wide variety of clinical circumstances: subclinical latent infection, clinically apparent warts, neoplasia or carcinoma, with normal or abnormal genital cytology[44] (Fig. 59.3). Condyloma acuminata and condyloma lata are often caused by low-risk HPV types 6, 11 or related types. Most tend to regress naturally and are rarely associated with malignant progression. They are often multifocal, can be large and have a high rate of recurrence after treatment.

Cervical intraepithelial neoplasia that exhibits nuclear atypia possesses potential for progression to invasive carcinoma if not removed. The transformation zone of the cervix has neither the protective qualities of the keratinized squamous cells nor the vascular and immunologic milieu of the columnar epithelia and is particularly susceptible to HPV infection and subsequent neoplastic transformation. The usual cervical lesion is flat with warty colposcopic and cytopathic features and may be visualized when painted with 5% acetic acid to reveal acetowhite thickening. Both CIN I and II may precede CIN III and carcinoma in situ, and a small proportion progress to invasive carcinoma.[1] The majority of lower-grade CIN regresses to normal. The trend in cytopathology and gynecologic pathology is to use full descriptions of biopsy results, acknowledging the limitations of the CIN system that may not capture the complexity of a given cervical specimen. In addition, the 2001 Bethesda System for cytology results merges CIN II and III, as did the older system, since interobserver reliability in distinguishing these is poor.[45]

High-risk HPV types have been associated with all grades of CIN, whereas low-risk HPV types have segregated primarily in condylomata and CIN I (Table 59.1). However, discrepancies between HPV type and morphology do exist, and cytology and histology provide variable and, at times, conflicting, information. Cells with inflammatory changes but no neoplastic characteristics are termed abnormal squamous cells or atypical cells (Fig. 59.4) and their management was the topic of the clinical trial (ALTS) described earlier.[46,47]

HPV infections occasionally are clinically recognized by the characteristic raised appearance of the accompanying warts. Acetowhitening is helpful to clinicians examining the cervix, vagina, vulva, anus or penis, especially under magnification. This is done following an abnormal cytology result in order to identify lesions requiring biopsy. A valid reference standard for grading HPV-related anogenital pathology remains elusive, as clinical and pathologic results are prone to observer interpretation and misclassification (Figs 59.5–59.9).[3]

On microscopic examination of a Pap test or biopsy, the diagnosis of HPV infection can sometimes be made by finding neoplasia or dysplasia. The term koilocytotic dysplasia is still used widely if cells pathognomonic for HPV infection are seen. Koilocytes are large cells with a clear enlarged cytoplasmic space. The nucleus is hyperchromatic, often irregular and larger than normal. It may appear smaller than normal, though it is not, because the nucleus is within a larger-than-normal cell and cytoplasmic space.[3]

Table 59.1 Human papillomavirus types and associated diseases

HPV-associated diseases	HPV types
Skin warts	1, 2, 3, 4, 7, 10, 26, 27, 28, 29, 41, 48, 57, 60, 63, 65, 75, 76, 77, 78
Epidermodysplasia verruciformis benign lesions	3, 5, 8, 9, 12, 14, 15, 17, 19, 20, 21, 22, 23, 24, 25, 36, 47, 49, 50
Epidermodysplasia verruciformis squamous cell carcinoma	5, 8, 14, 17, 20, 47
Periungual squamous cell carcinoma	16, 34, 35
Laryngeal papillomas	6, 11
Oral focal epithelial hyperplasia	13, 32
Squamous cell carcinoma (tonsil)	16, 33
Anogenital warts	6, 11, 40, 42, 43, 44, 54, 55, 74
Low-grade anogenital intraepithelial neoplasia	6, 11, 16, 18, 30, 31, 33, 34, 35, 39, 40, 45, 51, 52, 56, 57, 58, 59, 61, 64, 66, 67, 68, 70, 71, 72, 73, 74
High-grade anogenital intraepithelial neoplasia	16, 18, 31, 33, 34, 35, 39, 45, 51, 52, 56, 58, 59, 68
Squamous cell carcinoma (cervix mostly)	16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68
Adenocarcinoma (cervix mostly)	16, 18

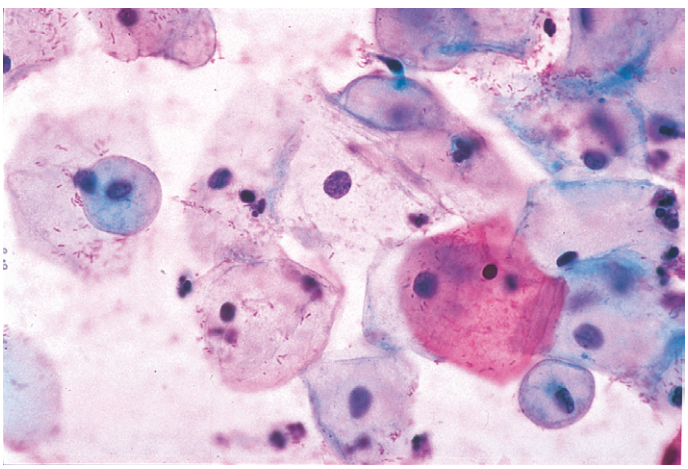


Fig. 59.3 Normal squamous cells and inflammatory cells. (Pap stain.) Courtesy of Dr William H Rogers.

DIAGNOSIS

As HPV cannot be cultured *in vitro* in animal models, detection relies on molecular analyses of presence and sequencing of HPV DNA.[48] Early HPV tests used nucleic acid probes labeled with radioactive phosphorus (^{32}P) in a slot/dot-blot hybridization format and nucleic acid *in-situ* hybridization. On the other hand, newer target nucleic acid amplification techniques can detect individual HPV genotypes based on specific primer sequences.[49] Polymerase chain reaction is the

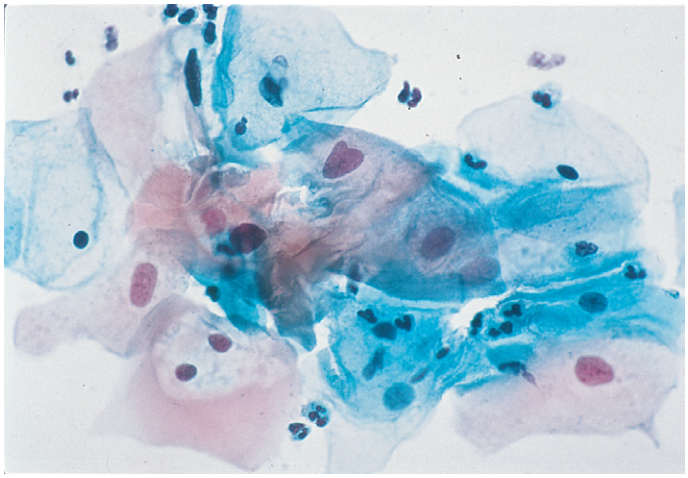


Fig. 59.4 Atypical squamous cells of undetermined significance. Here the cells are slightly enlarged and irregular relative to the cells in Figure 59.3 and contain perinuclear clear areas suggestive, but not diagnostic, of HPV infection. (Pap stain.) Courtesy of Dr William H Rogers.

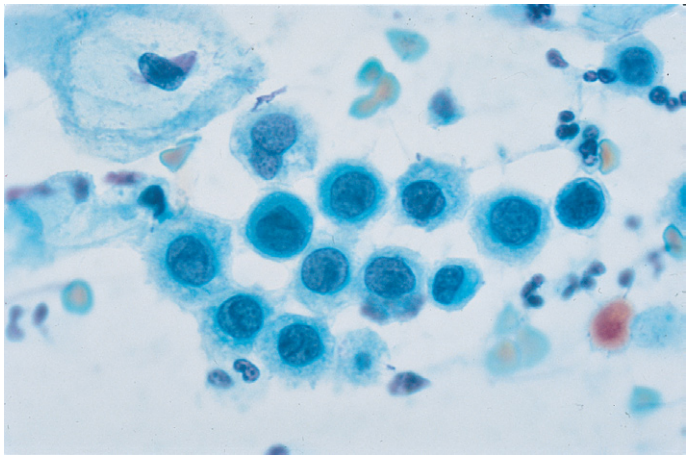


Fig. 59.6 High-grade squamous intraepithelial lesion. This contains small cells with an increased nuclear to cytoplasmic ratio and marked nuclear hyperchromasia; in the classic terminology, this would be considered a severe dysplasia or CIN III. (Pap stain.) Courtesy of Dr William H Rogers.

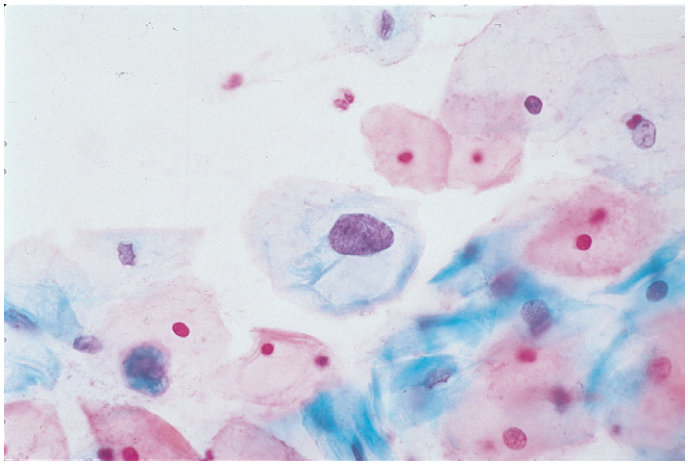


Fig. 59.5 Low-grade squamous intraepithelial lesion. In this case, the lesion would classically be called a mild dysplasia; the cell in the center of the photograph has a nucleus that is enlarged more than four times the size of the surrounding normal squamous cells; in addition, the nucleus has irregular nuclear outlines and hyperchromasia. (Pap stain.) Courtesy of Dr William H Rogers.

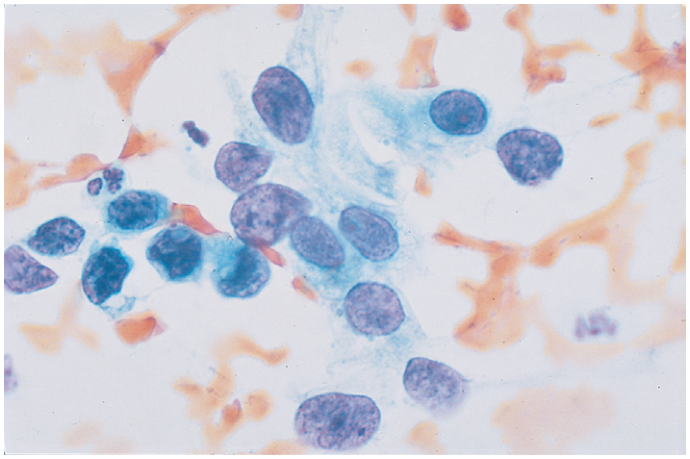


Fig. 59.7 Carcinoma in situ. The abnormal hyperchromatic cells have indistinct cell borders and form a pseudosyncytial arrangement. (Pap stain.) Courtesy of Dr William H Rogers.

'gateway technology' to all genotyping methods and can be harnessed for multiple applications, including HPV viral detection, quantitative measurement of HPV viral load, HPV DNA sequencing, analyzing mutations, etc.[49]

The L1 gene segment is used most often in genotyping assays based on PCR, wherein primers are selected from conserved or consensus sequences that flank polymorphic, type-specific sequences. Once PCR using consensus primers is performed, genotype determination can be accomplished by several methods, including restriction fragment length polymorphism (RFLP) reverse hybridization using a line-probe/dot-blot assay or cycle sequencing and assignment of genotypes by sequence comparison.[48] Polymerase chain reaction has been used as a diagnostic tool in epidemiologic investigations of HPV, but associated costs and technology requirements are often inappropriate for large screening programs. Although PCR-based methodology can detect very small amounts of HPV DNA, strict laboratory procedures and controls are critical in reducing contamination-related false-positive findings. Various PCR techniques can allow detection of more than 37 genotypes, including high-risk (HR) HPV types (16, 18, 31, 33, 35,

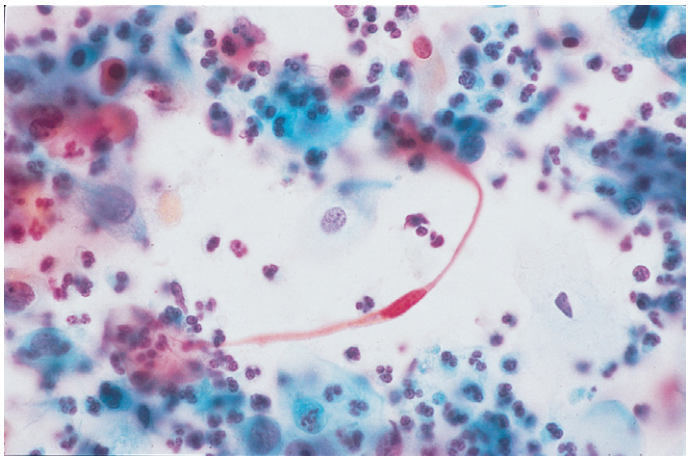


Fig. 59.8 Squamous cell carcinoma. This shows highly atypical, enlarged, abnormal keratinized cells. (Pap stain.) Courtesy of Dr William H Rogers.

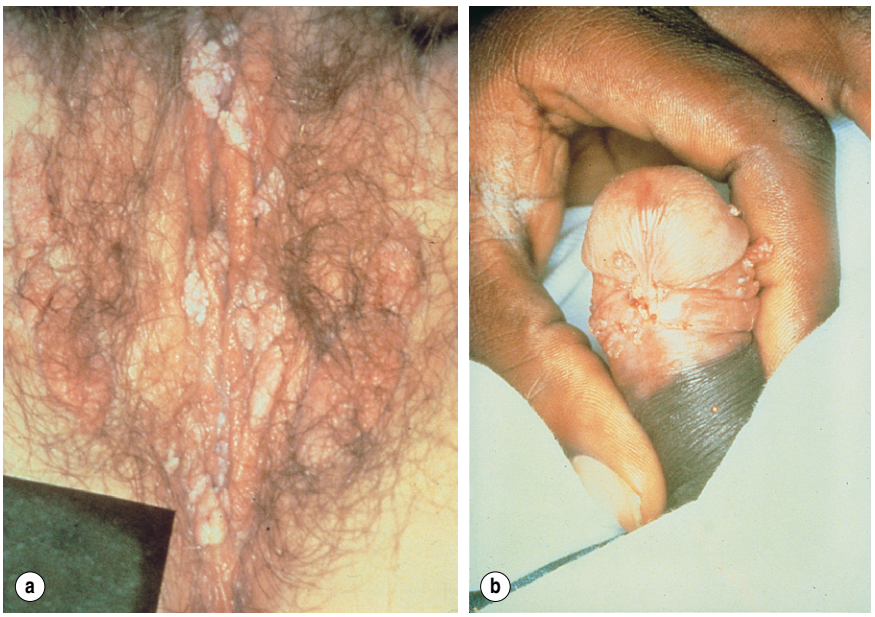


Fig. 59.9 Genital HPV infection. (a) Vulvovaginal HPV infection. (b) Penile HPV infection.

39, 45, 51, 52, 56, 58, 59, 66, 73, 82), probable high-risk (PHR) types (26, 53, 68), low risk (LR) types (6, 11, 40, 42, 54, 55, 61, 70, 72, 81, and CP6108) and HPV types with as yet undefined risk (UR) (62, 64, 67, 69, 71, 83, 84, and IS39).

The sensitivity of PCR can result in DNA detection in a very large proportion of sexually active women (often 20–60%). Although it is the most sensitive technique for identifying HPV, a loss of specificity for predicting cervical disease means that the appropriate use of PCR in clinical screening remains to be determined. Cell sampling strategies include scrape, swab, brush, cervicovaginal lavage, home lavage and even sampling from vaginal tampons. In many epidemiologic and clinical investigations, high sensitivity of HPV assessment is desired to avoid false-negative assessments. In such circumstances, use of the cervicovaginal lavage increases sensitivity method related directly to cellular yield. In routine cervical screening, the Pap test-style approach of cervical scrape and swab, using DNA-based diagnostics, seems sufficiently sensitive for clinical purposes.

Serology may become a tool to evaluate treatment successes, but it is not yet an adequate clinical diagnostic tool due to its suboptimal sensitivity and specificity. Seroepidemiologic studies have assessed antibody response to HPV-16 and HPV-18, and HPV-6 and HPV-11 for the most part. A variety of test formats have been employed, including ELISA, Western blot, radioimmunoprecipitation assays and IgG and IgA levels in serum and in cervical secretions. Standardization is in progress for antibody and DNA measurements to harmonize laboratory procedures; an international standard reference reagent could improve test performance and comparability.[50]

MANAGEMENT

An accurate diagnosis and appropriate treatment plan can help eliminate the long-term sequelae of HPV disease. Current forms of treatment attempt to ablate the pathologic lesions and eliminate HPV. The first goal is realistic in a large majority of patients, whereas the second is elusive given multifocal infection and the ease of re-infection with HPV. Ablation of frank warts or pathologic lesions identified visually or by biopsy can be achieved by topical application of chemicals, cold (cryosurgery), heat (loop electrosurgery, laser) or surgery (cone biopsy, hysterectomy). Topical treatments include salicylic acid, cantharidin, podophyllin liquid, podophilox gel, trichloroacetic acid and topical 5-fluorouracil.[51,52] Success in treating condylomata can be increased if the area is first soaked with 5% acetic acid to show the extent of the local infection.[53] Topical therapies work better against warts occurring on moist mucosal surfaces than on lesions found on heavily keratinized epithelia. Cryosurgery applies pressurized liquid nitrous oxide or liquid carbon dioxide with a swab, freezing superficial squamous epithelium and killing many of the HPV-inhabited cells. Loop electrosurgical excision procedure (LEEP), also called large loop excision of the transformation zone (LLETZ), has gained repute over the past few years as a treatment modality for high-grade CIN, since it is a safe and effective outpatient procedure carried out by removing broader areas than can be ablated and also providing tissue for histopathologic analysis. LEEP is much preferred over cold knife conization (removal of a large, cone-shaped part of the lower cervix), especially for cryotherapy-ineligible cervical lesions. Warts of the vagina, urethral meatus, anus and oral mucosa are often treated with cryotherapy initially, while topical agents may be used to treat warts of the vagina, vulva, anus, and urethral meatus and penis in men, when the evidence implicates low-risk HPV types.[52] Recurrence rates associated with all modalities are high because these methods often fail to eradicate the subclinical or latent reservoir of HPV remaining in adjacent epithelial cells and mucous membranes. Other methods such as laser vaporization have been studied and show adequate efficacy yet are limited in their widespread availability due to high costs. Surgery is reserved for patients with either extensive or refractory lesions.

During pregnancy, removal of visible warts is often advisable due to their propensity to proliferate and become friable.[54] Similarly, high-grade CIN may need intervention even during pregnancy. In contrast, treatment for subclinical genital HPV infection and lower-grade CIN during pregnancy is not recommended since spontaneous improvement postpartum is common.[54]

Immunologic therapy with interferons can be directed against all sites of infection, including clinical, subclinical and latent disease. Interferons have been used successfully as monotherapy or in combination with traditional modalities to treat anogenital condyloma acuminata. Persons with severe or recurrent disease are far more likely to receive interferon therapy in combination with other therapeutic modalities than monotherapy alone. Cost and side-effects inhibit the widespread use of interferon therapy.[55]

In the absence of HIV co-infection, currently available therapeutic methods (all modalities) are moderately successful in the treatment of frank genital warts, with recurrence rates of 25% within 3 months,

and in clearing up HPV. Recrudescence is higher in HIV-infected and immunocompromised individuals. Fortunately, cytologic successes are higher than HPV clearance rates. Patient follow-up is based on colposcopy and cytology, while the best use of HPV diagnostics for post-therapeutic prognosis and management remains to be defined.

Chemoprevention

Chemoprevention is the use of agents to prevent or retard neoplastic progression. The desired effect for chemopreventive agents is complete regression or at least the prevention of progression. However, spontaneous high regression rates, the subjective nature of CIN diagnosis, the impact of biopsy on regression and the low threshold for ablative intervention complicate evaluation of such approaches.

Several processes in the HPV infection cycle are appropriate targets for the development of antiviral and/or antineoplastic agents.[56] The development of chemical compounds active against HPV could prevent the benign and malignant diseases associated with HPV infection. Use of retinoids such as dietary vitamin A (retinol) and carotenoids is being studied early in the neoplastic process (either systemically or locally) to maintain normal cervical cell function and inhibit disease progression. Retinoids do not seem to act to inhibit proliferation of HPV-immortalized cervical cells via effects on HPV E6 and E7, but they may act to inhibit cervical proliferation by suppressing the activity of epidermal growth factor and insulin-like growth factor signaling pathways. Combined interferon–retinoid therapy might provide an enhanced anticancer benefit due to the fact that each agent inhibits cervical cell proliferation.

SUMMARY

Human papillomavirus is a ubiquitous, often transient infection that can cause anogenital carcinoma under conditions favoring persistence of high-risk genotypes. Simpler screening approaches now enable expansion of programs to high-prevalence, resource-limited settings. Availability of preventive HPV vaccines gives additional hope for radical reductions in cervical and other anogenital cancers. However, control of HPV-related cancers is realistic only with a concerted global effort to expand screening, treatment and vaccination to all women.

REFERENCES



References for this chapter can be found online at http://www.expertconsult.com

Chapter 60

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D William Cameron

Lymphogranuloma venereum, chancroid and granuloma inguinale

Cutaneous lesions are common on the external genitalia.[1] Such lesions may be restricted to the genital region, part of a generalized skin eruption or a local manifestation of a systemic disorder (see Table 60.1).

A thorough medical history and physical examination often provide the necessary keys to correctly diagnosing genital lesions. A history of multiple or new sexual partners or contact with commercial sex workers increases the likelihood of venereal disease. A history of recent drug intake or recurrence of a lesion at the same location upon re-introduction of the causative drug is characteristic of a fixed drug eruption or erythema multiforme. A history of trauma, such as from shaving, scratching or vigorous masturbation may suggest traumatic ulcers. A history of trauma also helps to distinguish the white lesions of postinflammatory hypopigmentation from those of vitiligo. Contact dermatitis is usually accompanied by a history of contact with an irritant or allergen. These can include soaps, lotions, lubricants, condoms or spermicides or perhaps less obvious irritants if they have been inadvertently transferred to the genital region by the hands. A history of pruritus may be obtained with contact dermatitis, scabies, lichen planus or lichen sclerosis. Local tingling or paresthesia may precede the appearance of recurrent lesions due to herpes simplex virus (HSV). A history of systemic involvement will usually accompany the diagnosis of Behçet's syndrome and Crohn's disease.

On physical examination, genital lesions can be broadly categorized as raised, flat or ulcerative (see Table 60.1). Raised lesions such as condylomata lata, pearly penile papules or molluscum contagiosum may be mistaken for genital warts. Hyperpigmented lesions, though usually benign, should be biopsied to rule out squamous cell carcinoma, Kaposi's sarcoma or malignant melanoma. Flat hypopigmented lesions that are not morphologically characteristic of vitiligo or postinflammatory hypopigmentation also should undergo biopsy to seek a diagnosis of lichen sclerosis or lichen planus. Ulcerative

Table 60.1 Differential diagnosis of cutaneous genital lesions

Category		Lesion	Usual morphology	Comments
Flat lesions	Erythematous	Contact dermatitis	Red edematous patch, may be vesicular	Recent contact with an irritant or allergen
		Seborrheic dermatitis	Ill-defined, erythematous, scaly patches or plaques with crusting	Idiopathic inflammation of the sebaceous glands
		Tinea cruris (dermatophytosis)	Well-demarcated scaling plaques, with an erythematous border, over the inner thighs	See Chapter 179
		Psoriasis	Well-demarcated erythematous plaques with scales on keratinized skin, and without scales on nonkeratinized skin	Typically involves the scalp, elbows, knees, back and buttocks
		Candidal balanitis	Red papules or plaques on penile shaft	See Chapter 178
		Plasma cell (Zoon's) balanitis	Glistening, moist, erythematous patch over the glans penis or coronal sulcus	Benign, chronic balanitis
	Hyperpigmented	Pigmented nevi	Well-demarcated lesions with regular borders and homogeneous pigmentation	Biopsy to rule out malignant melanoma
	Hypopigmented	Lichen sclerosis	Hypopigmented plaques of atrophic skin with progressive scarring over the vulva or penis (balanitis xerotica obliterans)	Benign chronic dystrophic disease, more common in postmenopausal women
		Lichen planus	Reticulated, white branching striae on nonkeratinized skin	Presents as inflammatory papules on keratinized skin
		Vitiligo	Depigmented well-demarcated patches with no skin surface changes	Absence of melanocytes, possibly autoimmune
		Postinflammatory hypopigmentation	Flat ill-defined demarcated patches with partial or total loss of pigment	Follows healing of some inflammatory lesions

Table 60.1 Differential diagnosis of cutaneous genital lesions—cont'd

Category		Lesion	Usual morphology	Comments
Raised lesions	Normal pigmentation	Genital warts	Cauliflower-like (condylomata acuminata), flat-topped or rounded papules	See Chapter 59
		Secondary syphilis	Moist, hypertrophic, flat-topped papules (condylomata lata)	May also be ulcerative; see Chapter 57
		Molluscum contagiosum	Smooth, firm, shiny umbilicated nodules	See Chapter 159
		Pearly penile papules	Crownlike arrangement of dome-shaped papules around the corona of the penis	Benign angiofibromas usually appearing in adolescence
		Prominent sebaceous (Tyson's) glands	Clustered pale papules on the corona and inner surface of prepuce	Aberrant sebaceous glands, normal anatomic variant
		Cysts	Dome-shaped nodules or papules	Common, benign growths
		Fordyce spots	Purplish or reddish telangiectatic papules on scrotum, penis or vulva	Occurs in late puberty, normal anatomic variant
		Sclerosing lymphangitis	Firm translucent cord encircling the penis proximal to the corona	Result of trauma or friction
	Erythematous	Scabies	Elongated papules (female's burrow) with surrounding excoriation	Look for corresponding lesions on wrists or in fingerwebs; see Chapter 11
		Hidradenitis suppurativa	Tender inflamed nodules at base of hair follicles	Recurrent abscesses of apocrine glands
		Pyogenic granulomas	Pedunculated vascular masses usually on the scrotum	Benign tumors
	Hyperpigmented	Seborrheic keratosis	Brown, keratotic, verrucous lesions	Benign lesions usually occurring after age 40
		Lichen planus	Pruritic, flat-topped violaceous papules on keratinized skin	Idiopathic, inflammatory eruption, often associated with oral lesions
		Squamous cell carcinoma *in situ* (three forms)	Large, red-brown scaly or crusted verrucous lesion, usually solitary	Bowen's disease
			Smaller, flat verrucous lesions, usually multifocal	Bowenoid papulosis
			Erythematous, raised, irregular plaques	Erythroplasia of Queyrat
		Kaposi's sarcoma	Violaceous indurated plaques or nodules	See Chapter 95
Ulcerative lesions	Nonvenereal diseases	Aphthous ulcers	Painful irregular ulcers with erythematous borders and a white fibrin base	Commonly associated with oral ulcers
		Behçet's syndrome	Painful, shallow, irregular ulcers with erythematous borders and a white fibrin base on the glans penis or labia minora	Associated with oral ulcers, uveitis, arthritis, vasculitis or chronic meningoencephalitis
		Reiter's syndrome	Painless, serpiginous, shallow erosions of the penis with raised, erythematous borders (circinate balanitis)	Typically associated with urethritis, arthritis or conjunctivitis
		Erythema multiforme	Superficial vulvar, vaginal or penile erosions	Associated with stomatitis or bullous eruption over palms and soles
		Fixed drug eruption	Well-demarcated dusky red shallow erosion	History of drug intake
		Erosive lichen planus	Nonspecific shallow ulcerations	Commonly associated with oral lesions
		Trauma	Variable morphology	History of recent trauma
		Crohn's disease	Ulcerative perineal granulomas; may form scars, sinuses or fistulas	Inflammatory bowel disease; anal fissures may be present
		Squamous cell carcinoma	Irregular, friable ulceration	Elderly patients with an indolent nonhealing ulcer
	Venereal diseases	Primary syphilis	Single painless ulcer with indurated edges and clean base	See Chapter 57
		Secondary syphilis	Multiple painless shallow ulcers	See Chapter 57
		Herpes simplex virus	Painful shallow ulcers, may be crusted	See Chapter 76
		Chancroid	Painful irregular, nonindurated ulcer with necrotic base; may have multiple ulcers	Commonly associated with inguinal buboes
		Granuloma inguinale	Painless nonindurated, 'beefy-red' ulcer with a clean base; may have multiple ulcers	May develop groin abscesses (pseudobuboes)
		Lymphogranuloma venereum	Primary lesion usually unnoticed but may present as a small painless ulcer or papule	Usually presents with painful inguinal lymphadenopathy

lesions may be classified as painful or painless. Syphilis and granuloma inguinale are typically painless, unless secondarily infected by other bacteria. Painful ulcers include chancroid, HSV, aphthous ulcers and Behçet's syndrome. In addition to genital ulceration, Behçet's syndrome may manifest with oral ulcers, panuveitis, large joint arthritis, erythema nodosum or chronic meningoencephalitis.

HIV and genital ulcer disease have reciprocal and synergistic effects. By disrupting the epithelial barrier and drawing immune cells (including $CD4^+$ cells) to the site of infection, genital ulcers facilitate the transmission of HIV.[2,3] Conversely, the immune suppression caused by HIV infection may alter the presentation and course of genital ulcer disease as well as the response to conventional therapy. Individuals with HIV may present with multiple, larger and more invasive genital ulcers which are slow to resolve. Ulcers due to HSV, for example, may become chronic and present as painless lesions due to destruction of local nerve endings.[4,5] Conventional therapies for genital ulcer disease in HIV-infected individuals may be complicated by slower healing and increased risk of treatment failure. While many advocate longer courses of therapy for HIV-positive individuals with genital ulcers, guidelines on appropriate durations are lacking. In most cases, therapy should be continued until complete resolution of the ulcer with close follow-up after discontinuing antibiotics.

The management of cutaneous genital lesions generally depends on making an accurate diagnosis. However, a syndromic approach to the management of genital ulcer disease has proved both feasible and useful in light of the cost and logistics required to make a definitive microbiologic diagnosis in resource-limited settings.[6] The management of syphilis and HSV is discussed elsewhere in this text. The remainder of this chapter will deal with lymphogranuloma venereum, chancroid and granuloma inguinale.

Lymphogranuloma Venereum

Lymphogranuloma venereum (LGV) is a sexually transmitted disease caused by three serovars of *Chlamydia trachomatis*: L1, L2 and L3.

EPIDEMIOLOGY

Lymphogranuloma venereum is endemic in West and East Africa, India, South East Asia, South America and some Caribbean islands. Until recently it occurred only sporadically in North America and Europe, usually in returning travelers. Beginning in 2003, outbreaks of LGV have been reported from across Western Europe[7,8] as well as from the United Kingdom, Canada, the United States and Australia. More than 98% of cases have been in men who have sex with men (MSM) and the majority of these appeared to be from high-risk sexual networks. In all jurisdictions, risk factors have included older age (median 40 years), white race and co-infection with HIV and other sexually transmitted infections, most notably gonorrhea. Most men infected with LGV have reported both receptive and insertive anal intercourse without condoms with multiple sexual partners. Whether the high prevalence of HIV among men diagnosed with LGV represents co-transmission, more symptomatic disease among those co-infected with HIV, or introduction of LGV into HIV-assortative sexual networks is not clear. Recent data from the UK suggest that LGV infection among MSM may have settled to a stable endemic rate.[9]

PATHOGENESIS AND PATHOLOGY

Lymphogranuloma venereum is acquired sexually by direct contact with infected lesions although transmission through contaminated fomites has been documented. Unlike other *Chlamydia trachomatis* infections of the urogenital system (due to serovars D through K) which are confined to the mucosal layer, the LGV serovars invade the submucosal connective tissue and lymphatic system. After exposure, epithelial abrasions allow the organism to penetrate into the submucosal tissues where they are taken up by macrophages and subsequently carried to the regional lymph nodes.

CLINICAL FEATURES

Lymphogranuloma venereum begins in the genital region and spreads through the regional lymphatics. Clinical disease classically occurs in three stages. The transient primary lesion is a small, painless genital papule which may ulcerate, and occurs at the site of inoculation. It appears 3–21 days after exposure and generally goes unnoticed. It usually occurs on the coronal sulcus in men and on the cervix, posterior vaginal wall or vulva in women. Urethral involvement may cause urethritis with discharge and dysuria.

The second stage generally occurs 2–6 weeks after the primary infection. It is characterized by painful regional lymphadenopathy and systemic symptoms. In men, the inguinal lymph nodes are affected and node enlargement on either side of the inguinal ligament produces the characteristic 'groove sign'. In women, lymph drainage from the rectum and vagina results in pelvic lymphadenopathy. Involvement of these deep nodes causes lower abdominal and back pain. Initially, the lymph nodes are mobile and discrete, but with progressive inflammation they become fixed and suppurative with bubo formation. Buboes, enlarged fluctuant nodes, may spontaneously rupture or form chronically draining sinuses. Hematogenous dissemination has been documented by the recovery of organisms from the blood and cerebrospinal fluid. Such patients experience nonspecific constitutional symptoms or, less commonly, present with meningoencephalitis, pneumonitis, arthritis or hepatitis.

The third stage is marked by subsequent scarring and fibrosis of affected tissue and results in lymphatic obstruction and genital edema (Fig. 60.1). This can lead to severely disfiguring conditions such as thickening and fibrosis of the labia in women (esthiomene) and distortion of the penis in men. Regional spread of LGV to the pelvis in women may cause salpingitis, pelvic adhesions and infertility.

Anorectal infection with LGV is predominantly seen in women and homosexual men and occurs by direct inoculation from either anal intercourse or contaminated fomites. Patients present with fever, rectal pain and mucopurulent or bloody anal discharge. Mucosal ulcerations and friable granulation tissue within the rectum are evident on sigmoidoscopy and resemble inflammatory bowel disease. The rectal ulcers are sharply demarcated with a mixed inflammatory infiltrate in the adjacent lamina propria and occasional crypt abscess formation. Crohn's disease, syphilis and herpetic proctitis should be considered in the differential diagnosis. Untreated LGV infection of the anorectum can result in perirectal abscesses, anal fissures, fistulae, and fibrosis causing rectal stricture.

Interestingly, the majority (70–96%) of LGV infections reported among MSM in western countries have presented exclusively with proctitis. The urogenital symptoms typically associated with LGV



Fig. 60.1 Lymphogranuloma venereum causing unilateral vulvar lymphedema and inguinal buboes.

have been notably absent and testing of urethral swabs and urine by polymerase chain reaction (PCR) for serovars L1, L2 and L3 in the majority of cases has been negative. This absence of genital ulcers and enlarged inguinal lymph nodes may mislead the clinician from considering LGV as the cause of proctitis and thereby delay diagnosis and treatment. While the preponderance of rectal cases among men who report experiencing both receptive and insertive anal intercourse may suggest a failure to recognize penile lesions and inguinal adenopathy due to LGV, the propensity for LGV to remain asymptomatic or present as a typical 'non-gonococcal' urethritis may also be unappreciated. Notably, in one study from the Netherlands, 32 men were found to have LGV infection of the rectum on routine screening, yet 40% had no anoscopic abnormalities and 56% reported no anal discharge.[10]

Conjunctival or oropharyngeal infection occurs rarely and may be a result of autoinoculation or orogenital sex. The regional mandibular and cervical lymph nodes are involved and subsequent spread to supraclavicular and mediastinal nodes has been reported to cause pericarditis.

DIAGNOSIS

LGV is diagnosed clinically with the exclusion of other causes of genital ulcer disease. While detection of LGV serovars by nucleic acid amplification followed by DNA sequencing or restriction fragment length polymorphism (RFLP) analysis is highly sensitive and specific, the availability of these assays varies. Where these tests are available, swabs of the primary lesion as well as vaginal or urethral swabs should be stored dry at –20 °C and sent immediately to the laboratory. Aspirates of inguinal nodes and urine should also be stored frozen. Although not currently approved for use with rectal or oropharyngeal swabs, some reference laboratories may offer nucleic acid testing from these sites for research purposes. Growth of the organism in cell culture is labor intensive, with a recovery rate of only 50%[11] and is generally not offered by most laboratories.

Serology is often the mainstay for the diagnosis of LGV. Complement fixation is currently the most sensitive and widely used test and a titer of 1:64 or greater in the appropriate clinical setting provides a probable diagnosis. Other *Chlamydia* infections may result in a positive complement fixation test, but a titer of less than 1:16 essentially excludes acute LGV. Microimmunofluorescence is more specific but less sensitive than complement fixation and titers of 1:256 or greater are required to provide a probable diagnosis.

MANAGEMENT

Although LGV infections resolve spontaneously, this occurs over several weeks and may be complicated by fibrosis, stricture formation or superinfection. Antimicrobial therapy decreases the incidence of complications, but has not been shown to affect the rate of healing. The recommended therapy irrespective of site of infection is oral doxycycline 100 mg twice daily for 3 weeks. Treatment recommendations for HIV-infected patients are the same. Oral erythromycin 500 mg four times daily is the treatment of choice during pregnancy. Successful treatment with oral azithromycin 1 g once weekly for 3 weeks has been reported but clinical studies are lacking. Trimethoprim–sulfamethoxazole (TMP-SMX; co-trimoxazole) is generally not recommended as it is not sterilizing and treatment failures have occurred. Patients should be followed to document clinical resolution. Anyone who had sexual contact with a patient with LGV within 60 days of the patient's onset of symptoms should be evaluated and, if asymptomatic, treated with doxycycline 100 mg twice daily for 7 days. Repeated aspiration of buboes may be necessary to relieve pain and prevent rupture. Surgical intervention may be required for fistula or stricture, or for management of chronic local edema.

Chancroid

Chancroid is an acute genital ulcer disease typically associated with regional lymphadenitis. The etiologic agent is *Haemophilus ducreyi*, a fastidious, pleomorphic, Gram-negative coccobacillus.

EPIDEMIOLOGY

Chancroid, endemic in Africa, Asia and the Caribbean, is the most common cause of genital ulcers in many developing countries and accounts for 23–56% of cases.[12] It is much less frequent in North America and Europe where it occurs in sporadic outbreaks associated with prostitution, illicit drug use, travel and returning military personnel.

Chancroid occurs predominantly in heterosexuals and outbreaks are related to frequent sexual partner change and the number of sexual partners of an infected person, as occurs in prostitution. Indeed, female sex workers appear to be the main reservoir of infection.[13] Women are often asymptomatic and commercial sexual activity may continue despite active infection and ulceration.[13] Also, PCR testing on cervical specimens from high-risk women without genital ulcers has confirmed that asymptomatic carriage of *H. ducreyi* does occur.[14] Likely the result of exposure to mucosal surfaces, uncircumcised men are more susceptible to infection.

PATHOGENESIS AND PATHOLOGY

Chancroid is transmitted from person to person by direct contact. A break or microabrasion in the skin or mucosa is necessary for the organism to penetrate the epidermis and establish infection. Virulence factors have not been well defined. Pili have been demonstrated, but their role in cellular adhesion is unknown. The production of superoxide dismutase and hemolysin may contribute to tissue damage and ulceration, while evasion of immune defenses and phagocytosis may be mediated in part through lipo-oligosaccharides and outer membrane proteins.

Once within the epidermis, *H. ducreyi* incites a cellular immune response with infiltrates consisting of $CD4^+$ and $CD8^+$ lymphocytes along with macrophages. The inflammatory lesion appears first as an erythematous papule which eventually pustulates and undergoes central necrosis, creating the pathognomonic painful, nonindurated ulcer. Three distinct histologic zones have been classically described on biopsy:

- the superficial zone, in which the organism is most readily seen, contains necrotic debris, fibrin and degenerated neutrophils;
- the middle zone is characterized by edematous inflammatory tissue with neovascularization; and
- the deep zone exhibits a dense cellular infiltrate.

CLINICAL FEATURES

Patients who have chancroid generally present with painful genital ulcers within 4–7 days of exposure. The initial tender, erythematous papule develops into a pustule and progresses to form a painful ulcer about 1–2 cm in size (Fig. 60.2). The ulcer margins, typically undermined and raised, are irregular and sharply demarcated. Because the ulcer edge is not indurated, it is known as a 'soft chancre'. The friable, granular base is often covered with a gray or yellow necrotic exudate. Approximately one-third of patients develop multiple lesions, which may coalesce to form giant ulcers. Autoinoculation may result in 'kissing lesions' on opposing surfaces.

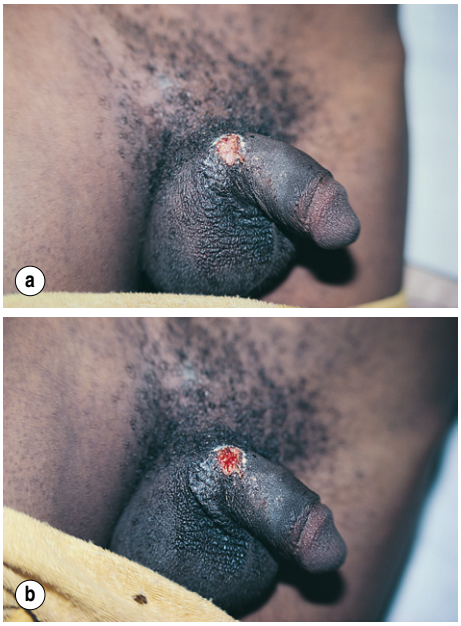


Fig. 60.2 Chancroid ulcer. (a) Before and (b) after the performance of a swab, demonstrating the friability of the ulcer base.



Fig. 60.3 Typical chancroid ulcer. Unilateral lymphadenitis and demonstration of the aspiration of a bubo.

The clinical picture varies with gender, and men tend to be more symptomatic than women. In men, the ulcer is visible and usually located on the prepuce, urethral meatus, glans, coronal sulcus or penile shaft (Fig. 60.3). The scrotum and perineum are less frequently involved. In women, the clinical presentation may be atypical, with symptoms of dyspareunia or dysuria. Ulcers in women may be found on the fourchette, labia, vaginal wall, perineum, perianal region and the medial aspect of the thigh. Involvement of the cervix is rare. The ulcers may resemble those of genital herpes or granuloma inguinale (GI).

Inguinal lymphadenitis is seen in about 50% of men and 35% of women and may be bilateral. Lymph nodes progressively enlarge to become necrotic and fluctuant (buboes), and may spontaneously rupture forming draining sinuses or inguinal ulcers. Systemic symptoms are characteristically absent. Extragenital ulcers are rare, but have been described in the mouth, and on the fingers and breasts. Patients co-infected with HIV are more likely to have multiple genital lesions and may experience a delayed response to treatment. Disseminated infection does not occur even in the immunocompromised. Although untreated ulcers usually heal spontaneously, they can be complicated by secondary bacterial infection, extensive tissue destruction and scarring (Figs 60.4, 60.5).

DIAGNOSIS

H. ducreyi is a fastidious, temperature-sensitive organism that survives for only a short time outside the human host. Growth *in vitro* requires specialized culture conditions and achieves a sensitivity of only 70–80%. In spite of this, culture remains the gold standard for diagnosis.

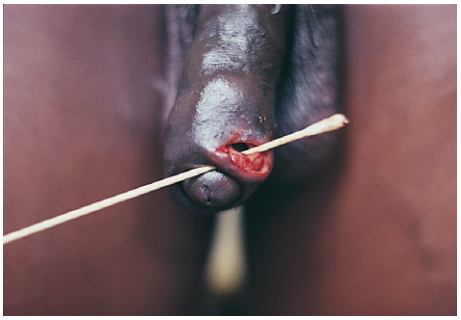


Fig. 60.4 Phagedenic chancroid with extensive tissue destruction.



Fig. 60.5 Healed inguinal bubo with scar formation from previous chancroid infection.

Specimens are obtained by first cleansing the ulcer by flushing with saline and then exudate is collected on a swab from the base of the ulcer. Ideally, specimens should be inoculated onto culture media at the bedside. If this is not possible, specimens should be transported to the laboratory promptly as *H. ducreyi* will survive on swabs for only 2–4 hours.[15] Bacterial contamination reduces the accuracy of the Gram stain and its overall sensitivity and specificity are less than 50%. Specific media are required to grow *H. ducreyi in vitro* and gonococcal agar supplemented with 2% bovine hemoglobin plus 5% fetal calf serum, or Mueller–Hinton agar supplemented with 5% chocolatized horse blood have been used.[15] Culture plates are incubated at 33 °C in 5% CO_2. Growth is usually seen within 72 hours, but plates should be kept for 5 days before being reported negative. Colonies are raised, nonmucoid, yellow-gray in color and very self adherent. *H. ducreyi* is oxidase-positive and catalase-negative. A positive culture may be confirmed by demonstrating the presence of nitrate reductase, cytochrome oxidase, alkaline phosphatase and the requirement for heme.

PCR techniques have been developed to detect *H. ducreyi* with a sensitivity of 83–96% and a specificity of 100%.[16] A commercially available multiplex PCR assay for the simultaneous detection of *H. ducreyi*, *Treponema pallidum* and herpes simplex virus simplifies the diagnosis and management of genital ulcer disease.

MANAGEMENT

The treatment of chancroid has been complicated by antimicrobial resistance and HIV co-infection. There are wide regional variations in antimicrobial susceptibilities, but increasing global resistance to tetracycline, aminoglycosides, sulfonamides and amoxicillin–clavulanic acid has been documented.

Current recommendations for treatment include oral azithromycin 1 g in a single dose, intramuscular ceftriaxone 250 mg in a single dose, oral ciprofloxacin 500 mg twice daily for 3 days or oral erythromycin base 500 mg four times daily for 7 days.[6] Ease of administration and compliance make single-dose therapies preferable and there is good evidence that single-dose ciprofloxacin 500 mg orally is effective regardless of HIV serostatus.[17] Erythromycin and ceftriaxone may be used in pregnancy.

Response to treatment is characterized by a decrease in pain within 48 hours. Complete ulcer healing may take approximately 10 days,

depending on the size of the ulcer. The development of fluctuant buboes may occur on treatment and these should be aspirated by needle or drained by incision.

Granuloma Inguinale

Granuloma inguinale (or donovanosis) presents as a painless genital ulcer that bleeds easily. It is caused by the Gram-negative pleomorphic bacterium *Klebsiella granulomatis*.

EPIDEMIOLOGY

Granuloma inguinale is endemic in tropical and subtropical regions such as Papua New Guinea and India. It is also found in South Africa, parts of Brazil, the Caribbean, and among aboriginals in central Australia. Granuloma inguinale is rare in North America and Europe.

The prevalence of granuloma inguinale is highest among adults aged 20–40 years. It is widely believed to be sexually transmitted because of its predominance in sexually active persons, especially those with multiple sexual partners or contact with prostitutes, its predilection for the genital region and the high incidence of co-infection with other STDs. The possibility of nonsexual transmissibility has been inferred from its occurrence in persons such as children and the elderly presumed sexually inactive.[18]

PATHOGENESIS AND PATHOLOGY

K. granulomatis invades epidermal and dermal tissues causing local inflammation but little is known about its virulence factors or the immune response to infection. On histopathology, large mononuclear cells containing inclusion bodies (Donovan bodies) are characteristic. The associated epithelial changes include ulceration, microabscesses, acanthosis, irregular elongation of the rete pegs and pseudoepitheliomatous hyperplasia. Untreated lesions may become hyperkeratotic. The dermal layer exhibits inflammatory changes, with a dense cellular infiltrate and varying degrees of fibrosis and edema.

CLINICAL FEATURES

The initial lesion of granuloma inguinale (GI) is a small firm papule at the site of infection which appears after an incubation period of 3–50 days. This typically erodes to form a painless ulcer (Fig. 60.6). The ulcer is granulomatous and 'beefy-red' with a nonpurulent base and bleeds readily when manipulated. Occasionally the ulcers may become necrotic and foul smelling with significant tissue destruction. Multiple lesions may develop on opposing surfaces or along skin folds. In men, ulcers are generally found at the coronal sulcus and are more common in uncircumcised men. In women, the labia minora and fourchette are most commonly affected but lesion may appear in the upper genital tract, including the cervix. Vaginal bleeding or discharge, pelvic inflammatory disease or a pelvic mass are not uncommon presentations. Less typically GI may present as a raised, hypertrophic ulcer with an irregular edge. The dry appearance of these lesions may be confused with genital warts.

Infection of extragenital sites accounts for 6% of cases and include the oropharynx, nose, larynx and neck. Abscess formation in the groin mimics lymphadenitis (pseudobuboes), but the lymph nodes themselves are rarely involved. The typical clinical picture may be altered if the genital lesions become secondarily infected, resulting in pain, purulent exudate and tender lymphadenopathy.

Without treatment, GI is slowly progressive, often resulting in soft tissue destruction and extensive scarring (Fig. 60.7). Possible sequelae include genital adhesions, stenosis of the urethral, vaginal or anal orifices, and rectovaginal fistulas. Contiguous pelvic spread may result in a 'frozen pelvis' and hematogenous spread to the bones or liver may occur albeit rarely. During pregnancy, GI is often more aggressive, with a higher rate of dissemination and a slower response to treatment. Perinatal infection is rare, but prophylactic antibiotics should be administered to the infant.



Fig. 60.6 Granuloma inguinale. The chronic, granulomatous, beefy-red ulcer without suppuration is typical. Photo kindly supplied by J K Maniar.

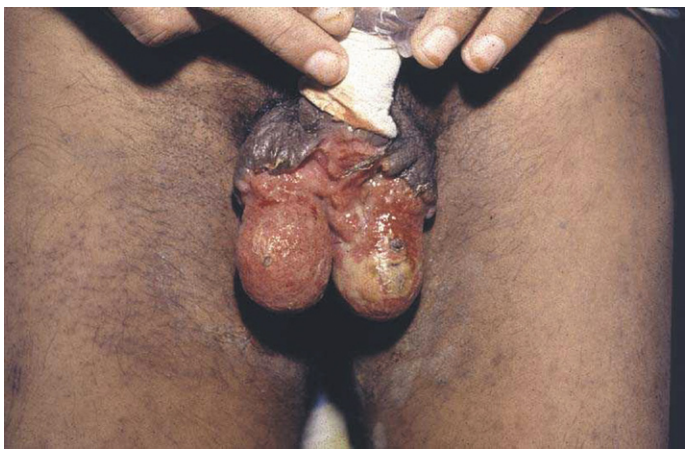


Fig. 60.7 Granuloma inguinale. Lack of treatment has permitted progressive destruction of the scrotum. Photo kindly supplied by J K Maniar.

DIAGNOSIS

The diagnosis of GI is made clinically and confirmed by the presence of intracellular Donovan bodies seen within large mononuclear cells on smear or biopsy. Reliable culture techniques and serologic tests are not available. To obtain a smear for diagnosis, debris should first be gently removed from the base of the ulcer with a cotton swab. A second swab is then used to scrape the surface of the ulcer without causing bleeding. The granulomatous tissue is spread directly onto a slide, air dried, and stained with Wright or Giemsa stain. Donovan bodies are identified as darkly staining, intracellular ovoid organisms, with or without a capsule. The smear is more likely to be negative if the lesion is early, sclerotic or secondarily infected. In such cases, a histologic diagnosis should be sought.

MANAGEMENT

Treatment regimens are empiric, as *in-vitro* susceptibility testing is not available. Currently recommended regimens include oral azithromycin 1 g followed by 500 mg daily, oral doxycycline 100 mg twice daily, a double-strength tablet of TMP-SMX twice daily, oral ciprofloxacin 750 mg twice daily or erythromycin 500 mg four times daily.[6,19] Erythromycin is the drug of choice in pregnancy. The rapid clinical response achieved with azithromycin and once daily administration has made this drug the treatment of choice.[6,20] Intravenous or intramuscular gentamicin may be added briefly in treatment of lesions that resolve slowly, and for HIV-infected persons. Duration of therapy has not been established but a minimum of 3 weeks or until all lesions have completely resolved is recommended.

Relapses are common and may occur up to 2 years after apparently successful treatment, requiring an additional course of antibiotics. Complications such as strictures, sinus formation, extensive superinfection or disfiguration may require surgical intervention.

REFERENCES



References for this chapter can be found online at http://www.expertconsult.com

Practice point | 29 |

Mary M Horgan

Management of gonorrhea

INTRODUCTION

Gonorrhea is caused by *Neisseria gonorrhoeae*, a Gram-negative intracellular diplococcus which is an obligate human pathogen. It is the second most commonly reported bacterial sexually transmitted infection (STI). Those individuals most at risk of gonococcal infection are women younger than 25 years, a previous diagnosis of gonorrhea or other STI, new or multiple sex partners, inconsistent condom use, commercial sex work, drug use and overseas travel to countries of higher prevalence.

CLINICAL MANIFESTATIONS

There is a broad spectrum of clinical manifestations of gonorrhea: asymptomatic and symptomatic, local infection of the anogenital area and pharynx, and disseminated infection. The incubation period is short, ranging from 1 to 14 days, with males likely to present with symptoms within 5 days and females within 10 days in those who become symptomatic.

Urethral infection in males is usually symptomatic and presents with a mucopurulent discharge followed shortly by dysuria in 20% after a single sexual exposure. The severity of symptoms is determined by the infecting strain. Common complications of urethritis are epididymitis and acute or chronic prostatitis. In the pre-antibiotic era, gonococcal urethritis in the male resolved over several weeks, with 95% of cases becoming asymptomatic within 6 months. Subsequent episodes of gonorrhea in those not treated are more likely to result in urethral stricture. However, as males are more likely to be symptomatic and present for care, such complications are now rare.

In females the endocervical canal, urethra, periurethral and Bartholin's glands are the most common sites of infection. The transmission rate to females after a single encounter is high at 50%. If symptoms are present, the patient will present with vaginal discharge, dysuria, postcoital and intermenstrual bleeding. Females are more likely to be asymptomatic and therefore screening of those at high risk of infection is an important aspect of control. Local complications include pelvic inflammatory disease (PID) in 10–20% of infections. Long-term sequelae of PID are ectopic pregnancy, infertility and chronic pelvic pain. Pregnant women with gonorrhea have an increased risk of spontaneous abortion and premature delivery. Gonococcal ophthalmia neonatorum is the most common complication in the newborn. Although *Chlamydia trachomatis* is the most common cause of neonatal infectious conjunctivitis, there is an overlap in incubation period and symptoms and therefore both diagnoses should be considered.

Rectal infection may be the only site of infection in up to 40% of men who have sex with men and presents with pruritus, mucopurulent discharge and rectal pain. Symptomatic infection in females is uncommon.

Gonococcal infection of the pharynx is transmitted by orogenital contact and occurs in 10–25% of patients with gonorrhea at another site. Over 90% of pharyngeal infections are asymptomatic and its transmission to others is inefficient and rare.

The most common systemic complication is disseminated gonococcal infection (DGI) which occurs in 0.5–3% of untreated local infection. Clinical manifestation is a triad of migratory polyarthritis, tenosynovitis and dermatitis. As the bacteremia is not continuous, only 20–30% of patients have positive blood cultures; however, 80% will have evidence of local infection at one of the sites outlined above. Gonococcal endocarditis, meningitis and perihepatitis are all rare.

LABORATORY DIAGNOSIS

The diagnosis of gonorrhea is dependent on the identification of the organism by Gram stain, culture, nucleic acid hybridization tests and nucleic acid amplification techniques (NAAT).

Gram staining showing intracellular Gram-negative diplococci in urethral specimens of symptomatic males is highly sensitive and specific for *N. gonorrhoeae*; however, as staining of specimens from endocervical, rectal and pharyngeal swabs is less sensitive, other diagnostic methods are necessary.

Culture and nucleic acid hybridization testing can be used on male urethral and endocervical specimens. Culture is best for rectal and pharyngeal specimens and has the additional benefit of antimicrobial susceptibility data. In cases of persistent gonococcal infection, culture is essential. NAAT can be used in a wide variety of specimens; however, limitations are cost, contamination and cross-reaction with nongonococcal *Neisseria* leading to false-positive results.

The recommendation is that patients being tested for gonorrhea should be offered testing for all other STIs, particularly *C. trachomatis*, HIV and syphilis. Co-infection with *Chlamydia* can occur in 25–38% of patients with gonococcal infection and gonorrhea facilitates the transmission of HIV.

TREATMENT

Control of gonorrhea has depended on the use of effective single-dose therapy administered at the time of diagnosis to ensure maximal patient compliance. This is done in conjunction with the diagnosis of other STIs and treatment of sexual partners. The rates of dual infection with *C. trachomatis* in those infected with *N. gonorrhoeae* are high. If *Chlamydia* results are not available or if a non-NAAT is negative for *Chlamydia*, patients should be treated for both gonorrhea and *Chlamydia*.

The availability of antimicrobial agents for the treatment of gonorrhea in the late 1930s has been persistently followed by sequential development of drug resistance to antimicrobial classes commonly used for treatment. The ongoing problem of antimicrobial resistance was recognized and in 1986 the Centers for Disease Control and Prevention (CDC) set up the Gonococcal Isolate Surveillance Project (GISP) to monitor trends in antimicrobial susceptibility and to provide a rational basis on which to select gonococcal therapies. This project has been instrumental in determining key policy changes in treating and thus controlling this infection over the past two decades.

Before 1976 antimicrobial resistance was not usually checked as the isolates were invariably susceptible to penicillin which was the drug of choice for treatment of the infection. The types of penicillin resistance that subsequently emerged were chromosomally mediated penicillin- and tetracycline-resistant *N. gonorrhoeae* (CMRNG), plasmid-mediated penicillinase-producing *N. gonorrhoeae* (PPNG) and tetracycline-resistant *N. gonorrhoeae* (TRNG), leading to abandonment of these drugs for eradication of infection. Since the 1990s, chromosomally mediated high-level resistance to quinolones (QRNG) resulting from mutations in gyrA (DNA gyrase) and par C (topoisomerase IV) has increased dramatically. The quinolones, as a class of drugs, are no longer recommended for the treatment of gonorrhea. Based on susceptibility patterns, current CDC guidelines for the treatment of gonorrhea are confined to a single class of drugs, the cephalosporins.

Uncomplicated gonococcal infection

Gonococcal infection of the urethra, cervix and rectum

- Ceftriaxone 125 mg intramuscularly in a single dose *or* cefixime 400 mg orally in a single dose *or* spectinomycin 2 g intramuscularly in a single dose, *plus*
- treatment of chlamydial infection if not excluded as a diagnosis.

Gonococcal infection of the pharynx

Gonococcal infection of the pharynx is more difficult to eradicate than from the anogenital area and the treatment recommendations differ. The treatment of choice, which clears infection in over 90% of cases, is:

- ceftriaxone 125 mg intramuscularly in a single dose, *plus*
- treatment of chlamydial infection if not excluded as a diagnosis.

Patients treated for uncomplicated gonococcal infection with one of the above regimens who have symptomatic relief do not need a test of cure. Those who are persistently symptomatic despite appropriate treatment require re-evaluation by culture to determine antimicrobial susceptibility patterns as well as sex partner evaluation.

Complicated gonococcal infection

Disseminated gonococcal infection

A cephalosporin-based regimen is recommended for DGI for a minimum of 1 week with parenteral administration for the first 24–48 hours until there is clinical improvement, followed by a switch to an oral regimen as outlined below:

- Ceftriaxone 1 g intramuscularly or intravenously q24h *or* cefotaxime 1 g intravenously q8h *or* ceftizoxime 1 g intravenously q8h *or* spectinomycin 2 g intramuscularly q12h, *followed by a switch to*
- cefixime 400 mg orally twice daily *or* cefpodoxime 400 mg orally twice daily.

As epididymitis can be caused by *N. gonorrhoeae* or *Chlamydia*, particularly in those under 35 years of age, antimicrobial therapy should cover both infections. The recommended regimen for sexually acquired infection is:

- ceftriaxone 250 mg intramuscularly in a single dose, *plus*
- doxycycline 100 g orally twice daily for 10 days.

Gonococcal conjunctivitis

This is a rare complication often resulting from autoinoculation. The treatment recommendation is based on a consensus opinion as there is only one published study on its treatment:

- ceftriaxone 1 g intramuscularly in a single dose, *plus*
- saline solution lavage of the infected eye.

Pelvic inflammatory disease

The mode of antimicrobial administration in women with PID is determined by the severity of the infection. Parenteral and oral regimens appear to be equally efficacious and a switch to oral therapy is recommended after 24 hours of symptomatic improvement. Duration of therapy is 14 days.

Parenteral therapy

- Cefotetan 2 g intravenously q12h *or* cefoxitin 2 g intravenously q6h *plus*
- doxycycline 100 mg orally *or* intravenously q12h

or

- clindamycin 900 mg intravenously q8h *plus*
- a loading dose of gentamicin 2 mg/kg intravenously or intramuscularly, followed by a maintenance dose of 1.5 mg/kg q8h.

Oral therapy

- Ceftriaxone 250 mg intramuscularly in a single dose *or* cefoxitin 2 g intramuscularly and probenecid 1 g orally, *plus*
- doxycycline 100 mg orally twice daily for 14 days, *with or without*
- metronidazole 500 mg orally twice daily for 14 days.

There are few data to support the use of an alternative regimen to cephalosporins in the treatment of gonorrhea. In the uncommon event that an individual has a history of a serious adverse event to cephalosporin, desensitization is recommended. If this is not possible, azithromycin 2 g orally as a single dose can be used in uncomplicated infection; however, there are concerns over emerging macrolide resistance, it causes significant gastrointestinal symptoms and it is costly. Use of azithromycin should be strictly limited to rare situations. Fluoroquinolones should only be considered as an alternative treatment option if antimicrobial susceptibilities are available on specimens.

PREVENTION

Public health programs designed to control gonorrhea aim to reduce harm to the infected individual and their sex partners by early detection and treatment at the time of diagnosis with contact tracing. In addition, public health policy at a population level aims to educate on prevention, promote screening programs and facilitate easy access to treatment clinics. Correct and consistent use of male latex condoms markedly reduces the risk of transmission of discharging diseases including gonorrhea.

At an individual level, an important aspect of care is the treatment of patients' sex partners to prevent further transmission or re-infection. Any sex partners within 60 days of the onset of symptoms or diagnosis of gonorrhea should be screened and treated. If the sexual contact is greater than 60 days then the most recent sex partner should be treated. Patients and their sex partners should avoid sexual contact until they are treated and their symptoms have resolved. Treatment regimens are outlined in treatment of uncomplicated gonorrhea above.

FURTHER READING

Further reading for this chapter can be found online at http://www.expertconsult.com

Practice point **30**

Jonathan M Zenilman

Persistent/recurrent vaginal discharge

INTRODUCTION

Vaginal discharge is one of the cardinal symptoms of lower tract gynecologic exudative infections. These disorders are classified by anatomic site of origin. For example, gonorrhea and chlamydial infections are cervical infections; bacterial vaginosis (BV), trichomoniasis and vaginal yeast infections are vaginal disorders. Herpes simplex and human papillomavirus can cause epithelial lesions on the external genitalia, but they can also cause cervicitis with discharge (see Chapter 49).

Initial steps

The first step in assessing a complaint of recurrent vaginal discharge is to differentiate between a normal physiologic discharge, a vaginal discharge and cervical infections. Many women have a small amount of vaginal discharge (physiologic leukorrhea), which is clear or white, does not have an odor, and is composed predominantly of squamous epithelial cells; this may vary with the menstrual cycle.

Patients do not differentiate from cervical and vaginal disorders, but often report 'vaginal discharge', which may represent either cervical or vaginal pathology. Therefore, initial evaluation of a patient who has recurrent or persistent discharge should include assessment for cervical infection. 'Recurrent' chlamydial and gonococcal infection is most often due to patient noncompliance with treatment regimens, re-exposure to an untreated sexual partner or re-exposure (i.e. new-incident infection). New-incident chlamydial and gonococcal infection is particularly common among adolescents.

Assuming that gonococcal and chlamydial infection has been ruled out and that the problem has been localized to the vagina, the next step in managing recurrent vaginal discharge is to determine the specific etiology of the vaginitis. This involves taking a careful history, with attention to recent douching, antimicrobial use (including use of over-the-counter antifungal medications) and reproductive history, followed by a clinical examination, which should include microscopic evaluation of vaginal discharge and determination of vaginal pH. The differential algorithm proposed by Sobel (see Further reading) is the standard in clinical assessment. The clinical and microscopic evaluation of recurrent vaginitis is shown in Figure PP30.1.

RECURRENT VAGINITIS DUE TO INFECTION

Recurrent trichomoniasis

Recurrent infection with *Trichomonas* spp. is most commonly due to re-infection from an untreated male partner or to re-exposure. Trichomoniasis in men is usually asymptomatic, and it is therefore imperative that all male partners of women who have trichomoniasis be treated with metronidazole, 2 g orally, as a single dose.

Metronidazole-resistant trichomoniasis is rare (<1% of clinical isolates) and should be considered only after re-infection has been ruled out and the patient has failed two courses of therapy. In these patients, metronidazole resistance should be confirmed, and they will respond frequently to a prolonged course of metronidazole (2–4 g daily for 10–14 days). Alternatively, tinidazole has been used successfully to treat resistant *Trichomonas* infection, at 2–3 g orally for 7–10 days.

Recurrent candidal vaginitis

Candidal vaginitis is extremely common and recurrences are frequent, in up to 45–50% of cases in some studies. Recurrent candidiasis is found more frequently in women who are taking antimicrobial agents and in those who have uncontrolled diabetes mellitus. Immunosuppressed

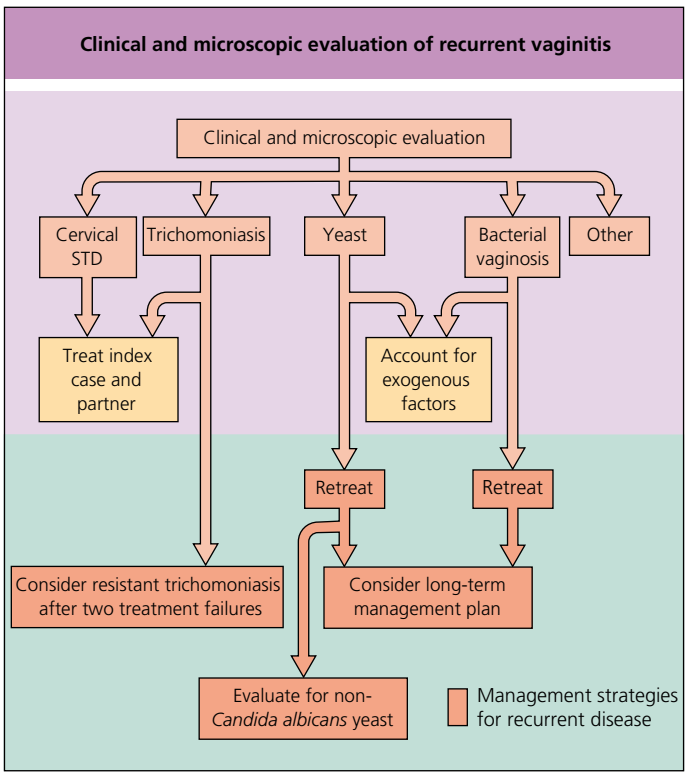


Fig. PP30.1 Clinical and microscopic evaluation of recurrent vaginitis.

patients, such as persons who have advanced HIV disease, are also at a modest risk of recurrent candidiasis as well as recurrent BV (see below).

After potential precipitating factors have been considered, there remains a subset of women who continue to get frequent recurrences. Careful microbiologic evaluation, with identification of the fungal species, may rarely reveal a yeast that is resistant to the commonly used imidazole and triazole therapies, such as *Candida glabrata* or *Candida tropicalis*. These investigations are expensive and should be reserved for use only after the clinical and epidemiologic risks have been identified. Persons who have chronic, recurrent candidiasis that is known to be caused by *Candida albicans* benefit from a course of long-term prophylaxis with fluconazole, 100–200 mg weekly (see also Chapter 49).

Recurrent bacterial vaginosis

Bacterial vaginosis is a disorder due to ecologic disturbances among the vaginal flora. Bacterial vaginosis is particularly prone to relapse. As above, the management of recurrent BV should account for the following:

- assurance that appropriate antimicrobial treatment was given initially – for example, many practitioners currently use a single-dose regimen of metronidazole (2 g orally) which has a treatment failure rate of up to 20%; persons who have suspected recurrence should be treated with a full multiday regimen of metronidazole 500 mg orally every 12 hours, or metronidazole vaginal gel (0.75%) 5 g every 12 hours for 5 days, or 2% clindamycin cream 5 g every 12 hours for 7 days;
- removal of exogenous factors that contribute to the pathogenesis of BV, including douching – douching is particularly associated with the development of BV because of the disruption of the local mucosal surfaces and flora, and women who use spermicides are at higher risk for BV and must balance the risk and benefits of their use;
- ruling out the presence of cervical infection – infections in the lower genital tract induce an inflammatory response, which in turn can cause BV; therefore, as above, ruling out cervical gonococcal and chlamydial infection is important early in the evaluation of persons who have recurrent BV.

The majority of women have primary BV (i.e. BV without an identifiable cause). Primary BV responds to treatment, but recurs frequently in these patients. Recurrence rates are as high as 20–30% 1 month after treatment. In these cases, long-term treatment with topical metronidazole should be considered. In nonpregnant women, treatment should take into account the impact of symptoms on the patient's lifestyle. In pregnant women, because of the potential for perinatal complications, periodic evaluation and treatment for recurrences is recommended.

Other causes of recurrent vaginitis

Bacterial vaginosis and yeast vaginitis are the most common types of recurrent vaginitis. Uncommon causes include hypersensitivity vaginitis, especially to latex, which is managed by avoiding latex exposure. This is one situation in which natural membrane condoms may be appropriate. Desquamative inflammatory vaginitis is an uncommon disorder that is diagnosed by a high vaginal pH, a negative amine test, the absence of clue cells, and the presence of polymorphonuclear leukocytes and parabasal cells. These patients may respond to intravaginal 2% clindamycin cream, 5 g every 12 hours for 1 week; however, they are prone to frequent recurrence.

USE OF BIOLOGIC REMEDIES

Women who have frequently recurrent vaginitis occasionally turn to biologic remedies obtained from health food shops or over the Internet. These have included (among others) yoghurt douches, lactobacilli for vaginal instillation and prescriptions for eating large quantities of yoghurt. Controlled studies to date have failed to demonstrate any benefits of these remedies.

PATIENT COUNSELING

By the time they see a medical specialist, patients who have recurrent vaginal discharge have typically been seen by many medical care providers with limited results, and they are therefore often frustrated. Counseling on the etiology and pathogenesis of the disorder with frank explanation of the expected impact of therapy is a critical component of management. Issues of sexuality should also be explored, because patients may find intercourse either uncomfortable or embarrassing.

SUMMARY

Important points to remember are:

- take a complete history;
- account for exogenous factors;
- account for systemic diseases;
- rule out cervical sexually transmitted diseases;
- perform a complete evaluation including microscopy;
- consider episodic management and suppressive management; and
- counsel the patients intensively.

FURTHER READING

Further reading for this chapter can be found online at http://www.expertconsult.com

Practice point | 31 |

Mettassebia Kanno

Persistent or recurrent nongonococcal urethritis in men and women

DEFINITION

There are an estimated 2 million cases of nongonococcal urethritis (NGU) in the USA per year, of which about 30–50% are caused by *Chlamydia trachomatis*; however, in the remaining *Chlamydia*-negative NGU cases, the microbiologic etiology is not well understood. It had been suspected that a proportion of *Chlamydia*-negative NGU cases may have been false-negative cases of *Chlamydia* urethritis; however, several lines of evidence have suggested that this is not the case, or is at least uncommon. Cervical cultures of female partners of men with *Chlamydia*-negative NGU have been repeatedly negative. In 25–30% of *Chlamydia*-negative NGU, no organisms have been recovered, although some additional etiologies have been proposed (Table PP31.1).

Chlamydia-negative NGU has been less well described in women. Women with persistent symptoms of a lower urinary tract infection yet with negative urine specimens from the bladder are all too often considered to have psychological problems, and thus are not adequately evaluated.

There are no widely accepted definitions for persistent or recurrent NGU. The 2006 Centers for Disease Control and Prevention (CDC) treatment guidelines for sexually transmitted diseases (STD) do not provide any definitions for these two clinical syndromes. In reviewing the literature, there are some 'loosely' applied defining terms for both entities:

- persistent urethritis refers to urethritis that fails to resolve or substantially improve within 2 weeks of initiating appropriate treatment regimens; and
- recurrent urethritis is defined as return of urethritis within 6 weeks following an initial response to appropriate treatment.

Most of the time these terms are used interchangeably since the duration of symptoms in relation to prior treatment may be unclear, although the median time for recurrence after completing treatment has been estimated at 2 weeks. Most men with recurrent NGU are culture negative for *Chlamydia* unless the interval following treatment is more than 2 weeks and they have had sexual intercourse with an untreated partner. The likelihood of resistant organisms playing a role in persistent or recurrent NGU is not known, with only infrequent cases reported over the past 10 years.

Table PP31.1 Etiologic agents for nongonococcal *Chlamydia*-negative urethritis (NGU) in men

Etiologic agent	Estimated prevalence	Preferred method of diagnosis	Comments
Mycoplasma genitalium	7–50%	Polymerase chain reaction (PCR)	In the 1990s, the advent of PCR-based assays for detecting *M. genitalium,* several studies reported the prevalence of *M. genitalium* being significantly higher in symptomatic men with acute *Chlamydia*-negative NGU than in asymptomatic men (13–42% versus 0–15%).
Trichomonas vaginalis	6–20%	PCR	*T. vaginalis* is not routinely diagnosed in men because of the apparent limited ability of culture to detect this organism in the male genital tract. One study found *T. vaginalis* in 17% of urethritis cases and 12% of those without evidence of disease. Another study reported a prevalence of *T. vaginalis* urethritis in men of 17%, but in men with *Chlamydia*-negative NGU, the prevalence reported was 19.9%.
Ureaplasma urealyticum	2–43%	PCR	The association between *U. urealyticum* and NGU remains controversial; it is suggested that serovar 2 is associated with NGU. The most convincing evidence linking *U. urealyticum* to NGU comes from human experimental studies. Results from a volunteer experiment showed that *U. urealyticum* may cause disease the first time it gains access to the urethra, but subsequent invasion results in colonization, without disease
Herpes simplex virus (HSV)	3–10%	PCR Serology	The possibility of HSV urethritis should be considered in persistent urethritis since symptoms from primary HSV urethritis last for about 2 weeks. Both HSV types 1 and 2 can be a cause of *Chlamydia*-negative NGU and urethral symptoms can occur without any obvious vulvar or penile lesions.

CLINICAL FEATURES

In contrast to the lack of definitions for the two clinical syndromes, the clinical presentations are much more specific, and similar to any acute inflammation of the urethra. Symptoms are prominent and detected earlier in men than in women, and include dysuria and urethral discharge, ranging from clear to mucopurulent. It is important to note that urethral discharge may not be present in all patients. In men, a spontaneous urethral discharge is frequently present prior to voiding.

DIFFERENTIAL DIAGNOSIS

Chronic bacterial prostatitis is a consideration; however, less than 5% of patients with persistent or recurrent *Chlamydia*-negative urethritis have microbial etiologies that can cause prostatitis. The clinical signs and symptoms of chronic prostatitis may resemble persistent or recurrent urethritis, and the prostate could possibly function as a sanctuary site for an indolent infectious process; however, the actual role of the prostate in persistent or recurrent urethritis is unknown. There is very little benefit in placing these patients on prolonged antibiotic therapy.

Acute bacterial cystourethritis may occur, mostly in patient with anatomic and functional lower urinary tract anomalies, but these patients tend to present with dysuria, urinary frequency and suprapubic pain, but without urethral discharge. Pyuria and positive urine cultures are common, although bacterial culture numbers may not reach 10^5 cfu/ml of urine. Treatment of this entity requires at least 7 days of antibiotics targeting uropathogens as well as attention to the urethral anomaly.

Rarer problems such as retained urethral foreign bodies, periurethral fistulas and periurethral abscesses should be excluded by palpation.

LABORATORY INVESTIGATIONS

Documenting laboratory evidence of urethritis is critical. The laboratory criteria for the diagnosis of urethritis are the presence of five or more polymorphonuclear cells (PMNs) per high power field in a urethral specimen and the absence of Gram-negative intracellular diplococci. If a urine sample is obtained, the presence of leukocyte esterase and of more than 10 white blood cells (WBC) per high power field on spun urine sediment are suggestive of urethral inflammation.

Testing for *N. gonorrhoeae* and *C. trachomatis* should be performed to exclude the possibility of re-infection with these organisms but not be repeatedly undertaken. In most clinical settings, testing for *Ureaplasma urealyticum* and *Mycoplasma genitalium* is not widely available. Routine testing for herpes simplex virus (HSV) is not recommended; however, polymerase chain reaction (PCR) testing or type-specific serology can be undertaken if there is a clear urethral discharge in the context of recent contact with a known HSV-positive sex partner, or if there is a urethral discharge at the same time as a genital HSV outbreak. The diagnostic tool of choice for suspected *Trichomonas vaginalis* urethritis is PCR testing either of a urethral specimen or urine.

MANAGEMENT OF PERSISTENT OR RECURRENT URETHRITIS

The management of persistent or recurrent urethritis is largely empiric and frustrating since often no organism is identified; however, it is important to confirm the presence of urethritis clinically and by laboratory criteria. This is a critical step to help exclude patients with functional complaints, who have urethral symptoms but no evidence of urethritis, who may benefit from reassurance. Revisiting the sexual exposure history and medication compliance may shed light into the possibilities of re-infection from an untreated partner and relapses secondary to inadequate treatment courses, as well as the presence of potentially resistant organisms.

Table PP31.2 Recommended treatment regimens for persistent and recurrent urethritis

Persistent/ recurrent urethritis	Important steps	Recommended treatment regimens
First episode	Look for indicators for re-exposure and re-infection; if present, repeat testing for gonorrhea and chlamydia	Metronidazole 2 g or tinidazole 2 g po (one dose), plus either azithromycin 1 g po (one dose) or erythromycin 500 mg po q6h for 7 days
Second or any subsequent episodes	Look for indicators for re-exposure and re-infection; if present, repeat testing for gonorrhea and chlamydia	Erythromycin 500 mg po q6h for 3 weeks, or ofloxacin 300 mg po q12h for 3 weeks

Management of the initial persistent or recurrent episode of urethritis includes re-evaluating for the presence of gonococcal and/or chlamydial organisms, and empirically treating for *T. vaginalis*. The optimal regimen for covering potentially tetracycline-resistant organisms (i.e. mycoplasmas and ureaplasmas) has not been defined, and different regimens have not been compared. Table PP31.2 outlines the recommended regimens for first and subsequent persistent or recurrent urethritis episodes.

If symptoms persist after 3 weeks of empiric treatment with no infectious agents identified, continued antibiotic therapy is not beneficial. Moreover, there are no data to support retreating previously treated asymptomatic sex partners. Many cases resolve after 3 weeks of antibiotics.

Reassuring and encouraging patients that symptoms will resolve spontaneously over time, that most recurrences will arise independent of resumption of sexual activity, that the risk of transmission to a partner is low, and that the likelihood of long-term sequelae such as infertility or cancer appears to be exceedingly low, is important.

FURTHER READING

Further reading for this chapter can be found online at http://www.expertconsult.com

Chapter 61

Aric L Gregson
Philip A Mackowiak

Pathogenesis of fever

HISTORICAL CONSIDERATIONS

Akkadian cuneiform inscriptions confirm that fever has been recognized as a sign of disease since at least the 6th century BC.[1] Although various theories to explain the source of fever were advanced over the centuries, it was not until the 1850s that Claude Bernard correctly attributed the source of fever to metabolic processes within the body itself.

Our ability to measure body temperature progressed more quickly than our understanding of its source and regulation. Early devices used the expansion property of air to measure temperature, probably first in the 2nd century BC. In 1592, Sanctoria Sanctoria, a colleague of Galileo's at Padua, employed such a device in clinical studies, leading him to hypothesize the existence of a 'normal' body temperature. These early devices were prone to changes in barometric pressure until a closed thermometer employing alcohol was invented by Cornelius Drebbel in 1608. Some of the earliest closed liquid thermometers used scales based upon the freezing point of water and the oral temperature of a healthy human.[2] It was not until the early 18th century that Gabriel Daniel Fahrenheit popularized a reliable mercury-based thermometer with a scale based on the freezing and boiling points of water.

Clinical thermometry was not fully integrated into medical practice until Carl Reinhold August Wunderlich published his sentinel work, *Das Verhalten der Eigenwärme in Krankenheiten* (The course of temperature in diseases), in 1868. In it he reportedly examined some 25 000 patients from whom he obtained nearly 1 million individual measurements. These observations led him to propose 98.6°F (37°C) as the 'normal' body temperature and temperatures of 100.4°F (38°C) or above as fever.[3,4] Despite the fact that these observations were made more than 130 years ago, were based on axillary temperatures and were obtained using thermometers calibrated 2.6–4.0°F (1.4–2.2°C) higher than contemporary thermometers, many continue to regard Wunderlich's observations as definitive.[5]

FEVER VERSUS HYPERTHERMIA

Fever is defined as 'a state of elevated core temperature, which is often, but not necessarily, part of the defensive responses of multicellular organisms (host) to the invasion of live (micro-organisms) or inanimate matter recognized as pathogenic or alien by the host'.[6] More simply, fever is a regulated rise in core temperature in response to a physiologic threat to the host. The febrile response, of which fever is a component, is characterized by a cytokine-mediated rise in core temperature, accompanied by increases in acute-phase reactants and a host of other immunologic, endocrinologic, neurologic and physiologic changes.

'Hyperthermia' is defined as a disturbance in temperature regulation when core temperature is above the range specified for the normal active state of the species.[6] It may be regulated (e.g. as in fever) or forced (as in heat stroke) when total heat production exceeds the capacity for heat loss. Forced hyperthermia is characterized by a sustained elevation of core temperature lacking the diurnal fluctuations characteristic of both fever and normal body temperature, and does not respond to antipyretic drug therapy.[7,8]

Heat stroke, a form of forced hyperthermia, often occurs in association with exertion and drugs, such as phenothiazines, anticholinergics or cocaine, which alter the body's ability to regulate heat. Classic heat stroke is most often seen in persons at the extremes of age with co-morbid conditions. It is associated with a lack of sweating, moderate rhabdomyolysis and lactic acidosis, and rarely causes renal failure. In the exertional form of heat stroke, sweating is profuse, making its detection more difficult. Rhabdomyolysis may be severe in exertional heat stroke and, along with marked lactic acidosis, leads to a significant risk of renal failure. Common to both types of heat stroke is extreme hyperthermia, in which core temperatures may exceed 106°F (41.1°C).

Malignant hyperthermia, induced by general anesthetics in predisposed persons, and the closely related neuroleptic malignant syndrome are other types of forced hyperthermia. Malignant hyperthermia is a rare autosomal dominant disorder, usually triggered by inhalational anesthetic agents and often fatal, which is the result of heat production in skeletal muscle which accumulates because of limitations of active heat dissipation during anesthesia. It manifests clinically as elevated core temperature, lactic acidosis, myoglobinemia and muscular rigidity. Because muscular rigidity follows the development of hyperthermia in a majority of cases, the increased core temperature is believed to be due initially to increased metabolism and disturbances of cellular permeability, not muscle contraction. Temperature increases rapidly, at approximately 1.8°F (1°C) each minute. Core temperatures reaching 114.8°F (46°C) have been reported.[9] Hyperthyroid storm can also lead to hyperthermia, although in such cases temperatures rarely exceed 104°F (40°C).

CLINICAL THERMOMETRY

In clinical practice, fever is defined as a core temperature above the normal range. Presently, no physical examination is considered complete without some measurement of body temperature. When interpreting the significance of such measurements, clinicians should consider important variables such as the anatomic site of measurement, the time of day and the type of instrument used to obtain the temperature recording. Thermometers must be tested periodically according to the manufacturer's recommendations to ensure proper calibration.[10,11] When measurements are taken, thermometers must

be allowed adequate time to equilibrate with the test site. For mercury thermometers, rectal measurements require 1–5 minutes for optimal readings, whereas axillary and oral measurements require up to 11 minutes.[12,13] Newer thermistor-based electronic thermometers have shorter equilibration times than traditional mercury thermometers. Tympanic membrane thermometers equilibrate in seconds and are convenient to use. Unfortunately, their results are inconsistent and correlate poorly with concurrent rectal, axillary and oral measurements.[2,14–16]

Core temperature is an amalgam of temperatures derived from the various internal organs, all having different metabolic rates. Under normal physiologic conditions such differences are small. However, during shock or in the face of extreme thermal stress, regional and organ-specific differences in temperature can be marked.[17] Because core temperature readings are difficult to obtain, peripheral measurements are used to approximate core temperature. Mean rectal temperatures exceed oral by 0.8°F (0.4°C) and tympanic membrane temperatures by 1.6°F (0.8°C).[18,19] During shock, rectal temperature may underestimate an elevated core temperature because of poor rectal perfusion.[20] Moreover, rectal measurements are associated with a small but significant risk of infection due to cross-contamination.[21]

Oral temperature measurements have been standard practice because of accessibility and reasonable accuracy. Mastication and smoking both increase oral temperature. Ice-water ingestion decreases oral temperature, although only briefly.[19] Two studies of the effect of tachypnea and open-mouth breathing on oral temperature found that open mouth breathing, but not tachypnea per se, lowers oral temperature.[22,23] Because oral measurements require the subject's cooperation, they are difficult to obtain in critically ill and uncooperative patients.

NORMAL BODY TEMPERATURE

What constitutes normal body temperature remains the subject of debate. A temperature of 98.6°F (37°C) continues to be regarded as normal by many physicians and health care professionals, as a result of Wunderlich's original studies. 'Normal body temperature', however, is not a specific temperature but a range of temperatures, the parameters of which vary from one person to another.[24] One of the largest studies of oral temperature in healthy subjects yielded a mean temperature of 98.2°F (36.8°C).[24] Only 8% of the temperatures recorded were 98.6°F (37°C). The mean temperature varied diurnally with a nadir at 0600 h and a peak at 1600–1800 h, a mean amplitude of variability of 0.9°F (0.5°C), and a range of 0.1–2.4°F (0.05–1.3°C). Women had slightly higher mean temperatures than men [98.4°F (36.9°C) versus 98.1°F (36.7°C), p <0.001] but did not have greater diurnal variability. In this study population, 98.6°F (37°C) had no special significance with regard to normal body temperature (Fig. 61.1). The upper limit of normal temperature (i.e. greater than the 99th percentile) varied according to the time of day, from an early morning low of 99.0°F (37.2°C) to an evening peak of 100°F (37.8°C).

THERMOREGULATION

Mammals employ various thermogenic mechanisms to increase heat production during episodes of cold exposure and during the initiation of fever. Shivering is a principal thermogenic mechanism, except in neonates. Nonshivering thermogenesis is most closely linked to brown adipose tissue, so named because of its brown color, which is due to a profuse vascular supply and a high concentration of mitochondria. Brown adipose tissue is most prominent near the shoulder blades, neck, adrenal glands and deep blood vessels. Noradrenaline (norepinephrine) induces enzymatic hydrolysis of triglycerides to glycerol and free fatty acids in brown adipose tissue. These free fatty acids are the primary substrate oxidized by mitochondria to produce adenosine triphosphate (ATP) and heat. They also act as a signal to uncouple oxidative phosphorylation, thereby generating excess heat.

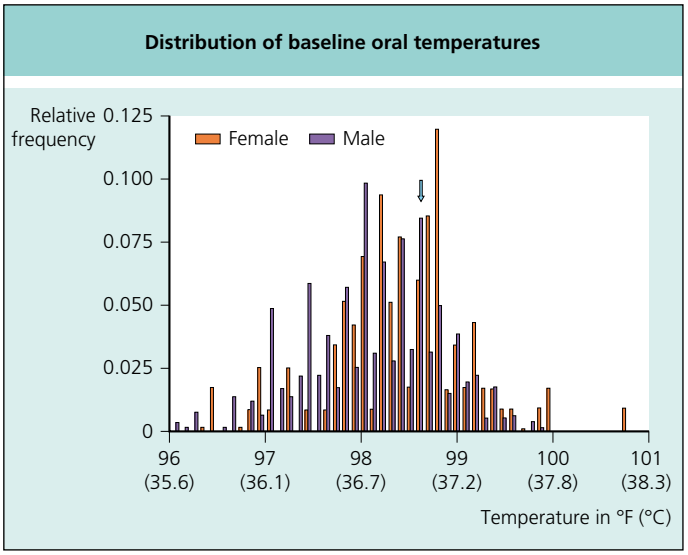


Fig. 61.1 Distribution of baseline oral temperatures in healthy men and women. Frequency distribution of 700 baseline oral temperatures obtained during two consecutive days of observation in 148 healthy young volunteers. Arrow indicates location of 98.6°F (37°C). With permission from Mackowiak *et al.*[24] Copyright 1992, American Medical Association.

In the resting state, approximately 72% of basal metabolic heat is produced by vital organs. Heat is distributed throughout the body via the circulatory system. Variations in cutaneous blood flow determine the amount of heat lost at the skin surface by radiation, evaporation, convection and conduction.[25] When environmental temperature exceeds body temperature, evaporation is the most effective means of heat dissipation. The balance between heat production and heat loss, largely under control of the autonomic nervous system, maintains body temperature near the hypothalamic set-point of 98.2°F (36.8°C). When it is necessary to decrease core temperature, blood flow is preferentially directed to the skin and sweating mechanisms are activated. In cold environments or at the onset of fever, blood flow is directed away from the surface towards the internal vital organs to minimize heat loss.

More than 60 years of investigation have suggested that no single, central location is responsible for temperature regulation. Rather, a series of hierarchical structures appear to control body temperature (Fig. 61.2). This hierarchical system includes a continuum of structures from the hypothalamus and limbic system extending through the brain stem, reticular formation, spinal cord and sympathetic ganglia. The rostral hypothalamus, referred to as the 'preoptic' region, is actually composed of the medial and lateral aspects of the preoptic area, the anterior hypothalamus and the septum. Many, although not all, thermophysiologists consider the preoptic region to be a central thermoregulation region, having greater thermosensitivity than other structures. The preoptic region orchestrates thermoregulatory responses in other effector areas via signals transmitted through the median forebrain bundle, a bidirectional pathway within the lateral hypothalamus.[26] Indeed, the median forebrain bundle has been shown to manage efferent signals controlling shivering and cutaneous blood flow.[27] Afferent signals ascend to the brain stem reticular formation via the lateral spinothalamic tract. The preoptic region of the hypothalamus, in turn, receives this information and integrates afferent and efferent signals to maintain the appropriate body temperature.

Thermoregulation is coordinated via a subset of temperature-sensitive preoptic neurons. There are three basic types of such neurons, which differ according to their responses to temperature changes. So called 'warm-sensitive neurons', accounting for approximately 30% of the preoptic neuronal population, increase their firing rates in response to increases in preoptic temperature. Their efferent output initiates heat

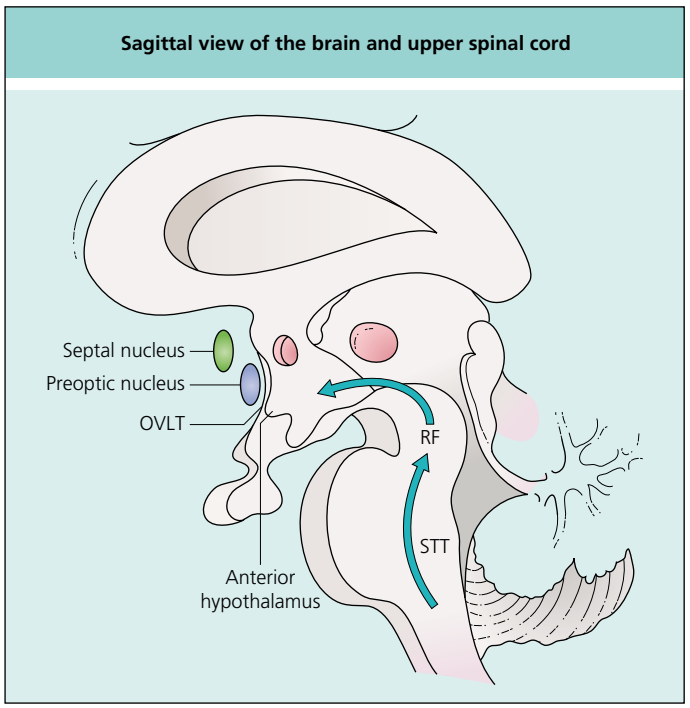


Fig. 61.2 Sagittal view of the brain and upper spinal cord. The figure shows the multisynaptic pathway of skin and spinal thermoreceptors through the spinothalamic tract (STT) and reticular formation (RF) to the anterior hypothalamus, the preoptic region and the septum. OVLT, organum vasculosum laminae terminalis. Redrawn with permission from Mackowiak.[1]

loss responses proportional to increases in body temperature over a certain threshold. A smaller number of 'cold-sensitive neurons' present in the preoptic region comprise less than 5% of total neurons. These neurons increase their firing rates as body temperature decreases. Cold-sensitive neurons are under synaptic inhibition from warm-sensitive neurons and, as the firing rates of warm-sensitive neurons decrease, so does the synaptic inhibition on cold-sensitive neurons. The net result is an increase in the firing rates of the cold-sensitive neurons[26,28] (Fig. 61.3). It may be that only the warm-sensitive neurons are truly thermosensitive and that thermoregulation is accomplished through their inhibitory and excitatory signals.[29–31] The majority of preoptic neurons do not demonstrate changes in firing rates in response to changes in preoptic temperature.

Pyrogens

Although peripheral neuronal input is of paramount importance during normal homeostatic thermoregulation, soluble 'endogenous pyrogens' (pyrogenic cytokines) exert a pronounced effect on temperature regulation during fever. These pyrogens have long been thought to mediate fever by elevating the hypothalamic set-point temperature (see also Chapter 2).

Traditionally, pyrogens have been classified as either 'endogenous' or 'exogenous'. Pyrogenic cytokines are endogenous immunoregulatory polypeptides, such as interleukin (IL)-1β, IL-6 and tumor necrosis factor (TNF). They are primarily derived from stimulated mononuclear cells and directly interact with the anterior hypothalamus to elevate the core temperature set-point. Exogenous pyrogens are the usual stimuli that induce mononuclear cells to produce and release pyrogenic cytokines. They include substances such as lipopolysaccharide (LPS), peptidoglycan, lipopeptides and numerous other microbial products. The distinction between endogenous and exogenous pyrogens and their ability to initiate fever is artificial, as some endogenous molecules can themselves induce the production of pyrogenic

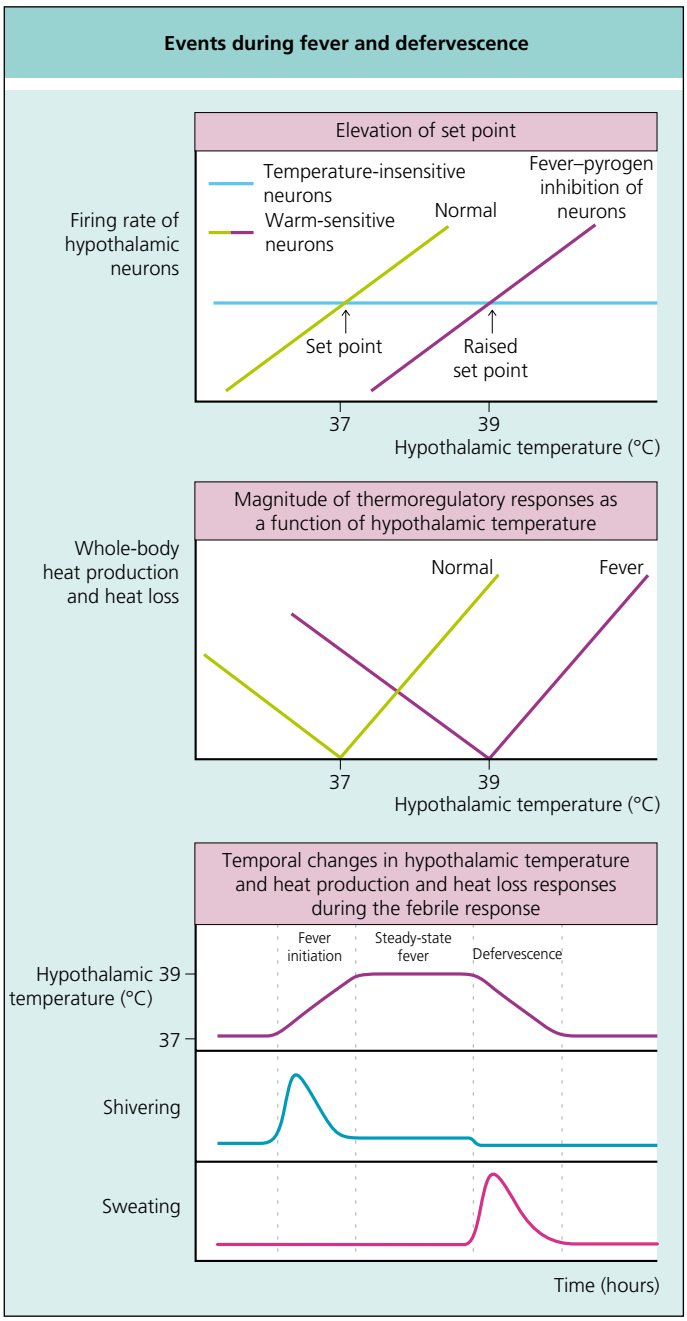


Fig. 61.3 Events during fever and defervescence. When pyrogens are present in the preoptic region the whole body and neuronal responses are as shown by the purple lines. Upper: Normally the firing rates of warm-sensitive and temperature-insensitive neurons functionally overlap at 98.6°F (37°C), the set-point of thermoregulatory neurons. During pyrogen inhibition of warm-sensitive neurons, this overlap occurs at the raised set-point of 102.2°F (39°C). Center: During fever, heat production is initially great but, as body temperature rises towards 102.2°F, heat production diminishes and should cease at 102.2°F. Lower: During fever initiation, shivering causes an increase in hypothalamic temperature and then ceases as the temperature reaches 102.2°F. During defervescence the hypothalamus activates heat loss responses such as sweating. Adapted with permission from Boulant.[28]

cytokines. Antigen–antibody complexes, complement, inflammatory bile acids and androgenic steroid metabolites are examples.

Pyrogenic cytokines have numerous activities other than pyrogenesis, including autocrine, paracrine and endocrine functions that

determine the character of the febrile response. As such, they coordinate gene expression and cellular activities that drive the inflammatory response to infection (and other forms of injury). However, preliminary evidence suggests that they might also prevent immune destruction of certain pathogenic micro-organisms[32] and stimulate the growth of others.[33]

Although expression of Toll-like receptors (TLRs) in the organum vasculosum laminae terminalis (OVLT) raises the possibility that exogenous pyrogens induce fever directly, independent of proinflammatory cytokines, considerable data have suggested that exogenous pyrogens primarily exert their pyrogenic effect indirectly by increasing circulating pyrogenic cytokines, which in turn elevate the set-point of the thermoregulatory center (Fig. 61.4).

Because pyrogenic cytokines are large, hydrophilic molecules, they do not easily cross the blood–brain barrier, and it is unclear how they interact with central regions in the brain. One way in which they might gain access to thermoregulatory sites within the brain is through the relatively leaky blood–brain barrier associated with the OVLT. However, even in this region, and with the assistance of active transport, passage of pyrogenic cytokines across the blood–brain barrier is too slow and modest to account for the initial rapid onset of fever seen following the intravenous administration of LPS, IL-1 or IL-6.[34] Endothelial and microglial cells at the blood–brain barrier have IL-1 receptors and, in response to IL-1 and LPS, synthesize TNF, IL-1β and IL-6, as well as cyclo-oxygenase (COX)-2 mRNA.[30,35]

Interaction of pyrogenic cytokines with cells at the OVLT might also induce responses that route the febrile signal inward toward the preoptic area via secondary cytokine production, phospholipase A_2/COX-2 activity, a nitric oxide secondary messenger, or via direct neural input to the preoptic region.[36] Prostaglandin (PG) E_2 readily crosses the blood–brain barrier and inhibiting PGE_2 synthesis blocks cytokine-induced fever, supporting its primary role in the pathogenesis of fever. Nitric oxide is generated by endothelial cells in the blood–brain barrier in response to IL-1 and IL-6. However, its role as a secondary messenger of the fever response is uncertain.[37]

Multiple pyrogenic mechanisms likely activate the OVLT. In certain experimental models, circulating levels of IL-1 do not correlate with the initial fever peak, although IL-6 may correspond to subsequent peaks.[30,38] Such observations suggest that IL-1 and TNF are paracrine modulators and IL-6 an endocrine modulator.[39] Yet other models suggest that bacterial superantigens, such as staphylococcal enterotoxin B, induce cell-associated cytokines that activate endothelial and microglial cells at the OVLT in the absence of high levels of circulating, cell-free cytokines.[40] Thus, it is possible that different combinations of mechanisms control fever in different situations.

In the case of endotoxin-induced fever, the febrile response seems to be initiated when Gram-negative bacteria and/or their products arrive in the liver, are taken up by Kupffer cells and activate the complement cascade to generate C5a.[34] This, in turn, rapidly stimulates Kupffer cells to release PGE_2. In such models, TNF and other pyrogenic cytokines are produced later and do not appear to be the immediate triggers of fever. One theory is that the Kupffer cell-generated PGE_2 is transported by the bloodstream to the ventromedial preoptic anterior hypothalamus (POA), and diffuses into the thermoregulatory regions to raise the thermal set-point. If this is so, PGE_2 would be the final, central fever mediator. However, it is also possible that PGE_2 activates hepatic vagal afferents projecting to the medulla oblongata, thence to the POA via the ventral noradrenergic bundle. Noradrenaline (norepinephrine) consequently secreted might then stimulate α_1-adrenoceptors on thermoregulatory neurons to evoke a rapid initial rise in core temperature not associated with any change in POA PGE_2.

Although these models address the mechanisms responsible for the induction phase of fever, the mechanisms responsible for the plateau or descending phases of fever remain obscure. The explanation for the tendency of fevers to oscillate in circadian fashion, even during infections such as bacterial endocarditis, remains unclear. In endocarditis the circulating levels of exogenous pyrogens (bacteria) remain remarkably constant throughout the day and night.

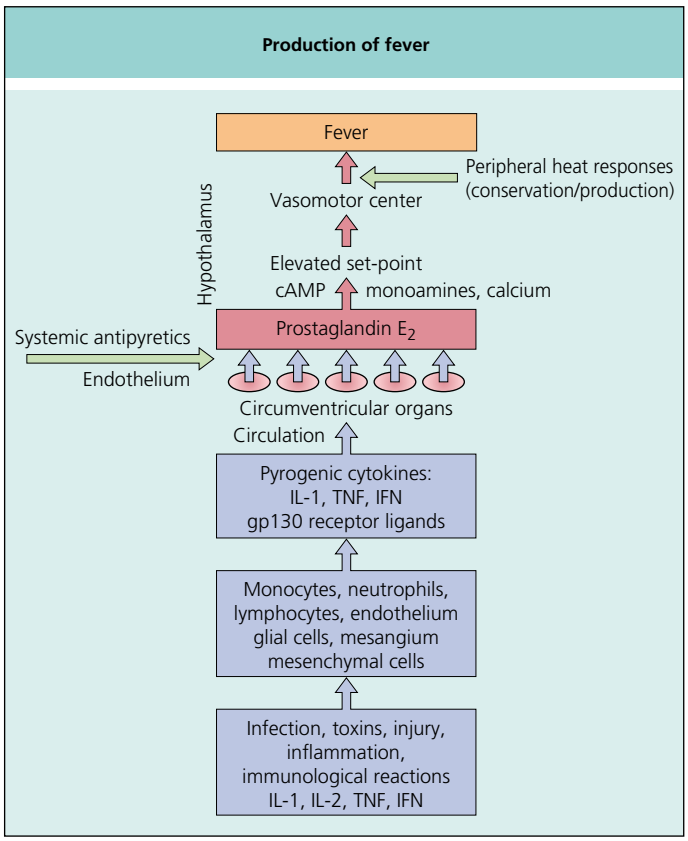


Fig. 61.4 Production of fever. Microbial agents, inflammatory agents and some cytokines induce the synthesis and release of pyrogenic cytokines from a variety of cells. These cytokines, in turn, trigger specialized endothelial cells of the hypothalamic vascular organs, which release prostaglandin E_2 (PGE_2). Elevated PGE_2 then brings about increases in cyclic adenosine monophosphate (cAMP) monoamines and calcium in the thermoregulatory center of the anterior hypothalamus, resulting in a resetting of the thermostatic temperature from normothermia to febrile levels. These neurotransmitters then activate the vasomotor center, which brings about vasoconstriction (heat conservation) and increased heat production, both resulting in an increase in blood temperature (fever). Regardless of the cause of fever, antipyretics inhibit this pathway by preventing the increase in cytokine-mediated PGE_2 production in the hypothalamus. IFN, interferon; IL, interleukin; TNF, tumor necrosis factor.

Acute-phase response

Fever is just one component of the febrile response, collectively referred to as the 'acute-phase response'. Often fever and the acute-phase response are linked, as in trauma or infection. However, they can also exist independently, suggesting that, although pyrogenic cytokines have the capacity to elicit both responses, fever and the acute-phase response are, nevertheless, independently regulated entities (see also Chapter 2).

A wide array of physiologic and endocrinologic alterations occurs during the febrile response. These include anorexia and sleepiness, and altered synthesis of glucagon, insulin, adrenocorticotropic hormone, cortisol, catecholamines, growth hormone, thyroid-stimulating hormone, thyroxine, aldosterone and arginine vasopressin. Inhibition of bone formation, negative nitrogen balance, gluconeogenesis and altered lipid metabolism also occur. Serum levels of zinc and iron fall,

Table 61.1 Acute phase proteins

Positive acute-phase proteins*	
CRP	Ferritin
Serum amyloid A	Phospholipase A_2
Haptoglobin	Plasminogen activator inhibitor-1
α_1-Acid glycoprotein	Fibronectin
α_1-Protease inhibitor	Hemopexin
Fibrinogen	Pancreatic secretory trypsin inhibitor
Ceruloplasmin	Inter-alpha protease inhibitor
Complement (C3 and C4)	Mannose binding protein
C-1 esterase inhibitor	LPS binding protein
C4b binding protein	
α_2-Macroglobulin	
Negative acute-phase proteins†	
Albumin	Transferrin
Transthyretin	α_2-HS glycoprotein

*Proteins exhibiting increased plasma concentrations during the acute-phase response.
†Proteins exhibiting decreased plasma concentrations during the acute-phase response.
Adapted from Kushner & Rzewnicki.[42]

whereas copper levels rise. Decreased erythropoiesis, in part responsible for 'anemia of chronic disease' (or, more appropriately 'anemia of chronic inflammation'), thrombocytosis and leukocytosis are additional features.[41]

Like fever, the acute-phase response is a tightly regulated response activated by a variety of stimuli (e.g. surgery, trauma, infection, cancer, burns). It is further characterized by production of an array of acute-phase proteins. These proteins are designated 'positive' or 'negative', depending on whether their levels rise or fall during the acute-phase response (Table 61.1). Those belonging to the former category are substantially more prominent than those belonging to the latter. The major positive acute-phase proteins are C-reactive protein (CRP) and serum amyloid A. Negative acute-phase proteins include albumin, pre-albumin (transthyretin) and transferrin. C-reactive protein and serum amyloid A normally exist in only minute concentrations but increase more than 1000-fold during the acute-phase response. C-reactive protein enhances phagocytosis by binding phosphocholine, present on foreign pathogens and damaged host cells, and activates complement. In certain situations, CRP stimulates thrombosis and promotes inflammatory cytokine production and the release of tissue factor by monocytes. Nevertheless, considerable evidence suggests that, *in vivo*, CRP functions primarily as an anti-inflammatory mediator.[43,44] Serum amyloid A enhances phagocyte adhesion and chemotaxis. Complement components, another group of acute-phase proteins, influence chemotaxis, opsonization and vascular permeability. The antioxidants and anti-inflammatory molecules, haptoglobin and ceruloplasmin, also appear to function as both positive acute-phase proteins and modulators of the inflammatory response. The thrombocytosis, decrease in iron and zinc levels and other features of the acute-phase response all appear to be important components of the defense against bacterial pathogens.[45]

Endogenous antipyretics

Core temperature rarely if ever reaches, far less exceeds, 106.0°F (41.1°C) during fever. This suggests that fever has a thermal ceiling, perhaps designed to protect the host against the deleterious effects of temperatures higher than 106.0°F (41.1°C).[38] Indeed, the warm-sensitive neurons in the preoptic region reach their peak firing rates and the cold-sensitive neurons in the same region are maximally suppressed at 107.6°F (42°C), suggesting that the anterior hypothalamus is incapable of thermoregulation at temperatures higher than 107.6°F (42°C).[46] Various mechanisms, including pyrogen tolerance, circulating cryogens and modification of effector synthesis, appear to work to prevent core temperature from rising above 106°F (41.1°C). The pyrogenic effects of IL-1 and TNF are attenuated after repeated or prolonged exposures. Moreover, an important feature of tolerance to LPS is a decrease in pyrogenic cytokine production by macrophages.[38,47]

The best characterized endogenous antipyretics are α-melanocyte stimulating hormone (αMSH), arginine vasopressin (AVP), lipocortin-1, glucocorticoids and IL-10.[48–50] IL-10 has been shown to prevent LPS-induced fever by attenuating the production of TNF, IL-6 and IL-1 and increasing release of soluble IL-1-receptor antagonist, and through direct interaction with microglial cells in the central nervous system.[51] Arginine vasopressin appears to exert its antipyretic effects as a neurotransmitter of specialized neurons projecting into the ventroseptal area. Firing of such neurons increases during fever.[52] Moreover, neutralization of AVP during experimental fever is associated with increases in both the height and the duration of fever, all of which suggest an antipyretic role for AVP.[53] The melanocortin αMSH is a neurotransmitter of neurons with projections to the hypothalamic paraventricular nucleus, the integrative output nucleus for fever and numerous autonomic stress responses. Under experimental conditions, peripheral and intracerebral administration of αMSH blocks IL-1- and LPS-induced fever. Centrally administered αMSH-receptor blockers inhibit the antipyretic effect of αMSH.[53]

Arachidonic acid liberated from cell membranes by the action of phospholipase can be metabolized either by COX to pyrogenic PGE_2 or via an alternative cytochrome p450-mediated pathway to antipyretic eicosanoids (epoxyeicosanoids). Induction of cytochrome p450 shifts arachidonic acid metabolism away from COX and toward the cytochrome p450 pathway, thereby reducing core temperature through a combination of diminished PGE_2 production and an increase in production of antipyretic epoxyeicosanoids.[54]

ANTIPYRETICS

Aspirin and acetaminophen (paracetamol) have been a mainstay of antipyretic drug therapy since the turn of the 20th century. A host of nonsteroidal anti-inflammatory drugs (NSAIDs) also possess antipyretic properties. Despite the popularity of these drugs among both health-care workers and the general public, it is still not clear that reducing core body temperature benefits febrile patients. Although antipyretic therapy assumes that fever is, at least in part, noxious and that suppression of fever will eliminate or reduce its noxious effects, neither assumption has been validated experimentally. Consequently, rational guidelines for suppressing fever have been slow in coming.

Fever creates a number of potential metabolic challenges for the host. During the chill or ascending phase of fever, activation of the sympathetic nervous system causes peripheral vasoconstriction and an associated increase in mean arterial pressure.[55] Oxygen consumption increases, as does carbon dioxide production.[56] External cooling can attenuate these effects, but only if shivering is prevented.[57] If shivering is not prevented, external cooling reduces skin temperature faster than core temperature and initiates vasoconstriction and shivering, which can paradoxically increase core temperature, oxygen consumption and the respiratory quotient. Importantly, external cooling can also cause vasospasm of diseased coronary arteries.[58] Unfortunately, certain antipyretic drugs also cause coronary vasoconstriction in patients who have coronary artery disease. Friedman *et al*.[59] observed significant increases in mean arterial pressure, coronary vascular resistance and myocardial arteriovenous oxygen difference

after administration of intravenous indometacin (0.5 mg/kg) in such patients. Mean coronary blood flow decreased simultaneously from 181 ± 29 to 111 ± 14 ml/min ($p < 0.05$). Thus, in this investigation, myocardial oxygen demand increased in the face of a fall in coronary blood flow following indometacin administration.

Aspirin, acetaminophen and the NSAIDs all inhibit COX-mediated synthesis of inflammatory thromboxanes and prostaglandins from arachidonic acid. Cyclo-oxygenase has at least two distinct isoforms, COX-1 and COX-2. Cyclo-oxygenase-1 was long regarded as a constitutively expressed cellular enzyme involved in various housekeeping functions, whereas COX-2 was touted as an inducible enzyme responsible for hypothalamus-mediated fever and produced as part of the inflammatory process by a variety of cell lines, including macrophages, synoviocytes and endothelial cells. However, this dichotomous concept of a constitutive COX-1 and an inducible proinflammatory COX-2 has proved to be oversimplified. Not only do some cells express COX-2 constitutively, but under certain conditions COX-2 has also been shown to promote healing of mucosal lesions and the resolution of inflammation.[60]

The distinctive affinities of the various categories of antipyretic drug for the different COX variants are thought to determine their relative antipyretic and analgesic potencies. Acetaminophen and aspirin, for example, are equally potent inhibitors of central COX but only 10% as potent in this regard as indometacin. Acetaminophen selectively inhibits a third COX variant, a close relative of COX-1 derived from the same gene and referred to as 'COX-3'.[61] The importance of the discovery of COX-3 is that it explained the pharmacologic actions of acetaminophen and other antipyretic analgesic drugs, which are weak inhibitors of COX-1 and COX-2 but penetrate easily into the central nervous system. NSAIDs, such as diclofenac or ibuprofen, are also potent inhibitors of COX-3 expressed in cultured cells, but being highly polar, are unlikely to reach brain COX-3 in effective concentrations.[62] Only aspirin irreversibly inhibits COX and does so via acetylation within the active site of the enzyme. Other NSAIDs and acetaminophen inhibit COX reversibly.[63]

Aspirin and the NSAIDs also have COX-independent antipyretic activity. Aspirin induces cytochrome p450, which might augment its antipyretic effect by shifting arachidonic acid metabolism toward cytochrome p450-mediated production of cryogenic epoxyeicosanoids. Additionally, acetylation of COX-2 by aspirin increases the production of 15R-hydroxyeicosatetraenoic acid, which neutrophils use to form aspirin-triggered lipoxins (ATL). These lipoxins have potent anti-inflammatory activity, independent of aspirin. Heat shock proteins have been shown to reduce transcription of IL-1β *in vitro*, and therapeutic doses of aspirin and certain NSAIDs increase heat shock factor-1 concentration *in vitro*. These same drugs also diminish the activity of transcriptional activator nuclear factor-κB.[63] The latter is involved in the transcription of pyrogenic cytokines, adhesion molecules, inducible nitric oxide synthase and COX-2 in certain cell lines. Production of adenosine, an anti-inflammatory mediator produced by leukocytes, is enhanced by aspirin and NSAIDs. The clinical implications of these alternative antipyretic pathways remain to be determined.

Antipyretic therapy is commonly administered to enhance patient comfort. General experience with antipyretic drugs, which are for the most part also analgesic agents, seems to support this rationale. However, carefully controlled efficacy studies have never quantified the degree to which antipyretic therapy enhances the comfort of patients who have fever. Moreover, the relative cost of such symptomatic relief, in terms of drug toxicity and adverse effects of the antipyretic agents on the course of the illness responsible for the fever, has never been determined. The importance of these negative drug effects is underscored by studies demonstrating prolonged time to crusting of varicella skin lesions in children,[64] and increased rhinovirus shedding and decreased neutralizing antibody formation following acetaminophen use in adults.[65,66] Paracetamol use has been reported to prolong parasite clearance time in children infected with *Plasmodium falciparum*, presumably by decreasing production of TNF and oxygen radicals.[67] The risks of antipyretic drug therapy, including direct and indirect drug toxicity, increased morbidity from infection and the masking of a possible underlying infection, must be weighed against any possible benefit.

Antipyretic therapy has never been shown to be effective in preventing febrile seizures, perhaps reflecting the fact that the etiology of such seizures has more to do with the disorders responsible for the fevers than fever per se.[68] Camfield *et al*.[69] conducted a randomized double-blind study comparing single daily-dose phenobarbital plus antipyretic instruction with placebo plus antipyretic instruction in preventing recurrent febrile seizures following an initial simple febrile seizure. In children treated with phenobarbital and antipyretics, the febrile seizure recurrence rate was 5%, whereas in those receiving placebo and antipyretics the rate was 25%, suggesting that a single daily dose of phenobarbital is more effective than counseling parents about antipyretic therapy in preventing recurrent febrile seizures. More recent studies in children have shown that, whether given in moderate doses or in relatively high doses, neither acetaminophen[70] nor ibuprofen[71] reduces the rate of recurrence of febrile seizures.

Antipyretic drugs to modulate the activity of pyrogenic cytokines have been studied in bacterial sepsis.[72] In certain animal models, COX inhibitors confer protection when given soon after bacterial challenge, presumably by blunting the adverse effects of TNF and IL-1. In a large clinical trial, Bernard *et al*.[73] found that 48 hours of intravenous therapy with the COX inhibitor, ibuprofen, lowered core temperature, heart rate, oxygen consumption and blood levels of lactic acid. However, ibuprofen did not decrease the incidence of organ failure or 30-day mortality rate in patients who have sepsis.

Several tentative conclusions regarding the benefits and relative risks of antipyretic therapy seem justified in light of the limited data available. Clearly short courses of approved doses of standard antipyretic drugs carry a low risk of toxicity. Most antipyretic drugs have analgesic as well as antipyretic properties. Therefore, if not otherwise contraindicated (as is, for instance, aspirin in young children because of the risk of Reye's syndrome), such drugs can be used to provide symptomatic relief in patients who have fever, to reduce the metabolic demands of fever in chronically debilitated patients and possibly to prevent or alleviate fever-associated mental dysfunction in the elderly. To minimize antipyretic-induced fluctuations in temperature and the risk of recurrent shivering and its increased metabolic demands, antipyretic agents should be administered to patients who have fever at regular intervals to preclude abrupt recurrences, rather than as needed for temperatures above some arbitrary level. When prescribing such medications, physicians must recognize that each carries its own risk of toxicity and may prolong the course of at least some infections. There is no compelling evidence that a response to antipyretic medications is useful diagnostically in distinguishing serious from self-limited illnesses, nor is there evidence that such medications are effective in suppressing febrile seizures, even if given prophylactically.

In view of the capacity of external cooling measures to induce a cold pressor response, it is questionable whether this form of antipyretic therapy should ever be administered to patients who have fever. If external cooling is used to treat fever, care must be taken to prevent shivering because of its associated increase in metabolic work. Intravascular cooling devices may reduce variation in body temperature as compared to external cooling devices, although their effect on shivering and oxygen consumption has not been evaluated.[74,75] Unfortunately, even if shivering is prevented, there is no guarantee that a cold pressor response will be averted. In view of indometacin's capacity to cause coronary vasoconstriction in patients who have coronary artery disease, NSAIDs should be used with caution, if at all, to suppress fever in such patients.

REFERENCES

References for this chapter can be found online at http://www.expertconsult.com

Chapter 62

Harold Lambert

Clinical approach to acute fever

Fever is one of the most frequent symptoms leading to consultation with a health professional. Vast numbers of febrile illnesses are of short duration and benign outlook, and few of these are diagnosed. However, in the midst of this mass of minor illness are patients whose illness is serious or likely to become so. Some of these serious illnesses also present a danger to others, and thus have a significance beyond that of the individual patient. This is the challenge of acute fever: to distinguish the threatening from the trivial in acute illnesses in which fever is a main feature.

In some cases, of course, the fever becomes prolonged and this topic of more long-lasting fever (fever of unknown origin; FUO) is discussed in Chapter 63. The distinction between fevers of short and those of longer duration is important in considering diagnostic possibilities. Many acute and short-lived fevers are of viral origin, and conversely viral fevers without particular diagnostic features rarely last longer than 1 or 2 weeks; thus, prolonged fever without distinguishing features is rarely caused by a virus, at least in immunologically normal patients.

HISTORY

As with any other medical problem, the history is the most productive component of the initial encounter. Hypotheses are generated from the patient's account and the physician's observations, and are successively pursued or rejected in the light of emerging data. In the case of acute fever, however, a few special features of the history stand out. One of these is the common difficulty in reaching agreement on the meaning of words and phrases used in this context. It is rare for the phrase 'I have a fever' to mean that the body temperature has been measured by a reliable method. More often, the phrase means that the patient has a subjective sensation of warmth, or a feeling of chilliness or undue sweating. 'Flu' is another word in common use and usually describes aching muscles, chills and shivering, but sometimes is used to denote upper respiratory symptoms such as a runny nose or scratchy throat, and sometimes to indicate a fever. And, of course, different meanings are attached to these and other words in different cultures and languages.

A second distinctive point when taking the history of a patient with acute fever relates to the time-honored 'systems review'. This is usually employed, if at all, at the end of a history taking, but often has so little to contribute that it is discarded. In the case of acute fever, however, because the range of possibilities is so much wider than for many presenting symptoms, the systems enquiry is useful early in the history taking; it often reveals the only relevant localizing evidence, as patients may have forgotten or thought insignificant symptoms that provide valuable clues. Among the apparently minor clues that may emerge on direct enquiry are minor respiratory, abdominal or urinary symptoms, a transient rash or previous episodes of illness of a similar nature. Apparent localizing features that are actually symptoms of the raised temperature may, however, give false leads. Thus, dark urine in a febrile patient may simply denote dehydration, and some patients, especially women, experience burning and discomfort passing urine when they are febrile. Muscle and joint pains are also hard to interpret during fever: severe pains suggest viral infections such as influenza or dengue, but they are also a feature of some enteroviral infections (Bornholm disease) and of leptospirosis. Neurologic features are especially difficult. Some patients regularly experience headache when febrile, and many children and some older patients become delirious with a high fever; whether such clinical features indicate a specifically neurologic involvement obviously needs careful observation.

A history of medication may be important in acute febrile disease. A large number of drugs may themselves cause fever, often without a rash or other clear indicators. Among antimicrobial agents, penicillins, cephalosporins and sulfonamides are especially notable, but many others may be implicated. Anti-infective agents may suppress or modify infections, and so confound a diagnosis, and antipyretic agents may greatly modify the pattern of fever in an infection.

Travel history

As rapid movements of vast numbers of people throughout the world have been made possible in the era of air travel, so have the possibilities of an infection developing in one country when it was acquired in another. A travel history must never be omitted in a patient who has fever, and many tragic deaths from *Plasmodium falciparum* malaria testify to its importance. The history should be accurate as to time and place. The name of a country is not enough; a stay in a four-star hotel in a capital city presents different risks from those of a camping trek in rural areas of the same country.

Timing is especially helpful, even though many infections have a wide range of recorded incubation periods. For example, an illness beginning more than about 10 days after return is unlikely to be one of the common acute respiratory infections, with the exception of *Mycoplasma pneumoniae* and perhaps Q fever; dengue, too, and other arbovirus infections would have developed by this time. An incubation period of more than 3 weeks excludes the hemorrhagic virus infections, such as Lassa fever, and almost excludes typhoid. On the other hand, longer periods still leave open the possibility of viral hepatitis, Katayama fever (acute schistosomiasis) and primary HIV infection. As to malaria, the incubation period of *P. falciparum* infection may be as little as 1 week, but after 6 or 8 weeks a first presentation of this form of malaria becomes uncommon. *Plasmodium vivax* and *Plasmodium malariae* infections may develop months or years after travel to a malarial area.

Other important aspects of the travel history are the immunization record and an account of medications, with special emphasis on antimalarial prophylaxis.

It is sometimes necessary to begin treatment before a definite diagnosis has been made, or when the causal organism but not its antibiotic susceptibility is known. The travel history is important here too, as the pattern of antibiotic resistance in many pathogens varies greatly from country to country, and will determine an appropriate choice of therapy. Notable examples are the differences in drug resistance in malaria in different countries, the spread of multiresistant typhoid and shigellosis and the erratic distribution of pneumococcal resistance to penicillin. In each of these examples, the area in which infection was acquired may limit the options available for chemotherapy.

Every physician who sees a febrile traveler cannot be expected to have an up-to-date knowledge of the precise infective risks, let alone the antibiotic resistance patterns of possible pathogens, and therefore easy communication with a Tropical and Infectious Diseases Unit and with one of the specialized information services dealing with travel medicine, which are available in many countries, is essential. Nevertheless, it is wise to keep up to date with prevalent trends; witness the large epidemic of chikungunya affecting large areas of Asia.[1] Free online resources such as ProMED (www.promedmail.org) provide enormously valuable real-time information on current outbreaks and ongoing epidemics.

What are the actual causes of acute fever in returning travelers? (As this chapter focuses on illnesses with a large element of fever, primarily diarrheal diseases, or sexually transmitted diseases with mainly local symptoms and signs, and many other health risks of travel are not discussed here, see Section 6.) Contrary to popular myth, the 'classic' tropical diseases are rarely acquired by short-term travelers, with the vital exception of malaria. This ranks first among diagnoses of acute fever in returning travelers, followed by a large group of short-lived fevers for which no etiology is ever established. Other diagnoses obviously vary in their frequency with the pattern of travel to and from a particular country. As the traveler is exposed not only to exotic pathogens, but also to a changing ecologic background of widely distributed pathogens, it is not surprising that ordinary respiratory infections, ranging from colds to pneumonia, are common. So too are initially febrile presentations of diarrheal diseases and prodromes of hepatitis. Urinary infection, as one of the most common causes of fever in women not always accompanied by localizing symptoms, must also be remembered. Some diagnoses encountered with widely variable frequency in different units are listed in Table 62.1.

Contact history

Contact history may be relevant in travelers and in people staying at home. Information about local endemic or epidemic infections should be sought. Most frequent of all, especially in the winter months, is a history of contact with acute respiratory infection, which is possibly relevant because so many respiratory infections begin with 1 or 2 days of indeterminate fever, but the very frequency of such infections makes this aspect of the history difficult to interpret. Even in places with high uptakes of routine immunization, measles is encountered, and tends then to be missed because of its low prevalence and because it may occur in older subjects in highly immunized populations. Measles is especially important when the patient, or a contact, is immunosuppressed. Rubella, a more difficult and uncertain clinical diagnosis, is clearly of the greatest import if the patient or a contact is pregnant. Known contact with meningococcal disease obviously demands immediate attention, although, owing to the vagaries of meningococcal carriage and immunity, few patients who have this disease have a direct contact history. A story of 'food poisoning' or diarrheal disease in contacts may be relevant as shigellosis, salmonellosis and *Campylobacter jejuni* infections may all begin with a febrile phase, while ingestion of raw milk and some cheeses raises the possibilities of brucellosis and listerial infection. The long incubation periods of most forms of viral hepatitis should be remembered in exploring possible contact history. Of those most commonly associated with travel, the incubation period of hepatitis A is from 2 to 6 weeks, that of hepatitis B from 4 weeks to many months.

Table 62.1 Diagnoses made in travelers returning from tropical countries with fever as a principal symptom

Common
Malaria
No diagnosis made
Respiratory infection
Diarrheal disease (fever before or accompanying gut symptoms)
Urinary tract infection
Viral hepatitis; febrile prodrome
Uncommon
Dengue
Typhoid
Tuberculosis
Acute HIV infection
Acute schistosomiasis
Rickettsial infections
Amebiasis

Occupational history

Many occupational exposures are not particularly relevant to acute febrile presentations, but many of the points about contact history just discussed are especially applicable to health workers and to those involved in child care. Other specific risks arising from occupation that may present as an acute fever include leptospirosis in sewage workers and fish farmers, and the many infective risks in veterinary and abattoir work, including brucellosis and *Streptococcus suis* infection. Fever may be the first clinical manifestation of tuberculosis relevant to health professionals, especially those involved with the care of patients who have HIV, and to carers of the homeless.

Some fevers associated with occupation are not infective. Fever and chills may be caused by inhalation of metal fumes or breakdown products of polymers. Many occupational lung diseases manifest with primarily respiratory features, but in extrinsic allergic alveolitis fever and influenza-like symptoms may dominate the picture. These conditions are characterized by recurrent episodes related to the particular exposure. Further detail is given in Chapter 67.

Bioterrorism and fever

Several agents that might be involved in bioterrorism are likely to cause nonspecific fever as their initial manifestation (Table 62.2). In smallpox, after the incubation period of 12 days, there is nearly

Table 62.2 Bioterrorism and fever

Agent	Main early features
Smallpox	Nonspecific fever for 2–3 days followed by rash slowly progressing to vesicles or pustules OR hemorrhages, system failure and death within a few days
Pulmonary anthrax	Rapidly progressive systemic illness with fever, dyspnea, sometimes wide mediastinum and pleural effusions
Pneumonic plague	Rapidly progressive systemic illness with respiratory features prominent from the second day. Extensive, often bilateral, pneumonia



Fig. 62.1 The early focal rash of smallpox. This is the fifth day of the fever and the third day since the rash began to appear.

always a period of 2–3 days of fever and chills, perhaps rigors, headache and backache, before the focal rash begins to emerge. In the most severe forms, hemorrhagic manifestations appear and the patient dies without ever developing the focal rash. An early focal rash is illustrated in Figure 62.1. The onset of plague is also very acute, with chills, rigors, headache and generalized pains. Signs of system failure including generalized hemorrhages become apparent early in the septicemic form, but are also often seen together with enlarged nodes in bubonic plague. Pulmonary anthrax usually has a very acute onset, with early respiratory and systemic symptoms, but less acute presentations are now known to occur. Chest radiography may show mediastinal widening. (For a further discussion of bioterrorism, see Chapter 71.)

PHYSICAL EXAMINATION

Temperature

Depending on the duration of the illness, few temperature measurements may be available for evaluation. Recorded temperatures of higher than 102.2°F (39.0°C) are more likely to be caused by a significant infection than are lesser degrees of fever, but very high fever must raise suspicion of a noninfectious cause such as heat stroke or substance abuse. The pattern of fever is much less valuable than is commonly supposed. This aspect is discussed more fully in Chapter 63, but a few points relevant to short-term fevers may be mentioned. A dramatic fever with wild swings between readings is suggestive of pyogenic infection (especially abscess formation) and of acute pyelonephritis, but may also be seen in other conditions, including malaria and disseminated tuberculosis, as well as in Still's disease and occasionally in drug fever. Perhaps the most common reason for this kind of chart, however, is the use of antipyretics in a febrile patient, which often gives rise to this feature.

The most important caveats relate to malaria, and they cannot be emphasized enough. The temperature pattern in *P. falciparum* infections is often quite erratic, and this diagnosis must be considered in all febrile and some nonfebrile patients coming from a malarial area. Regular tertian or quartan fevers (meaning every other day and every third day, respectively) are not found in the early stages of malaria, and are a feature of relapse rather than initial infection. On the other hand, when present they are very characteristic of malaria.

Rashes

Many acute febrile illnesses are accompanied by a rash, which aids greatly in establishing a diagnosis. A few, notably those of meningococcal sepsis, are of vital importance in determining the need for urgent treatment or the protection of contacts (Figs 62.2, 62.3). Some are pathognomonic, others only indicative (Figs 62.4, 62.5), and the features of the rash must be placed in context with other features of the illness. In hand, foot and mouth disease the distribution is pathognomonic. Tables 62.3–62.5 provide information about rashes associated with acute fevers, including those encountered in returning travelers, but a few specific points are worth emphasizing.



Fig. 62.2 Fully developed, almost pathognomonic hemorrhagic rash of meningococcal sepsis.

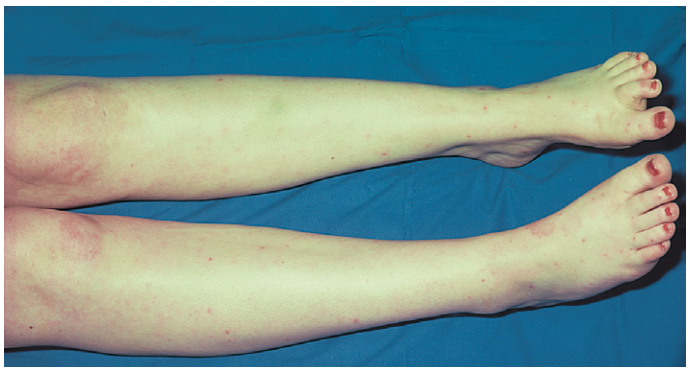


Fig. 62.3 Very early rash of meningococcal sepsis. A few petechiae only, but meningococcal sepsis nonetheless. It can progress to the appearance of Figure 62.2 within minutes or hours. This is the window of opportunity for early treatment.



Fig. 62.4 Hand, foot and mouth disease. This shows the scanty lax vesicles found at these sites. There is often a maculopapular rash too, especially on the buttocks.

In measles, easily forgotten in well-immunized populations but important to remember in older age groups, fever precedes the Koplik spots and the exanthem by 2 or 3 days but sometimes by as much as 1 week, although respiratory features are prominent for most of this time. In rubella and in enteroviral infections, general symptoms only rarely precede the rash, and then only by 1 day or so. In dengue, the



Fig. 62.5 Purpuric skin lesions in staphylococcal endocarditis.

Table 62.3 Rashes associated with acute viral infections

Virus	Syndrome	Comment
Measles	Measles	Maculopapular followed by staining
Rubella	Rubella/German measles	Macular, often general facial flush
Herpesvirus 6	Roseola infantum	Macular or maculopapular after several days of fever
Erythrovirus B19*	Erythema infectiosum	Slapped cheeks, lacy on trunk and limbs, often rubelliform or hemorrhagic
Varicella-zoster	Chickenpox Shingles	Vesicular, rarely hemorrhagic Neurologic distribution, premonitory pain, erythema
Herpes simplex	Disseminated herpes Eczema herpeticum	
Epstein–Barr virus		Occasionally macular rash; severe rashes usually ampicillin-induced
Enteroviruses		Usually macular or maculopapular, sometimes hemorrhagic and/or vesicular (hand, foot and mouth disease; see Fig. 62.4)
Primary HIV	Mononucleosis-like illness	(Maculopapular rashes in chronic HIV infection; see Chapter 89)
Viral hemorrhagic fevers	(See Chapter 126)	Purpura, ecchymoses

*Also called Parvovirus B19 (Chapter 158).

Table 62.4 Rashes associated with acute bacterial infections

Agent	Rashes	Comment
Neisseria meningitidis	Petechial/purpuric	Also non-hemorrhagic early rashes
Neisseria gonorrhoeae	Hemorrhagic vesicles, pustules	
Staphylococcus aureus	Pyogenic skin lesions Scalded skin syndrome Peripheral purpura Erythema	 In staphylococcal endocarditis In toxic shock syndrome
Streptococcus pyogenes	Erysipelas Erythema	Local erythema, bullae In toxic shock syndrome
Salmonella typhi	Rose spots	
Pseudomonas aeruginosa	Ecthyma gangrenosum Cellulitis ± blebs	Also in *Aeromonas* and other Gram-negative bacillary infections
Haemophilus aegyptius	Brazilian purpuric fever	

rash characteristically appears in the second phase of the biphasic illness. Infections associated with pathognomonic or at least characteristic rashes may, however, also occur with nonspecific rashes; the early rash of meningococcal sepsis may be macular or maculopapular, and Lyme disease may exhibit nonspecific rashes in addition to erythema chronicum migrans.

Mouth

The mouth may show useful signs in a febrile patient (Table 62.6, Fig. 62.6). Especially in infancy and childhood, the tongue and mouth give some indication of dehydration, although mouth breathing and tachypnea often produce a similar appearance. The tongue is notably raw and red in scarlatina, in Kawasaki disease and in toxic shock syndrome. Vesicles are found, especially on the soft palate and anterior fauces, in some enteroviral infections (hand, foot and mouth disease). Palatal petechiae are fairly nonspecific, but are common in infectious mononucleosis and rubella. Many important signs in HIV infection are to be found in the mouth and these are discussed in Section 5.

Eyes

Some degree of conjunctival suffusion is common in people with a high temperature. This is often prominent in measles, rubella, some adenovirus infections and in leptospirosis. In infections with a hemorrhagic rash, conjunctival hemorrhages may be present in addition to skin petechiae or purpura; this is especially helpful in patients with dark skin, when petechiae are difficult to see. Other ocular signs that may be important in acute febrile illness are uveitis in acute sarcoid (although many patients with acute sarcoid do not show ocular involvement) and in Still's disease. Choroiditis occurs in histoplasmosis and in toxoplasmosis. Although choroidoretinitis in toxoplasmosis is most frequently a late marker of congenital infection, it is now clear that a few patients with acute acquired toxoplasmosis (and not HIV-infected) do have acute choroiditis. Miliary tuberculosis, with

Table 62.5 Rashes associated with acute spirochetal and rickettsial infections

Agent	Rashes	Comments
Leptospirosis	Hemorrhages Also other rashes	Weil's disease
Borrelia recurrentis (relapsing fever)	Petechiae	Often no rash; sometimes severe hemorrhages
Borrelia burgdorferi (Lyme disease)	Erythema migrans	Sometimes secondary annular or nonspecific rashes
Spirillum minus (rat-bite fever)	Blotchy macular, papular and urticarial rashes, beginning near the bite and spreading	Rashes also in the *Streptobacillus moniliformis* form of rat-bite fever
Rickettsial infections	Macular, papular petechial	Primary eschar (*tache noir*) in some syndromes

Table 62.6 Oral signs in acute fever

Sign	Diagnosis
Dehydration	Any fever
Herpes simplex	Common in meningococcal and pneumococcal infection
Raw tongue	Scarlatina Kawasaki's disease Toxic shock syndrome
Ulcers, vesicles	Varicella Herpes simplex Enteroviruses (herpangina, hand, foot and mouth disease) Aphthous stomatitis Secondary syphilis Erythema multiforme
Palatal petechiae	Nonspecific, but common in infectious mononucleosis and rubella

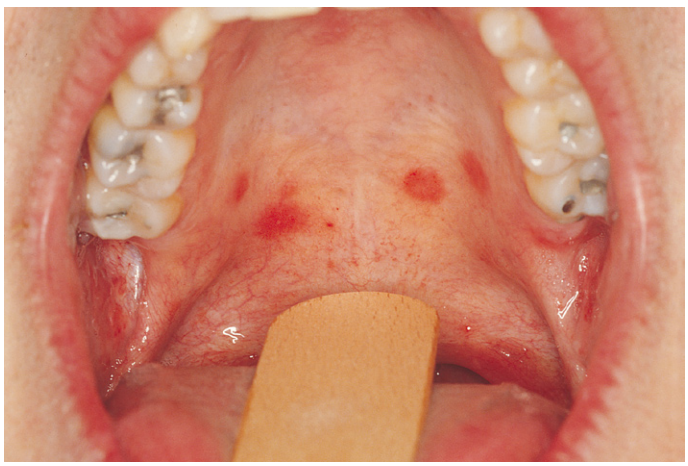


Fig. 62.6 Oral signs in hand, foot and mouth disease.

or without tuberculous meningitis, sometimes manifests as fever and general ill health; the diagnosis is immediately established if choroidal tubercles are seen. If, as often, they are scanty, they will be missed on perfunctory examination; a systematic search of both fundi with the pupils dilated is needed.

Lymph nodes

Generalized node enlargement is relatively uncommon in acute febrile illness. Among the common infections of children and young adults, rubella, Epstein–Barr virus mononucleosis and cytomegalovirus infection are notable causes, to which must be added cat-scratch disease and, in those at risk, secondary syphilis and primary HIV infection. These latter infections are especially important to remember in returning travelers. General node enlargement is also seen in some more specifically tropical diseases, of which dengue is the most likely to affect a short-term traveler. Acute histoplasmosis is another possibility after a first visit to an endemic area.

Focal nodes must direct a careful search of the relevant drainage area. For example, tender enlarged inguinal nodes may be more prominent than the source of infection, which may be insignificant-looking streptococcal lesions of the feet, perhaps superimposed on insect bites or fungal infection. Acquired toxoplasmosis in the immunocompetent host, although usually subclinical, manifests as a febrile illness with localized lymphadenopathy, most often in one or other cervical group but sometimes in nodes elsewhere. The persistent myth that toxoplasmosis is a cause of a 'glandular fever' syndrome must be firmly laid to rest. It is rare for the lymphadenopathy of acquired toxoplasmosis to be generalized, and atypical mononuclear leukocytes, if found at all on the blood film, are few in number.

Spleen

Acute and longer-term febrile illnesses make a contrast here. Splenomegaly is found in so many of the infections and other conditions causing FUO as to be of limited diagnostic value. By contrast, splenomegaly in a patient with fever of a few days' duration certainly merits further attention. It may indicate a particular infection, such as infectious mononucleosis, rubella, a hepatitis prodrome (in which splenomegaly is especially common in children) or, in a returning traveler, malaria. Splenomegaly may be attributable to an underlying hematologic condition, perhaps a hemolytic anemia or a lymphoma, itself the cause of the fever or a reason for increased susceptibility to infection.

MAKING A DECISION

Some specific factors in the history and examination suggest the need for a plan of management that goes beyond symptom relief. This may mean repeated observation, investigation or investigation combined with provisional treatment. The factors are:

- recent travel, especially to a malaria-risk country (see Chapter 111);
- chills and rigors;
- height of fever;
- fever and rash;
- extremes of age;
- any known or suspected immunosuppression;
- neurologic features;
- dehydration;
- parental or partner concern; and
- physician's impression.

Chills are common enough at the onset of respiratory infections, particularly at the onset of influenza. Nevertheless, the rapid rise in body temperature they denote is also common in some more serious infections, and this caution applies with greater force if the patient has rigors. A temperature of more than 102.2°F (39°C) is common enough in the early stage of an ultimately minor infection, but higher temperatures sustained for more than a short time are more likely to be associated with serious infections.

The combination of fever and any kind of hemorrhagic rash, be it only a few petechiae, is especially important.

Many patients on immunosuppressive therapy are living and working normally in the community but are at increased risk of infection. In some conditions, notably asplenia from any cause, infection may take a fulminant course and any fever demands very prompt attention (see Chapter 82). In addition, some forms of immunosuppression may initially manifest with a febrile illness, and the possibility of a first presentation of HIV infection has to be borne in mind.

Depression of consciousness, meningism or localizing neurologic signs are clearly important. Children with high fever may exhibit mild confusion and experience hallucinations; this is seen less commonly at older ages. Whether such clinical features denote specific neurologic involvement needs careful assessment.

Children and the elderly are at greater risk of dehydration, but patients of any age with fever, especially in warm conditions and if anorexic or vomiting, may become fluid deficient.

Whatever the level of anxiety in patient or carer, someone close to the patient may well have an accurate notion of whether the illness is out of the ordinary, and their opinions should be carefully considered.

The physician may form the impression that the illness is unusual, or serious, or likely to become serious. This is so common a problem as to merit more detailed discussion.

Is the patient ill?

Even after the most meticulous history taking, physical examination and attention to the issues just discussed, there are large numbers of patients with fever of short duration in whom no particular warning features are present. Fortunately, most patients who have fever of a few hours' or a few days' duration recover uneventfully without sequelae and without a diagnosis other than a meaningless attribution to 'viral infection'. How should one judge, in the home, in the health center or practice premises, or in the hospital Emergency Room, which of these patients should be further investigated, or investigated and given provisional empiric treatment? This must be one of the most frequent decisions that has to be made by clinicians all over the world and yet it is ignored in books about diagnosis. Decision theory is largely silent in this context because the elements of the decision involve multiple factors, usually heuristic in character, few of which can be assigned a numeric value.

It seems that the most common factor affecting the physician's decision is the impression that the patient has an infection with systemic and potentially hazardous features – for which the word 'toxic' is often used as shorthand – and this is the main determinant of further investigation and treatment in patients who have acute febrile illness. Indeed, algorithms on this problem often begin with the question: 'Is the patient toxic?'

Attempts to analyze what this means have been more often pursued in children than in adults. This work had its origin in the increased use of blood culture in infants and children, most of whom did not look ill, taken to 'walk-in' clinics or hospital Emergency Rooms in the USA. Positive blood cultures were found in 2.8–8% of these patients. In most places the main organisms were *Streptococcus pneumoniae*, *Neisseria meningitidis* and, before general immunization against this organism, *Haemophilus influenzae*. A few of these children, perhaps 4–7%, went on to develop meningitis or other focal infections.[1] These findings spawned a vast amount of work on the early detection of potentially serious illness in febrile infants and on developing management plans for their care. Observations which commonly raise concern in doctors and parents about the possibility of serious illness are the child's reaction to handling and to social overtures, the skin color and state of hydration,[2] while in one study the most sensitive predictors of serious illness were poor feeding and restlessness.[3]

A qualitative study of 83 cases of meningococcal disease seen in general practice in South Wales showed how, even in the absence of a rash, clinical and contextual features helped to differentiate these patients from the many with acute self-limiting febrile illnesses.[4] Many of the patients showed 'features not normally expected in children with acute self-limiting illnesses'; these included lethargy, poor eye contact, altered mental state and abnormal cry. Detecting early pointers of serious illness is especially important in meningococcal disease because an apparently mild illness may progress within hours to life-threatening sepsis. A large study of 448 children with meningococcal disease found that 72% had features of sepsis, leg pains, cold hands and feet, and abnormal skin color, preceding by many hours the classic signs of meningococcal disease.[5]

Attempts to analyze what experienced physicians do without conscious thought have tended to confirm the value of clinical judgment in predicting or excluding a high risk of serious disease in older infants and children, but more work of this kind is needed in adults. It seems impossible to dispense with the elusive but crucial clinical impression of illness, although even this may deceive in either direction. One reason for disasters associated with *P. falciparum* malaria and with meningococcal septicemia is the apparently good condition of the patient which may precede a rapid decline whereas, conversely, patients may look much more ill than they are after an exhausting long journey or a lively party. When, as so often, there is doubt about the assessment, a total leukocyte count of greater than 15×10^9/l has been valuable in some, but not all, studies in indicating an increased post-test probability of bacteremia.

Beyond the basic need for time and care in clinical assessment, a confident relationship between patient and physician, and easy access for further consultation are perhaps the most important factors in ensuring that major illness is not missed.

A decision tree for the management of acute febrile illness is given in Figure 62.7.

LABORATORY INVESTIGATIONS

With few exceptions, among them the classic infectious diseases such as measles and varicella, most acute and short-lived infections have no specific features that enable a clinical diagnosis to be made. Few of these illnesses are investigated. Facilities to do so are not available in most of the world and, when they are available, are unnecessary in patients who have mild and self-limiting illnesses. It follows that most of these infections remain undiagnosed, although a specific diagnosis can be assigned to a substantial number of them in research projects in which full virologic investigations are undertaken. This is the case especially in acute infections in children, who may experience a remarkably large number of infections, most of them viral, in the course of a year.

Investigations are certainly indicated when any of the 'danger points' just described are present, and in any febrile patient whose general condition gives concern. The precise range of investigations chosen will obviously vary depending on the facilities available and on the vagaries of the clinical situation, but even a small and frequently accessible range of investigations (Table 62.7) greatly increases the possibility of establishing a diagnosis. When the features of the illness give no specific direction, the most useful investigations are a routine blood count together with careful examination of a stained blood film, blood culture, urine examination, and posteroanterior and lateral radiographs of the chest. When blood is taken, a serum specimen should be saved for later study.

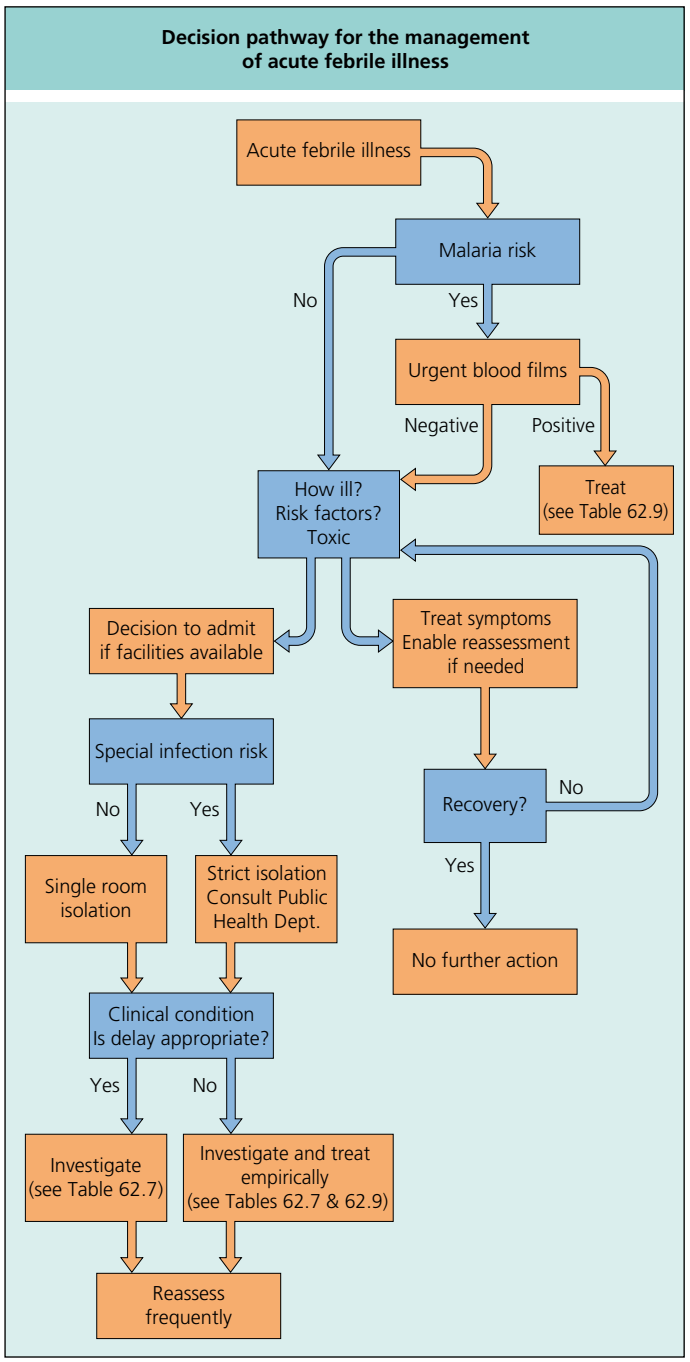


Fig. 62.7 Decision pathway for the management of acute febrile illness.

Initial investigations are often, of course, much more extensive depending as they do on available resources and initial clinical clues. These will most commonly include liver function tests, stool microscopy and culture, antigen detection and serologic tests for particular pathogens, and relevant imaging, especially abdominal ultrasound and CT scanning or MRI. The more extensive range of investigations is fully discussed in the context of FUO (see Chapter 63).

Table 62.7 Basic laboratory investigations in patients who have acute fever

- Routine blood count
- Stained blood film
- Urine microscopy and culture
- Chest radiograph
- Save serum

Total and differential leukocyte count

Modest degrees of neutrophilia, up to about 15 × 10^9/l, are of little help. More definite neutrophilia is principally found in pyogenic bacterial infections, but also in amebiasis, leptospirosis and in many noninfectious conditions such as thromboembolism, rheumatic fever, Still's disease, exacerbations of chronic liver damage and mechanical tissue damage (Table 62.8).

Neutropenia is common in many viral infections, including rubella and influenza, and, among infections of travelers, is often found in malaria, typhoid, brucellosis, rickettsial diseases and visceral leishmaniasis. Leukopenia is also a feature of severe and overwhelming sepsis, but the serious condition of patients who show this feature is usually only too evident. Thrombocytopenia is a very frequent feature of the blood film in malaria and in sepsis. A substantial number of atypical mononuclear cells is found most commonly in acute Epstein–Barr virus and cytomegalovirus infections, whereas eosinophilia points to the tissue-invasive stage of many parasitic infections.

Specific diagnoses from the blood film

Malaria is by far the most important finding from examination of the blood in those at risk, but other diagnoses that can sometimes be made in this way are listed in Table 62.9. In addition to examination of the blood, a diagnosis may occasionally be aided by direct examination of material from a skin lesion, for example in meningococcal sepsis.

Urine examination

Small degrees of proteinuria are of no significance in febrile patients. Dipsticks can be used to detect Gram-negative infections and pyuria, but should not be used alone in the diagnosis of fever because of the high false-negative rate of the nitrate test for bacteriuria. Urinary infection is best diagnosed by direct microscopy of a drop of urine with the finding of heavy pyuria, often some hematuria and visible organisms. The presence of organisms in association with pyuria in a freshly obtained specimen indicates significant bacteriuria and a Gram stain can help in distinguishing positive

Table 62.8 Value of total and differential leukocyte count in acute fever*

Neutrophilia	Neutropenia
Sepsis	Severe sepsis
Abscess	Malaria
Amebiasis (usually)	Typhoid
Leptospirosis (usually)	Brucellosis
Still's disease	Visceral leishmaniasis
Lymphoma (uncommon)	Rickettsial infections
Atypical mononuclear cells	**Eosinophilia**
Epstein–Barr virus	Schistosomiasis (Katayama fever)
Cytomegalovirus	Visceral larva migrans (toxocariasis, etc.)

*This table concerns acute illnesses in which fever is a principal feature; it does not include conditions such as tropical eosinophilia in which respiratory features are dominant.

Table 62.9 Specific diagnoses from the blood film*

- Malaria
- Babesiosis
- Trypanosomiasis
- Filariasis
- Leptospirosis (darkfield)
- Relapsing fever (darkfield or staining)
- Bartonellosis
- Ehrlichiosis
- Meningococcemia
- Histoplasmosis, candidemia

*Etiologies of acute fever are sometimes established by examination of the blood film.

cocci from negative bacilli. Urinary infection is one of the most common infections that can manifest as a febrile illness with no localizing symptoms or signs.

Chest radiograph

The most important findings in a febrile but previously healthy patient are areas of consolidation, since general symptoms and fever may precede respiratory symptoms and signs by several days, and radiographic changes may be surprisingly prominent in patients with few or no respiratory symptoms. This is especially likely in 'atypical' pneumonia, such as that associated with Q fever or *Mycoplasma pneumoniae* infection (Fig. 62.8). The other crucial finding is that of pulmonary tuberculosis, in some communities to be suspected throughout the population, and in others more especially in the indigent and those infected with HIV. Other diagnoses may also be made, such as *Pneumocystis jirovecii* (formerly *P. carinii*) infection in HIV infection before respiratory features become evident, allergic pneumonias, visceral larva migrans and pulmonary emboli. Chest radiography is especially important in older patients, who may develop serious pneumonia in the absence of any indicative clinical features.

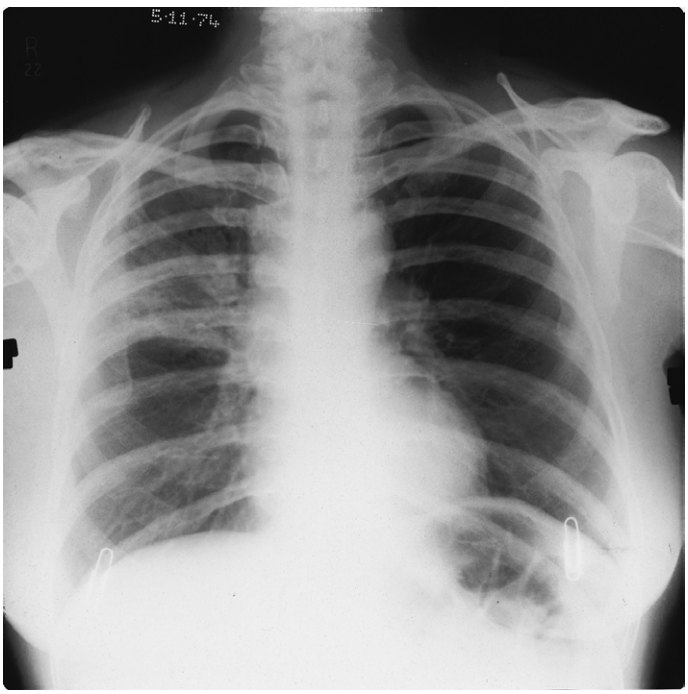


Fig. 62.8 Chest radiograph of a patient with *Mycoplasma* pneumonia. Respiratory symptoms are often scanty or absent in the first few days of the illness.

Blood culture

The importance of blood culture in febrile patients thought ill enough to need investigation is evident but, important as it is, the information gained is necessarily delayed and thus irrelevant to the immediate management decisions.

Serum

It is always sensible, if blood is drawn, to save a serum sample, especially for later comparative tests if the illness proves to be prolonged and remains undiagnosed.

ISOLATION

A few patients who have infections present a risk to other people. Because the diagnosis is often obscure at the time of admission to hospital of an acutely febrile patient, isolation is often advisable initially, if facilities are available. Sometimes, as for example in suspected Lassa fever, more elaborate measures involving the control-of-infection team in liaison with the public health authorities are indicated. These aspects of the management of infection are discussed in Chapter 6.

MANAGEMENT

Symptomatic treatment

Drug therapy is unnecessary in many acute fevers, but discomfort can be alleviated by agents such as aspirin, paracetamol and nonsteroidal anti-inflammatory agents, which act as cyclo-oxygenase inhibitors. There is little to choose between their effect on fever, but aspirin is avoided in infants because of its association with Reye's syndrome, and in many older patients because of its effects on the gastric mucosa.

The effectiveness of antipyretics in reducing fever does not seem to correlate with its cause. In a study of 1559 children with a temperature of more than 101°F (38.4°C) on arrival at the Emergency Room, reductions of temperature 1 and 2 hours after a single dose of paracetamol (acetaminophen) were slightly greater in patients who had a serious bacterial infection than in those who did not,[6] although it had been thought that fever with a serious cause might be less responsive to an antipyretic.

Sponging is still sometimes used as a method of reducing fever. A few studies confirm that it does this, but it is often very uncomfortable, and should be done with tepid and not iced water. The vasoconstriction and shivering causing distress do not, however, produce a rise of core temperature. Combining paracetamol (acetaminophen) with sponging gives a slightly greater reduction of temperature than sponging alone.[7]

It is useful to know the timing of antipyretic action, especially if the aim is to reduce the likelihood of febrile convulsions. Both antipyretic drugs and sponging show an appreciable effect within about 30 minutes and have their maximal effects in 2–3 hours.

Corticosteroids are effective antipyretics but should be used only for specific indications in conjunction with appropriate anti-infective therapy for the known or presumed cause of the fever.

Maintaining hydration is important and not always easy in a warm climate as patients who have fever are often anorexic and sometimes vomiting.

Empiric treatment

One of the most taxing and common decisions that has to be taken in acute febrile illness is whether to start empiric treatment based on one or more hypotheses about the diagnosis. On one side is the fear of rapid and perhaps irreversible deterioration, especially in *P. falciparum*

Table 62.10 Some dangerous features in patients who have acute fever

- Petechial/purpuric rash
- Travel involving risk of malaria
- Chills and rigors
- Extremes of age
- Neurologic signs
- Asplenia
- Hypogammaglobulinemia
- Post bone marrow transplant

malaria and in sepsis. On the other are the confounding effects of possibly inappropriate treatment, and the added problem of adverse drug reactions. If immediate treatment is given, it is nearly always possible to take blood before starting and this can be used for diagnostic tests.

Some indications for immediate treatment (Table 62.10) in acutely febrile patients can be firmly stated; others are contingent and depend on the precise details of the clinical and epidemiologic situation. The most urgent are the possibility of meningococcal sepsis and that of serious infection in asplenic subjects. Next in urgency are indications pointing toward streptococcal sepsis and, in returning travelers, the possibility of *P. falciparum* malaria. Immediate treatment should also be seriously considered if there is evidence of any immunologic disorder and, more generally, if other indicators of severe illness such as those already discussed are present. Table 62.11 gives some recommendations for action in these circumstances. The antibiotic choice, as always, must take account of local susceptibility patterns, and for this reason, more than one option is given. For example, penicillin is no longer the agent of first choice in many places for serious pneumococcal infection, and the spread of meningococcal resistance to penicillin would make future changes necessary in the national policy in the UK for the immediate treatment of suspected meningococcal sepsis.[8]

It has been written (by Garrison) that Wunderlich, the founder of clinical thermometry, 'found fever a disease and left it a symptom'. This chapter shows that fever remains an important and challenging symptom that demands meticulous analysis so as to achieve the best prospects for accurate diagnosis and treatment.

Table 62.11 Suggested empiric regimens for use when urgent treatment is indicated in patients who have acute fever

Presumed diagnosis	Action
Meningococcal sepsis	1. Blood culture if possible 2. Penicillin G (benzylpenicillin)
Septic shock in asplenia	1. Blood culture if possible 2. Penicillin G (benzylpenicillin) or cephalosporin*
Streptococcal sepsis	1. Blood culture if possible 2. Local lesion, Gram stain and culture if possible 3. Penicillin G (benzylpenicillin) ± clindamycin
Staphylococcal sepsis	1. Blood culture if possible 2. Local lesion, Gram stain and culture if possible 3. Flucloxacillin or similar agent*
Severe malaria	1. Blood films 2. Quinine (or artemether or artesunate; see Chapter 111)
Lassa fever	1. Strict isolation 2. Tribavirin (ribavirin) 3. Inform public health authorities

*Knowledge of local resistance patterns and antibiotic policy needed.

REFERENCES

References for this chapter can be found online at http://www.expertconsult.com



Chapter 63

Chinh Nguyen
Alan Cross

Fever of unknown origin

INTRODUCTION

Fever of unknown origin (FUO) is one of the most challenging tests of the diagnostic acumen of the physician. The workup can be long and arduous, leading to frustration on the part of both clinician and patient. There have been many reports spanning over 70 years (Table 63.1). The case mix of FUO patients has changed significantly over this period as technologic and radiographic advances have occurred.

The Petersdorf and Beeson criteria for FUO, which standardized its definition in 1961,[7] are:

- a body temperature of more than 101 °F (38.3 °C) for at least 3 weeks; and
- failure to establish a diagnosis after 1 week of investigation.

These criteria were designed to eliminate self-limited diseases that might have been represented in earlier series.[1–6] Because of changes in modern medicine, two modifications of the classic definition have been proposed:

- allowing 3 outpatient days to be the equivalent of 1 week's inpatient workup;[26] and
- shortening the 3-week febrile period to 2 weeks.[27]

These quantitative definitions are, by their nature, arbitrary. As such, recent literature has put an emphasis on changing the definition of FUO from a quantitative to a qualitative one, to specify a minimum negative diagnostic workup before the febrile illness can qualify as an FUO.[23] Patients who are neutropenic (see Chapter 73), who have HIV (see Chapter 92) or who acquired their fevers in a nosocomial setting (see Chapter 28) are now considered as separate categories, as the approach in these patient populations is different.

CLASSIC FEVER OF UNKNOWN ORIGIN

Disease categories

An etiology of FUO is identified in the majority of cases in each published series on FUO (average 73%, range 30–93% of cases). The principal disease categories include infection, neoplasms and collagen diseases, with the remaining diverse range of cases being grouped together as 'miscellaneous'. Table 63.1 summarizes the variations in select case studies on FUO which have been published over the years since its original description.

Figure 63.1 summarizes the relative contributions of the various disease categories to the final diagnosis. Trends are difficult to assess as they vary depending on the inclusion or exclusion of different series. The recommendation to change the definition of FUO to facilitate an ambulatory evaluation does not alter the relative contribution of various disease categories to the final diagnoses in one review.[23] This same review emphasizes 14 clinical entities that comprise the majority of final diagnoses: endocarditis, tuberculosis, abdominal abscess, Epstein–Barr virus (EBV) and cytomegalovirus (CMV) infection, lymphoma, leukemia, adult-onset Still's disease, systemic lupus erythematosus (SLE), polymyalgia rheumatica and giant cell arteritis, sarcoidosis, Crohn's disease, subacute thyroiditis, habitual hyperthermia and drug fever.

Infections

The major infections represented in older papers – tuberculosis (TB), endocarditis and abdominal abscesses – continue to make up a significant proportion of FUO in recent series.[13,14] Tuberculosis is a leading infectious cause of FUO, particularly in developing countries.[16,17,19,21,22,25,28] The types of infection causing FUO have become more diverse,[11–25] including HIV infection, Lyme disease, and diseases such as typhoid fever and amebiasis in patients who have resided in or traveled to developing countries.

In one series in which TB represented 40% of all cases of FUO,[22] TB manifested in all its myriad forms, including Pott's disease, TB lymphadenitis, primary and postprimary pulmonary TB, with miliary TB predominating. Current BACTEC technology allows for easier isolation of the organism than in the past. Occasionally, the diagnosis was established via clinical improvement with antituberculous therapy.[17]

In the diagnosis of miliary tuberculosis, the probability of a positive smear for mycobacteria increases with the number of sites sampled. On histopathology, granulomas are present in liver biopsies from 90% to 100% of cases, whereas they are seen in 60–70% of transbronchial biopsies (though more transbronchial biopsies are performed than liver). Anemia is present in as many as 50% of patients and leukopenia is not infrequent. Occasionally marked leukocytosis has led to the misdiagnosis of leukemia. Granulomas are seen in roughly 50% of bone marrow biopsies, but the yield increases to greater than 80% when hematologic abnormalities are present. Serial chest imaging may reveal infiltrates not initially present.

In earlier series, the most common cause of native-valve endocarditis was viridans streptococci; however, with the decreased incidence of rheumatic valvular heart disease, this etiology has become less common. More recent studies have seen an increase in *Staphylococcus aureus*, driven, in part, by intravenous drug abuse and the changing epidemiology of *S. aureus* in the community setting.[16,17] The continued finding of streptococcal species in more recent series places a premium on withholding antibiotics in clinically stable patients so that multiple blood cultures can be drawn.

Other infectious etiologies of FUO in recent series include occult osteomyelitis, intra-abdominal and retroperitoneal abscesses, pyelonephritis, enteroviral infections, rickettsial infections (including scrub and murine typhus, Mediterranean spotted fever), CMV, EBV

Table 63.1 Diagnoses in selected series of FUO reported in the literature

	Country	Total	Infection	Neoplasm	Collagen	Miscellaneous	Undiagnosed
Alt & Barker 1930[1]	USA	57	14	6	0	1	36
Hamman & Wainwright 1936[2,3]	USA	54	32	12	0	0	10
Keefer 1939[4]	USA	80	51	19	0	10	0
Bottiger 1953[5]	Sweden	68	16	11	4	4	33
Geraci *et al*. 1959[6]	USA	70	15	21	0	20	14
Petersdorf & Beeson 1961[7]	USA	100	36	19	15	23	7
Petersson 1962[8]	Finland	81	15	5	5	0	56
Sheon & Van Ommen 1963[9]	USA	60	12	11	8	6	23
Fransen & Bottiger 1966[10]	Sweden	60	8	19	2	4	27
Jacoby & Swartz 1973[11]	USA	128	51	26	19	22	10
Larson *et al*. 1982[12]	USA	105	32	33	9	18	13
Knockaert *et al*. 1992[13]	Belgium	199	45	14	42	47	51
Kazanjian 1992[14]	USA	86	28	21	18	11	8
De Kleijn *et al*. 1997[15]	Netherlands	167	43	21	33	20	50
Zhiyong *et al* 2003[25]	China	208	66	35	46	11	50
Liu *et al*. 2003[19]	Taiwan	78	33	5	16	6	18
Tabak *et al*. 2003[22]	Turkey	117	40	22	27	12	16
Vanderschueren *et al*. 2003[23]	Belgium	290	57	29	68	38	98
Saltogu *et al*. 2004[21]	Turkey	87	51	12	16	2	6
Arce-Salinas *et al*. 2005[20]	Mexico	45	19	8	12	1	5
Ertern *et al*. 2005[17]	Turkey	57	24	10	17	0	6
Ammari 2006[28]	Jordan	52	26	8	6	12	0
Chin *et al*. 2006[16]	Taiwan	94	54	8	7	8	17
Bleekers-Rovers *et al*. 2007[29]	Netherlands	73	12	5	16	3	37
Total		2416	780	380	386	279	591

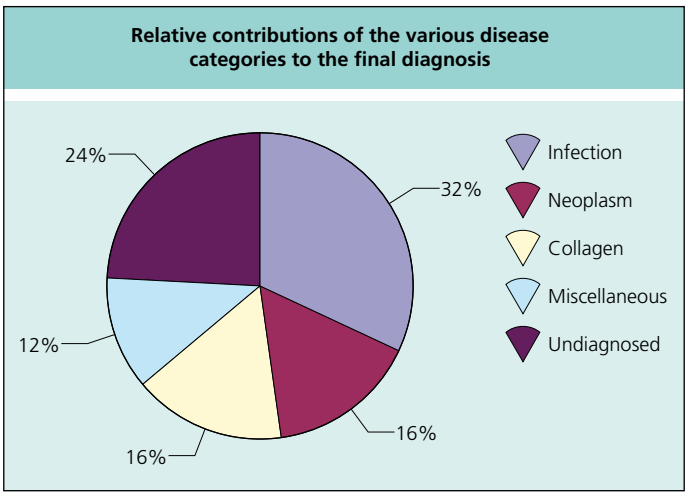


Fig. 63.1 Relative contributions of the various disease categories to the final diagnosis.

and parvovirus B19 infection, as well as fastidious bacteria, mainly brucellosis.[16–25,29] In many instances, the reliability of the microbiologic diagnosis is not always clearly defined.

Neoplasia

Lymphoma (both Hodgkin's and non-Hodgkin's) remains the most common malignant disease causing FUO.[12–15] Its insidious presentation and protean manifestations may explain why lymphoma continues to account for a sizable number of cases despite new diagnostic modalities. For example, fever in some cases persists for months before a lymph node becomes sufficiently large enough for detection. In addition, it may be difficult to locate an involved lymph node on physical examination and it is impossible when the retroperitoneal nodes or bone marrow are the only site of disease. A directed percutaneous needle biopsy of an involved lymph node or bone marrow may contain insufficient material to reveal diagnostic histopathology.[30] Thus, lymphoma may elude clinical detection for several weeks.

Other neoplasms and solid tumors are less common causes of FUO.[12–15] The peripheral blood smear of certain leukemias and

myelodysplastic syndromes may reveal no definitive findings (e.g. aleukemic leukemia). Consequently, establishing the diagnosis requires bone marrow examination.[31]

Renal cell carcinoma is a primary malignancy of the renal cortex. The classic triad of flank pain, hematuria and a palpable renal mass is present only in a minority of cases.[32] Up to 20% of patients will experience fever.[33] Metastatic adenocarcinoma in the liver, regardless of the primary site, may also cause FUO.[31] In most situations, but not all, there is elevated hepatic alkaline phosphatase, hepatomegaly, or both. Other solid tumors that can cause FUO include leiomyosarcomas of the gastrointestinal tract, pheochromocytomas, hepatomas, sarcomas and atrial myxomas.

Other rare causes of FUO from this category include malignant histiocytosis, angioimmunoblastic lymphadenopathy with dysproteinemia and a variety of CNS tumors.

Rheumatologic disorders

Adult Still's disease, for which there is no definitive laboratory test, remains the most common rheumatologic cause of FUO.[17,19,22,23,34] As there is no single diagnostic test for adult Still's disease, the diagnosis is largely clinical, requiring the presence of major and minor criteria, or a combination of both, as well as certain exclusions.[35] Proposed major criteria include fever of at least 102°F (39°C) lasting 1 week or longer, arthralgias and arthritis lasting 2 weeks or longer, characteristic rash, and a leukocytosis with granulocyte predominance. Minor criteria include sore throat, recent lymph node swelling, hepatomegaly and/or splenomegaly, abnormal liver function tests and a negative antinuclear antibody and rheumatoid factor. Temperature changes can be dramatic, sometimes as much as 4°C in 4 hours. The characteristic rash of adult Still's disease is described as an evanescent salmon pink, macular or maculopapular eruption, classically appearing only during febrile episodes.

Temporal arteritis, with or without polymyalgia rheumatica (PMR), should be considered in febrile patients over 50 years of age; the erythrocyte sedimentation rate (ESR) is nearly always elevated. While the diagnosis of PMR is largely clinical, the gold standard for temporal arteritis is temporal artery biopsy. Cardinal features, which are not always present, include jaw and tongue claudication, and visual and temporal artery abnormalities. The diagnosis of PMR rests on several diagnostic criteria, including elevated ESR, elderly age, and bilateral pain and stiffness in the shoulders, neck and hips, though these are not universally accepted.[36] The classic presentation of headache, jaw claudication, visual loss or a palpable temporal artery may be absent in one-third of patients who have temporal arteritis.

Systemic lupus erythematosus (SLE) has been variably present in more recent series on FUO, occasionally surpassing adult Still's disease.[20,29] With the widespread availability of the antinuclear antibody test, however, it has become less frequent as a cause of FUO. Lupus is a chronic inflammatory disease of unknown origin which can affect multiple organs, including the skin, joints, nervous system, lungs and serous membranes; the diagnosis is based upon a combination of compatible clinical and serologic criteria.

Other less common rheumatologic causes of FUO include Wegener's granulomatosis and cryoglobulinemia. Polyarteritis nodosa should be considered if there is mononeuritis multiplex, myalgias, skin lesions, abdominal pain (which is due to small intestinal ischemia) and azotemia.[37] A variety of other autoimmune diseases such as mixed connective tissue disease, small vessel and median vessel vasculitis, and erythema nodusum present initially as prolonged fever.

Miscellaneous

A diverse array of conditions, including granulomatous diseases, inflammatory illnesses and drug-related fevers may cause FUO. Crohn's disease and sarcoidosis are examples of granulomatous diseases.[38] Previous studies occasionally list granulomatous hepatitis as a final diagnosis in FUO. However, it should be regarded as a histologic finding, with its own broad differential diagnosis, including infections, like tuberculosis and brucellosis. It should not be regarded as a diagnosis unto itself.

Other conditions that must be considered include alcoholic hepatitis, pulmonary emboli, subacute thyroiditis, Sweet's syndrome and familial Mediterranean fever. Leukocytosis may occur with these conditions but it is often especially prominent in alcoholic hepatitis and familial Mediterranean fever. A number of hereditary periodic fever syndromes have become better elucidated within the past decade. Besides familial Mediterranean fever, other autoinflammatory diseases include TRAPS (tumor necrosis factor receptor-associated periodic fever syndrome, formerly known as familial Hibernian fever), HIDS (hyper-IgD syndrome), the cryopyrin diseases and all of their variants, including Muckle–Wells syndrome. These latter inflammatory disorders are a result of altered interleukin 1 production. Their clinical features are summarized in Table 63.2. Genetic testing for these conditions is now available to assist in the diagnosis, although it should be noted that these assays have not yet been validated.[39]

Subacute thyroiditis may be difficult to diagnose because there are usually no systemic features of thyrotoxicosis and the thyroid function tests are usually normal in its initial phase. The thyroid gland may be nontender; however, it is commonly diffusely enlarged. Schnitzler syndrome (hyperostosis, lymph node enlargement and monoclonal IgM gammopathy) is a rare cause of FUO.[40] In children, a new syndrome, PFAPA (periodic fever, aphthous stomatitis, pharyngitis and cervical adenitis), has been described.[41] This syndrome can be difficult to distinguish from familial Mediterranean fever but lacks serositis and episodes respond to steroids.

Hematomas and drug fever may cause FUO. Hematomas causing FUO may occur as a result of hemorrhages into the abdominal cavity or retroperitoneal space, but bleeding within the wall of an aneurysm or dissection of the thoracic or abdominal aorta has also been reported as being responsible. In these cases, persistent fever and anemia typically follow an episode of chest, back or abdominal pain that spontaneously resolves. Trauma may predispose to the formation of hematomas in extravascular spaces.

Kikuchi's necrotizing lymphadenitis is an infrequent cause of FUO. It is a benign, often self-limiting condition whose important clinical features include fevers and cervical lymphadenopathy. Fevers are typically low grade and can occasionally be accompanied by systemic symptoms. Diagnosis is usually established via lymph node biopsy.[42]

Drug fever can occur with virtually any medication, even those administered for long periods without previous problems (Table 63.3).[43] Despite common belief, eosinophilia, rash and/or relative bradycardia are not always present. There are no distinctive clinical features associated with drug fever that help to distinguish it from the other causes of fever mentioned above. The diagnosis often rests upon correction of fever upon discontinuation of the responsible drug.

Endocrinologic causes of FUO include hyperthyroidism and adrenocortical insufficiency.[40] Factitious fever comes in two forms: falsely elevated thermometer recordings without fever, and self-inflicted injury to induce fever or infection. Many of these patients have the features of Munchausen syndrome.[44] In order to provide objective evidence of fever, some patients may warm the thermometer when the health-care worker is not in attendance. Others may subject themselves to mutilation through such maneuvers as injecting themselves with specimens contaminated with micro-organisms. These entities appear almost exclusively in health-care workers.

Undiagnosed fever of unknown origin

Between 7% and 30% of cases remain undiagnosed despite intensive evaluations.[1–15] The long-term follow-up, available in two FUO series, shows that fever resolves in the majority of these patients within a short time.[45] In one series, follow-up was available for eight cases in whom no diagnosis was established.[14] Fever resolved within 3 weeks of the period of FUO in 87% of cases and within 4 months in all patients. The other series investigated the long-term follow-up of 49 cases of undiagnosed

Table 63.2 Familial autoinflammatory syndromes

	FAMILIAL MEDITERRANEAN FEVER (FMF)	MEVALONATE KINASE DEFICIENCIES			TUMOR NECROSIS FACTOR-RECEPTOR-ASSOCIATED PERIODIC SYNDROME		CRYOPYRIN DISEASES	
		Classic hyper-immunoglobulin D syndrome (HIDS)	**Mevalonic aciduria**	**Variant HIDS**		**Familial cold autoinflammatory syndrome (FCAS)**	**Muckle–Wells syndrome (MWS)**	**Chronic infantile neurologic cutaneous and articular syndrome (CINCA)**
Age at onset (yrs)	<20	<1	<1	<10	<20	<1	<20	<1
Cutaneous involvement	Erysipelas-like erythema	Maculopapular rash	Morbilliform rash	Maculopapular rash	Migratory rash, overlying area of myalgia	Cold-induced urticaria-like lesions	Urticaria-like rash	Urticaria-like lesions
Musculoskeletal involvement	Monoarthritis common	Arthralgia, occasional oligoarthritis	Arthralgia common	Arthralgia	Severe myalgia common; occasional frank monoarthritis	Arthralgia common; occasional mild myalgia	Lancing limb pain, arthralgia common; arthritis can occur	Epiphyseal bone formation
Abdominal involvement	Sterile peritonitis common	Splenomegaly, severe pain common	Splenomegaly, pain may occur	May occur	Severe pain very common	None	May occur	Hepatosplenomegaly
Eye involvement	Uncommon	Uncommon	Uncommon	Uncommon	Conjunctivitis and periorbital edema very common	Conjunctivitis	Conjunctivitis, sometimes optic nerve elevation	Papilledema with possible loss of vision, uveitis
Distinguishing clinical symptoms	Erysipelas-like erythema	Prominent cervical lymphadenopathy	Dysmorphic features, neurologic symptoms	Lymphadenopathy may occur	Migratory nature of myalgia and rash, periorbital edema	Cold-induced urticaria-like lesions	Sensorineural hearing loss	Chronic aseptic meningitis, sensorineural hearing loss, arthropathy
Gene involved	MEFV	MVK	MVK	?	TNFRSF1A	CIAS1	CIAS1	CIAS1
Protein involved	Pyrin (marenostrin)	Mevalonate kinase	Mevalonate kinase	?	Type 1 TNF-receptor	Cryopyrin	Cryopyrin	Cryopyrin

Reproduced and adapted from Simon *et al.*[39] with permission.

Table 63.3 Drugs associated with drug fever

Common	Less common
Atropine	Allopurinol
Amphotericin	Hydralazine
Penicillins	Isoniazid
Cephalosporins	Rifampin (rifampicin)
Phenytoin	Macrolides
Procainamide	Clindamycin
Quinidine	Vancomycin
Sulfonamides	Aminoglycosides
Interleukin-2, interferon	

FUOs taken from a larger cohort of 199 cases.[45] Fever resolved within a few weeks of discharge in 31 of the 49 cases (63%) and within 2 years of discharge in 83%. In the remaining 8 patients (17%), fever recurred and required repeated courses of nonsteroidal anti-inflammatory drugs (NSAIDs) and corticosteroids. Despite the lack of diagnosis in these patients, the mortality rate 5 years after discharge was small – only 3%. Thus, in most of these patients the fever abated without treatment, and rarely did a serious disorder emerge later.[14,45]

Despite advances in diagnostic techniques over the past decade, there continues to be a high proportion of undiagnosed cases in developed countries, sometimes exceeding 50%.[14,23,29,45] These same advances may allow for the diagnosis for a variety of conditions before they meet the definition of FUO. Moreover, the relatively good prognosis in patients with FUO may suggest the need for long-term clinical follow-up and diagnostic abstinence after life-threatening diseases have been reasonably ruled out.

FEVER OF UNKNOWN ORIGIN IN OTHER GEOGRAPHIC REGIONS

The clinician must consider diseases more common in specific geographic regions when evaluating patients from these areas. A compilation of all FUO series in Spain over 25 years, including 914 patients, demonstrated that brucellosis accounted for 16% of infectious diagnoses and that 8% were due to parasitic causes, including hydatid disease, leishmaniasis and fasciola infection.[46] Studies conducted in hospitalized patients who had fever in India and in Nigeria reported a *Brucella abortus* seroprevalence of 0.8–5.2%, leading the authors to speculate that brucellosis might account for a percentage of cases of undiagnosed FUO in these regions as well.[47,48] Studies of prolonged fever in Egypt note that 16% of cases were diagnosed with a parasitic infection.[49] In addition to typhoid fever and tuberculosis, melioidosis has been reported as a common cause of FUO in South East Asia.[50,51] HIV infection is an important consideration throughout the developing and developed world. Thus, patients from these parts of the world who present with FUO should be evaluated for infections caused by these organisms.

The literature on FUO in developing countries is limited. Several series have been published that characterize the specific diagnoses associated with prolonged fevers in other countries. Not all of the FUOs published from the developing world meet the revised Petersdorf and Beeson criteria requiring absence of a diagnosis despite an initial workup. Patients often present late in disease, and there is limited access to diagnostic tests, including accurate blood culture methods, limited treatment facilities, and variable patient follow-up. Endemic disease and inadequate immunizatons may complicate the workup of FUO in developing countries. For example, typhoid fever is often diagnosed by serologic methods (the Widal test). However, in endemic areas, 12–29% of patients may have a positive titer at baseline. Convalescent titers are difficult to obtain because of poor follow-up, and misinterpretations of these tests frequently occur.

In three series on FUO from Bangladesh, India and China, and in a pooled analysis of all the series from Turkey, infections were the leading cause.[25] In all of these, tuberculosis is the leading infectious diagnosis. Other diagnoses included typhoid fever, malaria, visceral leishmaniasis, amebiasis, syphilis, leprosy and filariasis. The finding that infectious diagnoses comprise a greater proportion of cases of FUO in developing countries is supported in other series as well.

GENERAL APPROACH TO DIAGNOSIS

There is no universally accepted approach to patients with FUO. Many series have proposed a variety of algorithms, and though many of them are prospective studies, no approach has been validated in randomized trials. The approach to the patient with FUO needs to be individualized on a case-by-case basis. Typical Bayesian theory may not apply, as there may be no high probability diagnosis, and thus one can expect a high false-positive rate.

It is necessary to perform a thorough history and physical examination with a baseline set of laboratory tests and imaging, with further testing being directed by potential diagnostic clues (PDCs) generated from this initial evaluation. The aggressiveness of the workup and the necessity of certain tests should be dictated by the clinical status of the patients, with the pacing accelerated to the use of more invasive studies in those individuals who are more ill.

In the elderly, an aggressive workup is often more warranted, as a diagnosis is possible in 80–90% of cases[52] (Table 63.4), though these data have not been updated recently and the case series in question were small. Similar to individuals with HIV, a separate definition for FUO in the elderly has been advocated based on several principles. The hypothalamus in the elderly is less responsive to pyrogens, and so the febrile response may be more blunted or delayed.[53] Further, the baseline temperature can be lower than in younger individuals, particularly in those who are more frail.[54]

History and physical findings

Thorough and repeated medical history and physical examations are essential. Particular attention should be paid to travel, both recent and remote, exposure to animals and pets, occupation, sick contacts, family history of similar illnesses, as well as a thorough drug list looking for potential causes of drug fever. Many of these elements, besides providing valuable tools to guide laboratory testing, can also change and add to the differential diagnosis of potential illnesses. For example, melioidosis, a disease typically restricted to South East Asia, can be latent for up to 29 years. A family history of similar illness may point to one of the growing number of periodic fever syndromes.

The patient should be questioned regarding symptoms such as myalgias, arthralgias/arthritis, rash and oral ulcers, as such clinical features can assist in the diagnosis of many diseases, including PMR and SLE.

Table 63.4 Pooled data from three studies of FUO in the elderly (%)

Diagnosis	Knockaert	Barrier	Esposito
Infections	25	41	36
Neoplasms	12	13	23
Multisystem	31	30	25
Miscellaneous	10	2	7
No diagnosis	12	13	5

Adapted from Tal *et al*.[52]

A thorough physical examination follows. For instance, in series on miliary tuberculosis, choroid tubercles were present in as many as 50% of patients on autopsy.[55] Particular attention should be paid to the presence of any rashes and their appearance, which is an important diagnostic clue to many infectious and noninfectious entities, including adult Still's disease and SLE.

The thorough history and physical examination is used to generate PDCs and pursue abnormalities with directed testing, or, in the words of the bank robber Willie Sutton, to 'go where the money is'. If the diagnosis is not made immediately, a thorough history and physical examination should be repeated, as many abnormalities which were not present initially or missed altogether may declare themselves during subsequent examinations. History and observation of the evolution of a clinical identity led to the diagnosis in 44% of patients in one series.[23] This number should not be regarded as 'low yield' effort, which is an arbitrary designation, as a 40% yield in instances where it is difficult to establish a final diagnosis should not be ignored. Another study found that PDCs, however, are frequently misleading.[29]

Table 63.5 Suggested minimum workup

- Complete blood count, including differential and platelets
- Routine serum chemistries, including assessment of hepatic and renal function
- Erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP)
- Antinuclear antibody (ANA) and rheumatoid factor (RhF)*
- Urinalysis with urine culture
- Three blood cultures separated in time by several hours
- Chest X-ray
- Abdominal ultrasound or CT imaging
- Tuberculin skin test*
- HIV ELISA with Western blotting for confirmation

*These tests can be ordered at the discretion of the consultant.

Verification of fever

Verification that a fever exists seems intuitive, but its importance cannot be overlooked. Up to 35% of patients referred for evaluation of prolonged fevers were found not to have fevers at all or to have factitious fevers. Temperature recordings should be made with a healthcare worker in attendance. The lack of physiologic responses associated with fever (chills, diapheresis, tachycardia, tachypnea) should alert the clinician to the possibility of factitious fever. Patients undergoing evaluation for FUO should measure and record their temperatures daily in a log book.

While the fever pattern was once considered useful in the diagnosis of FUO, such characterizations are highly nonspecific and cannot be used to make a diagnosis.[56] The only exception to this statement is relapsing temperature elevations. This is characteristic of malaria, trypanosomiasis, relapsing fever, brucellosis, cyclic neutropenia or fever patterns in lymphoreticular malignancies. Characterization of fevers as either continuous or intermittent may allow the clinician to at least provide the patient with some prognostic counseling, as a diagnosis is much more likely to be reached in the former than in the latter.[23,29]

Laboratory and imaging evaluation

While there is no agreed upon 'standard' set of laboratory studies required for the evaluation of FUO, a number of determinations have been frequently recommended:

- complete blood count, including differential and platelets
- routine blood chemistries, including assessment of hepatic and renal function
- erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP)
- protein electrophoresis
- antinuclear antibody (ANA) and rheumatoid factor (RhF)
- urinalysis
- at least three blood cultures separated in time by several hours
- chest X-ray
- abdominal ultrasound or CT imaging
- tuberculin skin test.

Additionally, all patients should be screened for HIV infection. In the following section select tests will be discussed in further detail. Table 63.5 summarizes the 'minimum' diagnostic workup as suggested by the authors of this chapter.

Erythrocyte sedimentation rate (ESR)

Several factors will elevate the ESR besides inflammation or infection,[57] including renal failure, hyperlipidemia, female gender, advanced age and even technical factors, such as tilting the test tube by an angle of 3° from the vertical, which may accelerate the ESR by as much as 30 mm/h. Conversely, factors that will lower the ESR include morphologically abnormal red cells and extremely elevated leukocyte counts. However, there are only a few infectious diseases that are known to drive the ESR to greater than 100 mm/h, and these include osteomyelitis and endocarditis.[58,59] Although a nonspecific test, many clinicians will use a low ESR to rule out a significant inflammatory disorder. Some individuals with giant cell arteritis can have a normal ESR.[60]

C-reactive protein

C-reactive protein (CRP) is an acute phase protein which plays a role in the recognition of foreign pathogens and phospholipid constituent of damaged cells. Advantages that CRP offers over ESR include less variation with changes in size, shape and number of red cells, more rapid changes with improvement in clinical condition, and less variation between gender.

The utility of ESR versus CRP in the diagnosis of FUO has never been systemically evaluated. This is likely to be more useful if there is a high pretest probability of a rheumatologic or hematologic disorder. It is probably also prudent to check these laboratory studies if the patient is older than 50 years, where the possibility of diseases such as PMR and temporal arteritis is higher.

Blood cultures

The earliest series on FUO emphasized endocarditis as one of the primary infectious etiologies of FUO, though it has become a less prominent entity in more recent studies, being absent from some altogether. Blood culture remains the primary method of detecting endocarditis. In the past, fastidious organisms such as the nutritional variant streptococci (e.g. now being renamed to the genus *Abiotrophia* and *Granulicatella*) and HACEK (*Haemophilus*, *Actinobacillus*, *Eikenella*, *Cardiobacterium*, *Kingella* spp.) organisms constituted a number of cases of culture-negative endocarditis. However, with modern technology, they are more easily grown and are isolated, on average, between 5 and 7 days.[61] This, along with the increased use of the Duke criteria and transesophageal echocardiography (TEE), and a decline in acute rheumatic fever and subsequent valvular heart disease, accounts for the decline in the overall cases of endocarditis which meet the definition of FUO.

Except for those individuals treated inappropriately with antibiotics, the most common causes of culture-negative endocarditis are *Bartonella*, *Brucella* and *Coxiella* spp., anaerobes and fungi. The primary method of diagnosis of Q fever is serologic, as isolating the organism in cell-free medium is difficult. However, it is still possible to attempt to grow *Bartonella* and *Brucella* species; hence communication with the laboratory in order to optimize collection and growth is still useful.

Filamentous fungi are also difficult to grow, though this entity is seen almost exclusively in intravenous drug abusers.

The overall yield of blood culture in the FUO patient population is low, often less than 5%, even in older series. Thus, unless there is a strong clinical suspicion of endocarditis, more than the requisite three blood cultures is probably not required.

CT imaging

Historically patients with FUO in whom no diagnosis was established were eventually referred for exploratory laparotomy. This practice has been almost entirely replaced in modern settings by CT scanning. CT scanning is effective for diagnosing many of the conditions which were etiologic in older series of FUO, including occult abscesses, hematomas and intra-abdominal lymphadenopathy. The diagnostic yield of abdominal imaging in a recent series on FUO was 20%,[29] comparable to older series that looked at the utility of this modality.

Some reviews use abdominal ultrasound in place of abdominal CT. In one such review, it was clinically helpful in 10% of cases where it was used.[62] Another study tabulated the number of abdominal imaging studies at more than three CT scans and/or ultrasound examinations per patient.[13]

Tuberculin skin test

It may be useful to place a tuberculin skin test (TST) in all patients with FUO. This should be done with the understanding that the presence or absence of a reactive skin test does not necessarily rule in or rule out the diagnosis of tuberculosis, which in the past was a leading infectious cause of FUO. In one series from Turkey, where there is a high incidence of tuberculosis, the TST was positive in 37% of patients overall, with 54% of these individuals having active tuberculosis and 24% without.[22] The TST is known to have a poor sensitivity in patients with miliary tuberculosis.

QuantiFERON-TB Gold test

This is an interferon gamma-based *in-vitro* T-cell assay, whose principle rests on the T cells of individuals previously sensitized to TB antigens producing interferon gamma on rechallenge. Thus far, it has only been studied for use in the diagnosis of latent TB, where it may have the advantage of being negative in those individuals with prior history of BCG vaccination[63,64] and less susceptible to false-positive results due to infection with nontuberculous mycobacteria. It also has the advantage of requiring only a single visit. What role it will play in algorithms for evaluating patients with FUO who are suspected of having TB is yet to be determined.

Additional investigations

Occasionally other forms of testing are warranted, including:

- serologies
- nuclear imaging
- biopsy.

Serologies

Antibody testing only indicates whether or not a patient has been exposed to a microbial agent, but does not indicate whether that agent is necessarily the cause of the FUO. In a study involving 73 patients, serologies were sent in 53, totaling 509 microbiologic serologic tests. With the exception of testing for *Yersinia enterocolitica*, serologic testing was never helpful in establishing the diagnosis.[29] This emphasizes that serologies should not be sent unless there is a high pretest probability that the patient has a given condition; even then, it is usually confirmatory of the diagnosis being made on some other basis. 'Blind' serologic testing may actually be misleading, resulting in further unnecessary diagnostic procedures. For example, serologic testing for zoonotic infections such as tularemia, Lyme disease or Rocky Mountain spotted fever should be performed only if the patient has been exposed to a relevant animal or tick. Brucellosis, bartonellosis, leptospirosis and some cases of hepatitis B or C, histoplasmosis or coccidioidomycosis can occasionally be detected by serologic diagnosis in FUO.

By contrast, antigen testing is usually more suggestive of active disease, as is the case with cryptococcal antigenemia. However, CMV antigenemia requires clinical correlation, as patients with HIV can be antigenemic without having CMV disease.

Radionuclide scans

The use of nuclear imaging in the diagnosis of patients with FUO has yielded variable results, in part because of low numbers, few randomized trials and differences in the timing of testing during evaluation.

Gallium scans are based on the principle that gallium is bound to transferrin, with increased blood flow and permeability at a given site of inflammation resulting in a subsequent increase in uptake of the radioisotope. Imaging is then obtained 18–72 hours after injection of the substance. In one study published before CT scanning came into widespread use, gallium 67 (^{67}Ga) scanning contributed to the final diagnosis in 14% of cases; however, false-positives led to unnecessary testing in 21% of patients.[12] In a more recent study of 145 patients with FUO, ^{67}Ga contributed to the diagnosis in 24–29% of cases.[65]

Leukocytes can be labeled with either indium 111 (^{111}In) or technetium 99m (^{99m}Tc). The process requires whole blood from the patient. The white cells are then isolated and incubated with radiolabel, washed and re-injected into the patient. Results over the years have been mixed, with sensitivity ranging anywhere from 45% to 82%, and specificity ranging from 69% to 86%.

Both CT and ultrasound can be done almost immediately, whereas radionuclide studies require anywhere between 24 and 48 hours between tracer injection and imaging.[66] Hence the delay implies a certain stability in the patient. Even in the event of an abnormal radionuclide scan, anatomic imaging such as CT is frequently required before intervention such as biopsy can be performed,[66] as was the case in many of the studies that examined the utility of nuclear imaging.[67]

The most promising nuclear study for the diagnosis of FUO is positron emission tomography using 2-(^{18}F)-fluoro-2-deoxy-D-glucose (FDG-PET). The application of FDG-PET is based on the observation of increased glucose metabolism in both malignant and inflammatory cells.[66] As part of a structured protocol, FDG-PET was deemed clinically helpful in 33% of cases.[62] Results in other studies have varied widely, with FDG-PET contributing to the diagnosis in 16–55% of cases.[62,67] Two prospective studies found FDG-PET to be superior to ^{67}Ga scanning, while another found it inferior to ^{111}In granulocyte scintigraphy, due to frequent false-positives.[68]

Some technical aspects may guide the selection of one form of nuclear scanning over another. Theoretically, ^{67}Ga scanning would be better able to detect both tumor and infection, whereas intuitively one would expect that leukocyte scanning would be more specific for infection. Malignancies have also been detected with white blood cell scans.[66] While the data are sparse as to whether one form of scintigraphy outperforms another, FDG-PET does offer the advantage that imaging can be completed within 2 hours of administration of the radioisotope.

Biopsy

Most PDCs are used to eventually point to biopsy of specific organs. The yield of such biopsies has varied depending on the series. Biopsy material can provide both culture and/or histologic evidence as a cause of FUO. Of note, in two clinical series of FUO published by the same investigator, tissue biopsy was required to make the diagnosis in the second series as often as in the first reported 20 years earlier.[12]

In a prospective study from the Netherlands, bone marrow biopsy established the diagnosis in 2 of 19 patients.[29] Other reports indicate a yield of anywhere from 0% to 18%.[69,70] In a pooled analysis of 857 published cases of FUO in Turkey, bone marrow biopsies were positive in roughly 30% of cases. In many instances in which biopsy was low yield, the biopsies were blinded and not guided necessarily by suspicion for a given condition. In areas with a low prevalence of tuberculosis, leishmaniasis, salmonella, brucellosis, etc., the yield of bone marrow biopsy for infectious etiologies of FUO would be expected to be low.

Liver biopsies in older series were stated to be useful without a number being given; more recent studies demonstrate a 14–17% yield.[71,72] Some reviews recommend liver biopsy in FUO investigations,[73] while others are against its routine use.[29,73] Physical or laboratory abnormalities have not been helpful in predicting which patients will have an abnormal liver biopsy.[71] The use of liver biopsy should again be dictated by the clinician and the entity to be diagnosed.

No study has evaluated the overall yield of temporal artery biopsy in those suspected of having temporal arteritis. Given that it is causative of up to 15% of cases of FUO in older patient populations, temporal artery biopsy should be considered in those older than 55 years with an elevated ESR.

Outside of the liver and bone marrow, biopsies of other sites should be guided by PDCs, i.e. if the patient has a rash or lymphadenopathy, then the site should be biopsied and sent for histopathology as well as appropriate cultures. The yield of such biopsies, even when there is palpable lymphadenopathy, has varied widely.

Other diagnostic studies in FUO

There are few data on the use of echocardiography or MRI in patients with FUO. In one study, echocardiography seemed to facilitate the diagnosis of endocarditis.[23] However, false-negatives have also been noted in other studies.[29]

No study has systemically looked at the overall yield of bilateral lower extremity ultrasound looking for deep venous thrombosis (DVT), even in the absence of symptoms. Given that DVT was etiologic of FUO in up to 6% of cases in some series, and the fact that the procedure is inexpensive and low risk, it could be given some consideration.[12,29] Pelvic CT is useful for the diagnosis of septic thrombophlebitis of pelvic veins when clinically indicated.

Drug-related fevers

When a drug-related fever is suspected, necessary drugs can be changed to alternatives of a different class. Upon initial presentation, a reasonable initial intervention is discontinuing as many drugs as possible. After stopping the agent that is responsible for the fever, the fever will usually resolve within 3 days, although it may take as long as 2 weeks. Persistence of fever beyond this time should direct the clinician to investigate an alternate source. If the fever remits, the clinician can definitively confirm the diagnosis by re-instituting the agent, which characteristically elicits fever again within a few hours. This procedure is safe unless drug-induced organ damage, such as interstitial nephritis or hepatitis, has occurred.

THERAPEUTIC INTERVENTIONS

The principle of a therapeutic trial is that the response to an intervention is both diagnostic and therapeutic. This requires that the intervention be as specific as possible. For instance, while isoniazid is fairly specific for mycobacteria, rifampin (rifampicin) has activity against a wide range of bacteria. The empiric use of agents such as these may be misleading for several reasons:

- medical intervention makes it difficult to determine whether a new finding has resulted from the treatment or from the underlying disease;
- fall of temperature may be fortuitous or it may result from the antipyretic effects of corticosteroids or NSAIDs; and
- improper use of antibiotics may lead to a false sense of therapeutic and diagnostic security and interfere with finding a diagnosis.

Thus, therapeutic trials should be discouraged in the early course of investigation in fevers of unknown origin.

The 'naproxen' test was used to differentiate fevers secondary to malignancies from those of infectious etiology.[74] This test has not been validated, and in a recent study of 77 patients with FUO,[75] though it caused a partial/complete response in 73% of patients with malignancies, this was also the case in 80% of patients with infections (including tuberculosis) and 55% of inflammatory states (including adult Still's disease). Overall, the sensitivity of the naproxen test was 55% for neoplastic fever with a specificity of 62%.

If patients are clinically suspected of having a pulmonary embolism or DVT as the cause of their fevers, defervescence should occur rapidly after the initiation of anticoagulation.

Prolonged empiric therapy may have multiple deleterious consequences beyond the unnecessary expense, inconvenience or iatrogenic complications for the patient. In addition, complications resulting from unnecessary interventions may confuse further diagnostic strategies based on abnormalities on physical examination or laboratory findings. For these reasons, therapeutic interventions should be discouraged in stable patients. If, however, a patient has a rapidly progressive, potentially fatal illness, empiric therapy becomes unavoidable.

PROGNOSIS

The prognosis of FUO is dependent on the patient population and the underlying diagnosis. In a disease process where a diagnosis may not be reached in up to 50% of cases, both clinicians and patients can easily get frustrated. Thus it is important to counsel patients about the relative positive outcome for those individuals in whom no diagnosis is reached after a reasonably thorough workup. In one study of 199 patients with FUO, 61 were eventually discharged with no diagnosis, of whom six died.[45] However, the deaths were thought to be related to the fever-causing process in only two of these cases. In a study of 34 patients discharged from hospital without a diagnosis and followed for up to 7 years, there was only one case of malignancy and two others had continuous illness. The diagnosis was found with certainty in only two cases, both within the first year.[76]

This is true in recent studies as well, though with shorter follow-up. In one prospective study of 73 patients with FUO, a diagnosis could not be reached in 37, of whom only one died at long-term follow-up.[29] The cause of death and whether or not it was related to the fever was not elucidated. In another study of 290 immunocompetent patients with FUO, follow-up information was available for 80 without a diagnosis.[23] Death occurred in three, but was presumably not linked to the initial febrile illness.

Prognosis is worse in those whose febrile illness is found secondary to a malignancy. In one of the above series, hematologic malignancies represented only 11.5% of the final diagnoses, but were responsbile for 58.3% of the fatalities.[23] Diagnostic delay has also been associated with adverse outcomes in such infections as miliary tuberculosis, disseminated fungi and intra-abdominal processes.

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References for this chapter can be found online at http://www.expertconsult.com

Chapter 64

Steven M Opal

The potential role of infectious agents in diseases of unknown etiology

INTRODUCTION

Ultimately all human diseases are the result of a deleterious interaction between our genomes and the environment in which we exist. A critical component of that environment are the other living things that share the biosphere with us. We are exposed to a multitude of microbial pathogens over a lifetime and carry with us a remarkably complex and highly variable complement of endogenous micro-organisms (the microbiota) with the potential to do harm if our defenses falter. The microbiota of the average adult human is estimated to contain 10^{14} organisms and consists of at least 1000 different species, of which nearly half have yet to be successfully isolated and cultivated.[1,2]

When Pasteur, Koch and many other microbiologists of the latter half of 19th century first confirmed the 'germ theory' of disease, they revolutionized medicine and the way we think about disease. The fatalistic perception of human illness as a passive, inevitable consequence of life was replaced by the notion that at least some diseases were active, and possibly even preventable and reversible processes caused by invading micro-organisms. The search for the microbial etiology of all human maladies was on and continues to the present time.[3] The most recent, dramatic example of this search is the discovery of the infectious cause of peptic ulcer disease. Generations of physicians and surgeons considered it axiomatic that ulcers were the result of excess gastric acidity ('no acid, no ulcer'). When Marshall and Warren[4] finally convinced a skeptical scientific and medical community in the 1980s that most cases of chronic gastritis and peptic ulcer were actually caused by spiral bacterial organisms (*Helicobacter pylori*) inhabiting the gastric mucosa, they revolutionized the management of peptic ulcer disease.[5] Are there other common, or not so common, chronic idiopathic disease states waiting to be revealed as infectious diseases? Many elements of enigmatic clinical illnesses such as chronic fatigue syndrome (Chapter 70) suggest a microbial etiology, yet the nature of these illusive, potential pathogens and proof of their causation in disease pathogenesis remain ill-defined.

The genomic era in both human and microbial genetics has rekindled an interest in the discovery of micro-organisms as a cause of human disease. Many chronic inflammatory, neoplastic, and neurologic diseases carry tantalizing hallmarks suggestive of an underlying infectious cause.[6] This renewed discovery process may force us to re-examine the dynamic relationship that exists between human physiology and the myriad of exogenous and endogenous organisms surrounding all of us.

THE DEFINITION OF DISEASE CAUSATION AND ETIOLOGIC AGENTS IN THE PRE-GENOMIC ERA

In 1890, Koch first formally presented his famous postulates to define the requisite evidence needed to confirm the causative role of a micro-organism in human disease.[3] This was the culmination of decades of seminal work by Koch and his mentor Friedrich Henle, along with many other scientists of the time, including Louis Pasteur and Edwin Klebs. Koch correctly surmised that such unambiguous guidelines were necessary to bring clarity and consistency into the newly emerging field of microbial pathogenesis. He initially proposed three essential elements to define a pathogen (or 'parasite' using his original terminology):

- Postulate 1: That the parasite is found in every case in which the disease occurs and accounts for the clinical and pathologic features of the disease;
- Postulate 2: That the parasite is not found in other diseases as a non-pathogen or fortuitous parasite; and
- Postulate 3: After being isolated from the body and repeatedly passed in pure culture, the parasite can induce the disease anew (in animal models or even in intrepid human volunteers).

A fourth postulate was subsequently added, apparently by others, necessitating the ability to re-isolate the same parasite from the diseased animal model. Koch's postulates have served us well and certainly helped solidify the germ theory of disease. The components are of particular value for obligate pathogens that are not highly species-specific, thereby allowing for animal models of human disease. Many microbiologists consider these postulates to be sacrosanct, even up to the present time. Yet it is worth noting that even Koch himself recognized from the beginning that these guidelines were not relevant for all pathogens. Koch famously observed that leprosy was likely caused by the mycobacterial-like bacillus (*Mycobacterium leprae*) that was readily identifiable on tissue stains of involved skin, yet he (or anyone else for the next century) was never able to grow the organism on artificial media. This violated his own third postulate; nonetheless, he was comfortable assigning a pathogenic role to these readily visible leprosy bacilli.

A myriad of well-known human pathogens, including many viruses, rickettsia, spirochetes and other pathogens, do not fulfill all of Koch's postulates and yet their etiologic identities are well established. The fact that many opportunistic potential pathogens colonize people for prolonged periods without causing disease (e.g. *Candida albicans*, *Staphylococcus aureus*, etc.) was not appreciated in the early days of clinical microbiology. This violates Koch's second postulate. Moreover, many human diseases caused by well-known microbial pathogens lack comparable animal models (e.g. rheumatic fever, progressive multifocal leukoencephalopathy) and therefore do not meet all of Koch's postulates.

ALTERNATIVE VIEWS OF MICROBIAL CAUSATION OF HUMAN DISEASE IN THE GENOMIC ERA

Many common infectious diseases result from a synergistic combination of variable numbers of different species of micro-organisms. Some examples include bacterial vaginosis, periodontitis or hepatitis D, a deficient virus requiring co-infection with a helper virus (hepatitis B

virus) for replication. These infectious diseases violate the principle of serial passage in pure culture and then reproducing the disease in animal models. Periodontitis is a good example of the complexity that underlies the pathogenesis of some common infectious diseases.

Periodontitis is among the most prevalent infectious diseases afflicting 30–70% of adults worldwide.[7] This oral infection has been extensively studied by both culture-dependent and culture-independent methods.[8] A consistent pattern of microbial pathogens has now emerged as the cause of periodontitis. Despite the fact that Koch's postulates could not be applied to define the etiology of this common pathologic process, the genomic evidence strongly supports this 'communal' model of periodontal disease. Biofilms consisting of *Streptococcus mutans* and other oral streptococci residing in a mono- or disaccharide-rich environment in the oral cavity express exotoxins that gradually induce dental caries over time. A synergistic combination of bacterial pathogens living within the biofilm lining the gingival crevice then gives rise to periodontal disease. A combination of *Treponema denticola, Porphyromonas gingivalis, Tannerella forsythia,* and *Actinobacillus actinomycetemcomitans* gradually leads to gingivitis, bone resorption and the full expression of periodontitis. Later disease progression often involves additional noncultivable bacterial strains and a methanogenic Archaea organism (the first example of a member of the Archaea kingdom associated with a human disease).[1,2]

Important human illnesses are the result of long term-sequelae of remote infections (the 'hit and run' hypothesis) that occur months or even years after the infection has cleared. Examples include Guillain–Barré syndrome following enteric infection with specific serotypes of *Campylobacter* spp., Sydenham's chorea as a late manifestation of the non-suppurative complications of *S. pyogenes* pharyngitis, or post-infectious reactive arthritis (formerly known as Reiter's syndrome) following enteric infections. The pathogen is usually no longer cultivatable from these patients when the disease is manifest and yet their immunologic footprint clearly identifies the etiologic organism in each case.[1,6] A large number of immune-mediated, inflammatory and neoplastic diseases may have, as their proximate cause, a specific pathogen or group of micro-organisms as an inducer or essential propagator of human illness.

Exposure of the susceptible host with the appropriate genotype, and the fortuitous concurrence of the necessary set of environmental and epidemiologic circumstances conspire together to initiate and maintain an aberrant host response leading to overt clinical disease. Sarcoidosis might be an example of a disease induced by environmental and generally nonpathogenic bacterial organisms in some genetically susceptible individuals.[9–11] A specific genetic locus has been recently identified that appears to be linked with sarcoidosis.[12] Excessive and aberrant granulomatous reactions to common micro-organisms such as *Propionibacterium acnes*[13–15] or tuberculous or environmental, non-tuberculous, mycobacterial antigens[16,17] might induce sarcoidosis in these susceptible patients. Similarly, genetic susceptibility to inflammatory bowel disease is well described in association with NOD2 polymorphisms and other mutations in pattern recognition receptors, resulting in defective clearance of enteric bacteria and microbial antigens and mediators along the mucosal epithelium of the small and large bowel.[18–20] This genetic predisposition to inflammatory bowel disease, in concert with alterations in the microenvironment and enteric microbial flora, is currently one of the favored hypotheses to explain the pathogenesis of Crohn's disease.[21–23]

Endemic Burkitt's lymphoma in children in sub-Saharan Africa provides another unfortunate example of a confluence of permissive environmental conditions and collaboration between two widely disparate pathogens to cause neoplasia. Burkitt's lymphoma is common in African children living in holoendemic regions where mosquito-borne falciparum malaria exists. Neoplastic transformation to lymphoma is generally attributed to an aberrant host response to Epstein–Barr virus (EBV) infection. Recent evidence indicates that *Plasmodium falciparum*, via its major erythrocyte protein PfEMP-1, induces general immunosuppression and profound B cell proliferation which activates productive infection with EBV. High level replication of EBV promotes the translocation and activation of the potent oncogene c-*myc*, resulting in malignant transformation to Burkitt's lymphoma.[24,25] The potential role of other viral, bacterial and parasitic infectious diseases in the pathogenesis of other neoplastic diseases is surveyed in Table 64.1.[26]

Table 64.1 Microbial disease associations and possible causations – neoplastic diseases

Possible pathogen	Neoplasia	Level of evidence of causation*
Helicobacter pylori	Gastric adenocarcinoma Gastric lymphoma	+++ ++
Epstein–Barr virus	Burkitt's lymphoma (with *Plasmodium falciparum*) Hodgkin's disease Nasopharyngeal carcinoma Primary CNS lymphoma Immunoblastic lymphoma	+++ ++ ++ +++ ++
Hepatitis B	Hepatoma	+++
Hepatitis C	Hepatoma	+++
Schistosoma haematobium	Bladder carcinoma	++
Human papilloma virus 16/18	Cervical carcinoma Colorectal cancer	+++ +
Helicobacter bilis	Biliary tract cancer	+
Human T-cell leukemia/ lymphoma virus-1	Acute T-cell leukemia/ lymphoma Invasive cervical carcinoma Small cell carcinoma of the lung	+++ + +
Human endogenous retrovirus-K10	Testicular cancer	+
Kaposi's sarcoma-associated herpes virus (human herpesvirus-8)	Kaposi's sarcoma Primary effusion lymphoma	+++ ++

*Level of evidence: epidemiologic link only +; some evidence of causation ++; clear evidence of causation +++.

There are a large number of clinical settings in which a specific micro-organism or group of micro-organisms might be necessary and sufficient to cause a variety of human illnesses, yet go unnoticed when relying upon standard diagnostic techniques.[1–5] A number of epidemiologic features and histopathologic findings suggest a microbial cause for a variety of idiopathic diseases. Such diseases as sarcoidosis,[9–11] Kawasaki disease,[27] multiple sclerosis, Kikuchi–Fujimoto disease[28–30] and numerous other diseases suggest an underlying infectious cause.

Caution needs to be exercised before it can be unequivocally concluded that an implicated organism is in fact the true etiologic agent.[3,6] Chronic disease syndromes and neoplastic diseases are frequently associated with a myriad of infectious diseases, yet the link between association as a mere bystander and actual microbial causation is often difficult to define clearly. Certain organisms could be highly associated to a specific disease entity, yet simply occupy a unique ecologic niche created by the disease itself. *Streptococcus bovis* (recently renamed *S. gallolyticus*) is highly associated with neoplastic lesions in the colonic mucosa, yet there is little evidence to suggest that this organism causes colon tumors. The organism takes the opportunity

Table 64.2 Mechanisms by which causative microbial pathogens might escape detection

Mechanism	Examples	Comments
'Hit and run hypothesis'	*Streptococcus pyogenes* – chorea minor in rheumatic fever Measles – subacute sclerosing panencephalitis	Disease manifestations induced by infection but separated from body site and in time by months to years
Difficult to culture or noncultivable	*Tropheryma whipplei* –Whipple's disease *Bartonella henselae* – cat-scratch disease	Genomic studies indicate enormous numbers of microbial genomes that have yet to be successfully cultured
Commensal organisms that rarely cause disease in susceptible hosts	*Sacrocystis* spp. – enteritis or myositis Non-tuberculous mycobacteria – respiratory infections	Difficult or impossible to prove Koch's postulates
Disease occurs with microbial communities	Periodontitis Bacterial vaginosis	Synergistic combinations of microorganisms cause disease
Micro-organism induces immunologic or neoplastic alterations	Kaposi's sarcoma-associated herpes virus (human herpesvirus-8) *Helicobacter pylori* Gastric carcinoma	Microbial pathogen may serve as promoter or essential inducer
Human endogenous retrovirus (HERV)	HERV-E and systemic lupus erythematosus	Widely prevalent in human genome – possible inducer or propagator for chronic diseases
Different micro-organisms trigger illness only in genetically susceptible patients	Possibly sarcoidosis Kawasaki disease Multiple sclerosis, etc.	A variety of possible microbial pathogens may induce disease at critical time intervals in susceptible individuals

to colonize the unique microenvironment adjacent to the neoplastic tissue. A survey of potential mechanisms by which the microbial cause of disease could go unrecognized using conventional diagnostic methods is provided in Table 64.2.

THE HYGIENE HYPOTHESIS AND THE PATHOGENESIS OF INFLAMMATORY DISEASES

It should also be acknowledged that, paradoxically, heavy exposure to some elements of the microbiota, particularly at a young age, may actually protect against a number of inflammatory and allergic diseases later in life. The incidence of asthma, Crohn's disease, diabetes and multiple sclerosis has progressively increased over the last half century in developed nations as vaccine-preventable and food and waterborne enteric infections have dramatically decreased.[31] These inflammatory diseases are much less common in developing countries where the incidence of childhood infection remains high. The 'hygiene hypothesis' suggests that improved hygiene fails to prime innate immune responses to common microbial antigens in early life. This impairs mucosal immune maturation, promotes a Th2-type cytokine response and facilitates inappropriate cellular tolerance to many common microbial antigens. The end result is excessive and disordered inflammation to many of these environmental antigens later in childhood and adulthood, with resultant increased risk of inflammatory diseases. The hygiene hypothesis seems plausible yet still lacks extensive experimental evidence in support of this appealing notion.[31–34]

CULTURE-INDEPENDENT TECHNIQUES TO DETECT NOVEL PATHOGENS

In the genomic age in which we now live, Koch's postulates need to be modified or even discarded as an obsolete system to define the essential elements of what identifies a microbial pathogen. The evolving definition of what constitutes a human pathogen has moved from a formal requirement to fulfill all of Koch's postulates to a genomic expression of Descartes' assertion, 'I clone, therefore I am.' This is best evidenced by the remarkable discovery of hepatitis C as the most common cause of non-A, non-B post-transfusion hepatitis by Choo and colleagues in 1989.[35] After decades of concerted, yet futile, efforts to isolate the implicated virus using standard virologic techniques, Choo *et al.* decided to attempt to clone the genome of the unknown agent directly from the serum of a chimpanzee experimentally infected and known by serial serum dilution and animal challenge to contain high titers of infectious non-A, non-B hepatitis.

After treating the serum with reverse transcriptase, they generated a large number of sequences of amplified nucleic acids to create a comprehensive cDNA library. It was not known at the time if the presumed hepatitis-causing agent was an RNA or DNA virus or perhaps a noncultivable bacterium. The resulting battery of lambda phage cDNA clones were then tested against the serum of a patient with chronic severe non-A, non-B hepatitis. It was hoped that this human serum would provide a source of antibodies that would recognize at least some of the expressed viral peptides derived from the cloned sequences. After screening nearly one million clones, the reactive peptide sequence was identified. They then showed that this was an RNAse- but not DNAse-sensitive genome and, by overlapping hybridization experiments, they confirmed that this was a positive-stranded RNA virus of approximately 10 000 base pairs. This newly discovered virus shared unexpected homologies with a number of arthropod-borne viral pathogens. The viral agent was confirmed to be a previously unknown member of the flavivirus family. The mystery of post-transfusion, non-A, non-B hepatitis was finally solved by cloning, unencumbered by the need to fulfill Koch's postulates.[3,35]

A similar cloning strategy has now been adapted from work done with genome searches for comparative microbial ecology, environmental biology and molecular phylogeny studies.[2,3] Microbial DNA sequences are isolated and amplified by polymerase chain reaction (PCR) using broad range bacterial primers for essential target sequences such as ribosomal RNA genes. These amplified, novel bacterial sequences are then cloned in a DNA library where comparative sequence analysis is performed with highly conserved and well-characterized sequences from known classes of micro-organisms. The genes for 16S RNA, other essential ribosomal RNA sequences, or highly conserved enzymes are scanned for sequence homologies throughout the Bacteria and Archaea domains.

Sequence homologies and genomic groupings can now be exploited to identify shared genetic space and evolutionary distance between essentially any unknown pathogen and all identifiable, genome-characterized micro-organisms. These culture-independent methodologies are now employed to search for novel, difficult to culture or noncultivable microbial pathogens that might cause human disease. Such unbiased genome searches have been instrumental to the work of Relman, Fredricks and others[3] discovering the etiology of longstanding diseases of known microbial cause but with noncultivable organisms such as the Whipple's disease bacillus (*Tropheryma whipplei*)[36,37] or newly recognized, emerging pathogens such as the causative agents of bacillary angiomatosis (*Bartonella henselae*) and human monocytic ehrlichiosis (*Ehrlichia chaffeensis*).[3] The resulting genomic evidence about the predicted metabolic requirements of *T. whipplei* was then exploited to develop a cell-free medium to successfully culture the Whipple's disease bacillus for the first time.[38] The basic strategy employed in these genome searches is depicted in Figure 64.1. Current sequencing machinery now permits the simultaneous sequencing of over 200 000 short unique nucleotides from small samples of DNA without the need for preparatory cloning.[3] This facilitates rapid genome screens with known sequences of viruses, rickettsia and numerous other difficult to culture microbial genomes for potential pathogens.

Another genomic technique that can be used to detect and define novel microbial pathogens is DNA subtraction techniques.

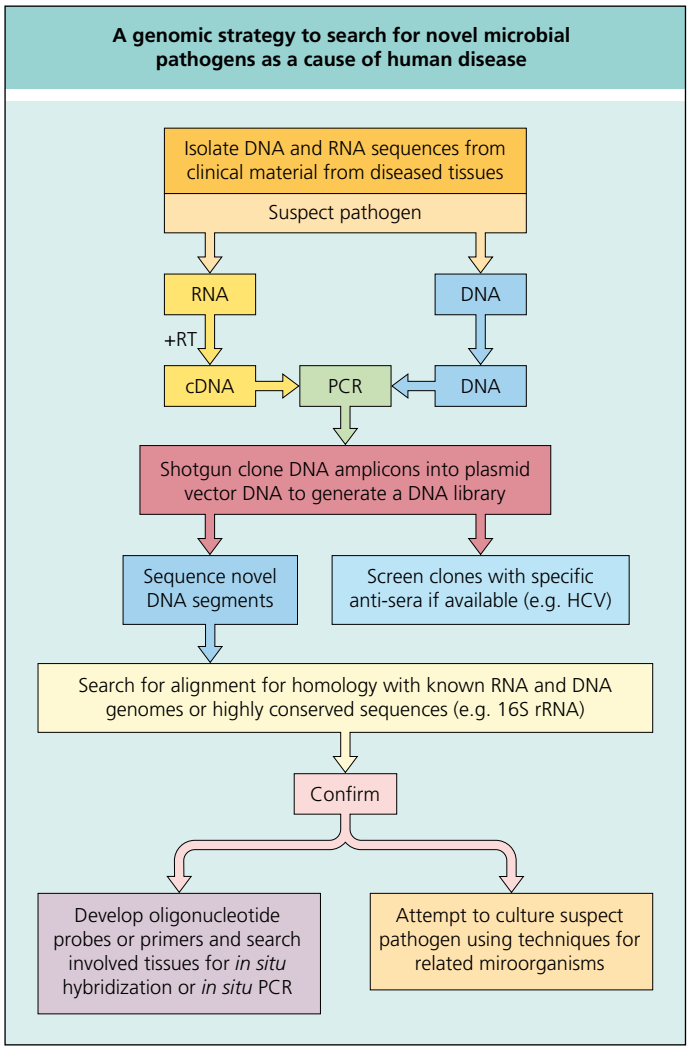


Fig. 64.1 A genomic strategy to search for novel microbial pathogens as a cause of human disease. cDNA, complementary DNA; HCV, hepatitis C virus; PCR, polymerase chain reaction; RT, reverse transcriptase.

This methodology was successfully employed to link a previously unknown herpes-like virus (HHV-8), now known as Kaposi's sarcoma-associated herpes virus (KSHV) as the cause of HIV-related Kaposi's sarcoma.[2,3] The DNA sequences derived from diseased tissues and a similar set of DNA sequences from normal tissues are subjected to subtractive hybridization and PCR amplification to enrich the DNA complement of DNA sequences found in the diseased sample only. Unique sequences are then analyzed to determine if any sequence homologies exist with known pathogens. As the known genomic universe of micro-organisms continues to expand, it is likely that this and related culture-independent methods will reveal new pathogens associated with human illness.

KOCH'S POSTULATES REDEFINED

A new set of more practical guidelines to assign causation of potential pathogens to diseases of unknown cause is needed in the genomic era of the 21st century. Numerous diagnostic schemes have been proposed to complement or replace Koch's postulates for defining pathogens. A modification of the Hill[39] and Evans[40] criteria to supplement Koch's postulates is a reasonable framework upon which to build a more logical and practical diagnostic system to define disease causation. This set of criteria can readily be modified to include genomic evidence for noncultivable pathogens (Table 64.3). Each criterion need not be met to determine disease causation of each suspect human pathogen. The more criteria that are satisfied, the more convincing the link becomes between the suspected pathogen as the etiologic agent responsible for the disease process. A more integrated system that takes into account variations in the host response along with the microbial agent responsible for disease causation may be needed in the future.[6]

THE ROLE OF HUMAN ENDOGENOUS RETROVIRUSES IN HEALTH AND DISEASE

The human genome is replete with endogenous retroviruses (HERVs, also known as retrotransposons) that have entered the human germline at various times in the evolutionary past and now occupy 8.3% of the genome.[41] They maintain the basic structure of retroviruses with long terminal repeats (LTR) flanking the open reading frames for polymerase (*pol*), including reverse transcriptase, envelope (*env*) and core matrix (*gag*) genes along with various regulatory genes. They have accumulated loss of function point mutations in structural and regulatory genes, rendering them incapable of exogenous viral production. It is possible to 'resurrect' some recently inherited HERVs from the human genome but this is unlikely to occur spontaneously.[42] However, these endogenous retroviruses are far from genetically or metabolically dormant. Some HERVs have physiologic roles including syncytiotrophoblast formation in placental development and intrinsic resistance to exogenous retrovirus infection.[43]

HERVs also have the potential to contribute to pathologic reactions in their hosts.[41] The LTR regions are transcriptionally active and serum antibodies are detectable to Env and Gag proteins from a number of HERVs, indicating that these structural genes are transcribed and expressed. Transposition of HERVs can inactivate cellular genes at other loci in the human genome. Moreover, the LTRs of HERVs can have polar effects in *cis* and promote transcriptional activation of adjacent cellular genes. Lastly, transcriptional activators are found within HERVs that might have the capacity to activate cellular genes in *trans*.

HERVs have been implicated in induction of malignant transformation by activating oncogenes or inactivating apoptosis-inducing genes. HERVs might contribute to immune-mediated diseases such as multiple sclerosis (MS), rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE) by the generation of HERV antigens that cross-react with endogenous antigens and break tolerance to normal autoantigens. The *gag* p30 of class I HERVs bear striking homology

Table 64.3 Koch's postulates revisited: microbial causation in the genomic era

Modified Hill[39] and Evans[40] criteria	Genomic criteria[3]
Prevalence of disease higher in patients exposed to suspect pathogen(s) than those not exposed	Nucleic acid sequence of suspect pathogen should be present in diseased tissues
Incidence of disease should be higher in patients exposed to suspect pathogen(s) than controls in prospective studies	No or few copies of nucleic acid sequences of suspect pathogen should be found in the absence of disease and normal tissue
The disease should be temporally linked to exposure to suspect pathogen(s)	If suspect pathogen sequences predate onset of disease, the copy number of sequences should increase with onset of clinical illness (temporal relationship)
A spectrum of disease should be found after exposure from mild to severe disease (dose–response relationship)	Copy number of suspect pathogen sequences should correlate to disease severity (gene copy–host response relationship)
A measurable host response should occur following exposure to suspect pathogen(s)	The suspect pathogen sequences should localize by *in situ* hybridization to affected tissues
Experimental reproduction of disease should occur in models or human volunteers	Suspect pathogen sequences should be reproducibly found in patients with similar illnesses and in animal models (if available)
Prevention of transmission of suspect pathogen should decrease incidence of disease and eradication of pathogen should decrease the disease	Resolution of clinical illness should be accompanied by reduction or elimination of suspect pathogen sequences
The whole process should make biologic sense and fit the epidemiology of disease	The suspect pathogen determined by nucleic acid homology searches should fit the biology and pathology of genetically related micro-organisms

with the human 70K ribonucleoprotein (RNP) U1 snRNP, a frequently recognized autoantigen in SLE.[44] It is tempting to speculate that SLE is induced in some patients by molecular mimicry when expressed HERVs Gag proteins induce antibodies that cross-react with epitopes found on the 70K RNP. HERVs may facilitate epitope spreading and loss of Fas-mediated apoptosis of self-reacting T cells which might further contribute to the progression of autoimmune disease.

HERVs may collaborate with other viruses such as EBV to induce the synthesis of endogenous superantigen motifs encoded by HERVs.[45] This process is hypothesized to contribute to the immune-mediated CNS pathology in MS. HERV-K18 has superantigenic activity in its envelope peptide; expression of this Env protein is transactivated by B-cell clonal expansion by co-infection with EBV. This type of immune dysregulation in the CNS is implicated in the pathogenesis of plague formation within the white matter in MS.[41,45,46] Finally, recent evidence suggests that the Env protein of HERV-W can act as a ligand for the pattern recognition receptors CD14 and Toll-like receptor 4 (TLR-4).[47] Activation of effector cells of the innate immune system via TLR-4 with cytokine and chemokine generation might further contribute to injurious CNS inflammation in MS and other demyelinating disorders. A brief review of the known and possible infectious diseases that are associated with or cause inflammatory and vasculitic diseases is presented in Table 64.4[48] and Table 64.5.[49–52]

Table 64.4 Microbial disease associations and possible causation – inflammatory diseases

Possible pathogen	Disease	Level of evidence of causation*
Propionibacterium acnes	Sarcoidosis	+ (environmental mycobacteria also implicated)
Human herpesvirus-6 (HHV-6)	Multiple sclerosis (relapsing–remitting type)	+/++ (HERV-W also implicated in association with Epstein–Barr virus or HHV-6)
Mycobacterium paratuberculosis	Inflammatory bowel disease	+ (high concentrations of enteric bacteria also implicated)
Human endogenous retrovirus (HERV)-W	Psoriasis	+
HERV-K18	Type 1 diabetes	+ (enteroviruses also implicated)
Bacterial superantigens	Kawasaki disease	+
Yersinia enterocolitica	Kikuchi–Fujimoto disease	++ (KSHV also implicated)
Kaposi's sarcoma-associated herpes virus (KSHV)	Multicentric Castleman's disease	++
Mixed enteric aerobes and anaerobes	Necrotizing enterocolitis[48]	+

*Levels of evidence: epidemiologic link only +; some evidence of causation ++; clear evidence of causation +++.

Table 64.5 Microbial disease associations and possible causations – vasculitis

Microbial agent	Disease	Level of evidence of causation*
Hepatitis B	Polyarteritis nodosa	++/+++
Hepatitis C	Mixed cryoglobulinemia	++/+++
Staphylococcus aureus	Wegener's granulomatosis Churg–Strauss eosinophilic vasculitis	++ +
Epstein–Barr virus (EBV)	Rheumatoid arthritis	++ (HERV also implicated)
Cytomegalovirus[49]	Atherosclerosis	+ (*Chlamydia pneumoniae* also implicated[50])
Human endogenous retrovirus (HERV)-E	Systemic lupus erythematosus	+ (EBV also implicated)
Parainfluenza virus	Giant cell arteritis[51]	+ (parvovirus B19 also implicated)
Bacterial lipopolysaccharide	Anti-neutrophil cytoplasmic antibody – associated crescentic glomerulonephritis[52]	+

*Levels of evidence: epidemiologic link only +; some evidence of causation ++; clear evidence of causation +++.

CONCLUSIONS

The number of potential pathogens that might cause disease in some susceptible human populations has expanded by an order of magnitude with the recognition of a multitude of noncultivable micro-organisms in our environment and even among our endogenous microbiota. It seems likely that at least some human diseases of unknown cause that currently exist will eventually be attributable to one or more of these as yet noncultured organisms. Synergistic combinations of viral, bacterial and even parasitic infections are now known to cause specific clinical disease entities and hopefully additional conditions, currently regarded as 'idiopathic', will be found to be of microbial cause. Aberrant host responses to common micro-organisms might explain many inflammatory and perhaps even some neoplastic diseases. The role of human endogenous retroviruses in human disease alone or in combination with other potential pathogens remains a fertile area of research in the coming years.

The discovery of previously unrecognized infectious diseases as a cause of human illness is certainly worth the time and expense. Microbial causation holds out hope for specific and improved treatments and even prevention of illness. This discovery work will be more complicated than that of the microbe hunters of the past century, but the new tools of systems biology and genomics offer unique opportunities to decipher human disease as never before. The challenge of discovery of new agents in microbial pathogenesis has been rekindled and significant advances in this field are fully anticipated in the coming years.

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References for this chapter can be found online at http://www.expertconsult.com

Chapter 65

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Infectious complications following surgery and trauma

Significant tissue injury substantially increases the risk of infection for several reasons. The net host response to injury is predominantly immunosuppressive; transgression of natural epithelial barriers (e.g. skin, respiratory mucosa, gut mucosa) creates a portal of entry for invasion by potential pathogens; tissue injury, foreign bodies and ischemia provide optimal conditions for bacterial proliferation; well-intentioned therapies can have adverse consequences; and lapses of infection control often occur under emergency conditions. These risks can be apportioned as patient-derived risks, environment-derived risks or treatment-derived risks. The polytrauma patient is often subjected to multiple surgical procedures, creating additive risks for adverse outcome.

Some infective complications are unique to surgery (e.g. surgical site infections (SSIs), infection of traumatic wounds and burns, post-operative intra-abdominal infections), whereas others are common to all critically ill patient populations (e.g. health-care related infection, catheter-related infection, etc.). Management of the surgical patient presents some special challenges:

- it can be particularly difficult to distinguish sterile inflammation with or without bacterial colonization from invasive infection; and
- prevention of infection at the site of tissue injury is of utmost importance, especially after severe trauma and before high-risk elective surgery.

The incidence of infection following surgery or trauma is context-sensitive. The incidence of SSI following minor, clean, elective surgery is approximately 2% and that of other infectious complications is practically nil.[1] In contrast, the overall incidence of infection following major trauma is about 25%.[1,2] Infection is common and the leading cause of late death after trauma accompanied by multiple organ dysfunction syndrome (MODS).[3–5] It is generally stated that the development of postoperative nosocomial infection, especially with a multidrug-resistant pathogen, increases the risk of death three- to five-fold;[5] however, the attributable risk of mortality from infection remains controversial and not universally accepted.[6]

PATIENT-DERIVED RISK FACTORS

Risk factors that result from intrinsic attributes of the host are listed in Table 65.1. Among these factors are age, chronic medical conditions (especially diabetes mellitus) and hypocholesterolemia. The contribution of age to infection risk is increasingly being recognized.[7] In a multicenter, preoperative risk assessment for the development of postoperative pneumonia, age was found to be the most powerful independent predictor of risk.[7] Substantial evidence indicates that perioperative hyperglycemia increases the risk of infection, even if mild or transient. Failure to maintain euglycemia during open heart surgery triples the risk of sternal wound infection.[8] While some controversy persists over the risk–benefit ratio in the maintenance of euglycemia in the critically ill surgical patient, avoidance of significant hyperglycemia (>150 mg/dl or 8.3 mmol/l) is advised. Hypocholesterolemia is rapid and dramatic after surgical stress, and has been associated with an increased risk of SSI and pneumonia[9] and independently with an increased risk of death in critically ill patients with systemic inflammatory response syndrome (SIRS). The possible mechanisms underlying this risk are several, including dysfunction of lipid-laden macrophages or decreased binding of endotoxin by apolipoproteins.

Table 65.1 Host factors that contribute to the development of nosocomial infection

Nonmodifiable	Modifiable
Ascites	Chronic inflammation
Obesity	Corticosteroid therapy (controversial)
Diabetes mellitus	Hypocholesterolemia
Extremes of age	Hypoxemia
Peripheral vascular disease (especially for lower extremity surgery)	Postoperative anemia
Extent of tissue trauma or burns	Prior site irradiation
	Recent operation
	Remote infection
	Skin carriage of staphylococci
	Undernutrition
	Transfusions
	Antibiotics

The surgical stress response

Surgical stress is associated with a stereotypical response.[10] The stress hormone response to surgical stress or tissue injury augments cardiovascular function through the sympathetic nervous system, enhances glycogenolysis, mobilizes peripheral lean tissue and fat for use as fuel, enhances coagulation to limit hemorrhage and stimulates a proinflammatory cytokine response to begin the process of tissue repair. Cellular immunity is depressed in large part by the actions of cortisol, catecholamines, a shift to Th2-type cytokines and the actions of regulatory T cells (T_{reg}). Three major events characterize the initial inflammatory response:

- activation of coagulation;
- increased microvascular endothelial permeability with tissue edema formation; and
- chemotaxis, margination and transvascular migration of neutrophils.

The first step in the process is recognition of a foreign antigen, either by elements of the adaptive immune system (e.g. T cells, preformed antibodies, classic complement pathway) or by innate immune elements (e.g. myeloid cells, Toll-like receptors, alternative complement and mannose-binding lectin pathways). The inflammatory response is then amplified in a complex process regulated by cytokines, plasma enzymes (e.g. complement, coagulation, kinin and fibrinolytic pathways), lipid mediators (e.g. prostaglandins, leukotrienes) and mediators derived from mast cells and platelets. Fast-acting mediators, such as vasoactive amines and bradykinin, modulate the immediate response. Several hours later, mediators such as leukotrienes, chemokines and platelet-activating factor are involved in the accumulation and activation of phagocytes. Once neutrophils have arrived at a site of inflammation, they release macrophage chemoattractants that, in turn, control the later accumulation and activation of monocytes and macrophages.

The acute-phase response is a dynamic homeostatic process that involves the immune, cardiovascular and central nervous systems. Normally, the acute-phase response lasts only a few days. Two physiologic responses are closely associated with acute inflammation: the first involves the alteration of the temperature set-point in the hypothalamus and the generation of fever; the second involves alterations in metabolism and gene expression in the liver. Interleukin (IL)-1, IL-6 and tumor necrosis factor regulate the febrile response through the induction of prostaglandin E2, possibly as a protective mechanism against bacterial infection (see Chapters 2 and 61). At the same time, IL-1 and IL-6 act on the hypothalamic–pituitary–adrenal axis to generate corticotropin and stimulate cortisol production. This provides a negative feedback loop, because corticosteroids inhibit cytokine gene expression.

The second important aspect of the acute-phase response is altered hepatic protein synthesis. Production of numerous proteins is upregulated, whereas synthesis of others is suppressed. Acute-phase reactants (APRs) thus may be 'positive' or 'negative'. Positive APRs include fibrinogen, clotting elements, proteases and some protease inhibitors, complement components, lipopolysaccharide-binding protein, serum amyloid A and C-reactive protein. Negative APRs (e.g. albumin, antithrombin, inter-α inhibitor, protein C) are decreased in synthetic rate to allow an increase in the capacity of the liver to synthesize the induced APRs. Although most APRs are synthesized by hepatocytes, some are produced by other cell types such as myeloid cells, endothelial cells, fibroblasts or adipocytes.

Acute-phase reactants contribute to host defense in several ways, including direct neutralization of inflammatory mediators, thereby minimizing tissue damage and facilitating tissue repair. For example, increased synthesis of complement proteins mobilizes neutrophils and macrophages. Fibrinogen plays an essential role in hemostasis and the promotion of wound healing. Protease inhibitors (e.g. α_1-antitrypsin) neutralize elastase and lysosomal proteases released following the infiltration of activated neutrophils and macrophages, thus mitigating the activity of the proinflammatory enzyme cascades. Increased plasma concentrations of metalloproteases help to prevent iron loss during infection and injury, minimize the amount of heme iron available for bacterial metabolism and scavenge reactive oxygen species.

PATHOGENESIS OF INFLAMMATION AND ORGAN DYSFUNCTION

The acute response to injury is characterized by a syndrome of:

- fever (≥100.4°F (38° C)) or hypothermia (≤96.8°F (36° C));
- leukocytosis (>12 000/mm³), leukopenia (<4000/ml) and excess bands (>10% immature neutrophils);
- increased heart rate (<90 beats/minute); and
- increased respiratory rate (>24/minute) or, if mechanically ventilated, $PaCO_2$ <32 mmHg.

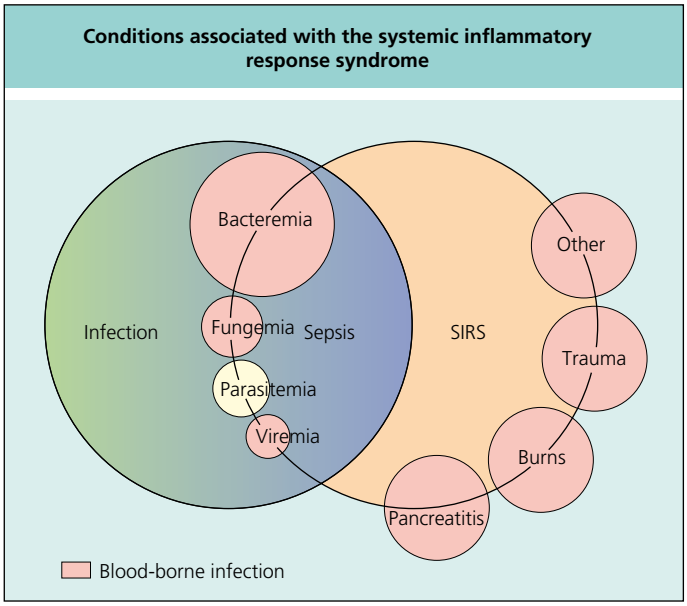


Fig. 65.1 The interactions between systemic inflammation, sepsis and multiple organ dysfunction syndrome. SIRS, systemic inflammatory response syndrome.

Systemic inflammatory response syndrome[11] is the name given to this constellation if at least two of the parameters are present. Among surgical patients, there are many causes of tissue injury-induced inflammation (e.g. trauma, burns, pancreatitis) (Fig. 65.1) that have no relation to infection on initial presentation. Recovery from general anesthesia can produce symptoms indistinguishable from true SIRS, but the effect is transitory. While the diagnostic value of SIRS is often criticized for being nonspecific, in the surgical setting the persistence of SIRS is important prognostically. The presence and magnitude of SIRS in the trauma bay has been associated with increased risk of nosocomial infection. Moreover, among critically ill surgical patients, persistent SIRS was associated with prolonged stay in intensive care units (ICU), worsening SIRS was associated with MODS and a higher mortality (see also Chapter 44).

The proinflammatory response can be incited by either tissue injury or infection. The same pattern recognition receptors of the innate immune system (the TLRs and receptor for advanced glycation end products) that detect infection-related, pathogen-associated molecular patterns (PAMPs) can also recognize numerous host-derived damage-associated molecular patterns (DAMPs) (see Chapter 44). Host-derived DAMPs include heat shock proteins, high-mobility group box 1 protein (HMGB1), heme, fibronectin extra domain A, calgranulins, heparan monomers, β-defensins and many other molecules released from damaged host cells and cells undergoing apoptosis.[12] Inflammation is modulated by a complex interrelationship of activation of the coagulation, kinin, complement and other systems, generating numerous already-known and probably yet-to-be-discovered mediators, including prostaglandins, leukotrienes, reactive oxygen and nitrogen species, lipid peroxides, coagulation factors, adhesion molecules and cytokines. Consequently, phagocytic cells, platelets and endothelial cells are activated, in part to contain the inflammatory response (e.g. to localize and eradicate a nascent infection). Containment is also the likely role of the counter-regulatory anti-inflammatory response, downregulating the potentially injurious, systemic, proinflammatory response. The anti-inflammatory response is mediated by cortisol, IL-4, IL-10, myeloid colony-stimulating factors, transforming growth factors and other mediators. The counter-regulatory response results in the activation of genetic programs ('suicide' genes) that cause phagocytic cells to undergo apoptosis (i.e. programmed cell death). Apoptosis causes inflammation to subside; several adverse clinical events such

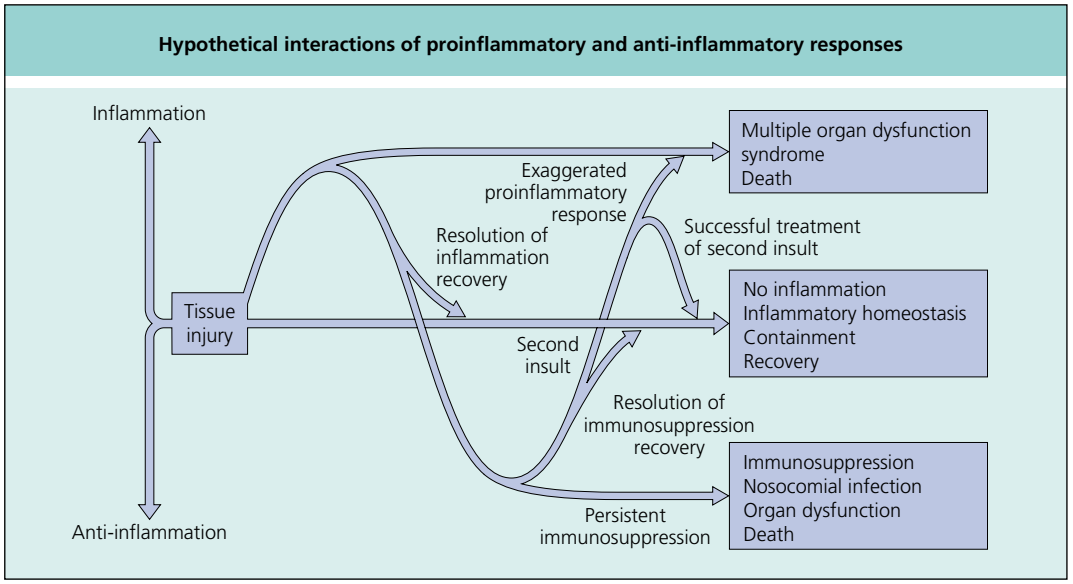


Fig. 65.2 Hypothetical interactions of proinflammatory and anti-inflammatory responses. Pathogenesis of immunosuppression, nosocomial infection, organ dysfunction and outcome.

as hypoxemia or blood transfusions may delay apoptosis, promote inflammation and contribute to adverse outcomes.[13]

Details of the regulatory control of the anti-inflammatory response over the proinflammatory response and the promotion of subsequent tissue repair remain incomplete. Elements that likely contribute to the inflammatory ebb and flow are depicted in Figure 65.2. There is substantial experimental evidence that an accentuated neutrophil proinflammatory response occurs by neutrophils that are first 'primed' by an inflammatory stimulus and then respond in an exaggerated, deleterious manner in response to a second insult. Examples of a primary insult include a period of hypoxia or blood transfusion, or the initial injury (e.g. a femur fracture), whereas an example of a second insult would be a postoperative nosocomial pneumonia.[13] The dysfunctional inflammatory response results in release of mediators that, among other effects, induce adherence of neutrophils and platelets to microvascular endothelium; entrap microthrombi of fibrin, platelets, neutrophils and cellular debris; and cause tissue hypoperfusion, ischemia and edema as a result of loss of microvascular integrity.[14] Resuscitation and natural fibrinolysis may restore microvascular flow before tissue necrosis occurs, but re-introduction of molecular oxygen to the ischemic bed may result in the generation of reactive oxygen species. These highly toxic mediators have innumerable deleterious effects, including the ability to oxidize lipid components of cell membranes.

The splanchnic bed is especially susceptible to ischemia, and ischemia and reperfusion of the gut may be central to the pathogenesis of inflammation and organ dysfunction.[13] The intestine is an immunologically active organ that serves to protect the host from ingested bacteria as well as the potentially lethal bacterial inoculum contained in feces. Ischemic reperfused intestine becomes dysfunctional, with ileus and the ensuing putative release of mediators, bacterial toxins (e.g. endotoxin) or live bacteria ('bacterial translocation'). Although the existence of bacterial translocation is well documented in murine models it does not appear to occur in humans. Increased intestinal permeability of luminal products does occur in humans and may account for the gut-derived, persistent proinflammatory stimulus following severe injury or hypotension. Under catabolic conditions, the dysfunctional gut no longer provides trophic support of hepatic function, and maintenance of hepatic reticuloendothelial host defense function is impaired.

The clinical manifestations of MODS are protean (see Fig. 65.2) and the consequences are grave. No longer conceptualized as an 'all-or-nothing' phenomenon in which organ 'failure' is recognized after a threshold of dysfunction is reached, MODS can affect several organs to varying degrees and the effects are cumulative.[11] As many as 50% of critically ill surgical patients and nearly 100% of critically ill nonsurvivors develop some degree of MODS. The development of MODS prolongs ICU stay even among patients who develop only minimal organ dysfunction, and mortality increases as it progresses.

ENVIRONMENT-DERIVED RISK FACTORS

The importance of colonization with bacteria or active remote infection as a risk factor for subsequent infection cannot be overemphasized. Patients who are chronic carriers of staphylococci and streptococci are at increased risk of SSIs after elective surgery,[15] as are patients with active soft tissue infections at tissue sites remote from the surgical wound. In hospitalized patients, colonization of the skin, lower airway and gut with pathogenic bacteria and fungi is common and occurs rapidly even among immunocompetent hosts. Aided and abetted by breaks in infection control measures, these potential pathogens colonize and then invade vulnerable hosts. The risk of SSI and intra-abdominal infection is increased by prolonged pre-event hospitalization. Nosocomial pneumonia is promoted by pre-existing colonization of the lower airway by the same pathogen.[16] The same can be said for invasive fungal infections of surgical patients, which are often preceded by multiple-site colonization and invasive infection with vancomycin-resistant enterococci. There is little doubt that antibiotic selection pressure leads to nosocomial infections with multidrug-resistant bacteria, which are a threat to vulnerable patients.[17]

TREATMENT-DERIVED RISK FACTORS

Modern therapy is often invasive and disruptive of homeostasis even as treatment tries to restore it (Table 65.2). Clinicians must be cognizant of the adverse consequences of therapies that may impair host defenses or disrupt commensal flora, increasing the risk of nosocomial infection. Among several factors of importance are the control of serum glucose concentration, the avoidance of blood transfusion, maintenance of normoxia and normothermia, minimized duration of mechanical ventilation, and rational antibiotic prophylaxis and therapy. Also crucial are maintenance of the operating room environment and adherence to the principles of infection control and universal precautions. There are published guidelines for the prevention of SSI[17] and vascular catheter-related infection (see also Chapter 45).[18]

Table 65.2 American Society of Anesthesiologists (ASA) physical status score

Score	Description	Examples
ASA 1	A normal healthy patient	
ASA 2	A patient with a mild to moderate disturbance that results in no functional limitations	Hypertension, diabetes mellitus, chronic bronchitis, morbid obesity, extremes of age
ASA 3	A patient with a severe systemic disturbance that results in functional limitations	Poorly controlled hypertension, diabetes mellitus with vascular complications, agina pectoris, prior myocardial infaction, pulmonary disease that limits activity
ASA 4	A patient with a severe systemic disturbance that is life threatening with or without the planned procedure	Congestive heart failure, unstable angina pectoris, advanced pulmonary, renal or hepatic dysfunction
ASA 5	A moribund patient not expected to survive with or without the operative procedure	Ruptured abdominal aortic aneurysm, pulmonary embolism, head injury with increased intracranial pressure
E	Any patient in whom the procedure is an emergency	

Blood transfusion

Evidence is substantial and increasing that allogenic transfusion of red blood cell concentrates is immunosuppressive and increases the risk of nosocomial infection. Transfusion requirements are understandable in the trauma and surgery environment and alternatives are few. Nonetheless, transfusions should be avoided if possible, especially in hemodynamically stable patients who are not bleeding. Leukoreduced red blood cell transfusion has reduced but not eliminated the adverse immune consequences of transfusion and now is considered the standard of care in developed countries.[19]

Protection of the hypoxic wound

A fresh surgical incision is a hypoxic, ischemic environment. Blood vessels are divided at the wound margins and use of electrocautery leaves a surrounding margin of coagulation necrosis. Maneuvers to support the oxygenation and perfusion of the incision have demonstrated benefit. Hypothermia (body temperature under 94.1°F (34.5° C)) leads to MODS and increased mortality after major abdominal vascular surgery and to an increased incidence of SSI.[20] Intraoperative hypothermia should be avoided at all costs with use of fluid warmers, forced-air blankets and other maneuvers to avoid body cooling.

ANTIBIOTIC PROPHYLAXIS AND THE RISK OF SURGICAL SITE INFECTION

The administration of antibiotics before surgery is commonplace and of proven benefit to prevent postoperative SSI. However, it is only the surgical incision that is afforded protection, and antibiotics are not a panacea. If not administered properly, antibiotic prophylaxis will not be effective and may be harmful.

Most commonly, the risk of SSI, as categorized by the National Nosocomial Infections Surveillance program, is defined by three risk factors:[1]

- a contaminated or dirty wound;
- poor overall medical condition of the patient; and
- a prolonged operative time (longer than the 75th percentile for operations of the type).

Surgical incisions are classified as clean (class I), clean–contaminated (class II), contaminated (class III) or dirty (class IV). A poor overall medical condition of the patient is categorized by an American Society of Anesthesiologists (ASA) score of more than 2 (see Table 65.2). Prolonged surgery has implications for the degree of tissue injury, antibiotic pharmacokinetics and intraoperative repeat dosing, and it provides a prolonged opportunity for wound inoculation. An increased risk of SSI occurs with an increasing degree of wound contamination (e.g. clean wounds have less risk than contaminated wounds), regardless of other risk factors (Table 65.3),[21] and as the number of risk factors increases for a given type of operation (Table 65.4).

Antibiotic prophylaxis is indicated clearly for most clean–contaminated and contaminated (or potentially contaminated) operations. Dirty operations are those in which surgery and antibiotics represent treatment for an infection, not prophylaxis against it. An example of a potentially contaminated operation is lysis of adhesions for mechanical small bowel obstruction – intestinal ischemia cannot be predicted accurately before surgery, and the possibility exists of an enterotomy during adhesiolysis, which increases the risk of SSI twofold. An example of a clean–contaminated operation in which antibiotic prophylaxis is not always indicated is elective cholecystectomy. Antibiotic prophylaxis is indicated only for high-risk biliary surgery; patients at high risk include those over the age of 70 years, diabetic patients and patients whose biliary tract has recently been instrumented (e.g. during biliary stenting). The vast majority of patients who undergo laparoscopic cholecystectomy do not require antibiotic prophylaxis. Laparoscopic clean–contaminated operations are generally at decreased risk of infection.[1] The potential reasons are several, including a diminished surgical stress response, decreased tissue injury and smaller incisions.

Table 65.3 Incidence of surgical site infection as a function of wound classification

	NNIS RISK INDEX				
Traditional class	**0**	**1**	**2**	**3**	**All**
Clean	1.0%	2.3%	5.4%	NA	2.1%
Clean–contaminated	2.1%	4.0%	9.5%	NA	3.3%
Contaminated	NA	3.4%	6.6%	13.2%	6.4%
Dirty	NA	3.1%	8.1%	12.8%	7.1%
All	1.5%	2.9%	6.8%	13.0%	2.8%

NNIS, National Nosocomial Infections Surveillance Program; NA, not applicable (by definition, because the traditional wound classification is one of the risk factors, patients with clean wounds cannot have three risk factors, nor can those with dirty wounds have no risk factors).
From Martone & Nichols.[21]

Table 65.4 Surgical site infection percentage rates for selected procedures

Procedure	Time cutpoint (hours)	NUMBER OF RISK FACTORS			
		0	1	2	3
Coronary artery bypass graft (chest or leg)*	5	1.20	3.57	5.68	9.63
Laparotomy	2	1.71	3.29	5.16	7.77
Open reduction and internal fixation of fractures†	2	0.73	1.35	2.51	4.85

*Pooled incidence of surgical site infection for both incisions.
†Overall incidence.
From National Nosocomial Infections Surveillance System.[1]

Antibiotic prophylaxis of clean surgery is controversial. When bone is incised (e.g. in a craniotomy or sternotomy) or a prosthesis is inserted, antibiotic prophylaxis is generally indicated.

Choice of antibiotic

Most SSIs are caused by Gram-positive cocci. The most common pathogens are *Staphylococcus aureus*, followed by *Staph. epidermidis*, *Enterococcus faecalis* and *Escherichia coli*, the latter two of which are common pathogens after clean–contaminated surgery. The antibiotic chosen should be directed against staphylococci for clean cases and high-risk clean–contaminated elective surgery of the biliary and upper gastrointestinal tracts. A first-generation cephalosporin is the preferred agent for most patients, with clindamycin preferred for patients with a history of anaphylaxis to penicillin. Although methicillin-resistant *Staph. aureus* (MRSA) has been isolated in the community from never-hospitalized patients, vancomycin prophylaxis is appropriate only in institutions where the incidence of MRSA is high. In regions where community-acquired (CA-MRSA) is high, screening patients for MRSA carriage before selected, high-risk, elective surgical procedures (e.g. total hip arthroplasty, neurosurgery with ventriculoperitoneal shunt placement) may be appropriate so that attempts at decontamination of CA-MRSA prior to surgery can be initiated.

Elective colon surgery is a special circumstance and one in which practices are in evolution. Mechanical bowel preparation to reduce bulk feces made colon surgery safe for the first time. Antibiotic bowel preparation, standardized in the 1970s by the oral administration of nonabsorbable neomycin and erythromycin base, reduced the risk of SSI further to its present rate of approximately 4–8%, depending on the number of ASA risk factors. Although outpatient mechanical preparation is now the norm, three doses of oral antibiotics are still given to most patients at approximately 18, 17 and 10 hours preoperatively so that fecal bacterial counts are minimal at the time of surgery. A dose of parenteral second-generation cephalosporin (or a quinolone or monobactam plus metronidazole for the penicillin-allergic patient) is given before skin incision, and the benefit appears to be additive.[22] Moreover, there is less reticence to operate on the less-than-perfectly-prepared colon, extrapolating from good results from primary repair of penetrating colon injuries.

Timing and duration of parenteral antibiotics

It is firmly established that the optimal time to give cephalosporin prophylaxis is within 2 hours before the time the incision is made (Table 65.5).[23] Antibiotics given sooner (except possibly for longer half-life agents such as quinolones and metronidazole) are not effective, nor are agents that are given after the incision is closed. Antibiotics with short half-lives should be re-dosed during surgery if the operation is prolonged or bloody, and there is still some benefit if the initial antibiotic dose is given intraoperatively.

Considering the exigencies of surgical hemostasis and that the fresh surgical incision is ischemic, the postoperative administration of antibiotics is questionable owing to hypoperfusion that results from divided vessels. Single-dose prophylaxis should be standard. Other than in solid-organ transplant surgery, in which therapeutic immunosuppression has made 48-hour regimens standard, there is no indication for prolonged antibiotic prophylaxis.

Topical antiseptics and antibiotics may also help prevent SSI. A preoperative shower with an antiseptic soap (e.g. povidone–iodine) is a mainstay of perioperative preparation. Topical mupirocin ointment applied to the nares of patients who are chronic carriers of *Staph. aureus* reduces the increased incidence of SSI that is characteristic of chronic staphylococcal carriage.[15]

Prolongation of antibiotic prophylaxis beyond 24 hours not only provides no benefit, but can also be associated with a number of complications. *Clostridium difficile*-associated disease (CDAD) has been associated with disruption of the normal balance of gut flora and overgrowth of the enterotoxin-producing *C. difficile*. The incidence and severity of nosocomial CDAD is increasing.[24] The spectrum of disease is broad, ranging from asymptomatic to life-threatening pancolitis with infarction or perforation. Although virtually every antibiotic has been implicated in the pathogenesis of CDAD, even after administration of a single dose, prolonged antibiotic prophylaxis

Table 65.5 Risk of the development of surgical site infection relative to the timing of antibiotic prophylaxis

Timing	No. patients	No. (%) infections	RR (95% CI)	OR (95% CI)
Early	369	14 (3.8)*	6.7* (2.9–14.7)	4.3* (1.8–10.4)
Preoperative	1708	10 (0.59)	1.0	
Perioperative	282	4 (1.4)	2.4 (0.9–7.9)	2.1 (0.6–7.4)
Postoperative	488	16 (3.3)*	5.8* (2.6–12.3)	5.8* (2.4–13.8)
All	2847	44 (1.5)		

Early, administration more than 2 hours preoperatively; Preoperative, administration during the recommended interval (≤2 hours before skin incision); Perioperative, administration within 2 hours after the skin incision; Postoperative, administration more than 2 hours after the skin incision.
*p <0.0001 compared with preoperative group.
From Classen et al.[23]

clearly increases the risk. Prolonged prophylaxis also increases the risk of later nosocomial infections unrelated to the surgical site and the emergence of multidrug-resistant pathogens. Both pneumonia and catheter-related infections have been associated with prolonged antibiotic prophylaxis, as has the emergence of SSI caused by MRSA.[25]

ANTIBIOTIC PROPHYLAXIS OF TRAUMA-RELATED INFECTIONS

Traumatic injury is profoundly immunosuppressive and injured patients are at very high risk of infection. The overall incidence of infection after trauma is approximately 25%,[4,10,13] with infection of a wound (or an incision made as treatment) and nosocomial infection equally likely. Certain patterns of injury are independently associated with infectious morbidity in particular, including hemorrhagic shock, the need for blood transfusion, heavy wound contamination, central nervous system injury, colon injury, combined thoracoabdominal injuries, four or more organs injured, and increasing injury severity.[4] Co-morbid factors of importance include hyperglycemia, hypothermia and hypoxemia.

Certain characteristics of trauma make the situation more complex. Antibiotics are administered after injury, but there is a period when injured tissues may be vulnerable before antibiotics are administered. Trauma patients may be hypotensive and vasoconstricted owing to shock and tissue penetration may be decreased. Ongoing blood loss may result in ongoing antibiotic loss, especially if the agent is highly protein-bound and tissue redistribution is slow, or if the antibiotic is administered before major hemorrhage is controlled. Postinjury fluid shifts and hypoalbuminemia can cause major fluctuations in volume of distribution that can be difficult to estimate. As a result, it has been postulated that higher doses of antibiotics should be administered for the prophylaxis of post-traumatic infection, although controlled studies to support this contention are lacking.

Despite the high risk, the basic principles of antibiotic prophylaxis still apply – use a safe, narrow-spectrum agent for a defined brief period (no more than 24 hours), preferably one that has a limited role in the therapy of infection (e.g. a first- or second-generation cephalosporin).[4] Multiple studies indicate unequivocally that 24 hours of prophylaxis with a cephalosporin is all that is necessary following penetrating abdominal trauma, even in the presence of a colon injury and shock.[3,4] Other injuries in which appropriate antibiotic prophylaxis is beneficial include high-grade open fractures, animal bites and, possibly, chest injuries when an emergency tube thoracostomy is required.[3]

Following a human bite, the risk of infection is so high that 7–10 days of therapy with an agent effective against oral anaerobic flora is commonplace (see PP34). Injuries in which there is demonstrated to be no benefit of antibiotic prophylaxis include clean lacerations (even if primary repair is delayed) and skull fracture with or without leakage of cerebrospinal fluid.

Tetanus prophylaxis should be administered for high-risk wounds, such as soft tissue wounds with fecal contamination (e.g. injury caused by farm implements). If tetanus immune status is inadequate or unknown in high-risk wounds, administration of tetanus toxoid alone will be insufficient and tetanus immune globulin should be co-administered (see Chapter 21).

Prophylaxis of postsplenectomy sepsis is another special circumstance, albeit an increasingly rare one owing to the development of successful protocols for the nonoperative management of blunt splenic injuries. Postsplenectomy sepsis can be fulminating and lethal, and is most commonly caused by encapsulated organisms such as *Streptococcus pneumoniae* (see Chapter 82). The incidence is sufficiently low in adults (<1%) for long-term antibiotic prophylaxis not to be indicated. In infants and children where the incidence is higher, daily oral penicillin prophylaxis may be indicated (see Chapter 82 for details). Polyvalent pneumococcal vaccine is administered both to adults and to children, usually postoperatively, although the asplenic host has a blunted immune response to vaccination.

THE POSTOPERATIVE PERIOD

Several circumstances conspire to make fever the most common postoperative complication. A proinflammatory response follows tissue injury. Pulmonary defense mechanisms are impaired during anesthesia and recovery, and small-volume pulmonary aspiration of gastric contents unquestionably occurs. Wound hematoma is one of many known noninfectious causes of fever,[26] and other potential postoperative complications, including venous thromboembolic disease, pancreatitis, myocardial infarction, visceral ischemia and atelectasis, can also cause fever in the absence of infection. In this milieu it is challenging, but crucial, to distinguish inflammation from infection so as to treat patients appropriately. Confounding the evaluation of patients further is that hospitalized patients become colonized rapidly with potential pathogens.[27] Colonization is known to precede invasive infection. However, the high incidence of colonization, especially of the upper aerodigestive tract, makes it impossible to equate the mere isolation of a pathogen with the diagnosis of nosocomial infection, especially in critically ill patients.

Postoperative prophylaxis of infection

Surgical patients are at especially high risk of nosocomial infection. Judicious preoperative antibiotic prophylaxis decreases the risk of infection, but this prophylaxis in the absence of other techniques will not accomplish much. Proper infection control practice cannot be emphasized enough. The use of fast-drying alcohol gels for hand disinfection improves compliance, and can be used in the operating room and at the bedside.[26]

Surgery is invasive and surgical patients are often immunosuppressed owing to illness or injury;[10,13] the surgical stress response augments the immunosuppression. Many standard interventions impair host defenses (see Table 65.1). Surgical care must minimize the possibility of iatrogenic infection, while supporting the patient until host defenses can recover.

Catheters and drains should be removed as soon as possible. Each time a natural epithelial barrier to infection (e.g. skin, respiratory tract mucosa, gut mucosa) is breached, a portal is created for potential invasion of the host by pathogens. Subcutaneous drains increase the risk of SSI.[28] Prolonged central venous catheterization increases the risk of bacteremia; prolonged intubation and mechanical ventilation increase the risk of pneumonia. Moreover, prolonged urinary catheterization increases the risk of urinary tract infection. These devices should be removed as soon as feasible.[4] Improved catheter designs and endotracheal tubes (e.g. silver impregnated tubes and tubes that aspirate subglottic secretions via an extra lumen) might decrease the risk of nosocomial infection further.

Although cumbersome and therefore not popular, topical antiseptics and antibiotics appear to decrease the risk of nosocomial infection in seriously ill, high-risk patients. Data demonstrate that topical 0.12% chlorhexidine mouthwash (a bactericidal, viricidal and fungicidal antiseptic), applied to the oropharynx and the exposed surface of the endotracheal tube, decreases the incidence of postoperative pneumonia. Selective digestive decontamination, described in various forms nearly two decades ago, utilizes an oral paste of multiple antibiotics, enteral administration of the same antimicrobial agents by gavage and sometimes a short course of intravenous antibiotics. Infection rates are reduced unequivocally[27] and it may be appropriate in high-risk situations.

Prophylaxis of fungal infections is controversial. Although surgical patients are immunosuppressed and are frequently exposed to antibiotics, invasive fungal infections are unusual. Although *Candida* spp. are frequently isolated from the peritoneal fluid of patients with peritonitis, specific antifungal therapy is generally not indicated unless *Candida* spp. are isolated in pure culture from blood, from peritoneal fluid or from an abscess. Fungemia can be associated with indwelling central venous catheters, but on the rare occasions where fungemia

complicates surgery (solid-organ transplant patients excepted), the patient is usually debilitated from a protracted serious illness and has already been exposed to multiple courses of antibiotics. Most surgical patients who are potential candidates for antifungal prophylaxis, apart from their routine use with organ transplant protocols, are those who become colonized with yeast at two or more sites (e.g. skin and urine). Empiric antifungal agents such as fluconazole are often used in this situation, despite convincing evidence of efficacy.[26] Fluconazole has numerous drug interactions (e.g. ciclosporin, macrolides, quinolones). Widespread use of fluconazole has been associated with the emergence of azole-resistant strains of *Candida*, especially *C. krusei*.[29]

Blood transfusion

In surgery and trauma, blood transfusions are given commonly and may be life-saving; alternatives to transfusions in the acute setting are few, but for hemodynamically stable postoperative patients hemoglobin concentrations >7 g/dl are well tolerated.[19] Erythropoietin administration may decrease transfusion requirements of the 'chronically critically ill' patient. An expanding body of evidence suggests that blood transfusion should be avoided, if possible. The immunosuppressive effects of allogeneic blood transfusions have been demonstrated in both solid-organ transplant recipients (in whom graft survival has been prolonged) and colon cancer surgery patients (in whom survival is decreased).

Observations that blood transfusions are associated with increased rates of nosocomial infection are numerous. Blood transfusions have been associated with an increased risk of infection following penetrating abdominal trauma independent of related factors such as shock or acute blood loss, and they have been related to increasing injury severity and increasing transfusion volume in unselected trauma patients. Data suggest that blood transfusion therapy of 6–20 units in the first 12 hours following multiple trauma is associated with an increased risk of nosocomial infection.[30] The risk of infection increased as the total transfusion volume increased, especially when units were transfused after more than 14 days of storage. The postulated 'storage lesion' is complex but includes changes in oxygen affinity, red blood cell deformability, shortened circulation time and the biologic consequences of cytokine generation and release. Recently, observational studies have suggested that transfusion of critically ill patients increases the risk of nosocomial infection,[31] and may worsen MODS and increase mortality.[32]

Hyperglycemia and control of blood sugar

Hyperglycemia has several deleterious effects on host immune function, most notably impaired function of neutrophils and mononuclear phagocytes. It is possible also that hyperglycemia is a marker of the catabolism and insulin resistance associated with the surgical stress response, and that exogenous insulin administration may ameliorate the catabolic state. Increasing evidence indicates that poor control of blood glucose during surgery and in the perioperative period increases the risk of infection and worsens outcome from sepsis. Diabetic patients have a higher risk of infection of both the sternal incision and the vein harvest incisions on the lower extremities.[8] Tight control of blood glucose by the anesthesiologist during surgery must be accomplished to decrease the risk, and that control must extend into the immediate postoperative period as well.

The need to manage carbohydrate metabolism carefully has important implications for the nutritional management of surgical patients. Gastrointestinal surgery may render the gastrointestinal tract unusable as a route for feeding, sometimes for prolonged periods. Ileus is common in surgical ICUs, whether from traumatic brain injury, narcotic analgesia, prolonged bed rest, inflammation in proximity to the peritoneal envelope (lower lobe pneumonia, retroperitoneal hematoma, fractures of the thoracolumbar spine, pelvis or hip) or other causes. Parenteral nutrition is relied on for feeding, despite evidence of a lack of efficacy[33] and the possibility of hepatic dysfunction; hyperglycemia may be an important complication as well. Every effort should be made to provide enteral feeding, as substantial evidence indicates that enteral feeding reduces the risk of nosocomial infection by nearly one-half among critically ill and injured patients.[34]

The diagnosis of postoperative infection

The evaluation of a patient for possible infection usually begins with the report of a fever. The clinical response is too often the reflexive ordering of multiple cultures of various body fluids, even at times when the likelihood of infection and therefore the yield of the cultures is low. The only intervention mandated by new-onset fever is a history and physical examination.[26] All additional evaluations, whether radiologic or microbiologic, should be dictated by the findings of the evaluation at the bedside.[35] Fever related to infection in the immediate postoperative period (<48–72 hours after the operation) is rare unless the patient was operated upon for an active infection. The exception is necrotizing SSI caused by *Strep. pyogenes* or *Clostridium* spp., for which inspection of the incision is mandatory. The yield of blood cultures is known to be very low in the immediate postoperative period, and obtaining cultures to evaluate fever less than 72 hours postoperatively is not recommended.[26] After 72 hours, the possibility of nosocomial infection increases, but even then there is still the possibility that fever may be due to a noninfectious cause (e.g. venous thromboembolic disease, wound hematoma) and diagnostic rigor must be maintained. Overall, postoperative fever may be due to a noninfectious cause in as many as one-half of circumstances.[26]

Intra-abdominal infection

Intra-abdominal infection is a recognized complication following abdominal surgery. After an abdominal operation, an existing infection may persist or recur. Potential causes include severe illness, inadequate antibiotic dosing, resistant pathogens, inadequate source control, technical shortcomings, tissue ischemia and complete failure of intra-abdominal host defenses. Most patients with community-acquired intra-abdominal infections (e.g. secondary peritonitis from, for instance, appendicitis or diverticulitis) are infected by sensitive Enterobacteriaceae and anaerobic Gram-negative bacilli such as *Bacteroides fragilis*. Such patients rarely harbor multidrug-resistant pathogens; if surgery is required for a good-risk patient and is performed properly, the patient will usually recover regardless of which of several appropriate antibiotic regimens may be chosen.[36]

Source control is an emerging concept in the management of intra-abdominal infection.[35] Simply stated, adequate source control is the correct operation performed at the correct time in the correct manner. In practice, however, it is much more difficult to define. Whereas the surgical management of complicated appendicitis has relatively few permutations, this is not the case for complex entities such as perforated diverticulitis. Issues surrounding whether to resect, perform an anastomosis or a colostomy, or place drains make it impossible to declare that there is one 'correct' surgical approach, but it may be possible to describe management attributes that are clearly not appropriate. Studies suggest that source control is inadequate in approximately 10% of cases of complicated intra-abdominal infection; therefore, failure of source control is more likely as a cause of failure of treatment of intra-abdominal infection than any shortcoming of the antibiotic regimen.

In circumstances in which involvement of the peritoneal cavity is generalized and at least one source control procedure has failed to control a nosocomial intra-abdominal infection, the patient may be considered to have tertiary peritonitis.[37] Diffuse peritonitis, infected serosanguinous fluid rather than pus, poorly localized collections and isolation of enterococci, coagulase-negative staphylococci, yeast and *Pseudomonas aeruginosa* are characteristic. Local peritoneal host defenses are nonfunctional. The management of tertiary peritonitis is controversial. Some experts believe that the peritoneal cavity is colonized rather than infected, and that peritoneal toilet should be

provided by daily saline lavage with the abdomen left open,[38] but randomized studies are nonexistent. What is clear, however, is that the typical mortality rate of about 30% is more than 10-fold higher than that usually reported for secondary peritonitis.

Ischemic enteropathies (e.g. acute pancreatitis, acalculous cholecystitis, ischemic colitis, ischemic hepatitis) can complicate the management of critical surgical illness. Patients have usually sustained a period of splanchnic hypoperfusion and the consequences can be devastating. Such patients usually manifest SIRS owing to tissue ischemia, and whether the patient is infected or becomes infected at some point can be very difficult to discern. Acute pancreatitis can complicate cardiopulmonary bypass or upper abdominal surgery (e.g. gastrectomy, splenectomy); most patients do not become infected and the administration of prophylactic antibiotics to patients with acute pancreatitis remains controversial.[26]

Outcomes of postoperative pancreatitis vary widely, depending on the severity of the attack. Acute acalculous cholecystitis has been reported as complicating virtually any operation but has a predilection for patients with trauma, burns, shock and emergency cardiac and peripheral vascular surgery.[39] The diagnosis is made most efficiently by bedside ultrasonography, and the treatment of choice is evolving to percutaneous cholecystostomy. The mortality rate of acute acalculous cholecystitis is approximately 30%.

Ischemic colitis is the most dangerous of these entities. The distribution and severity vary widely; sometimes the presentation can be as subtle as occult blood in the stool or an unexplained fever. When it does manifest itself, it usually does so within 72 hours of the insult that puts the patient at risk. The diagnosis is made most frequently by flexible lower gastrointestinal endoscopy but, because only the mucosa is inspected, endoscopy is not quantitative. Mild cases are probably noninfectious – there are no data to demonstrate that antibiotic therapy alters the course of mild ischemic colitis. Transmural colitis can lead to perforation; at worst, when severe ischemic colitis necessitates an emergency colectomy in the context of critical illness, the mortality rate may be as high as 80%.[40]

CT scan is probably the most useful modality for imaging the abdomen of seriously ill patients, particularly when there is substantial uncertainty as to the precise diagnosis. The benefits of imaging must be weighed against the formidable logistics and inherent risk of transporting a critically ill patient within the hospital for an imaging study. Moreover, it is possible to image the abdomen too soon (before about 7 days postoperatively) and thereby achieve a false-negative result. Fewer than one-half of abdominal-pelvic CT studies performed on critically ill patients yield meaningful information about diagnoses that are amenable to further intervention.[26]

Pneumonia

Although SSI is probably the nosocomial infection most closely associated with surgery, hospital-associated pneumonia (HAP) or specifically ventilator-associated pneumonia (VAP) is probably more common and certainly more dangerous. Long-term observational studies suggest that HAP is more common than SSI among surgical patients, and indicate clearly that the incidence of VAP is higher in surgical ICUs than in medical or pediatric ICUs.[1] In surgical subspecialty units (e.g. burns units, trauma units, neurosurgical units, cardiothoracic units), the incidence of VAP exceeds 15 cases/1000 days of mechanical ventilation (Table 65.6).[1] The increased incidence of VAP among surgical patients occurs despite comparable rates of mechanical ventilation in the ICU, which may reflect that intraoperative mechanical ventilation is not captured in ICU statistics if the patient is extubated early after surgery. However, surgical patients are at increased risk with respect to airway reflexes (because of anesthesia, analgesia and sedation), hypoventilation and atelectasis (because of the above factors plus painful chest or upper abdominal incisions), gastric intubation, ileus and numerous other factors. Trauma patients are at especially increased risk if they sustain a head or chest injury (e.g. pulmonary contusion, rib fractures) or are intoxicated with drugs or alcohol. The risk of VAP is increased by aspiration during endotracheal intubation, high severity of injury, blood transfusions, hyperglycemia, prolonged mechanical ventilation and the acute respiratory distress syndrome.

Table 65.6 Incidence of ventilator-associated pneumonia

Type of ICU	Ventilator utilization	Pooled mean	Median
Medical	0.49	7.3	6.0
Pediatric	0.45	4.9	3.9
Surgical	0.47	13.2	11.6
Cardiothoracic	0.47	10.5	9.5
Neurosurgical	0.38	14.9	11.9
Trauma	0.58	16.2	15.3
Burn	0.33	15.9	

Utilization, number of ventilator days per number of patient days; rates are per each 1000 days of indwelling artificial airway (e.g. endotracheal tube, tracheostomy tube).
From National Nosocomial Infections Surveillance System.[1]

Meticulous infection control to minimize the spread of pathogens around the unit, minimized sedation, daily sedation 'holidays', keeping the head of the patient's bed at an angle of 30° at all times, antiseptic or antibiotic decontamination of the oropharynx, endotracheal extubation at the earliest possible opportunity, early tracheostomy if extubation cannot occur promptly, early enteral nutrition and meticulous pulmonary toilet, including continuous aspiration of subglottic secretions, can all be part of a comprehensive program for the prevention of pneumonia (see Chapter 28).[40]

Bloodstream infection

Bacteremia is an unusual complication of most surgical infections. Bacteremia accompanies only 8% of intra-abdominal infections overall, although less common infections such as ascending cholangitis are characterized by positive blood cultures in about 90% of cases. Clostridial bacteremia can complicate emphysematous cholecystitis or occult perforation or neoplasm of the gastrointestinal tract.[41] Surgical site infections have been described as potential sources of staphylococcal or enterococcal bacteremias,[42] and staphylococcal or pseudomonal bacteremia can complicate pneumonia caused by those organisms. Suppurative phlebitis, a bacteremic complication of infected vascular access sites, usually a peripheral intravenous site, is a rare but potentially serious form of postsurgical bacteremia.[43]

Catheter-related bloodstream infection is relatively uncommon in surgical units compared with medical or pediatric units, despite the high reliance on central venous access and monitoring (Table 65.7).[1] According to the US Centers for Disease Control and Prevention, the operator inserting a central catheter must wear a cap, mask and sterile gown and gloves for all insertion procedures and the operative field must be draped widely.[18] Central venous catheter insertion under emergency conditions (e.g. trauma resuscitation, cardiac arrest) almost always has lapses of infection control, and therefore catheters inserted under such conditions should be removed (and replaced at a different site, if needed) as soon as the patient has been stabilized.

PRINCIPLES OF ANTIBIOTIC THERAPY

The decision to start empiric antibiotic therapy in the surgical patient should be made with great care. An occasional patient will develop an early infection, but when it occurs it is usually the delayed

Table 65.7 Incidence of central line-associated bloodstream infections, National Nosocomial Infections Surveillance System, 1995–2001

Type of ICU	Central line utilization	Pooled mean	Median
Medical	0.51	5.9	5.2
Pediatric	0.46	7.6	6.8
Surgical	0.66	5.3	4.9
Cardiothoracic	0.79	2.9	2.4
Neurosurgical	0.44	4.7	4.5
Trauma	0.63	7.9	7.0
Burn	0.49	9.7	

Utilization, number of central venous catheter days per number of patient days; rates are per 1000 days of indwelling catheter.
From National Nosocomial Infections Surveillance System.[1]

manifestation of a community-acquired infection that was occult at the time of admission. In the case of pneumonia that develops within 3 days after surgery, the clinician must be alert to the possibility of a pneumococcal pneumonia. However, 'atypical' pneumonia is almost unheard of in surgical practice unless the patient is profoundly immunosuppressed, and so there is seldom a consideration to administer a macrolide antibiotic for that indication.

Early-onset HAP is more likely to be caused by antibiotic-susceptible strains of *Staph. aureus*, *Haemophilus influenzae*, *E. coli* and *Klebsiella pneumoniae*, whereas late-onset HAP and VAP are more likely to be caused by multidrug-resistant pathogens such as MRSA, *P. aeruginosa*, *Acinetobacter* spp. and others. Considering that *Staph. aureus* and *P. aeruginosa* are the two most common health-care-associated pneumonia pathogens, initial empiric antibiotic therapy must account for both pathogens, meaning two-drug anti-MRSA and anti-pseudomonal treatment in institutions with a high prevalence of MRSA, until targeted therapy is feasible based upon the microbiology laboratory findings.

Because the consequences of overtreatment (primarily later nosocomial infections with multidrug-resistant bacteria or opportunistic pathogens such as fungi) can be as severe as undertreatment, ongoing antimicrobial therapy should be reviewed every day in every patient with a bias to stopping treatment as soon as the patient improves. It may be possible to withhold or truncate antibiotic therapy by the use of a propensity score, such as the Clinical Pulmonary Infection Score.[44,45]

It has been hypothesized that 'antibiotic heterogeneity' – changing the pattern of antibiotic use either randomly by computerized decision support or by scheduled change of antibiotic class (e.g. antibiotic 'rotation' or 'cycling' programs) – may be a valid alternative to antibiotic control or restriction programs to prevent the emergence of multidrug-resistant bacteria. Initial data in surgical patients provide support for the 'cycling' hypothesis. Quarterly cycling of cefepime, ciprofloxacin, imipenem–cilastatin and piperacillin–tazobactam resulted in decreased use of aminoglycosides, vancomycin and antifungal agents.[46] Moreover, infections caused by resistant bacteria were reduced, as was mortality. It is not known which attribute or attributes (drug, order of administration, duration of cycle, omission of other drugs for potential empiric use) contribute to the effect. Further clinical studies will be needed to confirm the safety and effectiveness of such antibiotic usage strategies in the surgical patient.

REFERENCES

References for this chapter can be found online at http://www.expertconsult.com

Chapter 66

Alastair Miller

Recreational infections

INTRODUCTION

At the end of the first decade of the 21st century we still eagerly await the arrival of the predicted age of leisure. However, even with major time pressures, people continue to spend their restricted leisure hours in more adventurous and imaginative ways. This chapter examines how these activities expose them to increased risks of infection and disease. Along with many other factors that predispose to clinical infection, recreational behavior may either expose the host to infective organisms or modify the immune response, thereby increasing susceptibility to infection and disease. Recreational infection can be classified by recreational activity or according to the particular infections (or systems infected – Table 66.1) Inevitably there is considerable overlap with other sections of this book, such as international medicine (see Chapters 101–128), sexually transmitted diseases (Chapters 57–60) and zoonotic infections (Chapter 164).

TRAVEL

Travel is a common recreational activity either as an end in itself or in order to participate in other recreations. Travelers may be exposed to infection either during the journey or at their destination. During travel there may be exposure to gastrointestinal pathogens in mass-produced food or exposure to respiratory pathogens from air-conditioning units and fellow travelers. In addition, the general fatigue of long-distance travel may perhaps lower resistance to infection in a nonspecific manner.

Table 66.1 Recreational activity associated with infection

Activity	Risk
Travel (especially 'adventure travel')	Infection during travel (gastrointestinal and respiratory pathogens) Geographic (tropical) infection
Animal contact: keeping pets, zoo/farm visits, etc.	Zoonotic infection
Outdoor activities: camping, trekking, barbeques, etc.	Zoonoses acquired by ingestion, inoculation, inhalation and arthropod transmission
Water contact: bathing, jacuzzi, canoeing, sailing, etc.	Ingestion, inoculation and inhalation
Contact sports: rugby, wrestling, football, etc.	Skin infection, blood-borne viruses
Vigorous exercise	Possibly respiratory infection

Outbreaks of food poisoning from airline food occur frequently and are well described,[1] and even cholera has been transmitted in this way.[2] An estimated 4.5 million North Americans travel on cruise ships each year and a recent review has drawn attention to the incidence of respiratory and gastrointestinal infection associated with this activity. Outbreaks of gastrointestinal illness have been caused by contaminated food and water,[3] and in addition there have been descriptions of more prolonged outbreaks of infection associated with the possibility of person-to-person transmission and environmental contamination. This type of outbreak is usually thought to be due to viral infection with organisms such as astroviruses, caliciviruses (norovirus) and reoviruses (rotavirus). The classification of these gastrointestinal viral pathogens is complex and changes rapidly.[4–7] Historically, a number of bacterial infections including cholera and typhoid have been associated with ship-borne spread.

Respiratory infection, including multidrug-resistant pulmonary tuberculosis, has also been transmitted during aircraft flight,[8,9] and there is a well-recognized association between outbreaks of *Legionella* infection and air-conditioning systems in holiday hotels.[10]

Western travelers increasingly seek more exotic destinations where, as a result of poverty and poor infrastructure in the local population, they may be at risk of infection with common pathogens (particularly of the gastrointestinal and respiratory tracts). They may also be at risk of more exotic infections that do not exist in their own country (e.g. malaria). By the nature of the travel, the patient with infection often may not have access to the level of diagnostic and therapeutic interventions that would be regarded as standard in the developed world, so empiric treatment of presumed infections is often required. These risks are discussed in detail in Chapter 102.

ZOONOSES

Zoonoses are infections of animals which can be transmitted to humans, who may act either as a dead-end host or may propagate the infection further. The resulting infection may or may not be clinically apparent. This topic is addressed in Chapters 110, 119–128, 160 and 164 but issues specifically related to recreation are discussed here. Many leisure activities increase the opportunity for contact between humans and animals, with consequent increased risk of infection. Keeping pet animals is a common recreational pastime and increases risks of zoonotic infection.

Hiking and camping (particularly light-weight 'backpacking') increase the risk of zoonoses. People may hike in a temperate climate or, increasingly, may choose to trek in a tropical or developing country.

These activities increase the potential for contact with infected animals. Infection can then be transmitted by a number of possible routes, such as:

- inhalation (e.g. Q fever, anthrax);
- ingestion of contaminated food or water (e.g. *Salmonella*, *Brucella*, *Toxoplasma*);
- animal bites (e.g. rabies, skin infections);
- exposure of skin to contaminated water (e.g. leptospirosis, schistosomiasis, mycobacteria)
- exposure of skin to sand (e.g. strongyloides, cutaneous larva migrans and jigger flea); and
- via arthropod vectors (e.g. arboviruses, Lyme disease).

Zoonotic infection acquired by inhalation

Inhaled zoonoses that can be acquired by the intrepid outdoor explorer include Q fever (caused by *Coxiella burnetii*) and brucellosis (more commonly acquired by ingestion). Rarer problems include plague, anthrax, tularemia and psittacosis.[11]

Zoonotic infection acquired by ingestion

Many of the common 'food poisoning' organisms are zoonoses, and these are discussed in detail in Chapters 34 and 109. However, there are certain recreational activities that particularly expose participants to increased risks of ingesting pathogenic organisms (which are often zoonotic, although they may be exclusively human parasites). Backpackers drinking inadequately boiled or purified water may become infected with *Cryptosporidium* spp., *Giardia* spp., hepatitis A, *Aeromonas* spp. and *Salmonella* spp. Barbecues are particularly notorious for leading to inadequately cooked meat (or fish) and consequent infection with *Salmonella* spp.,[12] *Campylobacter* spp.[13] and other more exotic organisms such as *Trichinella* spp. There have been well-documented outbreaks of cryptosporidial infection in children enjoying recreational visits to farm open days.[14,15]

Arthropod-borne zoonoses

Viruses that must spend some of their life cycle in a blood-sucking arthropod are known as arboviruses (see Chapter 164). Over 200 such viruses have been identified and over 70 have been reported as affecting humans. In 1994, 100 cases of presumed or confirmed arboviral disease were reported from 20 states of the USA.[16] These were all encephalitis viruses (mainly Californian and St Louis encephalitis), principally spread by mosquitoes. In 2001 there was a large outbreak of West Nile virus in the Eastern USA with 48 human cases and numerous infections of birds (principally crows)[17] and West Nile virus is now seen throughout the USA and Southern Europe. Yellow fever is a life-threatening mosquito-borne, zoonotic viral infection and the illness remains a risk for travelers and residents during outdoor activities in endemic regions of Africa and South America. In Europe, tick-borne encephalitis is regularly reported from Austria and southern Germany.[18] A major risk factor is outdoor recreation (in particular, walking through long grass while wearing short trousers). An inactivated vaccine is available.

Tick-borne rickettsiae (see Chapters 122 and 176) are also potential pathogens among those who enjoy 'the great outdoors'.[19] They are mainly of the spotted fever group. In southern Europe, Africa and India, the disease is called tick typhus or boutonneuse fever and is caused by *Rickettsia conorii*. In the USA, it is Rocky Mountain spotted fever, caused by *Rickettsia rickettsii*. New rickettsioses identified during the past decade include Japanese spotted fever, Astrakhan fever, Flinders Island spotted fever, California flea typhus, African tick-site fever and *R. slovaca* infections in central France.

Scrub typhus may affect the trekker in eastern Asia. The infective organism is *Orientia tsutsugamushi*. The reservoir is rodents and the vector is the larva (chigger) of the trombiculid mite. Clinically the disease resembles other rickettsial infections, and prevention and treatment strategies are similar.

Lyme disease (see Chapter 43), caused by infection with *Borrelia burgdorferi*, is another condition that may be acquired by recreational exposure, especially in the northern hemisphere. The reservoir consists of mammals such as rodents and deer, with infection being spread by hard ticks (the *Ixodes ricinus* complex).

INFECTIONS CAUSED BY EXPOSURE TO WATER

A large number of infections can be caused by exposure to water (Table 66.2), and some of these have already been discussed in the section on zoonoses. Exposure to water can take place in a variety of recreational contexts. Trekkers and fishermen may wade through infected water, people may bathe in fresh water or sea water, and people may undertake other non-bathing recreational activities in water (e.g. water skiing, sailing, canoeing). There is also an increasing popularity of spa baths, whirlpool baths and jacuzzis.

As with arthropod-borne infections, infections related to water may be acquired by a number of routes, including ingestion, aspiration, inhalation of aerosols, and penetration of skin or mucous membranes by invasive organisms. A variety of clinical infections, including gastrointestinal infection, hepatitis, conjunctivitis, pneumonia, skin and soft tissue infection, may result, and numerous diverse organisms have been implicated.

Table 66.2 Infections spread via recreational contact with water

Mode of spread	Bacteria	Virus	Protozoa	Helminths
Fecal–oral spread (accidental ingestion)	*Escherichia coli* *Salmonella* spp. *Vibrio* spp. *Aeromonas* spp. *Shigella* spp.	Enteroviruses (including polio) Hepatitis A Norovirus	*Cryptosporidia* *Giardia* spp.	
Direct inoculation	*Leptospira* *Mycobacterium marinum* *Pseudomonas* spp. *Vibrio* spp. *Aeromonas* spp.		*Naegleria* *Acanthamoeba*	*Schistosoma*
Aerosol or aspiration	*Legionella* spp. *Pseudomonas*	Adenoviruses		

Pathogenic organisms may enter the water from exogenous sources such as human contamination (e.g. sewage), animal and bird contamination, and farm effluent. Organisms may also come directly from aquatic animals or protozoa or be free living in the water supply.

Infection associated with whirlpools

Jacuzzis, whirlpool baths and spa baths, which are increasingly found in leisure resorts, are all based on the principle of being bathed in warm water through which jets of water and bubbles of air are blown in order to produce feelings of relaxation and pleasure. They therefore share the potential for the transmission of cutaneous, mucosal and respiratory infection. The main pathogens implicated in these infections are *Pseudomonas aeruginosa* and *Legionella pneumophila*. *Pseudomonas* infections of the skin were initially described in the early 1980s.[20] The first reports were of folliculitis, but infection of wounds, eyes, ears and urinary tract have now been described.[21] Fatal *Pseudomonas* pneumonia in an immunocompetent male has been associated with jacuzzi exposure.[22]

Legionella spp. (mainly *L. pneumophila*, but other species are also implicated) cause two distinct syndromes:

- legionnaires' disease (or legionnaires' pneumonia), which is usually a severe pneumonic illness requiring appropriate antibiotic treatment; and
- Pontiac fever, which is generally a more benign self-limiting illness causing myalgia, fever and headache.

The latter syndrome has frequently been associated with whirlpool use, although a prolonged outbreak of legionnaires' pneumonia among cruise ship passengers associated with exposure to a contaminated whirlpool spa has been described.[23]

Infection from bathing

Numerous case reports and reviews have associated bathing in swimming pools, natural fresh water and the sea with gastrointestinal, respiratory and cutaneous infection. In swimming pools there have been reports of infection with *Shigella*, *Giardia* and *Cryptosporidium* spp., and various viruses including hepatitis A virus.[24]

The association of sea bathing and disease is a major political issue as millions of dollars are spent in the developed world in an effort to improve sewage disposal and enhance the quality of bathing water. Microbiologic standards now exist for bathing water in Europe and North America. There is certainly a risk of infection from swimming in heavily contaminated water but the risk of minor symptomatic infection from swimming in less heavily polluted water remains contentious.[25] In the 1950s the UK Public Health Laboratory Service used a case-controlled method and showed no link between polio and sea bathing.[26] Cabelli's classic work under the aegis of the US Environmental Protection Agency in the 1970s suggested a dose–response relationship between the microbiologic contamination of bathing water and self-reporting of gastrointestinal symptoms.[27] These studies have been criticized for looking at self-reported symptoms in self-selected groups, with no control for other risk factors for gastrointestinal symptoms.

More recently, a large UK study attempted to address these criticisms by randomizing holiday makers to be 'swimmers' or 'non-swimmers'.[28] This study showed a significantly higher rate of gastroenteritis in the swimmers and demonstrated a dose–response relationship between occurrence of symptoms and concentrations of fecal streptococci (although only with the concentration measured at chest height). Currently, coliform counts are used to assess water quality and the authors of the above study could not demonstrate a correlation between symptoms and the coliform count.

In addition to gastroenteritis, an Australian study showed increased reporting of respiratory, eye and ear symptoms amongst swimming beach-goers as opposed to non-swimming ones. The incidence of the reported symptoms increased with increasing levels of pollution.[29] The authors of the UK study have now published their results of non-enteric illness acquired during bathing and their results are in broad agreement with those of the Australian study.[30]

Infection in non-swimming recreational water activities

In addition to the hazards of bathing detailed above, many people are exposed to infection by recreational use of water where swimming is not the primary purpose. Such activities include angling, canoeing, water skiing, sailing and white water rafting.

Leptospirosis (see Chapters 124 and 171) is traditionally regarded as a significant risk. It is estimated that on average in the UK there are 5 million recreational water users each year exclusive of bathers, and yet among this at-risk population there are only 2.5 cases of leptospirosis a year.[31] The annual total incidence of leptospirosis in England and Wales is more than 10 times that figure; it occurs principally among agricultural workers. Leptospirosis is a zoonotic infection that is mainly carried by rodents. It is estimated that about 25% of the rats in UK are infected. The risk of contracting infection relates less to the overall water quality than to the density of the local rodent population. Triathlon and other forms of 'adventure racing' have also led to outbreaks of leptospirosis. Sejvar *et al*. described the outbreak that occurred during the 2000 'Eco-Challenge' event in Sabah, Borneo. Of 304 competing athletes, 42% met the case definition for leptospirosis.[32] The authors suggest that taking 200 mg of doxycycline weekly during exposure may limit infection and disease (a strategy previously demonstrated to be effective by the US military).

By the nature of the sport, canoeing involves high level exposure to water and, in addition to leptospirosis, other infections can be acquired. There is an increased incidence of gastrointestinal symptoms, and it has been shown that more than 50% of canoeists had experienced 'flu-like' symptoms shortly after canoeing.[33] An outbreak of blastomycosis has been reported amongst canoeists in Wisconsin rivers.[34]

Miscellaneous water-related infections

Naegleria and *Acanthamoeba* spp. are free-living amebae with no insect vector or human carrier state. They have been isolated on a worldwide basis from water and soil, and rarely can produce a severe amebic meningoencephalitis that is usually fatal (see Chapter 182). Schistosomiasis is dealt with in detail in Chapter 112. The cercariae of human schistosomes penetrate intact human skin and then migrate to their favored site to commence their maturation. Within 24 hours the penetration of the skin can produce a pruritic papular rash that is called 'swimmers' itch'. Avian schistosomes are found in temperate climates, including in the Great Lakes of North America, and although they are unable to mature past the cercarial stage in a human host and therefore cannot give rise to later stage schistosomiasis, they can be responsible for producing a significant 'swimmers' itch'.

Katayama fever occurs typically 4–8 weeks after infection and is associated with fever, chills, headache and cough. There is hepatosplenomegaly and lymphadenopathy, and usually a significant eosinophilia. It is caused both by *Schistosoma japonicum* and *S. mansoni*, the latter being particularly recognized in swimmers who have bathed in Lake Malawi and the other rivers and lakes of East Africa.

INFECTION SPREAD BY DIRECT CONTACT

Many sports require close physical contact on the sports field and may also involve close contact in the changing rooms with shared towels, shaving equipment, etc. Tetanus is caused by contamination of a wound by the spores of *Clostridium tetani*. After contamination, the organism then elaborates a toxin that produces the clinical syndrome of tetanus. Although immunization against tetanus is widely practiced, there is still a risk to those playing contact sports (especially rugby and football), as well as to those pursuing more leisurely activities such as gardening.

The close contact in the scrum of rugby football may transmit herpes simplex virus and cause a condition called scrumpox or herpes gladiatorium. This is highly infectious and may spread rapidly between players. Aciclovir is effective treatment. Staphylococcal infection (including

methicillin-resistant *Staphylococcus aureus*) may also be spread in similar circumstances.

The moist atmosphere of changing rooms may promote the transmission of respiratory infections as well as a number of cutaneous infections such as verrucas, athlete's foot (*Tinea pedis*) and dhobie itch (*Tinea cruris*). The spread of these tineal infections may be facilitated by sharing towels and washing equipment.

Gardening is usually considered a fairly safe pastime, but tetanus is a potential risk and sporotrichosis (see Chapter 179) can be acquired by scratches from rose thorns and similar injuries.

Blood-borne infection transmission in contact sport

The risk of transmission of blood-borne pathogens during contact sport is thought to be extremely low. There were large outbreaks of hepatitis amongst orienteers in Sweden from 1956 to 1966, and on the basis of the clinical and epidemiologic picture these were assumed to be due to hepatitis B virus (HBV), although a serologic test was not available.[35] As part of these outbreaks, 568 cases of hepatitis occurred between 1957 and 1963. Several modes of transmission were postulated, including twigs contaminated with infected blood inoculating subsequent competitors, contaminated water in stagnant pools and transmission during washing after competition. It was established that 95% of orienteers received scratches or wounds during the competition. The outbreak was curtailed by the introduction of regulations that banned competitors who had hepatitis from competing for 15 months and that specified compulsory protective clothing. More cases were reported when these regulations were relaxed. There has also been a report of an outbreak of HBV infection among sumo wrestlers in Japan.[36] There is one report from Italy of an HIV-positive football player transmitting infection to another player during a collision when both players were bleeding profusely,[37] but the risks are generally considered to be negligible.

Numerous guidelines exist to limit the risk still further.[36] In rugby football, for example, a player with an open or bleeding wound must leave the field until the wound is covered and the bleeding controlled.

EFFECT OF EXERCISE ON THE IMMUNE SYSTEM

Although it is clear from the above that recreational activity can expose participants to numerous infective agents that they might not otherwise encounter, it is by no means so obvious whether recreation (in particular, vigorous exercise) has any clinically significant effect on immune function.[38] There is increasing evidence that physical exercise may bring benefit in terms of cardiovascular health. However, the evidence from the immunologic perspective is less obvious.

There are numerous anecdotal reports of increased incidence of upper respiratory infection in athletes and there have been attempts to examine this systematically. However, despite numerous reviews there is no consistent association between physical activity and incidence of clinical upper respiratory infection. Nor has any consistent immunologic abnormality been demonstrated in high-level athletes. This inconsistency has many parallels with the situation in chronic fatigue syndrome, and many top athletes who have recurrent infections develop a clinical condition indistinguishable from chronic fatigue syndrome.

The overall message from anecdotal reports, from case-controlled studies of symptoms, from animal studies and from laboratory testing of immune function seems to be that moderate regular exercise enhances immunity whereas sudden unusual exertion or consistent, very high-grade training may have a deleterious effect. This is described as the 'J-shaped curve' correlating exercise and immunity.[38,39] Animal studies suggest that exercising before infection is beneficial, whereas exercising when infected is harmful. Some of the reported immunologic effects of exercise are listed in Table 66.3.

It is generally advised that people who are suffering from acute infections do not participate in vigorous exercise. This seems common sense and most people would probably not feel like doing so, although definite evidence of harm remains contentious.

Table 66.3 Effects of exercise on the immune system

Symptoms/self-reported infections (anecdote and case-control)	Most studies suggest that moderate regular exercise reduces frequency and severity of upper respiratory tract infections but excessive training increases it Many studies are subjective and it may be that athletes are more aware of their symptoms than are controls Some increase in infection may be due to local factors such as mouth breathing rather than to any change in systemic immunity
Animal studies	Exhaustive exercise during experimental viral infection increases mortality and morbidity from that viral infection; the effect may be attenuated by exercise prior to infection Similar results have been shown in pneumococcal infection – exercise prior to infection protected against mortality but forced exercise after infection enhanced mortality
Immune function studies	Moderate exercise in HIV-positive people has been shown to produce some increase in $CD4^+$ lymphocyte counts Excessive training in non-HIV-infected people has been shown to suppress $CD4^+$ lymphocyte counts Heavy exercise decreases lymphocyte proliferation and levels of IgA; the decreased levels of IgA may correlate with increased incidence of upper respiratory tract infections Regular moderate exercise will increase levels of natural killer lymphocytes Exercise generally increases the release of proinflammatory cytokines and acute phase proteins

CONCLUSION

Recreational activities can expose participants to novel infectious agents that they are less likely to encounter in other contexts. In many of these the diagnosis may not be very obvious unless the condition is considered. Physicians need to add 'recreational history' to the already extensive list of travel, occupational and animal exposure details about which they need to enquire when evaluating a patient with a suspected infection. Whether recreational activity can alter immune function remains more controversial, although there seems to be increasing consensus that regular physical exercise may be of benefit but that excessive exercise may increase risks of infection.

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References for this chapter can be found online at http://www.expertconsult.com

Chapter 67

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Occupational infections

The traditional model of infectious disease causation is the epidemiologic triangle. It has three components: an external agent, a susceptible host and environmental factors that bring the host and the agent together to produce an infection.[1]

Occupational infections are defined by two of these components. Particular infectious agents or organisms may be associated with a workplace or occupational setting and specific work activities may predispose the worker to exposure, resulting in an occupational infection.

THE IMPORTANCE OF OCCUPATIONAL INFECTIONS

Although difficult to quantify, occupational infections are probably uncommon when compared to those that result from non-occupational activities or environments.

Infections can only be confidently attributed to occupational exposure as a result of careful epidemiologic investigation. Case reports, surveillance data and cross-sectional surveys may lead to a hypothesis that a particular infection is diagnosed more commonly in one or other group of workers. However, in order to accurately estimate an odds ratio or relative risk, a carefully designed, adequately powered, case-control or cohort study will be required. Assuming that bias and confounding factors can be adequately controlled, it may then be possible to satisfy the Bradford Hill criteria for causation. Few infections have been subject to this rigorous approach.

In the individual case where an occupational infection is suspected, it is important to take an adequate occupational history. A workplace visit to assess the system of work can help confirm the likelihood of the infection being acquired through workplace factors.[2] A high index of suspicion will ensure that occupational infections are not missed. If an occupational source is not recognized, there will be a continuing risk to other workers in the same work area, and the affected individual may be at risk of re-infection on return to work, especially if full immunity following the initial infection does not occur.

A number of occupational infections that are of historical interest in developed countries are still found in less developed parts of the world, and staff who are traveling to work in those areas may acquire these infections. As the working environment becomes ever more complex, there is the potential for new occupational infections to emerge.

Infections acquired occupationally may spread to other workers or the workers' families or social contacts. As with any infection, occupational infections are controlled by controlling the source of infection, its route of transmission and by protecting susceptible persons. Most occupational infections can be prevented if appropriate measures are implemented. Some occupational infections, especially those for which vaccines are available (e.g. hepatitis B), are more amenable to prevention than others (e.g. hepatitis C). Health education and preventive programs in the workplace provide a good system for minimizing the risk of occupational infections. The largest employer in the United Kingdom – i.e. the National Health Service – has a requirement for every health-care facility to have access to an occupational health service. This has helped in reducing the burden of occupationally acquired infections in the health-care community.[3] The system is not as well developed in other industries where there is a recognized risk of occupational infections (e.g. in farming).

This chapter is written from a United Kingdom perspective and describes the arrangements and structures for public health and occupational health practice that will be found in the UK. Similar arrangements can be expected in most industrialized countries. For example, the US Centers for Disease Control and Prevention (CDC) have published guidelines for preventing the transmission of infectious diseases in the health-care workplace. These are available at http://www.cdc.gov/ncidod/dhqp/worker.html.

The US National Institute for Occupational Safety and Health (NIOSH), which is part of CDC, has published research and recommendations on most aspects of work-related injury and illness including infections (see http://www.cdc.gov/niosh/topics/diseases.html).

The Australian Safety and Compensation Council (ASCC) provides policy advice on all aspects of occupational health and safety to allow local legislators to enact and enforce laws. A recent ASCC report focuses on the more common and important infections associated with occupations in Australia.[4]

CLASSIFICATION OF OCCUPATIONAL INFECTIONS

Mode of transmission, occupations and examples of infections are outlined in Table 67.1.

SURVEILLANCE OF OCCUPATIONAL INFECTIONS

Surveillance of occupationally acquired infection is problematic. A range of data sources are available. The examples described here are from the United Kingdom but similar systems may be found in other countries.

It is a legal requirement for clinicians to notify certain specified infectious diseases to local health authorities. The current list of notifiable infections for the UK covers common infections (including viral hepatitis and tuberculosis) that may be occupationally acquired, as well as rarities such as leptospirosis, rabies and anthrax. Microbiology laboratories also report micro-organisms of public health significance to local health authorities. Outputs from these surveillance schemes can be viewed at http://www.hpa.org.uk/infections/topics_az/noids/menu.htm.

Table 67.1 Occupational infections

Mode of transmission	Occupations	Examples of infections
Contact with animals and animal products (zoonoses)	*Source of infection and route of transmission*: Contact with material from infected animals, by inhalation, ingestion, bite or scratch Contact with contaminated animal product (carcasses, placental tissue, hair, wool or hides) from endemic area Contact with animal excreta by fecal–oral or percutaneous route or in water *Occupation types*: Farm worker[1] Poultry worker Veterinarian Butcher Slaughterman Wool and leather worker Zoo worker Animal handler Sewage worker	*Salmonella* Cryptosporidiosis *Escherichia coli* O157 *Campylobacter* *Yersinia* Brucellosis Leptospirosis Q fever Psittacosis Ovine chlamydia Cat-scratch fever Pasteurellosis *Capnocytophaga canimorsus* Anthrax Rabies *Echinococcus* Schistosomiasis Avian influenza Newcastle disease *Streptococcus suis* B-virus infection Monkeypox Glanders Hendra and Nipah viral diseases Rat bite fevers Rodents main reservoir, transmission by inhalation of excreta: • arenaviral hemorrhagic fevers • hantavirus infection • lymphocytic choriomeningitis • Lassa fever Exposure to sewage is not a risk factor for *Helicobacter pylori* or hepatitis A or E viruses in sewage workers who are properly trained and provided with personal protective equipment[2,3]
Exposure to vectors	*Source of infection and route of transmission*: Exposure to tick, flea, or mites through work in infested area or in rodent-infested building *Occupation types*: Farm worker Forestry worker Overseas worker Pest control worker	Borrelia infections (Lyme disease, relapsing fever) Babesiosis Ehrlichiosis Tularemia Plague Scrub typhus Typhus Tick-borne rickettsial infections Bartonella infection Arthropod-borne viral fevers (Over 100 arboviruses cause disease in humans, often as an incidental host in a zoonotic cycle. Infection can occur in those working in endemic areas and also through laboratory exposure) (see Chapter 164)
Care of patients	*Source of infection and route of transmission*: Contact with patients, respiratory or blood-borne Contact with human excreta Skin-to-skin contact with infected patient	HIV Hepatitis B Hepatitis C Staphylococcal infection, e.g. methicillin-resistant *Staphylococcus aureus* (MRSA), Panton–Valentine leukocidin (PVL)

Table 67.1 Occupational infections—cont'd

Mode of transmission	Occupations	Examples of infections
	Occupation types: Health-care worker[4,5] Dental worker Embalmer Teacher[6] Sewage worker Laboratory worker	Typhoid/paratyphoid Hepatitis A Cryptosporidiosis Norovirus Tuberculosis Mycoplasma infection Scabies West Nile virus infection[7] Influenza Measles Mumps Meningococcus Parvovirus[8] Cytomegalovirus[9] There is some evidence that working in a child day-care centre is associated with higher seroprevalence of antibodies to cytomegalovirus infection but not erythrovirus (formerly parvovirus) B19 infection, although both of these infections are common in the general population[10] Pertussis Varicella Rubella Adenovirus Diphtheria Ebola–Marburg viral infection Lassa fever Monkeypox Severe acute respiratory syndrome (SARS)
Environmental sources, exposure to soil	*Source of infection and route of transmission*: Ploughing, digging or excavating soil in endemic area Contact with dust containing rodent feces, bird roosts, chicken coops or bat-inhabited caves in endemic area *Occupation types*: Building cleaning worker Construction worker Archaeologist	Tetanus Listeria Histoplasmosis Coccidioidomycosis Paracoccidioidomycosis Blastomycosis Hookworm
Occupational skin infections	*Source of infection and route of transmission*: Cleaning pools or aquarium Dental work in patients' mouths Barefoot contact with contaminated soil in endemic area Working continuously with wet hands Touching infected farm animals, plants containing thorns, splinters or sphagnum moss, infected meat or poultry, infected fish or shellfish *Occupation types*: Dental worker Farm worker Veterinarian Florist Slaughterer Butcher Fisherman Aquarium worker	Orf Ringworm Herpetic whitlow Erysipeloid *Mycobacterium marinum* skin infection Viral warts *Candida paronychia* Chromomycosis Cutaneous larva migrans Sporotrichosis *Vibrio vulnificus* infection Cutaneous anthrax

In neither of these systems is the occupation of the case requested or recorded. Local and national health authorities may enhance the data that are collected as part of case investigation and management, and this may include occupation and other relevant risk factors. However, such additional data are not consistently collated, analyzed or disseminated and, when available, are susceptible to ascertainment and reporting bias.

To overcome these shortcomings, active surveillance of selected occupationally acquired infections is carried out by the Surveillance of Infectious Diseases at Work (SIDAW) Project at the University of Manchester. Data are contributed by local public health staff each month. Most reports relate to diarrheal disease and scabies in health-care workers but legionellosis, tuberculosis and cutaneous anthrax have been reported.

Other occupational surveillance schemes also occasionally report occupational infections. Outputs from these surveillance schemes can be viewed at http://www.hse.gov.uk/statistics/indexoftables.htm.

Acute illnesses due to biologic agents encountered during a specified work activity – for example Lyme disease, Q fever, rabies, *Streptococcus suis*, tetanus, tuberculosis, anthrax, brucellosis, avian chlamydiosis, ovine chlamydiosis, hepatitis, legionellosis and leptospirosis – are reportable to the Health and Safety Executive under Reporting of Injuries, Diseases and Dangerous Occurrences Regulations 1995 (RIDDOR 95).[5] This legal requirement to notify is intended to provide information on trends and to facilitate prevention. More information and links to outputs can be viewed at http://www.hse.gov.uk/statistics/sources.htm. Reports on infections are infrequent.

The Industrial Injuries Disablement Benefit (IIDB) Scheme provides benefits to employees if they develop a prescribed occupational disease. Diseases are prescribed when there is a recognized risk to workers in an occupation and where the risk is uncommon or absent in the general population. For some occupational diseases there is a strong association with occupation and the disease may rarely occur outside work (e.g. mesothelioma, coal miner's pneumoconiosis). However, most infections are common in the general population and it is difficult to establish a causal link with the occupation. In lay terms an infection will be attributed to an occupation if it is *more likely than not* to be caused by that occupation. In epidemiologic terms this means an attributable fraction (the proportion of the additional risk that can be attributed to the exposure in the exposed population) of 50% or more which equates to a relative risk of 2 (a doubling of the background risk caused by exposure).

Prescribed infections include:

- anthrax where work involves contact with animals infected with anthrax, or the handling of animal products or residues;
- glanders where work involves contact with equine animals or their carcasses;
- leptospirosis where work involves places liable to be infested by rodents or other small mammals, handling dogs, or contact with pigs or bovine animals or meat products;
- hepatitis A virus infection where work involves contact with raw sewage; and
- hepatitis B or C virus infection where work involves contact with human blood products or other sources.

Ancylostomiasis where work involves work in or about a mine is included in the list of UK prescribed diseases, although there is scant evidence that mining carries an increased risk of hookworm infestation. Earlier observations indicated a risk of anemia in Cornish tin miners, attributed to a lack of toilet facilities in the mines leading to spread of hookworm infestation.[6] Interestingly, this risk was not seen in coal mines. It has also not been reported as a risk in tin mines in other countries (e.g. Malaysia), even though hookworm infestation in the tropics is prevalent. The methods used for tin mining in different countries can, however, be different.[7] Most of the coal mines and tin mines in the UK are now closed, although ancylostomiasis remains on the prescribed diseases list. A complete list of prescribed infections can be viewed at http://www.dwp.gov.uk/advisers/db1/appendix/appendix1.asp.

The UK Labour Force Survey (LFS) is a national survey of 52 000 households on self-reported work-related illness. THOR-GP is a UK-wide surveillance scheme in which 270 participating general practitioners report cases of work-related ill health. Participants make a judgment as to whether a new case should be attributed to work on the balance of probabilities. Additional information and links to outputs can be viewed at http://www.hse.gov.uk/statistics/sources.htm. Again, reports on infections are infrequent.

PREVENTION AND CONTROL OF OCCUPATIONAL INFECTIONS

The control of any occupational infection requires a detailed knowledge of its epidemiology, clinical features, reservoir, mode of transmission, incubation period and communicable period. To prevent and control infection, measures are necessary to eliminate the source of infection and the route of transmission. Susceptible workers can be offered protection with antibiotics or immunization.

The Control of Substances Hazardous to Health (COSHH) Regulations 2002 require employers to assess the risks from exposure to all hazardous substances (including biologic agents) and to implement measures to protect workers and others from those risks as far as is reasonably practicable.[8] Following a workplace risk assessment, exposure to potential infection should be eliminated by changing working practices and removing hazardous products or waste. Residual risk is controlled by promoting good occupational hygiene and environmental hygiene, and by focusing on design and engineering controls. Staff training and provision and use of personal protective equipment (PPE) are key measures (Table 67.2).

Table 67.2 Control of occupational infection

Controlling the source of infection	In the case of zoonoses, best practice should be observed with respect to animal husbandry, biosecurity of animal houses, feed and water, hygiene of animal houses and equipment, inspection, testing and certification and quarantine. Codes of practice are available and some of these are backed by legal measures In the case of human sources, prompt action is needed to isolate the case while infectious and to treat if possible to render the case noninfectious.
Controlling the route of transmission	Guidelines are available which detail the measures that should be implemented to prevent transmission, including handwashing and use of appropriate personal protective equipment. In the health-care workplace, standard precautions are widely promoted in addition to enhanced measures for specific infections
Protecting susceptible workers	Antibiotic or antiviral chemoprophylaxis may be required. All staff should be up to date with their routine immunizations (tetanus; diphtheria; polio; measles, mumps and rubella). Immunization is cost-effective for some groups of workers, particularly health-care workers and laboratory workers. Health warning cards may be issued to at-risk workers

Immunization

All workers should be fully immunized according to the routine immunization schedule.[9] In UK this comprises diphtheria; tetanus; pertussis; polio; measles, mumps and rubella (MMR); *Haemophilus influenzae* b (Hib); meningitis C (MenC) and pneumococcal vaccines. See: http://www.immunisation.nhs.uk/Immunisation_Schedule for further details.

In addition, selective immunization may be recommended for groups of workers at increased risk[10] (Table 67.3).

Immunization for laboratory and pathology staff

Laboratory and pathology staff handle pathogens or potentially infected specimens, and mortuary staff are potentially exposed to infected cadavers.[11] Other laboratory personnel include cleaners, porters and administrative staff. Guidelines for morticians and embalmers can be viewed at http://www.hpa.org.uk/webw/HPAweb&HPAwebStandard/HPAweb_C/1200660060264?p=1200660029736.

All staff should have had all routinely recommended immunizations, which in the UK include tetanus, diphtheria, polio, MMR and MenC. Staff handling fecal specimens who may be exposed to polio viruses should have a reinforcing polio immunization every 10 years. Staff who may be exposed to diphtheria should have antibody levels tested 3 months after immunization. The recommended level is 0.01 IU/ml for those involved in routine diagnostic testing and 0.1 IU/ml for those exposed to toxigenic strains. A reinforcing dose is recommended every 10 years. Additional recommendations for laboratory and pathology staff are summarized in Table 67.4.

Table 67.4 Immunization of laboratory and pathology staff against specific occupational infections

Bacille Calmette–Guérin (BCG)	Recommended for microbiology and pathology staff, mortuary staff and others at high risk
Hepatitis B	Recommended for laboratory staff who have direct contact with patients' blood or tissues. Antibody levels should be checked after immunization
Hepatitis A, Japanese encephalitis, cholera, meningococcal serogroups A, C, Y, W135, smallpox, tick-borne encephalitis, typhoid, yellow fever, influenza, varicella, anthrax, rabies	Recommended for staff handling or carrying out research on specific organisms and those working in reference laboratories or infectious disease hospitals

Table 67.3 Immunization for groups of workers at increased risk

Immunization	Occupational groups
Anthrax	Those handling imported infected animal products or working with infected animals
Cholera	Relief or disaster aid workers
Diphtheria, polio, tetanus	Laboratory and health-care workers who may be exposed in the course of their work in laboratories and clinical infectious disease units
Hepatitis A	Laboratory workers who work with hepatitis A virus, staff of large residential institutions for those with learning difficulties, sewage workers, people who work with primates Consider for food packagers and handlers, staff in day-care facilities and some other categories of health-care workers based on risk assessment
Hepatitis B	Health-care workers in the UK and overseas, including students and trainees who have direct contact with patients' blood or tissues, laboratory staff who handle material that may contain the virus, staff of residential accommodation for those with learning difficulties, morticians and embalmers, prison service staff in contact with prisoners Consider for other staff groups such as the police and fire and rescue services based on risk of exposure
Influenza	Health and social care staff directly involved in patient care
Japanese encephalitis	Laboratory staff who may be exposed to the virus
Measles, mumps, rubella (MMR)	Health-care staff should be immune to measles, mumps and rubella for their own benefit and also to prevent them from spreading infection to patients
Rabies	Pre-exposure immunization should be offered to laboratory workers handling the virus, those who may handle imported animals, people who regularly handle bats in the UK, those working abroad whose work may bring them into contact with rabid animals and health-care workers who may be exposed to body fluids from a patient with rabies
Smallpox	Workers in laboratories where pox viruses (such as monkeypox or genetically modified vaccinia) are handled. Not recommended for people exhuming bodies in crypts
Tick-borne encephalitis (TBE)	In endemic areas recommended for those engaged in forestry, woodcutting, farming and the military. Recommended for laboratory workers who may be exposed to TBE
Tuberculosis	Bacille Calmette–Guérin (BCG) recommended for unvaccinated, tuberculin-negative persons aged under 35 with increased risk of exposure to persons with tuberculosis, including health-care workers, laboratory staff, veterinary and abattoir workers (who may handle infected animal species), prison staff, staff of care homes, staff of hostels for homeless people and refugees

Table 67.5 Selected infections with occupational significance

Infection	Description of infection in humans and public health importance	Risk factors, source and route of transmission	Surveillance/occurrence	Prevention and control
Brucellosis	Zoonosis. Acute febrile illness, fever or unknown origin or chronic bone or joint infection	Different species found in goats, sheep, cattle, pigs, dogs Contact with infected animals, animal tissues or consumption of unpasteurized milk Infectious aerosols occur in abattoirs No human-to-human spread	Approximately 20 cases reported annually in England and Wales (E&W) in recent years but still common worldwide Farmers and veterinarians at risk Prevalence of *Brucella* antibodies is elevated in Tanzanian abattoir workers[11]	Eliminate infection from domestic animals by testing, slaughter and immunization Pasteurize milk Precautions for those handling infected animals No immunization for humans
Varicella	Viral infection producing characteristic rash Occurs mainly in children; may have serious sequelae in adults, pregnancy and the immunocompromised	Direct and respiratory spread from human cases	Common; epidemics every 1–2 years 90% of adults are naturally immune as a result of childhood infection	Health-care staff with varicella and nonimmune health-care staff who have been exposed to varicella require active management to prevent spread to vulnerable patients Immunization is available and may be used universally or selectively In E&W varicella immunization is recommended for susceptible health-care workers who have direct contact with patients
Human seasonal influenza	Acute viral respiratory illness caused by influenza A or B viruses New strains may produce winter epidemics of varying size and severity Outbreaks can occur in hospitals, schools, prisons and other closed communities High attack rate with potential for staff absenteeism	Spread by respiratory secretions from human cases by large droplet or direct spread. Spread by airborne aerosol may also occur	Worldwide distribution In the UK in recent years primary care consultation rates for influenza-like illness have peaked at about 30/100 000 per week in January or February. In epidemic years rates may be 200/100 000 per week or higher	Affected staff should stay away from work during the infectious period (approximately 5 days in adults) Hygiene measures may reduce spread through coughing and contaminated hands and environmental surfaces. Guidelines on PPE should be followed Vaccines active against prevalent strains are manufactured each year and are used selectively Antivirals are available for treatment and prophylaxis and guidelines on their use have been published Annual immunization is recommended for health-care workers directly involved in patient care
Avian influenza	Zoonosis mainly affecting wild waterfowl and domestic poultry, caused by avian strains of influenza A virus Potential for transformation of avian strains into new human pandemic strain by genetic intermixing with seasonal human strains	Spread to humans occurs rarely through close contact with material from affected poultry or other birds Disease may be a severe respiratory illness (H5N1) or conjunctivitis (H7N2, H7N3)	Since 2003, H5N1 has been endemic in poultry worldwide The disease is rare in humans, albeit with a high case-fatality rate. To date 14 countries have reported 361 human cases of H5N1 with 221 deaths	Avian influenza is controlled in poultry by surveillance, testing and culling affected flocks Workers involved in culling activities should be provided with appropriate PPE; they should receive antiviral prophylaxis, seasonal influenza immunization and medical follow-up Detailed guidelines are available http://www.hpa.org.uk

Pandemic influenza	Global pandemics of influenza A occurred in 1918, 1957 and 1968 with high attack rates and significant morbidity and mortality, with effects on health services and other national infrastructure	Until a new pandemic strain emerges it is not possible to predict how it will behave in terms of attack rate, virulence and clinical features The World Health Organization and most national governments have published planning assumptions based on experience from previous pandemics	In the UK, planning assumptions are for a 50% clinical attack rate, with an initial wave lasting about 15 weeks with the peak in weeks 6 and 7, when 22% of cases will occur each week	In the UK, services are planning for business as usual for as long as possible Persons with influenza will be urged to stay at home, cough etiquette and enhanced hygiene will be encouraged, all affected persons will receive a course of antiviral treatment, business continuity plans will mitigate the effects of absenteeism, nonessential services will be suspended, social distancing measures may be introduced National strategic plans and detailed guidance can be viewed at http://www.dh.gov.uk
Hepatitis B and C viruses[12]	Hepatitis B is an acute viral infection of the liver. The initial illness may be severe and a chronic carrier state may develop, leading after some years to cirrhosis and hepatocellular carcinoma Hepatitis C is also a viral infection of the liver. The initial illness is often asymptomatic but 80% of those infected develop chronic infection that may result in cirrhosis and hepatocellular carcinoma	Human cases and carriers are the source of infection and transmission is by the blood-borne route. Hepatitis B is also transmitted by the sexual route and vertically from mother to infant Health-care workers and laboratory staff who are exposed to blood and tissues from infected patients are at risk. Other groups of workers such as tattooists and body piercers may also be at risk Nigerian butchers had an HBsAg seroprevalence rate of 9.4% compared with 3.3% in a control group[13] In the UK there is a reporting system for occupational exposure to blood-borne viruses	The incidence of acute hepatitis B in Europe varies from 1 to 30 per 100 000 per year. The prevalence of chronic infection varies from <1–2% Some seroprevalence studies have suggested a higher prevalence in health-care workers but this may be explained by confounding factors such as ethnicity and country of origin rather than risk of occupational exposure Reliable estimates of hepatitis C incidence are not available. Prevalence of past infection in the UK is 0.1% in blood donors and 0.2% in health-care workers In the UK there have been 11 cases of hepatitis C seroconversion in health-care workers following percutaneous exposure	Some groups of patients are screened for evidence of infection Standard infection control precautions should be followed (including use of PPE and safe handling of needles and sharp instruments) Hepatitis B vaccination is recommended for health-care workers who may have direct contact with patients' blood or body fluids. It is also recommended for workers who are at risk of injury from blood-contaminated sharp instruments, or of being deliberately injured or bitten by patients. Guidelines are available Guidelines are also available for the management of health-care workers who have had percutaneous exposures Infection may spread from health-care workers to patients and for this reason pre-employment screening has been introduced in some countries

Continued

Table 67.5 Selected infections with occupational significance—cont'd

Infection	Description of infection in humans and public health importance	Risk factors, source and route of transmission	Surveillance/occurrence	Prevention and control
Human immunodeficiency virus[14]	A chronic viral infection which leads to depletion of CD4 lymphocytes and immunosuppression resulting in AIDS	Humans are the source of infection and spread is by the blood-borne, sexual or vertical route Health-care workers and laboratory staff who are exposed to blood and tissues from infected patients are at risk	The incidence of HIV infection in the UK is not known. The cumulative incidence of HIV infection in mid-2007 was about 90 000 and there are about 7500 new diagnoses each year The prevalence of anti-HIV varies depending on the population subgroup that is tested. In pregnant women in the UK the prevalence is between 0.5% and 5% In the UK there have been five reports of HIV seroconversion following percutaneous exposure in health-care workers	Standard infection control precautions should be followed (including use of PPE and safe handling of needles and sharp instruments) Postexposure prophylaxis (PEP) with antivirals is recommended for health-care workers who may have contact with HIV-infected patients' blood or body fluids. Guidelines are available Spread of infection from an HIV-infected health-care worker to a patient has been reported and in some countries HIV-infected health-care workers are not permitted to carry out exposure-prone surgical procedures. Guidelines are available
Tuberculosis (TB)	Infection of lungs and other organs with *Mycobacterium tuberculosis* or rarely other species including *Mycobacterium bovis* TB can cluster in health-care settings and other closed communities	Transmission is by inhalation of respiratory droplets from an infectious case Bovine TB may be contracted by consumption of milk from or contact with an infected animal	In 2006 in E&W there were 8051 cases of TB. Regional rates vary from 5 to 45 per 100 000 per year Health-care workers have twice the expected incidence of TB, allowing for age, sex and ethnic factors[15]	Infection control procedures should be followed BCG vaccine is recommended for health-care workers who may have close contact with infectious patients but not for nonclinical staff. Guidelines are available
Leptospirosis	Zoonosis with wide clinical spectrum caused by one of many serovars of *Leptospira*	Many different animal reservoirs Transmission is percutaneous from urine of affected animals	In 2006 there were 44 laboratory-confirmed cases in E&W. Most cases were recreational but seven were farmers, all of whom reported contact with livestock or rats Other risks included clearing streams or drains	Those at risk should use appropriate PPE Guidelines are available
Anthrax	Infection in humans affects skin, respiratory and gastrointestinal tract	Spread from infected animals by spores of *Bacillus anthracis* through contact with animals or animal products Spores survive in the environment	Rare. Nineteen cases reported in the UK in 1975–96 in workers handling imported infected animal products or working with infected animals	Control anthrax in livestock and disinfect imported animal products Processing of products reduces risk of infection; bone meal used as fertilizer should be sterilized; PPE should be used Immunization is available for those at risk

Ovine and avian chlamydiosis (psittacosis)	Zoonosis. Potentially serious respiratory and systemic infection caused by *Chlamydophila psittaci*	Spread from psittacine birds (parrots etc.) and other mammals which may be asymptomatic by inhalation of aerosols of bird dropping and other material from infected species Human-to-human spread is very rare	Worldwide distribution 100 cases reported in E&W each year	Affected birds should be quarantined, treated or culled Caution is required when handling birds and cleaning cages
Avian and ovine chlamydiosis	Zoonosis. Respiratory infection and may lead to miscarriage in pregnancy, caused by *Chlamydophila abortus*	Spread by inhalation of aerosols from infected and aborting sheep. Cattle and goats may also be affected	Worldwide distribution, particularly in sheep-rearing countries Commonest cause of infectious abortion in sheep in the UK but human infection is rare (1–2 cases per year in E&W)	Pregnant women should not help to lamb or milk ewes; they should avoid contact with aborted or newborn lambs (and placenta) and should not handle clothing that has been in contact with ewes or lambs A live vaccine for use in sheep is available. This should not be handled by pregnant women or women of child-bearing age
Diarrheal disease	There are many causes of infectious intestinal disease. Some are zoonoses, others have only human reservoirs	Transmission is by the direct or indirect fecal–oral route.	Refer to surveillance data for specific infections. http://www.hpa.org.uk/infections/topics_az/gastro/menu.htm Norovirus infection is a common occupational infection amongst health-care staff	Infection control procedures should be followed, including handwashing and use of PPE Guidelines are available
Methicillin-resistant *Staphylococcus aureus* (MRSA)	A spectrum of infection from minor skin infection to life-threatening bacteremia caused by methicillin-resistant *S. aureus*	The reservoir is colonized or infected humans Spread is direct on hands, fomites, equipment or the environment Acquisition of MRSA infection has been described as result of occupational contact with pigs[16]	MRSA is common in hospitals and in other care settings In England in the most recent 12-month period there were 5366 cases of bacteremia, a rate of 1–2 per 10 000 hospital bed days Health-care staff may become infected or colonized with MRSA and may act as source of infection for nosocomial transmission	Infection control procedures should be followed, including handwashing and use of PPE Guidelines are available
Hepatitis A	Infection of liver caused by hepatitis A virus Asymptomatic disease is common in children; severity increases with age Improved standards of hygiene have resulted in a decline in incidence in the UK in recent years	Transmitted by the fecal–oral route through person-to-person spread or contaminated food or drink	In 2004 there were 669 cases in E&W A high proportion of adults and adolescents in developed countries are susceptible. Most cases are sporadic although clusters have been reported amongst men who have sex with men, intravenous drug users and hostel dwellers. Travel to endemic areas is also a risk factor In some studies work with sewage has been shown to be a risk factor but this has been disputed[17]	Observing appropriate infection control precautions including use of PPE will minimize occupational spread of hepatitis A infection Immunization is recommended for laboratory workers who may be exposed to hepatitis A, staff of large residential institutions for those with learning difficulties, sewage workers and people who work with primates Immunization may be considered for food packagers and handlers, staff in day-care facilities and some other categories of health-care workers based on local risk assessment

Continued

Table 67.5 Selected infections with occupational significance—cont'd

Infection	Description of infection in humans and public health importance	Risk factors, source and route of transmission	Surveillance/occurrence	Prevention and control
Orf[18,19]	Caused by parapoxvirus Presents as a self-limiting subacute papulovesicular cutaneous lesion usually on the hands	Associated with lambs, sheep and goats	Groups affected include farmers, children visiting farms, meat industry workers	Proper hand hygiene; use of gloves Quarantine of affected animals.
Q fever[20,21]	Caused by highly infectious bacterium *Coxiella burnetti.* Incubation period 2–3 weeks Acute cases present as high fever with malaise, myalgia, sore throat, chills, vomiting, and diarrhea. Some develop pneumonia, liver function abnormalities or hepatitis, and endocarditis 50% of infected humans are asymptomatic. Chronic cases are rare	Transmitted by inhalation of organisms in dried placental material and birth fluids from infected sheep Cattle, sheep and goats are main reservoirs Consumption of raw unpasteurized milk has been a cause of a few cases Individuals with pre-existing heart valve disease are especially vulnerable if they develop chronic Q fever	Notifiable disease in the USA and the UK Occupational groups at risk are farmers, veterinarians and abattoir workers	Vaccine available for groups at risk, and also for animals Preventive measures include proper disposal of products of conception from sheep, and quarantine of imported livestock
Nipah[22] and Hendra virus	Closely related paramyxoviruses Nipah virus causes encephalitis and both Nipah and Hendra virus cause respiratory effects High case-fatality rate	Nipah virus transmitted from infected pigs. Abattoir workers also at risk. Hendra virus from horses Certain species of fruit bats are reservoirs 2004 Nipah virus outbreak in Bangladesh suggests possible person-to-person transmission, although no specific evidence for this	First major outbreak of Nipah virus in humans reported in Malaysian pig-farmers in 1998 Hendra virus first isolated in 1994 following outbreak in horses and three cases in humans in Australia	In an outbreak, PPE advised for workers dealing with infected animals, and as a precautionary measure for health-care staff dealing with infected patients
Lyme disease	Infection with spirochaete *Borrelia burgdorferi* causing rash which may progress to polyarthritis and nervous system involvement	Deer and sheep are reservoir Transmission is bite of *Ixodes* tick	Widespread in Europe and North America In E&W in 2006 there were 768 reports	In endemic areas avoid tick bites Guidelines are available
Japanese encephalitis (JE)	JE is a mosquito-borne viral encephalitis Very common cause of childhood encephalitis in Asia Can spread in laboratory workers	Reservoir is pigs and birds Spread is by mosquito vector	There have been reports of laboratory-acquired JE virus infection	Immunization is recommended for laboratory staff who may be exposed to the virus

Rabies	Acute viral encephalomyelitis caused by Lyssavirus (classic rabies virus) or bat-related Lyssavirus	Infection is via the bite or scratch of a rabid animal Person-to-person spread does not occur although rabies has spread through corneal grafts and other transplanted tissues from infected persons	Worldwide there are 40 000–70 000 cases of rabies each year In the UK, deaths occur in people infected abroad, with 24 reports since 1902 Very rarely deaths are reported following exposure to bat-related Lyssavirus	Pre-exposure immunization with rabies vaccine should be offered to laboratory workers handling the virus, those who may handle imported animals, people who regularly handle bats in the UK, those working abroad whose work may bring them into contact with rabid animals and health-care workers who may be exposed to body fluids from a patient with rabies
Tick-borne encephalitis (TBE)	Flavivirus infection of central nervous system ranging from mild febrile illnesses to meningoencephalitis	Reservoir is small mammals, domestic livestock and birds Spread is by bite of infected tick or by ingestion of unpasteurized milk from infected animals, especially goats	The virus is restricted to parts of Central Europe and Asia Those working or traveling in forested parts of endemic areas during spring and summer are at risk	Awareness of risk areas is essential Minimize tick bites by covering arms, legs and ankles, and using insect repellents on socks and outer clothes Attached ticks should be removed as soon as possible; seek local medical advice Immunization is recommended for those engaged in forestry, woodcutting, farming and the military in endemic areas Laboratory workers who may be exposed to TBE should also be immunized
Meningococcal infection[23]	Meningitis, septicemia and rarely pericarditis and arthritis caused by *Neisseria meningitidis* Serogroups B and C are most common in the UK, overseas A, Y, W135 occur Overall mortality remains around 10% in the UK	Meningococci colonize the nasopharynx Transmission is by respiratory droplets from a person carrying the organism, usually requiring prolonged close contact	In E&W notified cases of meningococcal infection have declined from a peak of nearly 3000 cases in 1999 to 1275 cases in 2006 Seasonal epidemics of group A infection are common in sub-Saharan Africa. Epidemics of group W135 infection have occurred in association with Hajj pilgrimages to Saudi Arabia Health-care workers exposed to infected cases are at increased risk but absolute risks are very low	Persons aged under 25 years and others of any age at increased risk should be immunized with a single dose of MenC conjugate vaccine. It is also recommended for long-stay visitors to high-incidence countries who will be working with local people. This group should also be offered ACWY quadrivalent polysaccharide vaccine Health-care workers should minimize exposure by use of PPE when carrying out procedures that may produce aerosols Chemoprophylaxis is recommended if the mouth or nose is directly exposed to respiratory secretions from a case

BCG, bacille Calmette–Guérin; PPE, personal protective equipment.

CONCLUSIONS

There are many infections that can be acquired through work activities or from workplaces (Table 67.5). The recognition of occupational factors as an important component in the transmission of these infections will aid in the management of affected cases, and in prevention. Continuing vigilance for new occupational infections, advances in preventive measures and an experienced occupational health team working with infection control specialists are key to the successful prevention of these infections.

REFERENCES



References for this chapter can be found online at http://www.expertconsult.com

Chapter 68

Ellie JC Goldstein

Infections from pets

INTRODUCTION

Domestic pets, particularly dogs and cats, have dated back prior to Ancient Egypt. Currently, more than a third of US households keep some pet, including 53 million dogs, 57 million cats and a plethora of other animals from birds to fish to reptiles.[1] In Cheshire, England, 24% of households own a dog and 52% a pet of some type,[2] while in Australia, 66% of households have a domestic pet.[3] It is therefore not surprising that transmission of infection from animals to humans and humans to animals is common.

DOG-ASSOCIATED INFECTIOUS DISEASES

Dog bites

Table 68.1 lists some of the infectious organisms associated with dogs and canine contact. Of the over four million Americans bitten annually by a dog, only 15–20% will seek, or need, medical attention and 3–18% will become clinically infected. Dog bites account for 1% of all Emergency Department visits and result in ~10 000 hospitalizations and 1–10 deaths annually.[1] Most dog bites are inflicted by the victim's own pets or an animal known to them, often while separating the dog in a fight with another dog.[4] Half of dog bite wounds are to the hands and pose a special risk for penetration into the bones and joints and potential spaces of the hand. It is possible for nerves and/or blood vessels to be damaged. Approximately 15% of dog bites are to the head and neck, 20% to the leg or foot and 15% to the upper extremity.[1,3,4]

The incidence of dog bite wounds peaks in the summer months and on weekends. The median age of dog bite victims is 28 years old and more than 60% are male. Most dog bite wounds are punctures (~60%), lacerations (10%) or both (30%). Most patients attempt some form of self-therapy before presenting for medical attention, which include washing with soap and water, applying a topical iodophor or alcohol product. The median time for presentation for medical care is 35 hours post injury, usually after the onset of clinical signs of infection.

At presentation approximately 60% of wounds exhibit a purulent exudate, 30% have other signs of infection and 10% are abscesses. Approximately one-third of patients reporting to an Emergency Department will require hospitalization for intravenous antibiotics. When cultured, these wounds yield an average of five isolates, usually three aerobes and two anaerobes. Mixed aerobic/anaerobic infection occurs in 50% of dog bite wounds and 35% grow only aerobes. Common aerobic isolates include *Pasteurella* species, 50% (*P. canis* 26%; *P. multocida* subspecies *multocida* 12%; *P. stomatis* 12%; *P. multocida* subspecies *septica*, 10%), streptococci, 46%, staphylococci, 46% (half of which are *Staphylococcus aureus*) and *Neisseria* spp., 16%.[1,4] Methicillin-resistant *Staphylococcus aureus* (MRSA) carriage and infection in companion animals, including dogs and cats, is common and the isolates are indistinguishable from human isolates; transmission of MRSA from humans to animals and vice versa has been reported.[5,6] In addition, dog bite wounds with multidrug-resistant *Escherichia coli* and enterococci have also occurred. *Capnocytophaga canimorsus* from dog bites is able to escape from human immune surveillance[7] and has been associated with fatal sepsis, especially in asplenic patients and those with liver disease.

Common anaerobes isolated include *Fusobacterium* spp., 32%, *Bacteroides* spp., 30% (especially *B. tectum*), *Porphyromonas* spp., 28%, *Prevotella* spp., 28% (especially *P. heparinolytica*) and peptostreptococci, 16%.[1,3,4] Mycoplasmas (e.g. *Mycoplasma canis* and *Mycoplasma spumans*) and other atypical isolates may also be present in dog bite wounds.[8]

Table 68.2 outlines susceptibility patterns of common bite isolates. MRSA isolates are, at present, unusual. For those patients hospitalized,

Table 68.1 Infections transmissible from dogs

Bacteria	Fungi	Parasites	Viruses
Normal flora	*Blastomyces* spp.	*Giardia* spp.	Rabies
Pasteurella spp.	*Microsporum* spp.	*Babesia* spp.	Lymphocytic choriomeningitis
Capnocytophaga canimorsus	*Trichophyton* spp.	*Toxocara* spp.	Influenza
Tularemia		*Dipylidium canium*	Mumps
Ehrlichiosis		Echinococcosis	
Brucellosis		*Ancyclostoma* spp.	
Mycobacterium fortuitum		Scabies	
Campylobacter spp.		*Cheytelliella*	
Anaerobiospirillum thomasii			
Yersinia spp.			

Table 68.2 Activity of selected antimicrobials against animal bite isolates

	Pasteurella multocida	*Staphylococcus aureus*	Streptococci	*Capnocytophaga* spp.	Anaerobes
Penicillin	+	–	+	+	V
Ampicillin	+	–	+	+	V
Amoxicillin–clavulanate	+	+	+	+	+
Ampicillin–sulbactam	+	+	+	+	+
Dicloxacillin	–	+	+	–	–
Cephalexin	–	+	+	–	–
Cefuroxime	+	+	+	+	–
Cefoxitin	+	+	+	+	+
Tetracyclines	+	V	–	V	V
Moxifloxacin	+	+	+	+	+*
Erythromycin	–	+	+	+	–
Azithromycin	+	+	+	+	–
Clarithromycin	V	+	+	+	–
Trimethoprim–sulfamethoxazole	+	+	V	+	–
Clindamycin	–	+	+	–	+

+, active; –, poor or no activity; V, variable activity against listed pathogen.
* Except *Fusobacterium canefelinum*.

the mean length of stay is 3 days with ~30% requiring incision and drainage and 25% requiring debridement of the wound. Severely penicillin-allergic patients may need a combination of agents. Fluoroquinolones, tetracyclines and sulfa drugs are relatively contraindicated in pregnant patients; tetracyclines and fluoroquinolones should be avoided in patients younger than 18 years. Agents (monotherapy) associated with therapeutic failure include first-generation cephalosporins and macrolides.

The duration of therapy will depend upon the severity of the wound. For wounds that are seen less than 8 hours post-injury and require 'prophylaxis' (moderate to severe wounds), the duration of prophylaxis is usually 3–5 days. Therapy for a cellulitis is usually 7–10 days, while the therapy for septic arthritis and osteomyelitis is often 4–6 weeks. Cases of endocarditis and infection of prosthetic joints following bites have been reported, albeit rarely. The principles of wound care are noted in Table 68.3.

Rabies

Approximately 7000 animals per year test positive for rabies in the USA. Most are wild animals, such as raccoons, foxes and bats. Dog-associated rabies is uncommon in developed countries as a result of stray animal control programs and widespread rabies vaccine use by dog owners. Dog rabies has occurred in areas where endemic foci of infected raccoons and skunks are common. Canine transmission of rabies to humans infrequently occurs in the USA, but it is not uncommon in Latin American countries, Africa and particularly in the Indian subcontinent and other regions in Asia. The World Health Organization reported over 55 000 deaths/year due to endemic canine rabies worldwide (see Chapter 160).[9,10]

Enteric diseases from dogs

Giardiasis

Giardia lamblia is a flagellated protozoan parasite that is an important cause of diarrheal disease worldwide and can cause diarrhea in many mammalian species including dogs and humans. In many hospitals that have pet visitation programs, *G. lamblia* is the most frequent infectious agent identified on screening. The disease is transmitted by fecal–oral spread and causes flatulence, foul-smelling stools, abdominal discomfort and malaise in humans. Diagnosis is by stool examination. Therapy is with metronidazole; relapses are not infrequent.

Echinococcosis

Echinococcus granulosis is a dog tapeworm found in the small intestine, the ova of which are shed into the stool. The illness is associated with dogs found in sheep-raising areas, especially if the dogs are fed offal. These ova may remain viable for up to 1 year in appropriate climatic conditions. Humans can ingest the ova, which hatch in the small intestine into oncospheres that penetrate the bowel wall. Humans will act as an intermediate host. Usually these oncospheres reach the liver (60%) and occasionally the lung (25%) and other organs (15%).

Rupture of a hydatid cyst can cause catastrophic disease and anaphylaxis. A variety of serologic tests are available to assist in diagnosis. However, the presence of septation in a liver cyst (indicating daughter cysts) is considered diagnostic. Therapy for this disease includes surgery. Some suggest aspiration is acceptable, although there is a risk of spillage and complications. Scolicidal agents such as albendazole and mebendazole remain as adjunctive therapies.

Echinococcus multilocularis causes a severe form of alveolar hydatid disease, again often involving the human liver. This parasitic infection is found primarily in the artic regions of the northern hemisphere and often necessitates aggressive surgical resection for successful removal. *Echinococcus vogeli* is found in canines of Columbia, Ecuador, Panama and Venezuela, and can also cause human disease. The diagnosis and management of echinococcosis is further reviewed in detail in Chapter 114.

Miscellaneous enteric infections acquired from dogs

Isospora spp. infection of the gastrointestinal tract is common in dogs and while transmission to humans is not proven, it remains possible, especially in the immunocompromised patient. *Trichuris vulpis*, the whipworm of dogs, resembles the human whipworm *Trichuris trichuria*. However, the ova of the former are twice the size of the latter, and

Table 68.3 Components of care for animal bites

History	Situation, pet ownership/identity
Geographic location	
Examination	Nerve function
	Tendon function
	Blood supply (pulses)
	Presence of edema, crush injury
	Proximity to joint
	Bone penetration
Diagram (or photograph) of wound(s) for the record	
Wound care	Irrigation (important)
	Debridement (cautious)
	Elevation (important)
Antimicrobials	Prophylaxis, 3–5 days (po)
	Therapy for established infection (po vs im initial dose)
	Empiric vs specific (animal specific)
Management:	
Culture (if infected)	
Baseline radiograph	
Tetanus toxoid (0.5 ml im) if required	
Rabies prophylaxis (rabies immune globulin/human diploid cell vaccine) if needed	
Health Department report (if required)	
Decision regarding need of hospitalization	Fever >100.5°F (38°C)
	Sepsis
	Compromised host
	Advance of cellulitis
	Patient noncompliance
	Acute septic arthritis
	Acute osteomyelitis
	Severe crush injury
	Tendon/nerve injury or severance
	Tenosynovitis

have been reported in children, institutionalized patients, and rarely in immunocompetent adults associated with dog contact.

Toxocara canis is a ubiquitous roundworm in dogs that causes both cutaneous larva migrans and visceral larva migrans in humans, and affects many puppies (often less than 6 months old) and adult dogs (via ingestion of embryonated ova). Infection in humans is usually associated with children who have pica (1–6 years old) who acquire it from backyards and contaminated sandboxes. Most infection in humans is asymptomatic and eosinophilia (>30%) may be the only sign. However, the larvae may migrate anywhere in the human body and their final location determines the associated symptoms. Some patients develop an asthma-like illness; others present with pallor, weight loss, hepatomegaly or pruritic skin lesions. Diagnosis is based on a compatible history coupled with specific serology. Dogs may also transmit *Ancyclostoma canium* and *Ancyclostoma brazilense*, which also cause cutaneous larva migrans.

Infections from contact with dog urine

Leptospirosis

Leptospires are finely coiled, motile spirochetes that can be carried asymptomatically for many months by dogs, and the prevalence of leptospirosis increased significantly from 2002 to 2004, especially in suburban and rural areas.[11] Dogs may also become ill, manifested by fever, jaundice, conjunctivitis and hematuria. Dogs are often vaccinated for leptospirosis, but vaccine failures (>1 year postvaccination) have been reported. Humans may become infected through contact from the urine of an infected dog, from contact with infected tissues or indirectly from contaminated soil or water. Slightly alkaline or neutral pH, warm temperatures and moist soil favors survival of leptospires in the environment.

Most humans will have subclinical infection or an anicteric 'viral type' illness. Some may develop the classic biphasic illness and proceed onto Weil's disease. During the first phase, leptospires may be isolated from the blood, urine and spinal fluid. Later, antibodies may develop and recrudescent fever, arthralgia, hepatitis, skin rash and conjunctival suffusion may appear along with other complications (see Chapters 124 and 171). Some studies suggest that therapy with penicillin G or doxycycline may shorten the course of disease if started within the first 4 days of illness. Jarisch–Herxheimer reaction can occur after the first dose of therapy.

Brucellosis

Brucellosis is a disease of both wild and domestic animals that is transmitted to humans. Dogs may be infected with *Brucella canis*, a small Gram-negative coccobacillary organism that is found in kennel-raised dogs and is the least common cause of human brucellosis. Many infections with *B. canis* are laboratory animal acquired. Dogs may have persistent carriage of the organism and shed it in urine and gestational tissues. It can be transmitted between dogs during mating. Human disease occurs 2–4 weeks after exposure and has protean manifestations with both an acute and a chronic form (see Chapter 123).

Tick/flea-borne infectious diseases associated with dogs

Ehrlichiosis

Canine ehrlichiosis was first described in 1932 in Algeria. Since then it has been noted that dogs can be hosts to a variety of tick-borne, intraerythrocytic parasites including *Ehrlichia canis*, *Ehrlichia chaffeensis* (associated with human monocytic ehrlichiosis), *Anaplasma phagocytophilia* (associated with human granulocytic ehrlichiosis), *Ehrlichia ewingii* and *Ehrlichia platys* that cause canine pancytopenia and have a worldwide distribution. While dog to human transmission is unlikely, the disease has that potential. In humans, ehrlichiosis is contracted by a tick bite that inoculates the organism into the skin and which then spreads via the lymphatics. In humans it causes fever, headache, chills and malaise and has an associated leukopenia, thrombocytopenia and elevated liver enzymes. Therapy in humans is tetracycline or doxycycline.

Infections acquired from direct contact with dogs

Canine scabies due to *Sarcoptes scabiei* var. *canis* is also known as mange. The mites feed on the stratum granulosum of the skin and deposit the eggs in a burrow. After hatching, the larval forms migrate to the surface. Transmission to humans has been noted and is manifested as an erythematous, nonfollicular dermatitis, in part due to hypersensitivity. Skin scrapings will often not demonstrate the mite and diagnosis rests upon history and clinical response to scabicides. Dogs should also be treated with the application of a scabicidal agent. A similar condition known as 'walking dandruff' in dogs is due to *Cheyletiella* (mites). These mites do not burrow and disease is manifested in humans with erythematous macules, which may become pustular or vesicular or have other manifestations of allergic reaction such as urticaria and erythema multiforme (see Chapter 11).

CAT ASSOCIATED INFECTIOUS DISEASES

Cat bites

Cats can transmit a number of diseases to humans as listed in Table 68.4. Most physicians will encounter cat bites as the most common cat-related disease, with approximately 500 000 cat bites in the United States alone on an annual basis, usually when handling their own cat. Most wounds are trivial and do not need medical care. Cat bites affect more women (72%) than men, with an average age of 39 years for the victim.[1] At the time of presentation a nonpurulent wound with cellulitis (42%) is the most common condition encountered; however, the wounds may also develop into a purulent cellulitis (39%) and even have abscess formation (19%). An associated lymphangitis may be present in 28% of cases. An average of six bacterial species is isolated in the typical wound (range, 0–13). Approximately 85% of cat bite injuries involve puncture wounds, less than 5% are lacerations and ~10% are combinations of both.[3,4] Cats' teeth are small but sharp and easily penetrate the bones, tendons and joints of the hand and lead to osteomyelitis, tendonitis and septic arthritis, respectively.

The bacteriology of these wounds reflects the normal oral flora of the biting cat. Several recent studies[1,12,13] have shown *Pasteurella* species to be present in 75% of cases, with *P. multocida* subspecies *multocida* in 54% of wounds and *P. multocida* subspecies *septica* in 28%. These two subspecies are associated with more severe injury and have a propensity for bacteremia and central nervous system infections, respectively. Other common aerobic isolates include oral streptococci (46%), staphylococci (35%), *Neisseria* spp. (especially *N. weaverii*), *Moraxella* species (35%) and *Corynebacterium* spp. (28%). *Capnocytophaga canimorsus* is an unusual nonfermentative Gram-negative aerobic rod that is associated with bacteremia and significant mortality in compromised patients such as those with asplenia or liver disease.

Anaerobes are present in 63% of cases, usually in mixed culture, and when present were associated with more severe infection and abscess. Common anaerobic isolates include *Bacteroides tectum* (28%), *Fusobacterium* spp. (33%), especially *F. nucleatum* and *F. russii*, *Porphyromonas* spp. (30%) and *Prevotella* spp. (19%).[12–14] Most anaerobes isolated from cat bites are not producers of β-lactamase, but cat-associated *F. nucleatum* spp. are notably often resistant to fluoroquinolones and a new subspecies (*F. nucleatum* subspecies *canifelium*) has been proposed.[15]

In addition, *Erysipelothrix rhusiopathiae* has been isolated from cat bites. While usually a localized infection manifested by painful ulceration and a papular skin lesion, a disseminated form exists. Anthrax uncommonly infects cats and results from contact with infected soil, especially associated with herbivores. The differential diagnosis of the cutaneous papular lesion that starts as a vesicle and can progress to brawny edema and central necrosis also includes brown recluse spider bite, cat-scratch disease,[16] tularemia and plague. Tularemia has been associated with cat bites.

Antimicrobial selection is delineated in Table 68.2. Severely penicillin-allergic patients may need a combination of agents. The principles of wound care are noted in Table 68.3. Pain, out of proportion to the injury and in proximity to a bone or joint, suggests periosteal penetration.

Rabies

Rabies is a widespread zoonotic infection affecting numerous wild animals in many regions of the world. Domestic cats account for less than 5% of rabid animals and usually acquire it from a 'spillover effect' from exposure to infected wildlife. This disease is covered in Chapter 160.

Cat-scratch disease

Bartonella henselae is a fastidious Gram-negative rod that is the etiologic agent of cat-scratch disease (CSD) in the general population and bacillary angiomatosis in HIV-infected individuals. CSD is of worldwide distribution, has an autumn and winter prevalence in temperate climates and may be transmitted by direct inoculation (scratch or bite) and/or by fleas. Over 80% of cases are in people younger than 21 years old; children younger than 14 years old are typically infected after exposure to a newly acquired pet cat/kitten. Strays and cats from pounds have a higher frequency of infection and may be bacteremic (>40% in one San Francisco study) compared to household cats (6% in one New York City study). Most infections in cats are asymptomatic and can last for several months.[16] Domestic dogs, while accidental hosts, may also act as an important reservoir.

Approximately 1 week (range 3–10 days) after injury/exposure, 25–60% of patients may develop a primary inoculation papule at the site of injury, which may become vesicular or crust. In approximately 2 weeks (5–12 days) tender, regional lymphadenopathy may develop. These nodes may be the only manifestation in approximately half of the cases, often lasts more than 3 weeks (6–12 weeks) and usually resolves spontaneously but may suppurate in ~15% of cases. Accompanying symptoms include fatigue and malaise (28%), fever of 101–106°F (38.3–41.1°C) (12%), exanthem (4%), parotid swelling (2%) and seizures (<1%). Other manifestations may include ocular granuloma, erythema nodosum, thrombocytopenic purpura and osteomyelitis.[17] Endocarditis due to *Bartonella quintana*, a related organism, and rarely *B. henselae*, both associated with cat fleas, has been reported in homeless men (see Chapter 176).

Diagnosis is usually on clinical grounds coupled with a history of cat exposure. The organism is very difficult to isolate (chocolate media or CDC agar after 2–3 weeks) except in the research setting and serologies are of variable reliability. Biopsies are sometimes performed to exclude diseases such as Hodgkin's lymphoma, and show granuloma formation with stellate microabscesses. While the Warthin–Starry stain has been recommended, it is difficult to interpret, especially in the absence of a positive control specimen. Serology using EIA IgG antibodies may remain positive for over 1 year, while IgM antibodies usually resolve in less than 3 months.[18]

Table 68.4 Possible infections transmissible from cats

Inhalation	Vector-borne	Fecal–oral	Bites, scratches	Direct contact
Bordetellosis (*Bordetella bronchiseptica*)	Ehrlichosis	Campylobacterosis	Normal floral isolates (including *Pasteurella* spp.)	Plague
Plague	Cat-scratch disease	*Helicobacter* spp.	Rabies	Histoplasmosis
Q fever	Bacillary angiomatosis	Cryptosporidiosis	*Erysipelothrix rhusiopathiae*	Dermatophytosis
		Toxoplasmosis	Anthrax	Scabies
		Salmonellosis	Tularemia	*Cheyletiella* mites
		Anaerobiospirillum diarrhea		
		Yersiniosis		
		Toxocariasis		
		Opisthorchiasis		
		Dipylidiasis		

Therapy is usually supportive. Antimicrobial therapy with azithromycin has been reported to diminish the size and duration of the adenopathy. Doxycycline and rifampin (rifampicin) have been used for CSD-associated retinitis. *In-vitro* resistance to first-generation cephalosporins has been correlated with clinical therapeutic failure. Prevention is by control of fleas in pets and sometimes treatment of the pet at the time of acquisition.

Bacillary angiomatosis is CSD in a severally immunocompromised (usually HIV-infected) host that manifests either as purplish skin lesions resembling Kaposi's sarcoma or as colorless subcutaneous nodules. Biopsy of the lesions yields a characteristic histopathologic picture of vascular proliferation. DNA probes have also been used to diagnose this disease. These patients may also be symptomatic from disease and have associated fever and even peliosis hepatitis. Antimicrobial therapy, although its efficacy is poorly defined, is usually employed in this situation and includes macrolides, quinolones or doxycycline (Chapter 176).

Cat-associated enteric infections

Cats may acquire salmonellosis from infected foods, especially offal, live prey (such as songbirds), uncooked meat or fishmeal, or contaminated water. Kennel transmission has also occurred. Cats may shed *Salmonella* species via the feces, the conjunctiva or the oral route. In addition, their fur may become contaminated and pass infection as can their water dishes. Newly acquired young cats are more likely to carry *Campylobacter jejuni* than older cats. *Helicobacter bizzozeronii* and *Helicobacter felis* are found in cat stomachs and association with human cases have been noted, albeit rarely. *Helicobacter pylori* has also been isolated from cats and is thought to have been transmitted to them by human caretakers. Cryptosporidiosis can affect cats that, along with other animals, are definitive hosts. Naturally infected cats exhibit watery diarrhea and some cats can be colonized. Transmission between cats and humans has been reported.[19]

Cats are definitive hosts for *Toxoplasma gondii* and millions of oocysts may be excreted daily in their feces. Approximately 1% of US cats are thought to be excreting oocysts on any given day. While ingestion of undercooked meat is the usual mode of transmission of toxoplasmosis, infection may develop from exposure to fecal oocysts when changing litter boxes or gardening in soil contaminated with oocysts by cat feces. The oocysts that pass from the feces are noninfectious and nonsporulated. However, 2–3 days after shedding, the oocysts may sporulate depending on temperature (>39.2 and <98.6°F; >4 and <37°C) and climactic factors. These may remain infective in soil for up to 1 year.

Less common isolates include *Anaerobiospirillum thomasii*, which causes diarrhea in cats and is associated with bacteremia in humans. Cats may be asymptomatic carriers or infected with *Yersinia pseudotuberculosis*, which can cause diffuse diarrhea and abdominal pain in humans. *Toxocara cati* is a helminthic parasite that affects cats with which humans may become incidentally infected when ingesting infected cat feces (usually children in the ages of pica). Most human infection is asymptomatic but can present as asthma, abdominal pain, hepatomegaly and/or eosinophilia. The disease is usually self-limiting. The cat liver fluke, *Opisthorachis felineus*, can be transmitted to humans from ingestion of rare or raw fish infected with the parasite. *Dipylidium caninum* is a cat tapeworm that can be transmitted to humans, usually children, when they ingest infected fleas. Patients develop mild abdominal discomfort and eosinophilia. Demonstrating proglottids upon parasitologic examination of the feces makes the diagnosis (see Chapter 184).

Infectious diseases associated with direct contact with cats

Dermatophytosis

Domestic cats can harbor a wide variety of dermatophytes on the hair of their coat and skin. They may also acquire human dermatophytosis from their owners and transmit it to others with an incubation period of 1–3 weeks. Asymptomatic, as well as symptomatic carriage occurs. *Microsporum canis* has been found in up to 90% of longhaired show cats. Up to 50% of exposed humans will develop symptomatic infection, including ringworm and tinea capitis. Other organisms isolated include *Epidermophyton floccosum*, *Microsporum* spp. and *Trichophyton* spp. Infectious arthrospores can disseminate from the hair and skin to the local environment where they remain viable for months. Contaminated fomites may also act as vectors of transmission. In cats, dermatophyte infection can manifest as patchy alopecia or even a scaly dermatitis.

To break a cycle of transmission, cats may be treated with topical antifungals and on occasion oral antifungals. In addition, cleaning areas of cat hair and removal of dander from carpets and restriction of pet cats from the bedroom may facilitate control. Diagnosis is by skin scrapings and microscopic examination after potassium hydroxide addition or by use of a Wood's lamp. Human infection can be treated by topical or oral antifungals.

Cats may also directly transmit *Dermatophilus congolensis*, an actinomycete that can cause cutaneous exudative or pustular dermatitis. In cats, the hair around the lesion should be clipped and the lesion kept dry. The human dermatitis is usually self-limiting but on occasion antimicrobial therapy (penicillin) may be required if extensive skin involvement with cellulitis develops.

Cryptococcus neoformans can infect cats and cause feline disease that sheds viable organisms into the environment. However, human transmission from infected cats has not, as yet, been documented.[20]

Mites

Cats may become infected with *Sarcoptes scabiei* (scabies) and transmit this to humans. The mites cause a hypersensitivity reaction in humans, manifested by pruritic papular lesions and nocturnal itching. As cat scabies do not burrow into human skin, skin scrapings will not be diagnostic and diagnosis is by clinical presentation. Therapy is by removal of the mites from the cat and laundering the household bedding and clothing. *Cheyletiella* spp. are another type of animal mite that can be transmitted from cat to humans.

Cat infections acquired by inhalation

Bordetella bronchiseptica is a Gram-negative coccobacillus that can be found in the respiratory tracts of domestic cats and for which they should be vaccinated. In dogs it has been associated with 'kennel cough' but its clinical manifestations are often less prominent in cats. When cough appears in cats there may be an associated pneumonia. This organism can cause a pertussis-like (whooping cough) illness in humans, especially children or compromised hosts (HIV, etc.). Although cross-immunity may exist in humans from pertussis immunization during childhood, immunity wanes by adulthood. Human illness may range from a mild upper respiratory tract infection to frank pneumonia. The organism can be cultured but requires special media and can be misidentified in routine clinical laboratories.

Yersinia pestis is the Gram-negative coccobacillus that causes plague. Cat fleas are considered poor vectors for transmission but the cats themselves may contract the illness, especially in the summer months by exposure to rat fleas. Fatal cases in humans of inhalation plague and exposure to infected cats have been reported.

Humans directly exposed to infected material from parturient or aborted tissue from cats infected with Q fever (*Coxiella burnetii*) may also acquire disease. Cats may acquire infection from a tick bite or from infected material in the environment.

INFECTIOUS DISEASES ASSOCIATED WITH BIRD EXPOSURE

Contact with pet birds may vary from kissing or feeding the bird from the owner's mouth to cleaning cages or allowing the bird free range of a home or a yard. It is difficult to determine how often some of the

organisms, such as *Campylobacter jejuni*, *P. multocida* or *Mycobacterium tuberculosis*, are passed to humans, but *Chlamydia psittaci* is regularly transmitted, as are *Salmonella* spp.

Inhalation infections from birds

Psittacosis

Chlamydia psittaci can be carried by any bird – pet or wild – not just psittacine birds such as parakeets or parrots, and all birds that carry the organism can pass it on to humans. The disease is better called ornithosis rather than psittacosis. Ducks and turkeys have been responsible for outbreaks of ornithosis in humans as well as birds kept in the home. Although respiratory symptoms are usually the result of transmission from birds to humans, there have been reports of human-to-human transmission, but apparently this is not common. Too little is known about the incidence of infection with *C. psittaci* because few studies have been done of different human populations using modern, accurate serologic techniques. If the respiratory symptoms are mild, the infection is often undiagnosed. Even in patients who have pneumonia, the diagnosis needs to be confirmed by showing a fourfold rise in acute and convalescent serum titers 2–4 weeks apart, but this is seldom pursued.

The diagnosis of acute disease is dependent on a high index of suspicion, and therapy must be empiric, because rapid diagnostic techniques are not available. A history of contact with a sick bird is highly suggestive, but well birds can carry the disease and a number of cases have been reported without a history of bird contact, presumably from inapparent environmental contact with bird excreta. Pigeon and other birds' feces abound in many urban environments, as do chicken and duck feces in rural environments.

The pneumonia in humans can be severe, and is often lobar accompanied by high fevers and chills. The sputum may be purulent with a lack of potential pathogens on smear, because *C. psittaci* does not take the Gram stain. Once empiric therapy is decided upon, tetracycline is preferred, although erythromycin has been reported to be effective. A safe duration of therapy is 2 weeks, although a shorter period may be adequate. Complications such as meningoencephalitis, arthritis or endocarditis may occur.

A specific diagnosis is important because epidemiologic factors may need to be investigated. A bird dealer may be importing carriers without appropriate quarantine and/or treatment and cases of human-to-human transmission may be uncovered, including possible nosocomial spread. Control measures for *C. psittaci* infection of birds and humans have been detailed by the US Public Health Service.

Histoplasmosis

Histoplasma capsulatum has been found in bird droppings, especially from chicken and blackbirds. *Histoplasma capsulatum* has been found throughout the world, but it is especially common in river valleys of central North America, parts of Mexico and in the Caribbean, and is thus considered a 'regional' fungus. Histoplasmosis is usually not due to exposures to pets, with the exception of exposure to chickens, which are sometimes kept and regarded as pets. The fungus grows in the feces of chickens, but does not infect them. Most human infections are asymptomatic but others may result in an influenza-like illness or rarely a progressive pneumonia (see Chapters 31 and 178). The majority of symptomatic infections are self-limiting; however, treatment when necessary (especially in the immunocompromised host) can begin with amphotericin B followed by itraconazole or fluconazole for acute, progressive infections or itraconazole or fluconazole alone for more indolent disease. Prevention strategies should include advising immunocompromised people to avoid bird feces, especially from chickens.

Cryptococcosis

Cryptococcus neoformans in found in soil throughout the world, but it appears to thrive in pigeon feces.[20] Pigeon fanciers who keep flocks of pigeons for sport are exposed to *C. neoformans* more than the general population, as demonstrated by serologic testing; however, an increased incidence of disease has not been documented in these people. Cryptococcosis initially begins as a self-limiting respiratory infection. The fungus may disseminate widely to multiple organs including the central nervous system. It has a predilection for people who have $CD4^+$ T-helper-1 lymphocyte defects, and if untreated the meningitis it causes is associated with high mortality (up to 50%). There may be significant morbidity and sequelae (e.g. noncommunicating hydrocephalus, blindness) (see Chapter 91).

The diagnosis is aided by the presence of cryptococcal polysaccharide antigen in the blood or cerebrospinal fluid, and a decrease in antigen titer is usually associated with a response to therapy. Amphotericin B with or without flucytosine is the treatment of the acute episode, followed by fluconazole for more chronic therapy. Prevention should include advising immunocompromised people to avoid contact with pigeons.

Tuberculosis has been seen in patients in households where tuberculosis has been recognized in macaws.[21] It is difficult to determine whether spread is from birds to humans or vice versa. Other psittacine birds may become infected with *M. tuberculosis*.

Enteric diseases associated with bird exposure

All of the organisms listed in Table 68.5 have been isolated from birds; some, such as *Salmonella* spp. and *Giardia lamblia*, are clearly implicated in transmission to humans. Parasites that may spread to humans from birds include *G. lamblia* and *Cryptosporidium* spp., although direct spread from the latter is not as well documented as the former (see Chapters 180 and 181 for treatment guidelines). Mites may be spread by direct and indirect contact with infested birds.

Table 68.5 Organisms carried by small mammal pets

Bacteria	Fungi	Parasites	Viruses
Campylobacter spp. *Spirillum minus* *Streptobacillus moniliformis* *Salmonella* spp. *Leptospira interrogans* *Francisella tularensis* *Yersinia pestis* *Listeria monocytogenes* *Pasteurella multocida* *Burkholderia pseudomallei*	*Sporothrix schenckii* *Penicillium marneffei*	*Cryptosporidium* spp.	Lymphocytic choriomeningitis virus Hantavirus

Viral diseases from pet birds

Identical strains of influenza virus have been found in both humans and domestic ducks and chickens where contact has been documented. The incidence of ducks or other birds serving as reservoirs for influenza is not certain. Since 1998, when a large outbreak of avian influenza A (H5N1) in Hong Kong was associated with several human deaths, millions of birds have been slaughtered in an attempt to control the infection.

SMALL MAMMAL PETS

Small mammal pets include mice, rats, hamsters, gerbils, guinea pigs and rabbits. People may also keep more exotic animals such as mink, ferrets and ocelots. These animals carry organisms similar to those carried by mice and rats; ocelots carry organisms similar to those carried by cats. Table 68.5 lists organisms carried by small mammal pets.

Most pets, regardless of type, can carry *Salmonella* spp. and *Campylobacter* spp. Many, including rabbits, carry *P. multocida* as part of their mouth flora. There are some that are more likely to carry certain organisms.

Rat-bite fever

Rats can carry *Spirillum minus* and *Streptobacillus moniliformis*. Rat-bite fever due to *S. minus* (also known as spirillarly fever and sodoku) is seen worldwide but is most common in Asia.[22] A rash with reddish or purplish plaques accompanies the fever. The healed bite wound may reactivate when fever develops. The diagnosis requires highly specialized laboratories for confirmation. Treatment consists of penicillin or tetracycline.

Rat-bite fever due to *S. moniliformis* (also called Haverhill fever) is more common in the USA and may be due to a rat bite or to exposure to contaminated milk or water during an outbreak. The fever is usually accompanied by a maculopapular or petechial rash that is most pronounced on the extremities. Arthritis of large joints is common, as are relapses. Focal abscesses and endocarditis may occur. A specialized laboratory can confirm the diagnosis if a sterile site (i.e. joint fluid or blood) is positive on culture. Treatment is with penicillin or tetracycline.

Lymphocytic choriomeningitis virus (LCMV) is carried by mice and other rodents, including pet Syrian golden hamsters. It has been isolated from guinea pigs and dogs. Infection in humans results from exposure to the urine, feces or saliva of the rodent and may result in no symptoms, although a flu-like syndrome or meningitis may occur. The flu-like syndrome may be followed by recovery and then relapse with meningitis. Orchitis, parotitis and thrombocytopenia have also been observed. Diagnosis of LCMV is made by isolation of the virus from a sterile site such as the cerebrospinal fluid or acute and convalescent serum specimens showing a fourfold rise in titer. There is no treatment. If a case occurs in a pet hamster, the entire colony should be screened for LCMV.

MISCELLANEOUS PETS

Monkeys

The most insidious and disastrous viral infection that a pet monkey can pass to a human is cercopithecine herpesvirus type 1 (herpesvirus simiae; B virus). This latent infection of often-asymptomatic monkeys can be passed to humans by saliva or by a bite. It is almost always fatal in humans, in whom it causes progressive encephalitis which, if not fatal, will leave severe sequelae in most cases (see Chapter 155). If diagnosed early, treatment with aciclovir or other antiviral herpes agent, may result in improvement and even recovery. Although it is a legal requirement to screen imported pet monkeys, this does not always occur and may even miss carriers.

Nonhuman primates (and prairie dogs) can also transmit hepatitis A and B, and monkeypox, which is clinically indistinguishable from smallpox. Patients with bites or scratches are more likely to have a shorter incubation period (9 days vs 13 days) and more severe disease and require hospitalization than 'non-invasive' exposures from cleaning cages, etc.[23] Monkeys may also transmit salmonellosis, shigellosis, campylobacteriosis, amebiasis, strongyloides, giardiasis, yersiniosis, and dermatophyte infection.

Reptiles, amphibians and fish

Any reptile or amphibian may carry *Salmonella* spp., which are excreted in the feces and may infect humans caring for the pet. This has been best exemplified by outbreaks in humans who have pet turtles.

Fish may harbor *Salmonella* spp., including multidrug-resistant isolates, *Aeromonas hydrophila*, *Citrobacter diversus*, *Pseudomonas aeruginosa*, *Providencia stuartii* and *Vibrio* spp. including *Vibrio. vulnificus* (which can cause a rapidly fatal infection in patients with liver dysfunction) and other halophilic bacteria (salt water). In addition, *Mycobacterium marinum* has been found in fish tanks (both fresh and salt water) and can cause a purplish rash with granulomatous lesions, usually on the hands of those cleaning the tanks and handling the fish. The diagnosis is made when the skin lesion is biopsied and cultured. Treatment with rifampin (rifampicin) and ethambutol, minocycline or trimethoprim–sulfamethoxazole (co-trimoxazole) has been successful in eradicating this infection.

Erysipelothrix rhusiopathiae is an uncommon infection of food handlers, especially fishmongers. It causes erysipeloid, painful, indurated, irregular skin lesions, usually on the hands. It is very susceptible to penicillin. Penicillin-allergic patients can be treated with clindamycin.

REFERENCES

References for this chapter can be found online at http://www.expertconsult.com

Chapter 69

Daniel S Shapiro

Infections acquired from animals other than pets

Zoonotic infections may be acquired from farm animals, beasts of burden, fish and wild animals via a number of routes (Fig. 69.1). Many of the emerging infectious diseases are zoonotic in origin.

The approach to the patient with a potential zoonotic infection involves the generation of a reasonable differential diagnosis that includes those infectious agents that are potentially transmissible from the specific animal to which the patient was exposed. This may be a challenging task for the clinician when the exposure has been to a rarely encountered animal. Historical points worth considering are summarized in Table 69.1.

Although the number of infectious agents potentially transmissible from a specific animal to humans may be great, many of these infections are limited geographically and need not be considered in the differential diagnosis. Examples include the lack of plague transmission outside endemic areas such as the Western USA, countries that are free of brucellosis and the limitation of tularemia to the northern hemisphere. In some cases a good history of animal exposure will be elicited but a review of the medical literature will not be able to identify a published reference on zoonotic transmission of any relevant diseases from that specific animal. One additional difficulty is that the lack of an effective veterinary or human public health system in a given country may result in a lack of knowledge of zoonotic infections transmitted from even commonly encountered animals. In such cases, it is worthwhile considering similar animals with known flora or from

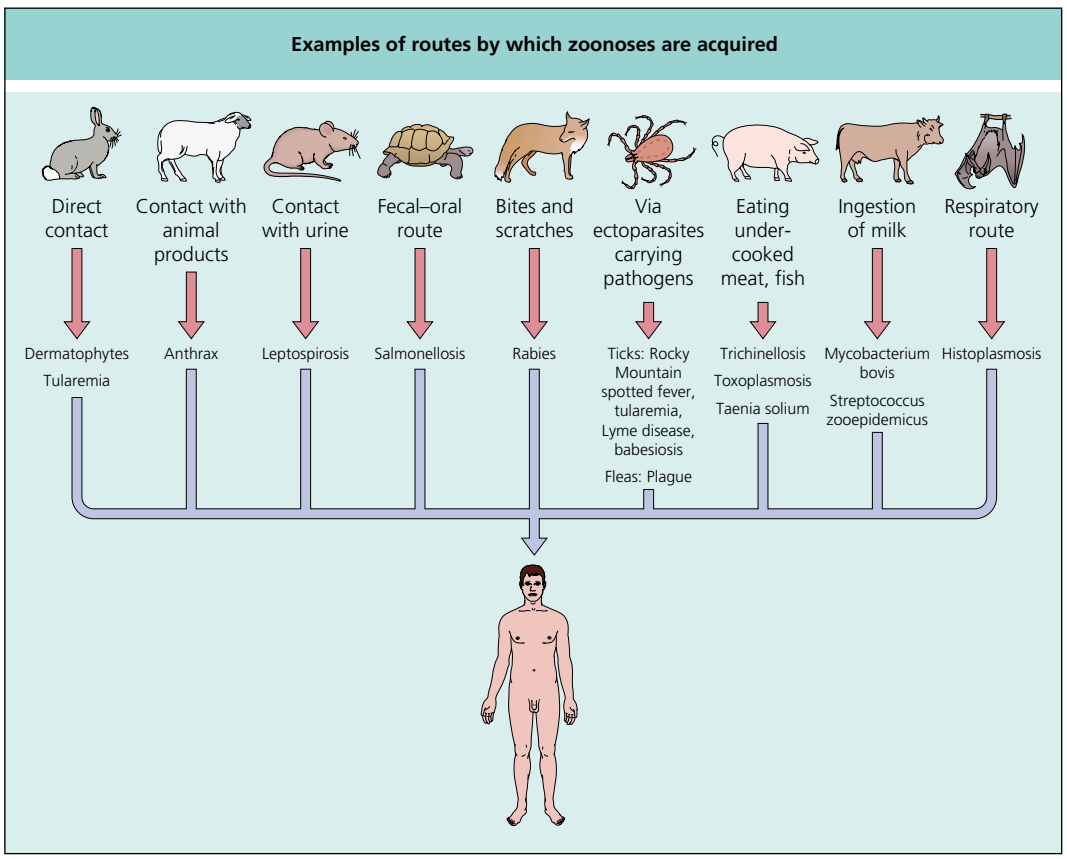


Fig. 69.1 Examples of routes by which zoonoses are acquired.

Table 69.1 Selected historical points in patient exposure history

Historical finding	Worth adding to differential diagnosis
Contact with any vertebrate, especially reptiles	Salmonellosis
Exposure to urine, either directly or via contaminated water	Leptospirosis, as essentially all mammals can become infected with *Leptospira interrogans* and shed infectious organisms in the urine
Bites from wild mammals, with the exception of those from rodents other than groundhogs (*Marmota monax*)	Evaluate risk of rabies and the potential need for rabies prophylaxis
Itching and a history of cutaneous contact with a mammal	Allergic reaction, dermatophyte infection or infestation with ectoparasites, such as species-specific varieties of *Sarcoptes scabiei*
Consumption of undercooked wild mammals	Trichinellosis and toxoplasmosis
Consumption of fermented fish or marine mammals	Botulism, most commonly due to the type E toxin
Consumption of uncooked fish	Any of more than 50 parasitic infections, depending upon the species of fish eaten and the geographic locale

which zoonoses have been acquired and infections of the animal with known zoonotic agents. Examples include the following.

- *Escherichia coli* O157:H7 infections have been most commonly transmitted to humans via the ingestion of undercooked ground beef. Deer, like cattle, are large grazing herbivores. They have transmitted this infection to humans via the consumption of venison.
- Camels have been noted to have serologic evidence of infection with *Coxiella burnetii*, but human cases of Q fever as a result of contact with camels have not been documented. It is possible that this is, in part, due to the lack of active case finding in those parts of the world in which camels are common.
- The environment of the animal, which may include exposure to fresh water or salt water, is important. For example, shark bite wounds may be infected with *Vibrio* spp., which are commonly found in salt water and as part of the normal oral flora of sharks, whereas fresh-water alligator bites are most commonly infected with *Aeromonas hydrophila*, an organism that is found in fresh water and as part of the normal alligator oral flora.
- Consider the diet of the animal. Cattle that have been fed material that includes nervous tissue are at increased risk of having bovine spongiform encephalopathy (BSE).
- Consider other species with which the animal has had contact, including contact with humans while in captivity. Tuberculosis, measles and shigellosis are not thought to normally be infectious agents of nonhuman primates. Rather, they are acquired from human contact. Similarly, the housing of camels indoors with cattle increases the risk that the camels will acquire bovine tuberculosis.

Good communication with the clinical microbiology laboratory is essential in the diagnosis of zoonotic infections. In some cases the diagnosis is established serologically, whereas in others a particular pathogen, perhaps one that requires special culture media or handling, may be found. In addition to increasing the probability of correctly identifying the etiology of the patient's illness, good communication is essential for safety, especially when infections due to *Francisella tularensis*, *Brucella* spp., cercopithecine herpesvirus type 1 (herpesvirus simiae; B virus) and other highly biohazardous agents are under consideration.

The following discussion is organized by type of animal, as this is helpful for the clinician who is attempting to generate a reasonable differential diagnosis.

DOMESTICATED HERBIVORES (CATTLE, SHEEP, GOATS, PIGS, CAMELS, HORSES AND RELATED ANIMALS)

Bacterial infections

Brucella melitensis is most commonly acquired from goats but has been acquired from sheep and dromedary camels. *Brucella abortus* is associated with cattle and, although horses can occasionally become infected, transmission to humans from horses, if it occurs, is very rare. *Brucella suis* can be transmitted to humans from both domesticated and feral pigs. Of note, the specificity of the association between the species of *Brucella* and the animal host is far from absolute. Examples include the occurrence of *B. melitensis* in cattle in parts of southern Europe, Israel, Kuwait and Saudi Arabia and the establishment of *B. suis* biovar 1 in cattle in Brazil and Colombia.[1] Many other animals have contracted brucellosis. Infection of bison in Yellowstone Park has been of concern to cattle ranchers in Western USA who worry that their cattle will be infected from contact with the bison. There have not, however, been documented cases of transmission of brucellosis in the area of the park from bison to cattle nor from bison to humans. In other geographic locations, *Brucella* infections of caribou have been known to spread disease to humans.

Transmission of anthrax by large domesticated herbivores is the normal means by which the infection is acquired by humans. Cutaneous anthrax, inhalation anthrax (woolsorter's disease) and gastrointestinal anthrax all are most commonly associated with the domestication of sheep, goats and cattle. In parts of the world in which water buffalo are domesticated, these animals have served as the source of outbreaks of human anthrax, as have oxen. Animal products can transmit the disease. For example, during the early 1900s cutaneous anthrax was transmitted via contaminated shaving brushes made of animal hair.

Yersinia pestis has been (rarely) transmitted to humans from both camels and goats in Libya and from camels in endemic areas of the former Soviet Union via direct contact and in individuals who consumed the infected animals.[2]

Epizootics of tularemia, associated with heavy infestation by the wood tick, *Dermacentor andersoni*, occur in sheep. Human cases of tularemia have included cases in sheep shearers, owners and herders. In a review published in 1955, 189 human cases of tularemia were reported in association with the sheep industry.[3] Tularemia has rarely been transmitted from pigs to humans.[4]

Tuberculosis due to *Mycobacterium bovis* subsp. *bovis* was the impetus for pasteurization of cow's milk. *Mycobacterium tuberculosis* infection can occur in cattle, but does not typically result in systemic infection. Infection with *M. bovis* is also associated with occupational exposure, as with slaughterhouse workers. The related *M. bovis* subsp. *caprae* has caused human infections after contact with cattle.[5]

Infection with *Listeria monocytogenes* may result from the ingestion of contaminated meat and dairy products or via direct cutaneous exposure during parturition. In the latter case, cutaneous listeriosis has been reported among veterinarians and other individuals delivering animals.[6] Infections transmitted by ingestion of animal milk are listed in Table 69.2.

Table 69.2 Agents transmitted via milk products and cheese

Disease	Source
Clostridium botulinum toxin	Yogurt, cheese
Brucella spp.	Many animals' milk and cheese
Campylobacter fetus	Cow's milk
Campylobacter jejuni	Cow's milk, cheese from goats
Campylobacter laridis	Cow's milk, contaminated by birds
Central European tick-borne encephalitis	Goat's milk, cheese from goats and sheep
Corynebacterium diphtheriae	Cow's milk
Corynebacterium ulcerans	Cow's milk
Escherichia coli O157:H7 and other strains	Cow's and goat's milk, cream, cheese
Listeria monocytogenes	Cow's milk, cheese
Mycobacterium bovis subsp. *bovis*	Cow's milk
Salmonella spp.	Many animals' milk, cheese, ice cream
Staphylococcus aureus	Cow's milk
Streptobacillus moniliformis	Cow's milk (single outbreak in 1926)
Streptococcus zooepidemicus	Cow's milk, cheese
Toxoplasma gondii	Goat's milk
Yersinia enterocolitica	Cow's milk

Yersinia enterocolitica, normally found in the fecal flora of pigs, has been transmitted from pigs to humans via contact and by ingestion of chitterlings (pig intestines).[7]

Erysipelothrix rhusiopathiae has been transmitted to humans from many different animals and animal products. It typically is an occupational illness, often acquired via a hand wound while handling animal material. Alerting the clinical microbiology laboratory to its possibility is of great help, as the organism's identification is not difficult if it is suspected.[8]

Streptococcus suis, especially type 2, a pathogen of pigs, is a common cause of bacteremia and bacterial meningitis among individuals working with pigs in Asia. *Rhodococcus equi* is commonly found in the feces of horses and in the soil. Exposure to farm animals, including horses, has been reported in some cases of human infection. The association of leptospirosis with swine is well known and one name for the illness is swineherd's disease. Cattle, goats, camels, dogs and rats are also potential sources of human infection.

Exposure of pregnant women to the birth products of sheep and goats that are infected with *Chlamydophila abortus* (formerly named *Chlamydia psittaci*, serotype 1) has resulted in illness. Human infection with this organism has been reported in both Europe and the USA, and can be severe, resulting in abortion.[9]

Salmonellosis has been transmitted to humans by each of these animals, including camels. Pigs have been documented as a source of human cases of multidrug-resistant *Salmonella enterica* serotype *typhimurium* definitive phage type 104 (DT104) infection.[10]

Escherichia coli O157:H7 is often present in the gastrointestinal tract of cattle. The most common route of transmission is via ingestion of undercooked ground beef. Transmission due to fecal contamination of food products can occur, such as via unpasteurized apple cider prepared from apples that were on the ground in a cattle pasture and used for cider production. Deer, like cattle, are large grazing herbivores and have been reported to transmit this infection to humans who have consumed venison. Outbreaks have been associated with visits to petting zoos. It has become increasingly understood that Shiga toxin-producing *E. coli* other than *E. coli* O157:H7 is responsible for approximately half of human Shiga toxin-producing *E. coli* infections.

Pasteurella aerogenes is the most commonly isolated organism from human infections following the bites of swine.[11] A number of other Gram-negative organisms have also been isolated from these infections. Camel bite injuries are typically infected and are particularly likely from male camels during the rutting season. Members of the genus *Actinobacillus* have been recovered from bites from horses and cattle and *Pasteurella caballi* has been isolated from wounds following horse bites. Rabies has been reported in all of these animals as well as in llamas.

Pig-bel, or enteritis necrotans, has been associated with the consumption of large quantities of pork in populations that have a low protein diet, such as in the highland Melanesians of Papua New Guinea and in Germany immediately following the Second World War. Evidence suggests that the major etiologic agent is *Clostridium perfringens* type C. Human cases of Q fever are acquired from sheep, goats and cattle, as well as from cats. Infection can be transmitted for significant distances via the wind. The data on human acquisition via contaminated milk are less compelling.

Glanders, due to *Burkholderia mallei*, has been transmitted to humans via equids. The disease is now quite limited geographically and the isolation of this agent from a patient in North America or Europe must be assumed to be due to bioterrorism until proven otherwise.

Viral infections

Localized cutaneous involvement can be due to infection with the parapoxviruses orf virus (which causes contagious ecthyma and is transmitted by sheep and goats either directly or via fomites), bovine papular stomatitis virus and pseudocowpox virus; and by the orthopoxviruses cowpox virus (which is more commonly transmitted to humans via cats than cattle) and buffalopox virus. The host range of influenza A virus includes many other mammals, including birds, swine and horses.

Variant Creutzfeldt–Jakob disease (variant CJD) has been reported from the UK, France, Japan and other countries and appears to be associated with the consumption of meat from cattle that were infected with BSE. Although cases of BSE have been identified in the USA, no cases of variant CJD have been found to be due to consumption of US cattle. Prion diseases of large herbivores in the USA, including chronic wasting disease of cervids, have raised the possibility of the introduction of prion diseases into the human food supply. A detailed discussion of the molecular aspects of prion-associated disease and the clinical manifestations of the spongiform encephalopathies are found in Chapter 22.

Rift Valley fever, which infects domestic ruminants, can be transmitted to humans both by mosquitoes and by contact with the tissues of slaughtered, infected animals such as sheep.[12] Similarly, Crimean-Congo hemorrhagic fever infects a variety of animals, including cattle and sheep, and is transmitted to humans via ticks (especially *Hyalomma* spp.), via contact with blood of infected animals and, if appropriate precautions are not taken, in the hospital setting.

Hendra virus, a paramyxovirus, caused an infection of horses and a few individuals in contact with these horses in Australia. The natural reservoir has been identified as a flying fox (bat). Nipah virus was the cause of an epidemic of encephalitis that affected more than 250 people in Malaysia and Singapore, killing 105 people. More recent outbreaks have occurred in India in West Bengal in 2001 when it killed three-fourths of the 66 infected people and in Bangladesh in 2004 when it killed 18 of 30 infected people. Subsequently, there have been additional outbreaks in West Bengal during 2007 and in Bangladesh in 2008. The vast majority of those infected had contact with pigs, which were culled in order to stop the epidemic. There has been concern about the possibility that

some cases were due to person-to-person transmission. The natural reservoir of Nipah virus, a paramyxovirus that is related most closely to the Hendra virus, has been identified as a bat. Menangle virus, a recently described paramyxovirus, caused an infection of pigs and infection in humans in contact with the pigs in Australia. The natural reservoir has been identified as a flying fox (bat).

Recent concern has been generated over the possibility of certain endogenous porcine retrovirus infections causing disease in humans following xenotransplantation of organ tissues from pigs. Some of these retroviruses can propagate in human cell lines and they could potentially induce immunodeficiency in experimental systems.[13] This poses a potential risk of activation of porcine retroviruses in the setting of an unnatural host such as an immunosuppressed, solid organ, human transplant recipient. Fortunately, it appears that commonly utilized porcine heterografts for heart valve replacement surgery are unlikely to be complicated by inadvertent activation of porcine retroviruses. The currently employed glutaraldehyde fixation and sterilization method for porcine heart valves eliminates infectivity of endogenous retroviruses.[14]

Parasitic infection

An epidemic of cryptosporidiosis occurred in 1993 in Milwaukee, Wisconsin, in which the public water supply was contaminated and more than 400 000 people were infected. The epidemic was traced to untreated water from Lake Michigan, from which the causative organism was incompletely removed by the water filtration process. Possible sources included cattle along two rivers, slaughterhouses and human sewage.[15] In addition to water-borne epidemics, human infections occur via direct contact with cattle and sheep (the disease primarily occurs in lambs), including infection as a result of farm visits.

Echinococcal disease, although not transmitted to humans from sheep, occurs in areas of the world in which sheep serve as an intermediate host and in which dogs ingest sheep viscera, subsequently excreting infective eggs in their feces. A different echinococcal strain occurs in dromedary camels.

The demonstration of the eggs of *Dicrocoelium dendriticum*, the lancet liver fluke of cattle, sheep and goats, in human feces may be due to a true infection (dicrocoeliasis) but is more often due to a pseudoinfection. In pseudoinfections, eggs obtained via the consumption of liver (sometimes eaten raw) are passed per rectum without causing an actual infection in the human.

The pig ascarid *Ascaris suum* has been reported to cause human infection. Illness, including pulmonary infiltrates as a result of larval migration, resulted in intubation in students who ingested intestinal contents of pigs as a result of a fraternity 'prank'.[16]

Taenia solium, the pork tapeworm, is acquired via the ingestion of undercooked infected pork. *Trichinella spiralis* is most commonly acquired from eating undercooked pork, but has also been acquired following the ingestion of bear and horsemeat.[17] *Taenia saginata*, the beef tapeworm, is acquired via the ingestion of undercooked beef. Toxoplasmosis can be acquired via the ingestion of undercooked meat, especially lamb, as well as from contaminated goat's milk.

Dermatophyte infection

Infection with zoophilic dermatophytes commonly occurs in people in contact with these animals. These include, for example, *Trichophyton verrucosum* spread from cattle to humans, and *T. equinum* from horses,[18] among others.

BATS

Rabies virus is known to occur in many species of bat and transmission to humans via bite, scratch and inhalation of aerosolized saliva has been reported. Bats also account for many cases of rabies in livestock. Other Lyssaviruses that have been transmitted to humans from bats include European bat Lyssavirus-1, European bat Lyssavirus-2 and, most recently, Australian bat Lyssavirus.[19] Most recent reports of human rabies from bat exposure find no clear evidence of a documented bat bite. Transmission apparently occurs from inadvertent bites or from unrecognized contact with the bat saliva. This is the rationale for the administration of rabies immune globulin and rabies vaccine when a bat is found in the room upon awaking from sleep, in the room of a small child or in the room of an intoxicated or mentally challenged person[20] (see Chapter 160). As noted above, bats have been found to be reservoirs of the zoonotic paramyxoviruses Nipah virus, Hendra virus and Menangle virus.[21]

Many outbreaks of histoplasmosis due to *Histoplasma capsulatum* have been associated with exposure to bat guano in caves. Other bat-associated outbreaks have been the result of disturbing piles of bat guano from old buildings[22] and clearing debris from a bridge.[23]

NONHUMAN PRIMATES

The numerous pathogens that have been isolated from nonhuman primates (NHPs) include many human pathogens. Documented transmission of human pathogens include bacterial (*Shigella* and *Salmonella* spp.), mycobacterial (*M. tuberculosis*), viral (hepatitis A virus), parasitic (*Entamoeba histolytica*) and fungal (dermatophyte) agents. In addition, there are infectious agents of human origin that infect NHPs and that have not been reported to be transmitted back to humans. These include measles virus and (human) herpes simplex virus type 1.

Of concern are those infectious agents that are not normally regarded as human pathogens. Many of these are particularly virulent. Historically, it is worth noting that molecular evidence suggests that HIV-1 was originally a pathogen of chimpanzees, *Pan troglodytes troglodytes*, and that HIV-2 was originally a pathogen of sooty mangabees. There are numerous simian immunodeficiency virus (SIV) strains and it is possible that one or more might be transmitted to humans via contact, ingestion or by growing the pathogen. Transmission of SIV to a human has occurred in a laboratory accident.[24]

The possibility of life-threatening infection with cercopithecine herpesvirus-1 must be considered in bites, scratches and contact with saliva from Asiatic macaques, especially from the rhesus monkey, *Macaca mulatta*. It is hypothesized that there are distinct genotypes of the virus and that the isolates from different species vary in their pathogenicity for humans. Institutions that work with macaques should have plans in place to prevent and treat infections with cercopithecine herpesvirus-1[25] and have access to testing by laboratories with special expertise in the isolation and serologic testing of this virus. The NIH B Virus Resource Laboratory at Georgia State University (phone (404) 413-6550, fax (404) 413-6556, website http://www2.gsu.edu/~wwwvir/Diagnostics/forms.html, e-mail bvirus@gsu.edu or jhilliard@gsu) is the reference laboratory for the USA.

Filovirus infections with both Ebola and the Reston strain of Ebola, which is less pathogenic for humans than other strains of Ebola, have been transmitted from NHPs to humans. Marburg virus, a filovirus causing hemorrhagic fever with high mortality, has been transmitted from vervet (or green) monkeys to humans. The reservoir in nature of Ebola and Marburg viruses appears to be bats.

Monkeypox, an orthopoxvirus, was initially identified in human cases of illness that were clinically consistent with smallpox. It is found in NHPs and in squirrels and other rodents in Africa and has been transmitted from human to human. Tanapox (benign epidermal monkeypox) has been transmitted to humans both via mosquitoes and by direct contact with monkeys in primate centers in the USA, but has not been transmitted from human to human. Yabapox virus has, rarely, caused subcutaneous growths at the site of inoculation.

Kyasanur forest disease virus, a member of the tick-borne encephalitis subgroup, is found in Karnataka State, India, and has a number of NHP reservoirs. The presence of dead monkeys in the endemic area may precede an epidemic.

Rabies has been reported in NHPs but, with the exception of a recent report in which the white-tufted-ear marmoset (*Callithrix jacchus*) was the source of eight human cases of rabies in Brazil,[26] there are only isolated case reports of transmission of rabies from NHPs to humans.

MUSTELIDS (FERRETS, SKUNK, OTTER, MINK, WEASEL, BADGER, MARTENS)

Influenza A virus has been transmitted in a laboratory setting in which a researcher was infected by a ferret that had been infected with a strain of influenza A virus and which 'sneezed violently at close range' while it was being examined.[27] Ferrets are susceptible to influenza A and B viruses. Mink on mink farms have been found to be infected with influenza A viruses.[28]

There is a report of *M. bovis* infection of the right palm more than 20 years following a ferret bite.[29] *Mycobacterium bovis* is known to infect wild ferrets and badgers. There is a case report of sporotrichosis complicating a badger bite. Rabies infection is known to occur in skunks, otters, badgers, weasels, mink and ferrets, including pet ferrets, in the USA. Transmission of rabies from skunks to humans has been documented.[30] A rabies vaccine has been licensed for use in ferrets and the National Association of State Public Health Veterinarians recommendations are for primary immunization at 3 months and booster immunizations annually.[31] The recommendations regarding a healthy ferret that bites a human are the same as those for dogs and cats with respect to confinement and observation for 10 days, with evaluation by a veterinarian at the first sign of illness.[31]

Rat-bite fever as a result of ferret and weasel bites was reported in the medical literature between 1910 and 1920. Only in a report of a weasel bite was there isolation of an organism from the patient's blood.[32] Trichinellosis has been reported in people who ate inadequately cooked or raw liver, spleen, blood and muscle of a badger.[33]

RODENTS

Yersinia pestis is transmitted in epidemics from rats to humans via the rat flea, *Xenopsylla cheopis*. Numerous rodents other than rats serve as reservoirs, some of which have been responsible for human plague. Similarly, tularemia is widely distributed in nature and has been transmitted to humans by many different rodents.

Leptospirosis is commonly associated with skin or mucous membrane exposure to water contaminated by the urine of rodents, including rats, mice and voles. It has rarely been reported to be transmitted via rodent bite.[34] Other uncommonly reported bacterial infections following rodent bites include *Pasteurella multocida*, the *Pasteurella* 'SP' group and sporotrichosis. Rat-bite fever can be due to either *Streptobacillus moniliformis* or *Spirillum minus*. The former has been transmitted to humans not only by wild rats but also by laboratory rats, mice and other rodents.

It is unclear how often rodents cause cases or outbreaks of human salmonellosis. However, given that *Salmonella* spp. are commonly recovered from rodent feces, the serotypes commonly recovered from rodents are similar to those recovered from cases of human disease, and rodents often infest human dwellings, restaurants and food production facilities, it is likely that they account for some fraction of human illness.

Many of the tick-borne relapsing fevers have wild rodents as reservoirs. This is also the case for *Babesia microti*, Lyme disease and human granulocytic ehrlichiosis. The reservoirs of Colorado tick fever include a number of squirrels, chipmunks and other rodents. Similarly, Powassan encephalitis, tick-borne encephalitis and Omsk hemorrhagic fever virus are transmitted via ticks and have small mammals as reservoirs. *Leishmania* spp. are transmitted by sandflies and often have rodents as reservoirs.

Those members of the Hantavirus genus that are known to cause hantavirus pulmonary syndrome (HPS) are carried by New World rats and mice, family Muridae, subfamily Sigmodontinae, and are transmitted via the inhalation of rodent excreta or saliva or, rarely, via rodent bite. In the USA and Canada, the viruses include Sin Nombre virus, the main cause of HPS, transmitted by the deer mouse (*Peromyscus maniculatus*); New York virus, transmitted by the white-footed mouse (*Peromyscus leucopus*); Black Creek Canal virus, transmitted by the cotton rat (*Sigmodon hispidus*); and Bayou virus, transmitted by the rice rat (*Oryzomys palustris*).[35] In South America, viruses include Andes virus in Argentina, Chile and Uruguay transmitted by the long-tailed pygmy rice rat (*Oligoryzomys longicaudatus*), a virus for which there is epidemiologic evidence of person-to-person transmission; Juquitiba virus in Brazil; Laguna Negra virus in Paraguay, transmitted by the vesper mouse (*Calomys laucha*); and Bermejo virus in Bolivia.[36] Hantaviruses that are associated with hemorrhagic fever with renal syndrome in Europe and Asia include Hantaan virus, transmitted by the murine field mouse (*Apodemus agrarius*); Dobrava virus transmitted by the murine field mouse (*Apodemus flavicollis*); Seoul virus, transmitted by the Norway rat (*Rattus norvegicus*) in Asia; and Puumala virus transmitted by the bank vole (*Clethrionomys glareolus*).[37]

Arenaviruses are transmitted from rodents via the excreta and urine. These include lymphocytic choriomeningitis virus, which is found worldwide and has been transmitted to humans by hamsters[38] as well as mice; Machupo virus, which causes Bolivian hemorrhagic fever and is transmitted by *Calomys callosus*; Junin virus, which causes Argentinian hemorrhagic fever and is transmitted by *Calomys* spp.; Guanarito virus, which is found in Venezuela; Lassa fever virus, which is found in Africa and is transmitted by the multimammate rat, *Mastomys natalensis*; and a recently described New World arenavirus that caused three fatal infections in California and shared 87% identity with the Whitewater Arroyo virus at the nucleotide level.[37]

Reservoirs of cowpox virus include several rodents. This is consistent with the epidemiology of cowpox in which cat contact is implicated. Cowpox, or a similar virus, has also been transmitted via rat bite.[39] A multi-state outbreak of more than 70 cases of monkeypox occurred in the United States following the importation of exotic rodents from Ghana and affected people who had contact with pet prairie dogs that had been in contact with the African rodents at an animal distributor.

Rickettsialpox has been associated with infestation of mice (*Mus musculus*) with mites which serve as the vector for human disease.[40] Rodents serve as reservoirs for many other rickettsial diseases, including murine typhus in which rats are the principal reservoir and the flea *X. cheopis* the principal vector; *Rickettsia prowazekii*, which has been associated with flying squirrels;[41] scrub typhus, in which rats are hosts of the trombiculid mite vectors; and members of the spotted fever group.

Although the issue of whether giardiasis is commonly zoonotic in origin is debated, beavers may have been the source of an outbreak of water-borne giardiasis.[42]

Ingestion of rodents has been associated with rare cases of trichinellosis, such as due to the ingestion of squirrel and bamboo rat.[43] There has been speculation on whether consumption of squirrel brains causes a spongiform encephalopathy, but data are limited.[44] Eating fermented beaver has resulted in botulism.[45]

Trichophyton mentagrophytes var. *mentagrophytes* is a common zoophilic dermatophyte, infecting humans and domestic animals. Rodents are regarded as the reservoir of infection.

LAGOMORPHS (RABBITS, HARES)

Tularemia, also known as rabbit fever, is acquired from rabbits and hares as a result of cutaneous contact and skinning of rabbits, presumably entering via microabrasions in the skin or conjunctiva, and via ingestion.[4,46] Transmission via infectious aerosol has also been reported as a result of mowing over a rabbit.[47] Tularemia transmission

to humans has not been reported from domesticated rabbits. Although uncommon, eight cases of human bubonic plague from 1950 to 1974 were reported as a result of contact (e.g. skinning) with rabbits and hares[48] in plague-endemic areas of the USA. Q fever has been transmitted to humans following contact with wild rabbits.[49]

A patient with *Bordetella bronchiseptica* respiratory infection was shown to have a strain that was indistinguishable by pulsed-field gel electrophoresis from the strain isolated from a respiratory tract isolate from one of 20 farm rabbits that slept with a cat with which she had contact.[50]

RACCOONS

The raccoon ascarid, *Baylisascaris procyonis*, has caused cases, including fatal ones, of meningoencephalitis, usually in young children who accidentally ingest infectious ova.[51] Ocular involvement has also been reported. Leptospirosis has been reported from contact with raccoons.[52] Rabies is common in raccoons, although direct transmission to humans in the USA has not been reported.

MONGOOSES

Leptospirosis is common among mongooses in Hawaii[53] and a number of Caribbean islands.[54] Rabies is quite common among many species of mongoose and accounts for a significant number of cases of human exposure to rabies in the Caribbean. It is the principal rabies reservoir in South Africa and it may be an important source of wildlife rabies in India.[55]

INSECTIVORES

Hedgehog contact has transmitted salmonellosis[56] and dermatophyte infections due to *Trichophyton erinacei*.[53] In an outbreak of leptospirosis in Italy in which 32 of 33 confirmed cases were contracted by drinking water at the same water fountain, a dead hedgehog was found in a water reservoir connected to the system, although isolation of *Leptospira* spp. from the hedgehog was not attempted.[57]

The Asian house shrew, *Suncus murinus*, has been found to be infested with the oriental rat flea, *Xenopsylla cheopis*, and infected with *Yersinia pestis*. It may well be important in the maintenance of plague between epidemics. Insectivores also appear to be reservoirs of tick-borne encephalitis and tularemia.

MARINE MAMMALS (SEALS, SEA LIONS, WALRUS, WHALES, DOLPHINS, PORPOISES, MANATEES)

At the case report level, there are several infections that have been transmitted from marine mammals to humans. Leptospirosis, which is commonly encountered in seals and California sea lions, has been transmitted from an infected sea lion pup to a human. Two people developed leptospirosis after performing a necropsy on a sea lion that died of leptospirosis.[58] Human infection with *Erysipelothrix rhusiopathiae* has been reported on a few occasions among veterinarians and veterinary students caring for or performing autopsies on cetaceans.[59] In these reports, the isolation of the organism was not made from the human cases. Two of three people who cared for affected gray seals developed 'single milker's nodule-like lesions' on the fifth finger of the right hand. The lesions from the seal handlers demonstrated virus particles that were identical with the virus particles from the seals' pox lesions and were characteristic of the paravaccinia subgroup of poxviruses.[60]

Pulmonary tuberculosis due to a member of the *Mycobacterium tuberculosis* complex that is similar to *M. bovis* has been transmitted from seals in a marine park in Western Australia to a seal trainer who developed pulmonary tuberculosis 3 years after his last exposure to the animals with an isolate of the *Mycobacterium* that could not be distinguished from the seal isolates on the basis of DNA restriction endonuclease analysis.[61] Seal trainers are in very close contact with seals who, by barking and coughing, are potentially able to transmit infection via the aerosol route.

Four people involved in necropsies of harbor seals from which influenza A virus A/Seal/Mass/1/80 (H7N7) was isolated, developed purulent conjunctivitis but did not have detectable antibodies in single serum samples 3–6 months after the exposure to the influenza A virus isolated from the seals.[62] A seal that was known to be infected with the influenza A virus sneezed into the face and right eye of a person who subsequently developed conjunctivitis from which the virus was isolated.[63] Influenza A virus has also been isolated from cetaceans.

Numerous cases of 'seal finger' have been reported in people who have been bitten or scratched by seals and from skinning or handling seals. Seal finger often responds to tetracycline therapy. The etiologic agent has not been established. Other organisms that have been transmitted via the bite of marine mammals include a single case report of *Mycoplasma phocacerebrale*, which was isolated from the drainage material from a patient's fingers and swabs from the seal's front teeth.[64]

Consumption of whale, seal and walrus meat is not uncommon among the Inuit in Canada, Alaska, Greenland and Siberia. There have been large epidemics of salmonellosis resulting from consumption of whale meat from floating and beached whale carcasses that have been used as the source of food. Trichinellosis (trichinosis) has been acquired following the consumption of raw or undercooked walrus meat. The clinical presentation in arctic trichinellosis due to *Trichinella nativa* differs from that of classic trichinellosis caused by *Trichinella spiralis* in that the most prominent clinical symptoms in arctic trichinellosis are gastrointestinal, with prolonged diarrhea.[65] Food-borne botulism, typically due to *Clostridium botulinum* type E, has been acquired from the consumption of fermented foods included beluga whale meat, seal meat, seal flippers and walrus meat.

ARMADILLOS

Both experimental and naturally occurring leprosy in nine-banded armadillos has been noted and there has been a body of literature (reviewed by Blake *et al.*[66]) which suggests that contact with armadillos may have been the source of leprosy in some patients in the USA and Mexico. Sporotrichosis has been found to be highly associated with armadillo contact in Uruguay.[67]

BIRDS

Psittacosis is transmitted to humans not only via pet birds but also via turkeys, wild and domestic pigeons, ducks and other birds.[68]

Salmonellosis has been acquired from contact with birds and from consumption of birds (e.g. chicken, turkey) and eggs.[69] *Campylobacter jejuni* and *C. laridis* infections have been associated with both the consumption of birds and, interestingly, consumption of milk that has been pecked by magpies (*Pica pica*) and jackdaws (*Corvus monedula*).[70] *Erysipelothrix rhusiopathiae* has been acquired from bird contact. Newcastle disease virus of fowl, an occupational disease, causes an acute conjunctivitis which may be associated with pre-auricular adenitis.[71]

Histoplasmosis, often in large outbreaks, has been the result of inhalation of bird excreta.[72] Infection with *Cryptococcus neoformans*, which is known to be found in bird droppings, has at the case report level been linked to exposure to pet birds[73] and fancy pigeons.[74]

Q fever has been reported in five members of a family as a result of exposure to either aerosolized, contaminated pigeon excreta or infected ticks, or both.[75]

Avian strains of influenza A virus represent a global concern, as the host range of the viruses may include humans. There exists the potential for pandemic influenza as a result of the introduction of an avian virus with a hemagglutinin to which humans lack immunity.[76] In 1997 in Hong Kong, there were 18 human cases of influenza A H5N1 infection and six deaths. The outbreak ended after the institution of control measures that included the culling of poultry in Hong Kong. In 1999 an H9N2 strain, closely related to a quail virus which co-circulated with H5N1 viruses in live poultry markets in Hong Kong in late 1997 and shown to possess a set of internal genes similar to those of the H5N1 viruses, infected two children.[77] More recently, avian strains have caused infections not only in birds in multiple countries but also in people, continuing to raise the specter of pandemic influenza. As of this writing, there has not been efficient person-to-person spread of these strains.

The recent epidemic of West Nile virus infection in the USA is largely attributable to the introduction of this flavivirus into a new ecologic niche in wild birds in North America. Blackbirds, crows, other wild birds and domestic chickens are susceptible to this viral illness and this forms the reservoir for this mosquito-transmitted infection that is responsible for a potentially lethal form of viral encephalitis.[78]

Tularemia has been, at the several case report level, acquired from wild birds. A case of Crimean–Congo hemorrhagic fever in an ostrich farm worker who was involved in the slaughter of ostriches, *Struthio camelus*, and handled the fresh blood and tissues of the birds, has been reported. There were numerous adult *Hyalomma* ticks on the ostriches and he likely was infected either directly due to skinning the ostriches or as a result of the presence of the ticks on the ostriches.[79]

FISH

In addition to the normal flora of the fish, a wound can become infected with environmental bacteria. The species of bacteria that live in water are dependent on both salinity and temperature. Free-living estuarine and fresh-water bacteria include the genera *Vibrio*, *Aeromonas* and *Plesiomonas*. As a result, the etiologic agents isolated from an infected wound from a fish bite, spine or fin injury that occurs in salt water may well be different from one that occurs in fresh water. The normal flora of teeth in salt-water sharks includes, for example, *Vibrio* spp., including *V. harveyi* (formerly *V. carchariae*), an organism that was the cause of infection following the bite of a great white shark.[80] By contrast, *Edwardsiella tarda* is commonly isolated from catfish injuries occurring in fresh water. Other organisms that have caused wound injuries as a result of injuries from fish include *Aeromonas* spp., *Erysipelothrix rhusiopathiae*, *Mycobacterium marinum*, *Mycobacterium terrae*, *Streptococcus iniae*, *Vibrio vulnificus* and *Vibrio vulnificus* serovar E (biotype 2; indole-negative) from eels.[81] *Vibrio alginolyticus*, *Photobacterium damselae* subsp. *damselae* (formerly *Vibrio damsela*), *Shewanella putrefaciens*, *Pseudomonas aeruginosa* and *Halomonas venusta* have all been isolated from fish bites and injuries. It is not always clear whether the source of the organism is the fish or the water.

Ingestion of fish or fish products can pose a significant risk of acquiring both bacterial and parasitic infections unless the fish has been well cooked.

Vibrio spp., including *V. fluvialis*, *V. hollisae*, *V. parahaemolyticus* and *V. cholerae* O1,[82] have all been associated with fish consumption, as has *P. shigelloides*. Eel consumption has been associated with *Photobacterium damselae* subsp. *damselae* (formerly *Vibrio damsela*).[83] *Listeria monocytogenes* infections have been associated with the consumption of fish, including vacuum-packed salmon and cold-smoked rainbow trout.[84]

Fish-associated botulism is usually due to type E toxin and in the USA is most common among Alaskans. Fermented fish eggs, fish eggs, home-marinated fish and dry salted fish have all been implicated. Consumption of apparently fresh (unpreserved and unfermented) fish in Hawaii resulted in three adults with botulism due to type B toxin.[85] Numerous parasitic infections have been reported following the consumption of raw, undercooked, pickled and lightly or cold-smoked fish. Selected cestodes, trematodes and nematodes acquired from the consumption of fish are listed in Table 69.3.

Table 69.3 Selected parasites transmitted via consumption of fish

Parasite	Type of parasite	Types of fish
Diphyllobothrium latum	Cestode	Salmon, pike, perch, burbot
Diphyllobothrium pacificum	Cestode	Marine fish
Diphyllobothrium ursi	Cestode	Salmon
Nanophyetus salminicola	Trematode	Usually salmonids
Heterophyes heterophyes	Trematode	Mullet, tilapia, mosquito fish
Haplorchis yokogawai	Trematode	Mullet
Haplorchis taichui	Trematode	Mullet
Clonorchis sinensis	Trematode	Fresh-water fish
Opisthorchis viverrini	Trematode	Fresh-water fish
Opisthorchis felineus	Trematode	Fresh-water fish
Metorchis conjunctus	Trematode	Fresh-water fish
Anisakis simplex	Nematode	Salmon, tuna, herring, mackerel, others
Pseudoterranova decipiens	Nematode	Cod, pollock, haddock, salmon, Pacific rockfish
Eustrongyloides spp.	Nematode	Killfish, estuarine fish, minnows
Dioctophyma renale	Nematode	Fresh-water, estuarine fish
Capillaria philippinensis	Nematode	Fresh-water, estuarine fish
Gnathostoma spinigerum	Nematode	Fresh-water fish

AMPHIBIANS

Contact with amphibians has transmitted sparganosis, due to *Diphyllobothrium* (*Spirometra*) *mansoni*, which has been transmitted by the use of contaminated frog flesh as a poultice (reviewed by Huang and Kirk[86]), and far less commonly with intraocular *Alaria* spp. in a woman with a long history of frog collection and food preparation.[87]

Ingestion of amphibians has transmitted sparganosis. Infection with the trematode *Fibricola seoulensis* has been reported from Korea, including a report in which 10 Korean soldiers who ate raw or undercooked flesh of snakes or frogs during survival training acquired the infection.[88] A single case of a fatal infection in a 24-year-old man due to *Alaria americana* has been attributed to the consumption of undercooked frogs' legs, although there was no documentation of consumption. Two cases of intraocular infection with an *Alaria* spp. were reported in Asian-Americans in California who consumed cooked frogs' legs in Chinese dishes.[89] Although frogs' legs have a very high rate of contamination with *Salmonella*, there are

few published reports of salmonellosis attributed to their ingestion. *Salmonella* infections remain a potential risk when handling reptiles, such as turtles, lizards and snakes.

BEARS

There is a single published report of transmission of leptospirosis to two zoo employees in which the most likely source was an ill polar bear cub.[90] There is a notable lack of published reports on infections following the bites of bears. A case report in which a man shot and killed a grizzly bear in Alaska and scratched his left index finger on one of the bear's teeth while removing the bear's tongue resulted in an infection with *Mycobacterium chelonae* subsp. *abscessus*.[91]

Consumption of undercooked bear meat has been associated in multiple reports with trichinellosis. Bear steaks are often served rare, in part because they are somewhat 'tough' if they are fully cooked. Bears are known to have a high rate of toxoplasmosis and the possibility of a dual infection (trichinellosis and toxoplasmosis) in a person who ingested undercooked bear meat has been reported.[92] It is worth noting that acute hypervitaminosis A has been reported following the ingestion of polar bear liver.

LARGE HERBIVORES (ELEPHANTS, RHINOCEROSES)

Few infections have been transmitted from elephants and rhinoceroses to humans. These include documented transmission of *M. tuberculosis* from elephants,[93] *M. bovis* from rhinoceroses[94] and an orthopoxvirus (possibly cowpox). It is likely that cases of tuberculosis in elephants, which are almost all reportedly due to *M. tuberculosis*, are the result of human-to-elephant transmission. In the United States, approximately 3% percent of elephants are infected with *M. tuberculosis*.[95]

REFERENCES

References for this chapter can be found online at http://www.expertconsult.com

Chapter 70

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Chronic fatigue syndrome

INTRODUCTION

Fatigue is one of the most common complaints of patients with all kinds of underlying disease, but physicians often tend to ignore the complaint because they are unable to deal with it properly.

Chronic fatigue syndrome (CFS) is a syndrome characterized by persistent and unexplained fatigue associated with severe impairment in daily functioning.[1] Patient organizations tend to prefer the term ME (myalgic encephalomyelitis) or CFIDS (chronic fatigue and immune dysfunction syndrome) which are both misnomers, because they refer to pathological processes that have not been proven (inflammation of the central nervous system; immunodeficiency).

Because a substantial proportion of patients with CFS (around 75%) report a sudden onset starting with or following an infection, infectious disease specialists are often consulted by patients with CFS. This also justifies a chapter on CFS in a textbook like this. However, not all patients attribute the start of the symptoms to an infection and a more gradual onset is not uncommon.

The pathogenesis of CFS is still poorly understood (see below) and among physicians and lay people some considerable controversy surrounds the syndrome. Some doctors feel that it is a nonexistent illness, others consider it a mental illness, whereas still others (among them many patients) are convinced that it is a somatic disorder. Because older textbooks did not deal with CFS or coin it as a psychiatric illness ('neurasthenia'), a number of doctors are not diagnosing patients as suffering from CFS; patients may return with the self-diagnosis of CFS or ME, and this may lead to friction within the doctor–patient relationship. Because of the controversies mentioned, many patients with CFS will have had negative experiences with doctors that voiced their doubts regarding CFS. Such negative experiences will often enforce the patient's attributions to a somatic explanation for the illness.

The literature on CFS reinforces this controversy; however, it also struggles with a number of mediocre studies in which unsubstantiated claims regarding pathogenesis, diagnosis and therapy are made. These studies are often readily accepted as true by patients and their organizations as they desperately look for solutions to their illness. Physicians who take care of CFS patients should be aware of these issues, and – as is stressed in the guideline from the UK National Institute of Health and Clinical Excellence (NICE) – need good communication skills to enable patients to participate as partners in decisions about their health care.[2] In addition, this guideline recommends a timely and accurate diagnosis and early symptom management.

CASE DEFINITION AND DIAGNOSTIC APPROACH

Descriptions of CFS-like illness can be found in the older literature, some dating back centuries.[3] Although there are several case definitions used around the world, the most widely accepted and used is the consensus definition published by Fukuda *et al*.[4] (Table 70.1). This case definition, which was primarily meant for clinical and epidemiologic research on CFS, has not been validated for use in individual patients. An intrinsic problem with classifications like this is that the way the criteria are scored makes a substantial difference; by spontaneous reporting the average score is considerably lower than when systems are systematically asked for, either orally or by questionnaire.[5]

A second problem of which clinicians should be aware, is that the number of positive criteria does not reflect the severity of fatigue and impairment.[5] This means that to assess the severity of illness other

Table 70.1 Diagnostic criteria for chronic fatigue syndrome (CFS)

To diagnose CFS the following criteria should be fulfilled:
- Clinically evaluated, unexplained, persistent or relapsing fatigue that is of new or definite onset (not lifelong) lasting 6 or more consecutive months
- The fatigue is not the result of ongoing exertion
- The fatigue is not substantially alleviated by rest
- The fatigue results in substantial reductions in previous levels of occupational, educational, social or personal activities

Four or more of the following symptoms are concurrently presented for >6 months
- Impaired memory or concentration
- Sore throat
- Tender cervical or axillary lymph nodes
- Muscle pain
- Multi-joint pain
- 'New' headaches
- Unrefreshing sleep
- Postexertion malaise

After Fukuda *et al*.[4]

questions must be asked. A better alternative is to use the validated questionnaires that are available. We prefer the Checklist Individual Strength for fatigue and the Sickness Impact Profile (SIP-8) or Medical Outcomes Survey Short Form-36 (MOS SF-36) to assess functional impairment.[5,6]

The criteria of the case definition[4] cover most of the symptoms that occur within the context of CFS. Most prominent are incapacitating fatigue and postexertional malaise lasting more than 24 hours; patients may further suffer from myalgia, arthralgia, headache, sleep disturbances (such as unrefreshing sleep, insomnia), and problems with memory and concentration. In addition, CFS patients have abdominal complaints (as in irritable bowel syndrome), hyperhidrosis, frequent micturition and slightly elevated body temperature.[7] Sore throat and painful lymph nodes, although listed in the American literature and in the Fukuda case definition,[4] are rare and usually not found by physical examination. It is not uncommon for patients to report that they have become allergic, but this also is hard to substantiate.[8] Patients with primary fibromyalgia often suffer from fatigue and accompanying symptoms similar to those of CFS patients.[9] Many authorities consider CFS and fibromyalgia part of a spectrum in which either fatigue or pain is most prominent.

A major difficulty for the physician is that CFS is – to a large extent – a diagnosis of exclusion. Over the years there have been a series of claims in the literature on diagnostic markers (e.g. RNAse-1, microbial serology or polymerase chain reaction, immunologic tests), but so far none has proven useful. Likewise, testing the function of the pituitary–adrenal axis or imaging the CNS (see below) is not fruitful outside the research setting as the abnormalities that have been reported are too subtle to be of diagnostic significance. Thus, there is no gold standard for the diagnosis and the differential diagnosis is a long one. In Table 70.2, a systematic differential diagnosis of chronic fatigue is presented. It is clear that most alternative diagnoses can be excluded by taking a thorough history from the patient and performing a meticulous physical examination and a limited set of diagnostic tests. If the duration of the illness is longer than a year, the number of additional investigations may be more limited, since a range of diagnoses (e.g. bacterial endocarditis, malignancy) are becoming highly unlikely in the absence of any specific accompanying signs and without overt deterioration of the somatic state. Extensive serology also becomes unnecessary since most infections do not remain symptomatic for such a prolonged period.

Table 70.2 Systematic differential diagnosis of chronic fatigue

Localized organ dysfunction	Endocrine: • Pituitary: hypopituitarism • Adrenal: adrenal insufficiency, hypercorticism • Thyroid: hypo- and hyperthyroidism • Parathyroid: hypo- and hyperparathyroidism • Diabetes mellitus Heart: heart failure Lung: emphysema, chronic obstructive pulmonary disease, other causes of respiratory insufficiency Liver: chronic hepatitis, cirrhosis Kidneys: renal insufficiency
Generalized disorder	Infection Noninfectious inflammatory disease Intoxication Storage disease Neoplasm Anemia
Neurologic	Multiple sclerosis Narcolepsy Myasthenia gravis
Psychologic/psychiatric	Mood disorder Sleep disorder Somatoform disorder
Drug-induced	Side-effect of drug Psychotropic agents
Idiopathic	Chronic fatigue syndrome

EPIDEMIOLOGY

Although CFS has received most attention in the western world, it seems to occur everywhere. In the older literature there are a number of small outbreaks of CFS, the causes of which have not been elucidated.[3] The recent literature deals merely with sporadic cases.

There is a clear female preponderance in CFS: at least 70% of the patients are female.[4] Most patients are in the third decade of life, but CFS occurs in the young (mainly adolescents) and also in people of older age.[4]

The prevalence of CFS has been found to vary between countries but this may be due to the different methodologies of the various studies, as well as ethnic and sociologic differences. With regard to the latter, perceptions of illness, attributions and the tendency of labeling a complex of symptoms CFS/ME greatly differ between countries. Through questionnaires sent to general practitioners, we found a prevalence of 1.95 per 1000 in the Netherlands.[10] From community-based studies in adults in the USA, prevalence rates of 2.3 and 4.2 per 1000 have been reported, whereas a prospective study in primary care in the UK found 26 per 1000.[11–13] After correction for co-morbid psychological disorders the prevalence in the latter study became 5.0 per 1000, which still is remarkably high.

ETIOLOGY AND PATHOGENESIS

There are many hypotheses regarding the etiology and pathogenesis of CFS, but few are supported by evidence. So far, the search for a unifying explanation (e.g. a chronic infection by persisting micro-organism) has not been successful. Some 15 years ago it occurred to us and others that – when investigating the etiology and pathogenesis of CFS – it is more fruitful to discern a series of factors:

- predisposing factors;
- precipitating factors; and
- perpetuating factors.

From a theoretical point of view, this does not preclude the possibility that ultimately the precipitating factors and perpetuating factors are the same, but in the research setting and for our current understanding this distinction is more appropriate.

Predisposing factors

Predisposing factors comprise genetic factors, some of which are emerging from studies using modern genetic techniques but still arouse considerable controversy.[14–17] So far these genetic factors have not led to greater insight into the molecular substrate of CFS. As mentioned, there is a strong gender predisposition for CFS: at least 70% of the patents with CFS are female. Personality characteristics such as neuroticism and introversion are considered to contribute to the risk of developing CFS.[1] Likewise, inactivity during childhood and infectious mononucleosis are contributing factors.[18,19] Childhood traumas are associated with an increased risk of CFS.[20]

Precipitating factors

Precipitating factors are the factors that start the illness. These precipitating factors, which are largely attributions of patients, seem to be heterogeneous: infection, physical trauma, intoxication (including medication), operation and/or anesthesia, and serious life events are all considered to be instigators of CFS. Although these attributions are often very plausible, there is only circumstantial evidence that they are the starting point of CFS. The typical history of the patient with CFS is that it all started with a flu-like illness that was accompanied by severe fatigue; when the flu resolved, the fatigue remained. Evidence for infection as a starting point comes from studies in infectious mononucleosis, caused by Epstein–Barr virus (EBV).[21,22] In the acute phase, this infection – when symptomatic – is often accompanied by fatigue that wanes over a period of weeks to months. A small minority of patients (approximately 10–15%) experience fatigue beyond 6 months and develop the characteristic chronic fatigue syndrome. How many of these patients spontaneously recover between 6 months and 1 year is not clear.

The severity of the acute infection but no other clinical, demographic or psychological factors have been found to be risk factors for developing CFS.[21] A more rapid IgG response to EBV-associated nuclear antigens and a slower development of the peak interferon gamma (IFN-γ) response to latent cycle EBV peptides seems to predispose to the development of CFS.[23] Recently, in a small study using microarrays, activation of mitochondrial function of a number of genes, fatty acid metabolism and cell cycle were found to predispose to CFS in EBV infection.[24]

Another relevant question is whether certain infections do lead more often to CFS than others. Although EBV infections are commonly incriminated as a starting point for CFS, other infections are also associated with CFS. In fact, fatigue after 6 months is as common in EBV infection as in Q fever and Ross River virus infection.[25]

Although a number of metabolic abnormalities (mitochondrial defects such as carnitine deficiency) have been claimed to underlie CFS, none of these has been supported by solid scientific evidence.[26]

Perpetuating factors

Perpetuating factors are responsible for the chronicity of CFS and can be divided into somatic and psychological perpetuating factors. With regard to the former, one may ask the question whether microbial pathogens, especially those that persist after acute infection, may act as perpetuating factors. For EBV there is no evidence in this respect: virologic studies[27] as well as interventions with antiviral drugs[28] do not point to EBV as a perpetuating factor. There is no evidence for a role of other micro-organisms (e.g. *Helicobacter pylori*, *Borrelia* spp., *Brucella* spp., *Coxiella burnetii*, *Candida* spp., *Mycoplasma* spp.) as a persisting cause. Nevertheless, the hypothesis could be entertained that an as yet unknown persistent microbe acts as the precipitating and perpetuating factor for CFS.

A number of claims have been made regarding immunologic factors in CFS but few have been independently reproduced. In well-controlled studies, only minor differences have been found.[29,30] One area of controversy is the sensitivity to dexamethasone and production of cytokines such as interleukin-10 and IFN-γ production; in these areas at least there are profound differences between adults and adolescents with chronic fatigue.[31,32]

In most studies the immunologic abnormalities are not great and such subtle changes do not automatically imply causality or a role as a perpetuating factor. It may well be that such findings are a consequence of change in activity and/or behavior.

There are more controversial findings regarding the pathophysiology of CFS. For instance, the finding of an abnormal RNAse-1 response has not been substantiated by independent investigations.[33,34]

There is a subtle derangement of the hypothalamic-pituitary-adrenal (HPA) axis, with a relatively low glucocorticoid response (albeit within the normal range) despite a relatively high adrenocorticotropic hormone (ACTH).[35,36] These changes are not present during the early stages of fatiguing illness and are reversed during successful cognitive behavioral therapy.[36] Intervention studies with low-dose steroids have not provided unequivocal answers[37,38] and are listed as strategies not to be used in the NICE guideline.[2]

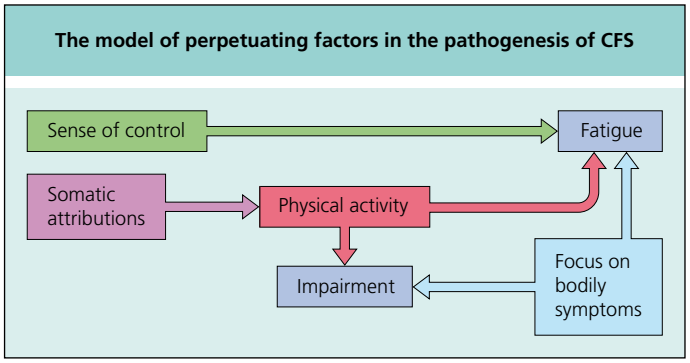


Fig. 70.1 The model of perpetuating factors in the pathogenesis of chronic fatigue syndrome. Using a Lisrel analysis of empiric data a model was established in which fatigue is a consequence of a lack of sense of control ('I don't have control over my symptoms'), decreased physical activity and a focus on bodily sensations (the patients are very tuned in to bodily symptoms and feel there is something wrong with their body). Strong somatic attributions ('I know this is a physical illness') leads to decrease in physical activity. The lack or avoidance of physical activity and the focus on bodily sensations also lead to the impairment. Reprinted with permission from Vercoulen *et al*.[47]

There are controversial reports in the literature regarding the growth hormone pathway, but no abnormalities were found in well-controlled studies.[39–41] Abnormalities within the serotonin pathway have also been implicated in the pathogenesis of CFS. Evidence for enhanced serotonin metabolism has been reported,[42–44] but interference with a 5-HT_3 inhibitor failed to demonstrate an effect in a placebo-controlled trial in well-defined CFS patients, despite an earlier positive pilot study.[45,46]

From the preceding paragraphs it may be clear that so far few, if any, somatic factors have been found to play a role in the pathogenesis of CFS.

With regard to psychological factors, Vercoulen *et al*.[47] were able to provide a model of CFS on the basis of a Lisrel analysis of a large number of measurements in patients with CFS (Fig. 70.1). This model appears to be specific for CFS, as it is statistically rejected for fatigue secondary to other diseases. Factors that were found to contribute to chronicity are strong somatic attributions ('I know that it is a pure somatic disorder'), a low self-efficacy ('I cannot influence my complaints') and a strong focus on bodily sensations. The latter is one of the most prominent features of CFS, and the avoidance of physical activity is a consequence of perceptions and expectations rather than of the actual physical condition. The role of perception will be discussed in more detail below.

In the older literature, CFS was sometimes considered to be atypical depression, but the current view is that CFS and depression are different conditions.[48] At time of diagnosis, depression is present in 25% of patients, while 50–75% of CFS patients have a lifetime history of major depression.[49] In patients with CFS a number of typical symptoms of depression, such as anhedonia, guilt and lack of motivation are not found, and the changes in the HPA axis are opposite. Antidepressants such as fluoxetine are neither effective against the fatigue nor against the symptoms of depression.[50]

CENTRAL FATIGUE IN CFS

The fatigue in CFS is considered centrally located as the subjective sense of fatigue is perceived at the level of the central nervous system.[51,52] There are few if any arguments for peripheral fatigue, the

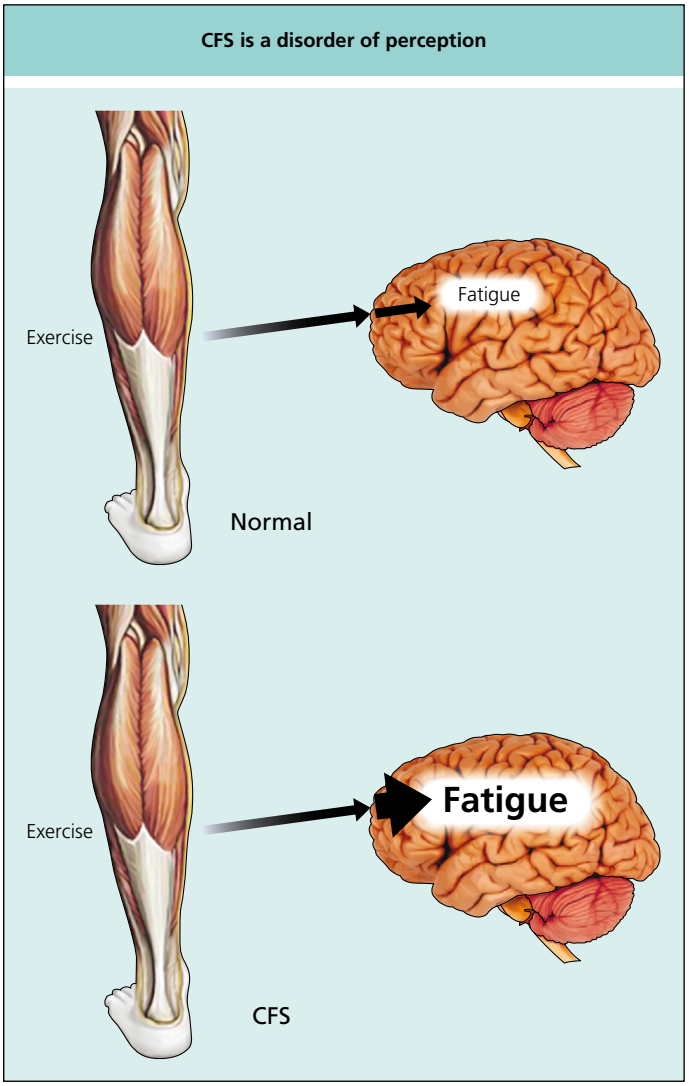


Fig. 70.2 Chronic fatigue syndrome (CFS) as a disorder of perception. The upper panel depicts the normal situation: muscle activity as in exercise is perceived as physiologic fatigue. In CFS the same signal from the muscle is perceived as severe fatigue; apparently the signal is amplified at the level of the brain. Similarly, other bodily signals are also perceived in an amplified fashion.

fatigability that occurs in disorders of muscle and neuromuscular junctions, as the motor power during voluntary muscle contraction is essentially normal.

The central localization of the fatigue is in agreement with the findings that point to a disturbed perception of fatigue (Fig. 70.2), as well as of other signals from the body (pain, sleep quality, memory). In well-controlled studies on exercise capacity, neuropsychological functioning, and sleep physiology, several investigators have found that CFS patients perceive the performance of their body as miserable or at least not to the level to be expected.[53–55] The finding of failing central activation of maximal voluntary contraction also fits into this concept.[56]

A key question in this respect is what the neurobiologic substrate is for this disturbance in perception. In the previous section we have already discussed a number of mediator systems (neurotransmitters, hormones) that could play a role, but it is clear that more research is needed here. A number of studies have addressed the question whether structural and functional abnormalities can be demonstrated in the brain of CFS patients, using modern high-resolution imaging techniques. Older studies reported white matter hyperintensities on MRI and cerebral blood flow abnormality using SPECT imaging, but

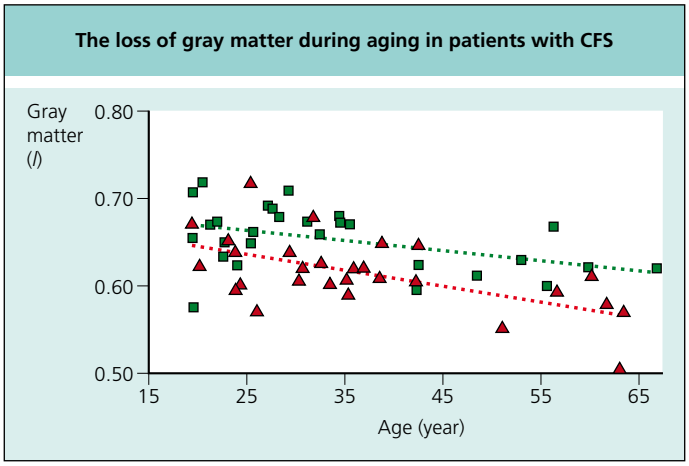


Fig. 70.3 The loss of gray matter during aging in patients with chronic fatigue syndrome (red symbols) compared to matched controls (green symbols). Adapted with permission from De Lange *et al*.[60]

well-controlled studies were not able to confirm these findings.[57,58] Recent MRI studies by two independent groups have detected diminished gray matter volumes in CFS patients compared to matched controls (Fig. 70.3).[59,60] These differences are, however, too small to be used for diagnosis in individual patients. The relevance of these findings for the pathophysiology of CFS is underscored by the observation that these changes are – at least to some extent – reversible with successful cognitive behavioral therapy (see below).[61]

On functional MRI it was found that in patients with CFS a motor imagery task evoked stronger responses in visually related structures than matched controls.[62] In addition, the ventral anterior cingulate cortex became active when healthy controls made an error, but remained inactive in CFS patients during erroneous performance.[62] These results point to dysfunctional motor planning and motivational disturbances in CFS.

In another functional MRI study, CFS patients were also shown to utilize more extensive areas of the brain to process auditory information than controls.[63] Likewise, greater activity in cortical and subcortical regions of the brain was found during a fatiguing cognitive task.[63] These imaging findings are corroborated by electrophysiologic data showing higher delta wave activity in the left uncus and parahippocampal gyrus and higher theta in the cingulate gyrus and right superior frontal gyrus.[64]

ABNORMALITIES OF THE AUTONOMIC NERVOUS SYSTEM

There have been speculations about abnormalities of the autonomic nervous system in CFS for many years as evidenced by some of the accompanying symptoms (hyperhidrosis, palpitations, dizziness and orthostatic intolerance). Research findings are somewhat controversial but it is probably safe to conclude that signs of postural tachycardia or (pre)syncope can be found in a minority of CFS patients.[65,66] The higher heart rate that may be encountered in many patients at baseline, together with a marked decrease in stroke volume in response to head-up tilt testing, may point to deconditioning.[65] Tests like the head-up tilt test should not be done routinely in the diagnostic process.[2]

PROGNOSIS AND TREATMENT

Without treatment, less than 10% of patients recover over time, even though some improvement is not rare.[67] A strong belief in a physical cause of illness and a long duration of illness are associated with a poor outcome.[68]

Although a great number of claims have been made regarding a variety of treatments for CFS, few are supported by results of solid clinical trials.[69] At least three systematic reviews and one meta-analysis have dealt with this subject and reached the conclusion that (only) cognitive behavioral therapy (CBT) and graded exercise therapy (GET) are of proven benefit.[69–72] There is no evidence-based drug treatment (hormones, immunomodulatory drugs, antimicrobials, psychotropic drugs, vitamins and other dietary supplements) for CFS.[2] The same holds for diets (e.g. the 'Candida diet') that are largely being advised by proponents of complementary medicine.

CBT is a form of psychotherapy directed at changing condition-related cognitions and behaviors. The key components of CBT according to Prins *et al*.[1] are:

- explanation of the pathophysiologic model (see Fig. 70.1);
- motivation for this form of treatment;
- challenging and changing fatigue-related cognitions;
- achievement and maintenance of a basic degree of physical activity, followed by
- gradual increase of physical activity; and
- planning work rehabilitation or rehabilitation in other personal goals.

With CBT patients should learn to gain control over their symptoms; graded increase of activity is an intrinsic part of the treatment. In that sense CBT for CFS always includes a graded activity component, which is the only therapeutic ingredient in GET while cognitions are not explicitly treated. The improvement rates for CBT are around 70%; in GET they are lower (around 50%). Indicators of failure are a strong focus on physical symptoms and a poor sense of control. Likewise, patients claiming disability-related benefits have a poor treatment outcome.[73]

An important issue with regard to efficacy is the aim of CBT. In many studies improvement and rehabilitation is the treatment goal. Our CBT studies[73–77] aimed to cure (i.e. disappearance of symptoms and functional impairment as its defined goal, ability to return to work and other activities and no longer considering oneself as a patient).[1] It should be realized that if cure is not the goal of treatment, it will never be attained. Of great interest is our recent observation that – as mentioned above – there is a regain of gray matter with successful CBT, underscoring that the loss of gray matter in CFS is neurobiologically important, that CBT induces morphologic changes that point to plasticity of the brain.[60]

Another important question is whether the effect of CBT is lasting; a number of follow-up studies have shown that there is a sustained effect.[78,79] However, there are a couple of problems with CBT. The first one is availability; although the term cognitive behavioral therapy is widely used, specific CBT, tailor-made for CFS is hard to get. A second major problem – fed by some patient organizations – is that many CFS patients have strong somatic attributions and reject the idea that a psychological intervention may help them.

CONCLUSION

Over recent years, although our insight into the pathogenesis of CFS increased and we have made progress with regard to our abilities to manage and treat the disorder, our knowledge of the syndrome is still too limited. Controversies about CFS between doctors, researchers and patients have not been particularly helpful in facilitating solid research and further solving the enigmas of the syndrome. In addition, such controversies are harmful to patients; health-care workers should do their utmost to facilitate effective management of the condition, as proposed in the NICE guidelines.[2]

REFERENCES

References for this chapter can be found online at http://www.expertconsult.com

Chapter 71

Andrew W Artenstein

Bioterrorism and biodefense

INTRODUCTION

Bioterrorism can be broadly defined as the deliberate use of microbial agents or their toxins as weapons against noncombatants outside the setting of armed conflict. The concept is analogous to biologic warfare in a combat theater. The broad scope and mounting boldness of worldwide terrorism was impressively demonstrated by the massive attacks on New York City and Washington DC on September 11, 2001; the multifocal anthrax attacks that followed shortly thereafter, while not known to be directly related to 9/11, awakened civilized society to the threats posed by these 'weapons of mass terror'. This realization, in concert with recent revelations regarding the apparent willingness of terrorist organizations to acquire and deploy biologic weapons, constitutes ample evidence that the specter of bioterrorism poses a persistent global threat.

Biologic weapons have been used against both military and civilian targets throughout history. It has been variously speculated that at least some of the plagues visited upon ancient Egypt, as documented in the biblical book of Exodus, represented natural outbreaks of endemic infectious diseases that were recast as supreme forms of bioterrorism. In the 14th century Tatars attempted to use epidemic disease against the defenders of Kaffa by catapulting plague-infected corpses into the city.[1] British forces gave Native American tribespeople blankets from a smallpox hospital in an attempt to affect the balance of power in the 18th century Ohio River Valley.[1] In addition to their well-described use of chemical weapons, Axis forces purportedly infected livestock with anthrax and glanders to weaken Allied initiatives during the First World War. Perhaps the most egregious period in the history of biologic weaponry involved the Japanese program in Manchuria from 1932 to 1945. Based on survivor accounts and confessions of Japanese participants, thousands died as a result of experimental infection with a multitude of virulent pathogens at Unit 731, the code name for the biologic weapons facility there.[2]

The USA maintained an offensive biologic weapons program from 1942 until 1969, when the program was terminated by President Nixon. The Convention on the Prohibition of the Development, Production, and Stockpiling of Biological and Toxin Weapons and on Their Destruction (BWC) was ratified in 1972 and formally banned the development or use of biologic weapons, with enforcement the responsibility of the United Nations.[1] Unfortunately, the BWC has not been effective in its stated goals; multiple signatories, including the former Soviet Union and Iraq, have violated the terms and spirit of the agreement. The accidental release of aerosolized anthrax spores from a military plant in Sverdlovsk in 1979, resulting in at least 68 human deaths from inhalational anthrax, verifies the existence of an active Soviet offensive biologic weapons program.

THREAT ASSESSMENT

Biologic agents are considered weapons of mass destruction (WMD) because, as with certain conventional, chemical and nuclear weapons, their use may result in large-scale morbidity and mortality. In a World Health Organization (WHO) assessment model of the hypothetical casualty estimates from the intentional release of 50 g of aerosolized anthrax spores upwind from a population center of 500 000 (analogous to Providence, Rhode Island, USA), nearly 200 000 people might be killed or incapacitated by the event.[3] Biologic weapons possess unique properties among all WMD. Unlike other forms, biologic agents are associated with a clinical latency period of days to weeks in most cases, during which time exposed individuals are asymptomatic and early detection is quite difficult with currently available technology. Additionally, specific antimicrobial therapy and, in select circumstances, vaccines are available for the treatment and prevention of illness caused by biologic weapons; casualties from other forms of WMD can generally only be treated by decontamination, trauma mitigation and supportive care.

Nations adhering to democratic principles are vulnerable to bioterrorism because of the inherent freedoms that their citizens and visitors enjoy. This freedom of movement and access to public and private institutions can be exploited by rogue nations, terrorist organizations or malicious individuals intent on untoward acts. When coupled with worldwide cultural tensions, geopolitical conflicts and economic instability, open societies provide fertile ground for terrorism.

Recent events have established bioterrorism as a credible and ubiquitous threat and, in some quarters, as a potential tool for political coercion. The intentional contamination of restaurant salad bars with *Salmonella* by a religious cult trying to influence a local election in The Dalles, Oregon, in 1984;[4] the revelations that Aum Shinrikyo, the Japanese cult responsible for the sarin gas attack in the Tokyo subway system in 1995, experimented on multiple occasions with spraying anthrax from downtown Tokyo rooftops; and the findings of the United Nations weapons inspectors of massive quantities of weaponized biologic weapons in Iraq during the first Gulf War and its aftermath[5] served as sentinel warnings of a shift in terrorism trends. The anthrax attacks in the USA in October and November 2001, following the catastrophic events of September 11th, elevated bioterrorism to the fore of the international dialogue.

The aims of bioterrorism are those of terrorism in general: morbidity and mortality among civilian populations, disruption of societal fabric, and exhaustion or diversion of resources.[6] A 'successful' outcome, from a terrorist standpoint, may be achieved without furthering all of these aims and, in fact, may be accomplished simply by the credible threat of action or by a small-scale agent deployment. The anthrax attacks in 2001 evoked fear and anxiety and diverted public health and health-care resources away from other critical activities despite the limited number of casualties associated with the event.

Biologic weapons offer other, significant advantages to terrorists:

- they are relatively inexpensive to acquire as compared with conventional or nuclear weaponry;
- they can be deployed in a stealth fashion due to a variable clinical latency period, thus allowing the perpetrator opportunity to escape if desired; and
- they clearly evoke anxiety and panic in a population that is, in some instances, out of proportion to their physical effects.

From a rogue government's standpoint, the technology for bioterrorism is 'dual use', i.e. it can serve legitimate functions such as vaccine or pharmaceutical production as readily as biologic weapons production, thus making detection by inspectors all the more difficult.

To be employed in large-scale bioterrorism, biologic agents must undergo complex processes of production, cultivation, chemical modification and weaponization. For these reasons state sponsorship or direct support from governments or organizations with significant resources, contacts and infrastructure would predictably be required in large-scale events.[6] However, some agents may be acquired by terrorist groups on the black market and in other illicit settings.[7] Although an efficient mode of delivery has traditionally been felt to be necessary, the anthrax attacks in the USA in late 2001 illustrated the devastating results that can be achieved with relatively primitive delivery methods, e.g. high-speed mail sorting equipment and mailed letters.

Numerous attributes contribute to the effectiveness of a biologic weapon:

- availability or ease of large-scale production;
- ease of dissemination, especially by the aerosol route;
- stability in storage and delivery;
- cost; and
- clinical virulence.

The last refers to the reliability with which the pathogen causes high mortality, morbidity or social disruption. The Centers for Disease Control and Prevention (CDC) have prioritized biologic agent threats based upon the aforementioned characteristics,[8,9] and this has influenced current preparedness strategies (Table 71.1). Category A agents, considered the highest priority, are associated with high mortality and the greatest potential for major impact on public health. Category B agents are considered 'incapacitating' because of their potential for moderate morbidity but relatively low mortality. Most of the category A and B agents have been experimentally weaponized in the past. Category C agents include emerging threats and pathogens that may be available for development in the future.

Another factor that must be addressed in assessing future bioterrorism risk and predicted agents is the historical track record of experimentation with specific pathogens, an area that has been informed from the corroborated claims of various high-level Soviet defectors and data released from the former offensive weapons programs of the USA and the UK.[1,7,10] It is apparent from these sources, combined with the burgeoning fields of molecular biology and genomics, that future risk scenarios may have to contend with genetically altered and 'designer' pathogens that may be equipped with enhancements in virulence, such as antimicrobial resistance or augmented toxin production, or modifications that enhance dissemination, such as prolonged aerosol stability. To this end the author has added a miscellaneous grouping of potential threat agents to the extant CDC categories (see Table 71.1). The most cautious approach to assessing risk may be to remain open to additional, novel possibilities.

BIOTERRORISM RECOGNITION

By definition bioterrorism is insidious; absent advance warning or specific intelligence information, clinical illness will be manifest before the circumstances of a release event are known. For this reason healthcare providers are likely to be the first responders to this form of terrorism. This is in contrast to the more familiar scenarios in which police, firefighters, paramedics and other emergency services personnel are deployed to the scene of an attack with conventional weaponry or a natural disaster. Physicians and other health-care workers must therefore maintain a high index of suspicion of bioterrorism and recognize suggestive epidemiologic clues and clinical features to enhance early recognition, disseminate information rapidly and guide initial management of casualties. This remains the most effective way to minimize the deleterious effects of bioterrorism on individual patients and on the public health.

Early recognition is hampered for multiple reasons. Terrorists have an unlimited number of targets in most open, democratic societies; it is unrealistic to expect that without detailed intelligence data, all of these can be secured at all times. Certain sites, such as government institutions, historic landmarks or large events may be predictable targets, but there are other, less predictable possibilities. Metropolitan areas are considered vulnerable, but owing to the expansion of suburbs, commuters, and the clinical latency period between exposure and symptoms inherent with biologic agents, casualties of bioterrorism are likely to present for medical attention in diverse locations and at varying times following a common exposure. An event in New York City on a Wednesday morning may result in clinically ill individuals presenting over the ensuing weekend to a variety of emergency rooms within a 60-mile radius. Additionally, our mobile society ensures that affected individuals will likely present for medical care thousands of miles away from the original release point of the bioterrorist's weapon. This adds layers of complexity to managing a bioterrorism event and illustrates the critical importance of surveillance, cooperation and real-time communication in this setting.

Further hindering the early recognition of bioterrorism is that initial symptoms may be nondiagnostic. In the absence of a known exposure, many symptomatic individuals may not seek medical attention early on or may be misdiagnosed with a flu-like illness if they do. Once beyond the early stages many of these illnesses progress quite rapidly and treatment may be less successful. Most of the diseases caused by agents of bioterrorism are rarely, if ever, seen in clinical practice; physicians are, therefore, likely to be inexperienced with their clinical presentation. Additionally, these agents, by definition, will have been laboratory-manipulated and may not present with the classic clinical features of naturally occurring infection. This was dramatically illustrated by differences in the clinical presentations of some of the inhalational anthrax cases in the USA in October 2001 as compared with those described in earlier outbreaks.[11]

Early identification of bioterrorism is facilitated by the recognition of epidemiologic and clinical clues. Clustering of patients with common symptoms and signs, especially if these are unusual or known to be associated with bioterrorism agents, should prompt expeditious notification of local public health authorities. This approach may not only detect malicious events but will also lead to the recognition of outbreaks of naturally occurring disease or novel, emerging pathogens. The recognition of a single case of a rare or nonendemic infection, in the absence of a travel history or other potential natural exposure, should raise the specter of bioterrorism and should prompt notification of public health authorities. Finally, unusual patterns of disease, such as unusual age distributions, more severe clinical forms of infection or concurrent illness in human and animal populations, should raise suspicions for bioterrorism or another form of emerging infection. In fact for some category A, B or C agents of bioterrorism, available evidence supports the potential role of animals as early warning sentinels of an attack or as markers of persistent exposure risks to humans.[12]

Infectious diseases specialists are uniquely suited to play pivotal roles in the recognition, investigation and mitigation of bioterrorism, based on:

- an understanding of epidemiologic principles and risk assessment;
- expertise in specific threat agents, their clinical presentations and diagnostic approaches;
- knowledge of communicability and infection control principles; and
- an understanding of the tenets of treatment and prophylaxis of infectious diseases.

Table 71.1 Agents of concern for use in bioterrorism

Highest priority: category A (based upon potential mortality, morbidity, virulence, transmissibility, aerosol feasibility and psychosocial implications of an attack)	
Microbe/toxin	**Disease**
Bacillus anthracis	Anthrax: inhalational, cutaneous
Variola virus	Smallpox and its variants
Yersinia pestis	Plague: pneumonic, bubonic, septicemic
Clostridium botulinum toxin	Botulism
Francisella tularensis	Tularemia: pneumonic, typhoidal
Viral hemorrhagic fevers	
Filoviruses	Ebola, Marburg
Arenaviruses	Lassa fever, South American hemorrhagic fevers
Bunyaviruses	Rift Valley fever, Congo–Crimean hemorrhagic fever
Flaviviruses	Dengue
Moderately high priority: category B (based upon potential morbidity, aerosol feasibility, dissemination characteristics, and diagnostic difficulty)	
Microbe/toxin	**Disease**
Coxiella burnetti	Q fever
Brucella spp.	Brucellosis
Burkholderia mallei	Glanders
Burkholderia pseudomallei	Melioidosis
Alphaviruses (e.g. EEE, VEE)	Viral encephalitides
Ricinus communis toxin	Ricin intoxication
Staphylococcal enterotoxin B	Staphylococcal toxin illness
Salmonella spp., *Shigella dysenteriae*, *Escherichia coli* O157:H7, *Vibrio cholerae*, *Cryptosporidium parvum*, *Listeria monocytogenes*, *Campylobacter jejuni*, *Yersinia enterocolitica*	Food- and waterborne gastroenteritis
Rickettsia prowazekii	Epidemic typhus
Chlamydia psittaci	Psittacosis
Epsilon toxin of *Clostridium perfringens*	*C. perfringens* intoxication
Emerging threat agents: category C (based upon potential for production and dissemination, availability, morbidity/mortality)	
Microbe/toxin	**Disease**
Hantaviruses	Viral hemorrhagic fevers
Flaviviruses	Yellow fever, West Nile virus
Mycobacterium tuberculosis	Multidrug-resistant tuberculosis
Nipah virus	Systemic flu-like illness
Miscellaneous (other examples of candidate threat agents that possess some elements of bioterrorism concern)	
Genetically engineered vaccine- and/or antimicrobial-resistant category A or B agents	
HIV-1	
Adenoviruses	
Influenza	
Rotaviruses	
Molecular hybrid pathogens (e.g. smallpox–plague, smallpox–ebola)	
Severe acute respiratory syndrome coronavirus	

EEE, eastern equine encephalomyelitis; VEE, Venezuelan equine encephalomyelitis.
Adapted from Patrozou & Artenstein.[26]

Nonetheless, an effective response to bioterrorism requires coordination of the medical system at all levels, from the community physician to the tertiary care center, with active engagement of public health, emergency management and law enforcement infrastructures.

THREAT AGENTS

This section will cover the biologic threat agents felt to be of major current concern, largely the CDC category A agents. Extensive coverage of specific pathogens can be found in related chapters in this text (cross-referenced in Table 71.2) and in other sources.[13,14] Data concerning clinical incubation periods, transmission characteristics and infection control procedures for agents of bioterrorism are provided in Table 71.2. Syndromic differential diagnoses for select clinical presentations are detailed in Table 71.3.

Anthrax

Anthrax results from infection with *Bacillus anthracis*, a Gram-positive, spore-forming, rod-shaped organism that exists in its host as a vegetative bacillus and in the environment as a spore. Details of the microbiology and pathogenesis of anthrax are found in Chapter 128 of this text. In nature anthrax is a zoonotic disease of herbivores that is prevalent in many geographic regions; sporadic human disease results from environmental or occupational contact with endospore-contaminated animal products.[15] Anthrax is uncommon in developed countries. In developing areas the cutaneous form of anthrax is the most common presentation; gastrointestinal and inhalational forms are exceedingly rare in naturally acquired disease. Cutaneous anthrax is rarely seen in current-day industrialized countries due to importation restrictions. The last known case of naturally occurring inhalational anthrax in the USA occurred in 1976.[16]

The recent attacks in the USA were on a relatively small scale, and nearly 40% of the confirmed cases were of the cutaneous variety.[17] The serious morbidity and mortality, however, were related to inhalational disease, as was noted in the Sverdlovsk outbreak in 1979. Therefore, planning for larger-scale events with aerosolized agent continues to be warranted.

The clinical presentations and differential diagnoses of cutaneous and inhalational anthrax are described in Table 71.3. The lesion of cutaneous anthrax may be similar in appearance to other lesions, including cutaneous forms of other agents of bioterrorism; however, it may be distinguished by epidemiologic as well as certain clinical features. Anthrax is traditionally a painless lesion, unless secondarily infected, and associated with significant local edema (Fig. 71.1). The bite of *Loxosceles reclusa*, the brown recluse spider, shares many of the local and systemic features of anthrax but is typically painful from the outset and lacks significant edema.[18] Cutaneous anthrax is associated with systemic disease and its attendant mortality in up to 20% of untreated cases, although with appropriate antimicrobial therapy, mortality is less than 1%.[16]

Once the inhaled endospores reach the terminal alveoli of the lungs, generally requiring particle sizes of 1–5 μm, they are phagocytosed by macrophages and transported to regional lymph nodes, where they germinate into vegetative bacteria and, subsequently, disseminate hematogenously.[15] The bacteria generate potent exotoxins, lethal toxin and edema toxin, which lead to hemorrhagic mediastinitis, systemic illness and death. Spores may remain latent for extended

Table 71.2 Epidemiologic characteristics for selected category A and B bioterrorism-associated diseases

Disease	Incubation period range (days)	Person-to-person transmission	Infection control precautions for patients	Case fatality rate
Inhalational anthrax (see Chapter 128)	2–43*	No	Standard	Untreated 100% Treated 45%
Cutaneous anthrax (see Chapter 128)	1–12	No	Standard	Untreated 20% Treated <1%
Botulism (see Chapter 21)	12–72 hours	No	Standard	6%
Primary pneumonic plague (see Chapter 120)	1–6	Yes	Droplet	Untreated 100% Treated ~50%
Bubonic plague (see Chapter 120)	2–8	No	Standard	Untreated 60% Treated <5%
Smallpox	7–19	Yes	Contact and airborne	Unvaccinated 30% Vaccinated 3%
Tularemia pneumonia (see Chapter 121)	1–21	No	Standard	Untreated 60% Treated <4%
Viral hemorrhagic fevers (see Chapter 126)	2–21	Yes	Contact and airborne	Marburg 25% Ebola 80% Other forms 2–30%
Viral encephalitides (see Chapter 19)	1–14	No	Standard	10–35%
Q fever (see Chapter 176)	2–41	No	Standard	3%
Brucellosis (see Chapter 123)	5–60	No	Standard	Untreated 5%
Glanders	1–21	Yes	Contact and droplet	Untreated – approaches 100% Treated – low

*Based on limited data from human outbreaks; experimental animal data support clinical latency periods of up to 100 days.
Adapted from Patrozou & Artenstein.[26]

Table 71.3 Syndromic differential diagnoses and clinical clues for category A agents of bioterrorism

Syndrome	Clinical presentation	Differential diagnosis	Bioterrorism-associated disease	Disease-specific clues
Influenza-like illness	Nonspecific constitutional and upper respiratory symptoms: malaise, myalgias, nausea, emesis, dyspnea, cough with or without chest discomfort, without coryza or rhinorrhea, leading to abrupt onset of respiratory distress, with or without shock, mental status changes, with chest radiograph abnormalities (wide mediastinum or infiltrates or pleural effusions)	Influenza, community-acquired bacterial pneumonia, viral pneumonia, *Legionella*, Q fever, psittacosis, *Mycoplasma*, *Pneumocystis* pneumonia, tularemia, dissecting aortic aneurysm, bacterial mediastinitis, SVC syndrome, histoplasmosis, coccidioidomycosis, sarcoidosis, ricin and staphylococcal enterotoxin B (pulmonary edema/ARDS), Nipah virus	Inhalational anthrax	• 3-day average symptom duration before presentation • Abdominal pain, headache, mental status abnormalities, hypoxemia common • Mediastinal adenopathy: ~90% (Fig. 71.2) • Hemorrhagic pleural effusions: ~70% • CT more sensitive than chest X-ray in early hemorrhagic mediastinal adenopathy • Meningoencephalitis: possibly ~50% • Blood cultures positive in untreated; pleural fluid cultures or antigen-specific immunohistochemical stain usually positive
Skin lesion(s)	Pruritic, painless papule on exposed areas leading to vesicle(s), ulcer, then edematous black eschar, with or without massive local edema and regional adenopathy and fever, evolving over 3–7 days	Recluse spider bite, staphylococcal lesion, atypical Lyme disease, orf, glanders, tularemia, plague, rat-bite fever, ecthyma gangrenosum, rickettsialpox, atypical *Mycobacteria*, cutaneous diphtheria, cutaneous leishmaniasis	Cutaneous anthrax	• Painless; spider bite is a painful lesion • Nonpitting local edema may be massive (Fig. 71.1) • If untreated, may progress to systemic involvement • Blood cultures, skin biopsy (from vesicular edge or erythema at edge of eschar)
Fulminant pneumonia	Abrupt onset of constitutional symptoms and rapidly progressive respiratory illness with cough, fever, rigors, headache, sore throat, myalgias, dyspnea, pleuritic chest pain, GI symptoms, lung consolidation, with or without hemoptysis, shock; variable progression to respiratory failure	Severe community-acquired bacterial or viral pneumonia, inhalational anthrax, pulmonary infarct, pulmonary hemorrhage, influenza, *Mycoplasma* pneumonia, *Legionella*, Q fever, bacterial pneumonia, SARS, tuberculosis, melioidosis	Pneumonic plague Pulmonary tularemia	• Lobar or multilobar involvement, with or without buboes • Hemoptysis common • Characteristic sputum Gram stain • Cough generally nonproductive • Pulse–temperature dissociation in 40%

(Continued)

Table 71.3 Syndromic differential diagnoses and clinical clues for category A agents of bioterrorism—cont'd

Syndrome	Clinical presentation	Differential diagnosis	Bioterrorism-associated disease	Disease-specific clues
Sepsis with bleeding diathesis and capillary leak	Sepsis syndrome, GI symptoms, mucosal hemorrhage, altered vascular permeability, DIC, purpura, acral gangrene, hepatitis, hypotension, with or without CNS findings, multiorgan system failure	Meningococcemia; Gram-negative sepsis; streptococcal, pneumococcal or staphylococcal bacteremia with shock; malaria; leptospirosis; typhoid fever; borreliosis; typhoidal tularemia; overwhelming postsplenectomy sepsis; acute leukemia; Rocky Mountain spotted fever; fulminant hepatitis; TTP; hemolytic uremic syndrome; SLE; hemorrhagic smallpox; hemorrhagic varicella (in immunocompromised); dengue.	Septicemic plague Viral hemorrhagic fever	• Occurs in minority of aerosol exposures • Cutaneous findings as late sequelae, with or without buboes • High-density bacteremia • Maculopapular rash in Ebola, Marburg • Certain organ systems preferentially involved with specific VHF etiologies
Febrile prodrome with generalized exanthem	Fever, malaise, prostration, headache, myalgias and enanthema followed by development of synchronous, progressive, centrifugal papular, leading to vesicular/pustular rash on face, mucous membranes, extremities more than trunk, leading to generalization with or without hemorrhagic component, with systemic toxicity	Varicella, drug eruption, Stevens–Johnson syndrome, measles, secondary syphilis, erythema multiforme, severe acne, disseminated herpes zoster or simplex, meningococcemia, monkeypox, generalized vaccinia related to smallpox vaccination, insect bites, coxsackievirus, vaccine reaction	Smallpox	• Palms and soles involved • Rash is denser peripherally even after fully evolved • Lesions are well circumscribed, firm and almost nodular • Secondary bacterial infection common • Hemorrhagic variant in pregnant and immunocompromised patients associated with severe systemic toxicity, bleeding diathesis, and early mortality
Progressive weakness	Acute onset of afebrile, symmetric, descending flaccid paralysis that begins in bulbar muscles, dilated pupils, diplopia or blurred vision, dysphagia, dysarthria, ptosis, dry mucous membranes leading to airway obstruction and respiratory muscle paralysis. Clear sensorium and absence of sensory changes	Myasthenia gravis, brain stem CVA, polio, Guillain-Barré syndrome variant, tick paralysis, chemical intoxication	Botulism	• Expect dearth of GI symptoms in aerosol attack as opposed to food-borne botulism • Low-dose inhalation exposure may delay symptom onset • Prominent anticholinergic effects.

ARDS, acute respiratory distress syndrome; CVA, cerebrovascular accident; DIC, disseminated intravascular coagulation; GI, gastrointestinal; SLE, systemic lupus erythematosus; SVC syndrome, superior vena cava syndrome; TTP, thrombotic thrombocytopenic purpura; VHF, viral hemorrhagic fever.



Fig. 71.1 Lesion of cutaneous anthrax. © Diepgen TL, Yihune G, *et al*. Dermatology Online Atlas (http://www.dermis.net). Reprinted with permission.

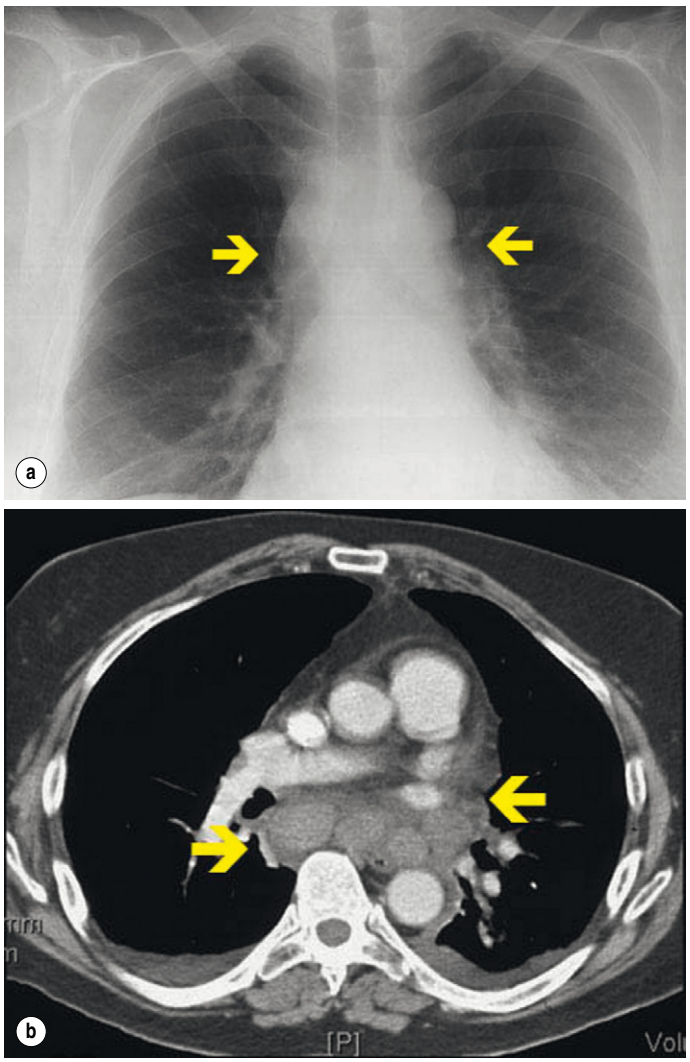


Fig. 71.2 (a) Chest X-ray, inhalational anthrax, United States, 2001 demonstrating mediastinal widening (arrows). (b) Chest CT scan demonstrating mediastinal widening (arrows) and bilateral pleural effusions. From Jernigan *et al*.[11]

periods of time in the host, up to 100 days in some experimental animal exposures[17] This has translated into prolonged clinical incubation periods following exposure to endospores; cases of inhalational anthrax occurred up to 43 days after exposure in the Sverdlovsk experience, although the average incubation period is 2–10 days, perhaps influenced by exposure inoculum.[15,17]

Prior to the anthrax attacks in the USA in October 2001, most of the clinical data concerning inhalational anthrax derived from Sverdlovsk, the largest outbreak recorded in humans. While the clinical experience derived from the US anthrax attacks in 2001 had much in common with the clinical manifestations of inhalational anthrax noted in the Sverdlovsk cases, more detailed data are available from the recent US experience and some novel findings were noted. There were 11 confirmed cases of inhalational anthrax, 5 (45%) of whom died. Although this contrasts with a case fatality rate of greater than 85% reported from Sverdlovsk, the reliability of reported data from the latter outbreak is questionable[17] and, perhaps more importantly, patients in the 2001 outbreak were more likely to receive appropriate treatment at an earlier stage. Patients with inhalational anthrax almost uniformly present for medical attention an average of 3.3 days after symptom onset with fevers, chills, malaise, myalgias, nonproductive cough, chest discomfort, dyspnea, nausea or vomiting, tachycardia, peripheral neutrophilia and liver enzyme elevations.[11,19]

Many of these findings are nondiagnostic and overlap considerably with those of influenza or other common viral respiratory tract infections. Data suggest that discrimination between inhalational anthrax and benign, influenza-like illnesses may be possible on the basis of presenting symptoms; shortness of breath, nausea, and vomiting are significantly more common in anthrax while rhinorrhea is uncommonly seen in anthrax but noted in the majority of community-acquired viral respiratory infections.[20]

Other common clinical manifestations of inhalational anthrax as informed by the recent attacks include abdominal pain, headache, mental status abnormalities and hypoxemia. Abnormalities on chest radiography appear to be universally present, although these may only be identified retrospectively in some cases. Pleural effusions are the most common abnormality; infiltrates, consolidation and/or mediastinal adenopathy/widening are noted in the majority (Fig. 71.2a). The latter is felt to be an early indicator of disease, but CT scan appears to provide greater sensitivity than chest radiographs for this finding (Fig. 71.2b). In the recent outbreak of inhalational anthrax, more than 80% of cases were noted to have mediastinal widening with or without pleural effusions or infiltrates.

The clinical manifestations of inhalational anthrax generally evolve to a fulminant septic picture with progressive respiratory failure. *B. anthracis* is routinely isolated in blood cultures if obtained prior to the initiation of antimicrobials (Fig. 71.3). Pleural fluid is typically hemorrhagic; the bacteria can either be isolated in culture or documented by antigen-specific immunohistochemical stains of this material (Fig. 71.4) in the majority of patients.[11] The average time from hospitalization until death was 3 days (range 1–5 days) in the five recent US fatalities, consistent with other reports related to the clinical virulence of this infection. Autopsy data typically reveal hemorrhagic mediastinal lymphadenitis and disseminated metastatic infection. Pathology data from the Sverdlovsk outbreak confirm meningeal involvement, typically hemorrhagic meningitis, in 50%[21] and, in fact, meningoencephalitis was the presenting diagnosis (Fig. 71.5) in the index case of the 2001 attacks.[22]

The diagnosis of inhalational anthrax should be entertained in the setting of a consistent clinical presentation in the context of a known exposure, a possible exposure or epidemiologic factors suggesting bioterrorism, e.g. clustered cases of a rapidly progressive systemic illness. The diagnosis should also be considered in a single individual with a consistent or suggestive clinical illness in the absence of another etiology. Table 71.3 delineates a detailed differential diagnosis with specific discriminating features. It should be noted that multiple bioterrorism threat agents are included in the differential diagnosis of inhalational anthrax.

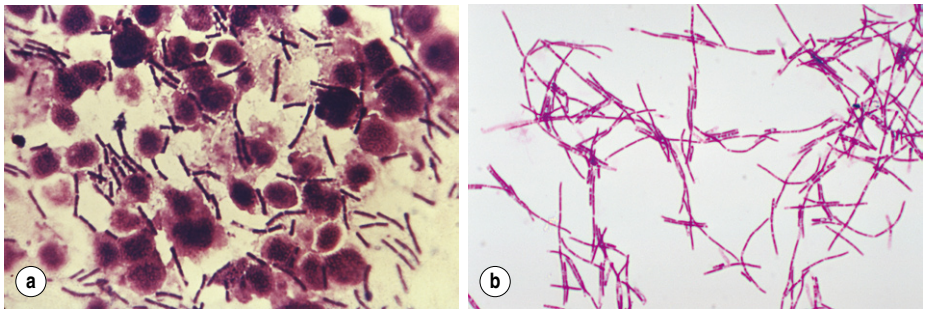


Fig. 71.3 *Bacillus anthracis*. (a) *Bacillus anthracis* appearing as Gram-positive bacilli. (b) The typical 'jointed bamboo-rod' appearance of the organism from blood cultures. Courtesy of CDC and Dr William A Clark.

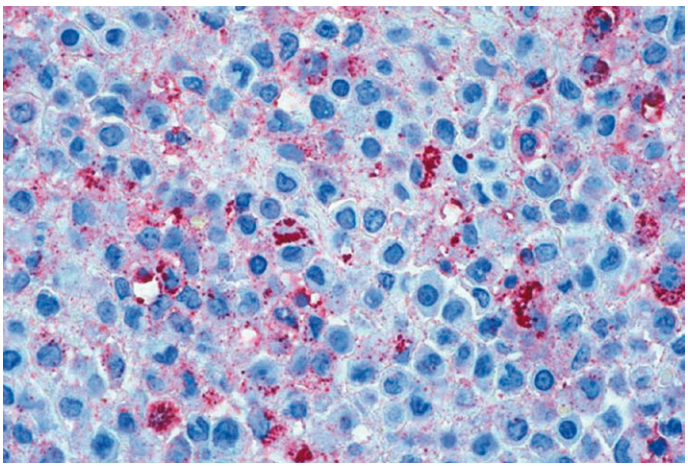


Fig. 71.4 Pleural fluid cell block immunohistochemical stain demonstrating *Bacillus anthracis* antigen (red) within a mononuclear inflammatory cell infiltrate. From Jernigan *et al*.[11]

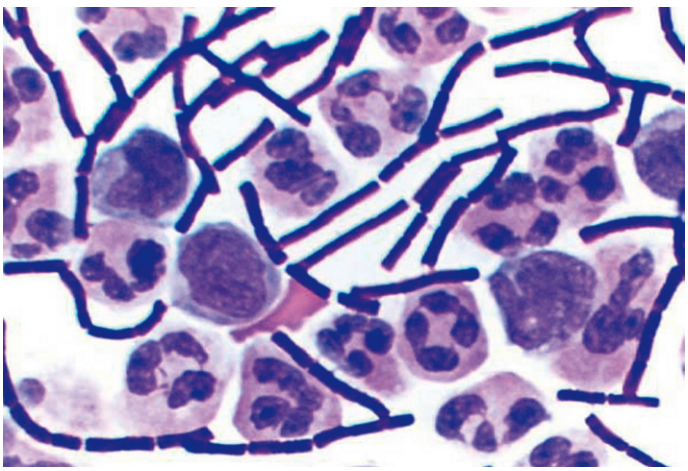


Fig. 71.5 Cerebrospinal fluid Gram stain from anthrax index case, United States, 2001, demonstrating numerous Gram-positive rods and neutrophils. From Jernigan *et al*.[11]

The early recognition and treatment of inhalational anthrax appear to be associated with a survival advantage;[11] in the US experience patients who received appropriate antimicrobials within 4.7 days of symptom onset had a mortality rate of 40% as compared with a mortality rate of 75% for those treated after that period.[23] Therefore, prompt empiric antimicrobial therapy should be initiated if infection is clinically suspected. Combination parenteral therapy is appropriate in the ill individual for a number of reasons:[11]

- to cover the possibility of antimicrobial resistance;
- to target specific bacterial virulence properties, e.g. the theoretical effect of clindamycin on toxin production;
- to ensure adequate drug penetration into the central nervous system; and
- to favorably impact survival.

In order to optimize the outcome in inhalational anthrax it is likely that novel therapies, such as toxin inhibitors or receptor antagonists, will need to be developed and deployed.[24] A variety of such strategies, guided by the pathogenesis of the organism and its disease-producing toxins, has shown promise in animal studies to date and will likely be components of effective therapeutic regimens in the future.[25]

Detailed therapeutic and postexposure prophylaxis recommendations for adults, children and special groups have been recently reviewed elsewhere.[17,26] Anthrax vaccine adsorbed (AVA), the current product in use for select indications, has been proven to be effective in preventing cutaneous anthrax in human clinical trials and in preventing inhalational disease after aerosol challenge in nonhuman primates.[27] The vaccine has generally been found to be safe but requires six doses over 18 months with the need for frequent boosting. Because of the aforementioned dosing issues and the limited availability of AVA, second-generation anthrax vaccines employing recombinant protective antigen and humanized antiprotective antigen monoclonal antibodies are in production.

Smallpox

The last known naturally acquired case of smallpox occurred in Somalia in 1977; in 1980, as the culmination of a 12-year, intensive campaign by the World Health Organization (WHO), the disease became the first in history to be officially certified as 'eradicated' as a scourge of humans.[28] However, because of concerns that variola virus stocks may have either been removed from or sequestered outside of their WHO-designated repositories, smallpox is considered to be a potential agent of bioterrorism. Smallpox is an attractive biologic weapon as its re-introduction into human populations would be a global public health catastrophe. It is stable in aerosol form with a low infective dose; case fatality rates approach 30%; secondary attack rates among unvaccinated close contacts are 37–88% and are amplified, especially in health-care settings; and much of the world's population is susceptible. Routine civilian vaccination was terminated more than two decades ago and vaccine-induced immunity appears to wane over time in vaccinees.[29] Vaccine supplies remain limited, and there are currently no antiviral therapies of proven clinical effectiveness against this pathogen.

Following an average incubation period of 10–12 days (range 7–19 days), patients experience the acute onset of a 2- to 3-day prostrating prodrome consisting of fever, rigors, malaise, vomiting, headache and backache. They subsequently develop a centrifugally distributed eruption that initially involves the face and extremities and then generalizes as it evolves through macular, papular, vesicular and pustular stages in synchronous (i.e. lesions progress concurrently and have

similar appearances diffusely) fashion over approximately 8 days, with umbilication in the latter stages (Fig. 71.6). Enanthema in the oropharynx typically precede the exanthem by a day or two; this represents high titer viral replication in the upper respiratory tract and correlates with high infectivity. The rash generally remains denser peripherally and typically involves the palms and soles early on, a potentially useful clue in narrowing the differential diagnosis (Fig. 71.7). The umbilicated pustules begin crusting during the second week of the eruption; separation of scabs is usually complete by the end of the third week, but the course of the systemic illness may be attenuated and the appearance of the exanthem milder in those with partial, preexisting immunity or more progressive and virulent in those with immunodeficient states.

The differential diagnosis of smallpox is extensive (see Table 71.3) but may be aided by a number of features of the disease: synchronous lesions, umbilicated appearance in the pustular stage, early involvement of palms and soles, and the centrifugal distribution of the eruption. Historically, varicella and drug reactions posed the most diagnostic dilemmas,[29] although the recent importation of monkeypox to the USA from its animal reservoir in Africa elevates this entity to a loftier position on the differential diagnosis list.[30] While the diagnosis of smallpox is suggested by clinical features, definitive diagnosis is accomplished by vaccinated clinicians acquiring samples of blood and lesional contents or scrapings from crusts for analysis by electron microscopy, viral antigen immunohistochemistry, polymerase chain reaction and viral isolation. Because processing and evaluation of specimens from a suspected case of smallpox requires high-level biocontainment facilities, collaboration with public health authorities is necessary.

Smallpox is transmitted from person-to-person by respiratory droplet nuclei and, less commonly, by contact with lesions or contaminated fomites. Airborne transmission by fine-particle aerosols has, under certain conditions, been documented[31] and should be assumed as a potential mode of spread in a bioterrorism event. The virus is communicable from the onset of the enanthem, generally one or two days prior to the rash, until all of the scabs have separated, although patients are felt to be most contagious during the first week of the rash due to high titers of replicating virus in the oropharynx. Household members, other face-to-face contacts and health-care workers have traditionally been at highest risk for secondary transmission; the last group is obviously of greatest concern with regards to amplification of infection, especially among medically vulnerable populations. Thus, hospitalized cases of suspected smallpox must immediately be placed in negative-pressure rooms with contact and airborne precautions; those not requiring hospital-level care should remain isolated at home in order to avoid infecting others.

The suspicion of a single smallpox case should prompt immediate notification of local public health authorities and the hospital epidemiologist. Containment of the disease is predicated on the 'ring vaccination' strategy, which was successfully deployed in the WHO



Fig. 71.6 Smallpox. (a) Third day of rash in smallpox. Additional lesions continue to appear and some of the papules are becoming obviously vesicular. (b) Fifth day of rash in smallpox. Almost all the papules have now become vesicular or pustular, the truly 'vesicular' stage usually being very brief. Some of the lesions on the upper arm show early umbilication. (c) Eighth day of rash in smallpox. This case is now clearly classified as discrete ordinary-type smallpox. In the confluent subtype of ordinary-type smallpox the lesions would have been confluent on the face and forearms: in the semiconfluent subtype they would have been confluent on the face but not on the forearms. (d) Twentieth day of rash in smallpox. The scabs have separated except on the palms of the hands and the soles of the feet, leaving depigmented areas. From Fenner *et al.*[28]

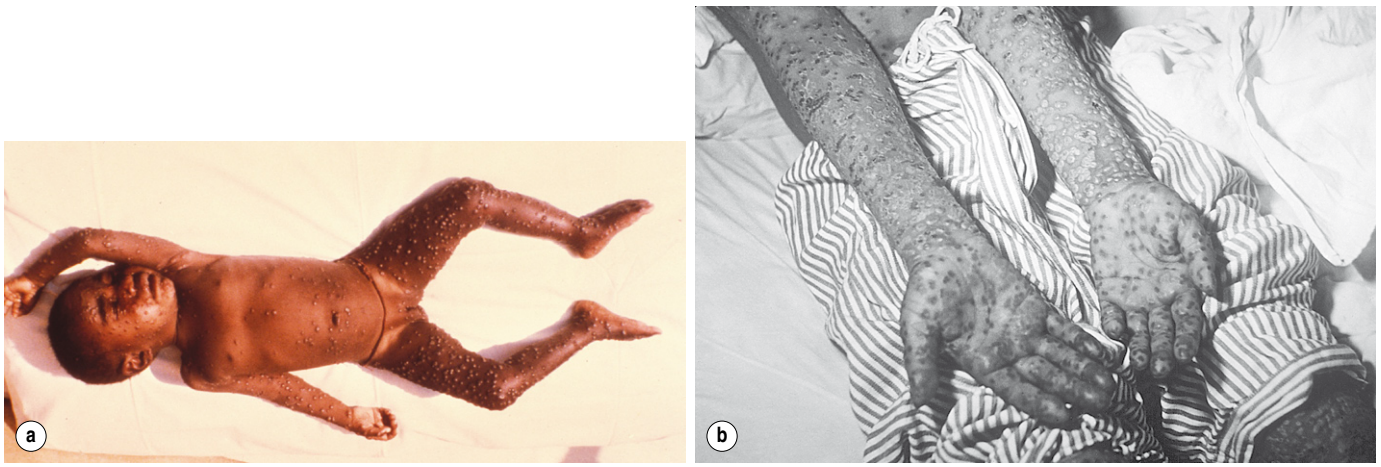


Fig. 71.7 (a) Typical centrifugal distribution of the rash in smallpox. (b) Patient with smallpox, Kosovo, Yugoslavia epidemic, March and April 1972. The scabs will eventually fall off leaving marks on the skin that will become pitted scars. The infection is transmissible until all scabs have fallen off. (a) Courtesy of CDC and Dr Paul B Dean; (b) Courtesy of CDC and Dr William Foege.

global eradication campaign and which mandates the identification and immunization of all directly exposed persons or those at high risk of exposure, including close contacts, health-care workers and laboratory personnel. Vaccination of infected individuals, if deployed within 4 days of infection during the early incubation period, can significantly attenuate or prevent disease and may favorably impact secondary transmission.[29] Because the disease does not exist in nature, the occurrence of even a single case of smallpox would be tantamount to bioterrorism and would warrant an epidemiologic investigation to ascertain the perimeter of the initial release, so that tracing of those initially exposed can be accomplished.[32]

Botulism

Botulism is an acute neurologic disease resulting from intoxication with *Clostridium botulinum* that occurs sporadically and in focal outbreaks throughout the world. Generally, the illness is associated with wound contamination by the bacterial form or ingestion of preformed, food-borne toxin. A detailed discussion of botulism is found in Chapter 21. Aerosol forms of the toxin, a rare mode of acquisition in nature, have been weaponized for use in bioterrorism although their actual use has never been documented.[5] Botulinum toxin is considered to be the most toxic molecule known; it is lethal to humans in minute quantities and acts by blocking the release of the neurotransmitter acetylcholine from presynaptic vesicles, thereby inhibiting muscle contraction.[33]

Botulism presents with the clinical features of an acute, afebrile, symmetric, descending, flaccid paralysis without mental status or sensory changes. The disease manifests initially in the bulbar musculature; fatigue, dizziness, dysphagia, dysarthria, diplopia, dry mouth, dyspnea, ptosis, ophthalmoparesis, tongue weakness and facial muscle paresis are early findings seen in more than 75% of cases. Progressive muscular involvement leads to respiratory failure in untreated cases. The clinical presentations of food-borne and inhalational botulism are indistinguishable in experimental animals.[33]

The diagnosis of botulism is based largely on epidemiologic and clinical features and the exclusion of other possible differential diagnoses (see Table 71.3); there is no commercial assay currently available to confirm intoxication. While sporadic or clustered cases occur regularly, albeit infrequently in developed countries, it must be recognized that any single case of botulism could be the result of bioterorism or could herald a larger scale 'event'. Certainly, large numbers of epidemiologically unrelated, geographically dispersed or multifocal cases should raise the specter of an intentional release of the agent, either in food/water supplies or as an aerosol.

The mortality from food-borne botulism has declined from 60% to 6% over the last four decades, probably as a result of improvements in intensive and supportive care. Because the need for mechanical ventilation may be prolonged in these patients, the finite resource of ventilators would be rapidly overwhelmed in the event of a large-scale bioterrorism event using botulism toxin, even though these devices are part of the Strategic National Stockpile in the USA for such incidents. New developments in ventilator technology may mitigate some of the predicted shortfalls. Treatment with an equine antitoxin is available in limited supply from the CDC and may ameliorate disease if given early.

Plague

Plague, a systemic disease caused by the Gram-negative pathogen *Yersinia pestis*, presents in a variety of clinical forms in nature as detailed in Chapter 120. Plague is endemic in parts of South East Asia, Africa and the western USA. While naturally acquired disease results from a variety of exposure modes, bioterrorism carried out using aerosolized preparations of the agent would likely result in cases of primary pneumonic plague occurring outside of endemic areas. As was the case with the anthrax attacks in the USA in 2001, however, unexpected forms of the disease, such as bubonic and septicemic plague, might also occur in an event.

Primary pneumonic plague classically presents as an acute, febrile, pneumonic illness with prominent respiratory and systemic symptoms; gastrointestinal symptoms, purulent sputum production or hemoptysis occur variably.[34] Chest roentgenogram typically shows patchy, bilateral, multilobar infiltrates or consolidations (Fig. 71.8). Untreated or inappropriately treated patients progress rapidly to develop respiratory failure, vascular collapse, purpuric skin lesions, necrotic digits and death. The differential diagnosis is essentially one involving etiologies of rapidly progressive pneumonia and includes clinical syndromes caused by a number of other agents of bioterrorism (see Table 71.3). The diagnosis may be suggested by observing the characteristic small, Gram-negative, coccobacillary forms in sputum specimens with bipolar or 'safety pin' uptake of Giemsa or Wright stain (Fig. 71.9).[35] Culture confirmation is necessary to confirm the diagnosis; the microbiology laboratory should be notified in advance if plague is suspected, as special techniques and precautions must be employed to prevent inadvertent transmission to laboratory personnel.

Treatment recommendations for plague have been reviewed elsewhere.[26,35] Pneumonic plague can be transmitted from person-to-person by respiratory droplet nuclei, thus placing close contacts, such as patients and health-care workers in the health-care setting,

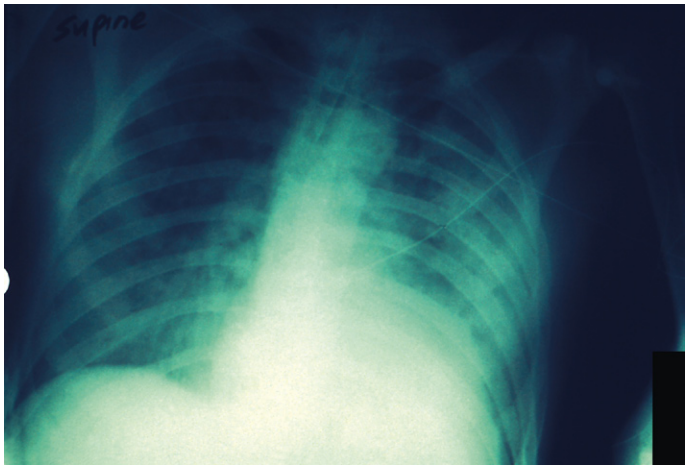


Fig. 71.8 Chest X-ray, pneumonic plague, demonstrating multilobar infiltrates. Courtesy of CDC and Dr Jack Poland.

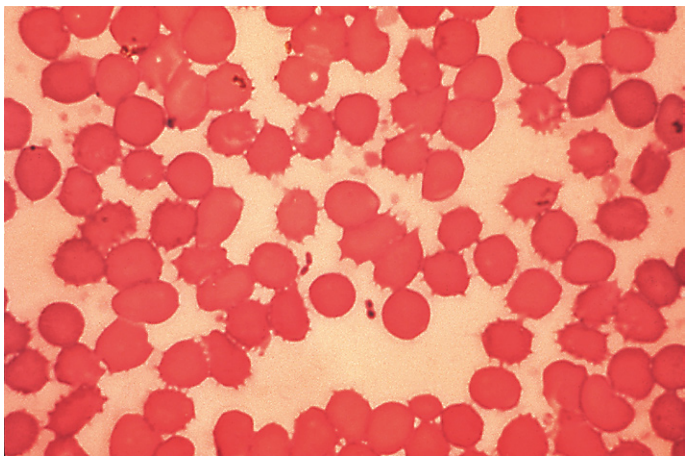


Fig. 71.9 Peripheral blood smear demonstrating bipolar uptake of stain, the so-called 'safety pin' appearance of *Yersinia pestis*. Courtesy of CDC and Dr Jack Poland.

at risk. Domestic cats may participate in maintaining a transmission chain during a bioterrorism event.[12] Prompt recognition and treatment of plague cases, appropriate deployment of postexposure prophylaxis, and early institution of droplet precautions for infected individuals will interrupt secondary transmission.

Tularemia

The causative agent of tularemia, *Francisella tularensis*, is another small Gram-negative coccobacillus that would be predicted to cause a primary pneumonic illness if delivered as an aerosol in a bioterrorism event. Once again, however, vigilance is necessary as naturally occurring disease can be acquired by a variety of routes and present in many clinical forms; therefore an intentional release of bacteria may also result in more than one form of tularemia. Pulmonic tularemia presents with the abrupt onset of a febrile systemic illness with prominent upper respiratory symptoms, pleuritic chest pain, and the variable development of pneumonia, hilar adenopathy and progression to respiratory failure and death in approximately 30% of inappropriately treated patients.[36] The diagnosis is generally established on clinical features, based on the differential diagnosis (see Table 71.3) and microbiologic data; laboratory personnel should be notified in advance if tularemia is suspected, as the organism can be very infectious when manipulated in laboratory conditions. This agent is discussed in depth in Chapter 121.

Viral hemorrhagic fevers

Pathogenic members of four distinct families of RNA viruses are potential agents of viral hemorrhagic fevers (VHF): the agents of Ebola, Marburg, Lassa fever, Rift Valley fever and Congo–Crimean hemorrhagic fever. These syndromes are discussed in detail in Chapter 126. VHF cause clinical syndromes with many common features: fever, malaise, headache, myalgias, prostration, mucosal hemorrhage and other signs of increased vascular permeability, leading to shock and multiorgan system failure in advanced cases.[37] Additionally, specific pathogens are associated with specific target organ effects.

Hemorrhagic fever viruses have generally been viewed as emerging infections in nature due to their sporadic occurrence in focal outbreaks throughout the world and environmental disruption by expanding human populations. These viruses are also potential weapons of bioterrorism for a number of reasons:[10]

- they are highly infectious in aerosol form;
- they are transmissible in health-care settings;
- they cause high morbidity and mortality; and
- they are purported to have been successfully weaponized.

Blood and other body fluids from infected patients are infectious. As such, person-to-person airborne transmission may occur and strict contact and airborne precautions should be instituted in these cases.[37] Transmission in health-care settings is a well-described risk with these agents. Treatment is largely supportive and includes the early use of vasopressors as needed. Ribavirin is effective against some forms of VHF but not those caused by Ebola and Marburg viruses. Nonetheless, this drug should be initiated empirically in patients presenting with a consistent clinical syndrome until an alternate etiology is confirmed.

ASSOCIATED ISSUES AND SEQUELAE OF BIOTERRORISM

Surveillance

Surveillance is perhaps the most critical element in the early recognition and identification of bioterrorism events. In the context of the individual clinician surveillance is analogous to clinical vigilance; in the broader context of communities and larger populations, it involves a public health system and infrastructure designed to detect perturbations in the baseline occurrence of either symptoms, as is the case with syndromic surveillance systems, or diseases, as is the case with a standard public health system of reportable diseases. Syndromic surveillance systems have been used recently for monitoring influenza activity and other emerging infectious diseases, and various real-time, electronic platforms are currently in use by a number of organizations to detect early, sensitive indicators of disease activity.

Quarantine

Quarantine, the physical separation and geographic restriction of groups of uninfected individuals potentially exposed to a communicable illness, has been variably considered to be one management strategy following bioterrorism. The potential effectiveness, feasibility, legality and consequences of quarantine have recently been reviewed.[38] The logistics of this approach are complex and impractical, and it can be associated with adverse consequences, such as increased risk of disease transmission among a quarantined group or riots. It seems clear that there are only limited scenarios in which the potential public health benefits of the imposition of quarantine may outweigh the potential problems engendered by this approach; these largely revolve around highly transmissible, lethal agents. In most situations a disease-specific containment strategy, based on transmission epidemiology and disease prevention approaches, is preferable.

Management of special patient populations

The approach to the management of diseases of bioterrorism must include provisions for children, pregnant women and immunocompromised individuals. Specific recommendations for treatment and prophylaxis of these special patient groups for selected bioterrorism agents have recently been reviewed.[16,26,35,36] A general approach requires an assessment of the risk of using certain drugs or products in select populations versus the potential risk of the infection in question, accounting for the extent of exposure and agent involved. Live virus immunizations such as the smallpox vaccine pose higher risk to these special groups than to others. This consideration will impact mass vaccination decisions and, like most other aspects of medicine, will require an assessment of risk versus benefit.

Psychosocial morbidity

An often overlooked but vitally important issue is that of psychosocial morbidity related to bioterrorism. These sequelae may take the form of acute anxiety reactions and exacerbations of chronic psychiatric illness during the stress of the event, or post-traumatic stress disorder (PTSD) in its aftermath, and may involve clinical victims of bioterrorism as well as health-care workers and other first responders. Nearly half of the emergency department visits during the Gulf War missile attacks in Israel in 1991 were related to acute psychological illness or exacerbations of underlying problems.[39] Data from recent acts of terrorism in the USA suggest that PTSD and/or depression may develop in more than 35% of those impacted by the events.[40,41] Although close proximity to an event and personal loss appear to be directly correlated with PTSD and depression, respectively, those indirectly involved also experience substantial morbidity.[41] The long-term psychosocial impact of these events and of the persistent threat of terrorism in general remains to be determined.

CONCLUSION

The response to bioterrorism is unique among weapons of mass destruction because it necessitates management strategies common to all disasters as well as the application of basic infectious diseases principles: disease surveillance, infection control, antimicrobial therapy and prophylaxis, and vaccine prevention. For these reasons, we, as physicians (and specifically infectious diseases specialists), are likely first responders to bioterrorism and must keep our diagnostic and clinical skills current and our clinical vigilance active regarding potential threat agents. We are expected to be reliable sources of information for our patients, colleagues and public health authorities.[42] As a group we must guard against the inexorable 'bioterrorism fatigue' that may otherwise result from a persistent state of heightened readiness without an actual event taking place.[6]

REFERENCES

References for this chapter can be found online at http://www.expertconsult.com

Practice point **32**

Shiranee Sriskandan

Management of candiduria in the ICU

INTRODUCTION

Candiduria can be defined as the presence of greater than 10^5 fungal cfu/ml urine, though as little as 10^3 cfu/ml can result in disease in certain 'at risk' groups. The prevalence of candiduria varies between 6.5% and 20% amongst hospitalized patients and presents a dilemma to clinicians, who must decide if the finding represents colonization alone, or is a feature of invasive fungal infection. Probably only 3–4% of cases of candiduria lead to candidemia, but 10% of all cases of candidemia are associated with a prior episode of candiduria. Indeed, studies based in the intensive care unit (ICU) have shown that candiduria can be associated with a rise in mortality from 19% to 50%.

PATHOGENESIS

Candiduria can arise in several ways. Contamination of specimens by perineal colonization is less common in the ICU, since most patients are catheterized, though this does not obviate the need for a confirmatory second specimen. Colonization of the urinary tract may occur in the catheterized patient and this is commoner in those who have had antimicrobial exposure. Local infection of both the lower urinary tract (cystitis, urethritis) and upper tract (pyelonephritis) with *Candida* spp. is encouraged by urologic instrumentation, including catheterization. Other factors predisposing to ascending infections include ongoing broad-spectrum antibiotic therapy, diabetes mellitus, renal insufficiency, and any obstructive anomaly of the urinary tract. Importantly, patients with disseminated candidiasis may seed the urinary tract from bloodstream spread; in up to one-tenth of patients, candiduria may herald candidemia (Table PP32.1).

MICROBIOLOGY

The majority (50–70%) of *Candida* isolates from urine in the ICU are *C. albicans*, which is sensitive to fluconazole. Indeed, provided that patients have not previously been exposed to fluconazole, it is reasonable to assume that any germ tube-positive yeast will be sensitive to fluconazole. However, increasing numbers of yeasts other than *C. albicans* occur in the ICU setting and the prevalence varies between units. In particular, *C. tropicalis* and *C. glabrata* account for 10–20% of such isolates, the latter species being notable for its resistance to azole drugs.

CLINICAL FEATURES

Candiduria alone does not cause symptoms; local infection can cause classic cystitis or urethritis and pyelonephritis may lead to flank pain. Patients who have candiduria as a feature of disseminated candidiasis may have evidence of systemic candidal disease, which should be assiduously checked for: clinical features include sepsis, fever, lesions of the optic fundi, skin lesions and hepatosplenomegaly.

INVESTIGATIONS

It is clear from Table PP32.1 that a repeat fresh urine sample must be sent to the microbiology laboratory to confirm candiduria. Microscopy for the presence of white blood cells may be useful in differentiating

Table PP32.1 Etiology of candiduria and laboratory investigation

Source of candiduria	Laboratory investigations
Inadvertent contamination	Repeat sample: clear
Colonization of lower tract	Repeat sample may be positive Patient well No WBCs in urine (catheterization will affect this) Check for *Candida* colonization at other sites
Infection of lower tract	Repeat sample will be positive WBCs in urine Ultrasound if not instrumented Screen for diabetes, renal disease Check for *Candida* colonization at other sites
Infection of upper tract	WBCs + casts in urine Ultrasound Screen for diabetes, renal disease Check for *Candida* colonization at other sites
Disseminated *Candida* infection	Check for *Candida* colonization at other sites Blood cultures/other sterile site: positive for *Candida* CXR, abdominal ultrasound High CRP

colonization from urinary tract infection, but the finding can be non-specific in a catheterized patient; the presence of hyphae-containing granular casts is a rare finding which would confirm true renal infection. If the patient has not been catheterized or had urologic instrumentation recently, it is prudent to screen for diabetes mellitus and renal insufficiency by biochemical testing, and anatomic anomalies using ultrasound. Ultrasound of the renal tract can also demonstrate the presence of fungal balls in patients who have persistent candiduria. Simple tests to screen for the possibility of disseminated candidiasis would include a chest X-ray, abdominal ultrasound, C-reactive protein, cultures of other potentially infected sites (e.g. tracheal aspirate or bronchial lavage, bile, surgical drains, intravascular line tips) and blood cultures.

The '*Candida* colonization index' (ratio of the number of '*Candida*-positive' samples to total number of surveillance samples sent to the laboratory) can be used to predict those that may benefit from pre-emptive systemic therapy; although the approach has not been extensively validated in all types of ICU, it provides a useful guide for borderline cases.

MANAGEMENT

Modern management of candiduria in the ICU setting is determined by the likely source of fungi (Table PP32.2). It is clear that colonization can be treated by simply replacing the urinary catheter or, better, permanent removal. In all cases, rational reduction in the spectrum of antibacterial agents administered to patients will help eliminate fungal colonization and infection. These simple measures allow up to 40% of patients with candiduria to clear fungi from the urine.

True infection of the urinary tract should be treated with a definitive 2-week course of an antifungal, usually fluconazole, in addition to catheter removal or exchange. Fluconazole can be administered intravenously in the ICU or via the nasogastric tube in the oral form, if the patient's gastrointestinal system is functioning. Isolation of a germ tube-negative *Candida* spp. raises the possibility of a fluconazole-resistant yeast, necessitating recourse to intravenous amphotericin B. Although of increasing favor in hospitals, neither voriconazole nor the echinocandin agents reach high concentrations in the urinary tract and therefore, at present, are not advised routinely. Despite anecdotal reports, there is no clear case for local intermittent or continuous bladder irrigation with amphotericin B; the procedure necessitates instrumentation of the urinary tract which might otherwise be unnecessary. Furthermore, although local irrigation with amphotericin B can achieve prompt clearance of funguria, the effect is short lived compared with clearance rates achieved by fluconazole.

Finally, the candiduric patient who may have invasive fungal infection warrants more aggressive 'pre-emptive' antifungal therapy. This must be based on careful assessment of combined clinical and laboratory findings; a *Candida* colonization index of above 0.5 is used by some to guide therapy. If *C. albicans* is isolated and the patient is stable, it is reasonable to treat with high-dose fluconazole for 2–6 weeks (400–800 mg/day for a 70 kg adult). However, if the same patient is clinically unstable, or if the isolate is non-*albicans*, it would be prudent to treat with amphotericin B, as indicated in Table PP32.2. Although newer agents such as voriconazole or the echinocandins provide alternative treatment options for non-*albicans* infection, urinary tract clearance may be more effective using systemic amphotericin. The appropriate duration of therapy in this setting is unclear, and must be determined according to the individual clinical situation and response to therapy.

Table PP32.2 Management of candiduria in the ICU

Suspected cause of candiduria	Action	Additional considerations
Contamination	None	–
Colonization	No antifungal Remove/replace catheter Stop antibacterials if possible	If patient is at risk of infection (e.g. neutropenia) or undergoing urologic procedure, low birth weight infant: fluconazole 200 mg q24h for 7–14 days
Lower UTI	Fluconazole 200 mg q24h for 7–14 days if *C. albicans*	–
Upper UTI	Fluconazole 6 mg/kg q24h for 2–6 weeks if *C. albicans*	Surgical drainage may be needed. Amphotericin B if patient unstable or if non-*albicans*
Disseminated infection likely	Fluconazole 6–12 mg/kg q24h (if *C. albicans*) *or* amphotericin B 0.7–1.0 mg/kg q24h, depending on severity, for at least 2–6 weeks	If dissemination confirmed: follow up for at least 3–6 months after discharge from ICU in case of distant seeding Use of lipid formulations of amphotericin B necessitates doses of 3–5 mg/kg q24h

FURTHER READING

Further reading for this chapter can be found online at http://www.expertconsult.com

Practice point 33

Alastair Miller

Infections associated with near drowning

At least 100 million North Americans use the marine environment for recreation each year. Recreational use of water is probably responsible for the bulk of the 8000 deaths from drowning per annum in the USA (1500 in children) and for many of the estimated 150 000 deaths worldwide. The epidemiology of 'near drowning' is less well known but is thought to be up to 500 times as common. Drowning is death from suffocation as a result of submersion in liquid. In the majority of cases water is aspirated into pulmonary air spaces. This produces a variety of pathologies depending on whether fresh or sea water is inhaled, but the end result is alveolar dysfunction, causing venous blood to be shunted into the systemic circulation past underventilated alveoli to cause hypoxemia. In a minority of cases hypoxemia can result from apnea caused by several different mechanisms. Although 'near drowning' by definition means that the victim survives the initial hypoxic insult (for more than 24 hours), a number of complications may then ensue from the aspiration and hypoxia, including pulmonary edema, convulsions and infective problems such as pneumonia or sepsis. There may be additional problems from hypothermia although paradoxically this may have a protective benefit against cerebral hypoxia. Many of these complications may result in an ultimately fatal outcome.

PATHOGENESIS

Lung damage following aspiration of either sea water or fresh water produces inflammation and edema, which damage alveolar defense mechanisms and enhance the risk of infection. The relatively anaerobic conditions may also favor infection. Infecting organisms may include those already colonizing the lungs or upper airways, which have been carried distally with the aspiration and have then taken advantage of improved conditions for growth. Alternatively, organisms in the aspirated water may give rise to infective problems. Finally, an ill patient with lung damage may be admitted to hospital (and to an intensive care unit) and therefore be exposed to all the risks of nosocomial pneumonia.

MICROBIOLOGY

The literature on the microbiology of near drowning consists mainly of single case reports rather than large-scale reviews but common themes do emerge. Organisms that have been implicated are shown in Table PP33.1, and these can be divided into those that are characteristically associated with pneumonia (either community-acquired or nosocomial) and those that are more specifically associated with immersion incidents. Gram-negative organisms predominate in the aquatic environment (both sea water and fresh water) but anaerobic organisms and *Staphylococcus* spp. can also be found. Some organisms are more likely according to whether immersion took place in sea water or fresh water and depending on whether the water was clean or contaminated. Certain organisms may be more common in particular geographic areas. For example, one might anticipate exposure to *Burkholderia pseudomallei* following a near-drowning episode in the paddy fields of South East Asia (see Chapter 119).

Several cases of infection with *Aeromonas* spp. exist in the literature and these are associated with a high proportion of positive blood cultures and a high mortality. Fungal infections can also cause problems and there are reports of *Aspergillus* pneumonia and disseminated aspergillosis particularly affecting the central nervous system (CNS) after immersion incidents. *Pseudallescheria boydii* is also reported. This is the sexual form of *Scedosporium apiospermum* and is also associated with frequent CNS invasion and a high mortality.

Infection is commonly polymicrobial and this is particularly reflected in the multiple case reports that emerged after the tsunami hit South East Asia in December 2004.

CLINICAL FEATURES

The clinical features of infection after near drowning are similar to those seen when the particular infection arises from more conventional causes and depend on the site of infection. The main complication is pneumonia (as might be predicted from the portal of entry) but there is often an associated bacteremia, which may produce clinical features of sepsis. There have also been case reports of meningitis after near drowning. Skin and soft tissue infection may also result from immersion, especially when associated with trauma such as after the tsunami.

Noninfective pulmonary edema is a common complication of near drowning and can progress to full adult respiratory distress syndrome. Pulmonary edema can be difficult to distinguish clinically and radiographically from pneumonia. In one series of 125 near drowning episodes, the incidence of pulmonary edema was 43% whereas the incidence of pneumonia was 14.7%. These figures are sensitive to changes in case definition and clearly many patients who initially have pulmonary edema may subsequently go on to develop pneumonia, which tends to be a later complication.

Most patients who have pneumonia have fever (although recognition of this may be confounded if there is any residual hypothermia from the immersion). They may have clinical features of pulmonary consolidation or edema, or both.

Table PP33.1 Micro-organisms implicated in pneumonia or sepsis after near drowning

Conventional respiratory pathogens (including atypical organisms and those associated with nosocomial pneumonias)
Staphylococcus aureus
Haemophilus influenzae
Streptococcus pneumoniae
Escherichia coli
Pseudomonas spp.
Moraxella spp.
Klebsiella spp.
Legionella spp.
Pathogens specifically related to immersion
Aeromonas spp.
Pseudomonas putrefaciens
Francisella philomiragia
Chromobacterium violaceum
Burkholderia pseudomallei
Vibrio spp.
Pseudallescheria boydii
Aspergillus spp.
Acinetobacter baumanii

INVESTIGATIONS

Near-drowning victims should have a chest radiograph on admission and this may well be clear or show nonspecific shadowing. They should also have a full blood count and arterial blood gas analysis. Leukocytosis is usual in patients who have pneumonia but is not specific for infection.

Pulmonary secretions must be examined microbiologically; these may include expectorated sputum or tracheal aspirates in intubated patients. There may be pus cells in the samples and it is common to find infecting micro-organisms by stain and by subsequent culture. Blood cultures must always be taken because there is a high rate of bacteremia. CNS involvement is a common complication and therefore there should be a low threshold for CNS imaging and obtaining of appropriate specimens by lumbar puncture or neurosurgery.

MANAGEMENT

Patients who have survived a near drowning episode require emergency evaluation to determine whether they are at risk of subsequent delayed complications. If they are asymptomatic, with no abnormalities on physical examination and with a normal chest radiograph, arterial blood gases and full blood count, they can be safely discharged because they are at low risk of pulmonary problems. However, any abnormality on this initial evaluation should prompt hospital admission for observation.

If hypoxemia is present, supplemental oxygen should be given. If this does not correct the situation, it may be necessary to admit the patient to an intensive care unit for further respiratory support.

In common with many other intensive care situations, there used to be a widespread practice of administering glucocorticoids to patients who had undergone aspiration. There has never been evidence of benefit in near-drowning incidents and this practice is not recommended.

Table PP33.2 Antibiotic regimens for pneumonia and sepsis associated with near drowning

	Dose for average adult patient
Clindamycin	900 mg q8h
Ciprofloxacin	400 mg q12h
Ticarcillin–clavulanate	3 g q6h
Gentamicin	5 mg/kg q24h
Ceftazidime	2 g q8h
Metronidazole	500 mg q8h
Meropenem	500 mg q8h

All these antibiotics are administered intravenously.

ANTIBIOTICS

Prophylactic antibiotics have been shown to be of no benefit and their use is not recommended. However, there should be a low threshold for instituting antimicrobial therapy if there is any suspicion of developing pneumonia or sepsis (Table PP33.2). Features giving rise to concern include deteriorating arterial blood gases, new infiltrates on chest radiograph, hemodynamic disturbance or the development of fever or leukocytosis. It is likely that antibiotics will have to commence before any microbiologic information is available from the laboratory (although initial Gram stains may be helpful). Therefore, broad-spectrum empiric cover with good pulmonary penetration is indicated. However, given the unusual microbiology with resistant organisms and the polymicrobial nature of the infections discussed above, it is essential that appropriate samples are taken prior to starting anti-infectives.

Numerous antibiotics have been used, including aminoglycosides, monobactams, carbapenems, cephalosporins and extended-spectrum penicillins (with and without β-lactamase inhibitors). There are no large-scale trials to guide rational therapy. I suggest the use of clindamycin, which has good penetration and will provide good Gram-positive cover as well as treating anaerobic infection. This should be combined with ciprofloxacin to cover the Gram-negative organisms and also provide some cover against *Legionella* spp. Other reasonable combinations would be ticarcillin–clavulanate with gentamicin, and ceftazidime with metronidazole, although neither of these two regimens offers cover against *Legionella* spp. Clearly, the initial regimen may need to be modified in the light of subsequent information from the microbiology laboratory, but it is important to remember that polymicrobial infection is common. If there is no adequate response or fungi are isolated, it may be necessary to consider the use of antifungal treatment and intravenous voriconazole can be valuable in this context.

FURTHER READING

Further reading for this chapter can be found online at http://www.expertconsult.com

Practice point 34

Eleni Patrozou

Management of human bites

EPIDEMIOLOGY

As with animal bites, bites from humans are relatively common injuries that can lead to severe infection with significant morbidity. Furthermore, human bites have a higher complication and infection rate than animal bites. The epidemiology of patients with human bite infections presenting to emergency departments was outlined in a prospective multicenter study of 50 patients by Talan *et al.* (see Further reading). Seventy per cent of the patients were men and the median age was 27 years. The hands were involved in 86% of these bite-related injuries; 56% and 44% were due to clenched fist and occlusive bite injuries, respectively.

Hand bites are especially prone to infection because of the numerous compartments and the absence of significant soft tissue separating the skin from bones and joints. The reported rate of infectious complications in human bites of the hand is 25–50% and in bites of the face 2.5%. The majority of infections already exist when the patient first presents for care.

MECHANISMS OF INJURY

Human bites may occur from accidental injuries, purposeful biting or closed fist injuries. Wounds may be either occlusive injuries, in which the teeth actually bite the body part, or clenched fist injuries. The clenched fist injury is a ragged laceration most often found over the metacarpal joints of the middle and ring fingers. It results when an individual strikes another person's mouth with a closed fist. An apparently innocuous 3–5 mm laceration over a dorsal metacarpophalangeal joint may overlie a deep bacterial inoculum, tracked deep into the wound with extension. Penetration of the joint occurs in up to 62% of wounds; up to 58% involve injury to the bone.

MICROBIOLOGY

Infected wounds are polymicrobial, with an average of five organisms per wound (Table PP34.1). At least 42 different species of bacteria have been identified in human saliva, and 190 species have been found when gingivitis or periodontitis is present. Anaerobes include *Prevotella*, *Peptostreptococcus*, *Fusobacterium*, *Veillonella*, *Porphyromonas*, *Bacteroides fragilis* and *Clostridium* spp. Anaerobes are found in more than 50% of human bite wounds and frequently produce β-lactamase, unlike those from animal bites. Common pathogenic aerobes include β-hemolytic streptococci, *Staphylococcus aureus* and *Haemophilus* spp. *Eikenella corrodens*, a facultative anaerobic, slow-growing, Gram-negative rod, is

Table PP34.1 Bacteriology of human bite wounds: aerobic and anaerobic micro-organisms isolated from 50 patients with infected human bites

Micro-organism	No. of patients with pathogen (%)
Anaerobes	
Prevotella spp.	18
Fusobacterium spp.	17
Veillonella spp.	12
Peptostreptococcus spp.	11
Eubacterium spp.	8
Actinomyces spp.	4
Lactobacillus spp.	4
Bacteroides spp.	2
Microaerophilic bacteria	
Campylobacter spp.	8
Aerobes	
Streptococcal species	42
S. anginosus	26
S. oralis	7
S. pyogenes	7
S. intermedius	6
S. constellatus	4
Staphylococcal species	27
S. aureus	15
S. epidermidis	11
Other aerobic organisms	
Eikenella corrodens	15
Haemophilus spp.	11
Corynebacterium	6
Gemella morbiliform	6
Neisseria	4
Enterobacter cloacae	4
Candida spp.	4

Modified with permission from Talan *et al.*

frequently implicated in fight–bite injury infections and has been found in 10–29% of human bite wounds. It acts synergistically with aerobic organisms, most frequently streptococci, and is thought to account for greater morbidity in these wounds. *E. corrodens* is usually resistant to first-generation cephalosporins, macrolides, clindamycin and aminoglycosides, and these agents should be avoided as monotherapy.

MANAGEMENT

Human bites do not typically cause immediate symptoms besides the laceration injury. Infection can develop rapidly, however, because of direct inoculation of oral and skin flora into the wound. Signs and symptoms of infection, including redness, swelling and a clear or purulent discharge, may develop 24–72 hours after the bite. Lymph nodes adjacent to the wound may become enlarged and range of motion in the area may be reduced. Leukocytosis may occur.

The approach to the human bite depends on the location and the mechanism of injury. Important prognostic factors for the development of infection include the extent of tissue damage, the depth of the wound and which compartments are entered, and the pathogenicity of the inoculated oral bacteria.

Wound care

The mainstays of bite wound management are as follows:

- *Cleansing*: To reduce the high inoculum of oral flora, wounds should be vigorously cleansed with soap or a quaternary ammonium compound and water.
- *Exploration*: Under topical anesthesia, the wound should be explored in a bloodless field to look for foreign bodies, tendon laceration or joint penetration. It is essential that the wound be examined through the full range of motion, including the position at the moment of injury. Wounds that demonstrate tendon lacerations should be presumed to have joint involvement as well, and this finding should prompt consultation with a hand surgeon. Hand radiographs should be obtained, looking for fractures, dislocations, retained teeth or foreign bodies, or air in the joint.
- *Irrigation*: Wounds should be irrigated with 150 ml or more sterile normal saline or lactated Ringer's solution.
- *Debridement*: Devitalized tissue should be removed through debridement.
- *Delayed suturing* (*except for facial bites*): Although there are no controlled studies on suturing human bites, the high rate of infection and complications of human bites on the hand mandate that they be left open. The wound should be covered with a dry sterile dressing and the hand splinted in a position of function, either with a plaster splint or by packing the palm with bulky gauze and wrapping the hand in a mitten-type dressing. The wound can be closed later with sutures if needed after infection has cleared and granulation tissue is present.
- *Facial bites*: Facial wounds are at low risk for infection, and a large series of sutured facial human bites treated in a plastic surgery clinic had an infection rate of only 2.5%. Treatment should include aggressive debridement, irrigation and suturing. Cosmetic considerations are important.

Antiviral agents

Human bites have resulted in the transmission of herpes simplex, hepatitis C, hepatitis B and HIV. Victims of bites from persons potentially infected with HIV or hepatitis should receive rapid, vigorous and thorough wound cleansing with soap and water to remove saliva, and irrigation with virucidal agents such as 1% povidone–iodine. A baseline HIV blood test and hepatitis antibodies at the time of injury should be obtained or arranged, along with a follow-up test in 6 months. If the bite involved blood, hepatitis B and HIV prophylaxis may be warranted. The potential risk of HIV transmission from the bite victim to the person inflicting the bite should also be considered.

Prophylaxis

Human bites are tetanus-prone wounds. A tetanus toxoid booster should be administered if the patient has been adequately immunized in the past, with last dosage given within the past 10 years. Adults aged 19–64 years who require a tetanus toxoid-containing vaccine should receive combined tetanus, diphtheria and acellular pertussis vaccine (Tdap) instead of tetanus, diphtheria vaccine (Td), if they have not previously received Tdap. Adults who have never received tetanus and diphtheria toxoid-containing vaccine should receive a series of three vaccinations (Table PP34.2). Tetanus immune globulin is required if tetanus immunization has not taken place or is inadequate.

The role of antibiotic prophylaxis in uncomplicated bite wounds is controversial. In general, when the patient is seen within 3 hours, the wound is clean and does not involve the hand, antibiotic prophylaxis can be avoided. However, antibiotic prophylaxis is recommended for all human bites of the hand. One randomized, placebo-controlled study of hospitalized patients with uninfected human bites on the hand found no infections in patients who received antibiotics, whereas those who received placebo had a 47% infection rate. Antibiotics are indicated for high-risk human bite wounds elsewhere on the body, including deep punctures, severe crush injuries, contaminated wounds, older wounds and wounds in patients with underlying illnesses. The antibiotic selected should provide coverage for Gram-positive organisms and *E. corrodens*, such as a second-generation cephalosporin or amoxicillin–clavulanate, and should be given for 5 days with close follow-up.

Antimicrobial therapy

Infected human bites of the hand require both aerobic and anaerobic cultures, and the patient should be treated with antibiotics that cover Gram-positive organisms, *E. corrodens* and anaerobes.

Table PP34.2 Antitetanus prophylaxis

	Previously received three or more doses of Td or DT	Incomplete or unknown vaccination history
Clean or minor wound, age of wound <6 h	Td* if last dose administered more than 10 years ago	Primary vaccination series†
All other wounds	Td* if last dose administered more than 5 years ago	Tetanus immune globulin (260 units im) *and* primary vaccination series

*Adults aged 19–64 years who require a tetanus toxoid-containing vaccine as part of wound management should receive combined tetanus, diphtheria and acellular pertussis vaccine (Tdap) instead of tetanus, diphtheria vaccine (Td) if they have not previously received Tdap.
†Adults who have never received tetanus and diphtheria toxoid-containing vaccine should receive a series of three vaccinations. The preferred schedule is a single dose of Tdap followed by Td >4 weeks later and a second dose of Td 6–12 months later. Tdap can substitute for Td for any one of the three doses in the series. Tdap is not licensed for use among adults aged >65 years. The safety and immunogenicity of Tdap among adults aged >65 years was not studied during US prelicensure trials.

Treatment options include oral amoxicillin–clavulanate, or intravenous ampicillin–sulbactam or ertapenem. β-lactam-allergic patients can receive fluoroquinolones plus clindamycin, or trimethoprim–sulfamethoxazole plus metronidazole.

The duration and route of antibiotic therapy should be individualized based on the site involved, the culture results and response to treatment. All patients with infected human bites of the hand should be hospitalized. High-risk patients, such as those with delayed presentation or deep structure involvement, require prophylactic parenteral antibiotics and close evaluation. Localized infections from human bites not involving the hand can usually be treated without hospitalization if the patient is reliable and has no evidence of lymphangitis, tendon, joint or bone damage or systemic symptoms. Discharge instructions should include immobilization, elevation and sterile dressing changes every 6 hours, and a follow-up visit in 1–2 days.

When cellulitis is already present, a therapeutic course of 10–14 days may be necessary, extended to 3 weeks for tenosynovitis, 4 weeks for septic arthritis and 6 weeks for osteomyelitis. In practice intravenous therapy until the C-reactive protein (CRP) falls to less than 50 mg/l is a pragmatic and objective indication to change to oral antibiotics. If CRP does not fall rapidly or remains static, clinical reappraisal and a second debridement are advisable.

HUMAN BITE FORENSICS

Although many human bites are a consequence of mutual aggression, the physician must keep in mind that the patient may be the victim or perpetrator of child, spousal or elder abuse. All countries require reporting of suspected child abuse; laws vary for spousal or elder abuse. Bite-mark evidence has become accepted as a powerful tool in the investigation of crime. The examining physician should document the appearance of the bite carefully, including its shape, color and size. The physician must determine if a criminal act may have occurred; if so, the wound should be photographed. Counseling or referral should be offered when appropriate.

FURTHER READING


Further reading for this chapter can be found online at http://www.expertconsult.com

Practice Point **35**

Alice Chijioke Eziefula

Factitious fever

Obtaining an accurate clinical history is the essence of good practice in infectious diseases. When deception on the part of the patient is introduced into a consultation or an episode of care, there is a risk that the real needs of the patient are not addressed. The underlying psychopathology may go unrecognized and untreated. The costs to both the patient and the health-care provider can be substantial.

Factitious disorder is defined in the ICD-10 and the DSM-IV-TR (Table PP35.1) as the intentional production or feigning of symptoms, either physical or psychological, in order to assume the sick role. The patient is not conscious of the motive for the abnormal illness behavior. This is in contrast to malingering, where the production or simulation of illness produces an identifiable advantage to the patient, such as avoiding a criminal trial or an examination or discharge from hospital. In somatoform disorders, the symptoms themselves are produced unconsciously.

Factitious fever is either fraudulent or self-induced. In the former, there is manipulation of the processes of measuring the temperature and the latter is achieved by the injection or ingestion of an infectious agent or pyrogen (Table PP35.2). In the earliest case reports, the majority of cases were fraudulent fevers. With the routine use of electronic thermometers, there is a preponderance of self-induced infection.

Table PP35.2 Mechanisms of factitious fever

Fraudulent fever	Self-induced fever
Manipulation of thermometer Switching thermometers External heat source Shaking mercury column Friction at site of thermometer placement	**Injection of pyrogen** Contaminated body fluids Bacterial cultures Nonsterile substance, e.g. milk
Fraudulent documentation	**Ingestion of pyrogen**
Fever by proxy	Thyroxine Phenolphthalein Antibiotics Phenytoin Atropine

Adapted from Sarwari & Mackowiak (1997).

EPIDEMIOLOGY

The true incidence of factitious fever is unknown. The collection of accurate scientific data on the condition is hindered by patients' secrecy and by the unwillingness or lack of confidence of physicians to document or report their suspicions. Much of the medical literature consists of retrospective case series and case reports. Prevalence data are derived from case series in highly selected patient groups, such as patients with 'fever of unknown origin' or patients seen by tertiary or quaternary psychiatric referral services. These provide estimates that factitious fever represents 2.2–9.3% of fevers of unknown origin, depending on the setting or level of care (Table PP35.3). In a national survey in Germany, physicians perceived that 1.3% of their annual case load consisted of factitious disease and that 11% of these cases had factitious fever (Fliege 2007).

Between 70% and 95% of factitious fevers reported in the literature are in females, usually in their third or fourth decade and with a high level of education. Male cases are usually older and are more likely to present with fraudulent fever than with self-induced infection.

Associations with other psychiatric conditions, such as borderline personality, depressive or anxiety disorders and eating disorders have been observed by some authors.

Factitious fever by proxy (see Table PP35.1) is usually induced by the female parent.

Table PP35.1 DSM-IV-TR criteria for factitious disorders

Factitious disorder	Factitious disorder by proxy
• Intentional production or feigning of physical or psychologic signs or symptoms • The motivation for the behavior is to assume the sick role • External incentives for the behaviour are absent	The intentional production or feigning of physical or psychologic symptoms in another person who is under the individual's care for the purpose of indirectly assuming the sick role

PATHOPHYSIOLOGY

The tendency to factitious disease often starts in early adulthood, sometimes following a period of hospitalization for a genuine medical condition.

The majority of cases are in individuals employed in health care, which may be an effect of the preoccupation with illness, rather than a risk factor in itself.

The unconscious need to assume the sick role may result from family disruption or emotional or physical abuse in early childhood.

Table PP35.3 The prevalence of factitious fever (FF) in case series of fever of unknown origin

Author	Year of publication	Number of cases in series	Prevalence of FF (%)	Setting	Data collection
Petersdorf & Beeson	1961	100	3	University hospital	Retrospective
Rumans & Vosti	1978	506	2.2	University hospital	Retrospective
Aduan *et al*.	1979	343	9.3	University hospital	Prospective
Larson *et al*.	1982	105	2.9	University hospital	Retrospective
Knockaert *et al*.	1992	199	3.5	University hospital	Prospective
Zenone	2006	144	4.7	Nonuniversity hospital	Retrospective

PRESENTATION

Although fever may be the presenting complaint, often it develops over the course of an illness. The history is often elaborate but inconsistent and, upon direct questioning, vague. Some patients are conversant with medical terminology and may form an inappropriately strong bond with the physician. Factitious fever is a recognized presentation of an extreme form of factitious disease, Münchhausen's syndrome, a condition first described by Asher in 1951 and inspired by the exaggerated chronicles of the exploits of the German cavalry officer Baron Karl Friedrich Hieronymus von Münchhausen. Such patients exhibit pseudologia fantastica (pathologic lying), dramatic presentations and aggressive behavior. Often, they have a history of multiple hospital admissions and self-discharges against medical advice.

Physical examination may be surprisingly normal. There may be evidence of previous presentations (historical accounts refer to the 'grid iron abdomen' from multiple previous operations) or unusual scars from self-inflicted lesions.

Fraudulent fever has notable characteristics (Table PP35.4). Patients with self-induced fever may have a true bacteremia, but no identifiable source. There may be evidence of local infection at the site of injection (cutaneous abscess, nonhealing wounds).

INVESTIGATIONS

Standard blood tests and imaging may be unremarkable. The unusual and inconsistent history and examination can lead to extensive and risky investigations as the search for the underlying cause progresses. In the pursuit of the sick role, the patient undergoes these procedures willingly, despite their invasive nature.

DIAGNOSIS

The differential diagnosis is wide (Table PP35.5) and there may be some overlap between different syndromes. An expert psychiatric opinion should be sought.

Table PP35.4 Characteristics of fraudulent fever

- Lack of normal diurnal variation
- Absence of tachycardia during spikes of temperature
- Rapid defervescence, unaccompanied by diaphoresis
- Fever greater than 41°C

Table PP35.5 Differential diagnosis of factitious fever

- True fever
- Somatization
- Malingering
- Eating disorder
- Chemical dependency
- Personality disorder
- Psychosis

Prior to the advent of digital tympanic thermography, fraudulent fever was revealed by simultaneous readings of oral and rectal temperature, measuring the temperature of freshly voided urine or by close observation of the patient whilst the temperature is being taken. The diagnosis may be suspected early because of inconsistencies in the history and examination. Patients may be recognized from previous hospital admissions, but 'hospital hopping' is common and a history of multiple previous admissions can go unrecognized. A room search, if acceptable, may reveal injection paraphernalia and even bacterial culture media that the patient has used for auto-inoculation. Ultimately, if obtainable, a patient confession provides the diagnosis.

MANAGEMENT

Patients may present with genuine medical conditions (e.g. septic emboli or endocarditis), which coexist with or result from the abnormal illness behavior; their prompt management is the priority.

It is important to recognize that the patient's intention is to assume the sick role rather than to gain gratification from the deception of the caregivers. Therefore, management should be directed to address this need.

Opinions vary regarding the importance and risk of confronting the patient with the suspicion that their illness is factitious. There is consensus that early psychiatric consultation is advisable. However, outcome studies show that the uptake of long-term psychiatric treatment in this patient group is poor.

Intervention studies are hindered by the difficulty of case identification and the lack of a reliable control group.

PROGNOSIS/OUTCOME

There is a recognized mortality from factitious fever, particularly from direct intravenous injection of bacterial cultures or contaminated

body fluids. Patients may be left with permanent disability resulting from self-induced disease or from invasive investigations and interventions. Health-care-associated illness becomes a risk in the event of prolonged hospital admission.

Although not easily quantifiable, repeated assessments, hospital admissions, unnecessary investigations and legal proceedings constitute a considerable cost to the health-care provider.

An awareness and early recognition of factitious disease is important to direct appropriate psychiatric referral and treatment.

MEDICOLEGAL ISSUES

It is advisable to consult a medicolegal organization if factitious fever is suspected. If the fraudulent nature of the illness could readily be inferred from the evidence in a patient's medical notes, the physician may be held responsible for unnecessary treatments or investigations. Ultimately, the patient may seek to sue their practitioner in order to legally sanction their pursuit of the sick role.

FURTHER READING

Further reading for this chapter can be found online at http://www.expertconsult.com

Practice point **36**

Heidi Ammerlaan
Jan Kluytmans

Methicillin-resistant *Staphylococcus aureus* (MRSA) colonization

Since its initial report in 1962, MRSA has been considered a hospital-related phenomenon for a few decades. Mostly sporadic cases and small outbreaks were reported. In the late 1980s MRSA rates started to increase and nowadays MRSA has become endemic in hospitals worldwide, with only a few exceptions. This is associated with high morbidity, mortality and additional costs to society. In addition, there are increasing reports of community-onset disease that primarily affects younger people and may sometimes be fatal.

Staphylococcus aureus is a commensal micro-organism that is found in healthy people. Carriage of *S. aureus* in healthy individuals is mainly located in the nose (Fig. PP36.1).[1] A recent survey in the USA in a random sample of the population found that 32.4% carried *S. aureus* and 0.8% MRSA.[2] Carriage of *S. aureus* rarely causes disease in healthy people but is associated with an increased risk for the development of infections in various patient populations.[1] Carriage of MRSA is associated with an even higher risk than carriage of methicillin-susceptible *S. aureus* (MSSA). In patients colonized with MRSA at admission, 19% develop an infection with MRSA during the hospitalization period. In carriers of MSSA this is only 1.5% and in noncarriers 2.0%.[3]

Although the risk of carriage has been clearly established, there is an ongoing debate regarding the effects of eradication of carriage. There are two possible objectives to eradicating carriage:

1. *To control the spread of MRSA*: As most of the individuals that carry MRSA are asymptomatic, control of spread includes

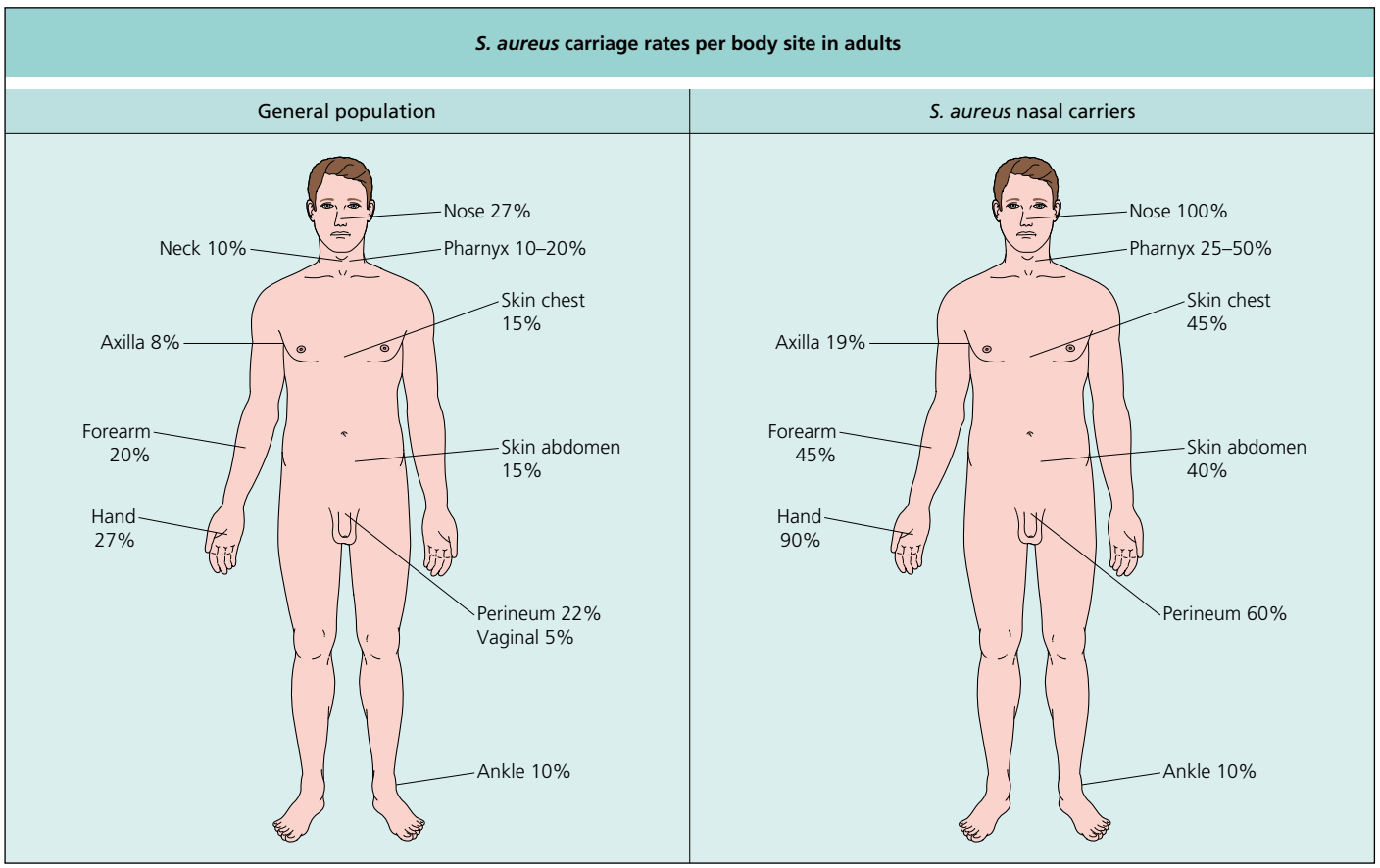


Fig. PP36.1 *Staphylococcus aureus* carriage rates per body site in adults. Redrawn from Wertheim *et al.*[1]

both infected and colonized individuals. This also includes health-care workers. Numerous reports have identified colonized health-care workers as the source of MRSA outbreaks.[4] Therefore, successful control of MRSA should include identification and treatment of colonized patients and health-care workers. Using this strategy, the MRSA rates in Scandinavia and The Netherlands have remained extremely low.

2. *To prevent the development of infection in patients who are at high risk* (e.g. patients in the intensive care unit or patients scheduled for high-risk surgery): Although this makes sense considering the proven risk of MRSA carriage, the strategy has not been studied sufficiently for its effectiveness.

The best studies in this field have included patients carrying *S. aureus* in general, mainly consisting of MSSA. A review integrating the results of these studies concluded that there is a significant reduction of the *S. aureus* infection rate in carriers treated before surgery or while on hemodialysis or peritoneal dialysis.[5] These studies used mupirocin nasal ointment. The study in surgical patients only showed a significant effect in carriers, although this was not the primary objective of the investigators. The protective effect of mupirocin in surgical patients is very likely; however, the final evidence should come from an intervention study treating carriers only. At present, the prophylactic use of mupirocin in patients is recommended in MRSA carriers undergoing high-risk surgery in several national guidelines. The studies in hemodialysis and peritoneal dialysis did show a significant reduction of the infection rate but the prolonged use of mupirocin carries a substantial risk for the development of resistance to this precious compound. To date, the optimal treatment schedule has not been defined and extremely careful monitoring for mupirocin resistance is mandatory when it is used in this setting.

Recently a guideline for the treatment of carriage has been developed, based on a systematic review of the literature and expert opinion.[6] This resulted in the following conclusions and recommendations.

The indication for eradication of carriage depends on the consequences of MRSA carriage for the individual and for others, the risk and the seriousness of side-effects of treatment and the estimated a-priori chance of successful treatment given the characteristics of the pathogen and the host.

In healthy individuals who do not work in the health-care setting, eradication therapy is usually not advised. In individuals with risk factors for the development of an MRSA-associated infection, eradication therapy is generally indicated. However, as this applies to patients with underlying diseases there are also often contra-indications because the risk of treatment failure is high. Therefore, a division is made into uncomplicated versus complicated carriage (Table PP36.1). Treatment strategies for uncomplicated and complicated carriage are outlined in Tables PP36.2 and PP36.3. In complicated carriers, topical therapy is combined with a combination of two systemic antibiotics. Details of the preferred agents are provided in Table PP36.4. This results in a treatment strategy as outlined in Figure PP36.2.

When treatment is complete, its effectiveness is measured by cultures. The first cultures are obtained at least 48 hours after completion of treatment. At least three sets of cultures from the nose, throat, perineum, sputum (in case of productive cough, ventilation or tracheostomy), wounds, skin lesions, insertion sites of catheters (if present) and urine (in the presence of a urinary catheter), taken with time intervals of at least 7 days, are needed before a carrier is considered to be treated effectively.

Table PP36.1 Definitions of uncomplicated and complicated MRSA carriage[6]

Uncomplicated carriage

MRSA carrier who:
- has no active infection caused by MRSA and no active skin lesions and no invasive material with a connection between the interior and exterior environment (e.g. urinary catheter, external fixator); *and*
- MRSA carriage is located in the nose (apart from other sites); *and*
- MRSA strain is *in-vitro* susceptible for mupirocin

Complicated carriage

MRSA carrier who:
- has active skin lesions or invasive material with a connection between the interior and exterior environment; *or*
- failed earlier treatment according to the guideline for uncomplicated carriers; *or*
- MRSA carriage is not located in the nose; *or*
- MRSA strain is *in-vitro* resistant to mupirocin

Table PP36.2 Recommendations for treatment of uncomplicated carriage[6]

Recommendations	Level*
Mupirocin nasal ointment in both nostrils q8h for 5 days	1
During treatment daily washing of skin and hair with a disinfecting soap (chlorhexidine soap solution 40 mg/ml or povidone–iodine shampoo 75 mg/ml)	3
Clean underwear, clothing, washcloths and towels daily, and a complete change of bedding on days 1, 2 and 5 during treatment	4
If therapy fails, explore the situation at home for reservoirs, i.e. humans and/or pets (a reservoir at home has to be treated simultaneously with the carrier)	3
If second treatment fails, the person becomes a complicated carrier and treatment as outlined in Table PP36.3 is recommended	4

*The strength of the recommendations given is according to the handbook of the Dutch Institute for Healthcare Improvement (CBO). Level 1: conclusion supported by at least two independent randomized clinical trials of good quality or by a meta-analysis; level 2: supported by at least two randomized clinical trials of mediocre quality or insufficient power or other comparative studies (cohort study, patient-control study); level 3: not supported by comparative studies mentioned in levels 1 and 2; level 4: expert panel opinion.

SUMMARY

To control the spread of MRSA, it is important to optimize control measures. One measure is the treatment of carriage, both in patients and health-care workers. Carriers of MRSA who are at risk of developing an infection or contaminating other susceptible individuals should be treated based on the relevant national guidelines.

Table PP36.3 Recommendations for treatment of complicated carriage[6]

Recommendations	Level
In the presence of active skin lesions, it is advised to treat the skin lesions first, if necessary by consulting a dermatologist	4
In the presence of foreign body material with a connection between the interior and exterior environment, postponing eradication therapy until removal is preferred	4
If wounds are present, eradication therapy is only advised after healing of the wound, unless there are reasons not to postpone	3
Application of mupirocin on the wound is discouraged, thereby preventing the development of resistance. Application of disinfectants is preferred, if necessary in combination with systemic antibiotics	4
If the complicating factor is eliminated successfully, the patient can be classified as uncomplicated and treated likewise	4
Treatment of carriage of mupirocin-susceptible MRSA	
Systemic treatment with a combination of two antibiotics for at least 7 days as outlined in Table PP36.4. The choice mainly depends on the *in-vitro* sensitivity of the MRSA strain. In principle, oral treatment is applied	3
Combine systemic treatment with mupirocin nasal ointment in both nostrils q8 h for 5 days	1
During treatment daily washing of skin and hair with a disinfecting soap (chlorhexidine soap solution 40 mg/ml or povidone–iodine shampoo 75 mg/ml)	3
Clean underwear, clothing, washcloths and towels daily, and a complete change of bedding on days 1, 2 and 5 during treatment	4
Simultaneous treatment of infected housemates is advised. If these housemates are considered uncomplicated carriers, the treatment recommended in Table PP36.2 will be sufficient	3
In the presence of intestinal and/or rectal carriage, there is limited experience with oral application of aminoglycosides and glycopeptides. Because of the risk of development of resistance against these important therapeutic means, this is not recommended	3
If therapy fails, referral to a centre with specific expertise is advised	4

Table PP36.4 Oral combination therapy for eradication of MRSA carriage[6]

Antibiotic 1	Antibiotic 2	Recommendation
Trimethoprim 200 mg q12 h Doxycycline 200 mg q24 h Clindamycin 600 mg q8 h Clarithromycin 500 mg q12 h Ciprofloxacin 750 mg q12 h	First choice: rifampin (rifampicin) 600 mg q12 h In case of resistance: fusidic acid 500 mg q8 h	Recommended
Fusidic acid 500 mg q8 h	Rifampin (rifampicin) 600 mg q12 h	Alternative

The dosages in this table are the recommended dosages for an adult patient of about 70 kg. Combination therapy is preferred because of better effectiveness and a decreased risk of resistance.

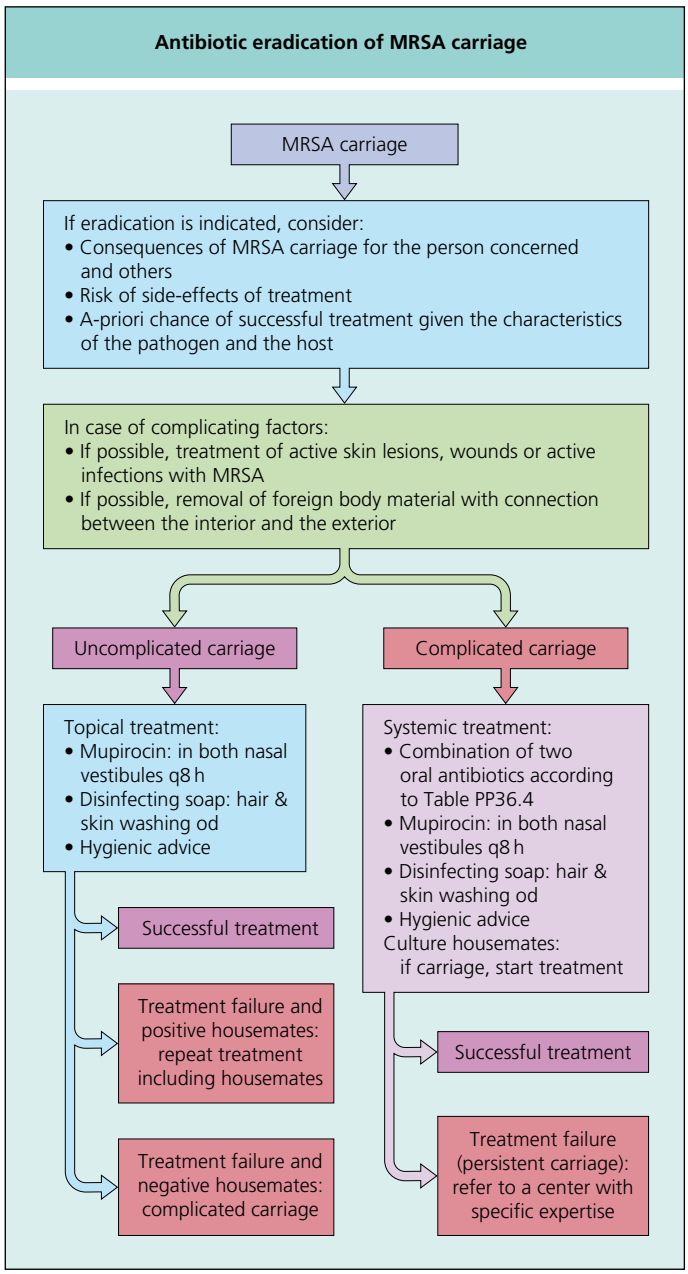


Fig. PP36.2 Antibiotic eradication of MRSA carriage.

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References for this chapter can be found online at http://www.expertconsult.com

Practice point | 37

David J Barillo
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Infection in burns

The opinions and assertions herein contained represent the private views of the authors and do not represent official policy of the US Army Medical Department, the US Army or the Department of Defense.

PATHOPHYSIOLOGY

Thermal injury results in a significant and sustained hypermetabolic response, possibly related to centrally mediated release of catecholamines, glucagon and cortisol.[1] The initial cardiovascular response is a decrease in cardiac output with an increase in systemic vascular resistance secondary to hypovolemia. During the second 24 hours, the patient becomes hypermetabolic and cardiac output increases to supernormal (2–2.5 times normal) levels, accompanied by a reciprocal drop in systemic vascular resistance to 40–80% of normal.[2] Cardiac output remains elevated until the burn wounds are closed, which may take weeks to months if the burn surface is large.[1] This pattern of elevated cardiac output and decreased systemic vascular resistance is a *normal response* to thermal injury, and is not, of itself, indicative of sepsis.

The hypermetabolism of burn injury results in increased heat production, elevation of core temperature and a central upregulation of the thermoregulatory set-point. Fever in the burn patient is thus often physiologic and not related to infection.[3] Burn centers do not consider burn patients as being febrile until core temperature exceeds 102.5°F (39.2°C) and do not perform 'fever workups' below this level. Burn patients may also become hypothermic secondary to heat loss to the environment as a consequence of lost skin integrity. Burn hypermetabolism causes increased clearance of many medications, including antibiotics and anticonvulsant, and dosing should be based upon serum measurements where available.

Thermal injury suppresses essentially all aspects of the immune system. The multiple immunologic consequences of burn injury are summarized in Table PP37.1 Leukocyte counts in the range of 14 000–18 000/mm^3 or higher are typically seen in burn patients in the absence of infection; the white blood cell (WBC) trend, rather than the absolute WBC count, should be followed if infection is suspected. Moreover, bacterial colonization of the burn wound is an expected and unavoidable consequence, which may or may not lead to wound infection.

In summary, the pathophysiology of burn injury makes the timely diagnosis of infection difficult. Elevated temperature, WBC count, and cardiac output combined with a low systemic vascular resistance and presence of bacteria in the burn wound may indicate serious systemic infection, or may simply represent normal physiologic response. Acute infection is best suggested by a *change* in hemodynamic status, body temperature or clinical condition, increased need for ventilatory support, new onset of ileus, glycosuria or glucose intolerance.

DIAGNOSTIC WORKUP OF THE POTENTIALLY SEPTIC BURN PATIENT

A septic workup starts with a complete physical examination with specific attention directed at the burn wound (see below) and to any current or previous site of intravenous cannulation. Rectal examination should be performed to rule out prostatitis in males and perirectal disease in both sexes. Cultures of blood, sputum and urine are obtained, along with a chest radiograph. A timeframe of initial presentation of infection by site is presented in Figure PP37.1

In addition to acalculous cholecystitis and pancreatitis, burn patients occasionally develop short-segment small bowel necrosis by a mechanism which remains unclear. An adjunct to abdominal CT is diagnostic peritoneal lavage, which has a sensitivity of 0.86, a specificity of 1.00 and an overall accuracy of 94% in the diagnosis of intraperitoneal infection in burn patients.[4]

Drug resistance is a continuing problem, with emergence of extended-spectrum β-lactamase-producing, enteric Gram-negative organisms as well as multidrug-resistant *Pseudomonas*. Multidrug-resistant *Acinetobacter* species are also becoming common, although the presence of this organism does not independently affect mortality.[5] For empiric coverage, a burn center-specific antibiogram should be developed as drug susceptibilities will differ from the remainder of the hospital. Empiric *Acinetobacter* coverage may necessitate inhaled colistin (for pneumonia) combined with oral minocycline and intravenous colistin for severe infection.

Children may develop idiopathic fevers in the 103°F (39.5°C) range early in the course of burn injury, usually resolving without identifiable source. Persistent fever suggests otitis media, urinary tract infection or burn wound cellulitis. Oropharyngeal colonization with *Streptococcus pyogenes* is a theoretical concern, as spread of streptococci to the burn wound results in a rapidly spreading cellulitis. Routine penicillin prophylaxis of pediatric burn patients has been advocated but is probably unnecessary. Alternately, penicillin can be administered until admission throat cultures return as negative.

Electrical burn injury may result in muscle necrosis (under intact or uninjured skin) along the route of current passage. Sepsis workup should include prompt surgical exploration of body regions known or suspected to be in the path of current flow if another source is not immediately apparent.

Positive blood cultures in the absence of wound infection should prompt examination of intravenous cannulation sites and consideration of endocarditis. Gram-negative bacteremia, but not Gram-positive bacteremia in burn patients is significantly associated with increased mortality.[6] The association of fungemia with increased mortality is equivocal.[6]

Table PP37.1 Impact of thermal injury on immune function

Circulating	IL-1	Initial increase in serum levels followed by decreased production Increased local production at sites of inflammation
	IL-2	Suppressed production
	TNF	Increased serum levels in severely infected burn patients
	IL-6	Increased serum levels in severely infected burn patients Increased local production at sites of inflammation
	Neopterin	Serum levels increased (nonspecific marker of macrophage stimulation)
	Circulating immunoglobulins	Decreased serum levels
	Prostaglandin E_2	Increased serum levels of prostaglandin E_2
	Thromboxane B_2	Increased serum levels
	Activation/depletion of alternative complement pathway	
	Secondary elevation of fibronectin levels	
	Reduction in serum opsonic activity	
Cellular	Initial leukopenia (margination) followed by leukocytosis	
	Generalized activation of circulating granulocytes by multiple pathways	
	Depression of neutrophil chemotaxis, phagocytosis and bactericidal activity	
	Depression of helper T cells	
	Generation of suppressor inducer T cells	
	Increased production of suppressor effector T cells	
	Suppression of IL-2 production by lymphocytes	
	Activation of macrophages	
Other	Loss of cutaneous skin barrier to infection	
	Smoke inhalation	Increased risk of pneumonia (impaired mucociliary clearance mechanisms, defective alveolar macrophage function, distal airway obstruction, alveolar collapse, segmental atelectasis, increased requirements for airway intubation and mechanical ventilation)
	Requirement for long-term intubation of bladder and vascular system	
	Nutritional deficits	
	Impairment of reticuloendothelial system function	
	Multiple blood transfusions	

IL, interleukin; TNF, tumor necrosis factor.

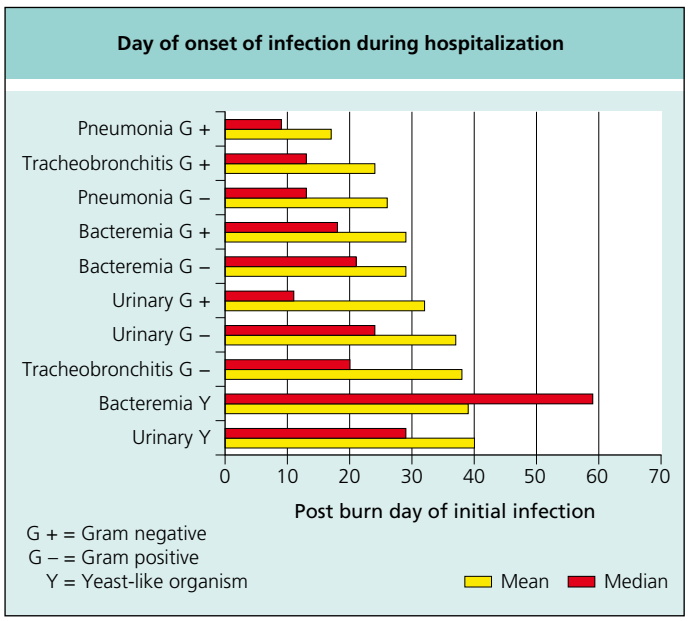


Fig. PP37.1 Day of onset of infection during hospitalization.

Radionucleotide studies such as indium or tagged leukocyte scans, in general, are not useful in burn patients. Burn wound or skin donor sites will frequently concentrate isotopes, making interpretation difficult.

EVALUATION OF THE BURN WOUND

The burn wound is the key to management of the burn patient. The physiologic, metabolic and immunologic changes seen in burn injury are proportional to the size of the wound, and do not return to normal until the burn wound is successfully closed. The burn wound is the first site to examine when sepsis is suspected.

The thick leathery nonviable coating of a burn, termed eschar, is warm, protein rich, moist and avascular, representing an excellent culture medium unaffected by parenterally administered antibiotics or by circulating elements of the immune system.[1] For this reason, the eschar of full thickness burns should be expeditiously excised. Eschar normally becomes colonized with the patient's own flora (Gram-positive bacteria) within 3–5 days of injury.[7] This initial colonization is subsequently replaced by Gram-negative flora over a variable interval. The principal concern is eventual colonization of the subeschar space. When bacterial densities at the interface of eschar and underlying viable tissue reach a level of $>10^5$ cfu/gram of tissue,

wound infection with systemic microbial spread is likely.[1] The goals of burn wound management are to contain microbial colonization to a manageable level with topical agents pending surgical debridement and wound closure with skin autografts or other modalities.[1]

Because the presence of bacteria in the burn wound per se is not a pathologic finding, specialized procedures and terms have been developed to quantify the potential for wound infection:

- *burn wound colonization* is the term given to the presence of micro-organisms within the eschar and does not imply active local or systemic infection;
- *burn wound invasion* occurs when micro-organisms invade viable tissue adjacent to the burn eschar, an infrequent event in contemporary burn practice; and
- invasion accompanied by a positive blood culture or by distant spread of micro-organisms or toxic products is termed *burn wound septicemia*.

Cultures of the burn surface or of debrided tissue are useful for epidemiologic purposes to document resident flora in the event of true infection. Quantitative wound cultures (cfu/gram of tissue) have limited diagnostic utility in burn wound care. A negative quantitative culture (bacterial density $<10^5$ cfu) correlates well (96.1% negative predictive power) with absence of invasive infection on histopathologic tissue evaluation; however, the agreement between positive cultures and positive histologic examination is only 35.7% (positive predictive power).[7] Thus, only a negative quantitative culture has clinical significance.

The *sine qua non* of wound evaluation is histopathologic examination of a biopsy specimen to determine the presence of micro-organisms in viable tissue.[1] This is performed as a bedside procedure under local anesthesia. A 500 mg sample of eschar (ellipse measuring 1–2 cm) is excised to include the underlying viable tissue. Frozen section technique provides results in 30 minutes but has a 3.6% false-negative rate, and should be confirmed by permanent sections.[8] A rapid section technique that produces permanent sections in 4 hours has been described.[9] Results with either technique are communicated to the clinician using standardized nomenclature (Table PP37.2).

Cellulitis is diagnosed by the presence of expanding erythema at the margins of the burn wound. Erythema surrounding second- or third-degree burn injury that does not increase in size may also represent areas of first-degree burn. Burn wound cellulitis usually responds to β-lactams. Occasionally, cellulitis is poorly controlled until the adjacent burn is excised.

True burn wound infection is suggested by early eschar separation, conversion of partial thickness to full thickness injury, subeschar hemorrhage, degeneration of granulation tissue, dark red, brown or black discolorization of eschar (Fig. PP37.2), violaceous discoloration of the unburned skin at the wound margins or ecthyma gangrenosum. Diagnosis is confirmed by wound biopsy and histologic examination. When invasive bacterial infection (Stage 2A, 2B or 2C) is diagnosed, the wound should be immediately excised. Gram-negative organisms are most frequently implicated.

Fungal infection of the burn wound is most commonly caused by *Aspergillus*, *Candida*, *Mucor* or *Rhizopus* spp. Invasive fungal wound infection is an independent predictor of mortality.[10] Wound colonization involving *Candida* spp. is commonly treated with topical nystatin or clotrimazole with little supportive evidence. *Aspergillus* spp. characteristically produce superficial colonization or infection of necrotic tissue, which should be promptly excised. *Mucor* spp. may produce a rapidly spreading fascial infection with early microvascular involvement and its presence in a burn wound is an indication for immediate, radical and repeated surgical excision.

Table PP37.2 Histologic classification of burn wound infection

Class 1 – Colonization	1A Superficial	Superficial bacterial colonization of the wound Micro-organisms present on wound surface
	1B Penetration	Penetration of the eschar by bacteria in variable thickness of eschar
	1C Proliferation	Multiplication of micro-organisms present in the subeschar space
Class 2 – Invasion	2A Microinvasion	Micro-organisms present in viable tissue immediately subjacent to subeschar space
	2B Generalized	Multifocal or widespread penetration of micro-organisms deep into viable tissue
	2C Microvascular	Involvement of small vessels and lymphatics

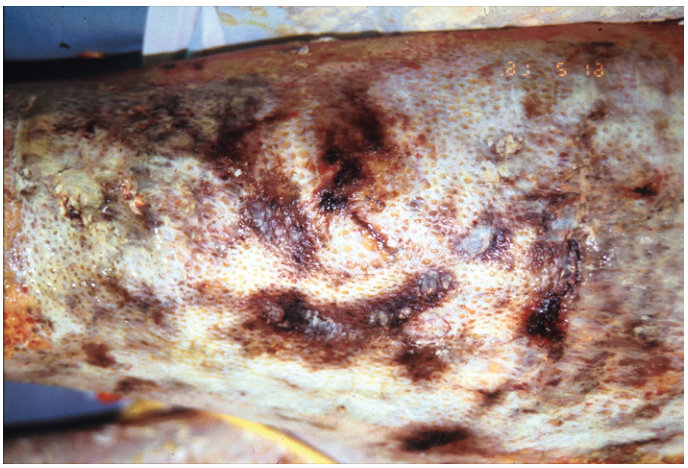


Fig. PP37.2 Invasive pseudomonal burn wound infection, stage 2C.

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Practice point **38**

Najam Zaidi

Transfusion-related infections

Disturbingly, transmission of HIV and the hepatitis viruses may occur from blood donors in an often asymptomatic phase after infection when neither the pathogen nor its antigens, nor host-specific immunologic markers are detectable by conventional screening tests. This is known as the window period (WP). The addition of nucleic acid testing (NAT) to screening tests has decreased the duration of the WP, resulting in less than one case of HIV transmitted per million units transfused in developed countries. Current risk estimates are based on mathematical models calculating the probability of donors presenting in the WP. Estimating infection risks by testing donor blood for all potential pathogens or observing the recipients for illness are impractical and prohibitively expensive because of the large sample sizes required (see Table PP38.1 and annotations).

Acellular 'manufactured' blood products, such as fresh frozen plasma (FFP), albumin, gamma globulins and clotting factor concentrates, are usually pooled from multiple donors. The risk of contamination of such large pools is offset by rigorous physicochemical sterilization procedures. In contrast, cellular products – packed red

Table PP38.1 Risk of transfusion-transmitted diseases in the United States

Pathogenic agent	Average estimated risk per unit
Hepatitis A virus (HAV)	Unknown, presumably <1:1 000 000
Hepatitis B virus (HBV)	1:205 000*
Hepatitis C virus (HCV)	1:1 935 000†
Human immunodeficiency virus-1 (HIV-1)	1:2 135 000†
Human T-cell lymphotrophic virus 1 and 2 (HTLV-1, HTLV-2)	1:2 993 000
Cytomegalovirus (CMV)	Infrequent‡
Parvovirus B19	Unknown, presumably <1:1 000 000
West Nile virus (WNV) and other arboviruses	Unknown, presumably <1:1 000 000†
Plasmodium spp. (agents of malaria)	1:4 000 000
Babesia	<1:1 000 000
Trypanosoma cruzi (agent of Chagas disease)	Unknown, presumably <1:1 000 000
Agent of Creutzfeldt–Jakob disease (CJD), variant CJD (vCJD)	Unknown, presumably <1 000 000
Bacteria associated with symptomatic sepsis	1:500 000 for red blood cell units 1:15 400 for apheresis platelet units§

Case of infection per unit of blood product transfused:
Calculated by the incidence rate/window period (IR/WP) model, which measures the residual risk of a given viral pathogen by multiplying the IR (new infections in the specific blood donor population per year) by the length of the WP in fractions of a year. These calculations assume that:

- timing of donation is independent of the time of infection;
- rate of transmission is nearly 100%;
- testing is flawless with no infections missed due to immunologically silent state or variant strains; and
- no infections are missed due to inherent decreased lifespan of transplant recipients because of co-morbidities.

*Estimates for HBV reflect risk projections before implementation of blood donor screening with nucleic acid testing (NAT) in 1999.
†Estimates for HIV and HCV indicate risk projections following implementation of NAT for these agents in 1999 and West Nile virus in 2003.
‡In association with CMV risk-reduced components.
§Risk estimate for symptomatic sepsis associated with transfusion of apheresis platelet units reflects the experience of a single US medical center (Johns Hopkins) before implementation of a bacterial detection system.
Data from Ness *et al.* (2001).

Table PP38.2 Methods for reducing risk of transmission

Method	Comment
Deferral of high-risk patients	Sole method available for CJD by exclusion of patients who have lived in the UK or Europe Important in prevention of malaria, babesiosis, HIV-2 and HIV serogroup O infection
Exclusion of patients with history of disease	Exclusion of patients with history of intravenous drug use or of jaundice prevents HIV-, HCV- and HBV-related illnesses
Screening of blood products with serologic tests	Selection of seronegative donors for the immunocompromised Effective in reducing transmission of viruses of the herpesvirus family (CMV, EBV, hepatitis viruses, HIV) Seronegative blood donors may harbor intracellular viruses such as CMV with undetectable antibody titers Transient viremia may bind circulating antibody, rendering it undetectable
Pathogen-reduction methods in the manufacturing process	
Heat (pasteurization) Nanofiltration Leukocyte reduction	Ineffective for prions and non-enveloped viruses Effective in viral illness Effective in reducing transmission of viruses of the herpesvirus family (CMV, EBV). Not as good as blood from seronegative donors Cells of granulocyte–macrophage lineage are a common source of CMV infection
Physicochemical processes	
Solvent/detergent treatment	Effective against lipid-enveloped viruses (HIV, HBV, HCV) Ineffective against non-enveloped viruses (HAV, parvovirus B19) Pooling of products with >2500 donors risks contamination of large batches
Photodynamic treatment	Allows single-unit treatment Photosensitive compounds (S-303, inactine, riboflavin, psoralens) activated by ultraviolet light inactivate pathogens by oxidation (methylene blue) or binding to nucleic acids (riboflavin and psoralens)
Selective-affinity resin	Adsorbs scrapie prion protein

CJD, Creutzfeldt–Jakob disease; CMV, cytomegalovirus; EBV, Epstein–Barr virus; HAV, hepatitis A virus; HBV, hepatitis B virus; HCV, hepatitis C virus.

blood cells (RBCs), platelets and granulocytes – are mostly single-donor derived and have limited sterilization options (Table PP38.2).

THE VIRAL PATHOGENS (TABLE PP38.3)

Hepatitis A virus (HAV)

Rare cases of HAV may occur in clotting factor concentrates treated with the solvent/detergent method (see Table PP38.2). The lack of a viral envelope enables this RNA virus to survive pasteurization and chemical inactivation by photodynamic treatment.

Hepatitis B (HBV)

In transcutaneous exposures, HBV is significantly more infective than HCV and HIV. In the USA, 5% of the population is seropositive and over a million people have chronic infection. Serum hepatitis B surface antigen (HbsAg) is detectable 30–60 days after transmission and persists up to 4 months following acute infection. IgM anti-HBc antibodies are present at symptom onset and signal high-level viremia. Anti-HBsAg antibodies (anti-HBs) become detectable after an often asymptomatic window period of 37–87 days. They protect against re-infection but cannot achieve tissue eradication. Anti-HBc is detectable in chronic carriers. It is possible for a donor to be HBsAg negative yet still be infective. Rare HBV mutants that do not produce HBsAg are undetectable by routine HBsAg and anti-HBc testing but are still pathogenic. Novel antibody and NAT-based assays detect these mutants.

Hepatitis C (HCV)

This RNA flavivirus has six genotypes. Genotype 1 responds less favorably to treatment and is predominant in North America. Overall, 3.9 million Americans are seropositive for HCV, resulting in chronic disease in 2.7 million and up to 40 000 new cases per year. Approximately two-thirds of HCV carriers have a history of intravenous drug use. Among blood donors, 0.07–0.09% have confirmed infection. Prior to seroconversion, fluctuating viral levels, often even below NAT thresholds, result in a residual risk of HCV transmission by blood transfusion.

Human retroviruses

HIV infections were first diagnosed in hemophiliacs and other transfusion recipients in 1982. Fortunately, timely policies of high-risk donor deferral by detailed history in 1983 and HIV antibody testing in 1985 decreased transmission dramatically, even before the disease became a part of the public consciousness. Improved screening methods with sensitive immunoassays, p24 antigen testing and RNA testing on pooled blood products have reduced transmission further since the 1990s (see Chapter 163).

While HIV-1 antibody tests may detect 60–91% of HIV-2 infections, these and HIV-1 group O (outlier group) are mainly prevented by donor deferral from high-risk areas.

Human T-cell lymphotrophic virus (HTLV)-1 and -2 infections

HTLV-1 and -2 have 60–70% sequence homology and a shared tropism for T lymphocytes. In contrast with HIV, HTLV is rarely present in cell-free plasma. Screening for HTLV-1 antibodies began in 1988. A sensitive HTLV-1/2 combination assay introduced in 1998 detects close to 100% of HTLV-2 infections, making these infections from blood transfusions very rare indeed. Prevalence of markers in screened blood has decreased recently.

Table PP38.3 The viral pathogens

Pathogen	Associated disease	Notes
Common hepatitis viruses		
HAV	Infectious hepatitis, 15- to 40-day incubation period Only 20–30% new infections are symptomatic Peak viremia occurs 2 weeks before jaundice Signs or symptoms may persist for 2 months	No chronic carrier state At risk: seronegative patients and travelers Infection prevented by donor deferral by history of acute disease, chemical evidence of hepatitis or serology
HBV	Incubation period ranges from 5 weeks to 6 months 30% of those infected are symptomatic Up to 10% of adults and 30–90% of infants develop chronic infection Acute hepatitis, vasculitis, glomerulonephritis Cirrhosis and hepatocellular carcinoma Chronic carrier state and chronic active hepatitis	Risk factors for the newly infected: • 40% infected partners • 15% MSM • 14% intravenous drug users • 1/3 no identifiable risk At risk are seronegative individuals by sexual, vertical, parenteral, transcutaneous routes Infection prevented by donor deferral by history of acute disease or chemical evidence of hepatitis
HCV	Chronic hepatitis develops in 75–85% of persons infected after 45 years of age and in 50–60% of those infected as children or young adults HCV causes hepatitis, vasculitis, rashes, cirrhosis and hepatocellular carcinoma	As HBV 3–7% of infants have vertically transmitted maternal infection from HCV Hepatocellular carcinoma yearly incidence rate 1–4% in cirrhotic patients with HBV
Other viral pathogens		
Deltavirus (HDV)	Acute on chronic (HBV) infection, fulminant disease	Parenteral, co-transmission with HBV, or superinfection of chronic carriers of HBV
CMV (HHV-5)	Mild viral illness with lymphadenopathy in the immunocompetent Pneumonitis, hepatitis, gastroenteritis, and retinitis in advanced HIV and immunosuppressed seronegative patients	Seronegative unit selection may prevent transmission Cell-depleted blood components (plasma, cryoprecipitate) do not transmit CMV; leukocyte reduction by filtration has a similar degree of protection Platelets also have a low risk of transmission
Neonatal CMV	Severe CMV disease including hemolytic anemia	Premature infants (<1200 g) born to CMV-seronegative mothers
Congenital CMV	Congenital malformation, jaundice, hepatosplenomegaly, microencephalopathy, thrombocytopenia Occasional severe malformation with high mortality rates	Fetuses of seronegative mothers
EBV (HHV-4)	Infectious mononucleosis associated with Burkitt's lymphoma, nasopharyngeal carcinoma and post-transplantation lymphoproliferative disease	Seronegative individuals
KSHV (HHV-8)	Kaposi's sarcoma, body cavity-based lymphoma and Castleman's disease	Seronegative individuals
HIV	These and other regionally limited variants such as HIV-2 and HIV-1 group O cause AIDS	Blood donors are deferred if they had risk exposures to HIV-1 group O in Central and West Africa Specific HIV-2 antibody test kits detect >99% of HIV-2
HTLV-1	Adult T-cell leukemia, lymphoma, tropical spastic paraparesis, chronic arthritis Mild immunosuppression opportunistic infections	Regional exposures (Southern Japan, Caribbean)
HTLV-2	As per HTLV-1 but no hematologic malignancy	HTLV-2 epidemic in intravenous drug users in the Americas and Europe in the past 50 years Parenteral exposures, and sexual and vertical transmission
Parvovirus B19	Fifth disease, a childhood exanthem Hypoplastic/aplastic crises and/or chronic severe anemia secondary to persistent infection	Patients with immunosuppression with HIV or hematologic malignancies or RBC abnormalities with shortened RBC lifespan; sickle cell anemia, thalassemia
Congenital	Fetal loss, malformation or congenital illness	Seronegative pregnant patients

CMV, cytomegalovirus; EBV, Epstein–Barr virus; HAV, hepatitis A virus; HBV, hepatitis B virus; HCV, hepatitis C virus; HDV, hepatitis D virus; HHV, human herpesvirus; HTLV, human T-cell lymphotrophic virus; KSHV, Kaposi's sarcoma-associated herpes virus; MSM, men who have sex with men.

Human herpesviruses (HHV)

These enveloped, double-stranded DNA viruses are associated with lifelong carrier states and reactivation. Herpes simplex and varicella-zoster viruses are rarely, if ever, transmitted by transfusion. Cytomegalovirus, Epstein–Barr virus (EBV) and HHV-8, widely prevalent in the adult population, may be transmitted in blood products and affect the seronegative young or immunocompromised.

Cytomegalovirus (CMV)

Anti-CMV antibodies are present in up to 50% of donors. Seronegative recipients of unscreened cellular blood components have an overall relative risk of approximately 1% of primary CMV infection. This ranges from zero to 50% of infants and 30% of bone marrow transplant recipients. In solid-organ transplant recipients, the risk of CMV by the transplanted seropositive organs overshadows the relatively smaller risk posed by transfusions. Second-strain infections remain theoretically possible even in seropositive recipients.

Other herpesviruses

About 90% of the adult population is EBV seropositive. Infection is deterred by host virus-specific cytotoxic T lymphocytes and transfusion-associated EBV infection is rare. HHV-8 infects B-lymphocytes and monocyte/macrophage cell types. Data about HH-8 infection risk from transfusion are sparse, but in a recent study of 991 transfusion recipients in Kampala, Uganda, 425 recipients received a blood product from a single seropositive donor. HHV-8 seroconversion occurred in 41 recipients.

Parvovirus B19

Parvovirus is present in 1/50 000 to 1/20 000 donors, with surges during epidemics. About 50% of the adult population has antibodies indicating exposure and immunity to this erythrotropic virus. Prospective studies have demonstrated a persistent 40% infection risk in untreated hemophiliacs who received virus-attenuated clotting factor concentrates. Either donor or recipient antibodies may prevent infection, however, as these antibodies are ineffective in large plasma pools and albumin preparations.

This virus is non-enveloped and hence resistant to most physicochemical sterilization methods. Exclusion of high viral load source plasma by NAT, and subsequent novel heating methods and nanofiltration may make these transfusion products safer. Fortunately, infection in most recipients is of little consequence.

West Nile virus (see Chapter 19)

In the 2002 West Nile virus (WNV) US epidemic, 23 infections were transmitted by blood components. Predictably, outcomes were severe in the elderly and the immunocompromised. Meningoencephalitis developed in 12 patients, and several patients died. In acute infection, viremia often lasts up to 2 weeks, making transfusion-related transmission possible. Due to swift policies implemented for product quarantine and improved NAT screening, the outbreak was contained. Potential arboviral epidemics with pathogens such as dengue may be halted by similar policies.

BACTERIAL CONTAMINATION OF BLOOD PRODUCTS

This is the second most frequent cause of transfusion-related fatalities reported to the US Food and Drug Administration (FDA) (Table PP38.4). Bacterial proliferation is more efficient in platelet concentrates typically stored at room temperature for up to 5 days than in RBCs stored for 42 days because they are refrigerated at lower temperatures. Bacteria enter blood products due to:

- inadequate disinfection during phlebotomy;
- transient bacteremia during donation;
- breaks in technique during pooling or sealing; or
- disruption of container integrity.

Table PP38.4 Bacterial contaminants and associated blood products

Contaminating pathogens	Comments
Yersinia enterocolitica *Pseudomonas fluorescens* *Staphylococcus epidermidis* *Serratia liquefaciens*	Red blood cell transfusions: *Y. enterocolitica* accounts for about 75% of all reported cases of transfusion of contaminated red blood cells Siderophoric growth enhanced by storage >25 days in cold storage (4°C)
Coagulase-negative staphylococci *Salmonella choleraesuis* *Escherichia coli* *Serratia marcescens,* *S. liquefaciens* *Bacillus* spp. *Enterobacter cloacae* *Brucella*	Platelet transfusions (1 in 4200): storage at room temperature for 4–5 days enhances growth of these organisms, mostly skin saprophytes 85% of fatal reactions are caused by Gram-negative organisms; predominant mechanism is endotoxic shock Prevalence: most common bacteria are 25% coagulase-negative staphylococci, 13.5% *Salmonella* spp.
Yersinia spp.	Desferrioxamine: *Yersinia* spp. are siderophoric (iron-loving) organisms and iron uptake is essential for growth Iron overload conditions have an increased risk of invasive yersiniosis

PARASITES

Malaria is not transmitted by RBC-free components. Interestingly, these tropical parasites remain viable both in platelet concentrates stored at room temperature and in RBCs stored at 4°C. They also survive cryopreservation and thawing. Post-transfusion incubation period is 7–50 days. Babesiosis is a malaria-like, tick-borne zoonosis (Table PP38.5). No screening test currently exists to detect asymptomatic carriers of these parasites. Donor deferral by history of residence in endemic areas prevents most transmission.

Approximately 20–25% of the US population is seropositive for *Toxoplasma gondii*, and screening for antibodies prevents transmission.

Visceral leishmaniasis is reported in 1990 Persian and 2003 Iraq war veterans. *Leishmania tropica* survives for at least 25 days in blood stored under standard conditions. Transfusion-transmitted *L. donovani* and *L. infantum* infection has been reported. Deferral policies from blood donation should prevent transmission from Gulf War veteran blood donation.

Chagas disease is widespread in Latin America. Low-level *Trypanosoma cruzi* parasitemia usually persists for life and may be transmitted by immigrants from endemic regions. Treatment is only effective during the acute stage. Of seven cases in immunocompromised patients reported in the US since 1989, five were due to platelet products. *T. cruzi* remains viable in refrigerated blood for 18 days

Table PP38.5 Parasitic diseases

Parasite	Causative organism and presenting symptoms	People at risk and risk reduction
Malaria	*Plasmodium falciparum*, *P. vivax*, *P. ovale* and *P. malariae* Chills, fever, splenomegaly, fatigue, nausea, vomiting, headache and diarrhea	Blood donation deferral of travelers, military personnel and immigrants from endemic countries
Babesiosis	*Babesia microti* in North America, *Babesia divergens* in Europe Usually asymptomatic or mild flu-like symptoms	Severe disease in the immunocompromised, asplenic patients and the elderly Risk reduced by preventive measures against tick bites during outdoor activity
Toxoplasmosis	*Toxoplasma gondii* causes transfusion-related infection, usually related to whole blood or WBC transfusion	May cause serious disease in the immunocompromised Risk reduced via serologic screening
Chagas disease	*Trypanosoma cruzi* infection causes an acute phase illness varying from no symptoms to fever, skin rash, conjunctivitis and hepatosplenomegaly A chronic phase occurs in up to 30–40% of infected patients and can be transmitted by blood donation	Screening donors by history of travel or residence, or antibody testing is not routine. Pathogen reduction with psoralens or other agents and blood filtration offer alternative approaches One-time serologic testing of donors with risk factors is a possible alternative to testing each donation
There are few reliable serologic tests for screening, and prevention is mainly based on donor deferral from endemic regions.		

and for more than 8 months in citrated samples at room temperature, and survives freezing and thawing. In South America where up to 49% of recipients of parasitemic blood become infected, 3–4% of donors with risk factors (see Table PP38.5) had *T. cruzi* antibodies. A study in California reported that 1 of 340 donors had a risk factor for Chagas disease but transmission risks are unclear. The American Red Cross tested nearly 149 000 blood donations between August 2006 and January 2007, with a *T. cruzi* enzyme-linked immunosorbent assay (ELISA) system. Sixty-three specimens from 61 donors (roughly 1 in 2365 donations) were positive. Based on early study analysis, the FDA has licensed this assay in the USA for blood donor screening.

CREUTZFELDT–JAKOB DISEASE

All forms of Creutzfeldt–Jakob disease (CJD) are transmissible by transplanted organ tissues including sporadic CJD. While classic CJD may not be transmitted by transfusion, the variant from of CJD behaves differently. A bovine spongiform encephalopathy (BSE) epidemic affecting 250 000 cattle occurred in the UK between 1980 and 1996 and was passed on to 145 patients who developed vCJD by consumption of tainted beef (see Chapter 22). Fifteen of the patients had donated blood before the diagnosis; two of the 48 recipients of these blood components have developed vCJD. Conventional pathogen reduction methods do not affect the transmission of vCJD.

Following discovery of additional vCJD in continental Europe, a policy initially implemented in 1999 for deferring donors from the UK was recently revised to include donors who spent more than 5 years in Europe between 1980 and 1996.

CONCLUSIONS

The risk of transfusion-transmitted infection is at an all time low in developed nations. Newer pathogen reduction methods promise further protection at an escalating cost. Decision making regarding blood screening policies must be based on accurate estimates of the incremental safety benefit, balanced with a need for affordability. Finally, in the era of global migration, it is critical to ensure overall safety of blood products by directing resources to help developing nations establish sustainable blood collection, processing and transfusion systems.

FURTHER READING

Further reading for this chapter can be found online at http://www.expertconsult.com

Practice point **39**

Kari Simonsen

Kawasaki disease

INTRODUCTION

Kawasaki disease is the most common cause of acquired heart disease in children living in the developed world. First described by Dr Tomisaku Kawasaki in Japan in 1967, the illness presents as an acute, self-limited, febrile vasculitis. It is not clear whether this entity newly emerged around the time of the initial report, or whether it was unrecognized as a distinct syndrome from common childhood febrile exanthems such as measles and scarlet fever prior to the era of vaccination and antibiotics. Although a host of potential bacterial and viral pathogens have been examined as the single causative agent for Kawasaki disease, the etiology of this important illness remains unknown.

EPIDEMIOLOGY

Kawasaki disease is a pediatric illness and over 80% of cases occur in patients under the age of 5 years, with nearly all cases occurring by the age of 8 years. The diagnosis is rare and may be delayed in older children and adults. A meta-analysis from 2005 described a total of 57 published reports of adult Kawasaki disease. Kawasaki disease has a male to female ratio of 1.5:1 and occurs in all racial and ethnic groups. Reported incidence rates vary widely from 3 per 100 000 in South America to 134 per 100 000 in Japan which reports the highest annual incidence.

After intensive search spanning four decades, a single etiology has not yet been identified as leading to Kawasaki disease, but many epidemiologic features suggest an infectious cause. The age distribution supports this hypothesis as Kawasaki disease is rare in infants under 3 months of age who might be protected by passively acquired maternal antibodies and rare in adults which suggests widespread immunity. Also, Kawasaki disease has a winter/spring seasonal predominance in temperate climates and has been associated with epidemics. The organisms listed in the differential diagnosis (Table PP39.1) are among those that have been studied as potential causative agents, but have not been found to be indisputably associated with the illness.

It is likely that a common exposure precipitates the syndrome only in genetically predisposed individuals. Whether that exposure is to a new, unidentified agent or is a response to bacterial toxins acting as superantigens continues to be widely debated. Some investigators have suggested that multiple causes may lead to the final common pathway of the clinical syndrome.

CLINICAL MANIFESTATIONS (FIG. PP39.1)

Kawasaki disease is a triphasic illness. The clinical signs and symptoms evolve over an acute febrile phase of 7–14 days then gradually spontaneously resolve even in the absence of directed therapy. Coronary artery aneurysms are the principal sequelae of the disease, and occur in 20–25% of untreated patients and in <5% of patients who receive appropriate therapy with intravenous immunoglobulin (IVIG).

There is no diagnostic test for Kawasaki disease, and diagnosis in the acute phase is based on the presence of fever for at least 5 days, and four of the five classic clinical findings. Other associated physical and laboratory characteristics may be supportive (Table PP39.2). The subacute phase occurs from about 10 to 25 days after the initial febrile period, and is marked by periungual desquamation, arthritis and thrombocytosis. The convalescent phase continues until all signs of illness have resolved and the erythrocyte sedimentation rate (ESR) has normalized, typically 6–8 weeks in total.

Adult patients with Kawasaki disease have been noted to present with similar findings, but arthralgia, lymphadenopathy and elevated liver enzymes occurred more commonly in adults, and cheilitis, meningitis and thrombocytosis were seen less often than in pediatric cases.

Not all patients present with four of the five classic features of Kawasaki disease, and these presentations are termed 'incomplete' Kawasaki disease. Incomplete presentations are most common in infants.

Table PP39.1 Differential diagnosis of Kawasaki disease

Viruses	Measles Echovirus Adenovirus Epstein–Barr virus
Bacteria	*Staphylococcus aureus* *Streptococcus pyogenes* *Rickettsia rickettsii* *Leptospira interrogans*
Drug reactions	Serum sickness Stevens–Johnson syndrome
Rheumatologic disease	Systemic-onset juvenile idiopathic arthritis
Heavy metal toxicity	Mercury hypersensitivity (acrodynia)

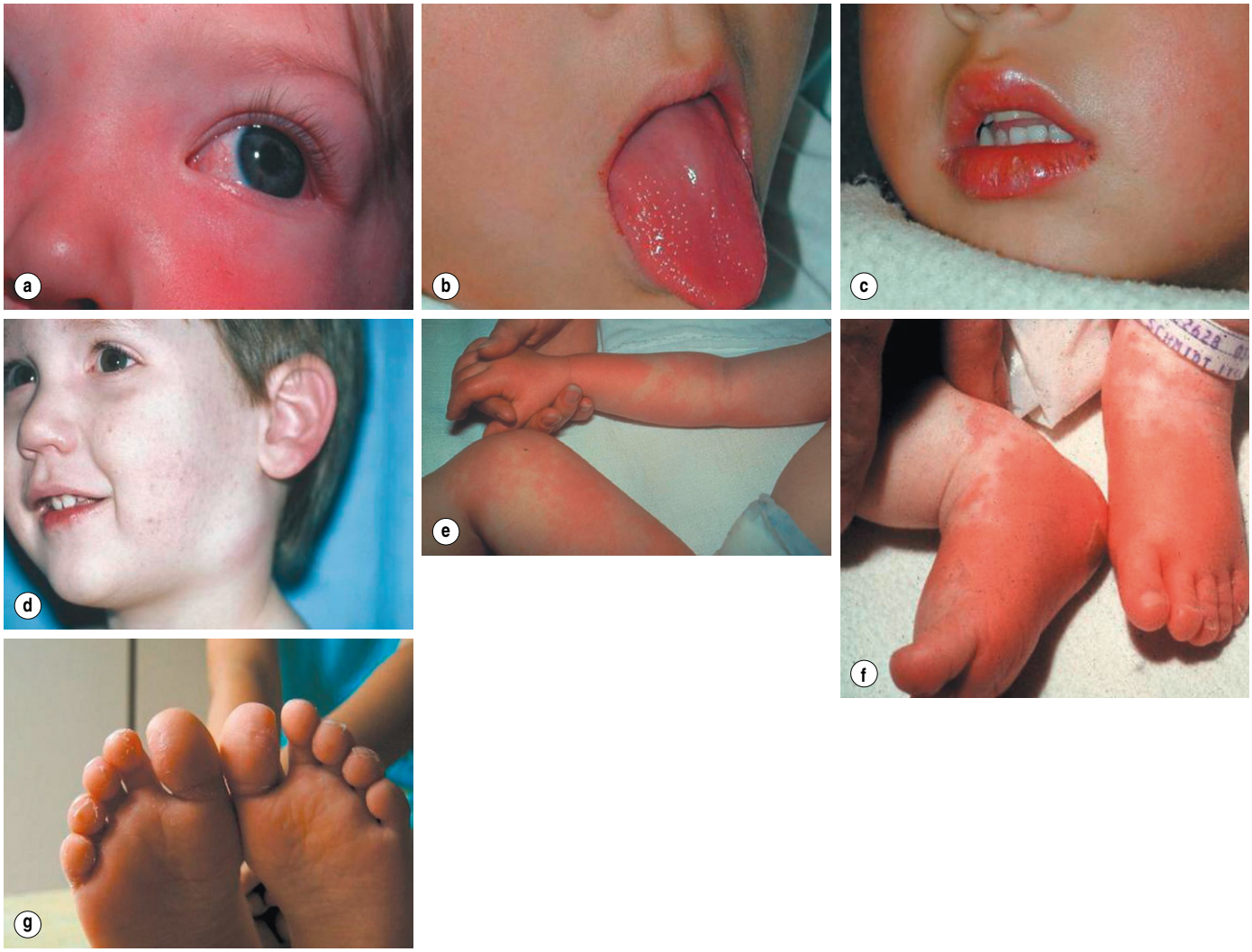


Fig. PP39.1 Features of Kawasaki syndrome. (a) Bilateral, nonexudative conjunctival injection with perilimbal sparing. (b) Strawberry tongue with loss of filiform papillae and persistence of fungiform papillae ('seeds' of strawberry). (c) Erythematous, fissured lips. (d) Unilateral enlarged left jugulodigastric nodes. (e) Erythematous rash. (f) Erythema of soles, swelling of dorsa of feet. (g) Periungual desquamation of toes in convalescent phase. Reproduced with permission from Burns & Glodé (2004). Reprinted from the Lancet. Copyright Elsevier 2004.

Although these patients do not fulfill the complete clinical criteria for the diagnosis of Kawasaki disease they do remain at risk for cardiovascular sequelae, and the syndrome must always remain a diagnostic consideration for children with prolonged, unexplained fever.

MANAGEMENT

IVIG is the mainstay of therapy, and is given as a single infusion of 2 g/kg. Aspirin is given adjunctively for its anti-inflammatory effects in the acute phase and antiplatelet effects in the subacute and convalescent phases of illness. The dosing for aspirin in the acute phase ranges from 30 to 100 mg/kg/day in four divided doses. The American Academy of Pediatrics/American Heart Association (AAP/AHA) guidelines suggest 80–100 mg/kg/day for 14 days or until the patient has been afebrile for 48–72 hours. This is followed by low-dose aspirin (3–5 mg/kg/d), typically for 6–8 weeks or until after convalescent echocardiography documents the absence of ongoing cardiac involvement and laboratory evidence of inflammation has resolved.

Despite their usefulness in other forms of vasculitis, corticosteroids have not been consistently shown to improve the outcomes of patients treated for Kawasaki disease and are not recommended as standard therapy.

There is clear evidence that delays in diagnosis and treatment contribute to the development of coronary artery aneurysms, and prompt treatment within the first 10 days of fever is recommended. In fact, United States and Japanese experts agree that treatment may commence after only 4 days of fever when the classic criteria are met. Importantly, treatment is still recommended for patients with evidence of coronary artery involvement or ongoing acute inflammation who present after 10 days of fever.

Most patients will respond with rapid clinical improvement and defervescence after IVIG infusion, but about 10% will have persistent or recrudescent fevers. These patients are at increased risk of coronary artery aneurysms. Although there is no clear evidence to establish the efficacy of further treatments, most experts recommend a second infusion of 2 g/kg IVIG for patients who remain febrile 36–48 hours after their initial infusion. Additional treatment approaches have been suggested for refractory cases including corticosteroids, plasma exchange,

Table PP39.2 Clinical and laboratory features of Kawasaki disease

Classic diagnostic criteria	Fever for at least 5 days *and* at least four of the following five physical findings: • polymorphous exanthem • conjunctival injection without exudate • oropharyngeal erythema, lips cracking, 'strawberry tongue' • extremity changes including erythema, edema and induration of hands and feet, periungual desquamation (subacute) • cervical lymphadenopathy >1.5 cm, typically unilateral
Other associated clinical findings	Cardiovascular: coronary artery ectasia or aneurysms, pericarditis, myocarditis, valvular regurgitation, aneurysms of noncoronary arteries CNS: aseptic meningitis, extreme irritability Gastrointestinal: hepatitis, diarrhea, gallbladder hydrops Genitourinary: urethritis, hydrocele Musculoskeletal: arthritis, arthralgia Dermatologic: erythema and induration at site of previous bacille Calmette–Guérin (BCG) vaccination, erythema with subsequent desquamation of groin Ocular: anterior uveitis
Associated laboratory findings	Blood findings: • elevated erythrocyte sedimentation rate, C-reactive protein • anemia (normocytic, normochromic) • leukocytosis with neutrophilia and band forms • thrombocytosis (subacute phase) • hypoalbuminemia • elevated serum transaminases Urine findings: sterile pyuria CSF findings: neutrophilic pleocytosis Synovial findings: neutrophilic leukocytosis

abciximab, infliximab, ulinastatin and cytotoxic agents, but their utility remains uncertain due to a paucity of controlled data.

LONG-TERM CARE

Cardiac follow-up for Kawasaki disease patients is individualized based on severity of coronary involvement. Coronary artery aneurysms will resolve in about 50% of patients, but functional and histologic changes in these affected segments remain. For patients with persistent aneurysms, progressive stenosis can lead to occlusion and myocardial infarction. Aneurysm rupture occurs rarely. Antithrombotic therapy is continued for patients with persistent coronary abnormalities.

It is necessary to defer any live-viral vaccines for 11 months after the infusion of IVIG, but other childhood immunizations need not be delayed.

FURTHER READING

Further reading for this chapter can be found online at http://www.expertconsult.com

Chapter 72

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Immunodeficiencies

INTRODUCTION

White blood cells (WBC) can be easily classified into lymphoid (T, B and natural killer (NK) cells) and myeloid (neutrophils, eosinophils, basophils and monocytes/macrophages) by virtue of their lineage-restricted progenitor's origin. Several recent, comprehensive reviews on the role of innate and adaptive immunity in host defense against microbial invasion are now available and interested readers are referred to these publications[1–3] and to Chapters 2 and 88 of this textbook. Defects in these cells lead to defects in their respective pathways of host defense, and specific and reproducible patterns of infection susceptibility. Therefore, the study of mechanisms of resistance to infection is best and most precisely approached through the study of monogenic immunodeficiencies.

MYELOID CELLS AND DEFECTS

Mature neutrophils develop in the bone marrow from a myeloid stem cell over about 14 days, but spend only 6–10 hours in the bloodstream before exiting by diapedesis to sites of inflammation. Myeloid disorders can be generally divided into those of number and those of function. Neutrophilia (>7500 neutrophils/μl in adults) is typically dependent on causes extrinsic to the neutrophils, such as acute or chronic infection, steroids and adrenaline (epinephrine). On the other hand, neutropenia (mild: <1500 neutrophils/μl, moderate: 1500–1000 neutrophils/μl, severe: <500 neutrophils/μl) can be intrinsic or extrinsic to neutrophils or their progenitors. Although neutropenia can accompany many immunodeficiencies, the most common causes are drug induced (e.g. chemotherapy, drug toxicity).

Myeloid disorders should be suspected in patients who have recurrent, severe, bacterial or fungal infections. Unusual organisms (e.g. *Burkholderia cepacia*, *Chromobacterium violaceum*) or uncommon locations (e.g. liver abscess) should always prompt questions about neutrophil integrity (Table 72.1). Severe viral and parasitic infections are not typically increased in these patients and should direct attention to disorders involving lymphocytes or monocytes. Laboratory evaluation should be guided by the clinical presentation and patterns of infection. Some assays, such as repeated WBC counts with differentials or microscopic evaluation of neutrophils, are relatively simple and can readily identify or exclude certain disorders. Assays such as oxidative burst testing, phagocytosis, chemotaxis or flow cytometry are more specialized, and few laboratories do them routinely.

SEVERE CONGENITAL NEUTROPENIA

Severe congenital neutropenia (SCN) (Online Mendelian Inheritance in Man (OMIM) #202700; see http://www.ncbi.nlm.nih.gov/omim) comprises a heterogeneous group of disorders with variable inheritance patterns that share bone marrow maturation arrest at the promyelocyte or myelocyte stage and severe chronic neutropenia (<200 neutrophils/μl). Some are associated with an increased susceptibility to acute myeloid leukemia. In 1956, Kostmann described a Swedish kindred with severe congenital neutropenia inherited in an autosomal recessive pattern.[4] However, the majority of cases of severe congenital neutropenia are sporadic or autosomal dominant due to heterozygous mutations in neutrophil elastase (*ELA2*, located at 19p13.3). Surprisingly, mutations in this same gene also cause cyclic neutropenia.[5]

The clinical manifestations of SCN appear promptly after birth: 50% are symptomatic in the first month and 90% within 6 months. Omphalitis, upper and lower respiratory tract infections, and skin and liver abscesses are common. Subcutaneous granulocyte colony-stimulating factor (G-CSF; 5 μg/kg per day; range 1–120 μg/kg depending on patient response) has dramatically reduced infections and hospitalizations and increased survival. Somatic mutations in the G-CSF receptor occur in some patients with elastase mutations and are associated with myeloid leukemias. The etiology of these mutations is unclear, but they are acquired and not germline. An elastase interacting protein, growth factor independent 1 (GFI-1, OMIM *600871), acts as a repressor of elastase production. Dominant GFI-1 mutations that block elastase repression are associated with neutropenia and abnormal lymphocyte function.[6]

An X-linked form of severe congenital neutropenia (XLN) due to discrete mutations in the Wiskott–Aldrich syndrome protein (WASP) has also been identified.[7]

CYCLIC NEUTROPENIA/CYCLIC HEMATOPOIESIS

Cyclic neutropenia (OMIM #162800) is an autosomal dominant disease characterized by regular cyclic fluctuations in all hematopoietic lineages, but symptoms are only due to reduced neutrophil counts. Neutrophil counts cycle about every 21 days (range 14–36 days); severe neutropenia (<200/μl) lasts 3–10 days.[5] Most patients become symptomatic in early childhood with aphthous oral ulcers, gingivitis, lymphadenopathy, pharyngitis, tonsillitis or skin lesions. Permanent teeth may be lost due to chronic gingivitis and periapical abscesses. Bone marrow aspirates during neutropenia show maturation arrest

Table 72.1 Infecting agents and myeloid defects

Disease or syndrome	Defect	Characteristic infections
Chediak–Higashi syndrome	CHS1/LYST	*Staphylococcus aureus* *Streptococcus pneumoniae* Other streptococcal infections *Haemophilus influenzae* Gram-negative rods (skin and lung)
Specific granule deficiency	cEBPe/others	*Staph. aureus* *Strep. pneumoniae* Other streptococcal infections *H. influenzae* Gram-negative rods (skin and lung)
Myeloperoxidase deficiency	MPO	*Candida* (when accompanied by diabetes mellitus)
Leukocyte adhesion deficiency 1	CD18	*Staph. aureus* *Pseudomonas aeruginosa* Gram-negative rods (skin and bowel)
Chronic granulomatous disease	NADPH oxidase	*Staph. aureus* (skin, liver, lymph nodes) *Serratia marcescens* (lung, skin, bone, sepsis) *Burkholderia cepacia* (lung, sepsis) *Chromobacterium violaceum* (skin, sepsis) *Nocardia* spp. (lung) *Aspergillus* spp. (other filamentous fungi; lung, bone)
Hyper-IgE syndrome (Job's)	STAT3	Primary pathogens: *S. aureus* (lung and skin) *Strep. pneumoniae* (lung) *H. influenzae* (lung) *Pneumocystis jirovecii* (lung) Secondary pathogens in lung cavities: *P. aeruginosa* *Aspergillus* spp.
Interferon-γ/IL-12 pathway	Multiple	Nontuberculous mycobacteria *Salmonella* spp. *Mycobacterium tuberculosis* Some DNA and RNA viruses

at the myelocyte stage or, less frequently, bone marrow hypoplasia. Granulocyte colony-stimulating factor lifts counts in cyclic neutropenia, dramatically improving quality of life and survival in these patients. Interestingly, infections and hospitalizations tend to lessen with age.

KOSTMANN SYNDROME

In 1956, Kostmann described a Swedish kindred with severe congenital neutropenia inherited in an autosomal recessive pattern.[4] Although the literature has been very confusing about whether Kostmann syndrome is caused by *ELA2*, and there has been a tendency in the past to use SCN and Kostmann syndrome interchangeably, the Kostmann gene has now been identified in the original family and in a large Kurdish cohort as being due to recessive mutations in *HAX-1* (1q21.3), the function of which is anti-apoptotic.[8]

IMMUNE-MEDIATED NEUTROPENIAS

Alloimmune neonatal neutropenia

Alloimmune neonatal neutropenia (ANN) is caused by the transplacental transfer of maternal antibodies against NA1 and NA2, two isotypes of the immunoglobulin receptor FcγRIIIb, leading to immune destruction of neonatal neutrophils.[9] This problem typically arises in otherwise normal children of apparently normal healthy mothers. The mothers do not express FcγRIIIb on their own neutrophils, leading to the elaboration of antibodies against FcγRIIIb expressed on fetal neutrophils to which the mother is sensitized during pregnancy. Antibody-coated neutrophils are phagocytosed and removed from the circulation, leading to neutropenia and infections. These antineutrophil antibodies can be detected in 1 in 500 live births and should be sought in all infants with neutropenia. Omphalitis, cellulitis and pneumonia within the first 2 weeks of life may be the presenting infections. Detection of neutrophil-specific alloantibodies in maternal serum is diagnostic. Parenteral antibiotics and G-CSF should be given; intravenous gamma globulin may not be effective in reversing ANN. With the normal waning of maternal antibody levels in the infant, ANN resolves spontaneously.

Primary autoimmune neutropenia

Primary autoimmune neutropenia (AIN) is the most common cause of chronic neutropenia (absolute neutrophil count <1500/μl lasting at least 6 months) in infancy and childhood.[10] It has a slight female preponderance and occurs in about 1:100 000 live births. Antibodies directed against different neutrophil antigens can be detected in almost all patients, almost 85% of which are IgG. Detection of granulocyte-specific antibodies may require repeated testing. Approximately one-third of these autoantibodies are directed against NA1 and NA2. Other antigens include CD11b/CD18 (Mac-1), CD32 (FcγRII) and CD35 (C3b complement receptor).

The average age at diagnosis is 8 months. The majority of patients present with skin or upper respiratory infections, but the diagnosis may be incidental, as patients may remain asymptomatic despite low neutrophil counts; severe infections are infrequent. Neutrophil counts are usually between 500 and 1500/μl at the time of diagnosis. The neutrophil count may transiently increase during severe infections and bone marrow may be normal or hypercellular. The prognosis of primary AIN is good, since it is usually self-limited. The neutropenia remits spontaneously within 7–24 months in 95% of patients, preceded by the disappearance of autoantibodies. Antibiotics for infections are usually sufficient. In severe infections or for emergency surgery G-CSF is used.

Secondary autoimmune neutropenia

Secondary AIN can be seen at any age but is more common in adults and has a more variable clinical course. Systemic lupus erythematosus, Hodgkin's disease, large granular lymphocyte proliferation or leukemia, Epstein–Barr virus (EBV) infection, cytomegalovirus (CMV) infection, HIV infection and parvovirus B19 infection have been associated with secondary AIN. Antineutrophil antibodies typically have pan-FcγRIII specificity, rather than to the FcγRIII subunits, making the resulting neutropenia more severe. Anti-CD18/11b antibodies have been detected in a subset of patients. Secondary AIN responds best to therapy directed at the underlying cause.

DEFECTS OF GRANULE FORMATION AND CONTENT

Chediak–Higashi syndrome

Neutrophil granules house critical enzymes for bacterial and fungal killing, and are mobilized to the phagosome immediately after ingestion of an invader (Fig. 72.1). This intracellular trafficking requires molecular motors which move granules inside the cell. The Chediak–Higashi syndrome (CHS) is a rare and life-threatening autosomal recessive disease, clinically characterized by oculocutaneous albinism, frequent pyogenic infections, neurologic abnormalities and a relatively late onset lymphoma-like 'accelerated phase'.[11] Affected patients show hypopigmentation of the skin, iris and hair. The latter is light brown to blonde, with a characteristic metallic silver-gray sheen. Under light microscopy, CHS hair shafts show pathognomonic small aggregates of clumped pigment that are disorderly spaced, instead of the central shaft distribution in normals. Giant azurophilic granules form from the fusion of multiple primary granules in neutrophils, eosinophils and basophils, but enlarged cytoplasmic granules are found in all granule-containing cells. Mild neutropenia is common and due to intramedullary destruction of neutrophils.

Chediak–Higashi syndrome is due to mutations in the lysosomal trafficking regulator gene, *LYST* or *CHS1* (1q42.1–q42.2; OMIM #214500),[11] but the mechanism and pathophysiology of CHS are still elusive. Monocyte and neutrophil chemotaxis are diminished. Phagocytosis is normal or increased, but bacterial killing is delayed, probably due to low levels of primary granule enzymes. Natural killer (NK) cells show very low cytotoxicity, but neutrophil and monocyte antibody-dependent cellular cytotoxicity is intact. B-cell function is usually unaffected. Progressive degeneration of the peripheral and central nervous systems is common, with neuropathy of the legs, cranial nerve palsies, seizures, mental retardation and autonomic dysfunction.

The 'accelerated phase' is one of the main causes of death in CHS and is clinically indistinguishable from other hemophagocytic syndromes. It is characterized by fever, hepatosplenomegaly, lymphadenopathy, cytopenias, hypertriglyceridemia, hypofibrinogenemia, hemophagocytosis and tissue lymphohistiocytic infiltration. Etoposide (VP16), steroids and intrathecal methotrexate have been effective. However, without successful bone marrow transplantation, the accelerated phase usually recurs. Unfortunately, although bone marrow transplantation cures the immune defect and the accelerated phase in CHS, it does not prevent the central or peripheral neurologic problems.[12]

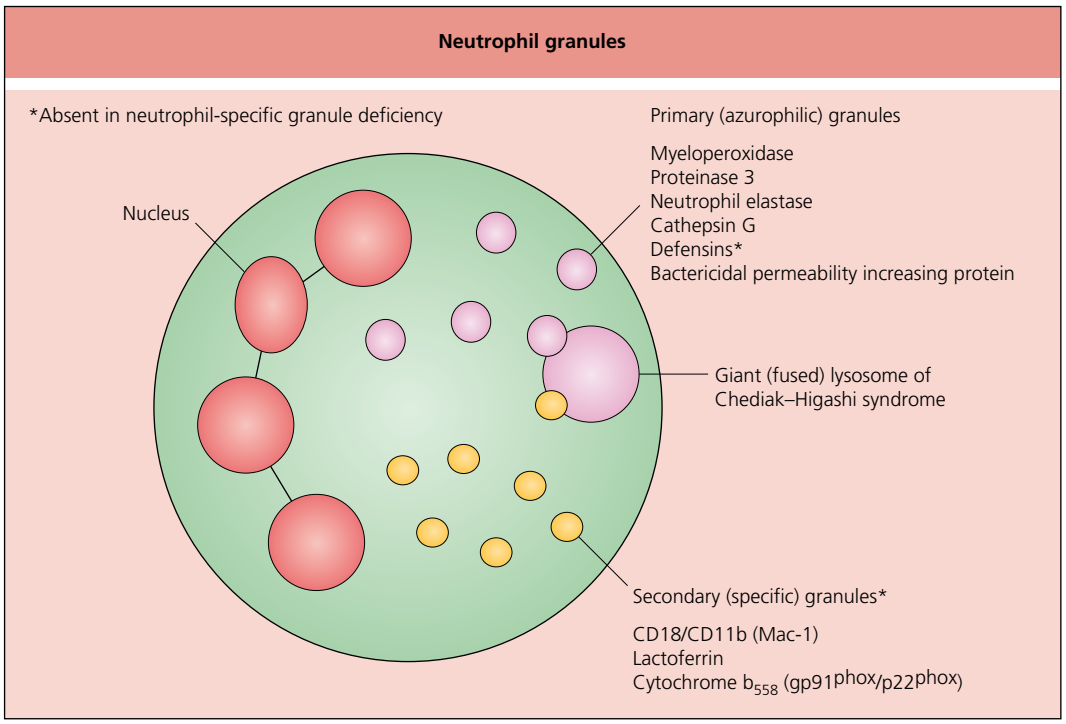


Fig. 72.1 Neutrophil granules. Neutrophils contain primary, secondary and tertiary granules, each of which has specific contents that are produced at different points in myeloid ontogeny. The larger, azurophilic primary granules contain a host of proteins, only several of which are listed here. Note that secondary granule deficiency affects both all secondary granule contents as well as the primary granule defensins. Primary granules fuse in Chediak–Higashi syndrome, along with a smaller number of secondary granules to give the characteristic cell inclusions.

Neutrophil-specific granule deficiency

The CCAAT/enhancer binding proteins (C/EBPs) are transcription factors that guide myelopoiesis and cellular differentiation. Neutrophil-specific granule deficiency (OMIM #245480) is a rare, heterogeneous, autosomal recessive disease characterized by the profound reduction or absence of neutrophil-specific granules and their contents, as well as the primary granule product, defensins. In several cases a homozygous recessive mutation was found in C/EBPε (14q11.2).[13] However, other cases do not have mutations in C/EBPε, suggesting genetic heterogeneity. These patients have markedly increased susceptibility to pyogenic infections of the skin, ears, lungs and lymph nodes. Neutrophils show bilobed nuclei (pseudo-Pelger–Huët anomaly). Electron microscopy shows absent peroxidase-negative granules in some patients and empty peroxidase-negative granules in others. Staphylococcidal activity may be reduced due to poor phagocytosis, but candidacidal activity and superoxide production are normal. Hemostatic abnormalities due to reduced levels of platelet-associated high molecular-weight von Willebrand factor and platelet fibrinogen and fibronectin, may occur.[13]

The diagnosis of specific granule deficiency is suggested by recurrent infections with Gram-positive cocci and Gram-negative rods particularly involving the skin. The peripheral smear shows large hypogranulated neutrophils and is confirmed by electron microscopy and specific granule enzyme detection by ELISA (e.g. lactoferrin or specific defensins). Eosinophils may not be detectable on routine smears because of the lack of specific granules. Management is complicated by poor inflammatory responses. Aggressive diagnosis of infections, prolonged therapy and early use of surgical excision are necessary. Bone marrow transplantation is appropriate to consider early.

DEFECTS OF OXIDATIVE METABOLISM

Chronic granulomatous disease

Nicotinamide-adenine dinucleotide diphosphate (NADPH) oxidation is the main process by which superoxide and its metabolites hydrogen peroxide and bleach are generated (Fig. 72.2). The nascent NADPH oxidase enzyme complex exists as two groups of components: a heterodimeric membrane-bound complex (cytochrome b_{558}) embedded in the walls of secondary granules and four distinct cytosolic proteins.[14] The structural components are referred to as *phox* proteins for phagocyte oxidase. Cytochrome b_{558} is composed of a 91 kDa glycosylated β chain ($gp91^{phox}$) and a 22 kDa nonglycosylated α chain ($p22^{phox}$). When bound together, these two proteins span the membrane and bind heme and flavin on the cytosolic side. The cytosol contains the structural components $p47^{phox}$, $p67^{phox}$ and the regulatory components $p40^{phox}$ and rac. On cellular activation the cytosolic components $p47^{phox}$ and $p67^{phox}$ are phosphorylated and bind tightly together. In association with $p40^{phox}$ and rac, these proteins combine with the cytochrome complex ($gp91^{phox}$ and $p22^{phox}$) to form the intact NADPH oxidase. Following assembly, an electron is taken from NADPH and donated to molecular oxygen, leading to the formation of superoxide. In the presence of superoxide dismutase, this is converted to hydrogen peroxide, which, in the presence of myeloperoxidase and chlorine in the neutrophil phagosome, is converted to hypochlorous acid (bleach). Phagocyte production of reactive oxygen species facilitates activation of the primary granule proteins neutrophil elastase and cathepsin G inside the phagocytic vacuole. This paradigm for NADPH oxidase-mediated microbial killing suggests that reactive oxidants are most critical as intracellular activation and signaling molecules.

Mutations in any of the four structural components of the NADPH oxidase cause chronic granulomatous disease (CGD), a disease characterized by recurrent life-threatening infections due to catalase-positive bacteria and fungi, and exuberant granuloma formation (OMIM #306400, 233690, 233700, 233710). Mutations in the X-linked $gp91^{phox}$ account for about two-thirds of cases.[15] The rest of the cases are autosomal recessive; there are no autosomal dominant cases of CGD. The frequency of CGD in the United States may be as high as 1:100 000. Clinically, CGD is quite variable with the majority of patients presenting in early childhood, but later presentations are increasingly common.[15]

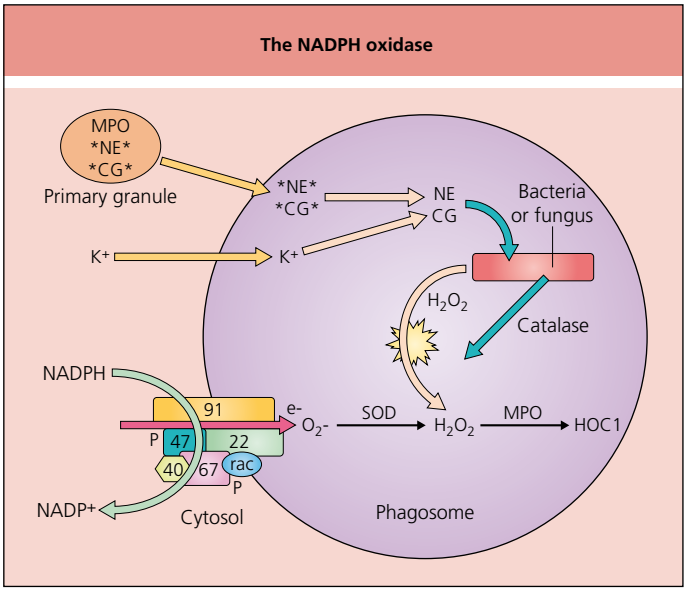


Fig. 72.2 The NADPH oxidase. Cellular activation leads to the assembly of the nascent NADPH oxidase, by joining of secondary granule membrane and cytosolic components. The generation of an intravacuolar charge is rectified by potassium influx, which in turn liberates neutrophil elastase (NE) and cathepsin G (CG) from their associated matrix (**). As classically conceived, an ingested organism is shown degrading its own hydrogen peroxide, thereby eliminating a supplement to the defective metabolic pathway in chronic granulomatous disease. However, the pathologic relevance of the role of catalase in bacterial or fungal virulence is unclear.

The lung, skin, lymph nodes and liver are the most frequent sites of infection (see Table 72.1). In North America, the overwhelming majority of infections in CGD are due to only five organisms: *Staphylococcus aureus, Burkholderia cepacia, Serratia marcescens, Nocardia* and *Aspergillus* spp. In other countries *Salmonella* and bacille Calmette–Guérin (BCG) infections are also common.[16] Trimethoprim–sulfamethoxazole (TMP–SMX) prophylaxis reduces the frequency of bacterial infections in general and staphylococcal infections in particular. Staphylococcal liver abscesses encountered in CGD are dense, caseous and difficult to drain, requiring surgery in almost all cases. With the success of antibacterial prophylaxis and therapy, fungal infections, typically those due to *Aspergillus* spp., became the leading cause of mortality in CGD.[15] Itraconazole prophylaxis and other highly active oral antifungal triazole compounds have significantly reduced fungal mortality in CGD.[17] CGD is the only primary disease in which invasive aspergillosis occurs in normal lung tissue.

The granulomatous manifestations of CGD are particularly troublesome, often involving the gastrointestinal and genitourinary tracts (Fig. 72.3). Esophageal, jejunal, ileal, cecal, rectal and perirectal involvement with granulomata mimic Crohn's disease. Gastric outlet obstruction is common and can be the initial presentations of CGD.[18] Bladder granulomata, ureteral obstruction and urinary tract infection are also common. Steroid therapy (e.g. prednisone 1 mg/kg/day then tapered to a low dose on alternate days) is quite effective and surprisingly well tolerated for treatment of obstructive lesions. The frequency of relapse/recurrence of gastrointestinal granulomatous disease is high, so prolonged low-dose maintenance is often necessary. Other therapies for severe granulomatous complications include cyclosporin A and colostomy for refractory rectal disease. Several cases have been treated with infliximab. The latter therapy must be used with great caution, as infliximab increases the rates of severe fungal and bacterial infections in CGD.

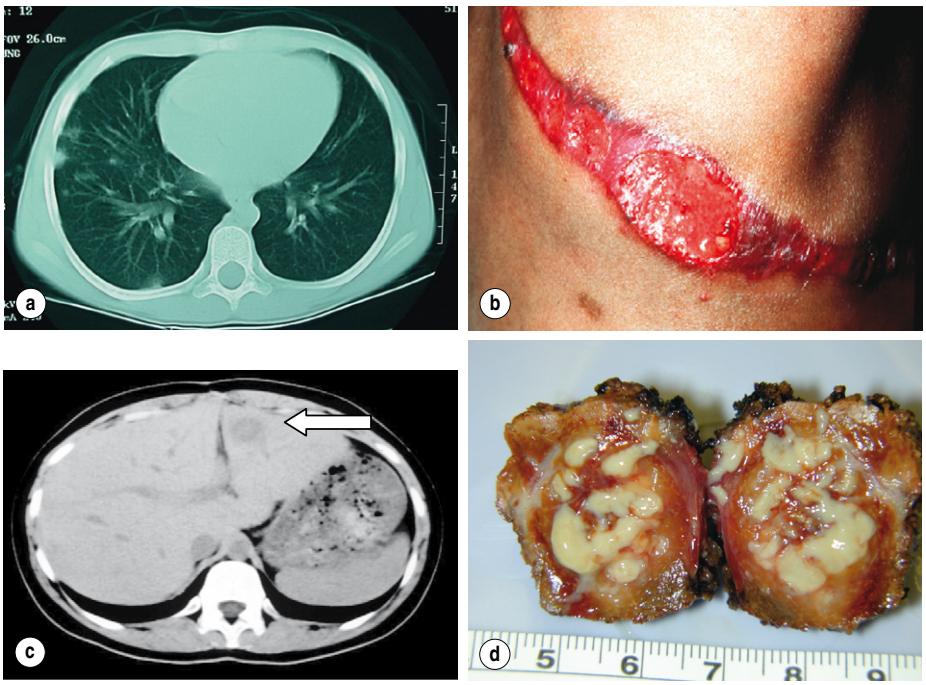


Fig. 72.3 Manifestations of chronic granulomatous disease (CGD). (a) Pneumonia due to *Aspergillus* can present subtly, both clinically and radiographically. This patient was asymptomatic but had multifocal pneumonia due to *A. fumigatus*. (b) Wound dehiscence typically presents 5–10 days postoperatively. It is an exuberant granulation tissue on biopsy and is best treated with short courses of corticosteroids. (c) Liver abscesses due to staphylococci are quite common in CGD (arrow) and typically require en bloc resection due to their dense, granulomatous nature (d).

X-linked carriers of gp91^{phox} have two populations of phagocytes: one that produces superoxide and one that does not, yielding a characteristic mosaic pattern on oxidative burst testing. Discoid lupus erythematosus-like lesions, aphthous ulcers and photosensitive rashes have been seen in gp91^{phox} carriers. Infections are not usually seen in these female carriers unless the normal neutrophils are below 5–10%, at which stage these carriers are at risk for CGD-type infections.[15]

The diagnosis of CGD is made by a measure of superoxide production. Currently, we prefer the dihydrorhodamine (DHR) assay because of its relative ease of use, its ability to distinguish X-linked from autosomal patterns of CGD on flow cytometry, and its sensitivity with low numbers of functional neutrophils.[19] Immunoblot and mutation analysis are required to identify the specific affected protein and genetic lesion, respectively. Male sex, earlier age at presentation and relatively severe disease suggest X-linked disease. Autosomal recessive forms of CGD (mostly p47^{phox} deficient) have a significantly better prognosis than X-linked disease. Mortality for X-linked CGD is about 5% per year, compared to 2% per year for the autosomal recessive varieties.[15] The precise gene defect should be determined in all cases, as it is critical for genetic counseling and is prognostically significant. Complete myeloperoxidase deficiency gives a DHR assay that looks like CGD, but has normal superoxide production.[20]

Prophylactic TMP–SMX (5 mg/kg per day based on TMX) reduces the frequency of major bacterial infections from about once every year to once every 3.5 years without increasing serious fungal infections in CGD. The greatest cause of mortality in CGD in developed countries remains *Aspergillus* pneumonia, but oral triazoles as prophylaxis can prevent fungal infection in CGD. A large, multicenter, placebo-controlled study showed that interferon gamma (IFN-γ) reduced the number and severity of infections in CGD by 70% compared to placebo regardless of inheritance pattern of CGD or use of prophylactic antibiotics, level of superoxide generation, bactericidal activity or cytochrome b levels.[21] Therefore, our current recommendation is to use prophylaxis with TMP–SMX, itraconazole and IFN-γ (50 μg/m^2) in CGD.[22] Azole antifungals (itraconazole, voriconazole, posaconazole) are preferred to amphotericin B for the treatment of active fungal infections.

A microbiologic or histopathologic diagnosis is critical in managing complications in CGD. In severe infections, leukocyte transfusions are often used, although their efficacy is anecdotal. Bone marrow transplantation leading to stable chimerism has been successfully performed in patients with CGD.[23] Bone marrow transplantation can be successful for refractory infection, predominantly from *Aspergillus*.[24] Gene therapy for p47^{phox} and gp91^{phox} deficiencies have been successful, but not durable.[25]

Myeloperoxidase deficiency

Myeloperoxidase (MPO; 17q23) is synthesized in neutrophils and monocytes, packaged into primary granules and released either into the phagosome or the extracellular space where it catalyzes the conversion of H_2O_2 to hypochlorous acid. Myeloperoxidase deficiency (OMIM #254600) is the most common primary phagocyte disorder: 1/4000 individuals have complete MPO deficiency and 1/2000 have a partial defect.[26] It is an autosomal recessive trait with variable expressivity. Despite *in vitro* studies showing that MPO-deficient neutrophils are markedly less efficient than normal neutrophils in killing *Candida albicans* and hyphal forms of *Aspergillus fumigatus*, clinical infection in MPO deficiency is rare. Of the MPO-deficient patients who have had clinical findings, candidiasis is the most common problem, and diabetes mellitus appears to be a critical co-factor.[26] Definitive diagnosis is established by neutrophil or monocyte peroxidase histochemical staining or specific protein detection. There is no specific treatment for MPO deficiency. Dihydrorhodamine gives an abnormal signal in complete MPO deficiency.[20]

THE LEUKOCYTE ADHESION DEFICIENCIES

For over a century, leukocyte movement from the bloodstream toward inflamed sites has been recognized as critical in preventing and fighting infections. Leukocyte adhesion to the endothelium, to other leukocytes

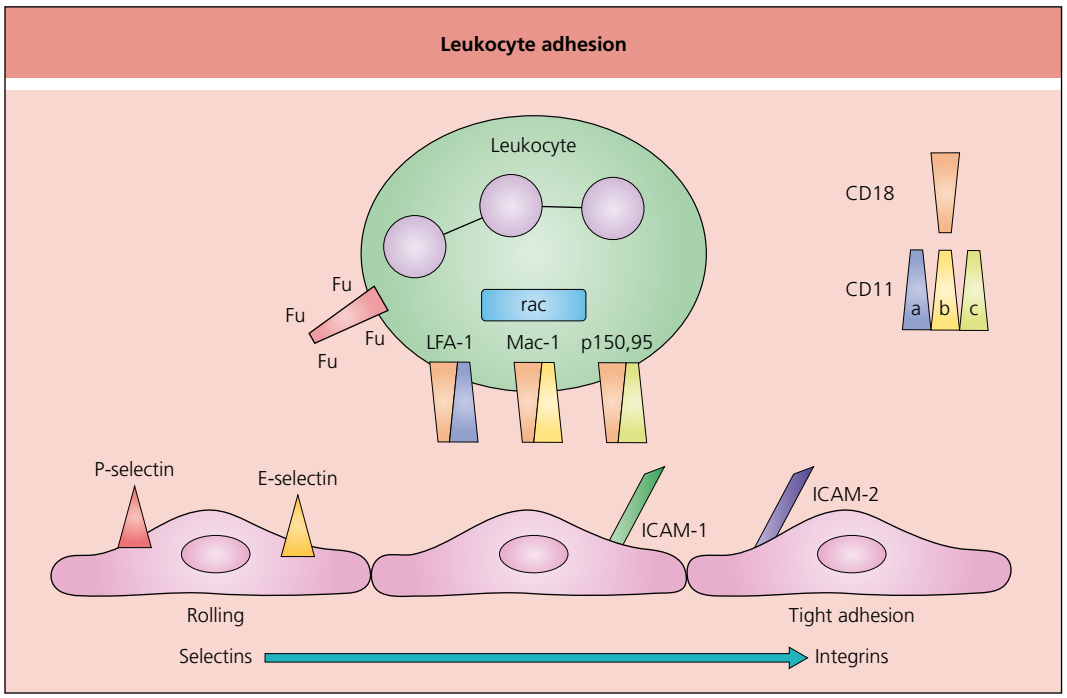


Fig. 72.4 Leukocyte adhesion. Neutrophils sample the endothelium in the postcapillary venules through a series of receptors. Depicted here are the selectin and integrin pathways, which are critical for neutrophil adhesion (monocytes and eosinophils have other ligand and receptor options). CD15s binds to selectins on the endothelium with loose adhesion, allowing closer sampling of the endothelium for tight adhesion in the setting of endothelial activation, mediated through the integrins. Neutrophil integrins bind to intercellular adhesion molecules (ICAMs).

and to bacteria is critical in the ability of leukocytes to travel, communicate, inflame and fight infection. Different families of adhesion molecules mediate these processes, critical among which are the integrins and selectins (Fig. 72.4).

Leukocyte adhesion deficiency type 1

The leukocyte β2 integrins are heterodimeric molecules on the surface of neutrophils, monocytes and lymphocytes that bind to intercellular adhesion molecules (ICAMs) on the endothelial surface in order to attach and exit the circulation.[27] ICAMs can also be expressed on other leukocytes, allowing for cell–cell adhesion. In addition, certain β2 integrins can bind directly to pathogens or to complement. Leukocyte integrins are composed of an α chain (CD11a, CD11b or CD11c), noncovalantly linked to a common β2 subunit, CD18, encoded by ITGB2 on 21q22.3. The αβ heterodimers of the β2 integrin family include CD11a/CD18 (lymphocyte function associated antigen 1 (LFA-1)), CD11b/CD18 (macrophage antigen 1 (Mac-1) or complement receptor 3 (CR3)) and CD11c/CD18 (p150,95 or complement receptor 4 (CR4)). Since CD18 is required for normal expression of the αβ heterodimers, recessive mutations in CD18 lead to either very low or no expression of CD11a, CD11b and/or CD11c, with resulting inability to bind to endothelium, each other, certain pathogens or complement opsonized particles. This disease is known as leukocyte adhesion deficiency type 1 (LAD1; OMIM #116920).[27]

LAD1 has severe (less than 1% of normal expression of CD18 on neutrophils) and moderate (up to 30% of normal expression of CD18 on neutrophils) phenotypes. In addition, patients with normal cell surface expression of a nonfunctional CD18 have been described, indicating that functional assays must be performed if the clinical suspicion of LAD1 is high.

Patients with the severe phenotype of LAD1 characteristically have delayed umbilical stump separation and omphalitis, persistent leukocytosis (>15 000/μl), destructive gingivitis and periodontitis with associated loss of dentition and alveolar bone. Recurrent infections of the skin, lung, bowel and perirectal area are common from *Staph. aureus* or Gram-negative bacilli. Necrosis and ulceration without pus accumulation or neutrophil invasion of the infected site are common (Fig. 72.5). Impaired wound healing is characteristic of LAD1: scars tend to be dystrophic and have a 'cigarette-paper' appearance. In contrast to the severe phenotype, patients with the moderate phenotype tend to be diagnosed later in life, have normal umbilical stump separation, have fewer life-threatening infections and live longer. However, leukocytosis, periodontal disease and delayed wound healing are still common. Aggressive medical management with antibiotics, and surgery when indicated, are requisite. Neutrophil transfusions may be helpful in severe cases.

Complement-mediated phagocytosis is severely impaired due to absence of CD18/CD11b (CR3/Mac-1) and antibody-dependent cell-mediated cytotoxicity (ADCC) is diminished. However, IgG-mediated phagocytosis is unaffected as is superoxide production and primary and secondary granule release. At present, bone marrow transplantation is the only definitive corrective treatment. Gene therapy of LAD1 is not yet of clinical benefit.

Other defects in leukocyte adhesion

Selectins are the molecules that mediate the loose, rolling adhesion of neutrophils along postcapillary venules.[27] Leukocyte adhesion deficiency type 2 (LAD2) is a metabolic defect now referred to as congenital disorder of glycosylation type IIc (CDG-IIc) due to recessive mutations in the GDP-fucose transporter (FUCT-1; 11p11q11; OMIM #266265).[28] This fucosylation defect is apparent on several molecules, most notably the neutrophil ligand sialyl-Lewis^x. Patients have infections of the skin, lung and gums, leukocytosis and poor pus formation, as well as mental retardation, short stature, distinctive facies, and the Bombay (hh) blood phenotype. *In vitro*, LAD2 cells show impaired neutrophil migration, aggregation and adherence to endothelial cells. Fucose supplementation may be helpful. Infections appear to moderate with age.

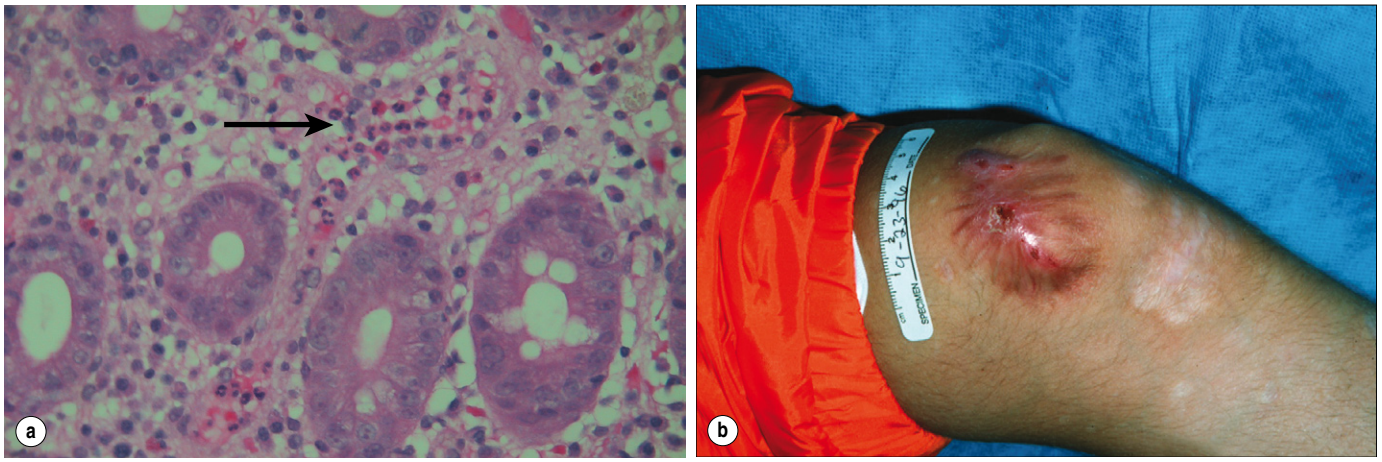


Fig. 72.5 Examples of leukocyte adhesion deficiency type 1 (LAD1). (a) A biopsy from the bowel of a patient with extensive inflammation of the intestinal ulceration. Note the abundance of neutrophils intravascularly (arrow), but the paucity of neutrophils in the parenchyma. (b) Charactristic dystrophic or 'cigarette paper' scarring following skin ulceration in a boy with LAD1.

Defects in endothelial expression of E-selectin[29] and in the Rho GTPase RAC2 (RAC2, 22q12.13–q13.2; OMIM #602049)[30,31] also lead to abnormalities in adhesion and increased infections.

INTERFERON-γ/IL-12 PATHWAY DEFECTS

The mononuclear phagocyte is critical for protection against intracellular infections. It mediates antigen presentation, lymphocyte stimulation and proliferation, and cytokine production and response. Mycobacteria infect macrophages leading to production of interleukin (IL)-12, which in turn stimulates T cells and NK cells to produce interferon gamma (IFN-γ). IFN-γ increases production of tumor necrosis factor (TNF), IL-12 and other cytokines as well as mediating mycobacterial killing, through unknown mechanisms. IFN-γ signaling depends on the signal transducer and activator of transcription 1 (STAT1), while TNF signaling depends on the nuclear factor kappa B (NF-κB) essential modulator (NEMO) (Fig. 72.6). Defects in several critical members of the pathway involving IFN-γ, IL-12, TNF and their respective receptors and signaling molecules have been clearly identified at the functional and genetic levels as being responsible for infections with mycobacteria, salmonellae and certain viruses.[32]

The IFN-γ receptor is composed of ligand binding (IFN-γR1) and signal transducing (IFN-γR2) chains. Autosomal recessive mutations in either chain that lead to abolition of IFN-γ signaling have severe infection phenotypes, predominantly with mycobacteria. Patients with complete defects present early in life, often after BCG vaccination. They have poor or absent granuloma formation (but normal tuberculin skin tests) and typically develop repeated, disseminated, life-threatening infections due to mycobacteria, salmonellae and some viruses. Mortality is overwhelmingly due to mycobacterial disease. Treatment relies upon antimicrobial agents and bone marrow transplantation if possible. Rare recessive mutations with partial function have intermediate phenotypes and more curable infections.[33]

The most common mutation in IFN-γR1 is due to a four-base deletion at or around base 818 (818del4), located just inside the intracellular domain of the molecule. This mutation allows the nonfunctional protein to remain stuck on the cell surface where it binds with the normal protein and impedes signaling[34] (Fig. 72.7). Patients with this autosomal dominant mutation in IFN-γR1 usually present in childhood with pulmonary nontuberculous mycobacterial infection and recurrent multifocal nontuberculous osteomyelitis. IFN-γ signaling persists, but at a greatly reduced level compared to normal. IFN-γ therapy, sometimes at high dose, can be effective.[33] Long-term prophylaxis against environmental mycobacteria with a macrolide seems prudent.

Recessive mutations in IL-12p40 and IL-12 receptor β1 and dominant mutations in STAT1 are typically not as severe as complete IFN-γ receptor defects. Mutations affecting IL-12 signaling may present with more *Salmonella* infections.[35] Because these defects have preserved IFN-γR function, IFN-γ can be used therapeutically.

NUCLEAR FACTOR KAPPA B PATHWAY DEFECTS

Immune signaling through the receptors for IL-1, IL-18, TNF, CD40 and the Toll-like receptors (TLRs), as well as signaling for ectodermal (teeth, hair, sweat gland) formation converge at the activation of NF-κB, a process dependent on the proper phosphorylation and degradation of its inhibitor, IκB. The X-linked gene for the NF-κB essential modulator (NEMO) is necessary for this process. Defects in NEMO may cause ectodermal dysplasia along with a complex and overlapping set of immunodeficiencies with dysfunction in the innate TLRs, tumor necrosis factor receptor (TNFR), IL-1R and acquired (CD40, IL-18) immune systems, due to disruption of their common signaling pathway.[36] These patients typically require intravenous immune globulin, due to ineffective immunoglobulin class switching, as well as antibiotics.[37] Macrolide prophylaxis to prevent the acquisition of environmental mycobacterial infection appears prudent.

While defects in NF-κB, which is at the convergence of TLRs, lymphocyte activation and ectodermal signaling, give a complex phenotype of immune and somatic abnormalities, defects in TLR signaling give phenotypes that are exclusively immunologic. Recessive mutations in interleukin receptor 1 associated kinase 4 (IRAK-4) lead to profound susceptibility to sepsis and meningitis with encapsulated bacteria (*Streptococcus pneumoniae, Neisseria meningitidis, Haemophilus influenzae*) early in life.[38] Clinically, these patients are remarkable for a paucity of inflammatory responses despite severe infection, due to inability to transduce IL-1 and TNF signals.[39] Recessive mutations in the more proximal signal transducing molecule MyD88 lead to a similar phenotype.[40] While IRAK-4 and MyD88 are required for bacterial defense, recessive mutations in TLR3 and Unc-93B predispose to cases of familial herpes simplex encephalitis.[41,42]

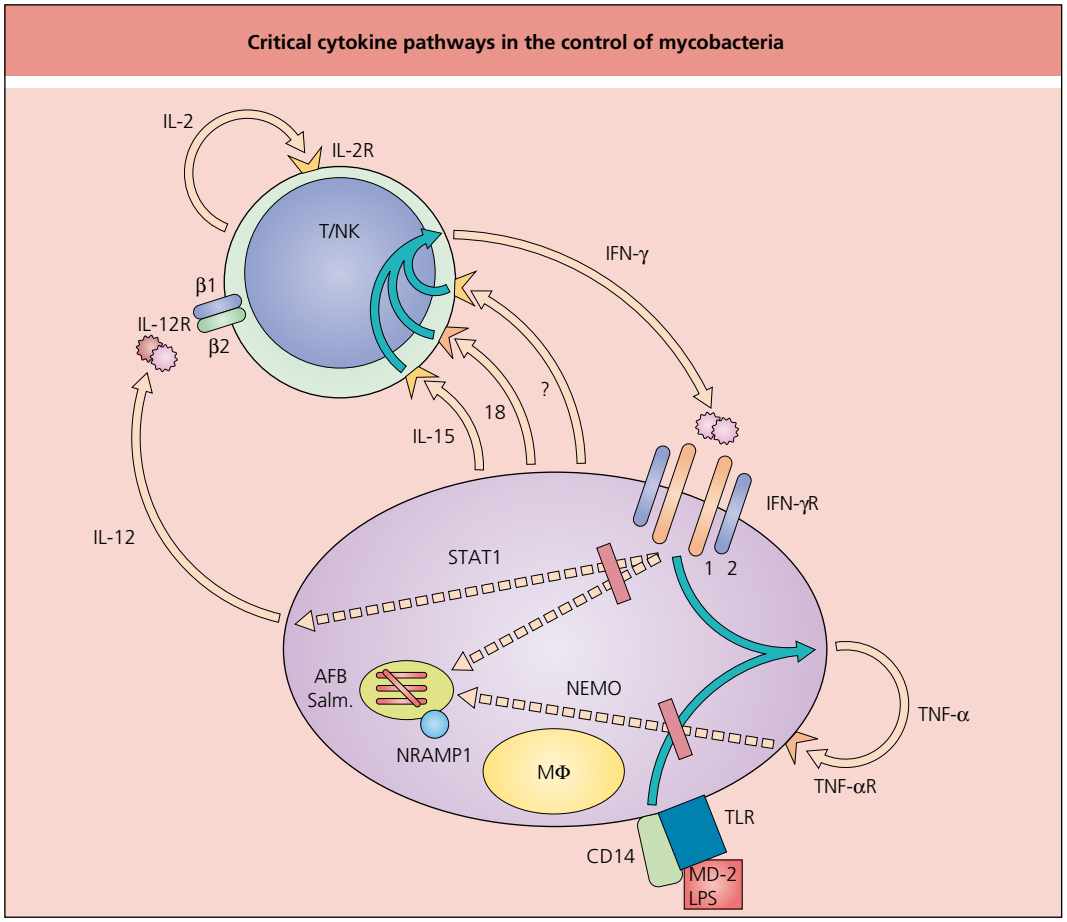


Fig. 72.6 Critical cytokine pathways in the control of mycobacteria. Mycobacteria and salmonellae stimulate the elaboration of IL-12 by infected macrophages, leading to the production of IFN-γ by T cells and NK cells. IFN-γ in turn stimulates macrophages to produce TNF and IL-12. The critical signaling molecules STAT1 and NEMO are also indicated. IL-12 independent pathways for IFN-γ production are also indicated (IL-15, IL-18), which work in concert with IL-12 for lymphocyte stimulation. Other, as yet undefined pathways are also suggested.

HYPER-IgE RECURRENT INFECTION SYNDROME

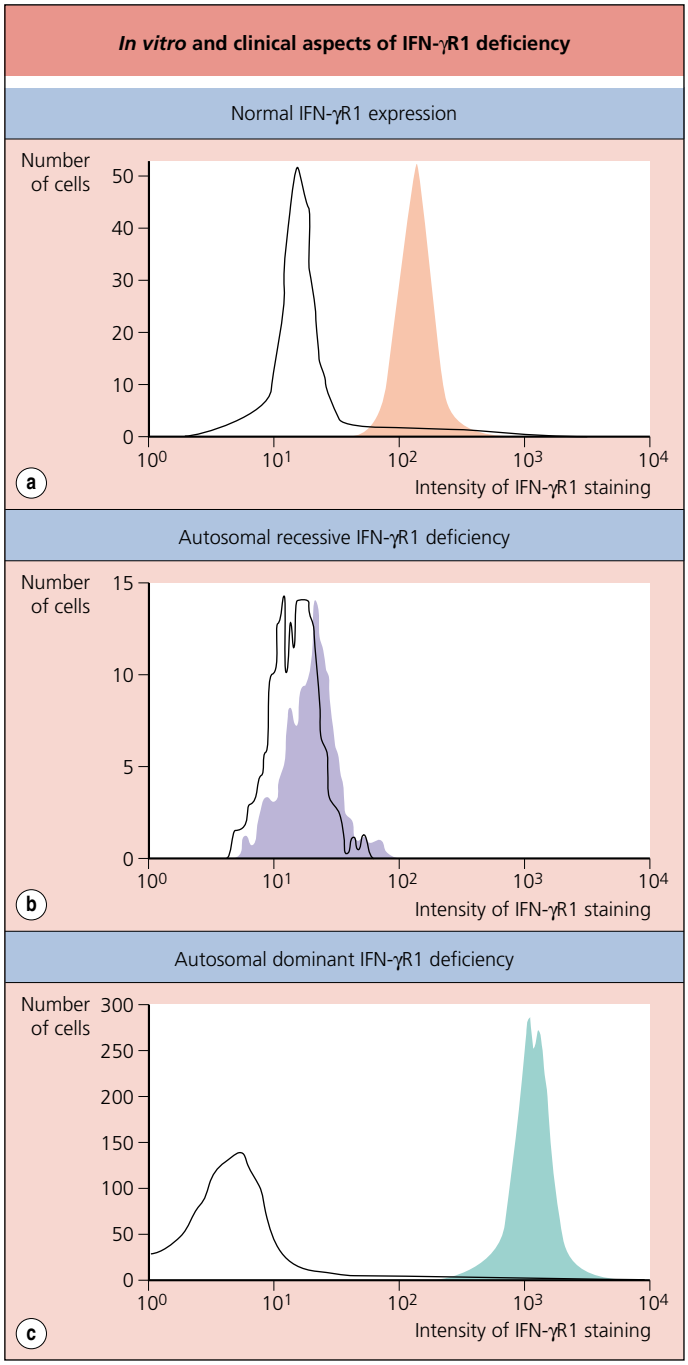
Immune signaling for activation of the inflammatory response is highly dependent on IL-6 for its upregulation and IL-10 for its down-regulation. The shared signal molecule STAT3 mediates not only signaling for IL-6 and IL-10, but also the key mediators of inflammation IL-17, IL-21 and IL-22. Therefore, mutations in STAT3 affect a large number of pathways that both increase and diminish inflammation, as well as control the expression of epithelial defenses, and cause the hyper-IgE recurrent infection syndrome (HIES; Job's syndrome).[43] It is an autosomal dominant disorder characterized by recurrent infections, typically of the lower respiratory system and skin, eczema, extremely elevated levels of IgE, eosinophilia, and abnormalities of the connective tissue, skeleton and dentition[44] (Fig. 72.8). The majority of patients have characteristic facial abnormalities including a broad, somewhat bulbous nose. Failure of primary dental deciduation, leading either to failure of secondary dentition eruption or retention of both sets of teeth is common. Many patients also have abnormalities of bone formation and metabolism, which may result in fractures, scoliosis, kyphosis and osteoporosis. HIES occurs spontaneously in all racial and ethnic groups.

IgE is greatly elevated at some point in the life of all patients with HIES, but about 20% have been observed to drop their IgE levels below 2000 IU/ml as they get older while retaining their susceptibility to infection.[44] The clinical manifestations of HIES are quite distinct (Table 72.2). Eczema usually presents within the first days to months of life. Other early signs include mucocutaneous candidiasis and severe diaper rash. Sinus or pulmonary infections, predominantly with *Staph. aureus* or *H. influenzae*, are common, as are postinflammatory pneumatoceles. Otitis media and externa are common. Other pathogens that have been recovered include *Aspergillus* spp., *Pseudomonas aeruginosa*, *Strep. pneumoniae*, group A streptococci, *Cryptococcus neoformans*, *Pneumocystis jirovecii* and *C. albicans*. Mucocutaneous candidiasis involving the mouth, vagina, intertriginous areas, fingernails and toenails affects about 50% of HIES patients. Bony abnormalities are frequent, as are pathologic fractures. IL-17, a critical proinflammatory cytokine made by CD4 T cells is STAT3 dependent, so is not produced in HIES.[45]

High-dose intravenous antibiotics for a prolonged course are required for eradication of infection and to prevent bronchopleural fistula formation and bronchiectasis. Empiric coverage should consider *Staph. aureus, H. influenzae* and *Strep. pneumoniae*. Infection of pneumatoceles and bronchiectatic lung with *P. aeruginosa* and *Aspergillus* spp. is especially problematic. Most experts use prophylactic antibiotics (e.g. TMP–SMX) to cover *Staph. aureus*.

LYMPHOCYTE IMMUNE DEFICIENCIES

Lymphocyte immune deficiencies have provided the paradigms for understanding immunology, tolerance and transplantation for decades. The nomenclature is dense and confusing because diseases originally



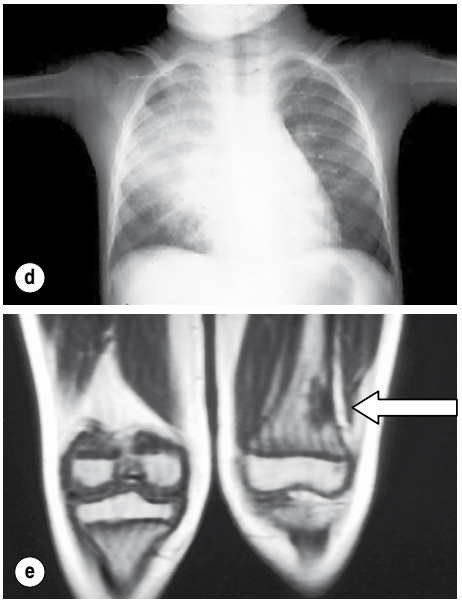


Fig. 72.7 *In vitro* and clinical aspects of IFN-γR1 deficiency. (a–c) Flow cytometry for the IFN-γR1 on peripheral blood monocytes. Dotted lines indicate the background fluorescence of the sample with an irrelevant antibody. (a) Normal IFN-γR1 fluorescence intensity. (b) Monocytes from a child with complete IFN-γR1 deficiency. Note the lack of specific staining for IFN-γR1. (c) Increased intensity of IFN-γR1 staining on monocytes from a patient who has MAC osteomyelitis. (d) Chest radiograph from the same patient showing extensive right lung infection with MAC. (e) The corresponding MRI of his distal femur (arrow indicates the infected lesion).

described phenotypically have turned out to be due to numerous separate genetic defects (e.g. interleukin 2 receptor common gamma chain, JAK3, interleukin 7 receptor alpha chain).[46] The confused terminology is unavoidable as the clinical syndrome is described first, followed by detailed genetics. The application of DNA diagnostics has demonstrated that defects in one gene may result in several different phenotypes (phenotypic heterogeneity) and that the same phenotype can be caused by different gene defects (genetic heterogeneity). Therefore, both syndromic and molecular diagnoses should be pursued in all cases.

DNA cleavage, polymerization and repair resulting in recombination are critical for lymphocyte diversity and antigen specificity.[47] Therefore, defects that affect lymphoid ligand binding, signaling or any of the steps of successful DNA recombination may present as defects in acquired immunity.

When considering the diagnosis of severe combined immunodeficiency (SCID), it is important to keep several things in mind:

- SCID is a medical emergency. If the patient with SCID is to survive transplantation, it is important to avoid community viral infections, which can be devastating.

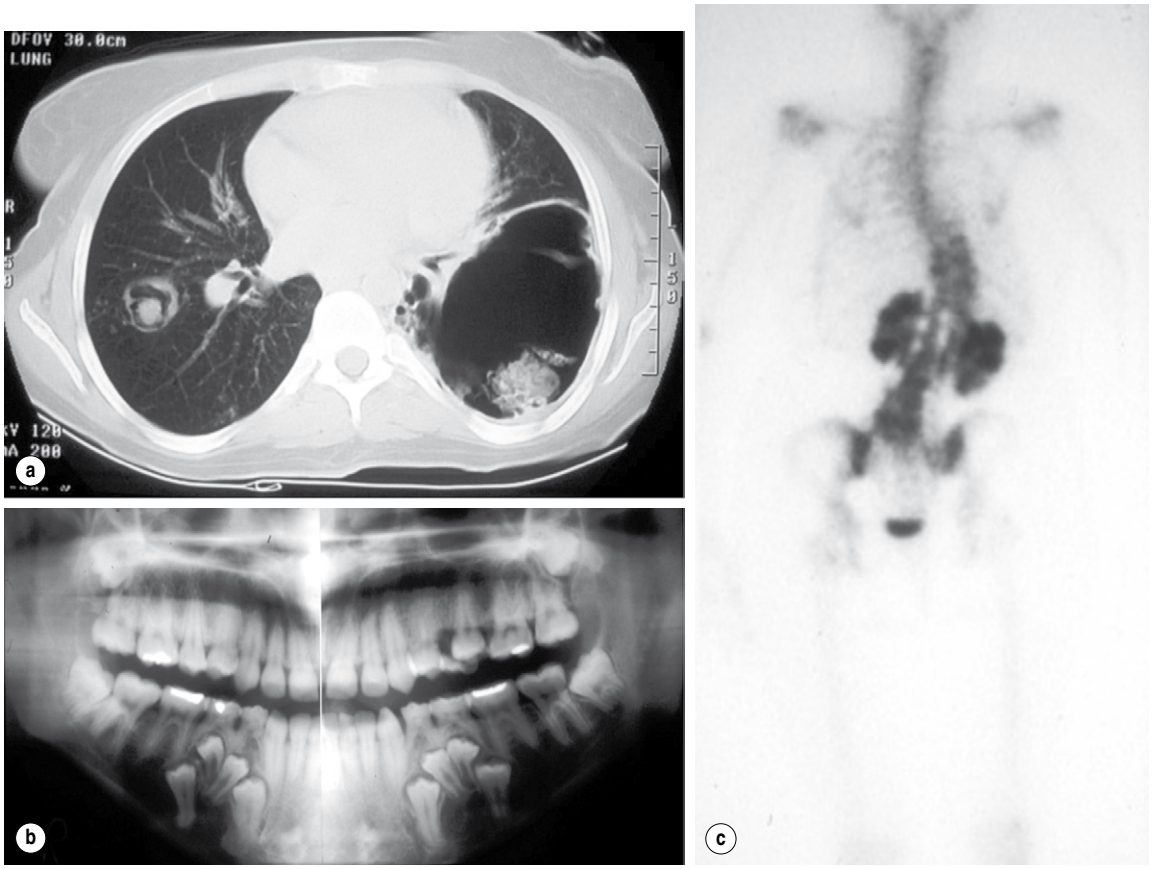


Fig. 72.8 Clinical manifestations of hyper-IgE (Job's) syndrome. (a) CT of the chest showing characteristic postinflammatory pneumatocele formation. Note the development of bilateral aspergillomata with inflammation of the cavity walls. (b) Panoramic radiograph of the dentition of a 33-year-old woman with HIES shows the characteristic retention of primary teeth due to failure of deciduation of the retained primary teeth. (c) Extensive scoliosis is demonstrated in this radionuclide bone scan in a 25-year-old woman with HIES. A list of some of the characteristic features of the syndrome is included in Table 72.2.

Table 72.2 Features of hyper-IgE syndrome

Feature	%
Eczema	100
Characteristic facies (>16 years)	100
Skin boils	87
Pneumonias	87
Mucocutaneous candidiasis	83
Lung cysts	77
Scoliosis (>16 years)	76
Delayed dental deciduation	72
Pathologic fractures	57

- Persistent maternal engraftment is both a sign of SCID and a cause of many of the symptoms, such as failure to thrive and rash.
- The phenotype of infections and clinical illness are the presentation of the patient; laboratory tests are efforts to confirm the genetics.
- Missense mutations may produce proteins that are still present in normal amount but nonfunctional.

Therefore, the clinical scenario, typically developed out of the infection profile and laboratory studies, must be used to guide decisions about which genes to sequence.[48]

T lymphocytes are not only crucial for resistance to intracellular pathogens, but also interact with B lymphocytes and antigen-presenting cells. Consequently, defects in T-cell development or function result in combined immune deficiencies. The forms with the most severe T-cell depletion are medical emergencies that lead to increased susceptibility to severe infections from birth. Failure to thrive, chronic diarrhea and interstitial pneumonia (due to opportunistic infections, such as *Pneumocystis jirovecii* or viruses) are typical (Fig. 72.9). The overall frequency of SCID is estimated to be about 1 in 50 000 live births. Lymphopenia in the setting of severe infection in an infant should raise suspicion. Quantification of the absolute $CD3^+$ cell count, analysis of HLA-DR expression and T-cell activation allow identification of almost 98% of all SCID babies (Fig. 72.10).

Vaccination with live attenuated viruses or BCG may lead to fatal disseminated infection (Fig. 72.11). Graft-versus-host disease (GvHD), due to transplacental passage of alloreactive maternal T lymphocytes or transfusion of unirradiated blood products, can cause skin rash, diarrhea, hepatitis or bone marrow failure. Untreated, patients with SCID die within the first years of life. Prophylactic TMP–SMX (to prevent *Pneumocystis jirovecii* pneumonia) and aciclovir (for herpes viruses), immunoglobulin replacement therapy and aggressive treatment of all infections prolongs survival, but only early allogeneic bone marrow transplantation or (in selected cases) gene therapy or enzyme replacement therapy offers a chance for cure.

In recent years molecular diagnosis has been achieved for most immune deficiencies (Fig. 72.12) and mutation databases have been established that are constantly updated and accessible via the world wide web.



Fig. 72.9 Typical severe combined immunodeficiency baby. Note the wasting and malnutrition.

Severe combined immunodeficiency

In typical SCID, severe intestinal infections with malabsorption and failure to thrive are prominent. The intestinal mucosa shows variable degrees of villous atrophy. The most frequently isolated pathogens include *Campylobacter, Salmonella, Shigella, Cryptosporidium parvum* and *Giardia lamblia*. The liver is often involved, ranging from hepatomegaly with elevated transaminases, to sclerosing cholangitis due to *Cryptosporidium parvum*. CNS involvement can also occur, mostly due to viral infections (herpes simplex virus, enteroviruses and adenoviruses). SCID has been classically defined by the numbers of T cells, B cells and NK cells. The advantage to this system is that with one simple flow cytometric study one can determine which pathways are most likely to be affected. The nomenclature for SCID is complex at a cellular, genetic and molecular level but has important implications for prenatal and carrier testing, transplant success and genetic correction.

X-linked SCID, γc deficiency ($T^-B^+NK^-$)

The most common SCID phenotype is absence of mature T and NK lymphocytes in the blood and peripheral lymphoid organs, with a normal to increased number of B cells (T^-B^+ SCID).[48] Most commonly, this disease is inherited as an X-linked trait (SCID-X1, OMIM #300400). SCID-X1 has an estimated incidence between 1:150 000 and 1:200 000 live births and accounts for 35–40% of all cases of SCID. It is caused by mutations in the *IL2RG* gene which encodes the chain of the IL-2 receptor that is common to multiple other cytokine receptors as well, hence its designation as the common gamma chain (γc). Located at Xq13, this cytokine receptor subunit is shared by the receptors for IL-2, IL-4, IL-7, IL-9, IL-15 and IL-21 and is therefore involved in the growth and maturation of T, B and NK cells[49] (Fig. 72.13). Since these cytokine receptors lack intrinsic catalytic activity, they partner with an intracellular signal transducer, JAK3, a member of the Janus associated kinase (JAK) family of protein tyrosine kinases. Therefore, absence of γc leads to marked impairment of signaling for multiple cytokines.



Fig. 72.11 Axillary BCGitis in a T^-B^+ severe combined immunodeficiency baby with generalized BCG infection. Note the extensive cutaneous ulceration.

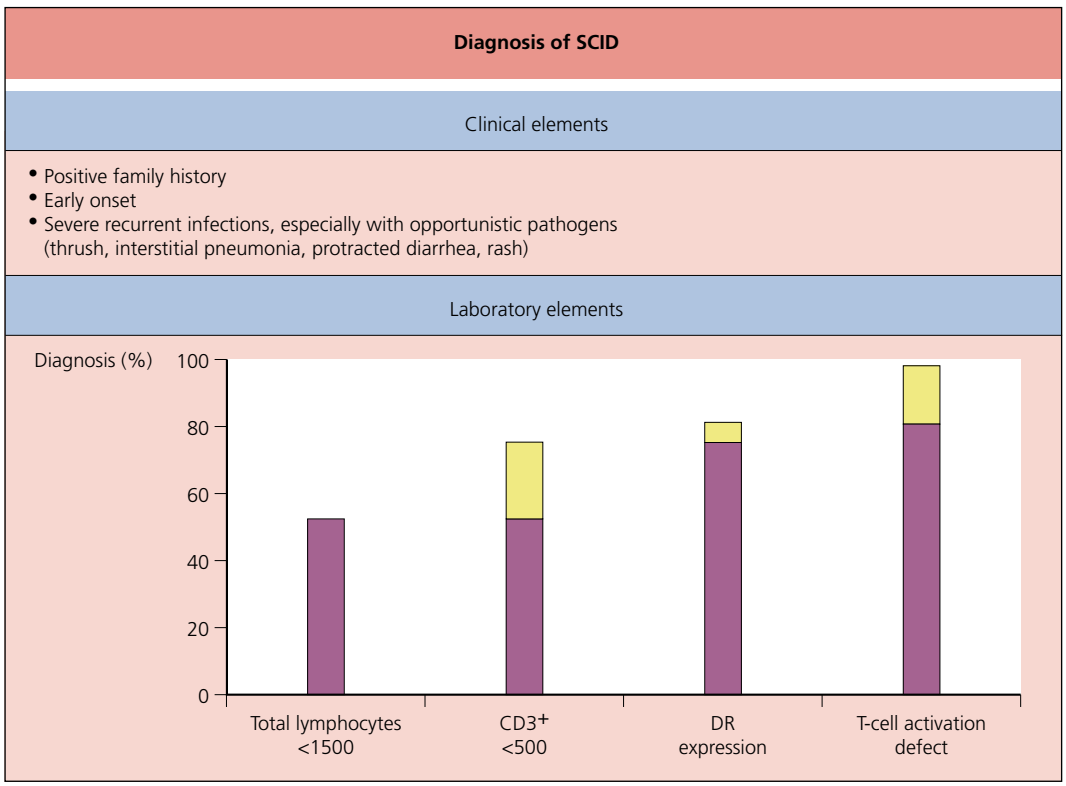


Fig. 72.10 Clinical and laboratory elements useful in the diagnosis of severe combined immunodeficiency.

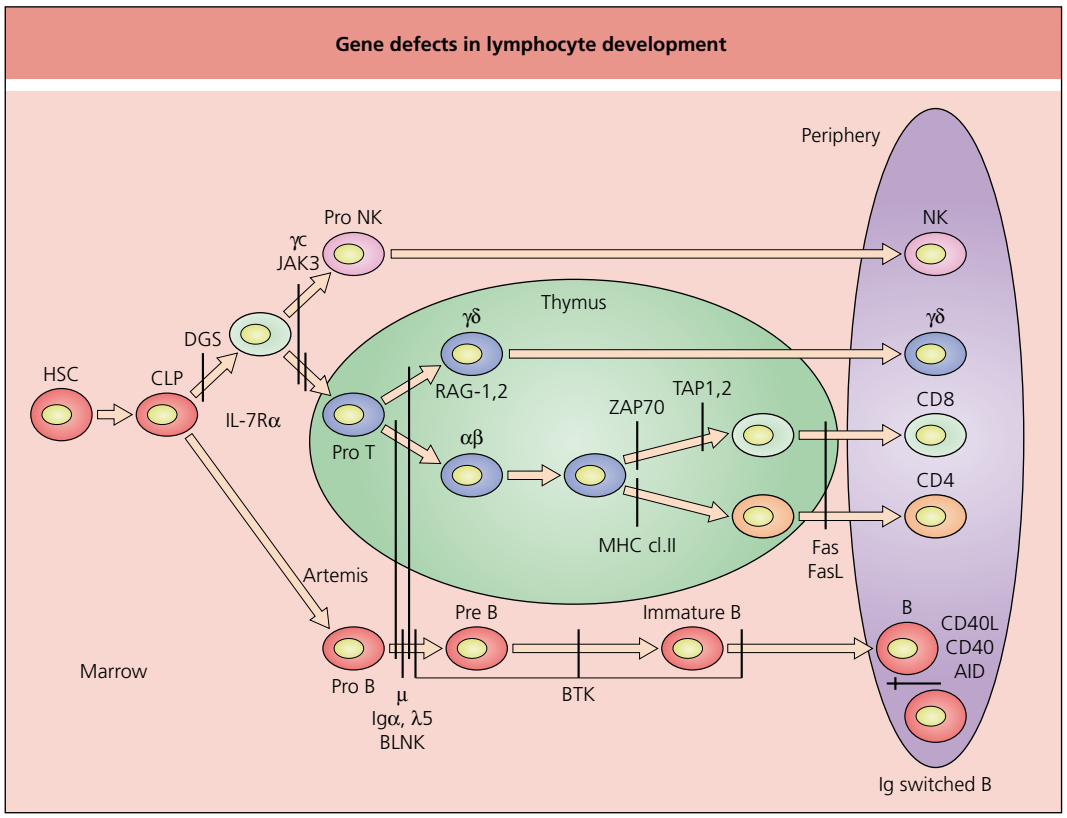


Fig. 72.12 Model of lymphocyte development with the relative gene defects that may lead to immune deficiencies shown in red (for details see text).

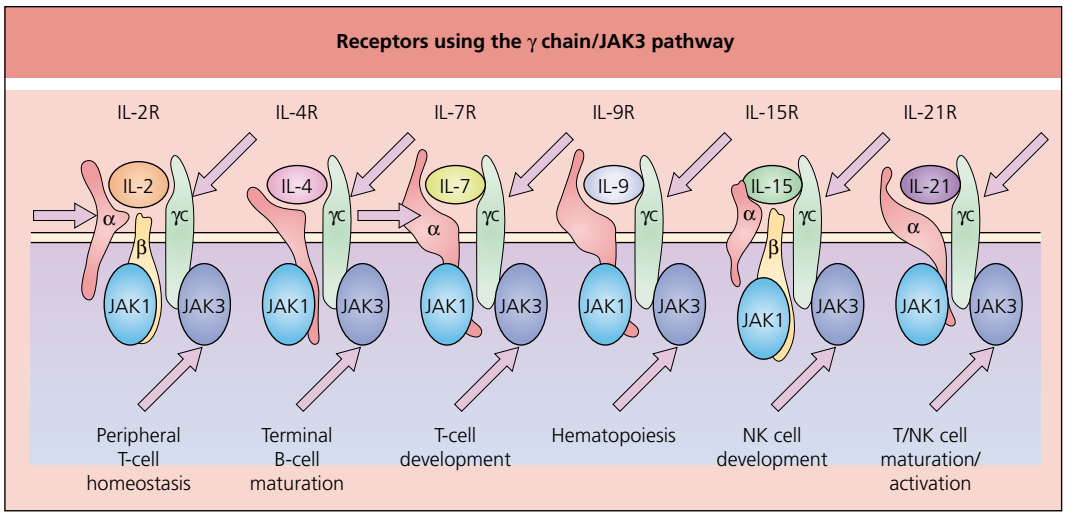


Fig. 72.13 Diagram describing the six interleukin receptors that use the common gamma-chain/JAK3 pathway for signaling.

As in all forms of SCID, clinical onset of SCID-X1 is usually within the first few months of life with persistent diarrhea, pneumonia, failure to thrive and severe or persistent candidiasis. The diagnosis is usually suspected because of lymphopenia, with low to absent T and NK cells and normal to elevated B cells. Mitogen-induced proliferation is virtually absent and serum immunoglobulin levels are low to undetectable. Serum IgG may initially be normal due to persistence of transplacentally passed maternal antibodies. The thymus lacks a clear cortical–medullary demarcation, Hassall's corpuscles are absent, and in general there is a severe depletion of lymphoid tissues with absent lymph nodes.

The diagnosis can be made by flow cytometry for γc expression on the surface of lymphocytes or monocytes. However, some *IL2RG* gene mutations allow expression of nonfunctional γc on the cell surface. Therefore, normal γc expression does not rule out a diagnosis of SCID-X1. Further complicating matters, engraftment of maternal T cells (observed in as many as 50% of cases of SCID) may lead to an atypical immunologic phenotype, representing a diagnostic challenge. HLA typing and molecular analysis at highly polymorphic DNA loci can identify the presence of maternal T cells. Other forms of SCID may present with the same cellular phenotype (T^-B^+ SCID) and should be considered if γc expression is

normal. Detection of a mutation in *IL2RG*, most often inherited from the mother, confirms the diagnosis. Mutations found in different families are listed at http://www.nhgri.nih.gov/DIR/GMBB/SCID/IL2RGbase.html.

The standard treatment for SCID-X1 is allogeneic human stem cell (usually bone marrow) transplantation (BMT), with success rates near 100% when an HLA-identical sibling is used as donor.[50] Excellent results are also achieved with BMT from matched unrelated donors, but since early treatment is crucial and the search for a compatible donor often takes several months without a guarantee of finding one, haploidentical family donors have been increasingly used.[50] GvHD prevention by elimination of T-cells from the donor marrow or the use of positively selected peripheral blood stem cells after mobilization is crucial to successful HLA-mismatched transplantation and is now successful in as many as 70–80% of patients. Even in the absence of myeloablation, engraftment of donor T (and often NK) cells is easily achieved. In contrast, engraftment of donor-derived B cells is facilitated by chemotherapy or irradiation conditioning of the recipient. When functional B-cell deficiency persists in patients without donor B-cell engraftment, regular intraveous immunoglobulin substitution is required. In utero stem cell transplantation has been successfully performed in affected fetuses with good clinical and laboratory T-cell reconstitution.[51]

Because functional γc expression provides a strong proliferative advantage to lymphocyte precursors, SCID-X1 is an ideal model for gene therapy. A number of patients have been successfully treated and have achieved normal T- and NK-cell counts and function after retroviral-mediated γc gene transfer into autologous $CD34^+$ bone marrow cells.[52] However, the development of leukemias due to gene insertion in some of the recipients has raised concern about the safety of gene therapy.[53]

JAK3 deficiency ($T^-B^+NK^-$)

Similar to the case with other transmembrane receptors, γc signals through an intracellular cascade including JAK3 and STAT5 molecules (Fig. 72.14). After the X-linked form, autosomal recessive *JAK3* mutations are the second most common form of T^-B^+ SCID (JAK3 deficiency, 19p12–13.1, OMIM #600173).[54] Because γc and JAK3 are linked components in the same signaling pathway, JAK3 deficiency is clinically and immunologically similar to SCID-X1. Diagnosis of JAK3 deficiency is established by immunoblot for JAK3 in patient lymphocytes or cell lines and/or demonstration of *JAK3* gene mutations. Since JAK3 deficiency is compatible with the presence of significant numbers of circulating, although poorly functioning, T lymphocytes, JAK3 deficiency should be considered in all undefined cases of combined immune deficiency, especially those presenting with a high proportion of peripheral B lymphocytes. Prenatal diagnosis based on DNA analysis of chorionic villus biopsies has been performed. As is the case for SCID-X1, definitive treatment of JAK3-deficient SCID is allogeneic BMT. *JAK3* gene transfer into hematopoietic stem cells in *jak3* knockout mice has been successful, indicating the possibility for *JAK3* gene therapy in humans. The mutations in JAK3 are listed at http://bioinf.uta.fi/JAK3base.

IL-7Rα (CD127) deficiency ($T^-B^+NK^+$)

Interleukin-7 is produced by stromal cells in the bone marrow and the thymus and provides survival and proliferative signals to IL-7 receptor bearing ($IL\text{-}7R^+$) cells. The IL-7 receptor consists of two subunits, the γc chain and the IL-7Rα chain, which maps to chromosome 5p13. IL-7Rα is specific for the IL-7R, is expressed by lymphoid precursor cells and is essential for differentiation of early thymocytes. Consequently, mutations that impair expression of IL-7Rα result in an early block in T-cell development, leading to SCID with absent T cells but normal to elevated B cells.[55] NK cells are preserved due to retained IL-15 signaling. The diagnosis is suggested by flow cytometry for CD127 on T, B, NK cells or macrophages, and confirmed by mutation detection. The clinical picture is similar to other forms of SCID. Allogeneic stem cell transplantation is the only curative treatment.

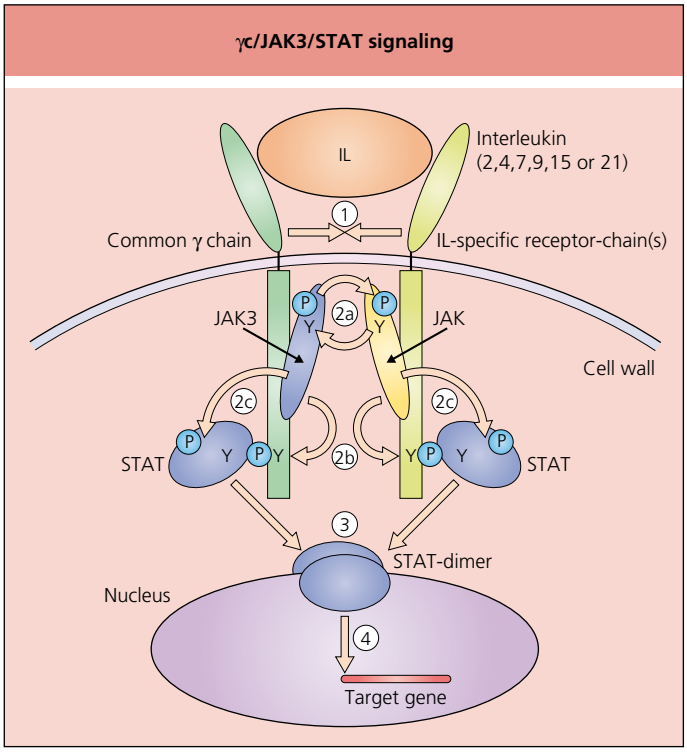


Fig. 72.14 γc/JAK3/STAT signaling is initiated by binding of the cytokine to its receptor. Heterodimerization (1) of the receptor chains (one interleukin-specific chain and the γc) allows for reciprocal tyrosine phosphorylation (2a) of JAK3 and other JAK molecules leading to their activation. Activated JAKs then phosphorylate (2b) tyrosine residues on both receptor subunits creating docking sites for the STATs. These signaling elements are themselves tyrosine phosphorylated by the JAKs (2c) in order to dimerize and translocate to the nucleus (3), where they bind to consensus sequences within regulatory regions of cytokine-inducible genes, and act as transcription factors driving transcription of the target genes.

IL-2Rα (CD25) deficiency ($T^-B^+NK^+$)

IL-2Rα (CD25) is a low affinity IL-2 receptor and another member of the complete IL-2 receptor complex required for IL-2-mediated signaling and peripheral T-cell homeostasis. Therefore, the defect caused by loss of IL-2α is relatively limited to T lymphocytes, leaving a normal number of B and NK cells[56] (OMIM *147730). This also makes for persistent lymphadenopathy and abnormal apoptosis. Unlike the case for γc and JAK3 defects, CD25 deficiency has significant autoimmunity. These patients can have a progressive lymphoproliferative syndrome reflecting disturbed peripheral immune homeostasis.

CD45 deficiency ($T^-B^+NK^-$)

CD45 is a protein tyrosine phosphatase, also known as the leukocyte common antigen since it is expressed on all nucleated hematopoietic cells, and makes up to 10% of the cell surface area of T and B cells. Not only does it govern T-cell receptor (TCR) signaling through regulation of the Src family kinases p56lck and p59fyn, it also downregulates integrin-mediated adhesion and dephosphorylates JAK kinase complexes. The immunologic phenotype is one of complete lack of T cells, with normal to increased B-cell counts.[57]

Defects of the p56lck/ZAP-70 pathway

Stimulation of T cells through the CD3/T-cell receptor (CD3/TCR) complex (Fig. 72.15) results in a complex series of activations focusing on p56lck, an Src tyrosine kinase, immunoreceptor tyrosine-based activation motifs (ITAMs) and ZAP-70, an intracellular tyrosine kinase.[58] ZAP-70 itself becomes phosphorylated by Src family protein tyrosine kinases. In humans, ZAP-70 is required for development of $CD8^+$ T cells in the thymus and peripheral blood T-cell proliferation in response to mitogens and antigens. Defective expression of p56lck leads to hypogammaglobulinemia, lymphopenia with a reduced proportion of $CD4^+$ T cells, and reduced *in vitro* proliferation. Defects in ZAP-70 result in impaired T-cell development and function.[58,59] Infants with ZAP-70 deficiency present with typical clinical features of SCID, virtual absence of $CD8^+$ T cells, and nonfunctional $CD4^+$ T lymphocytes. Both p56lck and ZAP-70 are autosomal recessive.

DNA repair defects (T^-B^- SCID)

Recombination of DNA in T cells and B cells is required for the generation of the immune diversity that is the hallmark of the mammalian immune system.[60] This process requires specific gene products that cut and recombine DNA as well as specific sites to be acted upon. The genes that control these processes are responsible for phenotypes of SCID causing complete absence of both T and B lymphocytes in the periphery. The majority of these patients have a defect in the recombination process.[61] To recognize foreign antigens, B and T cells use specialized receptors: the immunoglobulin (Ig) and the TCR, respectively. These are characterized by highly polymorphic antigen-recognition sites, which are the coding products of variable (V), diversity (D) and joining (J) gene segments that undergo somatic rearrangement due to a mechanism known as V(D)J recombination.[60] V(D)J recombination is crucial for the differentiation of T and B lymphocytes and is triggered by the lymphocyte-specific proteins recombinase activating genes (RAG)1 and RAG2. These gene products act together to recognize specific recombination signal sequences (RSS) that flank each of the V, D and J gene elements in the Ig and TCR genes and break the DNA double strand there. Several ubiquitously expressed DNA repair proteins (including Ku70, Ku80, DNA-PKcs, XRCC4, DNA ligase I and IV, and Artemis – the last involved in another form of SCID) then mediate the final steps of the V(D)J recombination process.

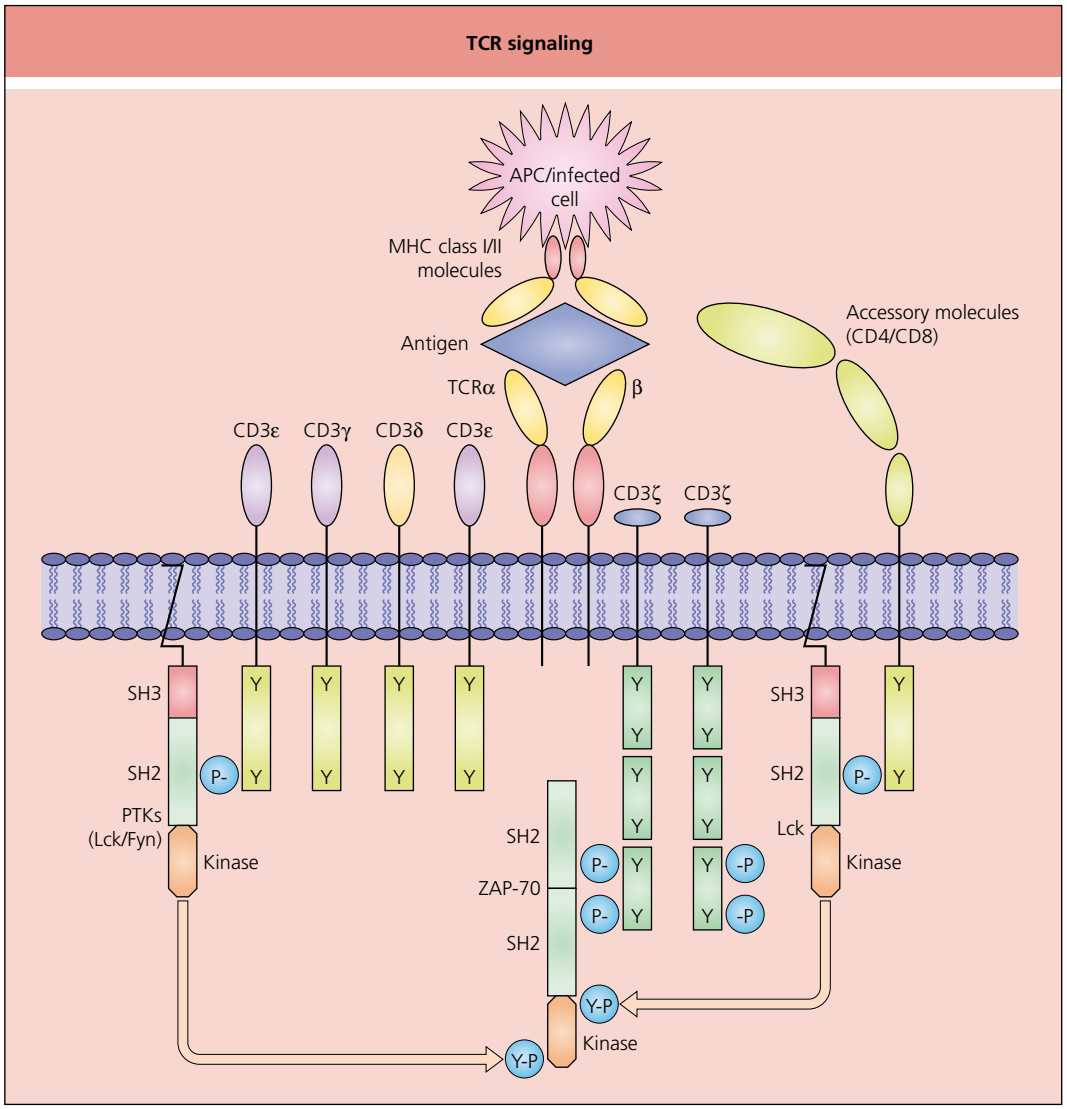


Fig. 72.15 TCR signaling. Stimulation of T cells through the CD3/T-cell receptor (CD3/TCR) complex results in activation of p56lck, an Src tyrosine kinase, that mediates tyrosine phosphorylation of immunoreceptor tyrosine-based activation motifs (ITAMs) in the CD3-γ, -δ, -ε and -ζ chains. ZAP-70, an intracellular tyrosine kinase, is then recruited into the CD3/TCR complex through binding of its SH2 domains to phosphorylated ITAMs of the ζ chain. ZAP-70 itself becomes phosphorylated by Src family protein tyrosine kinases. Phosphorylation triggers ZAP-70 activation, allowing phosphorylation of downstream signaling molecules such as linker for activation of T cells (LAT) and SLP-76.

Complete RAG1/RAG2 deficiency ($T^-B^-NK^+$)

The clinical presentation of complete RAG-deficient SCID (11p13, OMIM #601457) is very similar to the other autosomal recessive forms of SCID with early onset severe respiratory infections, chronic diarrhea leading to failure to thrive, and persistent candidiasis.[61] Frequent transplacental passage of maternal T lymphocytes leads to cutaneous manifestations suggestive of GvHD. The immunologic phenotype is characterized by severe lymphopenia, with virtual absence of T and B lymphocytes. Almost all autologous circulating lymphocytes are NK cells, since they do not require any DNA rearrangement. No specific immunoglobulins can be produced. Curative treatment is bone marrow transplantation.

Omenn syndrome ($T^+B^-NK^+$)

Omenn syndrome (OS, OMIM #603554) affects infants of both sexes who present with a prominent, generalized papular skin eruption or scaling exudative erythroderma, alopecia, enlarged lymph nodes, hepatosplenomegaly, hypoproteinemia with edema, and eosinophilia, associated with severe respiratory infections, chronic diarrhea and failure to thrive[62] (Fig. 72.16). It is an important example of genotype–phenotype correlation in immune function, since this syndrome is clinically distinct from complete RAG1/2 deficiency, but is due to 'leaky' mutations in *RAG1/RAG2*. This clinical phenotype may mimic histiocytosis X of the Letterer–Siwe type, or GvHD, and may occasionally be seen in SCID infants with transplacental passage of alloreactive maternal T cells (referred to as Omenn-like syndrome).

The molecular pathogenesis of OS long remained a mystery. However, the demonstration of a variable number of autologous, oligoclonal, autoreactive, activated T cells in OS infants, and the simultaneous occurrence of OS and T^-B^- SCID in two siblings suggested a genetic relationship. This hypothesis was proven when mutations in *RAG1* and *RAG2* were demonstrated in OS patients.[63] In contrast to T^-B^- SCID, each patient carried at least one mutant allele that allowed expression of a partially functioning RAG protein resulting in a leaky V(D)J rearrangement defect, with reduced, but not abolished, intrathymic T-cell differentiation.

In contrast to other forms of SCID, OS may have leukocytosis with marked eosinophilia and variable (up to normal) T-cell counts. A characteristic finding in OS is the coexpression of activation/memory markers (HLA-DR, CD45RO, CD25, CD95, CD30) on the surface of T lymphocytes, which predominantly secrete T helper (Th)2-type cytokines, such as IL-4 and IL-5. B lymphocytes are typically absent from peripheral blood and lymphoid tissues and immunoglobulin levels are markedly diminished, but IgE is usually elevated. Lymph nodes show lymphoid depletion and an increased proportion of interdigitating reticulum cells, eosinophils and histiocytes. Thymus shows lymphoid depletion, lack of corticomedullary demarcation and lack of AIRE staining, a critical protein for the induction of self-tolerance.[64]

OS is diagnosed by its characteristic features after engraftment of allogeneic T cells has been excluded. Mutation analysis of *RAG1* and *RAG2* confirms the diagnosis and can be used for prenatal diagnosis in affected families. Interferon gamma may reduce the Th2-type T-cell activity and ameliorate the clinical status, but unless treated by BMT, OS is usually fatal within the first year of life. Steroids and cyclosporin A may be used for treatment of the GvHD-like skin reaction.



Fig. 72.16 A baby with Omenn syndrome. Note the diffuse erythema.

Radiation-sensitive SCID (Artemis deficiency; $T^-B^-NK^+$)

Mutations in the RAG genes do not account for all V(D)J recombination defects in T^-B^- SCID patients. A subgroup of patients also show increased cellular radiosensitivity (even in nonlymphoid lineages) due to mutations in *Artemis* (10p, OMIM #602450) that participates in the later phases of V(D)J recombination.[65] This latter form of T^-B^- SCID is relatively common among Athabascan-speaking Native Americans (e.g. Navajo), among whom the incidence is estimated to be approximately 1 in 2000 live births. The encoded protein seems to be involved in the opening of the hairpin necessary to join the selected V(D)J regions. To date, *Artemis* mutations have been severe, resulting in a complete T^-B^- SCID phenotype. However, hypomorphic mutations have been associated with an Omenn-like phenotype and others with lymphomas.[66]

DNA ligase IV deficiency (OMIM #606593) also leads to defects in DNA recombination and radiation sensitivity, known as the LIG4 syndrome.[67] Patients also have developmental delay and chromosomal instability.

Purine metabolic defects

Adenosine deaminase (ADA) and purine nucleotide phosphorylase (PNP) work in series to handle the products of purine metabolism. ADA is ubiquitously expressed and transforms adenosine into inosine and deoxyadenosine to deoxyinosine. PNP then converts inosine and deoxyinosine to hypoxanthine, which can either enter the purine salvage pathway or be excreted as uric acid.

Adenosine deaminase deficiency ($T^-B^-NK^-$)

Adenosine deaminase (ADA) deficiency (20q13, OMIM *102700) is autosomal recessive and has a highly variable disease presentation depending on the degree of residual ADA activity.[68] Complete ADA deficiency presents early in life, with low enzyme activity, high accumulated adenosine levels, marked panlymphopenia without significant lymphoid tissues, and only maternally transferred IgG. SCID-type infections are common. Partial ADA defects may result in less severe clinical presentations with delayed (in infancy) or late (during adolescence or even adulthood) onset. Since the enzyme is ubiquitously expressed, nonhematopoietic organs such as kidney, bone, cartilage and the central nervous system may also be involved. Half of cases have characteristic bony abnormalities including flared, cupped costochondral junctions, best seen on lateral films, leading to a 'rachitic rosary' (bony prominences at the costochondral junctions, reminiscent of the beads of a rosary), similar to that seen in rickets.

Optimal treatment is BMT from an HLA-identical family donor. When this is not available, enzyme substitution treatment with pegylated bovine ADA (PEG-ADA) results in effective and sustained reduction of toxic metabolite levels and immune reconstitution.[69] *ADA* was the first SCID-causing gene to be cloned and ADA deficiency was the

first human disease to be treated with gene therapy, although it was not curative.[70] Significant improvements in vector design, cell targeting and recipient preparation have resulted in improved outcome of gene therapy for ADA deficiency.[71]

Purine nucleoside phosphorylase deficiency

Purine nucleoside phosphorylase (PNP) works downstream of ADA to convert guanosine to guanine and deoxyguanosine to deoxyguanine. PNP deficiency (14q13, OMIM +164050) is an autosomal recessive disease characterized by the accumulation of deoxyguanosine and guanosine (dGTP in particular) that are directly toxic to lymphocytes. The accumulation of these metabolites inhibits ribonucleotide reductase, whose activity is essential to DNA synthesis. Although PNP is widely expressed, its deficiency is particularly deleterious to lymphoid development, especially T cells. Consequently, patients with PNP deficiency experience a dramatic and progressive T-cell lymphopenia during the first years of life, leading to a SCID phenotype.[72] Toxicities of the metabolites also involve the CNS, with symptoms ranging from behavioral problems, low cognitive function, to ataxia or tetraparesis. BMT is the only therapeutic option, since enzyme replacement therapy is not yet available.

DEFECTS IN ANTIGEN PRESENTATION

Major histocompatibility complex class II deficiency

T cells recognize foreign antigens in the context of self-MHC class II molecules expressed by antigen-presenting cells. MHC class II molecules are critical for numerous aspects of immune function, including antibody production, T-cell-mediated immunity, induction of tolerance and inflammatory responses. Therefore, MHC class II deficiency leads to an inability of T cells to recognize foreign antigens that would normally be presented by MHC class II molecules and a combined humoral and cellular immune deficiency. This autosomal recessive immunodeficiency (OMIM #209920), also referred to as the bare lymphocyte syndrome type II (BLS II), is genetically heterogeneous and may be due to mutations in any of four components of transcription factors that control MHC class II gene expression: class II transactivator (CIITA), RFXANK/RFX-B, RFX5 and RFXAP.[73] The hallmark of MHC class II deficiency is the absence of HLA-DR, -DQ and -DP molecules on B cells, monocytes and dendritic cells (which constitutively express HLA class II), and IFN-γ activated cells. $CD4^+$ T-cell counts are low, whereas $CD8^+$ T-cell counts are normal to increased. *In vitro* response to mitogens is often reduced and immunoglobulin levels are low. In the absence of allogeneic BMT, patients generally die between 5 and 18 years. A moderate phenotype has also been described with survival into adulthood.[74]

TAP deficiency

HLA class I molecules are polymorphic cell surface glycoproteins that play an essential role in presenting antigenic peptides to cytotoxic T lymphocytes and in modulating the activity of NK cells that bear HLA class I-binding receptors. HLA class I molecules are composed of a polymorphic heavy chain, encoded by *HLA-A*, *HLA-B* and *HLA-C* genes, associated with the invariant β_2-microglobulin (β_2M). The assembly of HLA class I molecules occurs in the lumen of the endoplasmic reticulum (ER), where they are loaded with peptides derived from the degradation of intracellular organisms or proteins. These peptides are transported into the ER via transporter-associated-with-antigen presentation (TAP) proteins.[75] The TAP complex consists of two structurally related subunits (TAP1 and TAP2), which interact to form a functional peptide transporter system. Defects in either TAP1 or TAP2 result in impaired peptide-HLA class I/β_2M complex formation and cause reduced surface expression of HLA class I molecules.

Patients with defective HLA class I molecule expression due to defects in either TAP1[75] or TAP2[76] have a far less severe clinical phenotype than observed in MHC class II expression. They often have nasal polyposis and bronchiectasis with recurrent sinopulmonary infections caused by *H. influenzae*, *Strep. pneumoniae*, *Staph. aureus*, *Klebsiella* and *P. aeruginosa*. Deep skin ulcers may also occur. The diagnosis is made by demonstration of profoundly low expression of HLA class I molecules on mononuclear cells.

IMMUNE DEFECTS WITH PREDOMINANT IMMUNOGLOBULIN DEFICIENCY

B-cell differentiation is a multistage process regulated by transcription factors which orchestrate a complex scheme of gene activation resulting in specific intracytoplasmic and membrane-bound proteins. While B-cell differentiation in the bone marrow is antigen independent, later stages of B-cell development in the periphery depend upon contact between the B cell and its cognate antigen (Fig. 72.17).

X-linked agammaglobulinemia ($T^+B^-NK^+$)

X-linked agammaglobulinemia (XLA), the prototype humoral immunodeficiency, was first described in 1952 by Bruton (OMIM *300300).[77] The most important clinical features are shown in Table 72.3. A block in B-cell differentiation at the pre-B-cell stage due to a mutated tyrosine kinase of the Src family (named Bruton tyrosine kinase, BTK) leads to a complete lack of circulating B lymphocytes and virtually no immunoglobulin production.[78] XLA should be considered in males with recurrent bacterial infections, low immunoglobulin levels and absent B cells. A positive family history on the maternal side is suggestive. T-cell count and function are usually normal. Diagnosis can be confirmed by analysis of BTK protein expression (immunoblotting or flow cytometry) and DNA mutation analysis. No genotype–phenotype correlation has yet emerged, and this diagnosis can be made later in life. Therapy consists of lifelong immunoglobulin replacement, usually 400–500 mg/kg every 3 weeks. Despite this, patients remain at risk for certain enteroviral infections of the CNS that can be especially devastating. The mutations are listed at http://www.uta.fi/laitokset/imt/bioinfo/BTKbase.

Autosomal recessive agammaglobulinemia

The recognition of females with agammaglobulinemia and the fact that in some kindreds boys and girls are equally affected, led to the search for autosomal recessive causes of agammaglobulinemia, which account for 5–10% of all patients with complete B-cell deficiency.

The clinical picture is similar to XLA, with a somewhat earlier onset and perhaps a more severe progression. The disease is genetically heterogeneous, with defects identified in the genes that encode for the μ heavy chain, the λ5-chain (part of the surrogate light chain), the Igα chain of the membrane expressed pre-B-cell receptor (BCR; involved in signal transduction) and in BLNK, an intracytoplasmic adapter that is phosphorylated by the tyrosine kinase Syk upon BCR signaling.[78]

Common variable immune deficiency

Common variable immune deficiency (CVID) has an incidence of 1:10 000 to 1:100 000 live births and is characterized by low IgG and IgA levels associated with defective specific antibody production in the setting of a normal to low B-cell count. In about 30% of patients, IgM levels are also low. The inability to produce specific antibodies puts patients at risk for frequent bacterial infections, predominantly in the respiratory and gastrointestinal tracts. The clinical picture is often complicated by autoimmune disorders

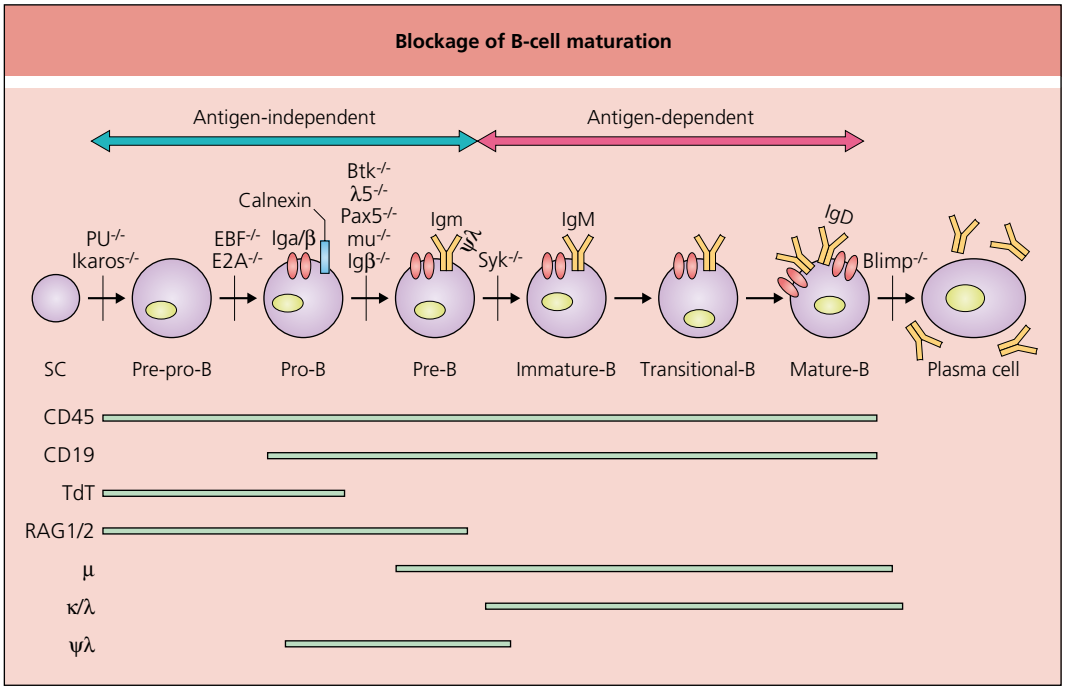


Fig. 72.17 Model of B-cell differentiation with the various proteins involved in the different stages shown below. The vertical red lines indicate a maturation block caused by the molecules indicated above.

Table 72.3 Characteristics of X-linked agammaglobulinemia

- Onset after 6 months of life (when maternal antibodies wane)
- Lack of tonsils and B lymphocytes (<2%)
- Recurrent bacterial infections of the upper and lower respiratory tract
- Risk of development of bronchiectasis
- Gastrointestinal *Giardia lamblia*
- Susceptibility to enteroviral encephalitis (echovirus, poliovirus)
- Susceptibility to arthritis caused by enteroviruses and mycoplasmas

like hemolytic anemia, thrombocytopenia, inflammatory bowel disease and others. There are early-onset (usually between 1 and 5 years) and late-onset (during adolescence) clinical phenotypes, suggesting that there may be different molecular mechanisms underlying CVID.[79] Recent work has identified causative mutations in the *CD19* and *ICOS* genes in a small number of cases. Sequence changes in *TACI* are widely recognized, but their role in CVID is still debated.[80]

Selective IgA deficiency

Selective IgA deficiency (IgAD) occurs in 1:700 live births and is characterized by low IgA levels in the serum (<5 mg/dl) and absent secretory IgA. IgG and IgM levels are normal, but defects in IgG_2 and IgG_4 have been described in a subgroup of patients. Circulating B cells are normal in number. In general, IgA deficiency is a benign disorder, with only 30% of affected patients being susceptible to bacterial infections, usually confined to the respiratory and gastrointestinal tract, or to autoimmune manifestations. A minor defect in IgA production (with levels ≤2 SD), partial IgA deficiency, is characterized by a good prognosis, with a tendency of IgA levels to normalize with age.[81] Little is known about the molecular basis of IgAD or CVID. However, given the fact that both may occur in the same family and that some cases of selective IgA deficiency have developed CVID, a common pathogenic mechanism for both forms of humoral immune defect has been hypothesized. Environmental factors, such as anti-inflammatory and antirheumatic drugs or perhaps viral infections may accelerate the development of IgAD and CVID.

The hyper-IgM syndromes

B cells start out producing IgD and IgM and then switch their antibody production to IgG, IgA or IgE depending on interactions with T cells and other mononuclear cells. Failure to adequately undergo class switch recombination leads to overaccumulation of B cells making IgM and hence the hyper-IgM (HIGM) syndromes.[82] However, IgM elevation is an inconsistent and unnecessary part of the syndrome; what is central is the interaction of B cells with mononuclear cells for the generation of mature antibody and the killing of intracellular pathogens. The four distinct genetic defects identified as HIGM are typically characterized by low IgG and IgA levels, normal to increased IgM and a normal number of circulating B cells. Both X-linked and autosomal forms of the disease are known. The clinical picture is variable but most patients suffer from recurrent bacterial infections of the respiratory and gastrointestinal tracts.[82] Some forms of HIGM convey susceptibility to opportunistic pathogens including *Pneumocystis jirovecii*, *Cryptosporidium parvum* and CMV.

HIGM1, XHIM, CD40L and CD154 deficiency

CD40 ligand (CD40L), encoded by the X-linked *TNFSF5* (OMIM #308230), is predominantly expressed by activated $CD4^+$ T cells. Interaction of CD40L with CD40 on B cells, dendritic cells, monocytes and some activated epithelial cells is necessary for immunoglobulin isotype switching, high affinity immunoglobulin production, somatic hypermutation and the formation of germinal centers in secondary lymphoid organs.[82] In addition, CD40L plays a crucial role in efficient interaction between T cells and

antigen-presenting cells, guiding the adaptive immune response to intracellular pathogens. HIGM1 is one of the two X-linked variants of HIGM. CD40L deficiency usually presents within the first year of life, and neutropenia is common. By the end of the first decade sclerosing cholangitis due to *Cryptosporidium parvum* infection is common. Later in life, the incidence of gastrointestinal tumors is elevated.[83]

The diagnosis is made by demonstration of absent CD40L on activated T lymphocytes and confirmed by mutation analysis of the *TNFSF5* gene. Mutations are catalogued at http://www.uta.fi/imt/bioinfo/CD40Lbase. Despite regular administration of immunoglobulins, mortality is as high as 40% by age 25. BMT should be pursued in appropriate cases. In murine models, unregulated CD40L expression has led to an increased incidence of autoimmune and lymphoproliferative diseases, making optimism for human gene therapy without the authentic regulatory elements premature. Trials with soluble trimeric CD40L are underway.

CD40 deficiency

CD40 is the cognate receptor for CD40L. Consequently, lack of CD40 expression (20q12, OMIM #606843) results in clinical and immunologic features very similar to those seen with CD40L deficiency.[84] Lack of isotype switching, impaired somatic hypermutation, defective memory B-cell generation and lack of germinal center formation are typical features. The diagnosis is made by analysis of CD40 expression and is confirmed by mutation analysis. Similar to X-linked HIGM1, treatment requires regular administration of intravenous immunoglobulin (IVIG), prophylactic antibiotics and use of sterile/filtered water to prevent *Cryptosporidium* spp. infection. Bone marrow transplantation should be considered in view of the poor long-term prognosis. Since CD40 is also expressed on some nonhematopoietic cells, unlike CD40L, patients with defective expression of CD40 are not fully corrected by BMT.[85] The clinical implications of this remain to be determined.

Ectodermal dysplasia with immunodeficiency

Nuclear factor kappa B essential modulator (NEMO, Xq28, OMIM #300291) sits at the juncture of innate immunity, acquired immunity and somatic development.[37] Affected boys have defects in the sweat glands, relatively thin hair, dental abnormalities (conical or peg teeth) and suffer from a wide range of infections such as pneumonia, osteomyelitis and meningitis. Since complete functional absence of NEMO in males leads to intrauterine death, those boys with the NEMO form of HIGM must have some degree of NEMO function due to hypomorphic mutations. In contrast, females heterozygous for nonfunctional mutations have incontinentia pigmenti, a syndrome that may include inflammatory lesions involving the skin, eyes and brain.[86] Skewed X-inactivation in hematopoietic cells in females usually, but not always, avoids manifestation of the immune defect.

Activation-induced cytidine deaminase deficiency

This autosomal recessive form of HIGM is characterized by a pure B-cell defect, with lack of immunoglobulin isotype switching, impaired somatic hypermutation and absence of high affinity antibodies (12p13, OMIM #606258). Activation-induced cytidine deaminase deficiency (AICD) encodes an mRNA editing protein that co-localizes with DNA repair enzymes and is selectively expressed in activated B cells in germinal centers.[87] Clinical symptoms include recurrent respiratory and gastrointestinal infections, but systemic bacterial infections such as meningitis and sepsis are also common. In contrast to the HIGM syndromes involving CD40–CD40L interactions, germinal centers are large, leading to hyperplasia of secondary lymphoid organs (tonsils).

SYNDROME-ASSOCIATED IMMUNE DEFICIENCIES

Immune deficiency may also be part of a broader clinical syndrome. In some of these disorders, signs related to immune deficiency predominate and direct the clinical evolution of the disease; in other cases, immune deficiency is variable and does not represent the most important clinical problem. In spite of their complexity, most of these diseases are monogenic, with the affected gene encoding a protein with pleiotropic functions. Alternatively, as in DiGeorge syndrome, the disease may result from a chromosomal microdeletion.

Immune deficiencies due to DNA repair defects

In addition to Artemis deficiency, immunodeficiencies due to DNA repair include ataxia telangiectasia (AT) and several related disorders.[88] Ataxia telangiectasia is due to mutations in the ataxia-telangiectasia-mutated (*ATM*) gene (11q22–23, OMIM #208900; mutations catalogued at http://ccr.coriell.org/Sections/BrowseCatalog/GeneDetail.aspx?PgId=414&gene=ATM). AT is typically associated with increased chromosome radiosensitivity and defective DNA repair, leading to somatic translocations that involve the T-cell receptor and immunoglobulin loci. Translocation between chromosomes 7 and 14 is particularly common. IgA, IgG_2, IgG_4 and IgE levels are low, and a progressive decline in T-cell number and function is typically observed. Most patients do not survive beyond young adulthood due to lymphomas and leukemia. Neurologic problems are a predominant sign of the disease.

The same clinical phenotype can be caused by mutations in the nearby gene *hMRE11*, which accounts for the so-called AT-like syndrome (OMIM #600814).[89] *hMRE11* encodes a protein involved in the earmarking of DNA double strand breaks. Mutations in *Nbs* (or nibrin) are the cause for the Nijmegen breakage syndrome (OMIM #251260), another immunodeficiency characterized by growth failure, microcephaly and a characteristic bird-like facies. The DNA ligases are also involved in DNA repair, and mutations can cause immunodeficiency.[90]

DiGeorge syndrome; velocardiofacial syndrome

This syndrome (22q11.21–23, OMIM #188400) has a wide range of clinical presentations, including thymus and parathyroid defects (hypocalcemia), overt facial abnormalities, growth failure, neurologic and neuropsychiatric manifestations, and heart defects, such as tetralogy of Fallot.[91] The predominant immunologic feature is a variably low T-cell count predisposing to *Candida* and other infections. The molecular defect lies in a heterozygous microdeletion on chromosome 22 that contains many genes (in 90% of the patients this occurs de novo). A critical gene in this cluster is TBX-1. However, this is a very common syndrome with quite variable expressivity. The frequency in the general population is thought to be 1:3000 live births and it can be transmitted as an autosomal dominant trait.

Wiskott–Aldrich syndrome

The Wiskott–Aldrich syndrome (WAS) protein is expressed selectively by hematopoietic cells. It is involved in the activation-induced reorganization of the cytoskeleton, particularly actin polymerization. Eczema, thrombocytopenia and immunodeficiency are the main features of WAS (Xp11.2;, OMIM #301000).[92] Microthrombocytes (low mean platelet volume) in a male with thrombocytopenia and eczema are highly suspicious, even before the immune deficiency characterized by recurrent bacterial airway infections is manifest. Immunoglobulin responses to polysaccharides are typically impaired and immune globulin replacement is often needed. Later in life there is an elevated incidence of leukemia, lymphoma and autoimmune diseases.

A relatively benign variant of WAS, X-linked thrombocytopenia (XLT), occurs without eczema or immunodeficiency. Most patients with this variant show missense mutations in *WASP* exons 1 or 2 that permit some gene expression. In some less severe cases, thrombocytopenia is intermittent,; however, mean platelet volume is consistently low.

X-linked lymphoproliferative syndrome (Duncan's syndrome)

Patients with X-linked lymphoproliferative syndrome (Xp25, OMIM *308240) show an increased susceptibility to severe manifestations after EBV infections.[93] Hepatitis, lymphomas and/or hemophagocytic lymphohistiocytosis (HLH) kill about 70% of affected males in their first decade. Those who survive, mostly affected by a milder variant, may present with hypogammaglobulinemia. The defective gene, *SH2D1A*, encodes the SLAM-associated protein (SAP), an SH2 domain containing protein expressed selectively in lymphocytes. The mutations are cumulated at http://www.uta.fi/imt/bioinfo/SH2D1Abase. SAP has a regulatory effect on cytokine production and is essential for the cytotoxic activity of NK cells.[93] Herpes viruses other than EBV have also been shown to trigger XLP. Several mutational hot-spots have been identified, but so far no genotype–phenotype correlation has been recognized. BMT should be considered before EBV infection if an HLA-compatible donor is available.

Familial hemophagocytic lymphohistiocytosis

Similar to X-linked lymphoproliferative syndrome, familial hemophagocytic lymphohistiocytosis is an autosomal recessive disease characterized by hepatosplenomegaly, acute bone marrow failure with histiocytic activation, coagulopathy and hypertriglyceridemia, often in response to viral infection. Onset is during the first few months of life; accelerated phases occur repeatedly, often with life-threatening hemorrhagic episodes.[94] The clinical presentation can be very difficult to differentiate from acute infection, as it is often accompanied by fever and hematologic signs of inflammation. The immunologic defect consists in virtually absent NK cytotoxicity due to a functionally deficient perforin (encoded by *PRF1* at 10q21–22), a protein normally released from the intracytoplasmic granules after the membranes of effector NK or T cells have fused to target cells. The resulting prolonged contact between infected and 'intended' cytotoxic cells leads to increased cytokine production that is thought to activate macrophages and histiocytes. Forms of this disease that do not have clear genetic predispositions may be triggered by various infections or by drugs. Accelerated phases of hemophagocytic lymphohistiocytosis (HLH) are true medical emergencies that may be brought into remission by chemotherapy and maintained with immunosuppression; however, they generally require BMT.[95]

Autoimmune lymphoproliferative syndrome (Canale Smith syndrome)

At the core of normal development is the proper coordination of cell death. When cell death occurs in a programmed way (i.e. not due to lysis by infecting agents or necrosis due to toxic events), it is referred to as apoptosis. Proper Fas/Fas ligand (FasL) interaction is critical for normal apoptosis (Fig. 72.18). The correct interaction Fas/FasL leads to the formation of the death inducing signaling complex (DISC), which acts through different caspases to induce mitochondrial damage and finally proteolytic cell death. Fas (CD95), encoded by *APT1* (*TNFRSF6*), is a transmembrane protein with an intracellular death domain. Death signaling requires assembly of homotrimers.

The autoimmune lymphoproliferative syndrome type I (ALPS I) is caused by heterozygous mutations in the receptor Fas that lead to disruption of normal homo-oligomerization resulting in deranged lymphoid homeostasis due to defective apoptosis.[96] In this autosomal dominant disorder, only one in eight homotrimers will have three normal Fas molecules. However, the story remains quite complex, as the mutations in Fas have variable penetrance and expressivity. ALPS usually presents with lymphadenopathy, hepatosplenomegaly and hematologic autoimmunity such as hemolytic anemia, autoimmune neutropenia and immune thrombocytopenia. There is a markedly increased risk (up to 50 times) of lymphoma and leukemia.[97] Diagnostic findings include increased numbers of T cells that lack both CD4 and CD8 molecules, referred to as double negative T cells.

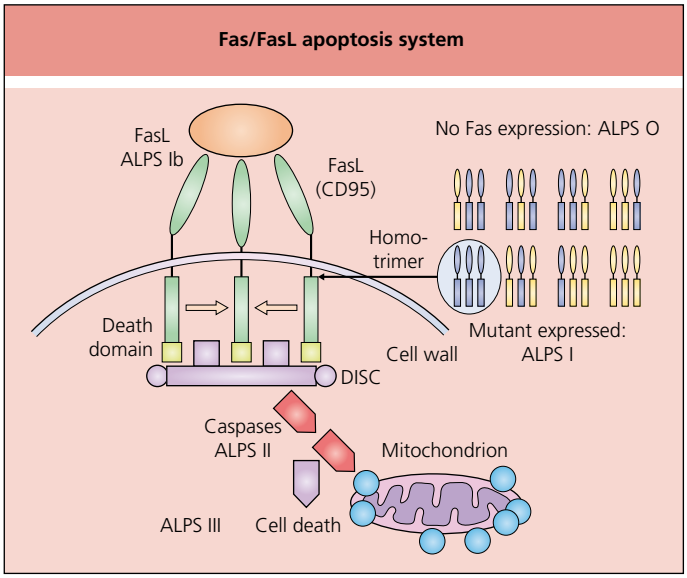


Fig. 72.18 The Fas/FasL apoptosis system and the various forms of ALPS caused by defects in it.

ALPS Ib is caused by mutations in the ligand for Fas, FasL (*TNFSF6*). Defects in certain caspases lead to a similar failure of lymphocyte apoptosis denominated ALPS II. ALPS III is the name currently used for the ALPS phenotype for which genetic defects have not been identified. ALPS-causing mutations have been found to have occurred outside of the germline, indicating somatic mutation leading to ALPS.[98]

IPEX

The clinical entity characterized by *i*mmunodysregulation (anemia, lymphadenopathy), autoimmune *p*olyendocrinopathy (diabetes, thyroiditis), *e*nteropathy and *X*-linked transmission is named IPEX and is due to defects in *FOXP3*, which encodes scurfin, a protein expressed predominantly in the thymus and spleen (OMIM: #304930, #304790).[99] It appears to have a key role in the generation of T regulatory cells, which repress T-cell activation and cytokine expression. Severely affected boys usually die in childhood. There is a somewhat milder phenotype associated with mutations sparing the coding sequence but affecting the polyadenylation signal. Opportunistic infections are not a typical part of IPEX, indicating that this is less a defect in protective immunity than a defect in regulation of immune response and cytokine production. Immunosuppression has proved beneficial in some patients with IPEX, underlining the importance of *FOXP3* in the control of autoreactive cell clones.

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Chapter 73

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Thierry Calandra

Infections in the neutropenic cancer patient

INTRODUCTION

Cancer occurs in one of every four people and is one of the leading causes of death in developed countries. Cancer can be subdivided in two main categories: solid tumors and hematologic malignancies, which include leukemias, lymphomas and multiple myeloma. Solid tumors account for more than 90% of all new cancer cases and hematologic malignancies for the remaining 5–10%.[1] Over the past decades, joint efforts of basic science and clinical research have resulted in substantial improvements of prevention, early detection and treatment of cancer. Indeed, overall 5-year survival rates in cancer patients improved from 39% in the 1960s to 60% in the 1990s.[1] Solid tumors are frequently treated with combined treatment modalities including surgery, radiation therapy and chemotherapy. In contrast, chemotherapy is the cornerstone of the management of patients with hematologic malignancies. New therapeutic options, such as immunotherapy and gene therapy, are being developed.

Infections frequently occur during treatment of cancer. Many factors contribute to increase the risk of infection: poor clinical and nutritional status, mechanical obstruction of natural passages, damage to anatomic barriers (surgery, use of prosthetic and intravascular devices), and defects of humoral and cell-mediated immunity, that are either disease associated or secondary to radiotherapy or chemotherapy. Cytotoxic agents exert their effects on both malignant cells and normally replicating progenitor cells and thus also cause major toxicity to normal tissues with high turnover (i.e. bone marrow and mucous membranes) resulting in myelosuppression and alteration of physiologic barriers. Historically, hemorrhage and infections have been major complications and leading causes of chemotherapy-related mortality (10–20% and 50–80%, respectively).[2] In the 1960s, both the severity and duration of granulocytopenia were identified as major determinants of infectious complications.[3] In the early 1970s, prompt empiric antibiotic treatment became the cornerstone of management of febrile neutropenic patients, resulting in drastic reduction of the mortality of bacterial infections.[4] Since then, major progress has been made in the understanding of the pathogenesis, prevention and treatment of infectious complications of cancer patients. Development of novel diagnostic and treatment strategies continues to improve the outcome of febrile neutropenic cancer patients.[5,6] However, new developments in anticancer therapy, especially new immunomodulating agents, increase the risk of opportunistic infections and represent new challenges for the prevention, diagnosis and management of infectious complications in neutropenic cancer patients.

EPIDEMIOLOGY

The majority of infections in granulocytopenic cancer patients are caused by micro-organisms of the patient's endogenous flora.[5] However, exogenous air-borne and food-borne pathogens, acquired either in the community or in the health-care system, can also cause infection.

Bacterial infections

Gram-positive and Gram-negative bacteria are the predominant pathogens in this clinical setting (Table 73.1).[5] Polymicrobial bloodstream infections, often consisting of mixed aerobic and anaerobic infections,

Table 73.1 Most common pathogens in neutropenic cancer patients

Gram-positive aerobic bacteria
Coagulase-negative staphylococci *Staphylococcus aureus* *Viridans* streptococci Other streptococci (*S. pneumoniae*, *S. pyogenes*) *Enterococcus* species *Corynebacterium* spp. (*C. jeikeium*) *Bacillus* spp. *Listeria monocytogenes*
Gram-negative aerobic bacteria
Escherichia coli *Klebsiella* spp. *Pseudomonas* spp. Other Enterobacteriaceae (*Proteus*, *Enterobacter*, *Serratia*, *Citrobacter* spp.)
Anaerobic bacteria
Bacteroides spp. *Clostridium* spp. *Fusobacterium* spp. *Propionibacterium* spp.
Fungi
Candida spp. *Aspergillus* spp. Other molds (*Fusarium*, *Pseudallescheria boydii*, *Scedosporium*, *Rhizopus*, *Mucor* spp.) *Pneumocystis jirovecii*
Viruses
Herpes simplex virus Varicella-zoster virus Respiratory viruses (influenza, respiratory syncytial virus)
Parasites
Strongyloides stercoralis Other parasites in endemic areas (e.g. *Leishmania*)

occur in 5–20% of cases.[7] In past decades, most cancer centers have experienced major changes in the etiology of bacterial infections in the neutropenic host.[5–8] While Gram-negative bacteria were predominant in the 1970s and early 1980s, the frequency of Gram-positive bacteria markedly increased in the late 1980s and early 1990s, when they became the prevalent pathogens in many institutions. This trend has been recently confirmed by the results of a North American survey.[9] However, this trend reversed in the late 1990s in Europe, where Gram-negative and Gram-positive pathogens now account for an equal proportion of infections (Fig. 73.1).[8]

Many factors are involved in these epidemiologic shifts. The increasing incidence of infections due to coagulase-negative staphylococci and other Gram-positive skin colonizers has been associated with the increased use of intravascular access devices. The emergence of viridans streptococcal infections, sometimes associated with acute respiratory distress syndrome (ARDS) and septic shock, has been attributed to several factors, including the toxicity of high-dose chemotherapy with cytosine arabinoside on oral mucous membranes, the reactivation of oral herpes simplex virus (HSV) infection, and the use of fluoroquinolone prophylaxis.[10] Among Gram-negative bacteria, *Escherichia coli*, *Klebsiella* species and *Pseudomonas aeruginosa* are the most common bloodstream isolates. However, the incidence of *Pseudomonas aeruginosa* infections, a predominant cause of bacteremia in the 1960s and 1970s, has substantially declined over the last 30 years. The use of fluoroquinolone prophylaxis has undoubtedly played a major role in the decreasing incidence of Gram-negative infections observed in the late 1980s and early 1990s.[11]

The recent re-emergence of Gram-negative infections is probably due to the reduced use of fluoroquinolone prophylaxis in many centers out of concern for increased resistance.[12] Some centers have reported the emergence of infections due to multiresistant extended-spectrum β-lactamase (ESBL)-producing Gram-negative bacteria.[13] Superinfections due to *Stenotrophomonas maltophilia*, a nonfermentative Gram-negative microorganism likely to develop *in-vitro* resistance after extensive exposure to broad-spectrum antibiotics including β-lactams, have been associated with poor outcome in neutropenic cancer patients.[14]

Fungal infections

Fungal infections are a major threat to neutropenic cancer patients. Disseminated mycoses have been demonstrated in 10–40% of autopsies in patients with hematologic malignancies, especially in patients who have been treated with broad-spectrum antibiotics and corticosteroids.[2] Classically occurring as secondary infections in patients with prolonged and profound neutropenia, fungal infections also account for approximately 5% of initial infectious episodes.[15] Mixed fungal and bacterial infections may occur and the fungal infection may manifest as persistent fever after eradication of the bacterial pathogen.

Over 90% of all invasive mycoses are caused by *Candida* species (mainly *C. albicans, C. tropicalis, C. glabrata, C. krusei, C. parapsilosis* and *C. pseudotropicalis*) and *Aspergillus* species (mainly *A. fumigatus* and *A. flavus*), which account for 30–80% and 10–60% of cases of fungal infections, respectively. Azole-resistant non-*albicans Candida* species (*C. krusei, C. glabrata*) have emerged in some cancer centers, usually in conjunction with several predisposing factors including fluconazole prophylaxis.[16] Infections due to *A. terreus,* a species with reduced susceptibility to amphotericin B, have been recently reported in some centers.[17] *Fusarium*, *Pseudallescheria boydii*, *Scedosporium* and zygomycetes (e.g. *Rhizopus* and *Mucor*) are emerging fungal infections.[2] The occurrence of zygomycosis has been associated with the use of voriconazole prophylaxis.[18]

Other pathogens

Reactivation of latent herpes simplex virus (HSV) and varicella-zoster virus (VZV) infections are common in patients with hematologic malignancies, especially after chemotherapy or treatment with corticosteroids.[5] In contrast to other immunocompromised patients, especially transplant recipients, cytomegalovirus (CMV) infections play a minor role in neutropenic cancer patients, as acquired immunity is less severely suppressed than innate immunity. Other viral infections such as respiratory viruses (Influenza, Respiratory syncytial virus) and Erythrovirus (formerly Parvovirus) B19 occur occasionally. Primary parasitic infections as well as reactivation of latent infections, in particular those due to *Strongyloides* or *Leishmania* spp., only occur in patients who have lived in or visited endemic areas.

PATHOGENESIS

Underlying conditions

Microbial invasion and development of infection are facilitated by the presence of co-morbidities, immunosuppression and damage to anatomic barriers caused by the cancer itself or induced by chemotherapy

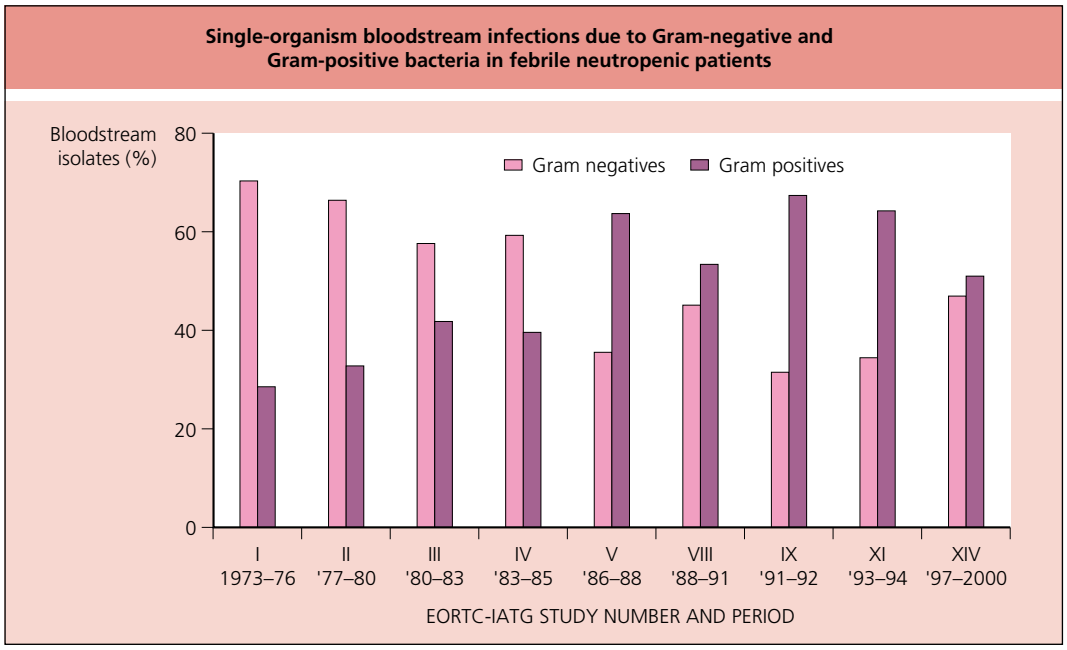


Fig. 73.1 Proportion of single-organism bloodstream infections due to Gram-negative and Gram-positive bacteria in febrile neutropenic patients: EORTC-IATG studies (1973–2000). Adapted from reference[8].

or invasive procedures.[5] Obstruction of the lumen of natural body passages (i.e. urinary, biliary, respiratory or digestive tract) by cancer impairs the flow of body fluids and secretions, creating conditions that promote microbial growth. Cytotoxic chemotherapy damages the epithelial tissue lining, resulting in loss of the integrity of the mucous membrane barrier. Development of mucositis therefore predisposes to infection by the patient's endogenous commensal flora and colonizing pathogens. Injury to the skin by venous puncture, presence of indwelling vascular access devices, bone marrow aspiration, lumbar puncture and other surgical interventions can also promote skin and soft tissue infections.

Defects of innate and acquired immunity

Neutropenia

Phagocytes (neutrophils, monocytes, macrophages and dendritic cells) are a critical component of the host innate immune defenses against infections. Thus, any alteration in function or number of these cells, especially neutrophils, will result in an increased risk of infection.[5] Neutropenia is defined as a neutrophil count <500 cells/mm^3 or <1000 cells/mm^3 with expected decrease to <500 cells/mm^3 within 48 hours. Studies performed in the 1960s have shown that there is an inverse relationship between the number of circulating neutrophils and the incidence of infections.[3] As the neutrophil count decreases to <1000 cells/mm^3, the incidence of infections increases markedly (Fig. 73.2). The risk of severe infectious complications such as bloodstream infections is greatest during agranulocytosis, i.e. when the neutrophil count drops below 100 cells/mm^3.

The duration of neutropenia is also a major determinant of the risk of infection.[3] As shown in Figure 73.2, the longer the duration of neutropenia, the greater the risk of infection. Profound and prolonged neutropenia (i.e. <500 cells/mm^3 for more than 10 days) is considered to be a major risk factor for both primary and secondary bacterial or fungal infections. Patients with neutropenia lasting less than 7–10 days are at low risk of complications.[5] Indeed, 95% of patients with neutropenia lasting less than 1 week respond to the initial empiric antibiotic therapy, while two-thirds of those with neutropenia for more than 2 weeks may require treatment modifications.[5,19] Moreover, the risk of recurrence of fever and infection is also substantially lower (<1%) in patients with a neutropenia of short duration than in those with neutropenia lasting more than 2 weeks (38%).[19] In addition, the risk of death associated with superinfections is five times higher than that of primary infections.[15]

Yet factors other than the severity and duration of neutropenia also help to identify those patients at low or high risk of infectious complications (see Risk assessment pp.12–13).

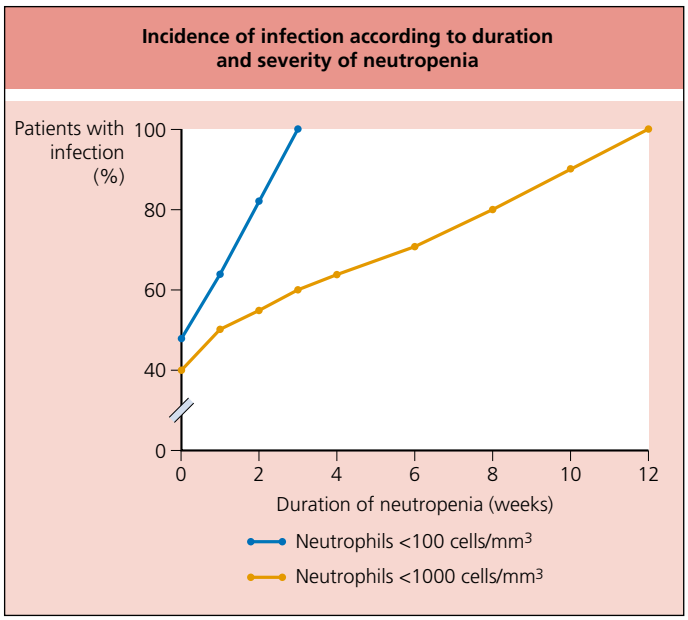


Fig. 73.2 Incidence of infection according to duration and severity of neutropenia. Adapted from reference[3].

Other immune defects

Neutropenia is the main immune defect of cancer patients. In general, defects of the humoral or cell-mediated components of acquired immunity are not predominant in these patients. However, specific immune defects directly associated with the underlying malignancy (e.g. hypogammaglobulinemia in chronic lymphocytic leukemia or multiple myeloma) or its management (e.g. therapy with high-dose corticosteroids) may occur and further increase the risk of infections in conjunction with neutropenia.[5]

New immunomodulating anticancer agents such as rituximab, fludarabine, imatinib, alemtuzumab and temozolomide have become available in recent years and are associated with cell-mediated immune defects, increasing the risk of infection. Temozolomide, a prodrug of dacarbazine, fludarabine, a purine analog and 2-chlorodeoxyadenosine induce selective CD4$^+$ T-cell dysfunction, resulting in an increased risk of opportunistic infections.[20,21] VZV reactivation, *Nocardia* infection and tuberculosis have been reported in patients receiving imatinib, a potent inhibitor of tyrosine kinase. Immunotherapy with alemtuzumab, an anti-CD52 monoclonal antibody, has been associated with increased incidence of viral, bacterial and fungal infections.[22] Combining rituximab with cytotoxic chemotherapy for non-Hodgkin's lymphoma has been associated with an increased risk of bacterial infections.[23]

Genetic immune defects also predispose cancer patients to infectious complications. Deficits in mannose-binding lectin, a critical component of the lectin pathway of complement, have been associated with severe infections during prolonged neutropenia.[24] Polymorphisms of Toll-like receptor 4 have been associated with an increased risk of invasive aspergillosis in allogeneic hematopoietic stem cell transplant recipients.[25] These observations highlight the impact that genetic screening may have in the prevention and management of infections in cancer patients.

CLINICAL FEATURES

Fever

During neutropenia, fever develops in virtually all patients with hematologic malignancies and in about half of those with solid tumors.[5] Although any temperature distinctly above baseline is indicative of fever, fever has been arbitrarily defined as a single temperature >101.3°F (38.5°C) or >100.4°F (38°C) on two or more occasions during a 12-hour period by the Consensus Expert Panel of the Immunocompromised Host Society, or as a single temperature reading >100.9°F (38.3°C) or >100.4°F (38.0°C) during at least 1 hour by the Fever and Neutropenia Guidelines Panel of the Infectious Diseases Society of America.[5] Temperature should be measured orally or by auditory canal probe. More than two-thirds of the febrile episodes are likely to be caused by infection, which may occur with or without focal symptoms or signs. Because of the impaired host response, the classic signs of inflammation (i.e. pain, heat, redness, swelling, and purulent discharge) are often reduced or may even be absent. Therefore, fever is generally the first and frequently the only sign of infection.

Fever occurring in the context of neutropenia is considered to reflect ongoing infection unless proven otherwise. However, there are noninfectious causes of pyrexia, of which the most frequent are the underlying malignancy itself, cytotoxic chemotherapy, transfusion of blood products, antifungal and occasionally other antimicrobial agents,

colony-stimulating factors or allergic drug reactions. Uncommonly, infection may develop in the absence of fever because of the lack of inflammatory response such as during therapy with high-dose corticosteroids or when caused by certain micro-organisms (e.g. *Clostridium septicum*).

Types of infection

Classically, infections have been subdivided into three main categories:[5]

- *Microbiologically documented infections* (MDI), subdivided into those with and without bloodstream infection. Bloodstream infections, caused predominantly by bacteria (bacteremia) and occasionally by fungi (fungemia), may be either primary (in the absence of a nonhematogenous focus of infection) or secondary to a proven focus of infection (e.g. pneumonia, cellulitis, catheter-related infection, urinary tract infection).
- *Clinically documented infections* (CDI) defined by the presence of a site of infection (e.g. pneumonia, cellulitis, oropharyngeal mucositis, enterocolitis, catheter-exit site infection) without microbiologic proof of the nature of infection.
- *Fever of unknown origin* (FUO), also designated as fever of undetermined origin, unexplained fever or pyrexia of unknown origin, and defined as a febrile episode that is not accompanied by clinical or microbiologic evidence of infection.

Figure 73.3 shows the proportions of MDI, CDI and FUO in febrile neutropenic patients with hematologic malignancies (mainly acute leukemia) or solid tumors in four consecutive multicenter studies conducted in the 1990s in Europe, the Middle East and North America.[26–30] Generally, MDI account for approximately 25–35% and 10–20%, CDI for 20–30% and 10–20% and FUO for 40–60% and 50–70% of the episodes of fever occurring in neutropenic patients with hematologic malignancies or solid tumors, respectively.[26–30] Most episodes of MDI consist of bloodstream infections (Fig. 73.4) that occur almost exclusively in patients with profound neutropenia (<100 cells/mm^3).[3,26–28] Although the etiology of fever remains by definition unclear in FUO, the fever was probably of infectious origin if it resolved with antimicrobial therapy.

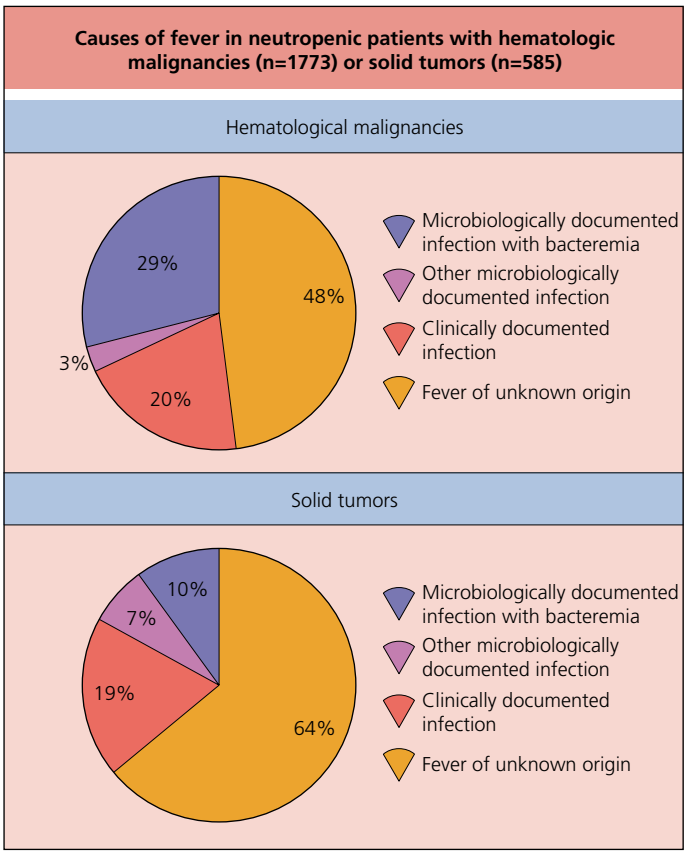


Fig. 73.3 Causes of fever in neutropenic patients with hematologic malignancies (n=1773) or solid tumors (n=585). Data are derived from four consecutive studies of the EORTC-IATG conducted between 1991 and 2000 and from a North American study conducted between 1992 and 1997.[26–30]

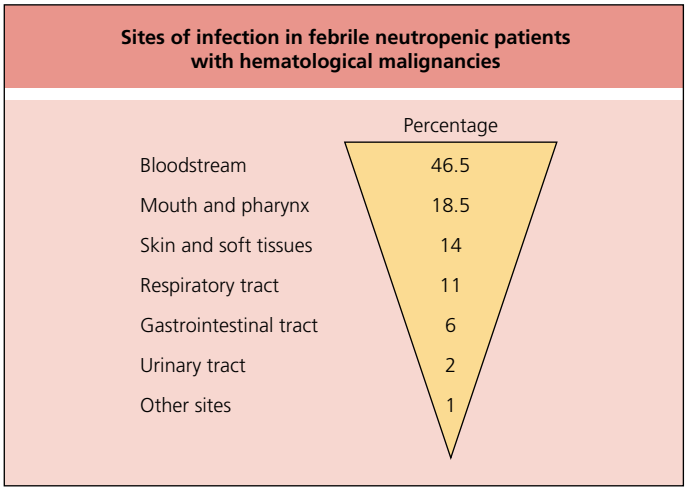


Fig. 73.4 Sites of infection in febrile neutropenic patients with hematologic malignancies (n=889). Data are derived from three consecutive studies of the EORTC-IATG conducted between 1991 and 2000.[26–28]

Sites of infection

In studies conducted in the 1990s the most frequent sites of infection in neutropenic cancer patients with hematologic malignancies or solid tumors with a first episode of fever were, by decreasing order of frequency, the bloodstream, the oral cavity and nasopharynx, the skin and soft tissues, the respiratory tract, the gastrointestinal tract and the urinary tract (Fig. 73.4).[26–28] Although similar data have been reported in recent randomized therapeutic trials, these may not reflect the whole spectrum of the clinical manifestations of infections during neutropenia. Different proportions of the site of origin of infection are expected in selected patient populations and in patients with secondary infections.

Bloodstream

Bloodstream infections account for 80–90% of microbiologically documented infections (Fig. 73.3) and for half of the febrile episodes for which a site of infection can be identified (Fig. 73.4). In primary bloodstream infections, the source remains unknown, but disrupted physiologic barriers (i.e. mucous membranes of the gastrointestinal tract and skin) are the most likely portals of entry. Bacteria are the most frequent blood isolates, accounting for over 90% of bloodstream infections. A single micro-organism is implicated in the majority of bloodstream infections, but polymicrobial infections occur in approximately 5–10% of cases.

As previously mentioned, the epidemiology of single-organism bacteremia in neutropenic cancer patients has changed over the last 30 years and may vary from center to center (see Fig. 73.1). Today, Gram-positive and Gram-negative bacteria cause an equal proportion of infections in many European centers. The most common Gram-positive bacteria are coagulase-negative staphylococci, viridans

streptococci and *Staphylococcus aureus*. Among Gram-negative pathogens, *E. coli*, *Klebsiella* species and other Enterobacteriaceae are predominant, while *P. aeruginosa* has declined progressively (Table 73.1). Resistant bacteria (such as ESBL-producing enteric Gram-negative bacilli or *Stenotrophomonas maltophilia*) or fungi are classically isolated from blood in secondary febrile episodes occurring during antibiotic treatment.[14] In some countries, multiresistant ESBL-producing Gram-negative bacilli have emerged as a frequent cause of infection in neutropenic patients.[13]

Bloodstream infections are potentially life-threatening, but with prompt administration of empiric antibiotics at fever onset, severe complications such as severe sepsis and septic shock rarely occur today. Mortality rates between 1% and 3% have been reported in recent studies in febrile neutropenic cancer patients.

Mouth and pharynx

Maintenance of good oral hygiene and proper dental care are essential for the prevention of oral and systemic infections. Dental septic foci, such as braces and periodontitis, which may promote or facilitate the development of local and systemic infections, should be removed or treated prior to the initiation of chemotherapy. Indeed, infections of the oral cavity (e.g. mucositis, gingivitis, periodontitis) and pharynx occur in 15–25% of neutropenic cancer patients (Fig. 73.4).

The frequency and severity of these infections is correlated with the degree of mucosal damage induced by cytotoxic chemotherapy and with the severity of neutropenia. Mucous membranes of the mouth and pharynx are heavily colonized with viridans streptococci, Gram-positive rods, and aerobic and anaerobic Gram-negative bacilli. Loss of integrity of the mucosal barrier is therefore a major portal of entry for infection. Neutropenic patients with no or minimal mucosal damage are at much lower risk of infection than patients with severe chemotherapy-induced mucositis. *Candida* spp. also play an important role in infections of the oropharynx, especially under the selective pressure of broad-spectrum antibiotics. Oral lesions caused by reactivation of HSV infection may mimic chemotherapy-induced mucositis and also serve as a portal of entry for bacterial infection.

Skin and soft tissues

Cutaneous infections are common in neutropenic cancer patients, causing about 10–20% of all septic episodes for which a source of infection is identified (Fig. 73.4). Primary skin and soft tissue infections often result from a disruption of the integrity of the cutaneous barrier caused by needle punctures (venous or lumbar puncture, bone marrow biopsy) or by the presence of intravascular access devices. The anal and axillary areas are frequent foci of cutaneous infections as moisture, high bacterial colonization, relative skin frailty and microtrauma all facilitate the development of infection. Anal fissures are often complicated by perirectal cellulitis, classically caused by the fecal flora, including Gram-negative bacilli (Enterobacteriaceae, *P. aeruginosa*), enterococci and anaerobic Gram-negative bacilli. Axillary hidradenitis is a classic infection of neutropenic hosts caused by skin commensals and Gram-negative bacteria, including *P. aeruginosa* (Fig. 73.5a).

Disseminated cutaneous infections may reflect the development of septic skin foci in patients with bacterial, fungal or viral bloodstream infections. Clinically, these skin lesions appear as papules or nodules (Fig. 73.6), which are usually associated with classic symptoms and signs of sepsis (i.e. fever, chills, headache, backache, myalgia and muscle tenderness). Ulcers, vesicles, hemorrhagic or crusted lesions that are either isolated (with or without dermatome distribution) or disseminated are typically associated with herpetic skin infections, but may occasionally occur with staphylococcal or streptococcal infections.

A bacterial (e.g. *Pseudomonas* and *Aeromonas* spp.) or fungal etiology (*Fusarium*, *Aspergillus*, *Mucor* and *Rhizopus* spp.) should be suspected in cases of disseminated necrotic skin lesions. For example, ecthyma gangrenosum is a classic cutaneous complication in patients with *Pseudomonas* sepsis (Fig. 73.5b). Necrotizing fasciitis and metastatic musculoskeletal infections complicating bacteremia or fungemia have also been reported, albeit infrequently (Fig. 73.7a).

Intravascular access devices

Indwelling intravenous catheters, especially those inserted for prolonged periods of time (e.g. Broviac, Hickman, Port-a-cath) are a major source of infections, which most commonly arise at the exit site, but may also occasionally affect the tunneled section of the catheter. Clinical manifestations include pain, erythema and tenderness with no or minimal swelling during marrow aplasia. Microbes of the skin flora (i.e. coagulase-negative staphylococci, *Propionibacterium*, *Corynebacterium* and *Bacillus* spp., and viridans streptococci), *S. aureus*, Gram-negative bacteria and *Candida* species are the most frequent pathogens. When skin necrosis occurs, infections due to *P. aeruginosa*, mycobacteria (especially *M. chelonae* and *M. fortuitum*) or to molds (*Aspergillus, Fusarium* or *Mucor* spp.) should be suspected. Infection of intravascular access devices may be complicated by septic thrombophlebitis.

Respiratory tract

Respiratory infections account for 10–15% of the identified sites of infection in febrile neutropenic cancer patients (Fig. 73.4). Infections of the upper respiratory tract (sinusitis, otitis, epiglottitis, laryngitis and tracheitis) are uncommon, occurring in only 1% of episodes. Sinusitis and otitis are mainly due to common community-acquired

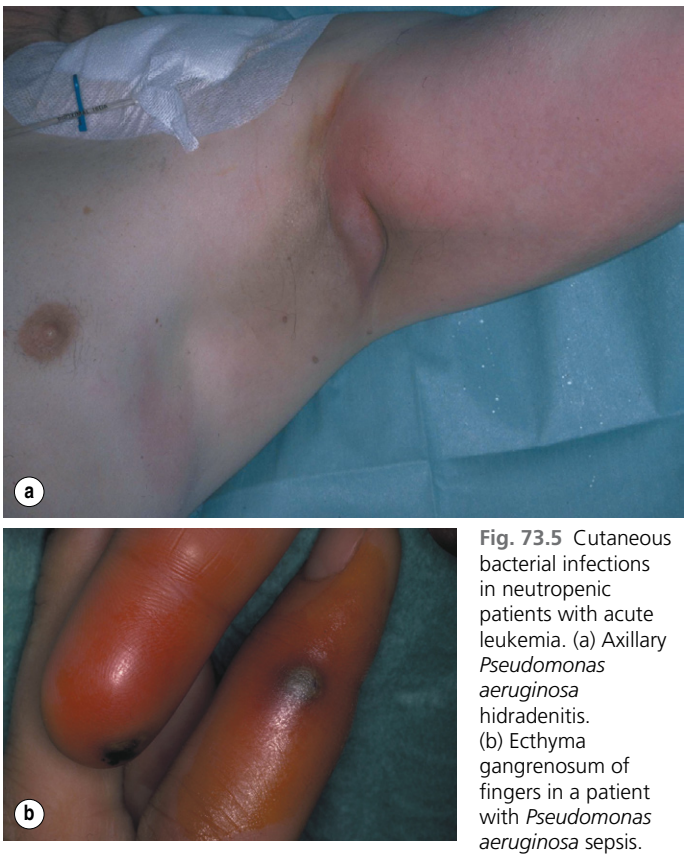


Fig. 73.5 Cutaneous bacterial infections in neutropenic patients with acute leukemia. (a) Axillary *Pseudomonas aeruginosa* hidradenitis. (b) Ecthyma gangrenosum of fingers in a patient with *Pseudomonas aeruginosa* sepsis.

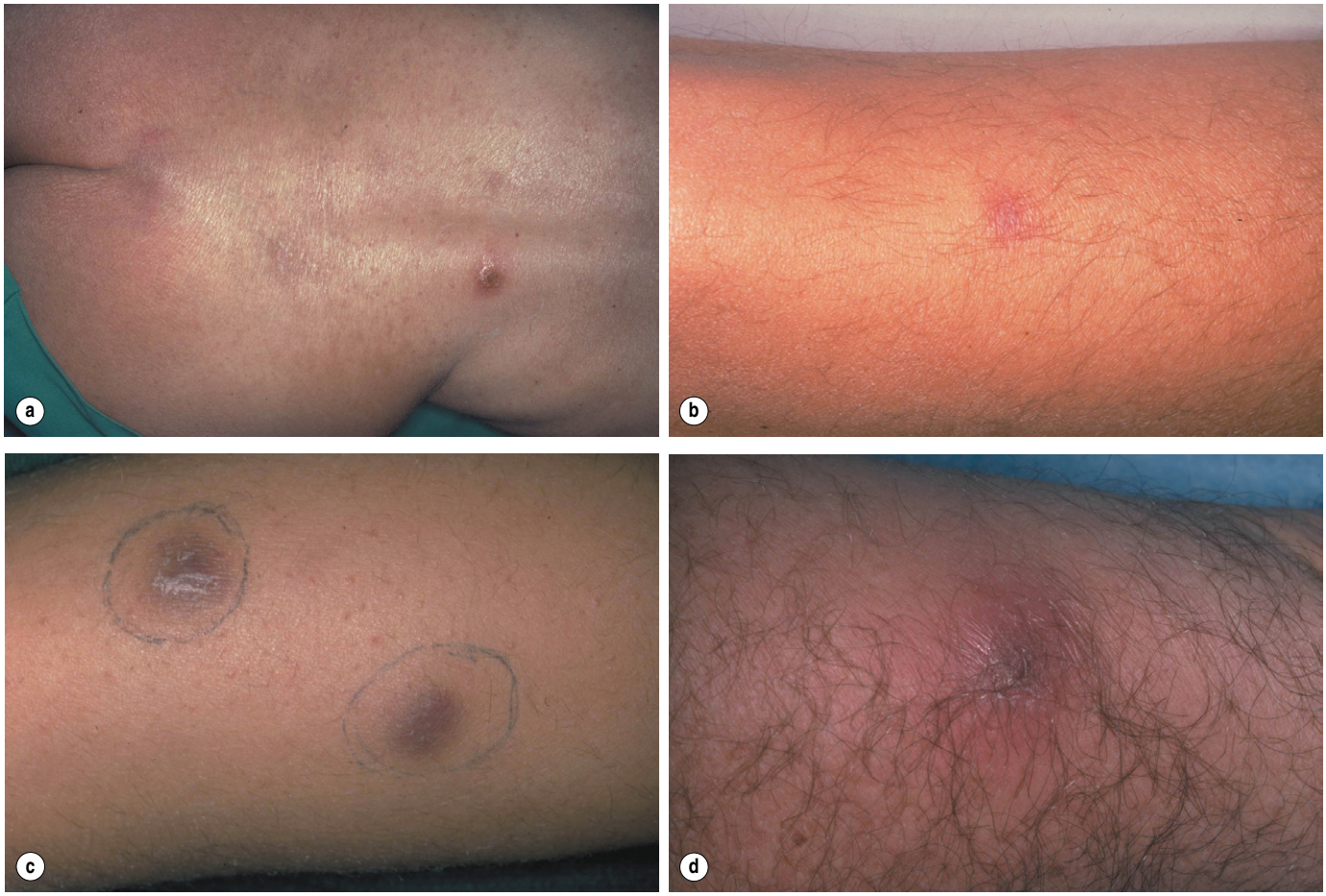


Fig. 73.6 Cutaneous manifestations of disseminated fungal infections in leukemic patients. (a) *Aspergillus terreus*, back. (b) *Candida tropicalis*, arm. (c) *Fusarium*, leg. (d) *Pseudallescheria boydii*, leg.

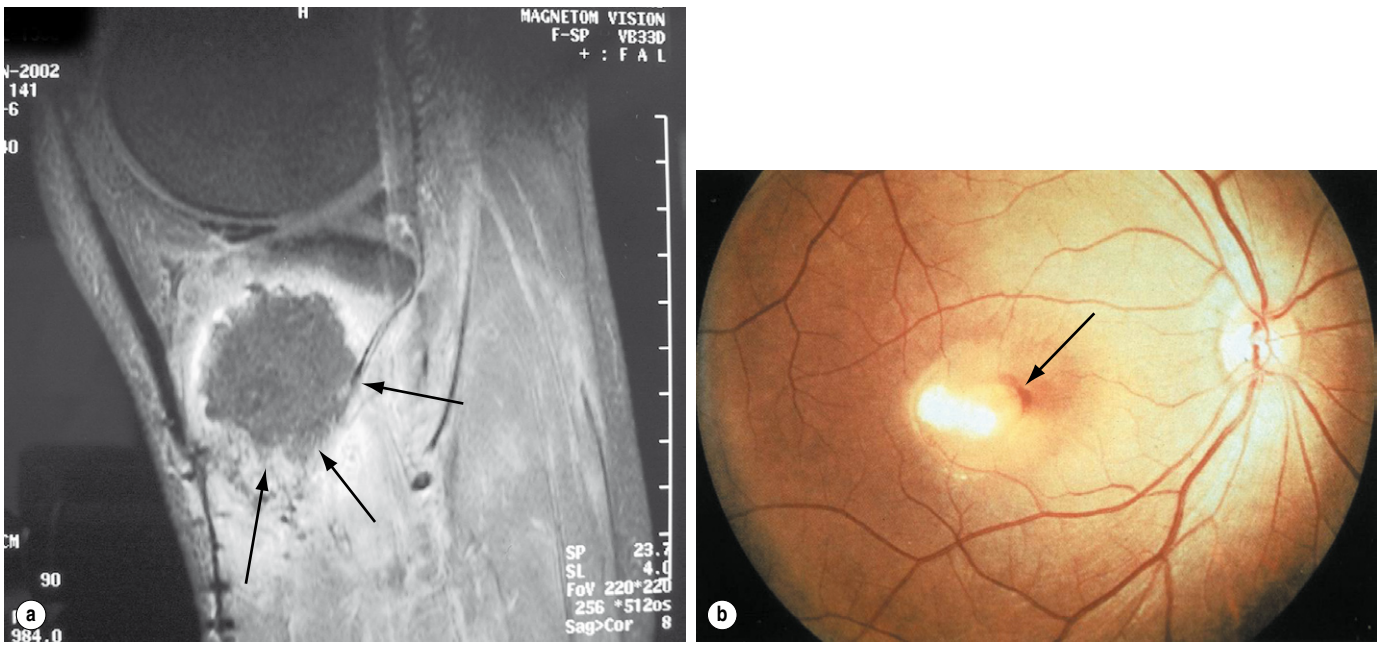


Fig. 73.7 Disseminated *S. aureus* infection in a leukemic patient. (a) Muscular abscess, MRI. (b) Retinal infectious lesion with macular involvement and secondary bleeding (arrow), fundoscopy.

bacteria, but infections due to *Pseudomonas* species and anaerobes can occur. Fungal (*Aspergillus*, *Mucor*, *Rhizopus* spp.) sinusitis is a severe and potentially life-threatening infection of patients with profound and prolonged neutropenia. It is often invasive and can infiltrate the nearby bone and skin and soft tissue structures of the orbit and extend into the central nervous system (Fig. 73.8).

Most frequently, lower respiratory tract infections (bronchopneumonia and pneumonia) are primary infections and account for about 10% of documented infections. However, they may occasionally be secondary to bloodstream infections. Due to the impaired inflammatory response, the classic symptoms and signs of pneumonia (such as fever, cough, dyspnea, chest pain, sputum and radiologic infiltrates) are often attenuated or delayed, and present in only half to two-thirds of the patients. Patients with profound neutropenia (<100 cells/mm^3) rarely produce sputum. The proportion of infections due to pneumonia tends to be lower in the first febrile episodes than in subsequent ones. Moreover, early-onset pneumonia is more likely to be of bacterial origin, while late-onset pneumonia is more likely to be due to fungi or other opportunistic pathogens (Figs 73.9, 73.10a). Respiratory viruses (respiratory syncytial virus, influenza virus, parainfluenza virus, adenovirus) are rare, but may be associated with severe morbidity and mortality (Fig. 73.11a).

Another classic pulmonary complication in febrile neutropenic cancer patients is the development of adult respiratory distress syndrome after viridans streptococcal bacteremia (Fig. 73.11b). This syndrome occurs despite appropriate antibiotic therapy and is associated with important morbidity (i.e. respiratory failure requiring mechanical ventilation) and high mortality.[10]

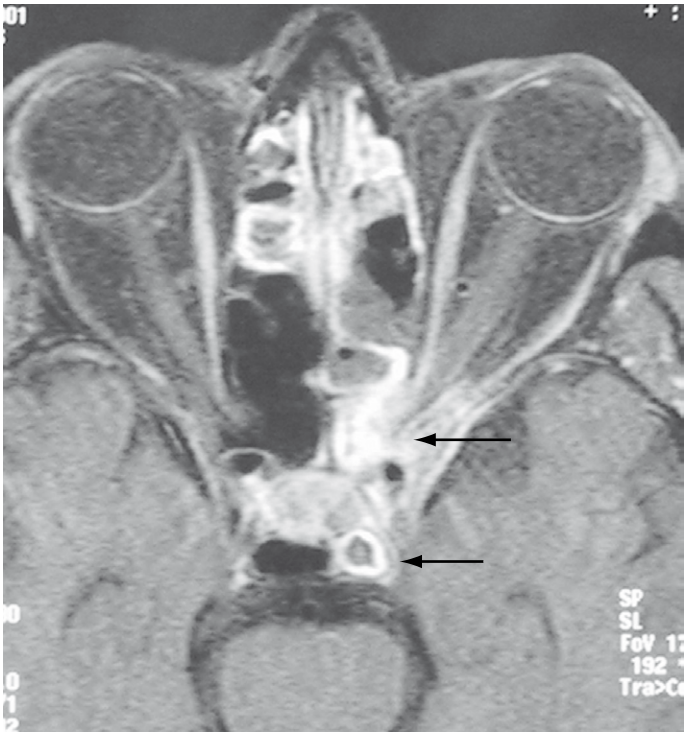


Fig. 73.8 Ethmoidal sinusitis due to *Aspergillus fumigatus* in a leukemic patient. The MRI shows bone destruction with invasion of the orbit, optical nerve (upper arrow) and central nervous system (lower arrow).

Gastrointestinal tract and intra-abdominal organs

The gastrointestinal tract is the largest reservoir of micro-organisms of the human body and the endogenous gastrointestinal flora plays an important role in the pathogenesis of infection in the neutropenic cancer patient. Extended chemotherapy-induced mucosal damage is therefore a major portal of entry of systemic infections.

The gastrointestinal tract itself accounts for 4–8% of documented infections (Fig. 73.4). Esophagitis and enterocolitis are the two most frequent gastrointestinal infections in the neutropenic host. Esophagitis presents with severe retrosternal pain and dysphagia and is usually caused by the commensal bacterial flora, *Candida* species or reactivation of HSV. Symptoms and signs of enterocolitis include nausea, vomiting, bloating, abdominal discomfort, cramps, pain, constipation or diarrhea (often severe in the case of antibiotic-associated colitis). Typhlitis, a life-threatening necrotizing enterocolitis affecting the cecum or other bowel segments, typically occurs in patients with severe and prolonged neutropenia postchemotherapy for acute leukemia (Fig. 73.12).[31] The clinical manifestations are fever, abdominal pain (initially localized to the lower right abdominal quadrant, but becoming diffuse as the infection progresses) and diarrhea, which are often associated with a profound alteration of the patient's

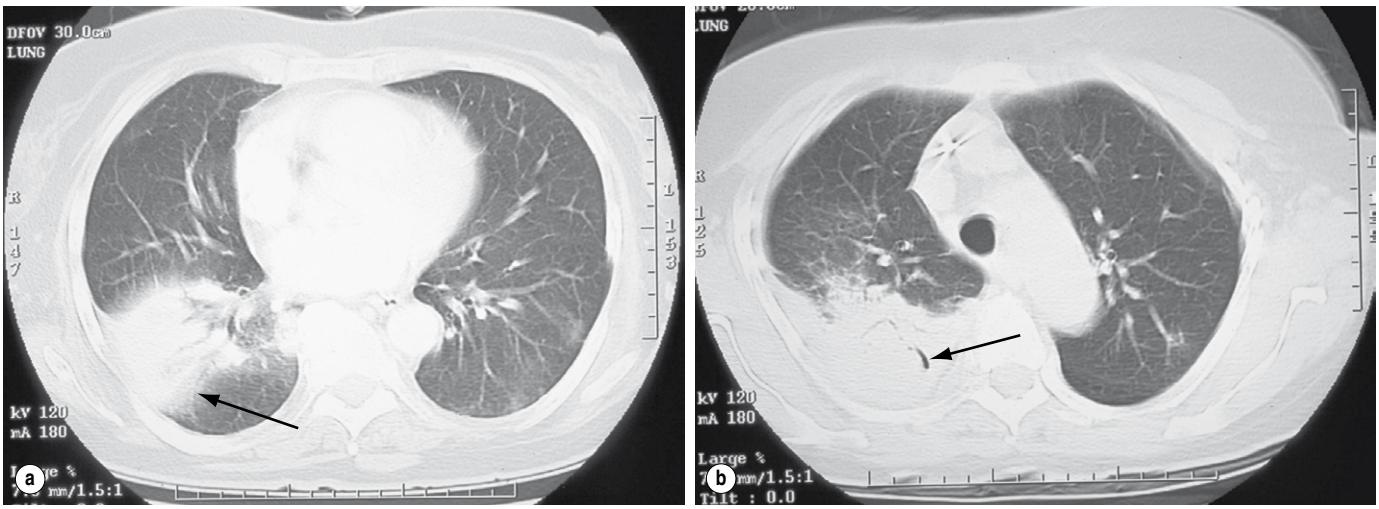


Fig. 73.9 Invasive pulmonary aspergillosis in a patient with acute lymphoblastic leukemia. (a) Early stage with halo sign (arrow) during neutropenia, CT scan. (b) Late stage with air crescent sign (arrow) after bone marrow recovery, CT-scan.

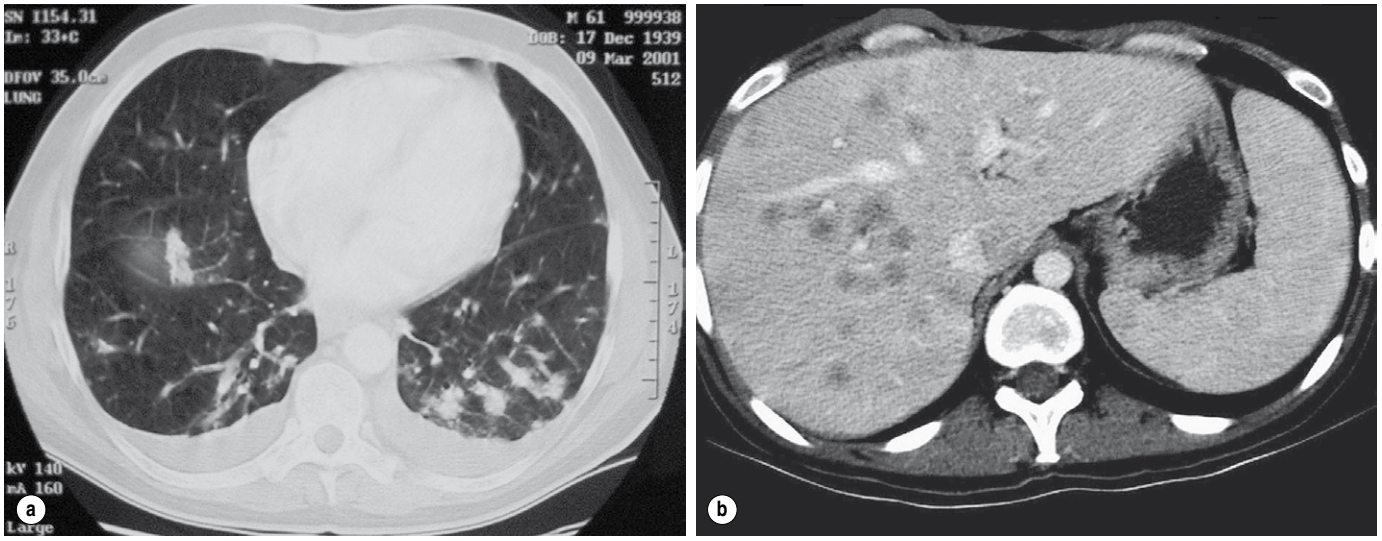


Fig. 73.10 Disseminated candidiasis in a patient with acute myelogenous leukemia. (a) Multifocal nodular lung lesions, CT scan. (b) Multiple hepatosplenic abscesses, CT-scan.

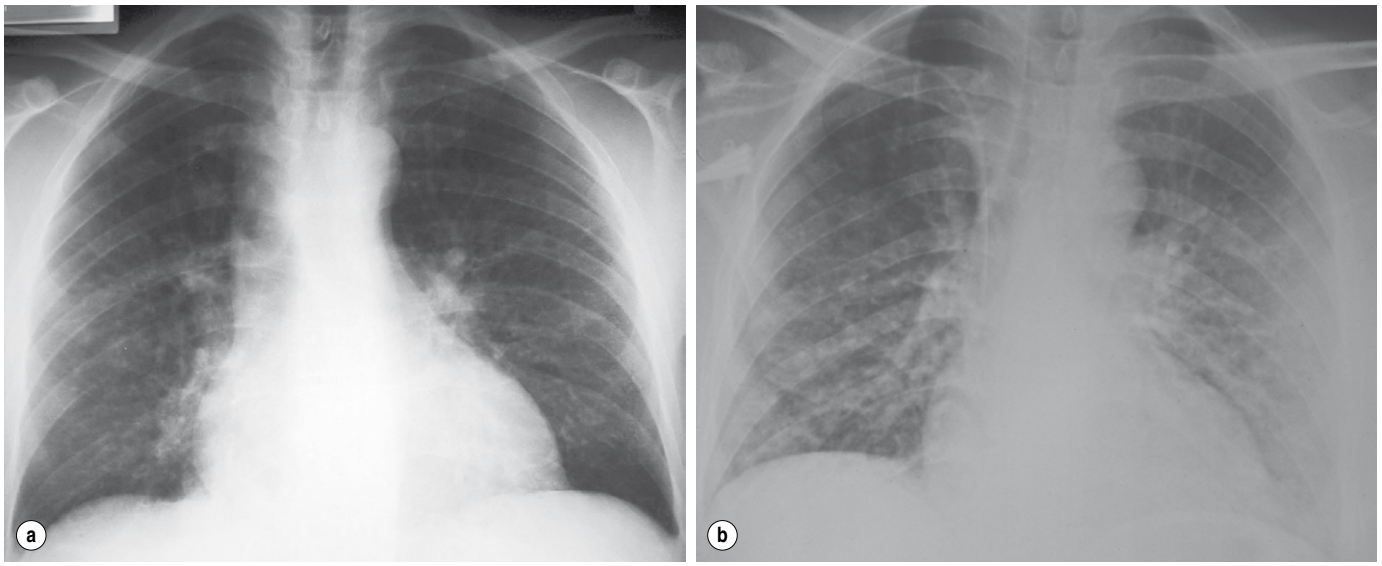


Fig. 73.11 Pulmonary infections in neutropenic patients with hematologic malignancies. (a) Interstitial pneumonia due to Influenza virus. (b) Viridans streptococcal bacteremia with acute respiratory distress syndrome.

clinical condition. Typhlitis is caused by the endogenous gut aerobic and anaerobic bacterial flora, but yeasts and molds may also be implicated.

In patients with neutropenic enterocolitis, the thickness of the intestinal wall as assessed by radiologic imaging has been correlated with morbidity and mortality.[32] Bleeding and perforation are the two major complications. Necrotizing fasciitis and septic shock can also occur, especially with clostridial infection, notably with *C. septicum*. Antibiotic-associated colitis due to *C. difficile* is a frequent cause of profuse diarrhea in neutropenic cancer patients exposed to multiple and prolonged antibiotic treatments. Severe complications such as bleeding, perforation or toxic megacolon occur in 5–10% of cases.[33]

Bacterial liver abscesses and viral hepatitis are rare in neutropenic cancer patients. Hepatosplenic candidiasis, a disseminated fungal infection mainly observed in patients with acute leukemia, classically becomes manifest at the time of marrow recovery after a prolonged period of neutropenia. Diagnosis should be suspected in patients with persistent fever, lack of appetite, abdominal discomfort or frank pain, hepatosplenomegaly and elevated alkaline phosphatase.[34] Imaging techniques – ultrasonography, CT or MRI – show typical multiple target-like or bull's-eye lesions of the liver and spleen (Fig. 73.10b).

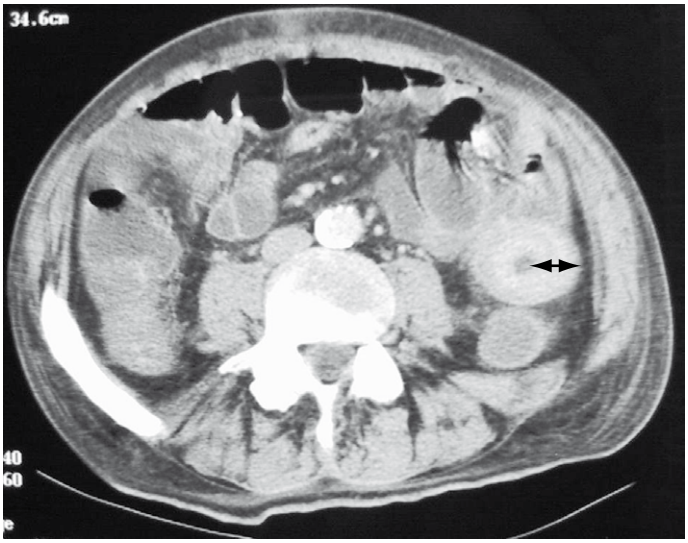


Fig. 73.12 Neutropenic enterocolitis in a leukemic patient. Prominent thickening of a segment of ileum (arrow), CT-scan.

Urinary tract

Urinary tract infections are rare (1–3%) in neutropenic patients (Fig. 73.4) and are mainly due to common uropathogens, such as Enterobacteriaceae. They occur with minimal symptoms, and dysuria is frequently absent as is pyuria because of neutropenia. Thus, diagnosis often relies on a positive urine culture in a febrile patient without evidence of another site of infection.

Other sites

Infections of the central nervous system, eye, heart, vasculature and bone are uncommon causes of sepsis in neutropenic patients with hematologic malignancies and solid tumors (1% of all episodes, Fig. 73.4). Infections of the central nervous system comprise bacterial meningitis and fungal or parasitic focal brain lesions. Acute primary bacterial meningitis occurs at a frequency similar to that of the general population. Bacterial meningitis also may occur in the context of bloodstream infections or as a complication of intrathecal chemotherapy. Infectious foci in the retina are observed in patients with disseminated bacterial and fungal infections (Fig. 73.7b). Endocarditis may occur even in the absence of risk factors, but is notably uncommon despite the frequency of bacteremia with organisms known to cause endocarditis. Clavicular osteomyelitis may be seen in patients with tunnel infections of subclavian intravascular access devices.

PREVENTION

Environmental measures

Preventive measures aimed at reducing the acquisition or transmission of nosocomial pathogens play an important role in reducing the risk of infection in neutropenic patients.[5] Special emphasis should be placed on careful hand washing by personnel, which can substantially diminish the risk of transmission of pathogens. Education of patients, family members, medical and nursing staff is essential. Contact with persons with overt respiratory or cutaneous infections must be avoided.

Patients should receive a well-cooked and low-microbial food diet and have access to safe water and ice supply. For patients with severe and prolonged neutropenia, air ultrafiltration may be desirable in settings where *Aspergillus* infections are frequently observed. When an optimal protective environment is required, clean air is provided by means of constant positive-pressure air flow and/or high-efficiency particulate air filtration. In addition, intensive disinfection measures are generally employed in high-risk patients. These include the use of antimicrobial mouth wash solutions, disinfectant soaps, creams and sprays. Proper oral and dental hygiene and careful nursing of the anal region are essential. Insertion and manipulations of intravascular access devices and bone marrow aspiration should be performed with great care and under strict aseptic conditions. Whenever possible, the use of nasogastric tubing and urinary catheters should be avoided.

Surveillance cultures of oropharynx, stools, urine and skin are of limited utility for the management of individual neutropenic patients, as they lack sensitivity and specificity for predicting and identifying the etiologic agent of infection and are costly and time-consuming. However, these cultures might be useful for epidemiologic studies and infection control purposes.

Antimicrobial prophylaxis

Prophylactic antimicrobial therapy has been used to suppress colonization by potential pathogens in high-risk neutropenic patients. Different strategies have been employed to prevent bacterial and fungal infections and reactivation of latent viral infections with mixed results. Thus, considerable controversy still surrounds the topic of antimicrobial prophylaxis for the prevention of infection in the neutropenic host.

Antibiotic prophylaxis

Oral nonabsorbable antibiotics have been used to achieve gut decontamination, since the bowel is the main reservoir of endogenous flora and an important source of infection in granulocytopenic cancer patients. However, the use of antibiotics such as vancomycin, gentamicin, polymyxin B, nystatin or colistin is associated with several problems. These antibiotics are nonpalatable and poorly tolerated, so that compliance is poor. Clinical efficacy has not been clearly demonstrated. Furthermore, administration of prophylactic oral antibiotics may induce resistance among endogenous bacteria, such as resistance to vancomycin among enterococci. Thus, prophylaxis with nonabsorbable antimicrobial agents has been abandoned in most institutions.

Alternatively, attempts have been made to 'selectively' decontaminate the alimentary tract using antibiotics such as trimethoprim–sulfamethoxazole (TMP-SMX; co-trimoxazole) to maintain the anaerobic flora, thereby preserving the 'colonization resistance' against aerobic bacteria and fungi. Results of initial studies suggested that TMP-SMX could decrease the incidence of infections, but this was not confirmed in subsequent studies. Furthermore, TMP-SMX is ineffective against *P. aeruginosa*, may prolong the duration of the bone marrow aplasia, and promote the emergence of resistant organisms. Current guidelines recommend the use of TMP-SMX only in patients at high risk of *Pneumocystis jirovecii* (formerly *P. carinii*) infection.[5]

In contrast to all other antibiotics used for prophylaxis of bacterial infections, fluoroquinolones have been unequivocally shown to reduce the incidence of Gram-negative infections, but the impact on the occurrence of fever, microbiologically or clinically documented infections, or infection-related mortality is less clear. The results of four meta-analyses are summarized in Figure 73.13.[35] Moreover, the use of fluoroquinolone prophylaxis was associated with an increased incidence of viridans streptococcal infections and emergence of resistance.[10,12] Levofloxacin, a fluoroquinolone with extended anti-Gram-positive activity, was recently found to reduce the incidence of febrile neutropenia when used as prophylaxis in patients with hematologic malignancies or solid tumors. The number needed to treat to prevent one febrile episode was 5 and 23, respectively.[36,37] However, the impact of levofloxacin prophylaxis on the morbidity and mortality associated with febrile neutropenia is limited, may vary from center to center and is in fact marginal in solid tumor patients.[38] Based on these data and on a recent meta-analysis, fluoroquinolone prophylaxis has been recommended for prevention of infection in patients with

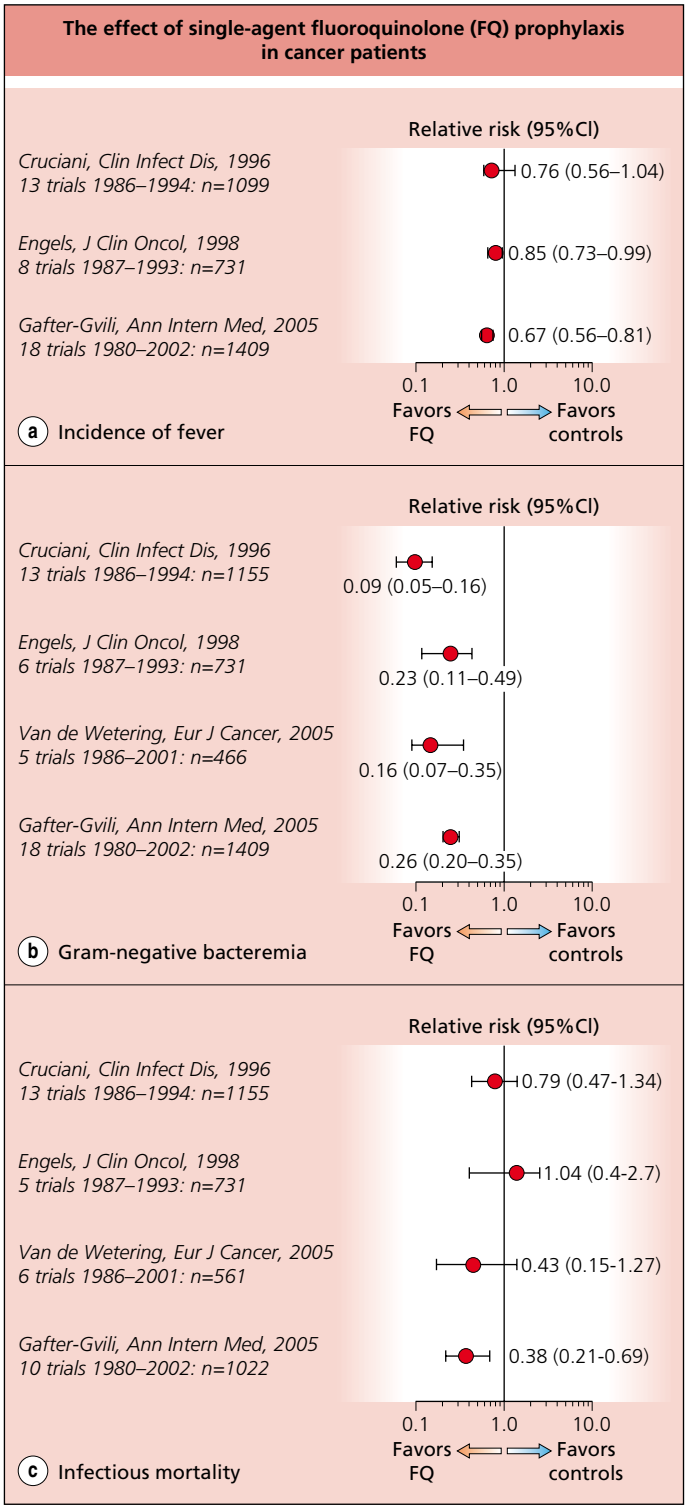


Fig. 73.13 Meta-analyses of the effect of single-agent fluoroquinolone (FQ) prophylaxis compared with that of placebo or no prophylaxis on the incidence of fever (a), Gram-negative bacteremia (b) and infectious mortality (c) in neutropenic cancer patients with hematologic malignancies. Red circles and black bars show relative risks with 95% confidence intervals in patients receiving fluoroquinolone prophylaxis compared to patients receiving placebo or other regimens. Adapted from references[11,35,40,81].

Table 73.2 Arguments in favor and against the use of fluoroquinolones and azoles for prophylaxis of infections in neutropenic patients

In favor	Against
Prevention of documented bacterial and fungal infections	Severe morbidity and mortality of bacterial and fungal infections infrequent in many centers
Prevention of febrile neutropenia	Exposure of large number of patients to routine antibacterial or antifungal prophylaxis: toxicity, resistance, costs
Reduced use of empirical antibacterial and antifungal therapy	Prophylaxis precludes use of the same class of agents for empirical therapy
Prevention of severe complications and mortality	New management strategies allow a targeted use of anti-infective agents

acute leukemia and haemopoietic stem cell transplantation (HSCT) recipients by experts of the first European Conference on Infections in Leukemia (ECIL-1).[39,40]

Clinicians remain divided on the utility and safety of antibiotic prophylaxis. While some consider that it should be strongly discouraged for the reasons mentioned above, others favor the use of fluoroquinolones, especially in high-risk patients with hematologic malignancies. Of note, once used as prophylaxis, fluoroquinolone antibiotics can no longer be used for empiric therapy of febrile neutropenia. This is a significant drawback for solid tumor patients in whom these agents are a key component of oral empiric regimens for outpatient therapy. The decision to use prophylaxis in a single center should therefore be based on a comprehensive evaluation of multiple factors including the frequency of severe complications. While this strategy may be efficacious in some settings, it may be of marginal utility in others. Arguments in favor and against the use of antibacterial prophylaxis are summarized in Table 73.2.

Antifungal prophylaxis

There is a lack of evidence suggesting a benefit of oral nonabsorbable antifungal agents and this practice has been abandoned in many institutions. In allogeneic hematopoietic transplant recipients, systemic antifungal prophylaxis with fluconazole (400 mg/d) has been shown to prevent infection with *C. albicans* and *C. tropicalis*, but not with *C. krusei*.[41] The activity of fluconazole against *C. glabrata* is unpredictable.

The utility of antifungal prophylaxis in patients with hematologic malignancies not undergoing bone marrow transplantation is less clear. Prophylaxis with ketoconazole, intravenous amphotericin B, fluconazole or itraconazole was found to reduce the incidence of superficial infections, but no consistent effects were observed on the incidence of invasive fungal infections, empiric use of amphotericin B or mortality of invasive fungal infections.[42] In some centers, the use of fluconazole prophylaxis was linked to a shift towards non-*albicans* *Candida* strains with either reduced *in-vitro* susceptibility (*C. glabrata*) or intrinsic resistance (*C. krusei*) to azoles, which may be associated with increased mortality.[16,43] Micafungin, a member of the echinocandin class of antifungal agents with activity against *Candida* and *Aspergillus* spp., was found to be superior to fluconazole for a composite end point (i.e. absence of suspected invasive fungal infection requiring empiric antifungal therapy and absence of documented breakthrough invasive mycoses) in hematopoietic stem cell transplant recipients.[44] However, this study failed to demonstrate a significant difference in the incidence of invasive mycoses or in mortality.

Few data are available on the use of other echinocandins (e.g. caspofungin, anidulafungin) as prophylaxis. Posaconazole, a new triazole with activity against *Candida* and *Aspergillus* spp. and emerging molds such as zygomycetes, was superior to fluconazole or itraconazole for the prevention of invasive fungal infections and reduced

fungal-related deaths in allogeneic hematopoietic transplant recipients with graft-versus-host disease and in patients with acute leukemia and neutropenia.[45a, 45b]

Despite these overall encouraging results, antifungal prophylaxis remains a matter of great debate among experts. The arguments in favor and against antifungal prophylaxis are summarized in Table 73.2.[18,46] Antifungal prophylaxis is reasonable in selected high-risk conditions such as allogeneic hematopoietic stem cell transplantation or induction chemotherapy for acute leukemia. The choice of the antifungal agent (coverage of *Candida* or of both *Candida* and *Aspergillus*) should be guided by local epidemiologic data and by the patient's condition.[5]

Antiviral prophylaxis

Viral serology tests should be performed at the onset of chemotherapy to determine whether patients with hematologic malignancies have been previously infected with HSV, VZV or CMV as these may reactivate during therapy. Reactivation of HSV infection occurs in 70–80% of patients with acute leukemia receiving chemotherapy. Aciclovir prophylaxis is therefore recommended for HSV-seropositive individuals.[47] Oral valaciclovir is an alternative to oral aciclovir. Patients unable to take oral medications should be switched to intravenous aciclovir. CMV-seronegative patients with leukemia who may benefit from allogeneic hematopoietic stem cell transplantation should receive leukocyte-depleted blood products to prevent CMV transmission. Those interested in further reading on antiviral prophylaxis should refer to Chapter 146.

Immunoglobulins

Replacement therapy with intravenous immunoglobulins may be indicated for patients with low serum concentrations of immunoglobulins (e.g. IgG <50% of the lower normal limit or <4 g/l) or a history of severe bacterial infections in the context of chronic lymphocytic leukemia, multiple myeloma or bone marrow transplant patients in the post-engraftment period. This topic, along with vaccine strategies in the immunocompromised host, is discussed in detail in Chapter 83.

Vaccinations

The routine vaccination of oncohematologic patients with the 23-valent pneumococcal vaccine is generally recommended; similar recommendations exist for patients following HSCT after immune reconstitution (i.e. within 12–24 months after engraftment). During the influenza season, the vaccination of cancer patients – and especially of family members, health-care workers and other close contacts – may reduce transmission and infection-related morbidity and mortality[48] (see Chapter 83).

Prevention of mucositis

Palifermin, a recombinant epithelial growth factor, has been shown recently to protect hematopoietic stem cell recipients undergoing total body irradiation against severe oropharyngeal mucositis and to reduce the incidence of associated febrile neutropenic episodes.[49] Whether this observation applies to other populations of neutropenic patients remains to be investigated.

DIAGNOSIS

Evaluation of the febrile neutropenic cancer patient follows the universal principles guiding medical practice, including a prompt and thorough medical history and physical examination and use of rigorous diagnostic measures. However, in contrast to the non-neutropenic patient, empiric broad-spectrum antibiotic therapy should be administered without delay after the onset of fever and before the results of the microbiologic investigations become available.

Medical history

It is critical that cancer patients are thoroughly investigated at presentation with a special emphasis on the assessment of risk of infection. Medical history should include information on:

- travels to, or residence in, areas where infectious diseases (such as tuberculosis, fungal or parasitic infections, e.g. leishmaniasis or strongyloidiasis) are endemic, as these may reactivate during neutropenia;
- immunization history; and
- presence of vascular access devices.

Upon development of fever, the physician must obtain a meticulous history, and be aware of the fact that symptoms may be modified because of the impaired inflammatory response.[5]

Physical examination

Physical examination should be particularly thorough and must be performed keeping in mind that the classic signs of infection are markedly attenuated (e.g. pain, redness and induration) or absent (pus) because of neutropenia.[5] The physician should look for subtle inflammatory signs at common sites of infection (e.g. oropharynx, esophagus, respiratory tract, skin, insertion site of intravascular catheters, gastrointestinal tract, perianal region). The mouth and pharynx should be examined for erythema, mucositis, gingivitis, white patches and pseudomembranes, vesicles and ulcerations. The anterior nares should be inspected for signs of congestion, rhinorrhea, crusts, bleeding and ulcers. The skin may present features of a localized (i.e. primary) infection or multiple lesions in distant areas suggestive of a disseminated infection (Figs 73.5b, 73.6). Special attention should be paid to the presence of erythema, swelling and tenderness in the anal, perineal, axillary and periungual regions (Fig. 73.5a). Insertion sites of intravascular access devices and sites of cutaneous injury due to needle punctures or bone marrow aspiration should be thoroughly examined. Fundoscopy should be performed as it may reveal the presence of signs of systemic infection (Fig. 73.7b). Physical examination should be repeated frequently as clinical signs of infection may become manifest only a few days after the onset of fever.

Microbiology, radiology and histopathology

At least two sets of blood cultures should be obtained prior to the administration of antibiotics in the febrile neutropenic patient. Blood cultures are recommended even in the absence of fever in any patient with suspected infection. Blood should be drawn from a peripheral vein and also from intravascular catheters, if present.[5] It is prudent to draw blood from every port of multilumen intravascular devices. Qualitative cultures are performed routinely, but quantitative cultures might be helpful for the diagnosis of catheter-related bloodstream infections.[50] However, the yield of blood cultures in patients already on broad-spectrum antibiotics is notoriously poor. New molecular diagnostic methods such as polymerase chain reaction (PCR) may improve our ability to document the etiology of infection in these patients or in patients infected with micro-organisms that are difficult to grow.

Specimens (aspirate or biopsy) should be obtained from any site suspected of infection. The physician should carefully evaluate the benefits and risks (mainly bleeding in the thrombocytopenic patient) of initial (i.e. at the onset of fever) versus delayed (i.e. in those patients who are not responding to empiric therapy) invasive procedures to diagnose infections in poorly accessible sites. Exit sites of catheters should be cultured for bacterial, fungal and nontuberculous mycobacterial pathogens. Skin lesions should be aspirated or, whenever possible, biopsied for culture and/or histopathologic examination. Lesions of the oral cavity, pharynx and paranasal sinuses can be brushed or

biopsied. In the presence of severe dysphagia, endoscopy with brushing and biopsy for cultures and histopathology should be considered. Stools from patients with diarrhea should be tested for *Clostridium difficile* toxins and cultured for bacteria (*Clostridia, Salmonella, Shigella, Campylobacter, Aeromonas* and *Yersinia* spp.) and protozoa (*Cryptosporidium*). Urinalysis and urine cultures should be performed routinely, even in the absence of urinary symptoms or of pyuria, a rare finding in neutropenic patients even when infection is present.

A chest radiograph is part of the standard workup of febrile neutropenic patients. Whether or not, and when, to obtain additional radiologic imaging is guided by the patient's clinical condition and response to empiric broad-spectrum antibiotics. For example, severe abdominal complaints in neutropenic patients will prompt thorough investigations with standard radiography, ultrasonography, CT or MRI scans. Patients with lesions of the liver and spleen suggestive of hepatosplenic candidiasis should undergo CT-guided or laparoscopic liver biopsy. The presence of lung infiltrates has important implications for the management of neutropenic patients. Several studies have shown that CT scanning is superior to conventional radiography for revealing the presence of lesions indicative of invasive aspergillosis that should prompt further investigations (bronchoalveolar lavage, transbronchial, transthoracic or open lung biopsy) to obtain samples for diagnosis.[51] Persistent or complicated sinusitis, otitis and suspected osteomyelitis or myositis are other indications for additional radiologic imaging and tissue sampling. Once again, it should be remembered that radiologic signs are attenuated and delayed in neutropenic patients, and may become apparent only after bone marrow recovery. Classic examples are hepatosplenic candidiasis and invasive pulmonary aspergillosis.[34]

Baseline viral (HSV, VZV, CMV, EBV) and parasitic (*Toxoplasma gondii*) serology should be obtained in patients with hematologic malignancies, especially when allogeneic hematopoietic transplant is contemplated, but not in those with solid tumors as they are not at high risk of developing severe viral or parasitic infections.

Novel nonculture diagnostic tests including molecular and serologic techniques have been described recently. These include the use of PCR for the diagnosis of bacterial and fungal infections and the serologic detection of circulating antigens (e.g. mannan for *Candida* species, galactomannan for *Aspergillus* species, and β-1,3-D-glucan for *Candida, Aspergillus* and other fungal species), metabolites (e.g. D-arabinitol for *Candida* species), and antibodies directed against fungal antigens (e.g. antimannan antibodies).[52–56] Variable diagnostic performances of these new tools have been reported. False-negative results have been associated with the use of antifungal prophylaxis, prompt initiation of antifungal therapy, localized infections and with infections caused by fungal species lacking or expressing low amounts of these markers. Conversely, false-positive results have been described in patients colonized by fungi at nonsterile sites or treated with semisynthetic penicillins. Whereas galactomannan is now routinely used in many institutions, additional studies are needed before the use of the other noninvasive tools can be recommended.

Circulating biologic markers of inflammation, such as C-reactive protein and proinflammatory cytokines (such as tumor necrosis factor, interleukin-1, 6 and 8) are not helpful for differentiating infectious from non-infectious causes of fever because of a lack of sensitivity and specificity. Procalcitonin, a circulating calcitonin precursor whose blood concentrations are markedly increased during sepsis and much less so during the course of inflammatory diseases, looks promising. Yet, at the time of onset of fever in neutropenic patients (i.e. when start of empiric antibiotic therapy is recommended), procalcitonin levels are not elevated in the majority of patients with bacterial infections. Moreover, procalcitonin remains low in infections due to coagulase-negative staphylococci. Clinical studies have suggested that procalcitonin might be useful when reassessing the etiology of persistent fever after the first 48 hours of empiric antibacterial therapy. However, further investigations on procalcitonin-guided management strategies in febrile neutropenic cancer patients are needed.

Other investigations

Hematology (complete blood count and differential) and chemistry tests (including electrolytes, tests of liver and kidney function) are an integral part of the monitoring of toxic reactions to cytotoxic and antimicrobial agents and should therefore be repeated at regular intervals. Moreover, measurement of blood levels of antimicrobial agents should be undertaken, especially in patients with renal or hepatic dysfunction. This recommendation is particularly important for agents with narrow therapeutic windows, such as aminoglycosides, glycopeptides, some β-lactams and some triazole antifungals, and when the patient does not respond to therapy despite demonstration of *in-vitro* susceptibility.[5]

MANAGEMENT

Neutropenic patients with suspected infection, whether febrile or not, must receive prompt empiric antibiotic therapy with broad-spectrum antibiotics. The concept of treating these patients with empiric antibiotics as soon they develop fever has radically changed the otherwise fulminant and almost uniformly fatal course of Gram-negative sepsis.[2,4] This is the single most significant advance made over the last 30 years in the area of supportive care for cancer patients.

Risk assessment

Serious and potentially life-threatening complications may occur during the course of bone marrow aplasia, including hemorrhage, disseminated intravascular coagulation, thrombosis, pulmonary embolism, organ dysfunction (such as congestive heart failure, renal insufficiency, respiratory failure) and septic complications (such as severe sepsis, septic shock, adult respiratory distress syndrome), that may require admission to the intensive care unit.[57–59] Until recently, all febrile neutropenic cancer patients have been treated in a uniform fashion. Today, risk assessment is an important aspect of the evaluation of the febrile neutropenic patient. It is important to determine whether the patient is at low or high risk of serious infections or other medical complications because this will influence the treatment modalities and will have an impact on the patient's hospital course and length of stay. Several factors have been identified that can be used to classify a given patient into a low- or high-risk category.

Factors associated with a *low risk* of complications are shown in Table 73.3.[5,57,58] Typically, a low-risk profile is defined by the following characteristics: cancer in remission, absence of severe co-morbidities, outpatient status at onset of fever and neutropenia not likely to last for more than 7 days. Conversely, uncontrolled cancer, the presence of concomitant medical conditions (severe mucositis, hemorrhage, dehydration, renal, hepatic, respiratory, cardiac or circulatory failure, altered mental status), inpatient status and neutropenia expected to last for more than 7 days are factors likely to be associated with a *high risk* of complications.

Different scoring systems have been proposed for risk stratification.[57,58] The Multinational Association for Supportive Care in Cancer (MASCC) score is based on seven clinical factors derived and validated from prospective analyses of 756 and 383 episodes of febrile neutropenia, respectively.[58] A MASCC score greater than or equal to 21, on a maximum score of 26, was used to identify patients at low risk (<5%) of severe complications (sensitivity: 71%, specificity: 68%, positive predictive value: 91%, negative predictive value: 36%) and who might be candidates for outpatient empiric antimicrobial therapy. However, the experience with this and other scoring systems is still limited.[57,58] According to a recent single-center study, about 50% of low risk febrile cancer patients, as assessed by the MASCC score, can be treated orally and 25% can be safely managed on an outpatient basis.[60]

Additional prospective studies are needed to more fully evaluate and finely tune the use of these risk assessment systems. It will be

Table 73.3 Factors associated with a low risk of severe complications in febrile neutropenic cancer patients

- Malignancy in remission
- Absence of co-morbidities (chronic lung disease, diabetes mellitus, congestive heart failure, hemorrhage, liver disease, renal disease)
- Absence of vomiting, diarrhea, dehydration
- Outpatient status
- Absolute neutrophil count >100 cells/mm^3
- Absolute monocyte count >100 cells/mm^3
- Anticipated duration of neutropenia <7–10 days
- Normal chest radiograph
- Peak temperature <102.2°F (39.0°C)
- Absence of shivering
- Stable condition
- Absence of neurologic or mental alterations
- Absence of abdominal pain
- Absence of intravenous catheter site infection
- Absence of septic complications (e.g. severe sepsis, septic shock, hypoxia, pneumonia or other deep organ infection)

Derived from references[5,57,58].

important to make sure that low-risk patients may be safely treated with oral antibiotics on an outpatient basis (see Oral antibiotic therapy, below). An algorithm for risk assessment may be used for guiding the choice of oral versus parenteral empiric antibiotic therapy (Fig. 73.14).

Empiric antibiotic therapy

Intravenous antibiotics

Over 90% of the first episodes of infection in neutropenic cancer patients are caused by Gram-positive and Gram-negative bacteria (Table 73.1 and Fig. 73.1). Empiric antibiotic regimens must be broad-spectrum and bactericidal, achieve high circulating and tissue levels and be nontoxic. For more than two decades, combinations of two or more intravenous antibiotics have been the 'gold standard' of empiric antibiotic therapy. Numerous combinations of antipseudomonal penicillins (e.g. mezlocillin, ticarcillin with or without clavulanic acid, azlocillin or piperacillin with or without tazobactam) or third- or fourth-generation cephalosporins (ceftazidime, ceftriaxone, cefpirome, cefepime) plus an aminoglycoside (gentamicin, tobramycin, netilmicin or amikacin) have been frequently utilized.[4,26,61] No regimen was shown to be superior to the others. For example, ceftazidime and amikacin was a popular antibiotic combination in the late 1980s and 1990s. However, aminoglycoside-containing regimens are associated with renal and auditory toxicity, especially in patients concomitantly receiving other toxic agents and often require monitoring of drug levels.

Thus, new treatment approaches have been explored to avoid these complications, such as once-daily dosing of aminoglycoside or β-lactam monotherapy. An international, multicenter trial of the EORTC-IATG showed that once-daily ceftriaxone and amikacin was as effective as, and at least no more toxic than, a combination of ceftazidime and amikacin given thrice daily.[62] Monotherapy with broad-spectrum and highly bactericidal agents, such as third- or fourth-generation cephalosporins (ceftazidime, cefepime, cefpirome), carbapenems (imipenem and meropenem) or antipseudomonal penicillins combined with a β-lactamase inhibitor (piperacillin–tazobactam) were found to be as effective as and less toxic than combinations of β-lactam and aminoglycoside.[28,63] The third-generation cephalosporin ceftazidime displays a limited activity against Gram-positive bacteria and no anti-anaerobic activity. Recent studies have suggested that ceftazidime may be less efficacious than carbapenems.[63,64]

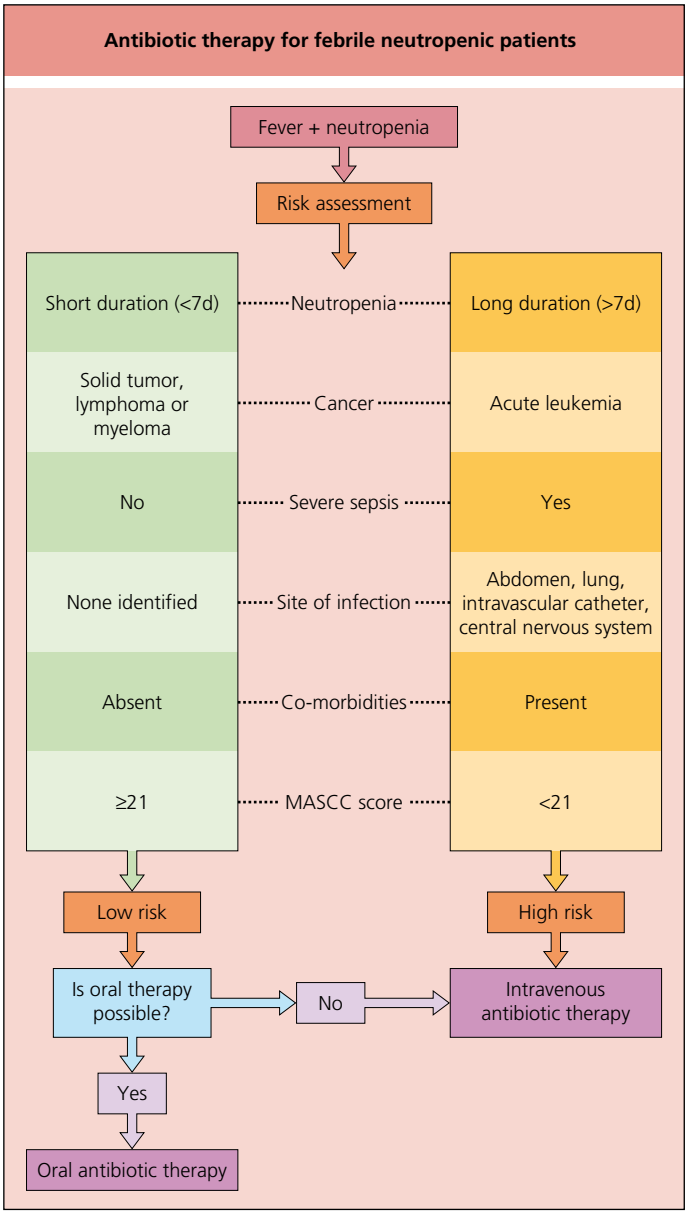


Fig. 73.14 Selection of intravenous or oral empiric antibiotic therapy based on risk assessment in febrile neutropenic patients. Severe sepsis and septic shock are defined according to reference[59]. Adapted from reference[6].

Choices of intravenous empiric antibiotic therapy for high-risk patients, typically those with hematologic malignancies and long-duration neutropenia, are shown in Figure 73.15. Choices should be guided by the patient's clinical condition and local epidemiologic data. Consider using an aminoglycoside-containing regimen in critically ill patients, such as those with severe sepsis or septic shock, when a *P. aeruginosa* infection is suspected or when resistant Gram-negative bacteria prevail, including ESBL-producing strains. Consider using a glycopeptide antibiotic (vancomycin or teicoplanin) in patients with catheter-related infections, when penicillin-resistant streptococcal or methicillin-resistant staphylococcal infections are suspected or in critically ill patients with severe sepsis or septic shock. However, outside of these well-defined clinical circumstances encountered in a minority of patients, routine use of glycopeptide antibiotics is strongly discouraged. Empiric use of vancomycin has not been shown to improve patient outcome and is associated with increased costs, toxicity and

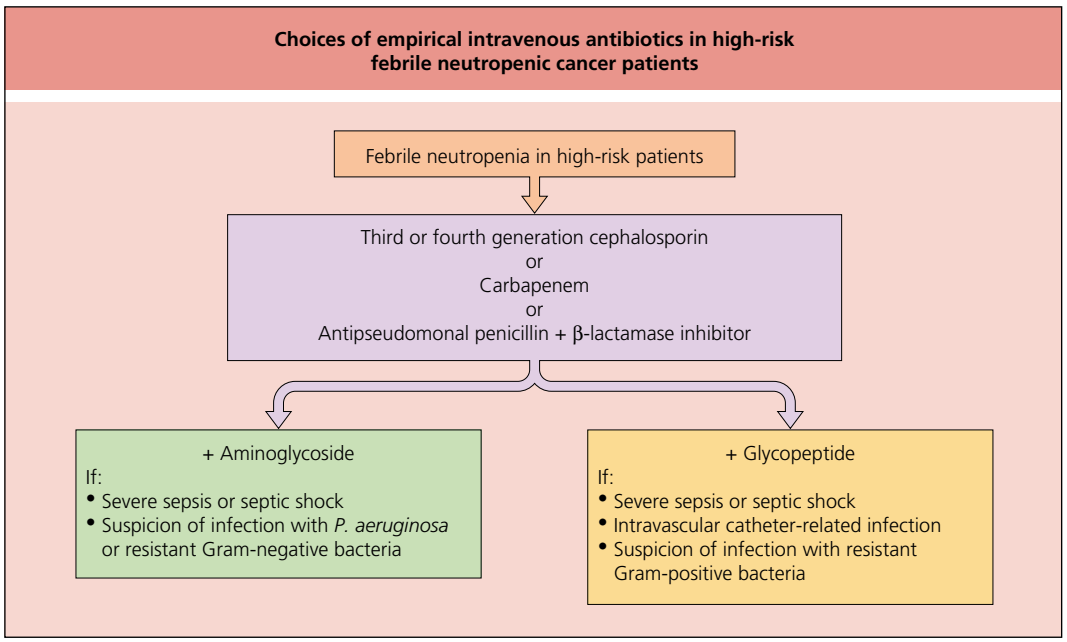


Fig. 73.15 Choices of empiric intravenous antibiotics in high-risk neutropenic cancer patients.

emergence of resistance. Whether new anti-Gram-positive agents (linezolid, quinupristin–dalfopristin, daptomycin, telavancin or dalbavancin) will provide new treatment options in neutropenic patients needs to be investigated.

After proper investigations and microbiologic cultures, it is reasonable to empirically use:

- an antifungal and/or antiviral agent in patients with esophagitis;
- a macrolide or 'respiratory' fluoroquinolone and/or TMP-SMX and/or an antifungal agent for coverage of opportunistic respiratory pathogens in patients with lung infiltrates;
- metronidazole in patients with an abdominal or perianal focus of infection and/or severe diarrhea; and
- TMP-SMX in severely ill patients with relapsing fever who are at risk of superinfection due to multiresistant *Stenotrophomonas maltophilia*.

The role of new parenteral broad-spectrum antibacterial agents such as ertapenem, doripenem, tigecycline, ceftobiprole, and telithromycin in febrile neutropenic patients remains to be determined.

Oral antibiotic therapy

Several studies have examined the role of oral absorbable antibiotics as empiric therapy of fever and suspected infections in low-risk adult patients with solid tumors and neutropenia expected to be of short duration (less than 7 days). Oral antibiotic therapy is possible when the patient is compliant, can swallow tablets, has normal gastrointestinal motility and function, and can be monitored for response to therapy, development of secondary infections and adverse reactions. Ofloxacin or ciprofloxacin given either alone or combined with amoxicillin–clavulanic acid have been studied in adult patients.[65,66] However, ofloxacin and ciprofloxacin have suboptimal activities against Gram-positive bacteria and should not be used as monotherapy. In contrast, oral ciprofloxacin plus amoxicillin–clavulanic acid was found to be as efficacious and safe as standard parenteral treatment in two large studies of low-risk patients in an inpatient setting.[29,30] New 'extended-spectrum' fluoroquinolones (i.e. moxifloxacin and gatifloxacin) with improved activity against Gram-positive bacteria are being investigated as monotherapy in febrile neutropenic patients. Of note, fluoroquinolone-containing oral regimens cannot be considered in neutropenic patients in whom fever develops during fluoroquinolone prophylaxis.

Despite preliminary favorable clinical observations, it has not yet been unequivocally demonstrated that management of low-risk febrile patients on a fully outpatient basis is safe.[60] At the present time, it is reasonable to discharge compliant patients who have responded to therapy, despite persistence of neutropenia, provided that the patient is not alone at home, is under careful medical supervision and lives within a short distance of a hospital.

There are no data on upfront oral antibiotic therapy of fever in children with neutropenia. Limited information is available on early discharge of selected children on oral cefixime 48 hours after intravenous antibiotic therapy in a hospital setting.[67]

Reassessment of therapy

The appropriateness of the empiric antibiotic regimen should be reassessed within 24–72 hours based on the results of microbiologic cultures and the patient's response to antibiotic therapy.

Therapeutic modifications based on culture results

If a pathogen has been isolated, antibiotic therapy is adapted based on *in-vitro* susceptibility tests and clinical response (Fig. 73.16). Vancomycin (or teicoplanin, a glycopeptide antibiotic not approved by the US Food and Drug Administration, which has been studied and used in some European countries) should be added if cultures grew methicillin-resistant staphylococci, penicillin-resistant streptococci or enterococci not covered by the empiric regimen. Recently introduced antibiotics such as quinupristin–dalfopristin (a streptogramin), linezolid (an oxazolidinone), daptomycin (a lipopeptide antibiotic), tigecycline (a tetracycline derivative) and other anti-Gram-positive drugs under development (e.g. telavancin, dalbavancin, ceftobiprole) exhibit excellent *in-vitro* activities against penicillin-resistant streptococci, methicillin-resistant staphylococci and vancomycin-resistant enterococci and might offer new therapeutic options for febrile neutropenic cancer patients. However, there is limited clinical experience with these antibiotics in these patients. Moreover, linezolid may cause thrombocytopenia and anemia when used for more than 2 weeks.

Three studies have now clearly shown that there is no indication for empiric use of vancomycin in centers where resistance of Gram-positive bacteria to β-lactam antibiotics is rare.[27,68,69] However, as

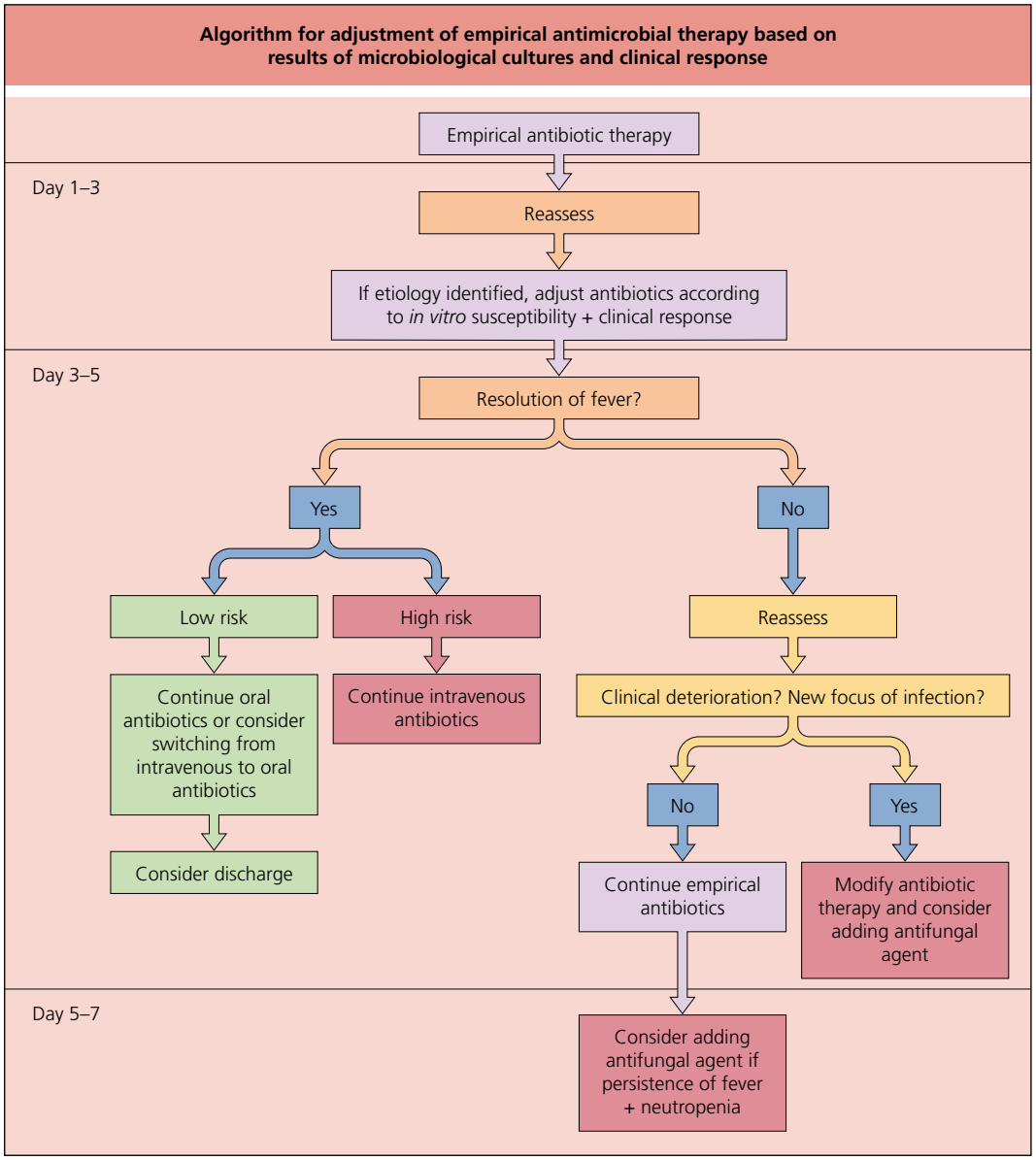


Fig. 73.16 Algorithm for adjustment of empiric antimicrobial therapy based on results of microbiologic cultures and clinical response.

mentioned previously, the empiric use of vancomycin is justified in critically ill patients with severe sepsis or septic shock, in the presence of a catheter-related infection and in centers with high levels of β-lactam resistance among Gram-positive bacteria other than common skin colonizers such as coagulase-negative staphylococci. If suspicion of resistant Gram-positive infection is disproved by culture results, empiric vancomycin should be rapidly discontinued. Likewise, empiric use of an aminoglycoside should be stopped as soon as β-lactam-resistant Gram-negative bacteria are ruled out.

Therapeutic modifications based on initial response to empiric antibiotic therapy

It is necessary to treat patients for 3–5 days before one can begin to evaluate the response to therapy with some degree of confidence. However, earlier treatment adjustments may be required if the patient's condition deteriorates. Treatment recommendations for patients who have become afebrile and for those who remained febrile within 3–5 days after initiation of empiric therapy are presented in Figure 73.16.

Resolution of fever

Intravenous (high risk) or oral (low risk) antibiotics should be continued in patients who have responded to therapy (Fig. 73.16). In low-risk patients receiving intravenous antibiotics, switching to oral antibiotics, such as ciprofloxacin and amoxicillin–clavulanic acid or monotherapy with new-generation fluoroquinolones with extended anti-Gram-positive activity (e.g. moxifloxacin, levofloxacin, gatifloxacin), should be considered. In the absence of microbiologic proof and identified site of infection (i.e. FUO), it is usually not necessary to continue broad-spectrum antibiotics for longer than 7 days, if fever resolved quickly and the neutrophil count has returned to normal levels. Antibiotics can be stopped when the patient is afebrile for more than 48 hours and the neutrophil count is >500/mm^3 for two consecutive days.[5]

Opinions differ regarding the optimal duration of therapy in patients with persistent neutropenia. Some physicians discontinue therapy in afebrile and clinically stable patients, while others prefer to continue broad-spectrum intravenous or oral antibiotics until recovery of neutrophils. Yet, the evidence supporting continuation of broad-spectrum antibiotics is not strong and is based on data obtained 20 years ago in a limited number of patients with FUO. In that study,

nearly half of the patients with persistent neutropenia became febrile again within 3 days of stopping treatment and breakthrough infections and severe septic complications occurred.[70] Patients with microbiologically or clinically documented infections are usually treated for a total of 10–14 days (i.e. until cultures become sterile and clinical response is complete). However, prolonged antibiotic courses are costly and may be associated with toxicity and emergence of resistant bacterial or opportunistic fungal infections.

Persistence of fever

It is important to keep in mind that the median time to defervescence is 5 days in high-risk patients with hematologic malignancies. Therefore, persistence of fever may not necessarily indicate that treatment is failing. If a specific causal organism or an infection site has been identified, treatment should be continued or modified, as appropriate.[5]

Considerable controversy surrounds the optimal management of a stable patient with unexplained fever after 3 days of empiric therapy. Persistence of fever may be due to a slow response, suboptimal levels of antibiotics, resistant pathogens, nonbacterial infection, development of a secondary infection or a noninfectious etiology (such as cancer, transfusion reaction or drug fever). A thorough clinical examination, follow-up microbiologic cultures, extensive radiologic imaging (including a CT scan of the chest and abdomen) and invasive investigations, whenever possible, are an integral part of the workup of the persistently febrile neutropenic patient.

If the patient's clinical condition is deteriorating, management depends on whether or not the patient has developed a clinically obvious focus of infection. If a focus of infection has been found, attempts should be made to identify the causative pathogen by culture or biopsy. The antimicrobial treatment should then be modified accordingly. Moreover, withdrawal of an infected intravascular catheter is indicated, when the local signs of infection are extending, when the patient is clinically unstable, when a septic thrombophlebitis is documented or when infection is due to pathogens other than common skin colonizers.[5] In contrast, the utility of antibiotic lock solutions or rotation of antibiotic delivery via the different ports of multilumen catheters is controversial.

If there is no obvious site of infection, the patient is not deteriorating and reassessment does not reveal a new focus of infection, the physician has the choice between several treatment options:

- *continue* the initial empiric antibiotics for 2 more days;
- *change the antibiotics* (change of broad-spectrum β-lactam, addition of a glycopeptide or of an aminoglycoside);
- *add empirically an antifungal agent* (intravenous amphotericin B deoxycholate, lipid formulations of amphotericin B, itraconazole, voriconazole or caspofungin).

In this situation, our approach is to continue the initial antibiotic regimen and initiate early (i.e. within 48–96 hours after the onset of fever) empiric antifungal treatment, especially in the following circumstances:

- expected long-lasting neutropenia (>10 days);
- recurrent episodes of fever during the same episode of neutropenia;
- prolonged therapy with broad-spectrum antibiotics;
- *Candida* colonization of the oropharynx or gastrointestinal tract and severe mucositis or neutropenic enterocolitis;
- presence of one or multiple lung infiltrates;
- positive circulating fungal markers such as galactomannan, β-1,3-D-glucan, mannan/antimannan; or
- epidemiologic data indicating a high incidence of mold infections.

Fungal infections due to *Candida* or *Aspergillus* species are a frequent cause of persistent fever in this setting. Unfortunately, early diagnosis is difficult and infections are *often diagnosed at autopsy*.[2]

Studies conducted in persistently febrile neutropenic patients in the 1980s have suggested that empiric intravenous amphotericin B may reduce the morbidity and mortality due to invasive fungal infections.[71] Yet, the patient population has changed (e.g. due to evolving myeloablative regimens, frequent use of antifungal prophylaxis and availability of new diagnostic tools) and no contemporary randomized controlled studies have definitively shown improved outcomes compared to placebo. Treatment guidelines recommend starting empiric amphotericin B after 4–7 days of unexplained fever, but this is purely arbitrary as the optimal timing for starting therapy is unknown.[5] The potential advantage of an early start must be balanced against the risk of unnecessary toxicity, emergence of resistant fungi and the not insignificant costs of a broad use of antifungal agents. Once started, it is recommended to continue empiric antifungal therapy until bone marrow recovery, at which time investigations (i.e. thoracic and abdominal CT scan) should be repeated to confirm or rule out the presence of an until then occult fungal infection. No further therapy is needed if an invasive fungal infection can be reasonably excluded. Conversely, any lesion suggestive of invasive mycosis after neutrophil recovery, such as cavitating pulmonary lesions or multiple hepatosplenic lesions, should, whenever possible, undergo extensive workup such as bronchoalveolar lavage and tissue biopsies for culture and histopathology. Treatment should be then reassessed if a fungal pathogen is isolated. Prolonged antifungal therapy is often necessary when an invasive mycosis has been diagnosed, as the patient is likely to undergo multiple cycles of chemotherapy. On the other hand, surgical resection should be considered in between two cycles of chemotherapy in the case of invasive pulmonary aspergillosis, especially in patients with a single lesion, with lesions at high risk of bleeding due to close contact with hilar blood vessels or in presence of hemoptysis.[51]

For decades, amphotericin B deoxycholate, a fungicidal agent active against most species of *Candida* and *Aspergillus*, has been the standard treatment for fungal infections. However, renal and infusion-related toxicity often lead to suboptimal dosing or discontinuation of therapy and emergence of resistant fungi has been reported. Lipid formulations of amphotericin B (lipid-complex, colloidal dispersion and liposomal form) are better tolerated, but costly.[72] Factors limiting the use of fluconazole as empiric antifungal treatment in persistently febrile neutropenic patients are its fungistatic activity for *Candida* species, lack of activity on *Aspergillus* and an increasing incidence of azole-resistant *Candida* species. Recent studies have shown that itraconazole and voriconazole are treatment alternatives to amphotericin B in this indication.[73,74] Voriconazole has been shown to prevent breakthrough fungal infections during neutropenia, to be efficacious for candidemia in non-neutropenic patients and to be a first choice therapy for invasive aspergillosis in immunocompromised hosts.[75] However, azole prophylaxis precludes the use of any agent of this class as empiric therapy for persistent febrile neutropenia. One should also keep in mind that azoles are fungistatic against *Candida*, which might reduce their efficacy in the context of neutropenia. Echinocandins, a new class of antifungal agents that inhibit fungal cell wall synthesis, show promising efficacy and toxicity profiles. Caspofungin has recently been licensed for salvage therapy of refractory invasive aspergillosis. A recent trial also showed that caspofungin was as effective as and less toxic than amphotericin B for the treatment of invasive candidiasis.[76] Finally, caspofungin has been shown to be as efficacious as and better tolerated than liposomal amphotericin B for empiric therapy of persistently febrile neutropenic patients.[77]

Evidence-based guidelines from the European Consensus Conference on Infections in Leukemia have proposed the following grading of recommendations for empiric antifungal therapy according to the grading system of the Centers for Disease Control:

- AI (strongly recommended) for liposomal amphotericin B and caspofungin;
- BI (generally recommended) for amphotericin B deoxycholate, lipid complex or colloidal dispersion, voriconazole and itraconazole;
- CI (optional) for fluconazole; and
- DI (generally not recommended) for amphotericin B deoxycholate in the presence of renal impairment or risk factors for increased nephrotoxicity.

Other new compounds, including posaconazole, anidulafungin and micafungin are approved for clinical use, but no data are so far available

on their empiric use in persistently febrile neutropenic patients. Moreover, the efficacy and safety of combinations of antifungal agents of different classes are being intensively investigated. Although there is no indication for their use in empiric therapy, combinations of liposomal amphotericin B or voriconazole with caspofungin have yielded promising results in case-control studies of salvage therapy of invasive aspergillosis.[78] These observations need to be confirmed in prospective, randomized studies in documented infections as the use of these costly strategies will have major economical implications.

Supportive care

Hematopoietic growth factors

The main goal pursued with the use of hematopoietic colony-stimulating factors (CSFs) is the acceleration of hematopoietic recovery to reduce the severity and duration of neutropenia and thus the risk of infections. Several studies have examined the role of granulocyte colony-stimulating factor (G-CSF, filgrastim) and granulocyte–monocyte colony-stimulating factor (GM-CSF, sargramostim) in the treatment of cancer patients not receiving bone marrow transplantation. Treatment with G-CSF or GM-CSF was found to moderately shorten the duration of neutropenia when given to patients with acute leukemia. Results were less striking in patients with solid tumors and other hematologic malignancies. In some, but not all, of these studies, a shorter duration of neutropenia was associated with a reduction of infections, use of antibiotics and length of stay, but without clear-cut benefit on the incidence of severe infections and infectious mortality. Moreover, no consistent effects on the duration of fever or antibiotic therapy, infectious morbidity or mortality, and costs have been demonstrated using CSFs in febrile neutropenic patients.

Recommendations for the use of CSFs have been published by the American Society for Clinical Oncology, the European Society of Medical Oncology and the European Organization for Research and Treatment of Cancer.[79–80] At the present time, the evidence supporting the use of CSFs in neutropenic patients not undergoing bone marrow transplantation is limited and opinions of experts remain divided.[5] Yet, high-risk patients with profound, long-lasting neutropenia and severe deep organ infections (e.g. pneumonia, typhlitis, invasive mycosis) not responding to appropriate antimicrobial therapy may benefit from an accelerated recovery of neutrophils. Although we occasionally use CSFs under such circumstances in our own institution, we acknowledge the fact that there is no definitive evidence to support the administration of CSFs in these clinical conditions. Intermittent administration of CSFs may be considered in patients with a myelodysplastic syndrome and recurrent infections due to chronic neutropenia.

Granulocyte transfusions

Some experts consider the transfusion of granulocytes in patients with profound neutropenia and life-threatening bacterial or fungal infections not responding to an adequate antimicrobial treatment. However, evidence of efficacy is lacking, and several problems are associated with this procedure, including transmission of CMV, alloimmunization, graft-versus-host reaction, and damage of lung capillaries with risk of acute respiratory distress syndrome.

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Chapter 74

Kieren A Marr

Infections in hematopoietic stem cell transplant recipients

HEMATOPOIETIC STEM CELL TRANSPLANTATION: BACKGROUND AND CURRENT PRACTICES

Hematopoietic stem cell transplantation (HSCT) has been increasingly used to treat hematologic and nonhematologic malignancies and inherited immunodeficiencies. Multiple practices, including conditioning regimens, stem cell sources and supportive care strategies have changed since the first successful bone marrow transplantations, owed principally to an evolving understanding of how overall success is achieved and to the development of new technologies. As these factors impact on the severity and duration of infectious risks, and sometimes even the clinical presentations and outcomes, clinicians involved in the care of HSCT patients benefit greatly from a baseline understanding of transplant practices.

In order to allow for donor hematopoiesis to establish in the recipient, a conditioning regimen must be applied before infusion of stem cells. The primary aim of the conditioning regimen is to suppress recipient T cells that could ultimately mediate rejection of the graft. It was also hypothesized that myeloablative conditioning regimens could effectively eliminate residual malignant disease. To accomplish these goals, conventional regimens have been highly myeloablative, employing total body irradiation (TBI) and high-dose chemotherapy (e.g. busulphan, cyclophosphamide). In this setting, severe and protracted neutropenia, and regimen-related organ toxicities can contribute to early infectious complications.

The type of conditioning therapy has been shown to impact on overall risks and timing of infectious complications. Recently, nonmyeloablative or reduced-toxicity conditioning regimens have been used, especially for patients who are not eligible for conventional myeloablative HSCT. Success of this strategy relies on the therapeutic potential of graft-versus-host (GVH) effects, which are produced when donor-derived immune cells recognize and eventually destroy host tumor cells and tissues.[1] Although graft-versus-host disease (GVHD) negatively impacts on overall survival by causing organ injury, necessitating the need for increasingly immunosuppressive therapies, GVH effects also induce the beneficial effects of 'graft versus tumor', sometimes to a degree that can result in complete remission.[2] A number of alternative conditioning regimens have been developed which typically are associated with fewer organ toxicities (e.g. gastrointestinal tract mucositis) and shorter (if any) duration of neutropenia.[3–7]

The most common causes of death in allogeneic HSCT recipients are infection (frequently in the setting of GVHD) and relapsed malignancy. One method transplantation groups use to minimize the risks for relapse and GVHD is manipulation of the cells that compose the infused graft. Cellular composition is dependent on stem cell source and treatment of the stem cell product before infusion. Peripheral blood stem cells (PBSCs) can be used instead of bone marrow; these are harvested from peripheral blood of granulocyte colony-stimulating factor (G-CSF or filgastrim)-mobilized donors. Peripheral blood stem cell products often undergo *ex-vivo* manipulation in order to decrease donor T cells (T-cell depletion), thereby decreasing the risk of GVHD, or to select for specific stem cell precursors, such as $CD34^+$ cells. The latter has been shown to reduce the incidence of relapse of malignancy by decreasing the number of contaminating tumor cells.[8,9] However, as expected, T-cell depletion through either method impacts on overall immune reconstitution and increases the risk for infections.

New technologies have allowed for the performance of more high-risk HSCTs, enabling transplantation from unrelated, human leukocyte antigen (HLA)-mismatched or even haploidentical donors. As these patients have relatively high rates of severe GVHD, the overall infectious risks and duration of susceptibility are expanded. Supportive care practices and the general approach to patients must then consider the primary risk periods, with consideration of the pace of immune reconstitution and how that impacts on the risk of infections.

RISK FOR INFECTIONS: IMPACT OF THE GRAFT, THE HOST AND TRANSPLANT COMPLICATIONS

The risk of infections post-HSCT is associated with a number of factors, defined largely by the type of graft received, the host and transplant complications – determinants of the 'net state of immunosuppression'. These risk factors vary according to the time after transplanation. Overall risks are dictated by the net state of immunosuppression, along with exposure to both exogenous and endogenous organisms.

The graft and type of transplant

Donor and HLA-matching of the graft impact on overall risks for infection, primarily by dictating the likelihood and severity of GVHD and the need for and intensity of immunosuppressive therapy. The cellular composition of the graft (stem cell source and *ex-vivo* manipulation) dictates the risks for infection through effects on immune reconstitution and the pace and severity of GVHD.

Transplantation with PBSCs may yield faster reconstitution of platelets, $CD4^+$ T cells, neutrophils and monocytes.[10–14] A large study compared immune reconstitution and coincident infection after myeloablation followed by either peripheral blood stem cell transplant (PBSCT) or bone marrow transplant. During the first year after transplantation, most lymphocyte subsets, especially $CD4^+$ T cells,

were higher in PBSCT recipients. This was accompanied by fewer bacterial and fungal infections in patients who received PBSCT than in recipients of bone marrow transplants.[14] However, there is concern that the success of PBSCT, especially from unrelated or HLA-mismatched donors, may be limited by a more rapid onset of severe GVHD.[10,11]

Cord blood transplantation, which is increasingly performed in patients who lack suitable related donors, is associated with delayed hematopoietic recovery. With this, slow engraftment and impaired neutrophil function may work together to increase the risk of infection, particularly early after transplantation.[15–18] Studies have reported high risk for aspergillosis, candidiasis and infections due to adenovirus and human herpesvirus (HHV)-6 in cord blood transplant recipients.[15,19,20] Patients who receive cord blood after nonmyeloablative conditioning regimens that incorporate antithymocyte globulin (ATG) appear to be at particularly high risk for Epstein–Barr virus (EBV)-related complications.[21] On the other hand, the low rates of GVHD may confer a relative protection from infection late after transplantation.[22]

Although T-cell depletion (TCD) decreases the pace and severity of GVHD, T-cell depletion itself is associated with delayed immune reconstitution and an increased risk for infections.[23–26] A recent large study comparing infectious complications in patients treated with TCD bone marrow from unrelated donors to those of patients treated with GVHD suppressive therapies found that TCD was associated with a particularly high risk for both cytomegalovirus (CMV) infection and aspergillosis.[27] Because $CD34^+$ selection results in removal of T cells, natural killer (NK) cells and monocytes, this practice may increase the risk for infections after allogeneic and autologous transplantation.[28,29]

It appears that immune reconstitution is similar after nonmyeloablative and myeloablative HSCT, although few large comparative studies have been performed, and reconstitution depends on multiple host and therapeutic variables.[30,31] Results of at least one study suggested that nonmyeloablative HSCT may be associated with more rapid reconstitution of immune responses assessed *in vitro*.[31] Results of another study suggested that although the total numbers of lymphocytes and antibodies was not different during the first 180 days after nonmyeloablative and myeloablative HLA-identical HSCT, recipients of nonmyeloablative regimens had relatively higher numbers of CMV T-helper cells during the early time period, raising the possibility of a protective effect of donor T cells that survive the less ablative conditioning regimen.[32] Of course, the short duration of neutropenia and differences in organ toxicities are important determinants of infectious risks. One case-control study suggested fewer early bacterial and candidal infections after nonmyeloablative conditioning.[4,33] Late-onset GVHD after nonmyeloablative HSCT poses increased risks for late CMV disease and aspergillosis.[4,33,34] Infections (e.g. CMV) may occur at increased frequency early after nonmyeloablative transplant in patients who receive certain conditioning regimens (e.g. the antibodies anti-CD52 or Campath-1H).[35]

Host factors that impact on infection risks

Underlying disease is one of the most important factors impacting on risks for infection post-HSCT by virtue of the immunologic defects associated with the hematologic condition itself and previous cytotoxic therapies. Patients who receive HSCT for a hematologic malignancy beyond first remission, for aplastic anemia and for myelodysplastic syndromes have higher risks for aspergillosis.[19,36–40] Patients who have protracted courses of primary immunodeficiencies may also have increased risks, although few large studies have been performed.

Multiple studies have reported that older people have increased risks for infections after HSCT.[19,36,39] Although it is not clear why older age predicts higher risks, factors that have been suggested include cumulative exposure to previous cytotoxic therapy, underlying disease, severity of GVHD, baseline organ dysfunction, previous microbial exposure and waning cellular immunity. On the other hand, youth may present increased risks for other infections; for example, adenoviral complications occur at increased frequency in young transplant recipients.[41,42] This may be explained by an increased likelihood of primary infection in the young, or even the eventual elimination of adenovirus infection with age.[41,43]

Other genetic factors may play a role in modulating risks for infectious complications. Factors that control innate immune responses in the host and donor may impact the risk for infection and for GVHD. This has become an active area of research. Polymorphisms in specific genes in stem cell transplant recipients, and in donor genes, have been associated with post-transplant risks for infection. For instance, risks for infectious complications after chemotherapy and stem cell transplantation have been reported to be associated with polymorphisms in the Fc gamma receptor, myeloperoxidase gene promoter, CXCL10, interleukin-10, DC-SIGN and multiple Toll-like receptors (TLR).[44–47] Studies to determine how host and donor defense polymorphisms impact on the risk for infection post-HSCT should provide novel therapeutic targets and allow for more personalized approaches to infection prevention in the future.

Transplant complications that impact on infection risks

Multiple complications that occur after HSCT alter the risk for infections. These complications may cause organ dysfunction and immune modulation and the need for potentially toxic and immunosuppressive therapies.

The most obvious organ dysfunction that occurs immediately following conditioning therapy is mucositis involving the gastrointestinal (GI) tract. Breakdown of the mucosa of the gut is a primary mode of entry for bacteria and *Candida* spp. that colonize the GI tract. Other, subtle manifestations of organ toxicities may also lead to subsequent infection risks. Patients with renal or hepatic dysfunction may not tolerate typical doses of prophylactic or empiric antibiotics or antifungals. Other biologic variables such as iron overload or metal chelating therapy may have an effect, especially on bacterial and filamentous fungal infections.[48]

Graft-versus-host disease and corticosteroid-based therapies are perhaps the most important post-HSCT complications leading to infections. Risk for all infections – bacterial, fungal and viral – are increased in patients with severe GVHD. These risks increase yet further in patients who receive high doses of corticosteroids. Most likely, this represents the combined impact of the direct immunosuppressive effects of GHVD, as well as the corticosteroid-induced impairment in neutrophil and monocyte/macrophage immunity and cell-mediated immunity. Corticosteroids administered for other post-HSCT complications, such as bronchiolitis obliterans, and 'idiopathic' pulmonary syndromes may also lead to equivalent, high risks for infections. The negative impact of corticosteroid exposure is not limited to the above risks; cumulative exposure to high-dose corticosteroids is an important variable predicting persistent infection and poor prognosis of treatment for viral or fungal disease.[49–51]

It has been hypothesized that infection with herpesviruses, especially CMV, may directly impact on the risk for other infections by modulating immune responses. Historically, the association between CMV disease and subsequent infections was largely attributed to the myelosuppressive effects of antiviral drugs administered for prophylaxis or therapy. However, high risk for subsequent bacterial and fungal infections have been noted in patients with active CMV or latent CMV disease, even after controlling for secondary neutropenia in multivariable models.[19,40,52] Cytomegalovirus-seronegative recipients of stem cells from seropositive donors (D+/R–) have increased risk for both bacterial and fungal infec-

Table 74.1 Approximate incidence of selected infectious complications in hematopoietic stem cell transplant (HSCT) recipients

Infection	Autologous HSCT	Allogeneic HSCT
Bacterial*		
Gram-positive	5–10	10–20
Gram-negative	5–10	5–10
Mycobacterial		
Mycobacterium tuberculosis	<2	<2
Nontuberculous	<2	<5
Fungal*		
Candida spp.	<2	<5
Aspergillus spp.	<10	5–15
Pneumocystis jirovecii	<2	<3
Viral		
Cytomegalovirus infection*		
R+ (auto)/R+/D+ or D– (allo)	25–40	70
R– (auto)/R–/D+ (allo)	3–5	15–25
R–/D– (allo)	NA	3–5
Cytomegalovirus disease*		
R+ (auto)/R+/D+ or D– (allo)	5–7	35–40
R– (auto)/R–/D+ (allo)	1–3	10
R–/D– (allo)	NA	1–3
Herpes simplex virus (HSV)*		
Without prophylaxis, HSV+	60–80	60–80
Varicella-zoster virus (VZV)*		
Without prophylaxis, VZV+	20–40	17–60
Influenza virus†	<5	<5
Respiratory syncitial virus†	<5	5–15
Parainfluenza virus†	<5	5–10
Adenovirus	<5	5–20
BK virus	<5	5–40
Epstein–Barr virus	<5	5–25
Human herpesvirus 6		
Infection	35–60	35–60
Disease	<3	<3 – 12
Parasitic		
*Toxoplasma gondii**	<2	<2

Shown are estimates of disease incidence (%), unless indicated otherwise.[55–68]
*Dependent on prophylaxis;
†Episodic incidence during the year; NA, not applicable.

tions, even in the absence of CMV-specific therapy.[53] Donor or recipient seropositivity may have the most impact on survival in patients who receive HSCT from unrelated donors.[54]

Thus, multiple factors related to both host and transplant contribute to the 'net state of immunosuppression'. This net state, which changes according to the timing of the transplant and to exposures, dictates overall infectious risk, as summarized in Table 74.1 and Figure 74.1.

The remainder of this chapter will discuss major issues regarding the epidemiology, manifestations and management of specific infections after HSCT. As many concepts that apply to neutropenic cancer patients (as outlined in Chapter 73 by Marchetti and Calandra) also apply in this setting, this discussion will focus primarily on issues that are specific to stem cell transplant recipients. Commonly employed prevention strategies for different types of infection are outlined in Table 74.2, and a more detailed discussion pertaining to diagnostic and therapeutic approaches for infectious complications are presented in other chapters.

BACTERIAL INFECTIONS IN HSCT PATIENTS: EPIDEMIOLOGY, MANIFESTATIONS AND MANAGEMENT

Patients are at highest risk for bacterial infections early after HSCT, with GI tract mucositis and neutropenia leading to translocation of endogenous pathogens, and during the later periods of GVHD, when risks for encapsulated organisms are particularly high. Bacteria most frequently become blood-borne through the GI tract and/or intravenous catheter, or through the respiratory tract. Risks are related to antibacterial prophylaxis patterns, degree of GI tract mucositis and presence of indwelling intravenous catheters. Late after HSCT, the epidemiology of infection largely depends on the severity of GVHD, application of prolonged prophylaxis and efficacy of immunization. There are numerous aerobic and anaerobic bacteria that cause infection in these patients, as outlined in more depth in Chapter 73. Overall risks are dependent on the type of transplant, degree of immunosuppression and post-transplant complications, and exposure; general estimates are provided in Table 74.1.

In patients with cancer and HSCT, the epidemiology of bacterial infections has changed over two decades, with large European and North American studies suggesting an increase in the incidence of infections caused by Gram-positive bacteria concurrent with a decrease in infections caused by Gram-negative bacteria.[72,73] Recent studies are demonstrating a potential reversal in trends, with more infections caused by Gram-negative bacteria.[74] Unfortunately, much of this increase appears to be associated with high rates of infection caused by multidrug-resistant (MDR) bacteria. A large multicenter Brazilian study showed that 37% of Gram-negative bacteria recovered from HSCT recipients were MDR; risks for these infections were increased with use of third-generation cephalosporins.[75] The outcomes of these infections can be particularly poor, especially in the setting of severe GVHD; for instance, *Pseudomonas aeruginosa* infections (lung and bloodstream) recur in 16% of patients who are treated with 14-day courses of appropriate antibiotics, with associated mortality estimated at 36%.[76]

Bacterial infections involving the GI tract are relatively common in these patients, as occurs in the setting of neutropenia after cytotoxic therapy; neutropenic enterocolitis occurs after conditioning therapy, with similar infectious causes. Diarrhea is also common after conditioning therapy for HSCT; recent reports suggest that 47–100% of autologous or allogeneic HSCT recipients develop diarrhea at some point during therapy.[77–81] Conventional bacterial pathogens, parasitic pathogens and viruses cause diarrhea in these patients; however, in allogeneic HSCT recipients, GVHD impacts the duration and magnitude of risks for infectious colitis and the differential diagnosis of diarrhea. For instance, GVHD has been reported to be an important risk factor for *Clostridium difficile*-associated disease (CDAD) in allogeneic HSCT recipients; in this population, complications such as CDAD-associated enteric bacteremia and mortality appear particularly high.[78,80] The contemporary burden of this infection is unknown in this population, as few large studies have been performed since recognition of highly toxigenic *C. difficile* strains.

Finally, management of allogeneic HSCT patients with acute diarrhea is more complex, as the differential diagnosis includes GVHD involving the GI tract; in a study of 169 HSCT recipients, an infectious cause of diarrhea was documented in only 45 (29%).[82] GVHD can be indistinguishable from infectious diarrhea using signs and symptoms alone; empiric immunosuppressive therapies often exacerbate and 'uncover' infectious causes (e.g. CMV, discussed below).

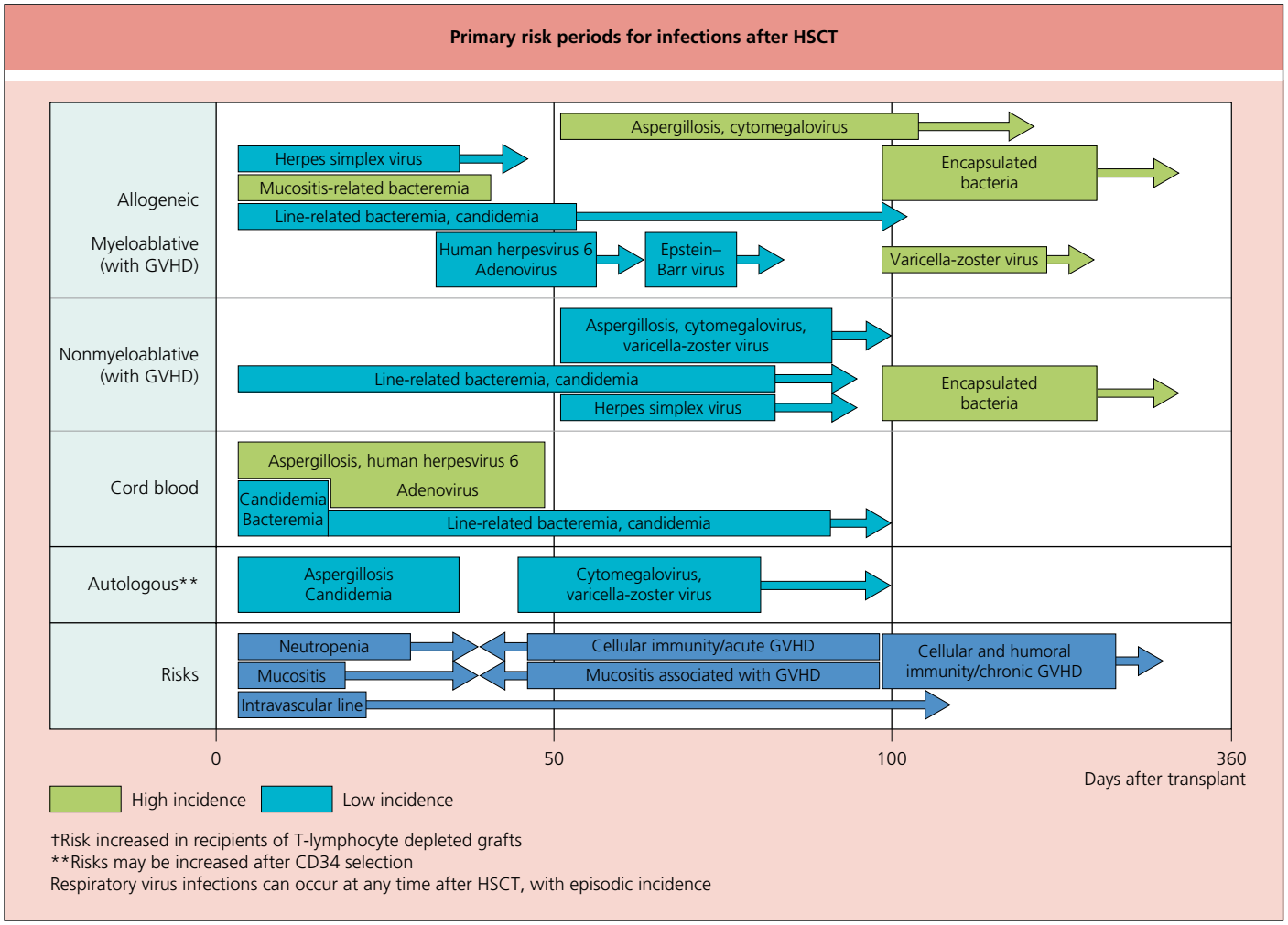


Fig. 74.1 Primary risk periods for infections after hematopoietic stem cell transplantation. Typical risk periods for the most common infections after each type of HSCT are shown. Risks are based on typical prophylaxis strategies, which include trimethoprim–sulfamethoxazole for *Pneumocystis jirovecii*, screened or filtered blood products and ganciclovir for cytomegalovirus, aciclovir for herpes simplex virus, and fluconazole for candidemia. Courtesy of R Bowden.[69]

VIRAL INFECTIONS IN HSCT PATIENTS: EPIDEMIOLOGY, MANIFESTATIONS AND MANAGEMENT

Infections caused by endogenous viruses (e.g. herpesviruses) and exogenous viruses (e.g. those acquired through the GI and respiratory tracts) are common in the HSCT population (see Table 74.1). The incidence of disease thus depends on mode of infection, recipient and donor CMV seropositivities, and immunosuppression. These host–pathogen interactions dictate the risk, timing and incidence of infection caused by latent herpes viruses, while exposure factors impact the epidemiology of exogenously acquired viruses. In the latter group, degree of immunosuppression more often impacts the severity of infection (i.e. whether a virus is contained within the upper respiratory tract or invades lung tissue).

Herpes virus infections

Herpesviruses are exceedingly important in HSCT recipients, with clinically significant infections caused by CMV, herpes simplex virus (HSV), varicella-zoster virus (VZV), EBV and HHV-6, -7 and -8. Perhaps the most important infectious complication is caused by CMV; historically, mortality caused by CMV disease was approximated at roughly 25%. The risk for primary infection by blood products has been significantly reduced by screening and leukocyte depletion. Currently, the risk of CMV disease is primarily related to reactivation of infected recipient or donor cells during periods of low CMV-specific T-lymphocyte function during immune reconstitution or to exogenous immunosuppressive therapies. Allogeneic HSCT recipients, who have the highest risk during GVHD, most commonly develop interstitial pneumonitis and gastroenteritis, and less commonly, other manifestations such as retinitis, isolated fever, pancytopenia, hepatitis and encephalitis. However, retinitis appears to be increasingly recognized, especially late after allogeneic HSCT in the setting of severe GVHD and recurrent CMV reactivation.[83]

Infection caused by CMV has also been associated with 'indirect effects', which include higher mortality related to other infections.[53] For these reasons, prevention strategies have been an active area of research, especially in recipients of allogeneic HSCT (see Table 74.2). Antiviral drug strategies, which include both universal prophylaxis and pre-emptive therapy, are effective and have lowered CMV-related mortality; however, both prophylaxis and pre-emptive therapies have specific limitations, including emergence of drug resistance and toxicities associated with antiviral drugs. Unfortunately, no strategy is 100%

Table 74.2 Prevention strategies commonly employed in HSCT recipients

Indication	Strategy
Decreased duration of neutropenia	Colony-stimulating factors WBC transfusions
Bacterial infections	Antibiotic prophylaxis during neutropenia Empiric therapy (fever during neutropenia) Antibiotic prophylaxis during GVHD IVIG Vaccination
Cytomegalovirus disease	Screening (or filtering) of blood products in seronegative recipients Antiviral prophylaxis PCR or antigen-based screening for reactivation with pre-emptive therapy IVIG Safe sex practices Vaccination (experimental)
Herpes simplex virus (HSV)-1 and HSV-2	Antiviral prophylaxis Safe sex practices
Varicella-zoster virus	Antiviral prophylaxis Postexposure prophylaxis IVIG Immunization
Epstein–Barr virus (EBV)/post-transplant lymphoproliferative disorders	PCR screening for reactivation, reduce immunosuppression if possible, consider rituximab and therapy with EBV-specific cytotoxic T lymphocytes (experimental)
Adenovirus infections	PCR screening for reactivation with pre-emptive therapy
BK infection	PCR screening for reactivation, reduce immunosuppression if possible, pre-emptive strategies
Respiratory virus infections	Enhanced infection control measures Prophylaxis, pre-emptive therapy for influenza Vaccination
Fungal infections	Antifungal prophylaxis Prophylaxis to prevent *Pneumocystis jirovecii* pneumonia
Toxoplasmosis	Prophylaxis with seropositivity and GVHD
Other	Infection control measures to prevent food-borne illness and respiratory acquisition of infection

Listed are strategies that are in common use or have suggested potential in recent clinical studies.
GVHD, graft-versus-host disease; IVIG, intravenous immunoglobulin; PCR, polymerase chain reaction; WBC, white blood cell.

effective, largely because the duration of time at risk for CMV disease is progressively extended in patients who receive suppressive therapies without effective immune reconstitution. CMV disease has largely become a 'late' complication of allogeneic HSCT, occurring most commonly during the period of GVHD.[84] CMV resistance to ganciclovir during prophylaxis or pre-emptive therapy has been reported, but appears to be less common than in other populations, such as in organ transplant recipients. The kinetics of viral reactivation, dictated by pathogen-specific cellular immunity and antiviral exposure, impacts the likelihood of viral resistance, especially in the settting in which resistance mutations impact viral 'fitness'.[85]

As control of CMV infection and risk for development of disease are primarily associated with both $CD8^+$ and $CD4^+$ T-cell immune responses, efforts are underway to develop strategies to enhance immunity, both as an effort to augment therapy of disease and to develop preventive strategies. Live-attenuated CMV vaccines, canarypox-vectored vaccines, recombinant gB vaccines and, more recently, DNA vaccines have been or are currently in development,[86,87] and may have efficacy in preventing both CMV disease and other 'secondary' manifestations associated with CMV immune modulation.

Both HSV and VZV infections occur and cause localized disease in seropositive hosts; reactivation and primary infection can also cause disseminated disease (e.g. hepatitis, pneumonitis, encephalitis). Prolonged antiviral prophylaxis is usually employed in seropositive patients; one randomized trial documented a decrease in VZV disease with 1 year of prophylaxis after allogeneic HSCT.[88] Rebound disease, and HSV and VZV antiviral resistance appear to be infrequent in this population.[89,90] An inactivated varicella vaccine given before receipt of autologous stem cells and 90 days thereafter has been shown to reduce the risk of VZV disease.[91]

Human herpes virus 6 (HHV-6), the cause of exanthem subitum, infects nearly everyone by 3 years of age and becomes latent in the brain. This virus (especially variant B) has been increasingly implicated in disease in adult and pediatric HSCT recipients. Although early reports associated HHV-6 with pneumonitis, rash, bone marrow suppression (especially involving megakaryotic lineage) and fever, most recent studies have shown more definitive associations with neurologic manifestations, including seizures, multifocal encephalitis and 'post-transplant acute limbic encephalitis'. The latter, 'PALE', is a recently described syndrome characterized by anterograde amnesia, syndrome of inappropriate production of antidiuretic hormone (SIADH), mild cerebrospinal fluid (CSF) pleocytosis and temporal EEG abnormalities.[92] Multiple issues surrounding HHV-6 including pathogenesis of disease, epidemiology and prevention, and the role of antiviral therapies remain under current investigation. One interesting observation is that this virus can be transmitted from a donor to an HSCT recipient by means of chromosomally integrated virus, also confounding the interpretation of HHV-6 viremia.[55]

Community-acquired respiratory viruses

Respiratory viruses, acquired within the community or nosocomially in association with institutional outbreaks, impose a significant burden of morbidity and mortality in this population. Biology of the viruses, pathogenesis of disease, diagnosis and therapies are discussed elsewhere in this text (Chapters 161 and 162). There are a few concepts specific to this population that are worthy of discussion.

Approximated incidence of respiratory tract 'infection' is listed in Table 74.1. However, incidence of pneumonia and related mortality is variable for specific viruses and is largely impacted by underlying immunity. Respiratory syncitial virus (RSV), influenza and parainfluenza (especially parainfluenza 3) have been reported to have the highest rates of pneumonia and related mortality in HSCT patients. These three viruses account for the bulk of infections in this patient population, progressing to pneumonia in 35% of patients.[93] Progression from upper to lower respiratory tract infection with RSV is dependent on lymphopenia, older age, allogeneic HSCT and degree of GVHD, but outcomes are also particularly poor when infection is established early after HSCT. In recent studies,[56,93,94] attributable mortality was estimated to be about 18%. Definitive recommendations of how to prevent progression of pneumonia are elusive in the absence of large randomized trials. Methods that have been explored and reported to

be safe include systemic and aerosolized administration of ribavirin, administration of intravenous immunoglobulin and use of neutralizing antibody, palivizumab.[56,95] Similarly, although no randomized trials have been performed to establish efficacy, oseltamivir is tolerated by HSCT recipients and used frequently to prevent disease caused by influenza viruses in outbreak settings.[96]

Human metapneumovirus has been recognized as a cause of previously undiagnosed 'idiopathic pneumonia' in HSCT recipients;[94,97] this virus, as well as parainfluenza 3, can persist in respiratory secretions for extended periods of time in asymptomatic HSCT recipients,[98,99] presenting challenges to both diagnostics and effective infection control. One caveat complicating the care of these patients is that diagnostic parsimony does not always apply in patients with diagnosed upper respiratory tract infections and pulmonary abnormalities. Both parainfluenza and influenza lower respiratory tract infections are frequently associated with 'co-pathogens', including bacteria and fungi, which require different therapies. In one study, parainfluenza 3 pneumonia was associated with other pathogens in 53% of cases.[100] Diagnostic bronchoscopy is wise, when feasible.

Adenovirus can cause disease after primary infection (gastroenteritis, interstitial pneumonitis) and after reactivation, with propensity to cause disease in the kidneys and liver. Incidence is variable and dependent on age, with higher rates in the young and older age groups, and degree of T-cell dysfunction related to cellular depletion or GVHD.[57]

Lower respiratory tract disease caused by respiratory viruses can present differently with different diagnostic imaging techniques. Disease may be inapparent on chest radiograph (Fig. 74.2a), with early pneumonia better shown on CT scan (Fig. 74.2b); CT scan can also show both nodular and 'ground glass' infiltrates, representative of bronchiolitis and pneumonia (Fig. 74.2c,d).

BK virus

Reactivation of polyoma BK virus (BKV) has been associated with post-engraftment hemorrhagic cystitis in allogeneic HSCT recipients.[101] Frequency of disease is dependent on the type of transplant and HLA match, type of conditioning agent, GVHD and pretransplant serologies.[58,102] Disease may be preceded by viral reactivation in urine and serum, presenting options for monitoring and 'pre-emptive' therapy.[103] Unlike renal transplant recipients, interstitial nephritis appears rarely (for a detailed discussion about BK virus, see Practice Point 43).

Hepatitis viruses

Hepatitis virus infections can be acquired from infected stem cell donors, although rates of transmission are variable and dependent on donor viral reactivation at time of stem cell recovery; effective prevention by treating donors with antiviral drugs and recipient vaccination has been reported.[104] Hepatitis C virus has recently been recognized as one of the two most common viral infections (with VZV) late after HSCT, in one series occuring in 10% of long-term survivors of allogeneic HSCT recipients from matched related donors.[105]

FUNGAL INFECTIONS IN HSCT PATIENTS: EPIDEMIOLOGY, MANIFESTATIONS AND MANAGEMENT

Overall risks for fungal infections are contingent upon variables that modulate the pace of immune reconstitution, organ toxicities and microbial exposures.

Infections caused by *Candida* species

Although the yeasts that cause infection in HSCT patients include multiple other organisms, such as *Cryptococcus neoformans*, the vast majority of superficial and invasive yeast infections are caused by *Candida* spp. Invasive *Candida* infections can be separated into two primary syndromes: acute candidiasis (bloodstream infection) and chronic candidiasis (hepatosplenic infection). The pathogenesis and clinical characteristics of candidiasis are discussed in Chapter 178. In this population, bloodstream infections occur either through an indwelling intravascular catheter or through a damaged GI tract. Acute infection usually manifests as fever and signs of sepsis. Adequate antifungal therapy is essential, not only to cure the acute episode, but also to decrease the likelihood of late embolic manifestations (e.g. chorioretinitis, endocarditis).[106]

The widespread use of fluconazole, which is supported by numerous randomized trials carried out in the early 1990s,[107,108] has decreased the incidence and mortality rate attributable to both acute and chronic infections caused by *Candida albicans*.[40,109,110] This practice has resulted in improved overall survival in allogeneic HSCT patients.[107] However, the decreased incidence of early candidiasis due to azole-susceptible species (*C. albicans* and *C. tropicalis*) and improved survival late after HSCT with GVHD has allowed for an increase in infections due to fluconazole-resistant *Candida* spp., such as *C. glabrata* and *C. krusei*.[40,110–113] There has also been a shift from *Candida* spp. to molds as the primary fungal pathogens. Thus, the appearance of azole-resistant organisms is not a failure of prophylaxis but reflects the success of supportive care strategies. In patients who underwent allogeneic HSCT from unrelated donors for chronic myelogenous leukemia (CML), two of the most important variables predicting HSCT survival in the 1990s were use of ganciclovir-based strategies and fluconazole.[114]

Infections caused by filamentous fungi

Infections with filamentous fungi are usually acquired through the respiratory route, although filamentous fungi can also invade through a damaged GI tract.[115,116] The most common cause of fungal infection in HSCT patients is currently *Aspergillus fumigatus*.[36,117] The day of onset

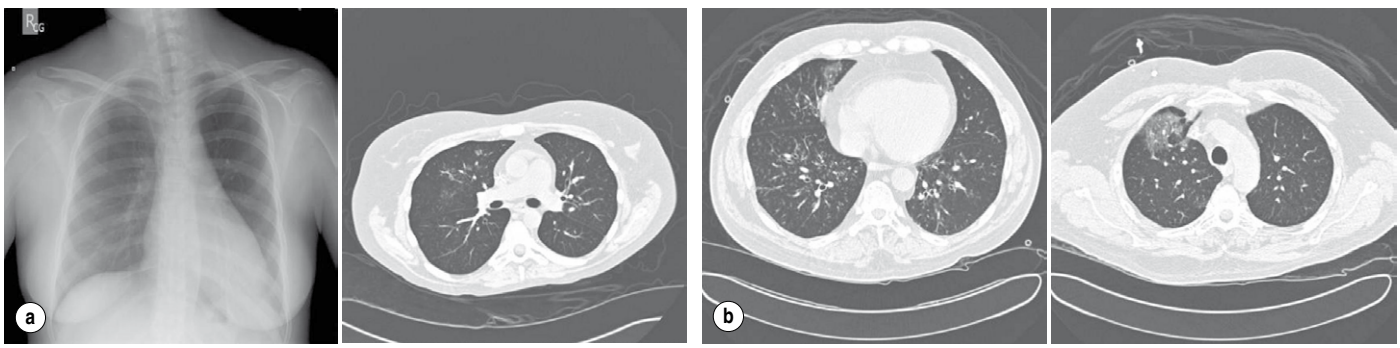


Fig. 74.2 Respiratory virus infections. (a) CXR and CT scan of documented human metapneumovirus infection in a patient with acute myelogenous leukemia. (b) CT scans of adenoviral infection complicating severe GVHD in a recipient of an HLA-matched unrelated donor transplant. In these scans, taken on the same day, both centrilobular nodules (left) and ground glass infiltrates (right) were apparent.

of aspergillosis has changed, from primarily the early neutropenic period to later after allogeneic transplant.[5,19,36] The risk factors for early and late disease are different; risks for early disease are primarily those that impact on the pace of engraftment, such as the specific stem cell product. There is also some indication that a portion of patients who present with aspergillosis early after HSCT may have been exposed to the organism before conditioning therapy. This may explain some of the impact of 'host' variables, such as age and underlying diseases on the risk of aspergillosis. Risks during the late period are largely those associated with GVHD and its therapies. Other infections, such as CMV disease, may pose both direct and indirect risks for subsequent disease. There has been an increase in invasive infections caused by zygomycetes and *Fusarium* spp.[117] Patients who receive transplants from an unrelated or an HLA-mismatched donor and patients with severe GVHD have particularly high risk for zygomycete infections.[117]

Most recent efforts have been directed towards preventing mold infections by using universal or targeted prophylaxis with different mold-active drugs (e.g. azoles, echinocandins or polyenes) or 'pre-emptive therapy' driven by screening for fungal antigens or nucleic acids. Success has been reported in large randomized trials applying azole antifungals, although there is concern of breakthrough infection with organisms that demonstrate innate or acquired drug resistance.[118,119] Specific concerns that have been reported in this context include infections with azole-resistant *Candida* species, zygomycetes with resistance to voriconazole, and *Trichosporon* and *Candida* spp. that have resistance to echinocandins. Currently, there are many issues of debate concerning preventative therapies, including which antifungal drug is best, which patients benefit and the optimal duration of therapy.

Fungal infections present with the typical appearance of nodular infiltrates on radiographs. In neutropenic patients, who have been studied most extensively, resolution of neutropenia may result in increased size of lesions and cavitation. Fungal pneumonia that occurs in non-neutropenic hosts can present with multiple radiographic abnormalities, including focal or multifocal consolidation. Nodular lesions and consolidations have multiple infectious and noninfectious etiologies (Fig. 74.3); bronchoscopic examination is necessary in order to optimize therapy. Recently, immune reconstitution syndrome has been recognized as a cause of progressive infiltrates in HSCT recipients with pulmonary aspergillosis.[120]

Pneumocystis infections

In the absence of prophylaxis, *Pneumocystis jirovecii* pneumonia (PCP) is a common cause of infection after allogeneic HSCT, particularly during lymphopenia and GVHD. Infections may manifest with multiple typical and atypical abnormalities, including ground glass infiltrates, pneumothorax, pleural effusions and nodular infiltrates.[121] Death attributable to PCP is particularly high after HSCT, emphasizing the importance of effective prevention. Trimethoprim–sulfamethoxazole is most frequently administered using a twice weekly algorithm, although the drug is only moderately tolerated due to allergic manifestations and bone marrow toxicity. Alternatives explored included pentamidine (aerosolized and intravenous), dapsone and atovaquone, although the latter strategies may not be as effective.[122] Importantly, patients who do not receive the trimethoprim–sulfamethoxazole formulation also do not have the added benefits of prevention of other pathogens, such as *Nocardia* spp. and *Toxoplasma gondii* infection; these patients can present with combined pulmonary and CNS lesions (Fig. 74.4).

SUMMARY

Infections remain a leading cause of death in HSCT patients, but the timing of onset and the spectrum of pathogens have evolved over the past two decades. Changes in transplantation practices, different hosts and the development of effective strategies to prevent early CMV disease and candidiasis have increased survival rates. Late after transplantation, severe

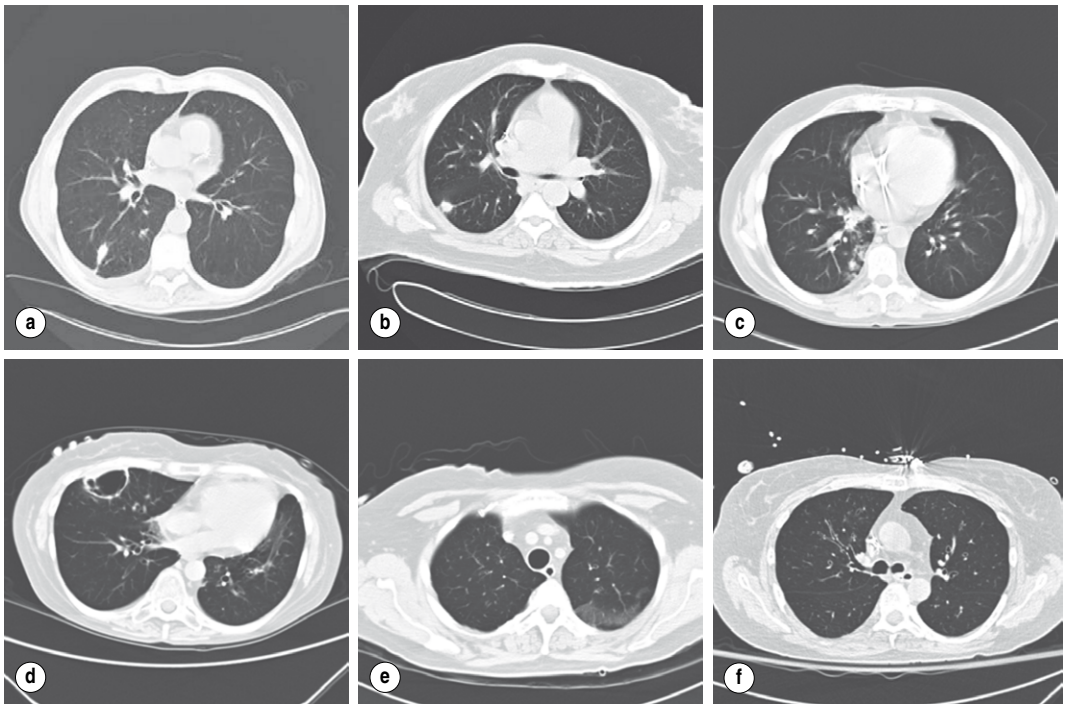


Fig. 74.3 Pneumonia. Multiple CT scans representative of documented infections. (a) *Stenotrophomonas* spp. infection presenting as a nodular lesion in patient with acute myelogenous leukemia (AML) and neutropenic fever. (b) Nodular lesion in a patient with AML and documented aspergillosis. (c) Cytomegalovirus pneumonitis presenting with focal, 'patchy', nodular infiltrate in a patient with graft-versus-host disease (GVHD). (d) Necrotic lesion diagnosed as *Aspergillus fumigatus* in a patient with Hodgkin's lymphoma. (e and f) Progression of scattered patchy peripheral ground glass infiltrate (e), followed by diffuse airway thickening and mild bronchiectasis (f) in a patient with GVHD after nonmyeloablative HSCT, who presented with obstructive symptoms and had *A. fumigatus* tracheobronchitis.

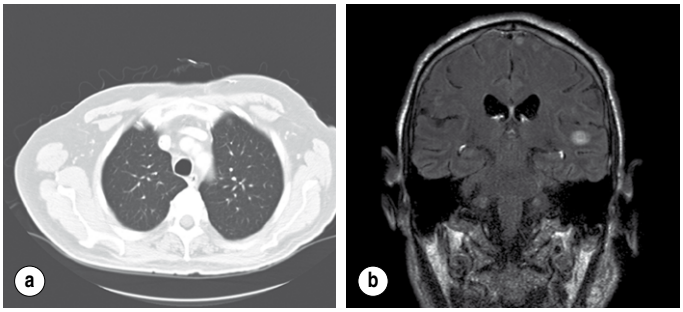


Fig. 74.4 (a, b) Toxoplasmosis. Nodular lung lesions and multiple foci of enhancement with associated edema in a patient treated with rituximab for mantle cell lymphoma who developed fevers and confusion as presenting symptoms of toxoplasmosis.

GVHD dominates the clinical presentation and is often accompanied by the emergence of other organisms, such as molds and other opportunistic pathogens. The approach to this patient population requires not only an understanding of the pathogens, but also a complete appreciation for the host and associated immunosuppression, which defines risk periods, differential diagnosis and appropriate therapies.

REFERENCES

References for this chapter can be found online at http://www.expertconsult.com

Chapter 75

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Infection in solid organ transplantation

INTRODUCTION

The remarkable success of organ transplantation today (~90% 1-year allograft survival for kidney, heart and liver grafts, with long-term survival approaching 20 years) is changing the approach to an increasing number of illnesses that produce end-organ failure – the best chance for many of these patients is now transplantation.[1]

Three major barriers to success in organ transplantation are closely linked: *availability of organs* for transplantation, *allograft rejection* and *life-threatening infection*. The worldwide shortage of organs results in attempts to extend eligibility and use of organs, despite possible injury and/or infection, which in the past would have disqualified a particular donor (or allograft). The use of non-heart-beating donors, older donors and those with pre-existing cardiovascular disease is becoming more common, which places even greater emphasis on the evaluation of potential donors. Successful organ transplantation is predicated on a number of factors, particularly surgical excellence, creation of a protected environment and careful use of both antirejection and antimicrobial agents (Table 75.1).[2–4]

The great irony in transplantation is that transplant patients are not so much at risk from their primary illness but rather have a new concern – microbial invasion. Immunosuppressive and anti-inflammatory therapy, the cornerstones of post-transplant therapy, amplify the extent and effects of invasive infection, while at the same time decreasing the signs and symptoms that could lead to early diagnosis (Fig. 75.1).

The current immunomodulating/anti-inflammatory agents are discussed in Practice Point 40.

Table 75.1 Advances in clinical transplantation

- Optimal tissue typing and matching of donor and recipient, with particular attention to presensitization issues
- Careful donor evaluation, technically impeccable procurement and preservation of the donor organ
- Precise attention to the surgical challenges, resulting in a minimum of tissue injury, secure vascular, bladder, ureteral, biliary or bronchial anastomoses, and the prevention and/or aggressive drainage of fluid collections
- Precise, individualized management of the immunosuppressive regimen: on the one hand, preventing and treating rejection; on the other hand, minimizing the adverse effects on host defenses and establishing the therapeutic prescription
- Prevention of infection whenever possible by instituting a program of environmental protection and preventive antimicrobial strategies, and early diagnosis/aggressive therapy of microbial invasion when prevention fails

Modified from Rubin.[2]

PRINCIPLES OF ANTIMICROBIAL THERAPY IN TRANSPLANTATION

Antimicrobial agents play a critical role in the therapeutic prescription for the transplant recipient. This prescription has two components: an immunosuppressive strategy to prevent and treat rejection, and an antimicrobial strategy to make it safe. Flexibility is built into this prescription: any increase in immunosuppression should be linked to an increase in the antimicrobial regimen, particularly in determining the duration of therapy.[2–4]

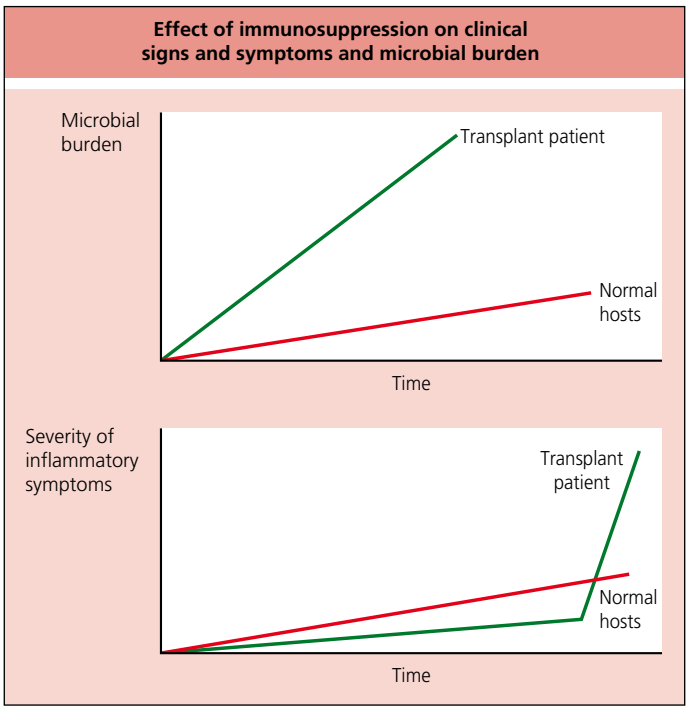


Fig. 75.1 Effect of immunosuppression on clinical signs and symptoms and microbial burden.

Antimicrobial agents can be prescribed in three different modes:[2,4]

- therapeutic mode, in which antimicrobial agents are administered as curative treatment for active infection;
- prophylactic mode, in which antimicrobial agents are administered to an entire population because of perceived risk. For such a strategy to be successful three criteria must be met: (1) the infection to be prevented must be common enough and important enough to justify the intervention; (2) the antimicrobial agent is nontoxic enough to make it safe; and (3) the use of this drug can be shown to be cost-effective; and
- pre-emptive mode, in which antimicrobial agents are administered to patients at risk for life-threatening infection, as determined by a clinical–epidemiologic assessment or a laboratory marker.

The most effective prophylactic program that has been tested is the use of low-dose trimethoprim–sulfamethoxazole post-transplant, which effectively eliminates *Pneumocystis jirovecii* from the differential diagnosis (as opposed to an incidence of 15% in the first 6 months post-transplant without prophylaxis). Significant protection against listeriosis, nocardiosis, toxoplasmosis and urinary tract infection is also provided at the same time.[2–4]

Pre-emptive therapy takes advantage of two separate approaches to the prevention of cytomegalovirus (CMV) disease. Clinical–epidemiologic studies have demonstrated that administration of OKT3 or ATG to CMV-seropositive patients results in reactivation of latent CMV infection and a significant rate of clinically important CMV disease. Initiation of ganciclovir therapy pre-emptively effectively limits CMV invasion. Alternatively, because viremia is usually demonstrable by polymerase chain reaction (PCR) assay 2–7 days before clinical disease is apparent, initiation of pre-emptive ganciclovir therapy on the basis of PCR results can be a useful way to manage antiviral therapy for those patients most in need of such an intervention.[2,4]

A similar situation exists when the active viral infection, such as that due to CMV, is present. Duration of therapy is determined by initial microbial burden, level of immunosuppression already present, and evolution of rejection while antiviral treatment is being administered. When immunosuppression is maintained at too high a level, resistance to antiviral therapy is particularly likely to develop; when antiviral therapy is administered for too short a period of time or at too low a dose, infection relapse occurs.[2,4]

RISK OF INFECTION

Factors have been defined that contribute to the occurrence and extent of infection in the organ transplant recipient (Table 75.2).[2]

Technical/anatomic problems

The occurrence of technical/anatomic problems can lead to devitalized tissue, formation of fluid collections, the need for invasive drainage catheters, indwelling vascular access devices and other foreign bodies that compromise the normal mucocutaneous barriers to infection. The incidence of such infections is determined by the complexity of the surgery being done, the wound healing capability of the patient and the technical skill of the surgeon. Antimicrobial prophylaxis provides some degree of protection for these patients, but nothing takes the place of technically impeccable surgery.

Table 75.2 Classification of infections occurring in organ transplant recipients

Infections related to technical complications	Transplantation of a contaminated allograft Anastomotic leak or stenosis Wound hematoma Vascular access device infection Iatrogenic damage to the skin Mismanagement of endotracheal tube leading to aspiration Infection related to biliary, urinary and drainage catheters
Infection related to excessive nosocomial hazard	*Aspergillus* spp. *Legionella* spp. *Pseudomonas aeruginosa* (and other Gram-negative bacilli, particularly extended β-lactamase producing Gram-negatives) *Nocardia asteroides* *Pneumocystis jirovecii* Methicillin-resistant *Staphylococcus aureus*
Newly emerging pathogens	*Fusarium* *Scedosporium* *Zygomycetes*
Infection related to exposures within the community	Dimorphic, geographically restricted, endemic mycoses: *Histoplasma capsulatum* *Coccidioides immitis* *Blastomyces dermatitidis* *Strongyloides stercoralis*
Community-acquired opportunistic infection resulting from ubiquitous saprophytes in the environment	*Cryptococcus neoformans* *Aspergillus* spp. *Nocardia asteroides* *Pneumocystis jirovecii*
Newly emerging infections	West Nile virus Avian influenza
Respiratory infections circulating in the community	*Mycobacterium tuberculosis* Influenza Parainfluenza Respiratory syncytial virus Adenovirus
Infections acquired by the ingestion of contaminated food/water	*Salmonella* spp. *Listeria monocytogenes*
Viral infections of particular importance in transplant patients	Herpes group viruses Hepatitis B and C Papillomaviruses and papovaviruses Human immunodeficiency virus

The incidence and severity of these infections and, to a lesser extent, the other infections listed are related to the net state of immunosuppression present in a particular patient.
Modified from Rubin.[2]

Environmental exposure

Environmental exposures, both in the community and in the hospital, can play an important role in the occurrence of clinically important infection in the transplant patient. In the community, inhalation of infection-laden air can be an important cause of morbidity and mortality. Thus, the geographically restricted, systemic mycoses (e.g. coccidioidomycosis and histoplasmosis) and acute infection with such respiratory viruses as influenza and respiratory syncytial virus (RSV) will have a greater impact on transplant patients than on the rest of the community. The incidence of disseminated infection, the occurrence of progressive lung disease and the importance of secondary infection with *Staphylococcus aureus*, *Pseudomonas aeruginosa* and other Gram-negative bacteria are all increased in transplant patients. [2,4]

Other exposures of importance within the community include the enteric pathogens, particularly *Salmonella* spp. and *Listeria monocytogenes*, and such opportunistic pathogens as *Aspergillus* and *Nocardia*, which can be acquired from aerosols created in gardening and other recreational activities.[2]

As important as community exposures are in the acquisition of infection, nosocomial exposures are both more common and more dangerous. Such infections tend to be opportunistic and relatively resistant to antimicrobial infection. Two patterns of environmental exposure have been identified: domiciliary and nondomiciliary.[2,4,5]

Domiciliary exposure

Domiciliary exposures occur in the room or on the ward where the patient is housed, and are usually caused by contamination of the air supply or the potable water by such opportunistic organisms as *Aspergillus* spp., *Scedosporium*, *Fusarium* and zygomycetes. Such contamination can occur because of construction; because of the presence of plants and flowers contaminated with Gram-negative bacteria within the patient's room; because of shower and toilet facilities that create aerosols of Gram-negative bacilli or *Legionella* spp.; and because of contaminated water systems or air handling systems. The net result is that these immunosuppressed patients inhale aerosols contaminated by excessive numbers of potential pathogens.[2,4,5] Outbreaks of this type are characterized by clustering of cases in time and space.

Nondomiciliary exposure

Nondomiciliary exposures occur when patients are taken from their rooms to such central facilities as the operating room, radiology suite, cardiac catheterization laboratory and other locations. Excessive exposures of this type are more common than domiciliary exposures and more difficult to identify because of lack of clustering of cases in time and space.

Both domiciliary and nondomiciliary exposures can expose patients to potential pathogens.

Net state of immunosuppression

The net state of immunosuppression is a complex function that integrates a number of factors frequently present in transplant patients that can influence the ability to fight infection. The prime determinant is the immunosuppressive regimen employed – the dose, duration and temporal sequence of the drugs prescribed. Table 75.3 delineates the various factors that can contribute to the net state of immunosuppression.

Table 75.3 The net state of immunosuppression

Immunomodulatory effects of the underlying condition	Effects of the anti-rejection regimen Neutropenia Metabolic factors Protein–calorie malnutrition Uremia Iron overload Hyperglycemia
Immunomodulating infection	Cytomegalovirus Epstein–Barr virus Human herpesvirus 6 Other herpes group viruses Hepatitis viruses
Other factors	Age (at the two extremes of life) Ethnic background (immune response gene effect)

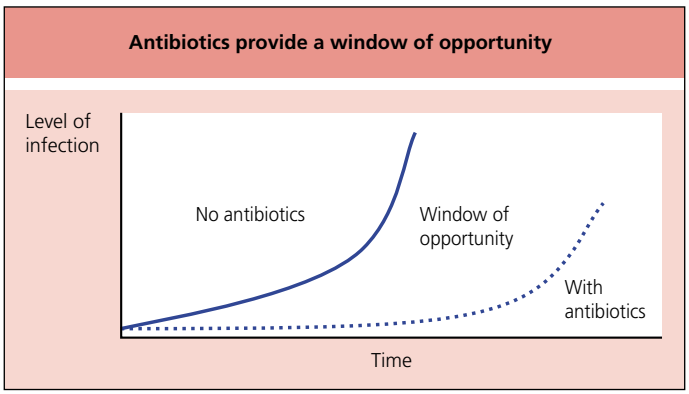


Fig. 75.2 Antibiotics provide a window of opportunity.

Particularly noteworthy in these patients are nutritional/metabolic factors and active infection with immunomodulating viruses such as CMV, Epstein–Barr virus (EBV), hepatitis viruses and human immunodeficiency virus (HIV). Thus, patients with protein–calorie malnutrition (as defined by a serum albumin <2.5 g/dl) have a 10-fold greater incidence of life-threatening infection; 90% of transplant patients with opportunistic infection do so in the context of systemic viral infection. Indeed, the exceptions are usually found to have been exposed to a previously unrecognized excessive environmental hazard.[2–4]

Darwinian factors

Bacteria and fungi compete with each other for nutrients and growth factors. These factors include glucose, iron and the products of red cell lysis. For example, the availability of iron will greatly increase the incidence of infection with such organisms as the zygomycetes, *Aspergillus* and *Listeria*, and other micro-organisms.[6] Similarly, hyperglycemia will greatly increase the level of *Candida* on the skin, in the gut, the pharynx and the female genital tract. A relatively minor break in the integrity of these tissues will lead to significant clinical infection because of the high microbial burden[2,4–6] (Fig. 75.2)

CHOOSING ANTIBIOTIC THERAPY

Choosing the best antibiotic regimen before the results of initial culturing are available can be a daunting process. The first consideration is whether the patient presents a 'therapeutic emergency' or a 'diagnostic dilemma'. If an emergent situation is present, then broad-spectrum therapy is indicated, and issues of cost and toxicity are of little concern. Such therapy is operative for the first 3–4 days, at which time culture information is usually available, the patient is clinically improved and a switch to narrower spectrum therapy, usually via the oral route, is appropriate.[2–4]

TIMETABLE OF INFECTION AFTER ORGAN TRANSPLANTATION

Based on the program of immunosuppression utilized, a 'timetable' (Fig. 75.3) can be constructed that delineates when in the post-transplant course different infections are likely to occur; that is, although pneumonia can occur at any point in the post-transplant course, the etiology will be very different at different times. Unexplained exceptions to the timetable should be regarded as clues to the presence of an unexpected environmental hazard that requires attention.[2–4]

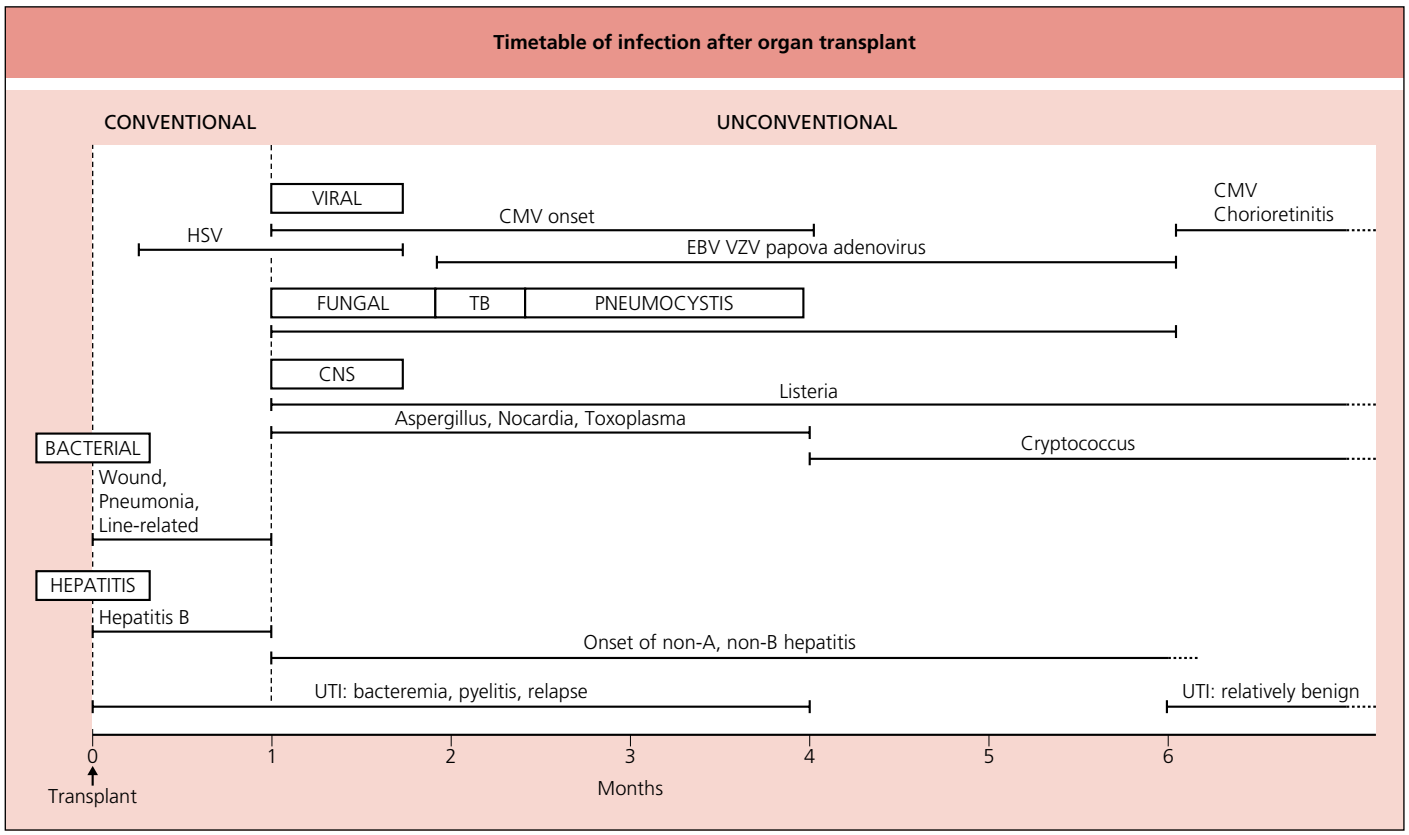


Fig. 75.3 Timetable of infection after organ transplant. Exceptions to this timetable should initiate a search for an unusual hazard. CMV, cytomegalovirus; CNS, central nervous system; EBV, Epstein–Barr virus; TB, tuberculosis; HSV, herpes simplex virus; UTI, urinary tract infection; VZV, varicella-zoster virus. From Rubin.[2]

Infection in the first month post-transplant

In the first month post-transplant, the three major causes of infection in the organ transplant recipient are:

- infection in the recipient present before transplant;
- active infection conveyed with the allograft; and
- the same wound-, catheter-, vascular access-related infection and pneumonia encountered in nonimmunosuppressed patients subjected to comparable amounts of surgery.[2,4]

Donor-derived infection

Donor-derived infection is a major concern. On the one hand, there is a severe shortage of organs; on the other, objective assessment of the risk of infection from a particular donor in a timely fashion remains a challenge. At present, a detailed epidemiologic history is probably the best tool available, with the following categories of infection requiring consideration:

- Bloodstream infection from the donor is usually related to intensive care support during the donor's terminal illness. Vascular access devices are particularly vulnerable to this form of infection, with such organisms as *Candida* spp., *Staph. aureus* and *Staph. epidermidis*, and Gram-negative organisms such as *P. aeruginosa* being particularly important. In addition to the expected consequences of bacteremia, infection of the vascular suture line, with the development of mycotic aneurysms at risk for catastrophic rupture, is a major concern.[2,4]
- Allograft infection, although less common, can have similar adverse effects. The organs commonly transplanted (kidneys, liver, heart, lung and pancreas) receive a significant portion of the cardiac output. This is reflected in the frequency with which disseminated infection seeds the allograft. Such micro-organisms as the endemic mycoses, the opportunistic fungi, mycobacteria and *Nocardia* can remain dormant for prolonged periods, only to become clinically important at a later time.[2,4]
- Viral infection has become the most important class of infection affecting organ transplant patients. The herpes group viruses, particularly CMV and EBV, hepatitis viruses B and C (HBV and HCV), HIV and community-acquired respiratory viruses are major pathogens for the transplant patient. Assessing the potential role of these viruses for both donor and recipient has become an important aspect of donor evaluation.[2,3]
- Newly emerging pathogens are playing an increasing role in transplant recipients. First, such pathogens as influenza (including avian flu and H1N1) and RSV have an increased incidence and severity in the transplant patient (see Fig. 75.1). What is new is the occurrence of clusters of fatal cases of rabies, lymphochoriomeningitis virus and the recognition of hitherto unrecognized viruses in the lungs. In addition, unrecognized lymphoma in the donor has been transferred via transplantation.[7–11]

Although we have entered an era of molecular diagnostics, which can provide the timely information required, the number of assays needed constitutes a formidable challenge. For the present, detailed clinical and epidemiologic evaluations remain the best tools available to prevent catastrophic donor-derived infection, with the available diagnostic assays serving as supplementary information.

Infection 1–6 months post-transplant

This is a period of great vulnerability to infection, a reflection of the fact that the net state of immunosuppression is markedly increased.

Table 75.4 Direct and indirect effects of cytomegalovirus infection in the organ transplant recipient

Direct effects	Mononucleosis/fever of unknown origin Pneumonia Enterocolitis CNS effects Aseptic meningitis Cerebritis Transverse myelitis Guillain–Barré syndrome
Indirect effects	Contributes to the net state of immunosuppression and the risk of opportunistic infection Contributes to allograft injury, with a particular emphasis on a vasculopathy Oncogenesis

There are two reasons for this: the sustained effects of exogenous immunosuppressive therapy now render the recipient particularly susceptible, and this is the time period when the immunomodulating viruses are most active. These include CMV, human herpesvirus (HHV)-6, EBV, HBV, HCV and HIV. The clinical syndromes that are important fit into two general categories:

- the direct and indirect effects of one or more of these viruses (Table 75.4); and
- opportunistic infection that is related to the increased level of immunosuppression, not to unusual environmental exposures.[2,4]

Infection more than 6 months post-transplant

Three categories of patients in this late period need to be considered:

- ~75% of patients will have had a satisfactory result from their transplant;
- 10–15% of patients have chronic liver disease; and
- ~10% of patients will have had a poor outcome from transplantation.

These patients are at particular risk for both opportunistic infections and community-acquired respiratory viruses.[2,4]

VIRAL INFECTIONS OF PARTICULAR IMPORTANCE FOR ORGAN TRANSPLANT RECIPIENTS

Herpes group viruses

The eight human herpes group viruses (CMV, EBV, herpes simplex virus (HSV)-1, HSV-2, varicella-zoster virus (VZV) and HHV-6, HHV-7 and HHV-8) share a number of properties, as follows.[2,4,5,12–16]

Latency

This describes the behavior of viruses when active replication is replaced by a nonreplicating state in a variety of cells within the patient. The viral genome is present in latently infected cells, but gene expression is limited, infectious virus is not produced and the virus is unavailable for immune attack. All patients with latent infection are seropositive for the particular virus. Latent virus is capable of being reactivated, producing fully functional virus. The stability of latency is an important consideration. Cytomegalovirus is stable in latency, requiring exposure to tumor necrosis factor (TNF) and other proinflammatory influences to begin full replication. Any cause of TNF release (e.g. rejection, ATG, sepsis) can be involved in this process. Other viruses, particularly EBV and HSV, can reactivate in response to minimal exposures or even spontaneously. Once viral reactivation occurs, the extent of the viral replication, and the subsequent clinical effects, are determined by the patient's net state of immunosuppression.[2,3,17–19]

Cell association

The term 'cell association' emphasizes that these viruses are spread between individuals by intimate contact, transfusion or transplantation. Spread within an individual is accomplished by the direct contact of infected cells with other susceptible cells.[2,4]

Oncogenicity

All herpes group viruses should be considered potentially oncogenic. In the case of post-transplant lymphoproliferative disease (PTLD) and Kaposi's sarcoma (KS), EBV and HHV-8 play a major role in the pathogenesis of these malignancies. In addition, CMV disease has been shown to increase the incidence of EBV-associated PTLD as much as 10-fold.[2,4,5,15–26]

Clinical significance

Both direct and indirect effects of these viruses on the transplant patient must be considered (see Table 75.4). Direct effects include viral pneumonia, mononucleosis, hepatitis, enterocolitis and meningoencephalitis. These are thought to be due to lytic viral infection, tissue invasion and the host response to these events. The term 'indirect effects' refers to those caused by the cytokines, chemokines and growth factors produced as a consequence of viral replication and invasion. These effects include immunomodulation, allograft injury and oncogenesis. In many patients, the indirect effects are at least as important as the direct effects.[2,4,12–16]

Indirect effects of particular importance are those that affect the allograft: rejection and vascular injury due to the consequences of endothelial cell infection. In lung transplants, bronchiolitis obliterans is also associated with CMV and can be limited by an effective antimicrobial strategy.[2,4]

Cytomegalovirus

Epidemiology

Cytomegalovirus (CMV) can be transmitted in three ways:

- transfusion (of viable, latently infected leukocytes);
- transplantation (of an organ from a seropositive donor); and
- reactivation of virus from a seropositive (and hence latently infected) individual.

Uncommonly, the virus can be acquired through intimate contact between an actively infected individual and a CMV-naive transplant recipient. Three epidemiologic patterns of CMV infection are recognized in transplant patients: primary infection, reactivation CMV disease and superinfection.[2,4]

- Primary infection involves the transfer of viable cells from a seropositive donor into a seronegative recipient; 90% of the time, the allograft is the source of the latently infected cells. In addition, viable leukocyte-containing blood products can be the source of the virus. This latter form of transmission should be avoided by using 'pedigreed' blood products that come from CMV-seronegative donors or those that are administered though highly efficient leukocyte filters. Overall, ~50% of patients at risk of primary infection as a result of transplantation become clinically ill.
- Reactivation CMV infection occurs when the transplant recipient is seropositive prior to transplant and the virus that is activated is of endogenous origin. The great majority of patients in this category will have some evidence of viral replication, but only 10–20% will have evidence of clinical disease.[2,4,16]
- Superinfection occurs when an allograft from a CMV-seropositive donor is transplanted into a seropositive recipient and the virus

that is activated is of donor origin. Superinfection appears to cause more symptoms than reactivation disease. In any case, if intensive immunosuppression is required, both reactivation and superinfection will have greater effects.[2,4,]

Another important clinical problem that is currently appearing is the late onset of CMV after prophylaxis.[27,28]

Pathogenesis of CMV infection

The pathogenesis of CMV infection has two key steps: reactivation from latency, and amplification and dissemination of actively replicating virus. The key mediator in initiating this process is TNF, which binds to the TNF receptor of latently infected cells, resulting in a downstream signaling process that includes the activation of protein kinase C and nuclear factor kappa B (NF-κB). Activated p65/p50 NF-κB heterodimer translocates into the nucleus and binds to the CMV immediate early enhancer region, which initiates viral replication. Such conditions as sepsis, fulminant hepatic failure, allograft rejection and the 'cytokine storm' associated with OKT3 and ATG administration are associated with the elaboration of large amounts of TNF (and other proinflammatory mediators, such as catecholamines and prostaglandins).[2,4]

Once replicating virus is present, the degree of amplification that occurs is determined by the net state of immunosuppression and the subsequent immunosuppressive program. The critical host defense is the major histocompatibility complex (MHC)-restricted, CMV-specific cytotoxic T-cell response. A rapid viral replication rate prior to antiviral intervention, a high viral load and a slow rate of viral clearance on therapy are all associated with a relatively poor outcome to conventional courses of therapy and a high rate of relapse. It has been suggested that the production of interferon gamma (IFN-γ) by $CD8^+$ T cells is reduced in patients with viremia, and that this deficit (termed 'specific interferon gamma impairment', SIGI) has predictive value prior to the initiation of antiviral therapy.[2,4,5,25–27]

Clinical effects of CMV infection

Direct effects Clinical CMV disease typically begins 1–4 months post-transplant, and is similar in all forms of organ transplantation, with one notable exception: the organ transplanted is more affec ted than the native organ (see Table 75.4).

The most common clinical syndromes include mononucleosis, interstitial pneumonia, bone marrow dysfunction, and both functional (poor motility) and structural damage to the gut (enterocolitis and poor gastric and small bowel motility). Perhaps most interesting is the possible bidirectional relationship between CMV and hepatitis C virus: infection with one results in an upregulation of the viral burden of the other.[2,4] However, a recent study argues against an association between HCV and CMV infections after transplantation.[29]

Indirect effects The indirect effects of CMV are of three types: allograft injury, promotion of opportunistic infection and oncogenesis. There is now a consensus that allograft injury can occur as a consequence of CMV infection. The most convincing data on this subject come from studies of heart transplant patients in whom both acute rejection and accelerated coronary atherosclerosis are linked to both symptomatic and asymptomatic CMV infection. CMV-infected endothelial cells are thought to play an important role in the vascular injury that can occur. Similar associations are found in liver and lung transplantation.[30–32]

Perhaps the most compelling information on this subject comes from human studies in which anti-CMV prophylaxis clearly decreased both acute and chronic rejection among kidney allograft recipients, and that ganciclovir therapy in cardiac transplant recipients decreased the incidence and severity of coronary artery atherosclerosis post-transplant. In summary, an ever-expanding body of evidence has linked CMV infection to allograft injury. There is a bidirectional relationship between these two processes, with a similar array of mediators being elaborated and a similar pathologic picture being produced by both; that is, although the initial stimuli may be different, the ultimate signaling pathway that causes injury is the same. A question that has long been debated has finally been answered – 'Which comes first, CMV or rejection?' The answer is either or both. What also becomes a reasonable hypothesis is that other infections (e.g. HHV-6) could have similar effects and that ultimately the extent of allograft injury is the integration of a number of different processes that include ischemia, infection and classic rejection, with room in this model for other as yet to be defined processes.[2–4,33–42]

The potential effects of herpesviruses on oncogenesis

Cytomegalovirus and several other herpesviruses have been thought of as potentially oncogenic for many years. Epstein–Barr virus and HHV-8, in particular, have been linked to post-transplant lymphoproliferative disease (EBV) and KS (HHV-8), respectively. In addition, the incidence of PTLD is increased 7–10-fold in individuals with symptomatic CMV disease. An uncommon form of malignancy linked to EBV is smooth muscle neoplasia of the gut.[20–23,25]

Clinical management of CMV: diagnosis and treatment

Diagnosis The three general approaches to the diagnosis of CMV are serologic, virologic and pathologic. Serologic approaches are useful in assessing the past experience with the virus and the possibility of latent infection (all patients with circulating antibody against CMV should be considered as harboring latent virus that is capable of being reactivated). There is little need for serologic testing post-transplant.[2,3]

The CMV antigenemia assay and a quantitative PCR assay both offer same-day diagnosis, with a sensitivity and specificity of >95%, as well as a useful measurement of microbial burden. There are, however, some drawbacks to these assays: lung and gastrointestinal infection do not yield reliable results (and hence colonoscopic and/or lung biopsy for both immunofluorescent and standard pathologic assessment is still needed for evaluation of the patient with signs and symptoms of inflammation at these sites).[2,3]

Whether it is determined by PCR or antigenemia, the key measurement is viral load. Viral load determinations are useful in several ways:

- as a stratification device in determining the dose and duration of therapy;
- serial viral load measurements can be used to monitor the response to therapy, to guide pre-emptive therapy; and
- to predict the chance of relapsing disease and the need for more therapy.[2,3]

In occasional cases renal dysfunction occurs with a biopsy picture revealing cells laden with CMV inclusions, typically in the tubular epithelium and interstitium. Renal biopsies without CMV at the time of acute functional deterioration of the kidney revealed classic tubulointerstitial findings of acute cellular rejection; however, a patient with viremic CMV had a distinctive glomerular lesion on biopsy. CMV-infected recipients who are viremic have about twice the frequency of glomerulopathy versus those who are nonviremic. Renal dysfunction associated with glomerular lesions is less likely to respond to antirejection therapy. In liver transplantation, this issue is even more complicated as the direct and possible indirect effects of CMV on the liver may be obscured by allograft rejection and hepatitis C infection.

Treatment Strategies for controlling CMV include both preventive and therapeutic approaches (Table 75.5). In the preventive category such interventions as 'protective matching', 'pedigreed blood products', hyperimmune gamma globulin and antiviral therapy have been shown to offer some degree of protection. The term protective matching applies to the allocation of organs so that the transplantation of an allograft from a CMV-seropositive donor into a seronegative recipient is avoided. This strategy is effective, but limits the pool of potential donors at a time when there is already a shortage of organs. At present this approach is largely reserved for children.[2–5,42–51]

There are three groups of antiviral compounds that can be used to prevent or treat the consequences of CMV infection: aciclovir and valaciclovir; ganciclovir and valganciclovir; and foscarnet.[2,3]

Table 75.5 Prevention and treatment of cytomegalovirus infectious dose in the organ transplant recipient

Intervention	Effectiveness
Prevention	
Hyperimmune anti-CMV globulin	2+ (attenuated by anti-thymocyte globulin)
High-dose oral aciclovir or valaciclovir	1+
Valganciclovir	4+
Therapy	
Hyperimmune anti-CMV globulin	1+
Valaciclovir	1+
Valganciclovir	3+

Number of + = degree of effectiveness.

Ganciclovir is the most useful anti-CMV drug currently available. Oral formulations of ganciclovir have the same bioavailability issues as oral aciclovir (<15% bioavailability, which rises to 50–60% when the valine ester is employed). Valganciclovir is the drug of choice for both the prevention and treatment of CMV disease. It is effective both pre-emptively and prophylactically.[2,3]

Primary resistance to ganciclovir is most unusual. However, when too low a dose and/or too short a duration of therapy are utilized, particularly when a high viral burden is present, secondary resistance can develop. In this circumstance, foscarnet becomes the treatment of choice, either alone or in combination with oral valganciclovir. Foscarnet is highly nephrotoxic, hence the hesitation for prescribing it unless resistance to the other antivirals is present.[2–4,43–52]

Epstein–Barr virus

The epithelial cells of the upper respiratory tract are the site of EBV replication and likely the natural reservoir for EBV infection. Transmission of the virus is effected by the exchange of virus-laden saliva. This infection is lytic in character, resulting in the release of replicating virus. These events result in continuing replication of the virus in the upper respiratory tract epithelium, with long-lived B lymphocytes becoming infected during their travel through the lymphoid tissues of the pharynx.[2,3,21–24]

In the nonimmunosuppressed host, clinical disease is initiated by infection of the epithelial cells and B lymphocytes, which then incites an intense, cytotoxic T-cell response to the latently infected B lymphocytes, which are widely distributed throughout the body. This immune response is responsible for most of the manifestations of infectious mononucleosis, as well as providing an explanation for the lack of efficacy of antiviral chemotherapy in dealing with the mononucleosis clinical syndrome.[2,3,21]

Although most adults who are EBV seropositive harbor latently infected, transformed B lymphocytes that have the potential for unlimited growth, lymphoproliferation is blocked by the activity of an active surveillance mechanism based primarily on an MHC-restricted, EBV-specific, cytotoxic T-cell response. High-dose immunosuppression, particularly with calcineurin inhibitors and with ATG, effectively blocks this mechanism, increasing the risk of PTLD. Similarly, primary EBV carries an increased risk of PTLD because the viral burden is greater and the immunologic response to the primary infection is still immature. There is increasing evidence that proinflammatory cytokines generated by such common events as CMV infection, allograft rejection and HCV infection increase PTLD risk considerably.

Table 75.6 Clinical syndromes due to Epstein–Barr virus in organ transplant recipients

Benign viral diseases	Mononucleosis Interstitial pneumonia Syndromes of fever and adenopathy Meningitis and meningoencephalitis Leukopenia/thrombocytopenia Hepatitis Gastrointestinal bleeding Guillain–Barré syndrome
Post-transplant lymphoproliferative disease	Continuum from benign polyclonal process to malignant disease with monoclonal cells Leukopenia/thrombocytopenia Adenopathy Gastrointestinal bleeding due to tumor mass in the wall of the gut with perforation and/or intussusception Pneumonia Focal and diffuse central nervous system disease

For example, CMV disease is associated with a 7–10-fold increase in the occurrence of PTLD and HCV increases the risk more than fivefold in liver transplant recipients.[20–25]

There are, then, two interacting factors that determine the incidence of PTLD in the transplant patient: viral load, and the ability of the EBV-specific, cytotoxic T-cell surveillance system to eliminate the transformed cells.

The clinical presentation of PTLD can be quite variable (Table 75.6). Management requires a marked decrease (including cessation) in immunosuppression, particularly of the calcineurin inhibitors and ATG. If further therapy is needed, anti-B cell monoclonal antibody has proven to be the treatment of choice for the more severe cases of PTLD.[2,20–25]

Herpes simplex virus

Virtually all the instances of HSV infection post-transplant are due to reactivation of latent infection in seropositive individuals, occurring 2–4 weeks post-transplant. Not only do the lesions persist longer than comparable lesions in the normal host, but also the severity is increased, with large, painful crusted ulcerations that may bleed or cause difficulties with aspiration. The typical vesicles on an erythematous base may be absent in the transplant patient, with ulcerations being the more common lesion recognized (both with anogenital and orolabial disease). Intraoral and esophageal infections occur predominantly in patients whose mucosa has been traumatized by endotracheal and/or nasogastric tubes. Lesions that are thought to be responding poorly to aciclovir should be considered for biopsy to rule out the presence of squamous cell carcinoma.[2–4]

Anogenital HSV-2 infection is less common than orolabial infection due to HSV-1. Unusually severe anogenital infection with large, coalescing, ulcerative lesions may be present in patients who are undergoing intensive immunosuppression. An unusual form of cutaneous dissemination (termed eczema herpeticum or Kaposi's varicelliform eruption) may be observed due to HSV, in which the skin lesions occur at sites of previous skin trauma – burns, eczema or atopic dermatitis. Rarely primary infection can occur due to allograft infection.[2,4,5]

Varicella-zoster virus

The most important information that relates to VZV infection in the transplant patient is whether the patient is seropositive for this virus.

Reactivation infection due to VZV (as with herpes zoster) or superinfection due to VZV are relatively mild illnesses; in contrast, primary VZV infection is a devastating illness characterized by hemorrhagic pneumonia and skin lesions, encephalitis, pancreatitis and hepatitis. The traditional approach to a seronegative patient with a significant exposure history to chickenpox is to administer zoster immune globulin, which has an efficacy of >80%. Delays in administering this agent can lead to attenuation of the preventive effects (e.g. the lack of the characteristic skin rash while dissemination and visceral invasion are occurring). Valaciclovir therapy is effective and can be combined with the vaccine in seronegative individuals.[2,3,12–14]

Human herpesviruses 6, 7 and 8

Human herpesvirus (HHV)-6 is a beta-herpesvirus closely related to CMV (66% DNA homology) that infects a variety of cell types, most notably $CD4^+$ cells. Replicating HHV-6 provides a strong stimulus for the release of an array of proinflammatory cytokines, most notably TNF, interleukin (IL)-1 and IFN-γ. The range of clinical effects includes febrile syndromes similar to those produced by CMV (e.g. fever and mononucleosis, hepatitis, bone marrow dysfunction, encephalitis and pneumonia). In addition, HHV-6 is thought to produce a series of indirect effects, particularly the potentiation of opportunistic infections due to an increase in the net state of immunosuppression, and allograft injury.[34,40–42]

HHV-6 primary infection is usually acquired in the first year of life, when it causes roseola (exanthem subitum); reactivation of latent HHV-6 is thought to be the major form of infection encountered thereafter. Possible central nervous system (CNS) effects of HHV-6 have received particular attention, and include mental status changes, seizures and headache. An association between multiple sclerosis and other demyelinating conditions has also been reported. Uncommonly, low attenuation white matter changes similar to those seen with immunosuppression-associated leukoencephalopathy can be identified.[34,40–42]

HHV-7 is another beta-herpesvirus that resembles CMV and HHV-6. Primary infection occurs under the age of 5 due to infection of CD4 molecules. In some ways, HHV-7 might be best thought of as an 'orphan virus' of unclear clinical importance.[15,40,41]

HHV-8 is a gamma-herpesvirus, which is the causative agent of KS. Both primary and reactivation infection with this virus have been linked with KS, particularly in those receiving corticosteroids and/or OKT3 antirejection therapy. Clinical manifestations of KS in transplant patients are similar to those seen in other immunosuppressed patients: violaceous/black nodules are common, often accompanied by visceral lesions, which include bleeding, perforation, obstruction and protein-losing enteropathy. Antiviral therapy with one or more of the following drugs may be useful in the management of transplant-related KS: ganciclovir, foscarnet, adefovir, cidofovir and HPMA [(S)-1-(3)-hydroxy-2-phosphonylmethoxypropyadenine].[20]

Papovaviruses

The papovaviruses are DNA viruses that can be divided into two genera: the polyomaviruses and the papillomaviruses. The polyomaviruses include BK virus, JC virus and simian virus 40 (SV40), which appear to cause asymptomatic infection during childhood; later in life, immunosuppression results in replication of these viruses, which are closely related antigenically and structurally.[53–57]

BK virus

BK virus (BKV) replication has been associated with a variety of renal and urinary tract disorders, including ureteral strictures, renal cell carcinoma and, increasingly, tubulointerstitial nephritis of the renal allograft. With present forms of immunosuppression, the incidence of BKV nephritis approaches 10% (see Practice Point 43).

JC virus

JC virus (JCV) has been shown to be the cause of progressive multifocal leukoencephalopathy (PML), a demyelinating disease of brain white matter. JCV causes progressive motor and sensory deficits, dementia and, usually, death in 3–6 months. As with BKV, a decrease in immunosuppression is usually advocated.[2,58]

Papillomaviruses

Papillomavirus infection is responsible for the development of warts, which can be so numerous in immunosuppressed transplant patients as to be disfiguring. Malignant transformation, particularly in sun-exposed areas, has been well documented. Human papillomavirus DNA has been linked to the skin cancers of these patients, and it is likely that such cancers arise as a result of the combined effects of the virus, immunosuppression and ultraviolet irradiation.[2,4,58]

Hepatitis viruses

Hepatitis B virus

Hepatitis B virus (HBV) is a DNA virus of the Hepadna group that has long been recognized as a significant problem in transplantation: HBV is efficiently transmitted by transfusion and transplantation, a reflection of its ability to sustain viremia for a prolonged period. Person-to-person transmission can also occur through intimate mucosal contact between virus carriers and susceptible individuals. Biomarkers for susceptibility to HBV infection are now in standard use – HBsAg in the blood is a marker for the presence of infectious virus, while anti-HBs is a marker for immunity. However, it should be emphasized that anti-HBs positivity can revert to HBsAg positivity and full transmissibility in the face of intensive immunosuppression. The presence of HBcAb in the serum of liver allograft donors (but not other types of organ transplantation) carries the risk of infecting the recipient. With the exception of this uncommon event, the rate of HBV transmission is estimated to be ~0.002% per transfusion or transplant. Therapeutic intervention with anti-HBS antibody for a prolonged period, beginning at the time of transplant, has had a favorable effect on the course of these patients. Even more promising is the emergence of lamivudine as an effective therapy (until resistance develops).[59–64]

Clinical impact of HBV infection

The major challenge with HBV infection in transplantation is the management of those who are already infected with the virus at the time of transplant. Corticosteroid therapy stimulates the level of virus replication, with a rapid increase in HBV DNA polymerase activity, HBeAg levels, HBV DNA and HBsAg being observed.[2]

Liver transplantation in patients with HBV infection has been particularly problematic. Recurrence of HBV infection was essentially universal, with a number of reports of excessive mortality in the first year post-transplant, as well as the occurrence of hepatocellular carcinoma within 4 years of successful transplantation. There was also an increased rate of sepsis during these first years post-transplant, with a particularly poor result (>80% mortality in the first year) in patients with both hepatocellular carcinoma and chronic HBV infection.[2]

Two major advances have changed this dismal picture. The first was prolonged immunoprophylaxis with anti-HBs hyperimmune globulin, beginning during the anhepatic phase of the transplant operation. One-year survival rates greater than 80% could now be achieved. In the face of these successes, two major problems emerged: cost (as high as $25 000/year with some administration schedules) and, more important, the selection of viral mutants that are still pathogenic but have changes in HBsAg such that the hyperimmune globulin and/or the hepatitis B vaccine is rendered inactive.[2,56–61]

The biggest advance, however, is the advent of effective antiviral chemotherapy with lamivudine. This nucleotide analogue is a potent

inhibitor of the viral polymerase/reverse transcriptase, is well tolerated, and is effective not only in lowering viral levels, but also in improving hepatic function and histology. Resistant virus begins to emerge after 1 year of therapy (these mutants are usually in a highly conserved region of the reverse transcriptase known as the YMDD motif). The higher the viral load, the more likely and more quickly resistance mutants will emerge. After 1 year of therapy, resistance rates of 14–31% are noted, and at 2 years this rises to 38%. Clearly, multidrug regimens will be necessary for complete control of HBV.[2–4,62–64]

Hepatitis C virus

Hepatitis C virus (HCV) is an RNA virus that is the cause of post-transfusion hepatitis, post-transplant liver disease and most of the cases of non-A, non-B hepatitis. HCV is a common cause of end-stage liver disease necessitating liver transplantation, may cause progressive liver disease following transplantation and is an important contributor to the net state of immunosuppression. The most effective therapy at present is the combination of pegylated interferon and ribavirin, which appears to favorably influence the course of ~50% of patients with chronic HCV infection.[65,66]

Clinical impact of HCV infection

Hepatitis C virus is the major cause of non-A, non-B hepatitis, as well as more than 80% of post-transplant liver disease. HCV is spread via parenteral contact with blood or by transplantation of an organ from an HCV-viremic donor. HCV appears to differ from hepatitis A virus (HAV) and HBV in rarely producing fulminant hepatitis. HCV infection carries a greater than 50% risk of chronicity, with end-stage liver disease or hepatocellular carcinoma developing as a consequence of this in ~20% of these patients.[2–4,65,66]

Risk factors that are correlated with increased morbidity and mortality include viral load; intensity of immunosuppression; allograft rejection; HCV genotype (genotype 1b having been associated with higher viral loads, a more accelerated course, and a poorer response to interferon-based therapy); the presence of quasispecies (the greater the heterogeneity in the HCV population, the greater the difficulty in containing the infection); CMV viremia; iron overload; and details of cytokine responses that are beginning to emerge from ongoing studies.[2–4]

Two uncommon complications of HCV infection can be devastating. The first – fibrosing cholestatic hepatitis – manifests as high levels of circulating HCV, rapidly progressive hepatic failure, mildly elevated serum aminotransferase level, extensive periportal fibrosis, intense cholestasis, minimal inflammatory infiltrate and no cirrhosis. The hepatocytes are choked with exceedingly high levels of HCV, which results in direct hepatocyte injury. This diagnosis requires immediate decrease in immunosuppression and the initiation of the best available antiviral therapy.[2–4]

The second of these complications is glomerulonephritis. The most common form of this is membranoproliferative glomerulonephritis, with or without mixed cryogobulinemia. Uncommon forms of renal injury associated with HCV infection include membranous glomerulonephritis, acute and chronic transplant glomerulonephritis and thrombotic microangiopathy with anticardiolipin antibody.[2–4]

Human immunodeficiency virus

Transplantation of an organ from an HIV-infected donor into an uninfected recipient is a highly efficient mechanism for transmitting the virus. Rarely, a false-negative HIV test can be obtained from a donor who transmits the virus with the allograft. A bigger issue is the donor who has received large numbers of transfusions in a desperate attempt to save the individual's life. By this time, blood bank blood will obscure the results of laboratory studies: false-negative results can be obtained in this situation since the blood actually tested does not reflect clinical events; that is, the allograft in this situation, despite negative serologies, is quite able to transmit both HIV and hepatitis B and/or C.

When primary HIV infection is acquired post-transplant, it not uncommonly produces a mononucleosis syndrome ~6 weeks post-transplant. The mean time for the development of overt AIDS in persons who acquire primary HIV infection at the time of transplant has been ~3 years. A far bigger issue is the optimal management of patients with asymptomatic HIV infection who present with end-stage renal, hepatic, pulmonary and/or heart disease. Thus far, the results of transplantation in these individuals have been remarkably good. Increasingly, transplant groups are establishing guidelines for transplanting the HIV-positive individual:[67–70]

- Transplantation of HIV-infected individuals should be carried out at centers with great expertise in both transplantation and HIV therapy, and where these skills can be brought to bear in the day-to-day care of these patients.
- HIV-infected patients accepted for transplantation should be on a stable highly active antiretroviral therapy (HAART) regimen, are known to be compliant and to have acceptable blood levels of virus.
- Careful ongoing assessment for opportunistic infection should be undertaken. Appropriate prophylaxis should be initiated (e.g. trimethoprim-sulfamethoxazole, fluconazole) as well as perioperative antibacterial prophylaxis.

FUNGAL INFECTIONS OF PARTICULAR IMPORTANCE FOR THE ORGAN TRANSPLANT RECIPIENT

Invasive fungal infection (Table 75.7) has become increasingly important to the transplant patient in recent years:[1]

- A wide variety of fungal species capable of invading the transplant patient are present in the environment, both in the community and in the hospital. Person-to-person spread of *Candida* spp. by medical personnel has increased the difficulty of patient management.[2]
- The net state of immunosuppression can be quite high in these patients, facilitating both transmission and amplification of infection.
- The anti-inflammatory (as well as immunosuppressive) effects of antirejection therapy tend to obscure the signs and symptoms of infection. Along with the bland nature of patient–fungal interactions, early diagnosis and treatment can be a major challenge.
- Several new therapies have been developed; however, guidelines for the treatment and prevention of invasive disease are still being established. Particularly important in formulating these guidelines is the therapeutic prescription – an antirejection program and an antimicrobial program to make it safe. Such an approach needs flexibility, in which changes in one of these will trigger changes in the other.
- Initial host defenses against these fungi are mediated by polymorphonuclear leukocytes and macrophages: definitive immune response mediated by MHC-restricted, fungus-specific, cytotoxic T cells – just the form of host defense most susceptible to antirejection therapy.

Environmental exposures play a major role in the occurrence of fungal infections, with the inhalation of infectious aerosols being the most effective means of acquiring invasive infection due to a wide range of fungi (the endemic mycoses, the opportunistic molds like *Aspergillus*, and newly emerging organisms such as *Fusarium* and *Scedosporium*).[2]

The other major route by which transplant patients acquire clinically important fungal infection is via candidal overgrowth on mucocutaneous surfaces. From that point, it is a relatively small step to penetration of the mucocutaneous barrier, with invasion followed by the possibility of disseminated infection.

Table 75.7 Clinical fungal infection in the organ transplant recipient

Challenges	Recognition of signs and symptoms of early fungal invasion Blandness of the host response to fungal invasion Anti-inflammatory effects of the anti-rejection therapy Mucocutaneous overgrowth with increased potential for invasion, dissemination and development of metastatic sites of infection Host responses: • Nonspecific ('innate immunity') – Polymorphonuclear leukocyte response – Macrophage and monocyte response • Specific (specific immunity) – Key host defense: major histocompatibility complex (MHC) restricted, microbial restricted cytotoxic T-cell response – Specific humoral response (B cell response)
Key prognostic sign	Microbial burden
Important clinical infections	Candidiasis (key species: *Candida albicans, C. tropicalis, C. krusei, C. glabrata*)
Mucocutaneous overgrowth with the potential for dissemination	Bloodstream invasion 5% rate of metastatic spread in nonimmunosuppressed host 20–50% rate of metastatic spread in transplant recipients Metastatic spread should be assumed in the presence of vascular access catheters and as a consequence of surgical manipulation of colonized sites Fluconazole susceptible (*C. albicans* and *C. tropicalis*) Fluconazole resistant (acquired resistance with prolonged exposure; person-to-person spread on hands of medical personnel; intrinsic resistance of *C. krusei* and *C. glabrata*)
Invasive opportunistic fungi (saprophytes from the environment)	Invasive aspergillosis (the model for molds, including the endemic mycoses and newly emerging fungi) Primary portals of entry: inhalation, damaged skin Vascular invasion Infarction, hemorrhage and metastatic spread Primary therapy: voriconazole ± echinocandin ± amphotericin
Endemic mycoses	Histoplasmosis, coccidioidomycosis and blastomycosis Inhalation of infectious particles Consequences: hypersensitivity syndromes, lung disease (primary disease, progressive disease, metastatic disease) Particular impact on transplant patients Therapy: induce remission with amphotericin (particularly a lipid formulation) followed by an azole for prolonged therapy
Newly emerging fungal pathogens	Similar events to invasive aspergillosis Examples: *Fusarium* spp., *Scedosporium* spp., zygomycetes Therapy: tend to be amphotericin resistant; therapy with either voriconazole or posaconazole

'Darwinian factors' are now recognized as contributing significantly to microbial pathogenesis. The most common example of this phenomenon is when broad-spectrum antibacterial therapy has been given in the setting of an anatomic problem that has not been corrected. Candidal superinfection is a common result, requiring surgical correction as well as systemic antifungal therapy. Another version of Darwinian influences is when excessive amounts of iron, an important growth factor, can potentiate mucormycosis, listeriosis and candidiasis. Elevated glucose levels will increase the microbial burden in diabetics, particularly on mucocutaneous surfaces, where candidal overgrowth can play an important role in the pathogenesis of invasive infection.[2,3]

Candidiasis

Candida albicans and *C. tropicalis* are the most common candidal species to cause human disease. Secondary infection due to azole-resistant species such as *C. glabrata* and *C. krusei* is common, particularly in circumstances where the underlying defect in mucocutaneous structures remains uncorrected. Typical sites of infection include a catheterized urinary tract, vascular access catheters, peritoneal dialysis catheters and drainage catheters, particularly in the setting of hyperglycemia. Removal of these foreign bodies in association with antifungal therapy has been strongly advocated. Normally present on mucocutaneous surfaces, the vagina and the gut, invasive candidal disease is associated with overgrowth and tissue damage. Bloodstream invasion is a not uncommon event, with an incidence of 5–60% of metastatic infection of the eyes, bone and other sites. Manipulation of sites colonized by these organisms is particularly associated with bloodstream entry and dissemination. For this reason, pre-emptive therapy in association with surgery is advocated. Fluconazole has long been the treatment of choice, but with the increasing incidence of resistance, the possible need for broader spectrum therapy should be considered.[2,3,5,71–73]

Cryptococcosis

Cryptococcosis is an important cause of opportunistic infection, being ubiquitous in the environment and easily inhaled when an aerosol is created. The portal of entry is the lungs, with infection causing minimal symptoms and most infections escaping medical attention. In the transplant patient, a variety of clinical syndromes may occur: in the

lungs, asymptomatic nodules are most common, although progressive pneumonia can occur. Systemic infection with metastatic seeding is common, with a particular impact on the CNS (with both meningitis and cerebritis being seen). Diagnosis is greatly facilitated by the ability to measure the presence of the polysaccharide capsule in the blood and/or cerebrospinal fluid. This is useful for both primary diagnosis and as a measure of the microbial burden. Initial therapy is usually with the combination of flucytosine and an amphotericin for 1–2 weeks to 'gain control', followed by a prolonged course of fluconazole.[2,4]

Invasive aspergillosis

Although a variety of hypersensitivity and colonization syndromes have been associated with *Aspergillus* infection, in transplant patients the concern is primarily with invasive disease following inhalation of aerosolized organisms that are ubiquitous in the environment. Three species account for 95% of the cases of invasive aspergillosis: *A. fumigatus*, *A. flavus* and *A. niger*. More than 80% of the patients with invasive disease have a pulmonary portal of entry, ~10% have a nasal sinus portal of entry, with cutaneous sites accounting for most of the rest.[2,3,74–76]

Both neutropenia and corticosteroid administration predispose to invasive aspergillosis. Environmental hazards leading to excessive exposure are the rule: within the hospital (usually representing construction) or in the community where such activities as gardening can lead to invasive disease. The clinical behavior of this entity is determined by its ability to invade blood vessels, so that the results of this infection include infarction, hemorrhage and metastatic spread.[2,3]

In more than 50% of patients with invasive aspergillosis, metastatic spread is already present at the time of first diagnosis; in addition, a site of metastasis may be the first sign of disseminated disease. The typical radiologic finding in these patients is focal lung disease, with either a nodule or a consolidation being present, often with cavitation. Unlike the leukemia or bone marrow transplant patient with invasive aspergillosis, halo signs and air crescent signs are uncommon. At present, biopsy is required for diagnosis. Preliminary evaluation suggests that the galactomannan assay may be a reliable biomarker for this condition.[2,3]

Therapy is continuing to evolve: voriconazole (or posaconazole; very few data on posaconazole for therapy) is the single drug of choice at present. Combination therapy is being further evaluated, with an echinocandin and/or an amphotericin B preparation being added to the azole. If the clinical state of the patient is appropriate and the anatomy is favorable, surgical resection in combination with the antifungal drugs will yield improved results.[77,78]

Invasive aspergillosis is potentiated by steroids and/or neutropenia, and occurs only in patients who are significantly immunocompromised and/or exposed to a major environmental hazard. Other associations include the presence of *Aspergillus* in marijuana, which can lead to invasive disease. Direct inoculation of damaged skin (e.g. vascular access sites, burns and sites of inoculation) can likewise require treatment. In lung transplant recipients two additional forms of *Aspergillus* infection have been noted: infection of the suture line, with subsequent necrosis and disruption, and tracheobronchial disease.[2]

Invasive aspergillosis, then, primarily occurs in situations of poor leukocyte function or a sustained exposure to steroids. However, even patients whose net state of immunosuppression is minimal can develop invasive aspergillosis if the environmental exposure is great enough. An important corollary to this observation is that a single case of invasive aspergillosis occurring in the first month post-transplant (a 'golden period' during which opportunistic infection only occurs under conditions of unusually intense environmental exposure) should trigger a search for environmental hazards before more cases occur.[2,3,74]

Clinically, invasive aspergillosis of the lungs, the most common presentation of invasive disease, can occur as the primary event or as a superinfection after pulmonary injury due to virus or bacteria, or pulmonary infarction. In as many as 50% of cases metastatic spread has already occurred by the time of diagnosis, and a site of metastasis may be the first presentation of invasive disease. The typical radiologic finding of invasive aspergillosis of the lungs is focal lung disease, with either a nodule or a consolidation being present, often with cavitation. Unlike the patient with leukemia and aspergillosis, halo signs and air crescent signs are uncommon in organ transplant patients. Specific diagnosis requires a biopsy procedure. Other diagnostic approaches such as the galactomannan assay appear to be quite promising. If the early promise is confirmed, then early pre-emptive therapy will become an important part of patient management.[2,3,73–76]

The traditional view of *Aspergillus* syndromes is that a colonization syndrome or allergic bronchopulmonary disease does not turn invasive. It is now clear, however, that on occasion this 'overlap' or 'crossover' occurs. Voriconazole now makes it possible to treat any evidence of invasive disease and, in many patients, avoid surgery. Combining voriconazole with surgery is also a useful strategy, preventing complications of surgical manipulation.[78]

The standard of care for invasive disease due to aspergillosis has long been a prolonged course of intravenous amphotericin B. Sustained therapy of this sort was rendered difficult by the toxicities of this regimen: an acute infusion toxicity, with cytokine release, fever, rigors and malaise; and chronic, progressive renal toxicity. Fortunately, there has been a major advance in the availability of alternative antifungal drugs. Antifungal agents, such as lipid-associated amphotericin B products, therapeutic azoles and echinocandins are discussed in Chapter 149.[71]

Pneumocystis jirovecii

Pneumocystis jirovecii (Table 75.8), previously known as *P. carinii*, was found to be the cause of interstitial pneumonia in Central and Eastern Europe in the mid-1940s among severely malnourished and premature infants. Long recognized as an important pathogen in transplant patients and other immunocompromised patients, the HIV epidemic amplified the importance of this pathogen. It is only in the past few

Table 75.8 *Pneumocystis jirovecii*

- Long recognized as an important pathogen, responsible for pneumonia (PCP) in immunocompromised hosts
- Exists in three forms: trophozoite, sporozoite (which is a precystic form) and cystic. For many years, mistakenly called a protozoan
- Although officially classified as a fungus, *Pneumocystis* does not respond to antifungal therapy
- Asymptomatic infection acquired before age 5.
- With immunocompromise, either reactivated or re-infection can occur.
- Patients with immunocompromise should be protected from person-to-person spread.
- Setting for *Pneumocystis* pneumonia
 - Children with malnutrition and hypoglobulinemia
 - HIV infection and/or AIDS
 - Organ transplant recipients
- In organ transplant patients not receiving prophylaxis, the incidence of pulmonary infection is 10–15% in the first year post transplant
- Prophylaxis indicated for ≥6 months
- *Pneumocystis* pneumonia clinically and radiologically very similar to CMV
- Clusters of patients with the exact same organism can easily be identified. Immunocompromised patients should be protected from nonimmunized patients
- Antimicrobial therapy for *Pneumocystis*
 - Trimethoprim–sulfamethoxazole
 - IV pentamidine (aerosolized form less effective)
 - Clindamycin
 - Dapsone ± pyrimethamine + leucovorin
 - Solid organ transplant
 - Atovaquone
 - Caspofungin ±

years that the taxonomy of this organism has been firmly established: it is not a trypanosome or a protozoan, as previously thought, rather it is a unicellular fungus that is usually acquired in the first decade of life and exists in three distinct forms: trophozoite (often found in clusters), sporozoite and a cystic form that often contains spores. Asymptomatic colonization is well recognized, with clinical disease occurring in patients with deficits in cell-mediated and humoral immunity. For example ~15% of organ transplant patients not receiving *Pneumocystis* prophylaxis will develop *Pneumocystis* pneumonia in the first 6 months post-transplant, particularly those patients with active CMV infection.

After long debate, it is now accepted that both person-to-person and hospital-based epidemics can occur due to *Pneumocystis*, although sporadic cases due to activation of longstanding lung colonization are even more common. Infection control activities to prevent outbreaks among immunosuppressed patients are appropriate. *Pneumocystis* clinically has a subacute presentation over several weeks, with fever, increased dyspnea, a nonproductive cough, tachypnea and pulmonary infiltrates. Rarely in transplant patients, but not uncommonly in HIV patients, extrapulmonary findings may be present, especially if aerosolized pentamidine is used. Other than tachypnea, abnormalities on physical examination are subtle or not present. The typical laboratory findings include an elevated lactate dehydrogenase level, diffuse bilateral infiltrates on X-ray and the presence of the organisms on microscopic examination of induced sputum. If this is negative, then bronchoalveolar lavage is indicated.

Therapeutic decision making is an important issue. Prevention is usually accomplished with trimethoprim–sulfamethoxazole; systemic treatment with the same drug for symptomatic *Pneumocystis* disease is the regimen of choice. Prednisone as adjunctive therapy should be considered for patients with significant hypoxemia. A wide variety of drugs (including pentamidine, atovaquone, trimetrexate, dapsone and pyrimethamine) are available for patients not tolerating trimethoprim–sulfamethoxazole. Drugs such as caspofungin, which act by inhibiting fungal cell wall synthesis in a fashion analogous to the effects of penicillin on bacterial cell wall synthesis, may add considerably to the efficacy of standard treatment. Clinical studies of the utility of this approach are currently in progress.[79–81]

Endemic mycoses

The three endemic mycoses (blastomycosis, coccidioidomycosis and histoplasmosis) share a number of characteristics in common: the organisms are dimorphic, growing as a mold in the soil of a particular geographic area, and the infectious particles, the conidia, are liberated from the mold into an aerosol that is inhaled. The host defense response to this challenge is nonspecific, consisting of polymorphonuclear leukocytes and alveolar macrophages. The more potent specific responses of cytotoxic T cells and specific antibodies then appear. The attenuation of the specific immune response caused by immunosuppression will lead to an increased incidence and severity of progressive pulmonary and disseminated infection, as well as metastatic spread to the skin, the CNS, bones and joints. Appropriate amphotericin B therapy followed by a prolonged oral course of an azole is the treatment of choice.[82]

Blastomycosis

Blastomyces dermatitidis, the causative agent of blastomycosis, has a geographic distribution similar to histoplasmosis (midwest and the south-eastern USA). Physical manifestations of this infection include radiologic findings of invasive pulmonary disease, hilar adenopathy and skin abnormalities. Disseminated infection can cause large nodular skin lesions that undergo necrosis and fibrosis. Therapy is with amphotericin B, followed by itraconazole.[83,84]

Coccidioidomycosis

Coccidioides immitis is dimorphic, growing as a mesh of septate hyphae that bear the arthoconidia that are inhaled and that mature into spherules, the definitive tissue pathogen. These events take place in the desert soil of the Lower Sonoran life zone, which encompasses the San Joaquin Valley of California, the south-western USA, northern Mexico and various sites of Latin America. The range of infectivity can be greatly extended by wind and dust storms, as well as on dust-laden packages sent from the endemic area. Risk factors for clinical disease include immunosuppression, pregnancy and non-Caucasian racial status. Following inhalation by a transplant patient the primary forms of disease encountered are progressive pneumonia, bloodstream invasion and meningitis.

A diffuse granulomatous meningitis encasing the base of the brain, causing hydrocephalus, cranial nerve palsies and a cerebral vasculitis with focal neurologic findings is the most important consequence of this infection, although other sites of metastatic infection can occur. The skin, the skeleton and the genitourinary tract are the most common other sites of involvement. During the first year of residence in the endemic area, an estimated 5% rate of infection is noted, with an additional 2–3% per year thereafter. Dissemination, often with CNS involvement, is found in as many as 75% of these individuals. Although hypersensitivity syndromes are common with this organism, these are rare in transplant patients because of the immunosuppression.

High-dose and prolonged amphotericin B therapy has long been the standard of care for this infection, with relapse being expected. This situation has been improved by the availability of fluconazole. The present approach is to gain control with amphotericin B and then switch to an extended course of oral fluconazole. Prophylaxis of transplant patients with fluconazole is usually recommended.[85,86]

Histoplasmosis

Histoplasma capsulatum is a dimorphic fungus that can be found in soil enriched with bird, bat and starling droppings in the Ohio and Mississippi river valleys, with extension into Maryland and Virginia. The inhalation of aerosolized organisms is the first step in the evolution of this infection, producing in the transplant patient both progressive pulmonary disease and systemic dissemination. Infected mononuclear cells are efficient carriers of the organisms, particularly to sites where large numbers of reticuloendothelial cells are normally found: liver, spleen, lymph nodes, bone marrow, gut and adrenal glands. In addition, mucocutaneous and CNS infections are not uncommon.

Studies carried out in Indianapolis during an epidemic resulting from urban renewal projects have shown a rate of infection of 5–15% among renal transplant patients. Disseminated infection is common in this situation, with CNS infection being particularly common. The treatment of choice is initial therapy with amphotericin (particularly with a lipid formulation that can target reticuloendothelial infection), followed by a prolonged course of itraconazole.[87,88]

Newly emerging mycoses

Unusual fungal species like the zygomycetes (the causative agents of mucormycosis), *Fusarium* and *Scedosporium* are becoming more common in transplant patients.[89] These are predominantly molds that produce a clinical picture similar to that seen with *Aspergillus*: invasive disease with vascular invasion and metastatic infection. Unless there is evidence to the contrary, these organisms should be considered amphotericin B resistant. The therapy of choice is voriconazole or posaconazole. Posaconazole is particularly effective against mucormycosis (an important point if voriconazole therapy selects for zygomycetes).

Zygomycosis (also termed mucormycosis) is a rapidly progressive fungal infection that causes tissue infarction. This necrotizing infection is observed in the following situations:

- primary infection of the skin at a site traumatized by extravasation of intravenous fluids, water immersion injury or pressure dressings contaminated by *Rhizopus* sporangiospores;
- primary pulmonary infections following inhalation of fungal sporangiospores; and
- rhinocerebral zygomycoses in which the fungal aerosol establishes disease in the nasal sinuses, with progressive extension into intracranial structures.

The clinical hallmark of zygomycosis is rapidly progressive, necrotizing infection, often with eschar formation in the skin and mucosa overlying involved tissues. Risk factors include acidosis (particularly diabetic ketoacidosis), steroids, over-immunosuppression and devitalized tissues that can be secondarily infected. Therapy is with posaconazole, with or without ablative surgery.

CONCLUSIONS AND SUMMARY

Invasive fungal infections remain a major problem for transplant recipients, causing pneumonia and bloodstream infection at a considerable cost and with greater consequences if not diagnosed early and treated aggressively. A major improvement in the treatment of fungal infection is the three separate classes of drugs (lipid-associated amphotericin B, antifungal azoles and echinocandins) that can be utilized. Particularly appealing is the possibility that combination therapy, bringing together different mechanisms of action, will result in even better and more effective therapy. For example, the combination of an echinocandin with trimethoprim–sulfamethoxazole appears to be more effective than either drug by itself. Similar observations are being seen with the treatment of *Aspergillus* spp. Early diagnosis, as with the galactomannan assay or a PCR-based technique, will improve the therapeutic results. Better diagnostics will help as well. High-definition chest CT scanning is proving to have a role in early diagnosis and is an example of utilizing existing technology in an innovative fashion. One word of caution: false-positive galactomannan assays are seen with piperacillin–tazobactam and amoxicillin–clavulanate up to a week after discontinuing the antibiotics.

REFERENCES



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Chapter 76

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Heart, lung and heart–lung transplantation

INTRODUCTION

According to the 2008 Registry of the International Society for Heart (HT) and Lung Transplantation (LT), >80 000 heart, 3342 heart–lung and 25 950 lung transplantation procedures have been performed worldwide. Approximately 5000 HT are performed each year. The slow decline in reporting of HT over the last 15 years appears to have finally stopped, with significant increases in the last 2 years.

The most common indication for adult *heart transplantation* is now noncoronary cardiomyopathy (50% vs 34% for January to June 2007) and the proportion of recipients aged more than 60 years has steadily increased, now accounting for almost 25% of all recipients. Congenital heart disease is the most common cause of HT in children. Patients arrive to the transplant procedure in a more critical situation: 41% were receiving intravenous inotropic support and 29% were on some type of mechanical circulatory support modality (22% on a left ventricular assist device, LVAD). These patients commonly receive perioperative antilymphocyte antibodies (51%), tacrolimus (57%), mycophenolate mofetil (77%) and prednisone (63%) at 1 year post-transplant.

The main indications for *lung transplantation* are chronic obstructive pulmonary disease (COPD, 36%), idiopathic pulmonary fibrosis (IPF, 20%), cystic fibrosis (CF, 16%) and α_1-antitrypsin deficiency emphysema (AAT, 8%). Bilateral transplantation accounted for 67% of transplant procedures in 2006 and its proportion has risen for each of the four major indications since 1994 (for CF, bilateral transplantation is the norm). The age of donors and recipients has shifted toward a larger portion of older recipients (in 2007 24% of recipients were >60 years of age). Induction therapy, usually with interleukin-2 receptor (IL-2R) antagonists and alemtuzumab (Campath) has risen (39–54%). Calcineurin inhibitor plus purine synthesis antagonist comprised the maintenance regimen for approximately 77% of recipients at 1 year. However, regardless of the maintenance drug regimen, acute rejection in the first year is common.

Overall, 1-year survival is 80% for both types of transplantation; after the second year, 3.5% of the patients die annually. Graft failure is the main cause of death in the first 30 days post-transplant (42% of deaths after HT and 28% after LT), followed by non-cytomegalovirus (CMV) infection (13% of deaths after HT and 20% after LT). From 31 to 365 days, non-CMV infection is the most important cause of mortality (33% of deaths after HT and 36.4% after LT), followed by graft failure (18% in both populations) and acute rejection (12% after HT and 1.8% after LT). Median duration of patient survival after HT is 10 years and 5.2 years after LT (13 years and 7 years for those surviving the first year, respectively).

Infection is a very important cause of morbidity and mortality in patients undergoing these procedures, and this is of special interest as infection is amenable to prevention and intervention. When one considers the cumulative incidences of individual causes of death, non-CMV infection represented the leading single cause of death from 6 months post-transplant through 10 years of follow-up. We will now briefly review some specific characteristics of infectious complications in these populations.

EPIDEMIOLOGY AND SPECIFIC RISK FACTORS FOR INFECTION

The incidence of infection after HT ranges from 30–60%, with a related mortality of 3–15%.[1] Patients undergoing lung or heart–lung transplantation are at a higher risk for developing infectious complications. These account for approximately 40–50% of all deaths in these patients.

The chronology of the infections after solid-organ transplantation (SOT) has been described in detail in other chapters (see also Chapter 75). In summary, early infections (≤1 month) are similar to those of other patients who have undergone cardiothoracic surgery (pneumonia, empyema, wound infection, mediastinitis, catheter-related infections, urinary tract infections, etc.). Intermediate infections (2–6 months) are usually caused by opportunistic micro-organisms (CMV, fungi, etc.). Finally, late infections (≥6 months) may be caused either by common community pathogens in healthy patients or by opportunistic micro-organisms in patients with chronic rejection (Table 76.1).

Risk factors for infection after thoracic transplantation are described in Table 76.2. They may be classified into factors related to the patient and those due to technical complications during the operative and perioperative periods. We also recommend ruling out hypogammaglobulinemia in these patients.[13–15] The need for ventricular assist devices is an important risk factor for infection in HT recipients. In some series, up to one-third of patients on biventricular assist devices died of sepsis; bacteremia (59%), driveline infection (28%) and pump infection (11%) are also common.

A unique feature of cystic fibrosis patients who are undergoing lung/heart–lung transplantation is that they may develop bacteremia with organisms that normally inhabit their airway (*Pseudomonas aeruginosa*, *Burkholderia cepacia*, *Alcaligenes xylosoxidans*, *Stenotrophomonas maltophilia*). Moreover, the pattern of infections of the late period is unique in lung/heart–lung transplantation compared to other organ recipients. The lung is the most important site of late infection in these patients and the presence of chronic rejection or bronchiolitis obliterans is a major risk factor. Pathogens found in these late pulmonary infections can be the same as those recovered from cystic fibrosis patients, regardless of their underlying disease (*P. aeruginosa*, *A. xylosoxidans*, *S. maltophilia* and *Aspergillus fumigatus*). The presence of one or more of these potential pathogens may be associated with asymptomatic colonization or fatal disease. Opportunistic infection with *Nocardia* or *Cryptococcus* spp. can also occur late after transplantation.

Table 76.1 Etiology, incidence and timing of infections in heart transplant patients

Etiology	Incidence (%)
Bacteria	44–60
Virus	40–45
Fungus	2–15
Pneumocystis jirovecii	0.5–8
Parasites	0.5–2
Chronology of infection	**Most common syndromes**
Early infection (first month)	Pneumonia Surgical wound infection Mediastinitis Urinary tract infection Catheter-related infection Bloodstream infection Antibiotic-associated diarrhea Herpes simplex stomatitis Infections transmitted with the allograft
Intermediate infections (2–6 months)	Opportunistic infections* (similar to other SOT)
Late infections (after 6 months)	Common community-acquired infections Respiratory tract infections Urinary tract infections Varicella-zoster infections Opportunistic micro-organisms

*See also Chapter 75. SOT, solid-organ transplant.

Regardless of the time period, differentiation between rejection and infection can often be difficult in thoracic transplant recipients. Accordingly, biopsy and cultures are essential for appropriate management.

SPECIFIC CLINICAL SYNDROMES

For the remainder of this chapter we will address only a few infectious problems of special interest in the thoracic transplant recipient.

Pneumonia

In recipients of thoracic transplantation, infections frequently involve the lung.[2,3] The patient's underlying lung disease impacts on the presentation and the type of infection that occur after these procedures. This is illustrated by the increased risk of early and severe pneumonia with multiple antibiotic-resistant bacteria seen in cystic fibrosis patients undergoing heart–lung or lung transplantation.

Unique among heart–lung and lung transplant recipients is the relationship between the presence of chronic rejection and bronchiolitis obliterans with chronic infection of the airway. This relationship may be explained in part by the fact that lung transplant recipients have altered lung immunity due to impaired ciliary clearance, poor cough reflex and abnormal lymphatic drainage that predispose these patients to lower respiratory tract infections. Clinical manifestations may be indistinguishable from rejection of the lung and may strongly resemble a classic 'pulmonary exacerbation' of cystic fibrosis: increased cough and sputum production with a measurable decline of pulmonary function.

Table 76.2 Risk factors for infection after thoracic transplantation

Recipient
Age
Diabetes, malnutrition, obesity
Hypogammaglobulinemia
Renal failure
Critically ill status and ventilation at transplantation
Pulmonary hypertension not responsive to vasodilators (heart transplant)
No immunity against CMV, *Toxoplasma*, EBV, VZV
Latent infection (TB, CMV, HSV, VZV, EBV, endemic mycosis)
Donor CMV-positive serology
Previous admissions and colonization with multi-resistant pathogens
Previous antimicrobial or immunosuppressive therapy
Transplantation
Allograft injury (ischemia, preservation)
Complexity and length of surgery
Bacterial or fungal allograft colonization
Allograft latent infection (*Toxoplasma*, CMV, other viruses)
Native lung colonization (unilateral transplantation)
Postsurgical care (mechanical ventilation, iv catheters, ventricular assist devices, drainages, indwelling bladder catheter)
Transfusions
Intensive care unit stay
Postsurgical technical complications
Re-intervention
Postsurgical renal failure
Immunosuppression
After transplantation
Immunosuppression
Immunomodulatory infections (CMV)
Rejection

CMV, cytomegalovirus; EBV, Epstein–Barr virus; HSV, herpes simplex virus; TB, tuberculosis; VZV, varicella-zoster virus.

In isolated heart transplants, the etiologic agents of pneumonia are similar to those of other SOT patients: 60% are caused by opportunistic micro-organisms (mainly CMV), 25% by nosocomial pathogens and 15% by community-acquired bacteria and mycobacteria.[2] Pneumonia is one of the leading causes of death after HT. Mechanical ventilation is required in 37% of these patients and death occurs in 23–31%. This rate varies widely depending on the etiology, *Aspergillus* pneumonia having the worse prognosis (50–62%), followed by nosocomial pneumonia (26%, even 50% for those patients receiving ventilatory support) and CMV pneumonia (13%).

Nodular lesions are detected in 10% of HT patients and are mainly caused by *Aspergillus*, *Nocardia* and CMV. The time of appearance and some clinical manifestations may suggest the etiology and may help

determine empiric treatment in selected cases.[4] Other pathogens such as *Nocardia*, *Rhodococcus equi* and tuberculosis (incidence in HT 1% in Spain) should also be considered.[5]

Postsurgical mediastinitis and sternum osteomyelitis

HT patients have a higher risk of postsurgical mediastinitis and sternal osteomyelitis than other heart surgical patients.[6,7] This complication may manifest as early bacteremia, sternum instability or dehiscence. The main causes are bacterial pathogens (staphylococci, Gram-negative rods), but *Mycoplasma*, mycobacteria, *Candida* and other less common pathogens should be suspected in 'culture-negative' wound infections. A bacteremia of unknown origin during the first month after HT should always suggest the possibility of mediastinitis. Risk factors include prolonged hospitalization before surgery, early chest re-exploration, low output syndrome in adults and the immature state of the immune system in infants. In a recent study, antibiotic prophylaxis with ciprofloxacin alone (OR 15.8; 95% CI 1.2–216.9) was independently associated with the development of incisional surgical infection.[8] Therapy consists of surgical debridement and repair, and antimicrobial therapy given for 3–6 weeks.

Cardiovascular infections

Infective endocarditis is a relatively rare complication of HT (1.5–6%).[9] Most of the cases are associated with previous nosocomial infections, mainly venous access devices and wound infections. *Staphylococcus aureus* and *Aspergillus* are the most common pathogens involved.[9] Interestingly, 80% of SOT recipients who developed endocarditis in one series had no previous history of valvular disease. CMV, *Toxoplasma* and parvovirus B19 may cause myocarditis in this population.

CONSIDERATIONS RELATED TO MICRO-ORGANISMS

Viral infections

Cytomegalovirus

Cytomegalovirus is the second most common cause of infection in thoracic transplant recipients, after bacterial infection. When no prophylaxis is given, 30–90% of patients will have laboratory data of infection and 10–90% will develop associated clinical manifestations (CMV disease). It can cause primary infection in a previously seronegative patient or secondary infection in a previously seropositive person. Primary infection is usually transmitted via the donor graft or blood products. For ethical aspects, seronegative donor/receptor selection is not indicated. However, if transfusions are needed, the use of leukoreduced blood products is recommended.

CMV infection typically occurs between 1 and 6 months after transplantation. Clinical presentation includes asymptomatic infection, CMV syndrome, with malaise, fever and bone marrow suppression, and focal organ disease, most frequently pneumonia (27%) or gastrointestinal disease (19%). CMV pneumonitis usually presents as fever, tachypnea and hypoxia, and should be differentiated from rejection in LT patients. Chorioretinitis due to CMV is infrequent in these patients. CMV disease is particularly severe in high-risk patients such as recipient negative/donor positive (R–/D+) individuals or those receiving OKT3, anti-thymocyte globulins or monoclonal antibodies for a rejection episode.

Symptomatic CMV infection may occasionally be associated with superinfection with bacteria or fungi, possibly as a consequence of viral modulation of immunity. Interestingly, CMV infection has also been associated with an increased frequency of acute and chronic allograft rejection (diffuse arteriosclerosis in HT and bronchiolitis obliterans in LT),[10,11] although there is still controversy around this point.[12]

Treatment and CMV prevention

Therapy and prevention of CMV disease in this population does not differ from other SOT patients; a full discussion is presented in Chapters 72 and 155. Some authors, including ourselves, recommend administering gammaglobulins to patients with pneumonitis, gastrointestinal disease and frequent relapses.

Prevention of CMV disease in thoracic transplantation is based on two types of strategy: universal prophylaxis and pre-emptive therapy. In *lung and heart–lung transplantation* most authors recommend initial universal prophylaxis followed by pre-emptive therapy. In the first 3–6 months, all patients should receive initial intravenous ganciclovir (5 mg/kg/q12h) followed by oral valganciclovir (900 mg/q12h). In high-risk patients (D+/R–), administration of intravenous gammaglobulins is recommended. Pre-emptive therapy based on follow-up with polymerase chain reaction (PCR) or antigenemia should then be performed. Weekly antigenemia assay is recommended until the sixth month. Late-onset CMV disease (>1 year) has been observed in lung recipients after long-term prophylaxis (6 months) and some authors recommend indefinite prophylaxis.[12]

After *heart transplantation*, mismatched recipients (D+/R–) should receive universal prophylaxis. Ganciclovir followed by valganciclovir (1–3 months), with or without gammaglobulins, seems to be the most effective combination. Pre-emptive therapy is recommended in seropositive patients. After corticosteroid-resistant rejection, a course of antiviral therapy is usually administered.

Epstein–Barr virus

The incidence of post-transplantation lymphoproliferative disease (PTLD) related to Epstein–Barr virus (EBV) in lung recipients varies between 1.6% and 20%, and is two to six times more frequent than in other SOT recipients. More than half of patients are symptomatic, presenting with a mononucleosis-like picture with lymph node swelling, isolated PTLD lesions in the lung or gastrointestinal tract, disseminated disease or lymphoma. Risk factors for PTLD are primary infection and therapy with OKT3 or anti-thymocyte globulins. When PTLD is suspected, CT of the neck, chest, abdomen and pelvis should be considered to identify occult lesions. Biopsies should be obtained from suspicious lesions. Patients with active EBV disease typically have an elevated EBV viral load, which may be detected before clinical onset and may remain persistently high even after resolution of PTLD.

The high rates of morbidity and mortality attributed to PTLD have prompted efforts aimed at the prevention or pre-emptive treatment of EBV. Serial monitoring of the EBV viral load using quantitative PCR assays has been shown to predict occurrence of PTLD in transplant populations and is increasingly being used to guide initiation of pre-emptive therapy. However, specific target levels of load and specific sites to sample (blood versus bronchoalveolar lavage), as well as therapeutic pre-emptive treatment regimens, need to be clarified in prospective, comparative studies. The management of patients with PTLD is controversial. Reduction of immune suppression is uniformly recommended in order to enhance a cytotoxic T-lymphocyte response. Concerns over the development of rejection can limit this approach. While frequently used, antiviral therapies (primarily nucleoside analogues – e.g. ganciclovir – and immunoglobulins) are probably of limited benefit for the treatment of EBV PTLD. The use of the new anti-CD20 monoclonal antibody (rituximab) appears very promising for these patients. The use of chemotherapy may be necessary for patients who fail to respond to reduction of immunosuppression or in whom the PTLD lesions are judged to be malignant.

Other viruses

Viruses other than CMV and EBV cause disease in up to one-third of all lung/heart–lung transplant recipients. An important feature of these infections is that they can mimic rejection.

Herpes simplex virus can reactivate or cause primary pneumonitis, which can be fatal. Aciclovir prophylaxis is effective in preventing this



Fig. 76.1 Kaposi's sarcoma in a heart transplant recipient. Primary infection by HHV-8 was demonstrated.

problem. The incidence of Kaposi's sarcoma (KS) is 0.75% after HT, higher than after kidney transplantation (0.28%). KS was diagnosed a median of 24 months after SOT and mortality was 28.5% (Fig. 76.1). Primary infection with human herpesvirus 8 (HHV-8) was found to be an important risk factor.[16]

The community respiratory viruses such as respiratory syncitial virus (RSV), parainfluenza virus, influenza virus and adenovirus are also important causes of disease after thoracic transplantation. They often involve the lower respiratory tract and may be associated with significant morbidity and mortality. In addition, the insult from these viruses (particularly adenovirus) can lead to chronic sequelae such as bronchiolitis obliterans in lung recipients. The mainstay of treatment for community-acquired viruses is decreasing the immunosuppression and providing supportive care. Antiviral treatments are controversial and, while licensed therapies are available for RSV (ribavirin) and influenza (e.g. amantadine, rimantadine, oseltamivir, zanamivir), no well-proven therapies are available for parainfluenza or adenovirus.

Annual immunization with influenza vaccine or prophylaxis with amantadine is recommended in thoracic transplant patients. Varicella-zoster virus (VZV) infection should be prevented in seronegative patients who have contact with individuals with varicella or herpes zoster by the administration of hyperimmune globulin. VZV vaccine should be given to seronegative patients before transplantation.

Thoracic organ transplantation in serum hepatitis B surface antigen (HBsAg)-positive and hepatitis B virus (HBV) DNA-negative recipients is followed by HBV reactivation in a high percentage of cases. However, the clinical outcome and the availability of lamivudine and other anti-HBV agents suggest that infection is not an absolute contraindication (see Chapter 148).

Bacterial infections

Bacterial infections are the more frequent infectious complications after thoracic surgery, accounting for more than 40–50% of all infections. They occur early after transplant and are related to:

- pre-transplant donor and/or recipient colonization;
- invasive procedures (surgery, intubation, intravascular devices, etc.); and
- prolonged hospitalization.

Late bacterial infections in lung recipients are related to the severity of the obliterans bronchiolitis. Nosocomial pathogens, principally *P. aeruginosa* and methicillin-resistant *Staph. aureus* (MRSA), may be implicated. Cystic fibrosis patients represent an important cohort of LT recipients. These patients, as well as those with a prolonged intensive care stay before transplantation, are at high risk of being colonized with resistant pathogens (*Burkholderia cepacia*, *S. maltophilia*, *Alcaligenes xylosoxidans*). It is therefore important to obtain baseline cultures prior to transplant. A 2-week course of appropriate antimicrobial therapy is recommended for all patients with cystic fibrosis based upon these culture results. In LT recipients, it is very important to check for ischemic lesions of the anastomoses, since they increase the risk of infection and dehiscence. Nebulized antibiotics are recommended in these cases.

Some 'opportunistic' bacteria such as *Listeria monocytogenes*, *Rhodococcus equi* or *Nocardia* should also be considered in this population. In a recent review of nocardiosis, SOT (117, 26.1%) was the most common underlying condition (36 heart, 35 lung).[17] The disease is less common nowadays due to the widespread use of trimethoprim–sulfamethoxazole (TMP–SMX) in this population. However, it is important to remember that transplant patients may develop late nocardiosis, when TMP–SMX prophylaxis has been withdrawn. On the other hand, TMP–SMX prophylaxis is not uniformly protective against *Nocardia* spp. Nocardiosis may appear in patients with chronic rejection and intensive immunosuppressive treatment.

Finally, there is a group of bacteria that cause late infection, such as encapsulated micro-organisms including *Streptococcus pneumoniae* and *Haemophilus influenzae* (group b). Preventive vaccines should be offered to this population. Pneumococcal vaccination may be repeated 5 years after the first immunization.

Tuberculosis

Mycobacterium tuberculosis deserves a special mention. Lung transplant recipients have a high risk of tuberculosis (TB): 2072 (565–5306) cases per 10^5 inhabitants compared to 512 (317–783) for all SOT.[18] The incidence of tuberculosis in HT patients in Spain is 1.35 cases/100 heart transplant-years, more than 20-fold the national average.[19]

On average, TB developed 76 days after transplantation and extrapulmonary disease was common. Fever, dyspnea and alveolar infiltrates were the most common findings in a series of LT recipients with TB.[20] Reactivation may be triggered by antirejection therapy, although it may be acquired in the hospital environment or even from the allograft in heart–lung transplantation. TB should be considered in HT patients with prolonged and culture-negative febrile episodes. If the patient's condition deteriorates, prompt specific therapy should be initiated after obtaining samples for culture.

Besides the potential difficulty in establishing a diagnosis, due to its atypical presentation, the major problem of management of TB in this population is multiple drug interactions.[20,21] The benefit of rifampin (rifampicin) must be balanced against the problem it causes by interfering with the metabolism of immunosuppressive drugs, especially ciclosporin and tacrolimus. When the use of rifampin is mandatory, immunosuppressant levels should be monitored daily until they are stable.[18]

Fungal infections

Invasive fungal infections (IFI) after SOT used to be a major cause of morbidity and mortality after thoracic transplantation, although the incidence now appears to be decreasing.

Aspergillus species

The incidence of invasive aspergillosis (IA) in lung and heart transplant recipients varies, estimated to range between 3.9% and 26%. *Aspergillus* and other filamentous fungi have also been identified as causing colonization and disease in patients who develop bronchiolitis obliterans. The presence of fungi preoperatively is not a contraindication to transplantation; however, in double lung transplantation, the exclusion of the presence of mycetomas with a thoracic CT is mandatory. A characteristic clinical presentation in LT recipients is tracheobronchitis which can present in a simple, ulcerative, pseudomembranous or nodular form. In most cases it affects the anastomoses and may cause dehiscence, mediastinitis, hemorrhage and disseminated disease. IA of the residual native lung in recipients of single lung transplantation is difficult to detect and manage. In HT patients, the isolation of *Aspergillus* from any respiratory sample has a 60–70% positive predictive value for invasive aspergillosis.[22] Although other species may be involved, in our series *A. fumigatus* was recovered from 74% of the samples, but caused all episodes of invasive aspergillosis. In the first 6–9 months after LT, the recovery of *Aspergillus* spp. from respiratory secretions is an indication to perform an endoscopic study of the airways.

Risk factors for invasive lung aspergillosis after LT are *Aspergillus* spp. colonization, CMV pneumonia, airway ischemia and single lung transplantation. Risk factors after HT are reoperation, CMV disease, post-transplant hemodialysis and high environmental load reflected by the existence of an episode of IA in the HT program 2 months before or after the transplantation date. Itraconazole prophylaxis showed an independent protective value against developing IA (RR 0.2; 95% CI 0.07–0.9, p=0.03) and also determined a significantly prolonged 1-year survival (RR 0.5; 95% CI 0.3–0.8, p=0.01).[23]

Prompt recognition of this fungal infection is essential for achieving a successful outcome. However, both clinical symptoms and radiologic manifestations may be nonspecific at early stages of the disease. The isolation of *Aspergillus* spp. from nonsterile respiratory samples may indicate invasive infection, colonization or laboratory contamination, thus making treatment of symptomatic transplant recipients difficult. However, as mentioned, the isolation of *A. fumigatus* is highly predictive of invasive disease and should always be considered very seriously in this population. In our experience, all respiratory samples other than sputum proved to have the same predictive value, as has already been suggested by other authors. The choice of one or other technique should be based on the availability and expertise in each hospital, on the extent and location of the infiltrate and on patient characteristics. In previous studies we have demonstrated that in HT recipients with pneumonia, the sensitivity of bronchoalveolar lavage ranged from 58% to 89% and transthoracic aspiration reached 100% sensitivity. Clinical and microbiologic information should been combined with early CT scan, which provides more specific information than conventional chest X-ray (Fig. 76.2).

Although detection of galactomannan in serum has poor sensitivity for the diagnosis of invasive aspergillosis in LT recipients, detection of galactomannan in the bronchoalveolar lavage with a compatible clinical illness is highly suggestive of invasive disease.[24] Treatment of IA is discussed in depth in Chapters 72, 73 and 178. Voriconazole is the recommended first-line therapy in most patients. Parenteral liposomal amphotericin B (AMB), inhaled AMB or combination therapy may be considered as alternative or co-adjuvant therapies.[25,26] Duration of therapy depends on the extent of disease, the clinical response and the immunologic status of the patient. Most authors recommend prolonging the intravenous treatment until clinical response and radiologic normalization have been obtained and then continue with oral voriconazole or itraconazole for another 3–18 months. Regular monitoring of liver enzymes and serum concentrations of calcineurin inhibitors is required to avoid hepatotoxicity and nephrotoxicity. Inhaled AMB, with or without itraconazole, is employed in LT recipients with evidence of *Aspergillus* colonization, until the normalization of respiratory cultures.[27,28] For tracheobronchitis, treatment includes inhaled AMB combined with systemic voriconazole or itraconazole until bronchoscopic evidence of disease resolution.

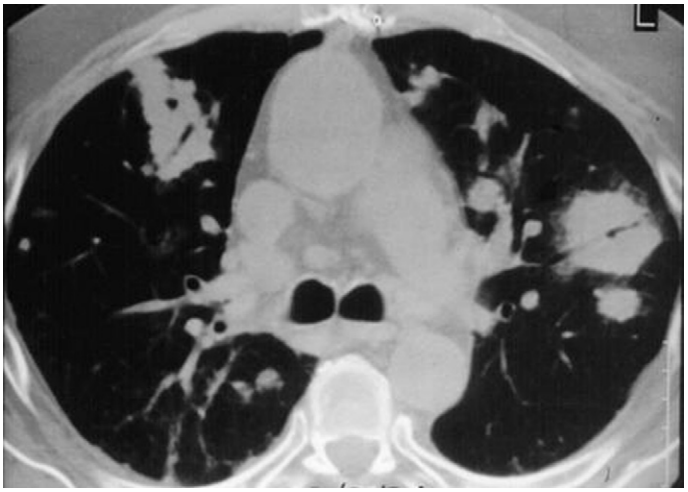


Fig. 76.2 Bilateral invasive aspergillosis in a heart transplant recipient.

Very few data on prophylaxis against *Aspergillus* in HT recipients are available. We have used oral itraconazole with good results and inhaled AMB is used at other institutions.[23,29] Inhaled AMB, with or without an oral antifungal, is predominantly used after LT.[27] A recent study has shown that universal prophylaxis with voriconazole was better than standard prophylaxis with itraconazole ± inhaled AMB (IA at 1 year 1.5% vs 23%, p=0.001) in patients at high risk (pre- or post-transplant *Aspergillus* colonization).[30]

Pneumocystis jirovecii

Pneumocystis jirovecii pneumonia is a severe pulmonary infection in immunocompromised hosts. Before the introduction of universal prophylaxis with trimethoprim–sulfamethoxazole, the incidence of *P. jirovecii* pneumonia in thoracic transplantation was 3–12% in heart recipients, and up of 80% in lung recipients. *P. jirovecii* pneumonia tends to occur within the first year after transplantation, but has occasionally been seen later, generally after rejection episodes. Symptoms range from isolated fever to serious respiratory distress leading to death. A summary of prophylactic strategies is presented in Table 76.3.

Table 76.3 Summary of the most commonly used prophylactic strategies in thoracic transplantation patients

Indication	Standard therapy	Dose and duration	Comments
Streptococcus pneumoniae, *Haemophilus influenzae*, influenza	Pre-transplant vaccination	As in other patients	*S. pneumoniae* vaccination should be repeated every 5 years Annual influenza vaccine
Perioperative prophylaxis	Cefazolin	1–2 g q8h iv for 48–72 h	LT recipients: active against *Staphylococcus aureus* and *Pseudomonas aeruginosa* (some centers recommend amoxicillin–clavulanate + ceftazidime) In cystic fibrosis patients, prophylaxis should include inhaled antibiotics (e.g. tobramycin) combined with systemic therapy selected with pre-transplant from donor and recipient cultures If cultures are negative, prophylaxis can be ended after 3–5 days; if positive, should be prolonged for 2 weeks

Table 76.3 Summary of the most commonly used prophylactic strategies in thoracic transplantation patients—cont'd

Indication	Standard therapy	Dose and duration	Comments
Pneumocystis jirovecii	Trimethoprim–sulfamethoxazole (TSX)	Double-strength TSX q24h or q12h on week-end days, or three times a week	May protect against *Nocardia* spp., *Listeria* spp., *Toxoplasma* and other bacteria Some groups recommend life-long prophylaxis for LT recipients For allergic or intolerant patients, see Chapter 75
Cytomegalovirus	Ganciclovir/valganciclovir	5 mg/kg/q12h iv for 15 days, followed by valganciclovir (900 mg/q12h) in D+/R– for the first 3–6 months	D+/R–: universal prophylaxis for 3 months; consider iv gammaglobulins R+: pre-emptive therapy in HT and universal prophylaxis in LT
Aspergillus spp. (Lung and heart–lung transplant)	Amphotericin B alone or with an oral antifungal	25 mg/q24h inhaled for 2–6 months, then once/15 days	Voriconazole, alone or combined with inhaled amphotericin, is also effective Patients who receive prophylaxis with voriconazole should be monitored for hepatoxicity
Mycobacterium tuberculosis	Isoniazid	300 mg/q24h po for 6–12 months	Low risk of toxicity
Toxoplasmosis (R–/D+)	Pyrimethamine plus folinic acid*	25 mg/q24h for 6 weeks	With folinic acid

D+, seropositive donor; HT, heart transplant; LT, liver transplant; R+, seropositive recipient; R–, seronegative recipient.
*Trimethoprim–sulfamethoxazole, three times a week, is effective protection for both *P. jirovecii* pneumonia and toxoplasmosis; high-risk HT patients should receive anti-*Aspergillus* prophylaxis.

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Chapter 77

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Liver transplantation

INTRODUCTION

Up to 70% of liver transplant recipients develop bacterial, viral, fungal and protozoal infections during the first year after transplantation.[1,2] The occurrence of these infections in liver recipients is influenced by two major factors: (1) the net state of immunosuppression, and (2) the epidemiologic exposures of the recipient and the donor. The complexities of liver transplantation surgery with the potential break in areas of high microbial load (i.e. gastrointestinal tract) and the common infectious indications for liver transplantation (e.g. chronic viral hepatitis) predispose to unique complications that may not be commonly observed among other solid-organ transplant (SOT) recipients.

PRETRANSPLANT INFECTIOUS DISEASE EVALUATION

Liver transplant candidates and their potential donors should undergo extensive infectious disease evaluation prior to transplantation (Table 77.1).[3] Among the many infections screened for are cytomegalovirus (CMV), herpes simplex virus (HSV) 1 and HSV2, Epstein–Barr virus (EBV), human immunodeficiency virus (HIV), human T-lymphotropic virus, hepatitis A–D viruses (HAV, HBV, HCV, HDV), *Treponema pallidum* and *Mycobacterium tuberculosis*. Individuals with identifiable risk factors such as residence in endemic areas undergo additional screening for *Coccidioides immitis*, *Trypanosoma cruzi* and *Strongyloides stercoralis*.

A unique characteristic of liver transplant candidates is the common occurrence of active infection during the pretransplant period. Cholangitis, peritonitis and abscesses are a few examples. In general, an underlying infection does not absolutely contraindicate liver transplantation, unless it is uncontrolled. However, all active infections should be controlled prior to, and if necessary, after transplantation. To illustrate, chronic HBV and HIV, which were once considered absolute contraindications to liver transplantation, are now adequately controlled after liver transplantation with the use of hepatitis B immunoglobulin (HBIg) and antiviral nucleosides (for HBV)[4] and highly active antiretroviral therapy (for HIV).[5]

Table 77.1 Suggested measures to determine risks and exposures of liver transplant candidates and the suggested preventive measures prior to liver transplantation

Screening	Control measures, if susceptible
Standard screening tests	
HIV 1/2 ELISA	Highly active antiretroviral therapy
Hepatitis A serology	Hepatitis A vaccine
Hepatitis B serology	Hepatitis B vaccine
Hepatitis C serology	Assess for need of interferon alpha with or without ribavirin
Cytomegalovirus IgG	Advise the patient of risk of transmission and reactivation after transplantation. If susceptible to primary infection, use antiviral prophylaxis after transplantation
Epstein–Barr virus (EBV) serology	Advise the patient of risk of transmission and reactivation after transplantation. If susceptible to primary EBV infection, closely monitor for virus replication after transplantation; others use antiviral and immunoglobulin prophylaxis after liver transplantation
Varicella serology	Varicella vaccine; role of booster in immune individuals is not established
Purified protein derivative (PPD) skin test	Treat for latent tuberculosis infection prior to or after liver transplantation
Rapid plasma reagin	Treat syphilis if detected
Targeted screening tests	
Strongyloides stercoralis antibody	
Coccidioides immitis serology	
Human herpesvirus-8 serology	
Human T-cell lymphotropic virus	

RISK FACTORS

Epidemiologic exposures, underlying diseases and patient characteristics

Unlike other SOT patients, infection-related indications for liver transplantation are common. Indeed, the single most common indication for liver transplantation is HCV-induced cirrhosis.[6,7] Liver failure from HBV with or without superimposed HDV infection or from acute fulminant HAV or HBV or disseminated HSV hepatitis are other infection-related indications for liver transplantation. In the absence of effective therapy, the majority of patients with HBV develop recurrent infection after transplantation. Likewise, almost all HCV-infected recipients develop recurrence after transplantation. Fulminant hepatitis, regardless of etiology, is particularly associated

with an increased risk for post-transplant viral and fungal superinfections.[8] Active or latent infection involving the donor or recipient liver (e.g. *Histoplasma capsulatum*, *C. immitis*, *Cryptococcus neoformans*, *Toxoplasma gondii*, *M. tuberculosis*) may be unrecognized prior to, and manifest with severe atypical disease after, liver transplantation. Interestingly, the risk of opportunistic infections is not significantly increased among HIV-infected liver recipients,[5] and the risk of infection is similar between recipients of liver allografts from deceased or living donors.[9]

Surgical factors and the hospital environment

A prolonged and complicated surgical procedure and the volume of blood loss directly correlate with infection risk after liver transplantation (Table 77.2).[2,8,10,11] The urgent nature of the procedure, such as during transplantation for fulminant hepatitis, could lessen the time to optimally prepare patients for liver transplantation. Abdominal re-exploration surgery (for re-transplantation, abdominal bleeding and other complications) and the type of biliary anastomosis (e.g. choledochojejunostomy) increases the risk of bacterial and fungal infections.[2,8,10,11] The length of hospital stay, the presence of indwelling vascular, genitourinary and percutaneous catheters, and nosocomial infections further contribute to infections after liver transplantation.

Net state of immunosuppression

The net state of immunosuppression is influenced by host factors, viral factors and pharmacologic immunosuppression. Muromonab-CD3 (OKT3), antithymocyte globulin (ATG), mycophenolate mofetil (MMF) and alemtuzumab increase the risk of CMV disease[12,13] and other opportunistic infections such as human herpesvirus (HHV)-6, *C. neoformans*, *Aspergillus* spp., and *Pneumocystis jirovecii*. The combination of OKT3 and MMF may accelerate the course of recurrent HCV.[7,14]

Contributing to the net state of immunosuppression are patient age, underlying diseases such as diabetes and HIV, and inherent immunologic deficiencies.[6,15,16] Drug-induced myelosuppression (i.e. leukopenia from trimethoprim–sulfamethoxazole, valganciclovir and MMF) and infections with immunomodulating viruses further increase the risk of infection.[17–19] Infections with HHV-6 and CMV, whose levels of replication are enhanced by immunosuppressive drugs, paradoxically increase immunosuppression and the risk of opportunistic superinfections.[17–21] Unique to liver transplantation is the interaction among CMV, HHV-6 and HCV, leading to the accelerated course of recurrent HCV.[7,14,22,23]

CLINICAL PRESENTATION

Characteristically, infections follow a temporal pattern that is predicted based on the time to onset after liver transplantation (Fig. 77.1). The post-transplant period is generally divided into three characteristic periods when certain types of infection typically occur. This period reflects the net state of immunosuppression, although the clinical course of many infections has been modified by antimicrobial prophylaxis.

First month

Liver transplantation is unique because the surgical procedure is performed in close proximity to areas of high microbial content. Spillage of gastrointestinal contents may cause intra-abdominal infections. Infected bilomas, intra-abdominal abscesses and surgical site infections caused by drug-susceptible and -resistant bacteria (e.g. *Staphylococcus aureus*, coagulase-negative staphylococci, enterococci, Gram-negative bacilli, anaerobic organisms) and fungi (e.g. *Candida albicans*, *Candida glabrata*) may occur. Clinical manifestations include fever and sepsis, although in many instances, atypical presentation may be observed such as isolated leukocytosis. Erythema, purulence and dehiscence of surgical wounds may occur. The risk of infection is particularly increased in patients who require abdominal re-exploration (i.e. hepatic artery or portal vein thromboses, biliary leak, re-transplantation). Prolonged hospitalization increases the risk of nosocomial pneumonia, catheter-associated urinary and bloodstream infections, and antibiotic-related *Clostridium difficile*-induced diarrhea.

HSV reactivation disease is the most common viral infection during this period, although antiviral prophylaxis has markedly reduced its incidence. Recurrence of HCV viremia is manifested during this period, and the degree of HCV replication has been correlated with the severity of recurrent HCV hepatitis and cirrhosis.

Second to the sixth month

This is the period when opportunistic infections, with CMV as the most common pathogen, typically occur.[24] CMV and other β-herpesviruses (HHV-6 and HHV-7) reactivate within 2–6 weeks after liver transplantation, although antiviral prophylaxis has delayed the onset of clinical disease.[24] HHV-6 and HHV-7 reactivations are mostly asymptomatic, although fever, myelosuppression and tissue-invasive diseases have been observed.[18] In contrast, CMV commonly presents with symptomatic illness characterized by fever and myelosuppression (CMV syndrome); in roughly 50% of cases, tissue invasion, particularly of the gastrointestinal tract and liver allograft, is a major manifestation.[12,24] If CMV hepatitis occurs, it can be confused with allograft rejection or drug toxicity.

Table 77.2 Risk factors for infections after liver transplantation

Preoperative period	Intraoperative period	Postoperative period
Lack of pathogen-specific immunity Severity of underlying clinical illness Fulminant hepatic failure Dialysis and renal insufficiency Anemia Previous or ongoing fungal infection (e.g. endemic mycoses) Chronic active infections (e.g. hepatitis B and C viruses) Latent infections (e.g. cytomegalovirus, herpes simplex virus, varicella zoster virus) Immune deficiencies (e.g. cytokine and chemokine deficiencies, immune receptor defects)	Presence of pathogens in the allograft (e.g. herpesviruses, fungal pathogens) Prolonged operative time Complicated surgical procedure Profound blood loss Choledochojejunostomy Infusion of large volume of blood products Prolonged ischemia time	Prolonged hospitalization Prolonged stay in intensive care unit Prolonged antibiotic use (e.g. resistant pathogens and fungi) Dialysis and renal insufficiency Gastrointestinal and biliary complications Vascular complications Allograft rejection Immunosuppressive drugs (e.g. muromonab-CD3, antithymocyte globulin, high-dose steroids, mycophenolate mofetil, alemtuzumab) CMV and HHV-6 reactivation Re-operation within 1 month after transplantation Re-transplantation

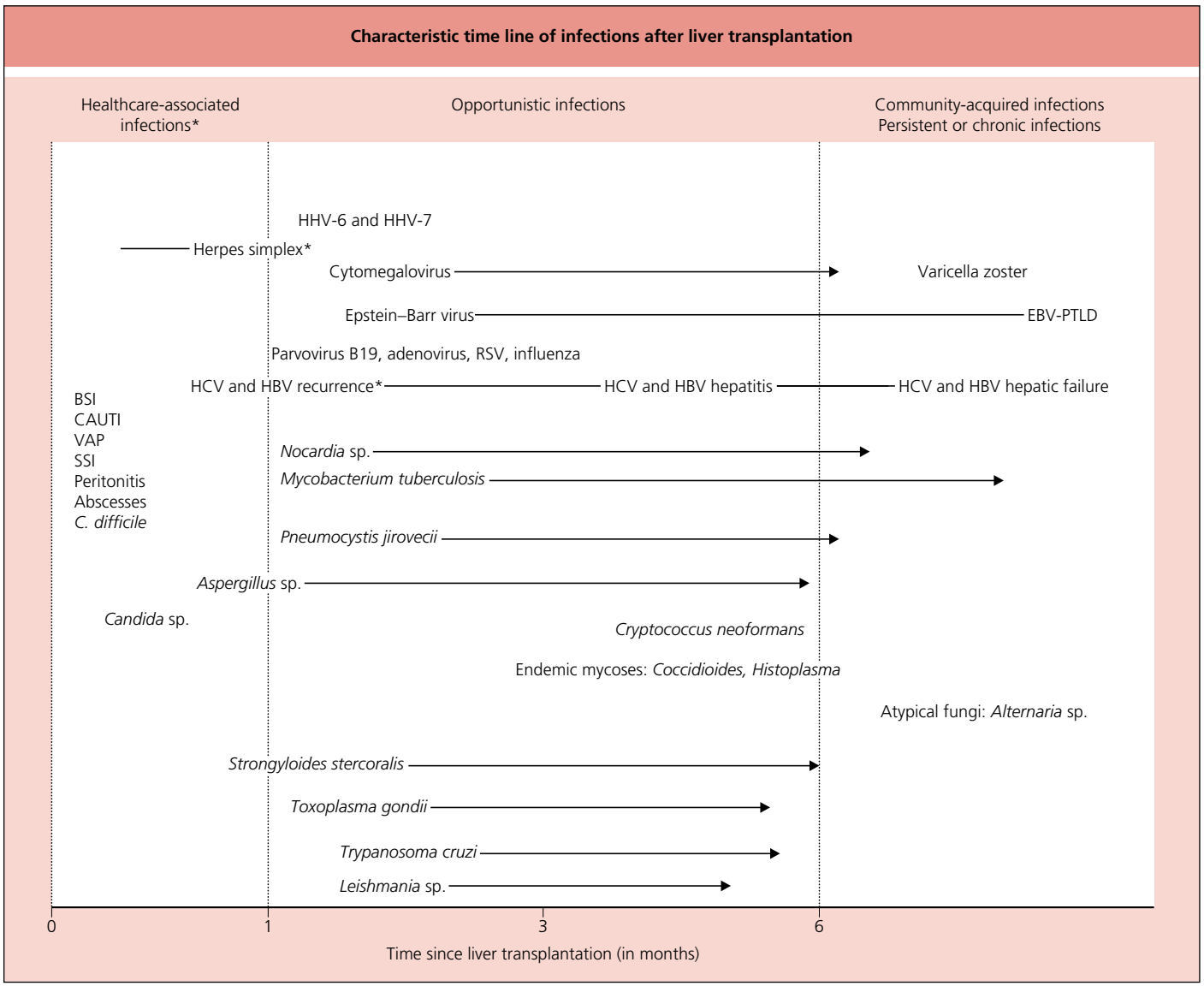


Fig. 77.1 Characteristic time line of infections after liver transplantation. BSI, bloodstream infection; CAUTI, catheter-associated urinary tract infection; EBV, Epstein–Barr virus; HBV, hepatitis B virus; HCV, hepatitis C virus; HHV, human herpesvirus; PTLD, post-transplant lymphoproliferative disorder; RSV, respiratory syncitial virus; SSI, surgical site infection. VAP, ventilator-associated pneumonia. *Healthcare-associated infections predominate during this period, with the exception of herpes simplex, HBV and HCV recurrence.

Hepatic dysfunction, from rejection, infection or drug toxicity, complicates the use of antimicrobial prophylaxis. In many instances, trimethoprim–sulfamethoxazole is withheld because of elevated serum transaminase levels, thereby predisposing the patients to *P. jirovecii*, *Nocardia* spp. and other bacterial infections. Other drugs that are often withheld because of hepatic dysfunction include isoniazid (for the prevention of tuberculosis) and antifungal medications (for prevention and treatment of fungal disease).

Invasive aspergillosis, most commonly due to *Aspergillus fumigatus*, is one of the most dreaded infections after liver transplantation. It is observed more commonly among liver recipients who were transplanted for fulminant hepatitis, those with profound immunosuppression, and those with epidemiologic exposures (such as close proximity to construction sites). Infection with endemic fungi (e.g. *H. capsulatum*, *C. immitis*) and *C. neoformans*, which have been transmitted occasionally through the transplanted allograft, may occur during this period. Cryptococcosis often presents as meningoencephalitis but can occur as an isolated pulmonary cryptococcoma or nonhealing cellulitis.

Virus-to-virus interactions are more commonly observed in liver compared to other SOT recipients.[7,17,19–23] The predisposition to a more severe form of CMV disease in patients with HHV-6 and HHV-7 co-infections is a classic example.[17,20,21] Also unique among the liver transplant population is the interaction between HCV, CMV and other herpes viruses, leading to rapid progression of recurrent HCV.[7,22,23]

Beyond the sixth month

Beyond the sixth month, the majority of liver recipients have good allograft function and their levels of immunosuppression have been reduced to the minimum. As a result, patients are no longer at high risk of opportunistic infections. In the minority of liver recipients, such as those with poor allograft function, those with recurrent chronic viral hepatitis, those with recurrent acute or chronic rejection, and those who remain on an intense immunosuppressive regimen, opportunistic infections similar to those observed during months 1–6 may occur.

Almost all HCV-infected liver recipients develop recurrent HCV viremia, and in many cases the severity of immunosuppression influences

the progression of HCV replication to clinical hepatitis, fibrosis and allograft failure. In the HCV-infected population, recurrent HCV-associated allograft failure is the most common cause of death during the first 5 years after liver transplantation. Likewise, in the absence of anti-HBV therapy, recurrence of chronic HBV is characterized by a rapidly progressive course that results in allograft failure.

VZV reactivation, which manifests as mono- or multidermatomal zoster, typically occurs during the period between 6 and 12 months after liver transplantation. EBV-related post-transplant lymphoproliferative disorder may occur at any time, although it often peaks between 6 and 12 months and between 2 and 4 years after liver transplantation. In some cases, as exemplified by CMV, the onset of the typical infections has been delayed (i.e. late-onset CMV disease) by antimicrobial prophylaxis.[24,25]

DIAGNOSIS AND SURVEILLANCE

Surveillance

Surveillance cultures or other methods such as polymerase chain reaction (PCR) to identify infection before its clinical manifestation are utilized. Surveillance using rectal swab and stool specimen can identify colonization with drug-resistant bacteria, such as vancomycin-resistant enterococci, in an effort to interrupt nosocomial transmission.[26] Surveillance culture of biliary fluid is not recommended.[26]

Screening for CMV

CMV screening, either with PCR or pp65 antigenemia, is usually performed based on the risk of disease.[27] Because of poor sensitivity, viral culture should not be used to guide pre-emptive therapy.[27]

Hepatitis virus surveillance

HBV surveillance should be performed on all HBV-infected patients before and after transplantation to document HBV replication and to monitor effectiveness of therapy. Surveillance for HCV recurrence is performed using molecular methods that measure HCV replication.

Diagnostic methodologies

The etiology of an infectious syndrome is identified using culture, serology, histopathology, antigenemia testing (such as pp65 for CMV and galactomannan for aspergillosis) and molecular methods (such as PCR to detect CMV, HCV, HBV, HSV, VZV and bacterial pathogens). Generally, the utility of serology is limited by the inability of some transplant recipients to mount a humoral immune response. In contrast, molecular techniques provide a rapid, sensitive method for diagnosis.

MANAGEMENT

Prevention: vaccination, prophylaxis and pre-emptive therapy

Vaccination

All susceptible liver transplant candidates should receive necessary vaccinations prior to transplantation (Tables 77.1, 77.3). Susceptible patients should receive hepatitis A and B vaccines, varicella-zoster vaccine and tetanus vaccine prior to transplantation. Live attenuated vaccines are generally contraindicated after liver transplantation.

Table 77.3 Suggested measures for the prevention of infections after liver transplantation

Prophylaxis	Indications	Dose and duration	Comments
Cefotaxime	Perioperative antibacterial prophylaxis	1 g q8h iv for 48 h	Should be adjusted based on resistance patterns
Trimethoprim–sulfamethoxazole	*Pneumocystis jirovecii*	80 or 160 mg of trimethoprim component po q24h for 6 months	May protect against *Nocardia* spp., *Listeria* spp. and other bacteria; alternatives are inhaled or iv pentamidine or oral dapsone
Aciclovir	Herpes simplex virus	200–400 mg po q8h for 4 weeks	Should be withheld when ganciclovir is used; alternative is valaciclovir
Ganciclovir	Cytomegalovirus	1 g po q8h for 3 months	Used as prophylaxis or pre-emptive therapy; may protect against HHV-6 and herpes simplex virus
Valganciclovir	Cytomegalovirus	900 mg po q24h for 3 months	Not approved for this indication in the United States; may be used as prophylaxis or pre-emptive therapy; may protect against HHV-6 and herpes simplex virus
Fluconazole	*Candida* spp.	400 mg po q24h for 4 weeks	Targeted to patients with complicated and prolonged surgery with profound blood loss
Amphotericin B	Fungal pathogens including the filamentous *Aspergillus* spp.	0.2 mg/kg iv q24h	Administered to patients with fulminant hepatic failure; risk of nephrotoxicity; role of triazole such as voriconazole or echinocandins such as caspofungin not established
Oral selective bowel decontamination	Gram-negative bacilli and fungi	Variable; may be given prior to liver transplantation and for up to 4 weeks thereafter	Provides selective pressure favoring an anaerobic environment, with the goal of reducing the risk of bacterial and fungal superinfection
Hepatitis B immunoglobulin	Hepatitis B virus	10 000 IU q24h for first week then every 4 weeks	Maintain serum hepatitis B immunoglobulin level >100 IU; used in conjunction with antiviral nucleosides such as lamivudine and adefovir

Influenza virus vaccination should be given annually, although the live influenza virus vaccine should be avoided.

Oral selective bowel decontamination

A controversial practice after liver transplantation is the use of oral selective bowel decontamination (OSBD), a solution containing a combination of antimicrobials such as colistin, gentamicin and nystatin.[28,29] OSBD is administered to apply selective pressure to intestinal microflora (i.e. to decrease colonization with Gram-negative bacilli and fungi), thereby decreasing bacterial and fungal superinfections after liver transplantation.

Cytomegalovirus and herpes prevention

The two major approaches for preventing CMV disease after liver transplantation are antiviral prophylaxis and pre-emptive therapy.[30] Generally, CMV D+/R– liver recipients receive prophylaxis with a ganciclovir-based regimen for 3 months after transplantation. In the United States, valganciclovir is not approved for this indication, although most centers use the drug for prophylaxis. Newer agents such as maribavir are currently under investigation. In patients at lower risk of CMV disease, a pre-emptive approach is recommended wherein patients are monitored for CMV by PCR or pp65 antigenemia; if CMV is detected, antiviral therapy is provided. CMV prophylaxis is administered to patients receiving OKT3 for acute rejection. When ganciclovir is not used for prophylaxis, aciclovir should be given to prevent HSV disease.

Antifungal prophylaxis

In some centers, prophylaxis with fluconazole or low-dose amphotericin B is given to patients with fulminant hepatitis. Because of the risk of invasive fungal disease, particularly with candidiasis, fluconazole may be administered to liver recipients who require re-transplantation or re-operation, and those with prolonged surgical time or profound blood loss. This approach is supported by a meta-analysis demonstrating that fluconazole reduced invasive fungal (mainly due to *C. albicans*) infections by 75%.[10] Nonetheless, antifungal prophylaxis is not a widely accepted practice after liver transplantation. The utility of newer antifungal drugs such as the echinocandins have not yet been established.

Hepatitis B virus prevention

Prevention of recurrent HBV after liver transplantation is essential since it recurs with a progressive and fatal course. This can be accomplished with HBIg, which acts by neutralization of circulating HBV antigens. HBIg is usually administered with or without antiviral nucleosides such as lamivudine and adefovir which decreases HBV replication. The dose of HBIg is usually 10 000 IU/day for the first week after transplantation and, because it provides temporary protection, it is given at 4-week intervals with the goal of achieving serum HBIg levels above 500 IU for the first year and over 100 IU thereafter. Reinfection with HBV is estimated to occur in less than 10% of patients who were receiving HBIg and lamivudine therapy.[31] Prolonged use of HBIg and antiviral drugs before liver transplantation is discouraged because of the risk of selecting resistant mutants, such as the surface antigen and YMDD mutants, respectively.

Hepatitis C virus prevention

HCV recurrence is almost universal and its course is accelerated after liver transplantation. Progression to cirrhosis and allograft failure occurs in 30% of patients within 5 years after liver transplantation. There is currently no widely accepted measure to prevent HCV recurrence.[32] Pre-emptive treatment of early HCV infection with interferon alpha (IFN-α) may be a reasonable option; however, the risk of interferon-mediated acute rejection, contraindications to IFN-α and ribavirin use during the early transplant period, and the poor tolerance to IFN-α and ribavirin therapy are major limitations.

Therapy of established infection

Early administration of effective therapy is key to the management of infections after liver transplantation. Common bacterial infections such as catheter-related bloodstream and urinary infections should be treated aggressively with antimicrobial therapy tailored to the offending micro-organism and guided by susceptibility tests.

CMV disease, which is one of the most common infections in liver recipients,[24,30] is currently treated with intravenous ganciclovir for a duration dictated by clinical and virologic responses.[24,30] Valganciclovir has now been demonstrated to be as effective as intravenous ganciclovir for the treatment of non-severe forms of CMV disease in SOT recipients.[33]

Treatment of recurrent HCV is often initiated during acute or chronic phases of recurrent hepatitis. Combination therapy with IFN-α and ribavirin has yielded better results when compared to IFN-α monotherapy.[32] Studies on the efficacy of pegylated IFN-α, which provides more sustained levels of circulating interferon, possibly with fewer side-effects, are anticipated.

Reduction in the degree of pharmacologic immunosuppression should complement antimicrobial therapy. Drainage of infected fluid collections (e.g. infected hematoma and abscesses), debridement of surgical infections and removal of infected catheters are essential components of therapy.

CONCLUSION

Liver transplant recipients have characteristics that differentiate them from other SOT groups. The complexity of the surgical procedure places the liver recipient at higher risk of bacterial and fungal infections. The higher prevalence of infection-related indications for transplantation, such as HCV and HBV infection, among liver transplant recipients presents as a challenge that is not seen in other SOT groups. Recently, liver transplantation among HIV-infected patients with end-stage chronic HCV cirrhosis has become a reality. These characteristics and the diversity of the clinical indications for liver transplantation require a tailored and individualized approach to prophylaxis, surveillance and management of infections after liver transplantation.

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Chapter 78

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Pancreatic transplantation

INTRODUCTION

Pancreas (more accurately pancreaticoduodenal allograft) transplantation is currently a widely accepted modality for the treatment of insulin-dependent diabetes mellitus.[1] While most patients undergo pancreas transplantation in combination with renal transplantation, selected nonuremic patients may also be candidates for pancreas transplantation alone. Although rejection rates have decreased with better immunosuppressive therapy, infections remain a source of major morbidity and are one of the leading causes of death in this population. In contrast to infections after kidney transplantation, much of the infection-related morbidity in the pancreas allograft recipient is related to postsurgical intra-abdominal infections. Common postsurgical infectious complications include urine leak, abscess, infected pseudocyst, infected pancreatocutaneous fistula, infected pancreatic or lymphatic ascites, leakage of pancreatic exocrine secretions or enteric contents, and, rarely, infected pseudoaneurysms.

To handle pancreatic exocrine secretions, drainage into the bladder (bladder drainage, BD) or the small intestine (enteric drainage, ED) are the two most commonly used techniques (Fig. 78.1). Bladder drainage is associated with metabolic acidosis, intravascular volume depletion, reflux pancreatitis and urinary tract complications, including hematuria, urethritis, urethral strictures and urethral disruption,

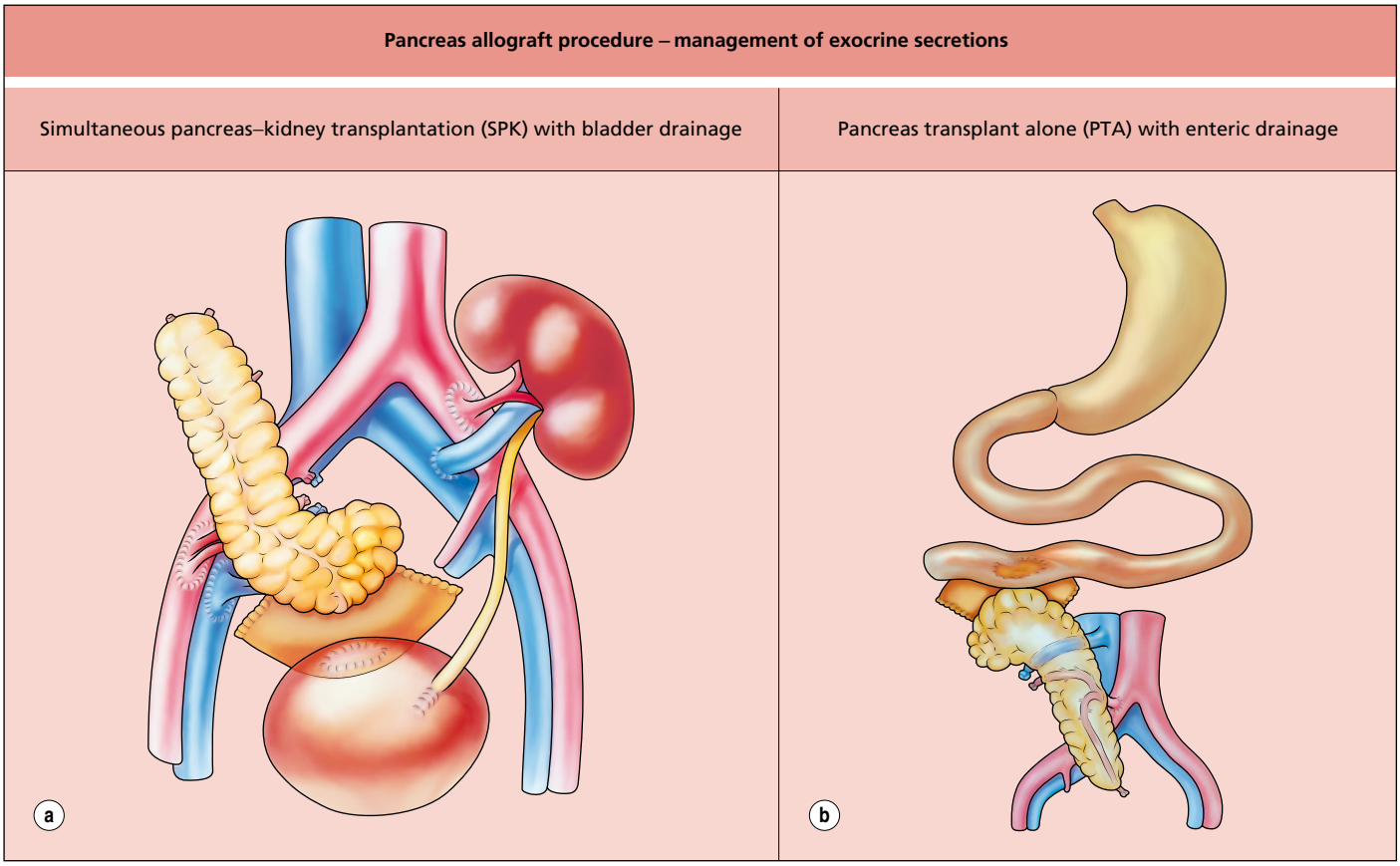


Fig. 78.1 Pancreas allograft procedure – management of exocrine secretions. (a) Simultaneous pancreas–kidney transplantation (SPK) with bladder drainage. (b) Pancreas transplant alone (PTA) with enteric drainage.

all of which may be compounded by infected urine and recurrent urinary tract infections (UTIs). On the other hand, ED is associated with significantly fewer urologic and metabolic complications than BD, and has therefore become the preferred technique.

Several factors contribute to the development of intra-abdominal infections after pancreas transplantation, including:

- intense immunosuppressive regimens;
- compromised defenses associated with diabetes;
- postreperfusion pancreatitis with local release of cytokines and digestive enzymes providing a favorable environment for micro-organisms;
- contamination of the operative field by micro-organisms present in the donor duodenum; and
- microbial translocation from the duodenum facilitated by postreperfusion edema, ischemia or rejection.

Infectious complications associated with pancreas transplantation include UTI, anastomotic leaks of the duodenal segment, intra-abdominal abscesses, mycotic aneurysms, infected pancreatic pseudocysts, infected pancreatocutaneous fistulae and infected abdominal ascites. In addition, cytomegalovirus may affect the pancreas allograft.

These infections typically present with nonspecific symptoms such as pain over the pancreas graft, dysuria, hematuria, fever, malaise, vomiting, etc., and signs such as elevated serum amylase, lipase and creatinine. Postoperative sepsis can be accompanied by mild hyperglycemia.

URINARY TRACT INFECTIONS

Urinary tract infections are a common problem in patients with primary BD of the duodenum segment in pancreas transplantation. Bladder-drainage pancreas transplants lead to significantly more UTIs than ED transplants do (63% vs 20% in the first postoperative year).[2] Urinary tract infections can occur either in the early or late post-transplant period, and they are frequently recurrent. The underlying pathophysiology includes impaired integrity of the bladder mucosa, alteration in urinary pH, urinary retention or stasis due to residual diabetic autonomic neuropathy, prolonged catheter drainage, microbial contamination by the contents of the donor duodenum, retained intravesical sutures and bladder stones. Activation of pancreatic enzymes in the bladder together with urinary alkalosis resulting from bicarbonate secretion appears to cause significant impairment of the mucosal defenses.[3]

The presentation of UTI in pancreas transplant recipients does not differ from the presentation in any other immunosuppressed patient. Symptoms of fever, urinary frequency, dysuria and urgency are common, although patients can be asymptomatic. Enterovesical fistula involving the duodenal segment of the pancreas allograft is a rare cause of recurrent UTI following ED pancreas transplant and can present with pneumaturia. The majority of UTIs are bacterial but fungal infections are also common. The most common bacteria encountered are *Escherichia coli* (27%) and other Gram-negative organisms, such as *Klebsiella*, *Proteus* and *Pseudomonas* spp.[4] Gram-positive organisms are present in approximately 20% of cases, primarily in males, and are probably related to the long-term use of indwelling catheters.[4] In BD pancreas transplants, 50–71% of patients developing a first UTI had a recurrence within the first 90 days. However, less than one-third of these recurrent infections were from the same pathogen as that responsible for the previous infection.[4] Occasionally, a simple lower UTI progresses to an upper UTI or pyelonephritis in the renal allograft, a process that may be accelerated by a diabetic neurogenic bladder and vesicoureteral reflux. Frequently, the donor duodenal segment is colonized with *Candida* spp., explaining the prevalence of fungal UTIs.

Most UTIs following pancreas transplantation can be treated successfully with appropriate antibiotics. Non-nephrotoxic antibiotics that concentrate in the urine, such as the fluoroquinolones, are first-line agents. Fungal UTIs can occasionally be successfully treated with fluconazole or amphotericin bladder irrigations; however, recurrence is common and systemic amphotericin B has been the mainstay of therapy. The role of newer triazole antifungals is undefined. Recurrent UTIs merit further investigation, including cystoscopy to exclude lower urinary tract pathologies such as bladder calculi, foreign bodies or suture granulomata at the duodenum–bladder anastomosis, or a fungus ball (see also Chapter 55). The diagnosis of enterovesical fistula involving the duodenal segment of the pancreas allograft can be made by cystoscopy (presence of bladder mucosal erythema), CT scan (Fig. 78.2) and a high urinary amylase level. In the absence of lower tract abnormalities, the upper tracts are examined by retrograde pyelography and CT scans of the transplanted and native urinary systems. Recurrent, problematic, resistant infections in BD pancreas transplant recipients or those causing renal allograft dysfunction are generally considered indications for conversion to ED.[5]

Multiple recurrent UTIs are associated with poorer long-term renal transplant outcomes.[6,7] Patients with chronic rejection have more UTIs per year, and an earlier onset of chronic rejection correlated with a higher incidence of UTI.[6] However, the pathophysiology uniting UTI

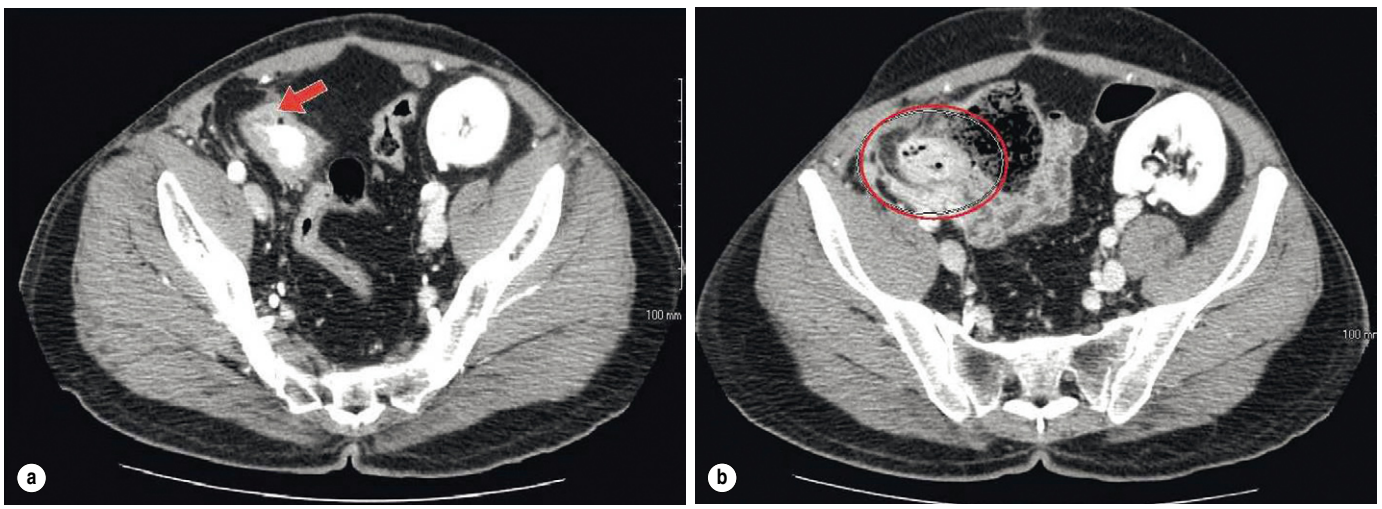


Fig. 78.2 Enterovesical fistula. (a) CT scan shows inflammation and air in wall of bladder. (b) CT scan shows a focus of air and fluid in a cavity adjacent to the bladder and neighboring duodenal segment of the pancreas allograft.

with chronic rejection remains unclear. Potential mechanisms include direct injury, mediated by inflammatory cytokines and chemokines, or enhanced alloreactivity, stimulated by inflammation.

Anastomotic duodenal segment leak: enteric drainage versus bladder drainage

Leakage of pancreatic exocrine secretions from the transplant results in severe life-threatening intra-abdominal infection requiring prompt surgical intervention. With greater experience and better immunosuppression, this surgical complication has fortunately become less common. A retrospective study at our center compared the incidence of pancreatic enzyme leaks with BD with the incidence with ED. In patients with ED of the duodenum segment the leak rate was 6.3% whereas in BD patients it was 18.8% (p=0.0001; Table 78.1). Most ED leaks occur early post transplant (<4 weeks). In contrast, many leaks in BD transplants occur late (>4 weeks), usually months to years after transplantation, suggesting different etiologies between the two groups. In both groups, early post-transplant leaks are largely technical, either related to ischemia of the duodenal segment or dehiscence of the anastomotic suture line. On the other hand, the precise etiology of late leaks in BD transplants is rarely identified and is probably multifactorial. Whereas early leaks are more common at the anastomotic suture lines, late leaks may occur anywhere in the duodenal segment and are possibly related to immunologic rejection, chronic distention, cytomegalovirus (CMV) disease or chronic urine irritation.

In addition to differing in their time of presentation, anastomotic duodenal segment leaks in ED and BD pancreas allografts differ in presenting signs and symptoms. Leaks in BD pancreatic transplants present with rapid onset lower abdominal pain, fever and occasionally hematuria, reflecting sudden spillage of urine into the peritoneal cavity. A sentinel episode of gross hematuria is not uncommon. Concomitant elevation of serum creatinine and serum amylase are classic laboratory findings. In contrast, leaks in ED pancreatic transplants generally cause abdominal pain, fever and hyperamylasemia. An elevated serum creatinine is not part of the usual symptom complex, unless sepsis is severe.

In patients with BD transplants, the diagnosis is made by either a ^{99m}technetium (^{99m}Tc) voiding cystourethrogram (VCUG) or a CT cystogram. A ^{99m}Tc VCUG is performed by administering labeled diethylene-triamine-penta-acetic acid through an indwelling urinary catheter. Persistent radioisotope in the peritoneal cavity on delayed images indicates a bladder leak (Fig. 78.3). In ED transplants the CT scan detects a fluid collection surrounding the pancreas allograft, frequently in proximity to the duodenal segment (see Fig. 78.3). The presence of air or contrast material in the fluid collection is the sine qua non for enteric leak. However, the absence of these findings does not exclude a leak and an infected fluid collection. Determination of sterility depends on sampling the fluid, usually by percutaneous aspiration or laparoscopy. The presence of fluid that is rich in amylase and bilirubin confirms the diagnosis of enteric content leak. Polymicrobial infections with enteric organisms are common and indicate an enteric leak until proven otherwise. On the other hand, if amylase content is high and bilirubin content is low, then pancreatic parenchymal injury causing leak of exocrine secretions is likely. This diagnosis is further supported if the fluid is sterile or contains only Gram-positive organisms.

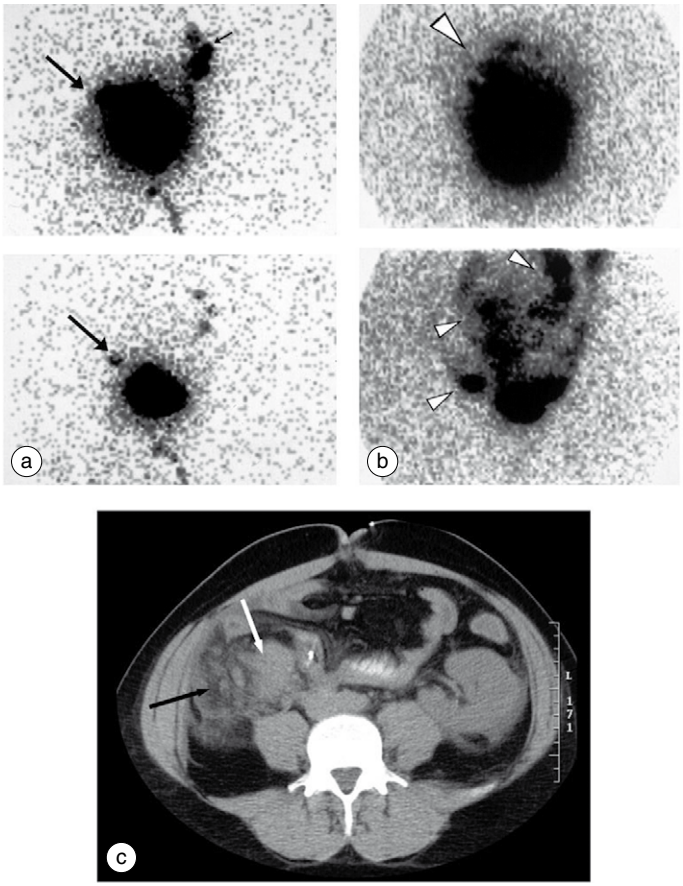


Fig. 78.3 ^{99m}Technetium voiding cystourethrograms. (a) Filling and voiding phases of normal ^{99m}Tc DTPA VCUG. The large arrow represents the duodenal segment of the bladder-drained pancreas transplant. The small arrow represents retrograde flow of radioisotope into the pelvis of the transplanted kidney. (b) Abnormal ^{99m}Tc VCUG. Persistence of radioisotope in the peritoneal cavity is consistent with bladder leak (multiple white arrow heads). Although sensitive for diagnosing urine leak, a radioisotope VCUG does not localize the site of leak. A follow-up contrast VCUG may provide localization, if necessary. (c) Abdominal CT scan demonstrating an enteric leak. The pancreas (white arrow) and a peripancreatic fluid collection (black arrow) are seen. Air and contrast material in the fluid collection are diagnostic of an enteric leak. The absence of these findings, however, does not rule out the possibility of a leak.

Small leaks in BD transplants can occasionally be treated by Foley catheterization alone for 4–6 weeks. However, if symptoms do not

Table 78.1 Leaks in 747 consecutive simultaneous pancreas and kidney transplants at the University of Wisconsin from October 1983 to July 2000

	Bladder drainage (n=446)	Enteric drainage (n=301)	p value
Patients with leak (n)	84 (18.8%)	19 (6.3%)	0.0001
Patients with leak by 60 days	5.30%	5.50%	
Patients with leak by 1 year	16.1%	6.6%	
Patients with leak by 5 years	20.5%	6.6%	
Patients with leak by 10 years	22.9%	6.6%	
Graft salvage rate after leak	83/84 (98.8%)	6/19 (31.6%)	0.001
Candida-associated infection	15/84 (17.8%)	7/19 (38%)	0.01

resolve completely or if they recur after removal of the Foley catheter, patients may require surgical conversion to enteric drainage.[5] Leaks in ED transplants usually require urgent surgical intervention. In our experience, the most common organisms associated with leaks in ED transplants are *Candida* spp., including *C. glabrata* or *C. albicans* (38%), and vancomycin-resistant enterococci (14%). The mainstays of surgical treatment are debridement of devitalized tissue, tension-free re-closure of the duodenal segment, placement of continuous suction drains in conjunction with the use of appropriate antibiotics and construction of a Roux-en-Y intestinal diversion if this was not performed at the time of transplantation. However, despite these measures, the rate of re-leakage or failure to control sepsis is rather high, even in experienced centers. These complications may ultimately necessitate pancreatectomy in a significant number of patients.

The impact of duodenal segment leaks and associated intra-abdominal infections on renal and pancreas allograft survival is significant. These infections and their antimicrobial treatment commonly contribute to renal allograft dysfunction. Furthermore, in the current immunosuppressive era, leak is a major cause of graft loss since ED transplants that develop a leak may require graft pancreatectomy.

INFECTED FALSE ANEURYSM OF THE ARTERIAL GRAFT

Mycotic aneurysms that occur in immunocompetent patients are often thought to be from septic emboli of cardiac origin. In contrast, the origin of mycotic aneurysms after pancreas transplantation is thought to be due to local infection around the iliac Y graft interacting with tissue digestion by activated pancreatic enzymes. A locally invasive infection promoted by enzymatic tissue digestion leads to disruption of the arterial anastomosis culminating in massive bleeding. Candidiasis is commonly seen in these patients; however, other organisms, such as *Staphylococcus aureus*, *E. coli*, *Enterococcus faecalis*, *Pseudomonas aeruginosa* and *Enterobacter* spp., can also be present individually or in mixed infections. Reduced cell-mediated immunity in transplant recipients, enzymatic digestion of tissues, and adhesiveness and invasion of *C. albicans*[8] may contribute to this infectious complication.

Patients with infected false aneurysms usually present in the immediate postoperative period with hypotension associated with intra-abdominal bleeding and hemorrhagic shock. In some cases, gastrointestinal bleeding may also be present. A sentinel episode of mild intra-abdominal or gastrointestinal bleeding frequently presages a more life-threatening event. It is also occasionally preceded by a significant peripancreatic infection. Patients may also present with generalized sepsis, unilateral iliac vein thrombosis and rarely a tender or pulsatile mass.

An infected false aneurysm of the transplanted pancreas is difficult to diagnose. In stable patients with peripancreatic infection and a sentinel bleeding episode, urgent iliac arteriography should be performed (Fig. 78.4). If unstable, an emergency laparotomy can be life saving. Ultrasound, CT and MRI are useful adjunctive tools to evaluate peripancreatic infection or bleeding, but only in the stable patient. Although they may show a dilated vessel in the region of the pancreatic arterial supply, CT angiography or conventional angiography is usually necessary to confirm the diagnosis (see Fig. 78.4). Samples of the aneurysm wall and contents should be cultured for aerobic and anaerobic bacteria and fungi.

The extent of active infection in the iliac artery or Y graft artery wall is difficult to determine at the time of surgery, and minimal intervention at the initial surgery is usually doomed to failure. Aggressive management is necessary in order to prevent death. Five general principles apply to the operative management of infected aneurysms:[9]

- control of hemorrhage;
- confirmation of the diagnosis by obtaining tissue specimens for culture;
- operative control of sepsis, including resection of the aneurysm and wide debridement of the infected tissue;
- prolonged antibiotic or antifungal therapy; and
- arterial reconstruction through uninfected tissue planes.

The recommended procedure for an infected Y graft associated with intra-abdominal bleeding or gastrointestinal bleeding is complete excision of the involved donor or recipient iliac arteries, and transplant pancreatectomy. Rarely, infected false aneurysms may present only as a pulsatile mass without peritoneal or gastrointestinal bleeding and this requires only aneurysmectomy without a pancreatectomy. In any case, limb blood flow is restored by surgical bypass of the recipient common iliac artery.[10] The conduit of choice is a blood-type compatible cadaver iliac graft from another donor or autogenous vein. Alternatively, an extra-anatomic femoral–femoral or axillary–femoral bypass with polytetrafluoroethylene can be used. The identification of *Candida* spp. in the peripheral blood or blood clot removed from the Y graft indicates systemic fungal involvement. Broad-spectrum antibiotic and antifungal therapy should be initiated immediately until organism-specific antibiotic therapy can be instituted.

INFECTED PANCREATIC PSEUDOCYST

Pancreatic pseudocyst is rare after pancreas transplantation. It is defined as a peripancreatic inflammatory fluid collection rich in pancreatic enzymes but devoid of enteric contents that is localized by a nonepithelial fibrous wall. Patients typically present with nonspecific abdominal complaints including generalized malaise and weakness, nausea, abdominal pain, fevers and weight loss; associated elevations of serum amylase and lipase are common. In cases of BD, malodor of the urine and a UTI may coexist. A CT scan should be obtained. Pseudocyst formation in the pancreas

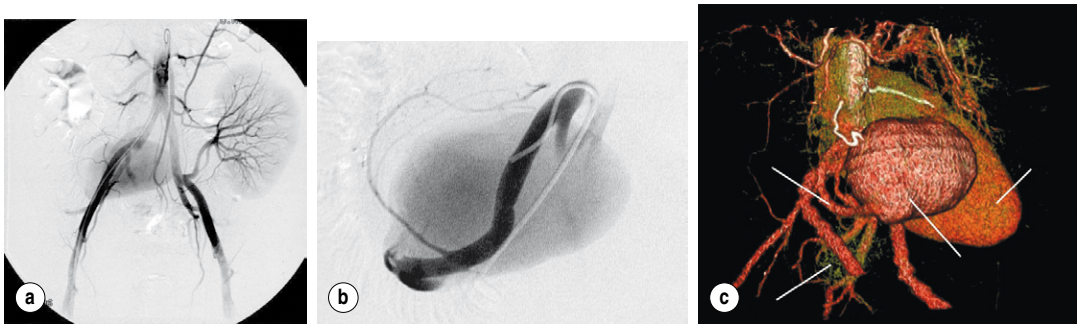


Fig. 78.4 Infected false aneurysm of a pancreas transplant. (a), (b) Arteriogram of an infected false aneurysm originating from the ligated superior mesenteric artery stump of the pancreas transplant. (c) Triphasic CT reconstruction (90° rotation) demonstrating the feeding vessels of the false aneurysm in relation to the pancreas transplant.

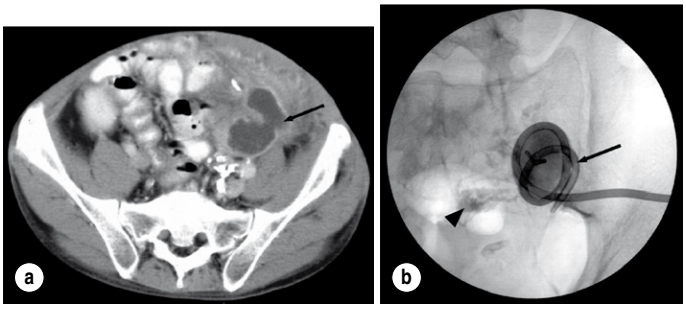


Fig. 78.5 Pancreatic pseudocyst. (a) CT scan demonstrating a pseudocyst of a pancreas transplant in the left pelvis (arrow). (b) Percutaneous drainage of a pancreas transplant pseudocyst. Injection of radiographic contrast material (arrow) demonstrates the pancreatic duct (arrowhead) in communication with the pancreatic pseudocyst.

transplant is thought to be similar in origin to that of the native pancreas – namely, inflammatory pancreatitis. Pancreatitis of the pancreas transplant is most commonly related to reflux and neurogenic bladder, alcohol or duct strictures. Biliary stone disease, while commonly contributing to native pancreatitis, is almost nonexistent in the transplant setting.

Loculated fluid encompassing the transplanted pancreas on CT scan suggests pancreatic pseudocyst (Fig. 78.5). A well-developed capsule of the pancreatic pseudocyst can sometimes be observed, as can a dilated pancreatic duct. Aspiration of the fluid collection is necessary to evaluate the presence of organisms as well as to determine the nature of the fluid (Fig. 78.6). The absence of bilirubin and the presence of amylase in the aspirate is the sine qua non in the diagnosis of pancreatic pseudocyst.

Management mimics that for pseudocyst of the native pancreas. If the pancreatic pseudocyst is infected, external drainage and antibiotic therapy are key. Sterile pseudocysts that are smaller than 6 cm in diameter in relatively asymptomatic patients can be managed expectantly. Persistent infection of the pancreatic pseudocyst can require prolonged antibiotic therapy (up to 6–8 weeks) and percutaneous drainage (Figs 78.5 and 78.6). Persistent high amylase drainage despite conservative measures suggests a possible communication with the pancreatic duct. To confirm this, a fistulogram through the percutaneous drainage catheter is indicated. If a communication with the pancreatic duct is identified and the infection has been treated, a definitive internal drainage procedure is indicated. Internal drainage of a pancreas transplant pseudocyst is optimally achieved via a Roux-en-Y cysto-enterostomy.[11] In BD cases, the pancreatic pseudocyst may alternatively be drained into the bladder by performing a pseudocyst–vesicostomy through a combined transurethral and trans-abdominal approach.[12] Enteric conversion may also be indicated. The long-term outcome from these procedures should be excellent.

INFECTED PANCREATICOCUTANEOUS FISTULA

Pancreaticocutaneous fistula (Fig. 78.7) is a rare complication that can occur after pancreas transplantation due to a pancreatic abscess, concomitant downstream flow obstruction of the pancreatic duct or to direct percutaneous drainage of a pancreatic pseudocyst in communication with duct of Wirsung. Treatment involves a switch to enteric drainage if the pancreas is bladder drained or, if enterically drained primarily, takedown of fistula tract and Roux-en-Y pancreaticojejunostomy. Endoscopic retrograde pancreatography (ERP) and a stent insertion may be effective therapy if a duct stricture exists in association with the enzyme leak. Octreotide may also help with closure of the fistula by diminishing the fistula output. If the pancreas is nonfunctional, pancreatectomy is the best option. Treatment of the underlying or concomitant surrounding infection is usually required for definitive treatment and closure of the fistula tract.

INFECTED ABDOMINAL ASCITES

Lymphatic ascites after pancreas and kidney transplantation in uremic patients occurs in up to 15–20% of cases. This complication is attributed to the extensive lymphatic dissection necessary for surgical exposure during the operative procedure. It is exacerbated by a poorly absorptive peritoneal membrane if there has been prior peritoneal dialysis complicated by peritonitis. In addition, impaired peritoneal macrophage function and accumulation of peritoneal effluent following peritoneal dialysis may explain the high incidence of deep wound infections in this population.[13] In a comparison study at our institution, the rate of postoperative fluid collections in patients previously on peritoneal dialysis was significantly greater than the rate in patients who had not previously received peritoneal dialysis (24% vs 13%, $p<0.01$; personal communication, Y. Becker). In many instances, fluid collections were associated with Gram-positive or Gram-negative bacterial or fungal contamination and required antibiotic or antifungal therapy. Peritoneal dialysis patients have a greater incidence of intra-abdominal fluid collections infected with micro-organisms that colonize human skin.[14]

The initial diagnosis of ascites is based on physical examination. Abdominal radiographs will demonstrate a paucity of air or a ground-glass appearance. CT scanning or ultrasound confirms the diagnosis. The ascites fluid should be sampled for bacteria and fungi and evaluated for cell count, amylase, total protein and chylomicrons. Elevated pancreatic enzymes and total protein are characteristic of pancreatic ascites, whereas amylase-poor ascitic fluid is characteristic of lymphatic ascites.

Treatment of ascites is based on the use of therapeutic paracentesis, placement of a soft, small-caliber intraperitoneal, indwelling drainage catheter or judicious use of diuretic therapy, or both (see Fig. 78.6). For pancreatic ascites, cessation of oral feedings with the use of hyperalimentation and parenteral octreotide is usual. For infected ascites, percutaneous drainage and appropriate antibiotics usually result in complete resolution and excellent long-term outcomes. Polymicrobial or fungal infection suggests possible leak of enteric contents. Investigation for this entity should include injection of the percutaneous drain with contrast, a CT scan with oral contrast, or enteroclysis.

CYTOMEGALOVIRUS INFECTION OF THE PANCREAS ALLOGRAFT

Cytomegalovirus continues to be the most common viral pathogen affecting organ transplant recipients. The 1-year rate of CMV disease is 13–17% in prophylaxis-treated simultaneous pancreas–kidney transplant recipients.[15,16] As in bone marrow and other solid organ transplants, the relative risk of CMV infection in pancreas transplantation varies according to the CMV antibody status of donor and recipient. Nearly 80% of cases of CMV occur in recipients in the high-risk donor-positive–recipient-negative cohort.[15] Symptomatic CMV infection and CMV disease increases the risk for subsequent renal graft rejection (relative risk, 2.11; $p=0.0032$) and non-CMV infections (relative risk, 2.22; $p=0.0011$).[16] Despite the high frequency of systemic CMV infection, CMV infection of the pancreas allograft is uncommon.[17]

The clinical presentation of CMV infection of the pancreas allograft is characterized by abdominal pain and fever, associated with elevation of serum amylase and lipase, and leukopenia. Distinguishing CMV allograft pancreatitis from acute rejection on clinical grounds alone is difficult.[17] Evaluation of allograft histology is essential in this setting. Cytomegalovirus pancreatitis demonstrates multifocal, predominantly acinar mononuclear inflammatory infiltrates associated with characteristic cytopathic changes. Marked cellular enlargement, intranuclear acidophilic inclusions with surrounding halos, and

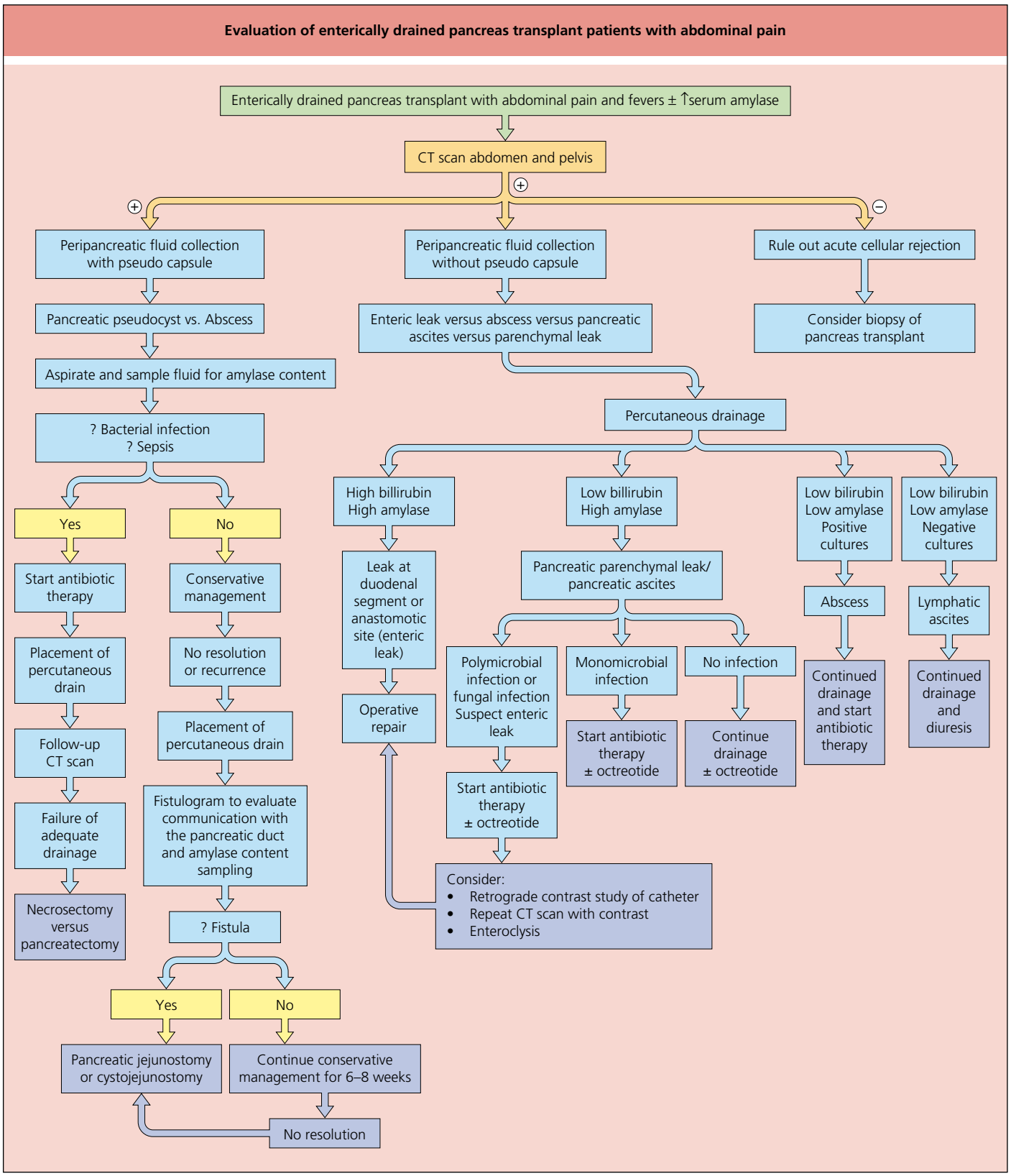


Fig. 78.6 Algorithm for evaluating enterically drained pancreas transplant patients with abdominal pain.

granular basophilic cytoplasmic inclusions are characteristic findings. Immunohistochemistry may be a useful adjunctive test in equivocal cases.[17]

Current CMV prophylaxis is based on the use of oral valganciclovir or intravenous ganciclovir for the first 10 days or while being treated with polyclonal antibody therapy. Doses are adjusted according to creatinine clearance. Long-term post-transplant prophylaxis (for 12 weeks) may be tailored to CMV antibody donor–recipient status, using valganciclovir or ganciclovir for high-risk patients, and aciclovir for those patients who have a low risk of CMV infection.



Fig. 78.7 Leakage of irritating pancreatic juices from the pancreas allograft into the surgical wound. Courtesy of Dr H Sollinger.

Treatment of tissue-invasive CMV disease, including allograft pancreatitis, includes lowering immunosuppression and using maximum antiviral treatment with either intravenous ganciclovir or oral valganciclovir. Persistent or recurrent cases may merit the addition of CMV immune globulin (starting dose of 100 mg/kg intravenously every other day for 3 days, then 100 mg/kg intravenously weekly as indicated).

In spite of adequate prophylaxis, CMV remains a problem following pancreas transplantation. Newer, more effective anti-CMV medications may help clinicians to achieve further reduction in symptomatic CMV infection and disease.

REFERENCES

References for this chapter can be found online at http://www.expertconsult.com

Chapter 79

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Intestinal transplantation

INTRODUCTION

Experimental models of intestinal transplantation (IT) in dogs were pioneered in the late 1950s; however, clinical trials in humans met with initial failure because of graft rejection, sepsis and/or technical problems.[1] The availability of tacrolimus in 1989 facilitated better control of rejection and allowed IT to become clinically feasible.[2,3] With more than 15 years of clinical experience, IT is being performed by a growing number of transplant centers.[4] However, both the procedure and postoperative care remain complex which likely accounts for the fact that more than 80% of patients undergoing these procedures have done so at only one of 10 major centers worldwide.[4,5]

EPIDEMIOLOGY

Intestinal transplantation is performed as treatment for intestinal failure, defined as a loss of its function manifest by an inability to maintain a normal state of fluid and electrolyte balance, nutrition, growth and development. Intestinal failure may be present on the basis of congenital (e.g. intestinal atresia) or mechanical (e.g. pseudo-obstruction syndrome) problems or as the consequence of an intestinal calamity (e.g. short gut syndrome following necrotizing enterocolitis or volvulus). While total parenteral nutrition (TPN) is available for patients experiencing intestinal failure, its use is associated with significant risks, including venous access complications, recurrent episodes of catheter-associated bloodstream infections and TPN-induced cholestatic liver disease.[6,7]

Intestinal transplantation can be performed as an isolated procedure, concomitant with a liver allograft or as a multivisceral (usually including stomach, duodenum, pancreas, liver and the small intestine) transplant procedure. The choice of procedure is individualized based on the underlying diagnosis associated with intestinal failure and the status of liver function in the presence of chronic TPN. Patient and graft survival vary according to which transplant procedure a patient receives; the best outcomes are in isolated IT and the worst are observed in recipients of multivisceral transplantation. Historically, 5-year survival for isolated IT, liver–intestine and multivisceral transplantation was around 50%. Improvements in surgical techniques along with innovations with immunosuppressive strategies have led to improved outcomes, with 5-year survival rates now more than 70% at some institutions.[5,8] The outcome of IT must be compared to the survival of potential candidates for this procedure without IT, which estimates 40% at 2 years,[9] depending on age and the presence or absence of liver disease. Given the poor outcomes without IT and the improving outcomes of IT itself, it is not surprising that the number of IT recipients in 2001–2005 rose fourfold compared to earlier time periods.[4]

Recipients of IT are, on average, younger than recipients of other types of organ transplantation; 66% of IT recipients are less than 18 years old[4] and 50% are less than 5 years.[1] The young age of these patients reflects the relatively high prevalence of intestinal failure in children due to congenital malformations or intestinal calamity during infancy. It also has important implications on the risk for infection. Patients may undergo IT prior to completion of standard vaccination regimens, putting them at increased risk for vaccine-preventable infections. Transplantation at a younger age also increases the likelihood that IT recipients will be immunologically naive against a variety of pathogens including cytomegalovirus (CMV) and Epstein–Barr virus (EBV). In addition, published experience suggests that younger children are at increased risk for the development of respiratory infections after IT.[10] The importance of this latter observation is further emphasized by the fact that respiratory infection accounted for 40% of infection-related deaths in one recent series of IT in children.[10] As a counterpoint to the potential risks associated with IT in children, undergoing this procedure at a younger age is associated with higher life expectancy and quality of life.[6]

PATHOGENESIS AND PATHOLOGY

Patients undergoing IT are at high risk for developing infectious complications. Unique among IT recipients is the relationship that has been observed between the presence of rejection of the intestinal allograft and the development of bloodstream infection.[11] This relationship may be explained in part by the development of breaks in the protective barrier of the intestinal mucosa that can be associated with rejection of the intestinal allograft. Historically, the overall incidence of rejection of the intestinal allograft has been extremely high (90%); an average patient experiences between one and five episodes per graft.[12] Accordingly, the high rejection rate is one of the major factors accounting for the high frequency of bloodstream infections seen in IT recipients. Recent modifications in immunosuppression, including the use of induction therapy, appear to have resulted in a decreased frequency of rejection[5,8] and a lower rate of bloodstream infections.[8]

Patients with rejection of the allograft can present with clinical signs and symptoms (including fever, abdominal pain or distention, nausea, vomiting and an increase in stomal output) that may be suggestive of infection. Because the presentation of intestinal rejection can mimic infection, it is critical to obtain endoscopic biopsy specimens to confirm the diagnosis. Empiric antibiotics should be considered in patients with severe rejection of the intestinal allograft until blood cultures are found to be negative. Bloodstream infections are also associated with the presence of EBV-associated post-transplant lymphoproliferative disease (PTLD) of the intestine in this patient population.[11] This association is likely also due to breaks in the protective

barrier of the intestinal mucosa. Infections in IT recipients can also be attributed to technical complications such as anastomotic leaks resulting in bacterial peritonitis.

The intense immunosuppression required by IT recipients puts these patients at high risk for opportunistic pathogens that are typical after solid-organ transplant, including CMV, EBV and *Pneumocystis jirovecii*, as well as more unusual pathogens such as *Cryptosporidium*.[13] Finally, IT patients frequently require prolonged use of central venous catheters, increasing the risk for catheter-associated bloodstream infections. As in other settings, strategies to reduce these catheter-related bloodstream infections, such as antibiotic locks, have been studied[14] but have yet to demonstrate significant reduction in catheter-associated bloodstream infections in IT recipients.

TIMING AND PATTERN OF INFECTIOUS COMPLICATIONS

In general, the timing of infectious complications after IT follows the typical pattern described for any solid-organ transplant recipient (Table 79.1).[15] However, some differences in the typical timeline of infection are seen following IT, related in part to prolonged and intense immunosuppression. The most important difference is that bloodstream infections occur at a higher rate and continue to occur for a prolonged (if not indefinite) period compared to other organ transplant recipients. A second difference is that IT recipients are at higher risk for morbidity and mortality from CMV and EBV disease than other transplant recipients.[16–19] CMV may present much later after IT compared to other organ recipients and these patients can develop chronic or recurrent CMV disease (particularly of their graft). These differences between IT recipients and other solid-organ transplant patients is largely attributable to the young age and increased likelihood that IT recipients will be seronegative for CMV and EBV at the time of transplant. Thus, they are at risk for developing graft-related primary infection, which tends to be more severe. The differences may also be explained by the large amount of lymphoid tissue in the intestinal allograft and the generally higher levels of immunosuppression needed to prevent rejection in these patients.

Table 79.1 Timing of infection in intestinal transplant recipients

	Frequency of infection (%)
Early (0–30 days after transplantation)	
Surgical site:	
• Intra-abdominal	10
• Superficial and deep wound	20–30
Catheter-associated infection	
• Bloodstream*	25
• Urinary tract	10
Ventilator-associated pneumonia	10
Rejection-associated bacteremia	5–10
Intermediate (1–6 months)	
Catheter-associated infection:	
• Bloodstream*	10–20
Rejection associated bacteremia	10–15
CMV*	20–50†
EBV/PTLD*	10–33‡
• Associated with bacteremia*	5
Late (> 6 months)	
Catheter-associated infection:	
• Bloodstream*	5–10
Rejection associated bacteremia	10–15
CMV*	<10
EBV/PTLD*	<5
• Associated with bacteremia*	<5
Community-acquired infection	Varies§

CMV, cytomegalovirus; EBV/PTLD, Epstein–Barr virus/post-transplant lymphoproliferative disease.
*Indicates difference from solid-organ transplant recipients.
†Incidence higher in adult intestinal transplant recipients.
‡Incidence higher in pediatric intestinal transplant recipients.
§Varies with age and community exposure.
Adapted from Fishman & Rubin.[15]

INFECTIOUS SYNDROMES

More than 90% of children with IT present with a bacterial infection following transplantation, with an average of approximately three per patient.[20] However, fungal and viral infections also contribute to the infectious burden after IT.[10]

Bacteremia

Clinical presentation

Bacteremia has been reported to be the most frequent infectious complication following IT.[10] In one series, 277 episodes of bacteremia were identified in 123 children undergoing IT.[10] The rate of bacteremia in IT patients is much higher (about two episodes per patient)[21,22] than that reported for patients undergoing other types of organ transplantation.[23] Bacteremia, typically presenting as fever alone or in combination with signs of sepsis, occurs most frequently during the first 4 months after transplantation. However, episodes can occur even years after IT. Sepsis has been identified as the major cause of death in nearly 50% of cases reported to the International Intestinal Transplant Registry.[4]

The high incidence of bacteremia may be linked to the prolonged presence of central venous catheters as well as translocation of microbiologic flora from within the lumen of the intestinal allograft. In most studies, an obvious source of bacteremia is not identified but abnormal histologic findings in the allograft (rejection, PTLD or both) are frequently present at the time of bloodstream infection.[12] Enteric organisms are the most frequently recovered pathogens and are commonly resistant to multiple antibiotic classes. Some centers have observed a correlation between bacteria identified in the stool and those recovered from the blood. Bacterial overgrowth (defined as a bacterial count $>10^9$ colony-forming units (CFU)/ml of stool) has been postulated to facilitate the development of translocation and has been recognized in nearly 90% of bacteremic episodes in these patients.[24] Bacterial overgrowth may be attributed to surgical manipulation of the transplanted bowel, absence of the protective ileocecal valve, abnormal gastrointestinal motility, lymphatic disruption, prolonged TPN and the use of antacid medication. In more than half the cases, the overgrowth includes both Gram-positive and Gram-negative bacteria.

Diagnosis

Blood cultures should be obtained from IT recipients presenting with fever or other clinical signs suggestive of systemic infection. If enteric pathogens are recovered, endoscopy should be performed to look for underlying causes such as rejection or PTLD.

Treatment

The initial empiric therapy for suspected bacteremia should take into account previous isolates obtained from that patient and their antimicrobial resistance patterns. Final treatment should reflect cultures and antimicrobial susceptibility testing performed at the time of clinical presentation. Attention should be paid to high rates of antimicrobial resistance present in bacterial isolates recovered from recipients of IT.

Abdominal infections

Due to mechanisms mentioned above (translocation, anastomotic leaks, increased rejection and mucosal damage), intra-abdominal infections (e.g. peritonitis, intra-abdominal abscesses or fluid collections) are more common in IT recipients than in other solid-organ transplant recipients. Staphylococci, enterococci, *Pseudomonas* spp. and a variety of different Enterobacteriaceae are the most frequent causative agents.[20] Imaging studies support microbiologic cultures obtained by targeted biopsies or other cultures to identify the infection. Again, attention should be paid to multidrug-resistant micro-organisms when starting antimicrobial therapy.

Cytomegalovirus

Epidemiology and clinical presentation

Experience in the 1990s identified that disease from CMV occurred in almost 25% of IT recipients and accounted for significant morbidity and mortality despite treatment with ganciclovir. However, rates of CMV disease have declined with aggressive use of CMV preventive strategies. One recent series reported that only one of 36 children undergoing IT developed symptomatic CMV disease.[8] When CMV disease occurs, up to 90% of patients may have involvement of their allograft as well as their native gastrointestinal tract.[16] Similar to other transplant recipients, CMV-negative recipients of organs from CMV-positive donors present with disease that is more likely to be invasive and more difficult to treat. Historically, primary CMV disease initially presents in the second month after IT, while reactivation episodes present later in the first post-transplant year.[16] However, the timing of both primary and secondary CMV disease may be delayed in patients who have been managed with CMV preventive strategies. Of importance, recurrent CMV disease has been observed frequently in IT recipients[16] and should be treated promptly with antiviral agents.

Diagnosis

Laboratory tests to diagnose viral infections are outlined in Table 79.2. Diagnosis of CMV disease is definitively made on histopathology but can be inferred with the finding of a positive pp65 antigenemia assay or detection of CMV DNA in the blood of a patient with a compatible clinical syndrome. Clinicians must be aware that results of viral cultures of the urine and even bronchoalveolar lavage specimens are difficult to interpret in previously infected patients since CMV is frequently shed asymptomatically in these secretions. Similarly, the presence of pp65 antigen and CMV DNA in the blood can be misleading, as these assays are often positive in asymptomatic patients. The specificity of these assays can be improved by quantitative determination of the pp65 antigen or CMV DNA. Because of the lack of specificity of these assays, histologic examination of involved organs to confirm the presence of CMV is critical when the diagnosis of invasive CMV is being entertained. The importance of histologic evaluation of the gastrointestinal tract should be further emphasized since antigenemia and/or CMV DNAemia may be absent from up to 50% of proven cases of CMV enteritis.[17]

Prevention

Strategies for the prevention of CMV disease vary amongst the various transplant centers. Current recommendations for the prevention of CMV in IT recipients at the Children's Hospital of Pittsburgh are shown in Table 79.3. CMV prevention is typically accomplished through the use of chemoprophylaxis with intravenous ganciclovir and/or valganciclovir, alone or in combination with CMV intravenous immunoglobulin (IVIG), or as pre-emptive therapy where antiviral therapy is initiated in response to a rising viral load, as measured by CMV antigenemia assay or nucleic acid amplification test. More intensive prophylaxis is recommended for patients at high risk for CMV (CMV-seronegative recipients of organs from CMV-seropositive donors).

At the present time, the use of oral valganciclovir is an alternative to prophylaxis with intravenous ganciclovir for adolescents and adult IT recipients. Pharmacokinetic studies for dosing oral valganciclovir suspension in children after transplantation are ongoing. Upon completion of these studies, use of valganciclovir suspension will likely be of merit as an alternative to intravenous treatment for younger children. Upon completion of ganciclovir chemoprophylaxis, some centers will continue to follow these patients using CMV viral load monitoring to inform pre-emptive antiviral therapy with ganciclovir (or valganciclovir). Finally, patients at risk for CMV (all patients except CMV-negative donor/CMV-negative recipient) treated for rejection with intravenous steroids or antilymphocyte antibodies should also receive a pre-emptive course of ganciclovir or valganciclovir.

Table 79.2 Laboratory evaluation of viral infections following intestinal transplantation

Organism	Frequency (%)	Diagnostic test	Follow-up
HBV	<1	HBV serologies, HBV PCR, histology	HBV PCR, liver function tests
HCV	<1	HCV serologies, HCV PCR	HCV viral load, liver function tests
RSV	Adults: <1 Children: 1–5	NP aspirate for antigen detection, culture, PCR	None
Parainfluenza	Adults: <1	NP aspirate for antigen, PCR	None
Adenovirus	30–50	Viral culture, histology, PCR	None
Enterovirus	Adults: <1 Children: 1–5	Viral culture, PCR	None
Influenza	1–5	NP aspirate for antigen detection, culture, PCR	None
HSV	1–5	Culture, antigen testing	Chronic aciclovir prophylaxis
CMV	>5	Culture, pp65 antigen, quantitative PCR, histology	Monitor pp65 antigen or quantitative CMV-PCR
EBV	>5	Quantitative PCR, histology, serology	Monitor EBV PCR, Imaging studies

CMV, cytomegalovirus; EBV, Epstein–Barr virus; HBV, hepatitis B virus; HCV, hepatitis C virus; HSV, herpes simplex virus; IS, immunosuppression; NP, nasopharyngeal; PCR, polymerase chain reaction; RSV, respiratory syncitial virus.

Table 79.3 Prophylactic strategies* in intestinal transplantation

Indication	Prophylaxis	Dose and duration	Comments
Cytomegalovirus			
Donor +, recipient –	Ganciclovir	5 mg/kg iv q12h for 14 days. Then 5 mg/kg iv q24h for 7–14 days if patient remains in hospital; start valganciclovir to complete 90–120 days of therapy once there is evidence of good gut function and absorption (adults: valganciclovir 900 mg po q24h; children: contact pediatric infectious disease specialist)	Adjust dose for renal function. Monitor CMV viral load after completion of prophylaxis until out 6 months from intestinal transplantation. Initiate pre-emptive ganciclovir if viral load becomes positive
	CMV-IVIG	150 mg/kg within 72 h of transplant and at 2, 4, 6 and 8 weeks post-transplant. 100 mg/kg at 12 and 16 weeks post-transplant	
Donor +, recipient +	Ganciclovir	5 mg/kg iv q12h for 14 days	Monitor CMV viral load after completion of prophylaxis until out 6 months from intestinal transplantation. Initiate pre-emptive ganciclovir if viral load becomes positive
Donor –, recipient –	No treatment		

*These are the strategies used at the Children's Hospital of Pittsburgh.

Treatment

Strategies for the treatment of CMV in IT recipients used at the Children's Hospital of Pittsburgh are shown in Table 79.4. CMV disease following IT should be managed using intravenous ganciclovir. Serial measurement of the CMV viral load using the pp65 antigenemia assay or a quantitative CMV PCR, along with follow-up endoscopic evaluation, should guide the duration of therapy. Use of CMV-IVIG in combination with ganciclovir should be considered for patients with involvement of the gastrointestinal tract (native or allograft) or of the lungs. Children have experienced a better outcome than adults after appropriate anti-CMV treatment, with successful outcomes approaching 90%.[25] The median time to resolution (defined as the absence of inclusions on biopsy) on ganciclovir treatment is approximately 20 days.[16] Immunosuppression should be maintained at baseline levels to avoid rebound rejection after treatment of CMV disease. Recurrence of disease is common, especially in CMV-positive donor/CMV-negative recipient patients. Long-term suppression with oral ganciclovir or valganciclovir may be helpful in patients who experience recurrent CMV disease, although few data are available to support this practice. Persistence of disease or of elevated CMV viral load despite treatment suggests the potential presence of ganciclovir resistance. The use of foscarnet or cidofovir should be considered in these cases.

Epstein–Barr virus

Epidemiology and clinical presentation

IT recipients are at high risk of developing EBV disease compared to other organ transplant recipients. EBV disease and PTLD are seen more commonly in children than adults and occur more frequently in recipients of multivisceral transplants > liver and intestinal transplant > isolated intestinal transplant. The International IT Registry identifies incidence rates of PTLD of 5.0% in adult compared to 15.3% in pediatric IT recipients.[4] However, the spectrum of EBV-related disease includes other clinical presentations in addition to PTLD (e.g. EBV enteritis, EBV hepatitis). Recent experience from our center found the current overall incidence of EBV disease to be approximately 20% although only half of these children met the histopathologic criteria for PTLD. These results represent an improvement from our earlier experience with EBV in IT recipients and likely reflect improved immunosuppression regimens and the use of an EB viral load monitoring protocol. Similar improvements in the incidence of EBV/PTLD have also been reported in the International IT Registry.[4]

EBV disease may present clinically as a febrile syndrome, mononucleosis, PTLD or malignant lymphoma. Diarrhea, which often contains occult blood, is a frequent finding in this patient population and may be associated with ulcerated nodular tumors of the intestinal tract. Nonspecific symptoms including fever, weight loss and malaise are common. Lymphadenopathy may also be present. Although the most frequent site of involvement is the intestinal tract, including the allograft, disseminated disease can also occur. Accordingly, it is important to note that EBV enteritis can be misdiagnosed as rejection. For this reason, it is recommended that Epstein–Barr encoded RNA (EBER) staining be considered on bowel biopsies with presumed rejection to rule out the possibility of EBV infection before treating for rejection.[26] This is particularly important in patients with a current or recent history of an elevated EB viral load in the peripheral blood.

There are three major differences between EBV/PTLD in IT recipients and other solid-organ recipients:

- EBV disease and PTLD are seen frequently in EBV-seropositive children undergoing IT. With other organ transplants EBV/PTLD is rarely seen in patients who are EBV-seropositive prior to transplant;
- EBV-associated disease including PTLD can occur concurrently with rejection after IT, whereas in other organ transplant recipients rejection tends to occur only after evidence of regression of the EBV disease; and
- as many as 30% of surviving IT patients will experience chronic and/or recurrent episodes of EBV disease in contrast to a recurrence rate of about 5–10% after other organ transplants.[27]

The last difference may be explained by the difficulty in reducing immunosuppressive treatment in the presence of concomitant rejection, limiting the body's ability to generate a cytotoxic T-lymphocyte response against EBV.

Diagnosis of Epstein–Barr virus post-transplant lymphoproliferative disease

When EBV disease (including PTLD) is suspected, CT scan of the neck, chest, abdomen and pelvis should be performed to identify occult lesions. Endoscopy should also be performed in IT recipients, especially

Table 79.4 Treatment strategies* in intestinal transplantation

Treatment	Dose and duration	Comments
Cytomegalovirus		
First-line therapy		
Ganciclovir	5 mg/kg iv q12h for minimum of 14–21 days	Maintenance therapy 5 mg/kg iv q24h or consider valganciclovir. Treat until pp65 or CMV PCR reverts to negative. Adjust dose for renal function
CMV-IVIG (for CMV pneumonitis, enteritis or retinitis)	100 mg/kg iv q48h for three doses	May repeat every 2 weeks depending on results
Alternative therapy		
Foscarnet	180 mg/kg iv q8h for 14–21 days	Maintenance therapy: 90–120 mg/kg iv q24h
Cidofovir	5 mg/kg iv once weekly for 2 weeks	Continuing therapy: 5 mg/kg iv once every 2 weeks as clinically needed
Epstein–Barr virus		
Initial treatment		
Reduction or elimination of immune suppression		Immune suppression reduced and maintained at low levels until resolution of disease unless rejection develops
Ganciclovir	5 mg/kg iv q12h for 14–21 days	Adjust for renal function. Treatment typically maintained until resolution of disease. Clinical efficacy unproven
Aciclovir	500 mg/m^2 iv q8h for 14–21 days	Adjust for renal function. Treatment typically maintained until resolution of disease. Clinical efficacy unproven
IVIG	300 mg/kg iv	May repeat every 2 weeks depending on results. Clinical efficacy unproven
Secondary treatment		
Rituximab	375 mg/m^2 iv once weekly for 4 weeks	The use of rituximab should be considered when patients fail to respond to initial treatment, or for those who develop concurrent rejection at the time of diagnosis or treatment for EBV/PTLD. Consultation with a subspecialist with experience in the management of EBV/PTLD (e.g. infectious disease, hematology/oncology) is recommended

*These are the strategies used at the Children's Hospital of Pittsburgh.

if the patient has diarrhea or other symptoms of gastrointestinal disease. Biopsies should be obtained from suspicious lesions and sent for histologic evaluation. The use of immunohistopathologic stains for the presence of EBV (e.g. EBER staining) is necessary to distinguish EBV-infected cells from nonspecific lymphocytic infiltrates. Patients with active EBV disease typically have an elevated peripheral blood EB viral load. However, results of these assays lack specificity as elevated EB viral loads also may be present in otherwise asymptomatic individuals.

Prevention of Epstein–Barr virus post-transplant lymphoproliferative disease

The high rates of morbidity and mortality attributed to EBV/PTLD have prompted efforts to prevent or pre-emptively treat EBV in IT recipients. Serial monitoring of the EB viral load using quantitative PCR assays has been shown to predict occurrence of PTLD. EB viral load surveillance is currently used to guide initiation of pre-emptive therapy at many centers.[19] However, specific target levels of viral load as well as therapeutic pre-emptive treatment regimens vary from center to center and remain to be evaluated in prospective, comparative studies. Pre-emptive therapy typically consists of reduction of immune suppression whenever possible. Ganciclovir and IVIG are also used by some centers although definitive data demonstrating efficacy of these latter two agents are lacking.

Treatment of Epstein–Barr virus post-transplant lymphoproliferative disease

Strategies for the treatment of EBV/PTLD used at the Children's Hospital of Pittsburgh are shown in Table 79.4. Reduction of immune suppression is the major therapeutic manipulation for transplant recipients with EBV/PTLD and allows the body to develop EBV-specific cytotoxic T-cell lymphocytes to control the infectious process. Unfortunately, the high rates of rejection observed during attempts at immunomodulation of EBV may limit this approach in IT recipients. While frequently used, ganciclovir or aciclovir as well as IVIG are of unproven benefit for the treatment of EBV/PTLD. The use of anti-CD20 monoclonal antibody (rituximab) has been increasingly used in this population. Although rituximab appears quite promising, evidence of efficacy is limited to registry and anecdotal reports.[28–31] Historically, the mortality rate for IT patients with PTLD has been reported to be as high as 50%. However, the use of EB viral load monitoring to facilitate earlier diagnosis in association with evolving treatment strategies have led to improving rates of patient and graft survival, with rates approximating 75%.[10]

Adenovirus

Epidemiology and clinical presentation

Adenovirus infection has been observed frequently in pediatric recipients of IT. Rates of adenovirus infection in this population have ranged from 20.8%[32] to 100%.[33] However, the latter rate may have been attributable to the fact that viral cultures were obtained as part of routine screening of graft biopsies and not all of the patients were symptomatic. The presence of adenovirus has been identified much less commonly in adult IT recipients. The interpretation of a positive culture or histologic finding for adenovirus is made difficult by the frequent absence of any associated symptoms in IT recipients from whom evidence of adenovirus infection is demonstrated. When symptoms are found they most often include high stool output, alone or in the presence of fever.

Adenovirus can also present as an invasive disease.[34] Risk factors for invasive disease include failure to clear virus, isolating virus from more than one site and intensified immunosuppression.[33]

Diagnosis

It is very difficult to presumptively diagnose infection due to adenovirus in IT recipients, as fever, hepatitis and pneumonia may be caused by a variety of other pathogens. In addition, high stool output after IT is nonspecific and can also occur with rejection. The presence of high-grade fevers and symptoms suggestive of adenovirus infection should prompt serial cultures for viruses (including adenovirus) or PCR investigation and evaluation of graft biopsies. Unexplained hepatitis should warrant consideration of a liver biopsy. Similarly, an increase in stool output, with or without fever, should prompt endoscopic evaluation of the intestinal allograft. Histologic examination for the presence of adenoviral inclusions as well as the use of immunohistochemical stains of biopsy specimens from either site should be undertaken to help confirm this diagnosis.

Treatment

Unfortunately, there is no definitive treatment for adenoviral infection at this time. The most important component of therapy is supportive care along with a decrease in immunosuppression. The role of antiviral agents is unproven. A small number of case reports describe the use of ribavirin, ganciclovir and cidofovir in the treatment of single patients with adenoviral infection after solid-organ or bone marrow transplantation.[35,36]

Other pathogens

Similar to other organ transplant recipients, recipients of IT are at risk for infection from a wide variety of pathogens. Infection may develop from nosocomial exposures or once the patient has returned to the ambulatory setting. Infectious pathogens of the intestinal tract that can mimic intestinal rejection include adenovirus, rotavirus, *Clostridium difficile*, herpes simplex virus and nontuberculous mycobacteria.[21] An effort should be made to search for these pathogens by culture, histology and/or immunoassay before treating the patient for rejection.

Respiratory viruses (parainfluenza, respiratory syncytial virus, influenza) can be fatal in IT recipients.[22] While the relative frequency of acquisition is greater in the intermediate to late time periods after transplantation (after exposure to community-acquired pathogens), occurrence of disease in the early post-transplant period is most dangerous. Additional risks for severe disease with respiratory viruses include age less than 1 year, pre-existing lung disease and exposure to augmented immunosuppression.

REFERENCES

References for this chapter can be found online at http://www.expertconsult.com

Chapter 80

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Kidney transplant patients

INTRODUCTION

Kidney transplantation is the preferred strategy to treat end-stage renal disease. Improvement in surgical techniques, more efficacious immunosuppressive protocols, and a more accurate selection process of donors and recipients, have led to a remarkable improvement in both patient and graft survival, as well as in quality of life.[1] In parallel, a progressive reduction of morbidity and mortality caused by infectious diseases after transplantation has occurred, mainly as a consequence of improved prophylactic regimens. Nevertheless, infectious diseases are still a major cause of hospitalization after transplantation.[2] New infections have emerged and clinical and epidemiologic patterns of old infections have changed. BK virus has become an important viral pathogen after kidney transplantation, mostly due to the higher net state of immunosuppression associated with modern antirejection regimens and the lack of effective antiviral therapy.[3,4] Recent reports have appeared of donor to recipient transmission of rabies, lymphocytic choriomeningitis virus and West Nile virus infection.[5] Although cytomegalovirus (CMV)-associated morbidity and mortality have decreased due to the introduction of better diagnostic methods and new antiviral strategies, late-onset CMV disease remains a significant problem after kidney transplantation, particularly in high-risk recipients.[6]

While many infections following kidney transplantation are similar in nature to other organ transplant recipients, there are also specific problems found in patients with kidney disease.

- The majority of transplant candidates are undergoing dialysis before transplantation (except in cases of pre-emptive kidney transplantation). Patients on dialysis have frequent hospitalizations and are exposed to multiple medical procedures. They are more frequently colonized with methicillin-resistant *Staphylococcus aureus* and other resistant organisms and are at an increased risk for developing urinary tract infections and invasive fungal infections after transplantation.[7]
- Compared to native kidneys, the kidney allograft is more susceptible to nephrotoxic drugs and antimicrobial drugs can potentiate the nephrotoxicity of calcineurin inhibitors (ciclosporin and tacrolimus). For example, as shown in Table 80.1, drugs such as aminoglycosides, amphotericin B, foscarnet and cidofovir increase the risk of nephrotoxicity from calcineurin inhibitors. In addition, antimicrobials that inhibit the CYP3A4 enzyme (e.g. some macrolides or azoles) increase calcineurin inhibitor plasma levels, with a subsequent higher risk of nephrotoxicity. Conversely, drug inducers of the CYP3A4 system (e.g. rifampicin) may decrease levels of calcineurin inhibitors, resulting in allograft rejection. Clinicians must be aware of these and other drug interactions before starting antibiotic therapy in kidney transplant recipients.
- There are some infections seen predominantly in kidney transplantation. Recurrent urinary tract infections are frequently seen after kidney transplantation and may be due to the recipient's specific diseases (e.g. reflux nephropathy) or to anatomic changes produced during surgery. Although BK virus has been detected in the urine of other organ transplant recipients, it has rarely been associated with clinical nephropathy.

URINARY TRACT INFECTION

Urinary tract infection (UTI) is the most common bacterial infection after kidney transplantation.[8] Asymptomatic bacteriuria, acute cystitis and acute pyelonephritis are usually included in the definition of UTI. The incidence of UTI in kidney transplant recipients may vary between 20% and 75%. Although the major impact of UTI is seen in the early post-transplant period, it can appear months after transplant and has been associated with varying degrees of allograft dysfunction and a higher mortality.[9] Risk factors associated with UTI are female gender, diabetes mellitus, vesicoureteral reflux, duration of vesical catheterization and use of ureteral stenting.[10] As post-transplant urologic complications such as urinary leak or ureteral stricture are significantly reduced by ureteral stenting, this procedure is now in common use. The higher risk of UTI due to ureteral stenting can be reduced by antimicrobial prophylaxis.[11]

The microbial etiology of UTI after kidney transplantation is similar to that observed in the general population. *Escherichia coli* remains the most common pathogen of UTI, followed by other Enterobacteriaceae, such as *Klebsiella* or *Proteus* spp.[8,10] Other series have also identified *Enterococcus* and *Pseudomonas* spp. as common pathogens involved in UTI. *Corynebacterium urealyticum* is the cause of a rare entity reported as encrusted pyelitis and cystitis, where mucosal inflammation with encrustation of the mucosa by struvite crystals can be seen. Although candiduria is common after kidney transplantation, symptomatic infection is seen less frequently.

Clinical manifestations of UTI after transplantation may be attenuated due to the anatomic particularities of the kidney allograft and the immunosuppression-associated impaired inflammatory reactions. In kidney graft recipients with fever but without an evident clinical source, the urinary tract should be initially suspected as the potential cause of the infection.

The first step for preventing UTI after transplantation is an appropriate and timely removal of the urinary catheter. In addition, antibiotic prophylaxis is used in the majority of kidney transplant programs during the first 4–6 months after transplant (Table 80.2). Because co-trimoxazole is effective against common uropathogens and as prophylaxis for *Pneumocystis jirovecii* pneumonia and other pathogens such as *Nocardia*, *Toxoplasma* and *Listeria* spp., this drug is widely used

Table 80.1 Interaction between antimicrobial agents and immunosuppressive drugs

Antimicrobial drug	Interaction with immunosuppressive drugs	Comments
Aminoglycosides		
Aminoglycosides	Increase nephrotoxicity of calcineurin inhibitors	Aminoglycosides should be avoided in patients receiving calcineurin inhibitors. No published data available on interaction between aminoglycosides and sirolimus
Quinolones		
Ciprofloxacin	May increase ciclosporin levels	Mild interaction. Quinolones may potentiate the tubular toxicity of calcineurin inhibitors
Macrolides		
Clarithromycin	Increases calcineurin inhibitor and sirolimus levels	Important interaction. Azithromycin may be a better option
Azithromycin	May increase ciclosporin levels	Mild interaction
Antifungal agents		
Fluconazole	Increases calcineurin inhibitor and sirolimus levels	Moderate interaction
Voriconazole	Increases calcineurin inhibitor and sirolimus levels	Important interaction. Calcineurin inhibitors and sirolimus dose should be decreased 50–80% after starting voriconazole
Caspofungin, anidulafungin	Calcineurin inhibitors may increase echinocandin levels	Clinical relevance of this interaction is unknown
Amphotericin B	Increases nephrotoxicity of calcineurin inhibitors	Possibly lower toxicity with liposomal formulation
Antiviral agents		
Foscarnet	Increases nephrotoxicity of calcineurin inhibitors	Incomplete clinical data
Cidofovir	Increases nephrotoxicity of calcineurin inhibitors	Incomplete clinical data
Other antimicrobials		
Rifampicin	Decreases calcineurin inhibitor levels	If possible, rifampicin use should be avoided, due to high risk of rejection. Rifabutin may be a better option
Co-trimoxazole	May increase nephrotoxicity of calcineurin inhibitors	The risk of nephrotoxicity is higher with therapeutic than prophylactic doses

Calcineurin inhibitors: ciclosporin, tacrolimus and co-trimoxazole (trimethoprim–sulfamethoxazole, TMP–SMX).

Table 80.2 Preventive strategies after kidney transplantation

Prophylactic strategy	Recommendation	Comments
Antimicrobial prophylaxis	Co-trimoxazole 400/80 mg q24h or 800/160 mg three times a week for initial 3–6 months after transplant [In case of allergy to co-trimoxazole, a quinolone (ciprofloxacin, levofloxacin or moxifloxacin) can be used, along with a drug effective for *Pneumocystis jirovecii* pneumonia prophylaxis (dapsone 50–100 mg q24h, atovaquone 1500 mg q24h or inhaled pentamidine 300 mg every 3–4 weeks)]	Efficacy in preventing urinary tract infection, *Pneumocystis jirovecii* pneumonia, toxoplasmosis, *Listeria*, *Salmonella* spp.
Antiviral prophylaxis	Valganciclovir 450–900 mg* q24h for initial 3–6 months after transplant in D+/R– and/or after antilymphocyte therapy for rejection Valganciclovir 450–900 mg* q24h for initial 3–6 months after transplant in D+/R+ and D–/R+ Aciclovir 200 mg* q8h, valaciclovir 250 mg* q12h, or famciclovir 250 mg* q12h for initial 3 months after transplant for herpes prophylaxis in CMV D–/R– or in patients followed by pre-emptive approach	Usually recommended Optional. Some authors prefer using a pre-emptive approach in this setting Usually recommended
Antifungal prophylaxis	Fluconazole 200–400 mg* q24h in case of high risk for *Candida* infection, i.e. surgical complications (e.g. urinary leak), long intensive care unit stay, *Candida* colonization, use of parenteral nutrition, use of broad-spectrum antibiotic	Systemic antifungal prophylaxis generally not recommended

*Dose to be adapted for kidney function. D, donor; R, recipient.

after kidney transplantation.[12] In case of drug allergy or a high rate of co-trimoxazole-resistant organisms, a quinolone can be useful for prophylaxis. The management of asymptomatic bacteriuria after kidney transplantation remains controversial. Studies have failed to show a higher rate of symptomatic UTI in patients with untreated asymptomatic bacteriuria. Therefore, screening for or treatment of asymptomatic bacteriuria in renal transplant recipients is not recommended.[13]

Management of UTI should integrate a medical and a surgical approach. In general, prompt empiric antibiotic therapy is necessary in order to avoid progression to a more severe infection. Usually, broad-spectrum β-lactams (e.g. piperacillin) or a carbapenem is the antibiotic of choice to treat Enterobacteriaceae, *Pseudomonas* and *Enterococcus* spp. This antibiotic therapy should subsequently be adjusted when the etiologic pathogen is identified. The duration of therapy may vary from 7 to 10 days for uncomplicated UTIs to 4–6 weeks in cases of septic shock, presence of anatomic abnormalities or infections with resistant micro-organisms. An ultrasound of the allograft is also recommended to check for urologic complications such as obstruction or urinary leak, especially in cases of recurrent UTI or breakthrough infection during antibiotic prophylaxis. In patients with polycystic kidney disease, the native kidneys may be the cause of the UTI. If anatomic abnormalities are suspected or identified, a surgical or urologic consultation is indicated.

POLYOMAVIRUS INFECTION

BK virus, JC virus and the simian virus SV40 belong to the Papovaviridae family.[3] The main pathogen implicated in polyomavirus-associated nephropathy is BK virus, although some cases of JC-associated nephropathy have also been reported. Primary infection with either BK or JC virus usually occurs during childhood, and serologic studies have shown a prevalence of BK virus infection in 60–80% of adults. The prevalence of BK virus active infection in kidney transplant recipients measured by polymerase chain reaction (PCR) in plasma has been estimated to be approximately 10–15%. The prevalence of BK virus-associated nephropathy varies depending on the series and the immunosuppressive regimen, but is estimated to be between 1% and 10%.[4]

Clinical manifestations of BK virus infection in kidney transplant recipients include tubulointerstitial nephropathy and ureteral stenosis. A rising creatinine level is usually the only manifestation of BK virus-associated nephropathy; if unrecognized, this may lead to graft loss in 10–80% of cases.[4] In centers where universal screening for BK virus is performed, current rates of graft loss due to BK virus-associated nephropathy are low because of appropriate management.

Identifiable risk factors are associated with BK virus-associated nephropathy. Although this nephropathy has occurred with all immunosuppressive regimens, it is more frequently associated with more intensive regimens such as those including tacrolimus, steroids and mycophenolate mofetil (MMF) at normal dosages. The use of antilymphocyte preparations as induction therapy and for treatment of acute rejection has also been suggested as a risk factor, but not in all series.[3]

The diagnostic certainty for BK virus-associated disease is classified at three levels – possible, presumptive and definitive BK virus-associated nephropathy.[4]

Possible Possible BK virus-associated nephropathy refers to a positive screening test, usually the presence of intranuclear viral inclusions in urogenital tract epithelial cells (decoy cells) in the urine. This test has an excellent negative predictive value, but lacks specificity.

Presumptive The term presumptive BK virus-associated nephropathy is used when an adjunct test is also positive (e.g. a quantitative BK viral load measured in urine or plasma by PCR). Plasma viral load appears to best correlate with an eventual diagnosis of BK virus-associated nephropathy.

Definitive The definitive diagnosis of BK virus-associated nephropathy requires a kidney biopsy to identify characteristic viral inclusions, as well as inflammatory infiltrates and cytopathic changes (Fig. 80.1). Histology of BK virus-associated nephropathy ranges from pattern A (minimal to mild viral cytopathic changes and inflammatory infiltrates) to pattern C (moderate to severe tubular atrophy and interstitial fibrosis, with variable inflammatory infiltrates).[4] Concurrent acute rejection can be present and is difficult to differentiate from the histologic changes due to BK virus.

The management of BK virus infection in kidney transplant recipients is discussed in more detail in Practical Point 43. Most programs recommend screening for BK virus, with or without urine cytology, every 3 months during the first 1–3 years after transplant, or when renal dysfunction occurs. If decoy cells are identified in urine, BK virus DNA should be quantified by PCR. Generally accepted thresholds predictive for BK virus-associated nephropathy are $>10^7$ DNA copies/ml in urine or $>10^4$ DNA copies/ml in plasma. Subsequently, a renal biopsy should be performed to confirm the diagnosis of BK virus-associated nephropathy.

There is no current etiologic treatment for BK virus-associated nephropathy. The most effective approach is to decrease immunosuppression, with close follow-up because of the risk of inducing acute

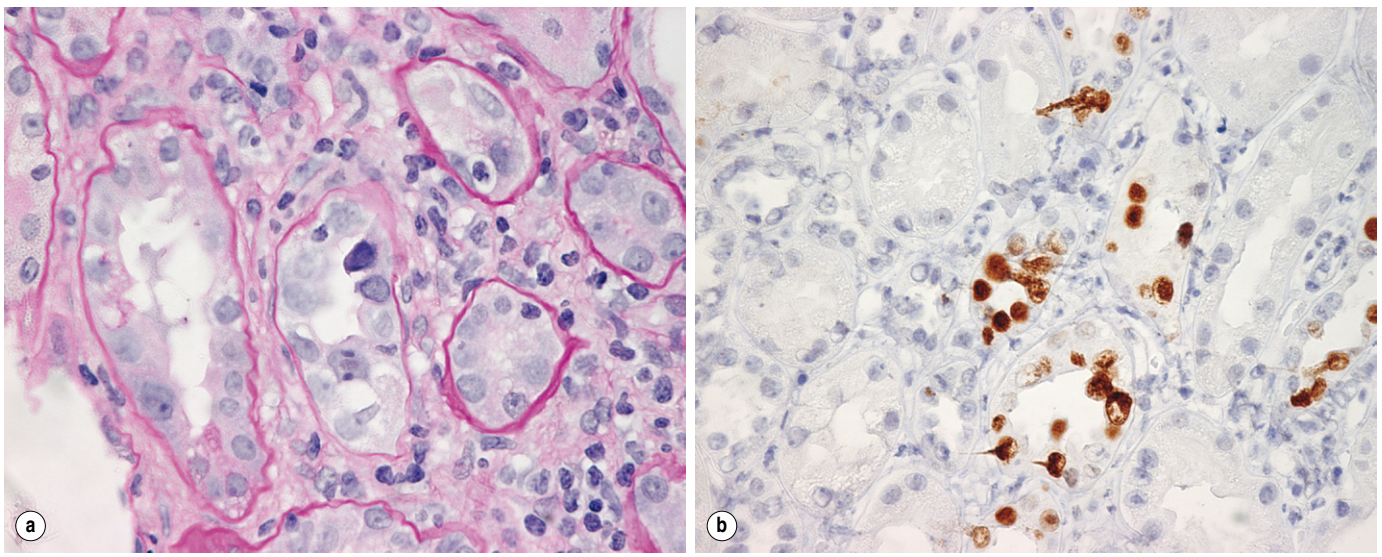


Fig. 80.1 Kidney biopsy of a patient with BK virus-associated nephropathy. (a) Mononuclear infiltrates. (b) SV40 stain is positive in some tubular cells (brown dots).

rejection. Some treatment options include switching from tacrolimus to ciclosporin, decreasing the dose of MMF (or replacing it by azathioprine) or discontinuing one of the drugs to maintain a two-drug regimen. Cidofovir at low doses (0.25–0.33 mg/kg given intravenously every 2–3 weeks) and leflunomide (a drug used for treatment of rheumatoid arthritis) have antipolyomavirus activity *in vitro* and some apparent benefit in small clinical series, but currently there is no consensus on their use.

HERPESVIRUS INFECTION

Cytomegalovirus infection remains a major problem in kidney transplantation.[14] In the absence of prophylaxis, around 50% of kidney transplant recipients may develop CMV active infection during the first 3 months after transplantation; this rate is as high as 80% in donor-positive, recipient-negative primary infections. Manifestations of CMV infection range from asymptomatic viral replication to symptomatic disease, including local tissue-invasive disease or generalized CMV syndrome. CMV syndrome is manifested by fever, malaise, leukopenia, thrombocytopenia and elevated liver enzymes. CMV tissue-invasive disease often presents as colitis or hepatitis. In kidney transplant recipients, CMV may produce allograft nephritis, although this is rare.

The major risk factor for developing CMV disease is the donor/recipient serostatus, the seronegative recipient with a seropositive donor being at the highest risk.[6] Other risk factors include the type of immunosuppressive regimen (e.g. the use of MMF), and the use of antilymphocyte agents as induction or antirejection therapy.[6]

Currently, there are two main strategies to prevent CMV disease: universal antiviral prophylaxis and pre-emptive therapy: universal prophylaxis (see Table 80.2) consists of administering an antiviral drug after transplantation to all at-risk patients, usually during the initial 3- to 6-month period; pre-emptive therapy consists of monitoring for CMV appearance in blood (e.g. with either CMV DNA PCR or antigenemia) and administering antiviral therapy only when significant CMV viremia is detected. Both strategies have not been compared in large-scale prospective randomized clinical trials. Drugs which have been used for the prevention of CMV infection in kidney transplantation are oral aciclovir or valaciclovir, and intravenous and oral ganciclovir. Recently, oral valganciclovir, a valyl-ester prodrug of ganciclovir with an improved bioavailability compared to oral ganciclovir, has been approved for CMV prophylaxis.[14,15] While intravenous ganciclovir (5 mg/kg twice a day) has been the preferred drug for the treatment of established CMV disease, recent reports show the equivalence of oral valganciclovir (900 mg twice a day, adapted for kidney function) for this indication.[16,17] However, caution is needed in cases of life-threatening infection and when oral absorption may be compromised. Monitoring of CMV viral load during treatment is a valuable tool to decide the duration of the antiviral therapy (usually 6–8 weeks), since the incidence of relapse is reduced if antiviral therapy is stopped when CMV viral load is undetectable.

Herpes simplex virus (HSV)-1 and -2 reactivation is common after kidney transplantation and its incidence is estimated to be approximately 50% in seropositive recipients. HSV may reactivate shortly after transplantation, and the lesions may be clinically atypical or more severe and may last longer, when compared to those seen in immunocompetent patients. HSV infection is seen less frequently in the era of CMV prophylaxis.

Several studies have described the epidemiology and clinical manifestations of varicella-zoster virus (VZV) infection after kidney transplantation. Incidence of VZV infection may vary between 3% and 10%, but primary infection (i.e. chickenpox) should be differentiated from secondary reactivation (i.e. herpes zoster). Since the majority of adult recipients are VZV seropositive, the most common manifestation of VZV infection after transplant is herpes zoster. Usually, zoster in kidney transplant recipients does not differ from that seen in the general population, although some series have highlighted a higher incidence of disseminated cutaneous reactivation (or visceral involvement) and relapse can occur in up to 15% of patients. Postherpetic neuralgia also seems to be more frequent in this population. Diagnosis is often clinical; however, microbiologic confirmation by immunofluorescence or PCR may be useful to help distinguish it from HSV infection.

A more difficult problem is VZV primary infection in pediatric kidney transplantation, characterized by a high rate of visceral complications and mortality. Recent studies, however, have shown a reduction of VZV-associated complications, likely due to universal VZV screening and vaccination prior to transplantation.

The potential clinical significance of human herpes virus (HHV)-6 and -7 infection has not been convincingly established after kidney transplantation. A number of case reports of HHV-6-associated encephalopathy in solid-organ transplant recipients have been described.[18] Some reports have suggested an association between HHV-6 and -7 infections and CMV infection and disease in kidney transplant recipients.[19] The appropriate approach for screening and management of HHV-6 and -7 infections is currently unknown.

HEPATITIS B AND C VIRUS INFECTIONS

Infections with hepatitis B and C viruses (HBV and HCV) have been recognized as important health problems in end-stage renal failure and, consequently, in the kidney transplant population.[20] Transplant recipients develop HBV- or HCV-associated liver disease after transplantation as a result of progression of pre-existing infection or by transmission through blood products or organs from infected donors.

The introduction of blood donor screening, the increased use of erythropoietin and the adoption of strict infection control practices have significantly reduced the prevalence of HBV and HCV in most dialysis units. The availability and routine use of HBV vaccination has dramatically decreased the prevalence of HBV in dialysis units to <2%.[21] Owing to the lack of effective vaccination, HCV is still found in 5–40% of kidney transplant recipients.[22]

Liver disease associated with HBV and HCV infections (now mostly with HCV) is an important cause of morbidity and mortality after transplantation.[22,23] Chronic hepatitis and its sequelae are the main forms of liver involvement in these patients. In addition, an unusual form of liver disease called fibrosing cholestatic hepatitis, characterized by severe cholestasis and rapidly progressive liver failure, has been reported with both viruses after kidney transplantation.[24,25] These viruses have also been implicated in the pathogenesis of de novo or recurrent glomerular disease in the allograft, which may impair graft survival.[26] More recently, various reports have suggested an association between HCV infection and new onset diabetes mellitus after renal transplantation.[27] In spite of the detrimental effects of HBV and HCV on patient and graft survival, kidney transplantation still remains the best long-term option for HBV- and HCV-infected dialysis patients on the waiting list. Combined liver and kidney transplantation should be considered in the presence of liver cirrhosis.[23]

The diagnosis of chronic HBV infection is made or excluded on the basis of serologic markers such as HBsAg, HBsAb, HBeAg and anti-HBc-IgM/G. Measurement of hepatitis B viral load by PCR is an important guiding tool for the administration and monitoring of antiviral therapy. The diagnosis and screening of HCV infection is based on serologic testing with third-generation anti-HCV assays. As false-negative results may occur in immunocompromised patients, HCV RNA testing by PCR should be strongly considered. Genotyping and determination of hepatitis C viral load are helpful to evaluate potential efficacy and monitoring of antiviral treatment. Serum transaminase levels and viral load are poor markers of the severity of liver disease in HBV- and HCV-infected patients. Therefore, liver biopsy remains the gold standard for accurate grading and staging in these patients. CT scanning or duplex ultrasonography of the abdomen should be performed at regular intervals for early identification of portal hypertension, ascites and hepatocellular carcinoma.

The recommended antiviral treatment strategies in the nontransplant setting cannot be simply applied to kidney recipients. Interferon alpha (IFN-α, standard or pegylated) has been associated with triggering

severe acute allograft rejection, often irreversible.[28] Therefore, antiviral treatment of HCV-infected transplant candidates while on dialysis remains the best option. It may be beneficial not only to prevent post-transplant liver disease, but also to possibly prevent post-transplant HCV-related glomerular disease and new onset diabetes. Currently, judicious use of combination therapy with pegylated IFN-α and low-dose ribavirin (adjusted according to hemoglobin levels and ribavirin plasma concentrations) is indicated when treating HCV-infected dialysis patients and is more effective than IFN-α monotherapy.[22,29]

Unlike interferon, lamivudine (100 mg/day) is safe and effective treatment for HBV after kidney transplantation.[23] However, the optimal timing to start or stop treatment remains obscure. Lamivudine use before transplantation has been shown to be associated with lower rates of reactivation than its use post-transplant in response to evidence of hepatitis. Survival post-transplant was also improved in patients who were treated with lamivudine in a pre-emptive strategy based on serial HBV DNA level measurements after transplantation.[30] The exact duration of antiviral therapy is not defined and long-term treatment is required in most of the patients. Unfortunately, the duration of antiviral therapy is related to both the likelihood of response and the development of resistance. Recently, adefovir (10 mg/day), tenofovir (300 mg/day) and entecavir (1 mg/day) have been used successfully for lamivudine-resistant HBV infection.[30,31] Adefovir therapy carries some risk of nephrotoxicity.

The doses of all antiviral agents need to be adjusted according to renal function.[30] Newer antivirals for both HBV and HCV are currently under study. These include antiproteases, immunomodulatory agents such as new interferons or therapeutic vaccines, alternatives to ribavirin for HCV and several new polymerase inhibitors including nucleosidic/nucleotidic inhibitors (e.g. clevudine, telbivudine, emtricitabine and valtorcitabine and their combinations) and peptide nucleic acids for HBV.[31,32]

Vaccination against HBV in nonimmune renal transplant candidates is essential and should be performed in all patients. Efficacious vaccines or specific immunoglobulin preparations are not yet available for HCV. However, hepatitis A and B vaccination should be recommended in HCV-infected patients before transplantation, since co-infection of HCV with other hepatotropic viruses results in worse clinical outcomes.[23]

OTHER INFECTIONS

The rate of tuberculosis in solid-organ transplant recipients has been estimated to be 50-fold higher than in the general population.[33] Management of tuberculosis is challenging in kidney transplant recipients. First, clinical manifestations can be atypical in immunocompromised patients and, therefore, delays in diagnosis are common. Second, therapy against *Mycobacterium tuberculosis* infection may be associated with significant toxicity and potential drug interactions with immunosuppressive agents. Thus, the diagnosis and treatment of latent tuberculosis infection in transplant candidates is of major importance.

In contrast with other organ transplants, invasive fungal infections are less common after kidney transplantation. The frequency of invasive aspergillosis has been estimated to be 0.7–4%, with the majority of cases occurring in patients with known risk factors, such as high-dose corticosteroids and graft failure requiring hemodialysis.[34] On the other hand, candidiasis is relatively more common, especially during the first month after transplant, in patients with surgical complications and long intensive care unit stay. Recommendations regarding antifungal prophylaxis are shown in Table 80.2. Arterial infection of the allograft by *Candida* spp. has rarely been reported and is associated with high morbidity and mortality due to hemorrhagic complications.[35] Asymptomatic candiduria is a common problem in kidney transplant recipients. A thorough search for invasive candidiasis and pre-emptive antifungal therapy is warranted in this setting. *Pneumocystis jirovecii* pneumonia has been practically eradicated since the introduction of universal prophylaxis with co-trimoxazole during the first months after transplantation. However, outbreaks of late-onset *Pneumocystis jirovecii* pneumonia have recently been described in patients without any new identifiable risk factor.[36]

Table 80.3 Vaccines in kidney transplantation

Timing of vaccination	Vaccine	Comments
Exclusively before transplant	Mumps, rubella, measles Varicella (chickenpox) Varicella-zoster (shingles)*	Live attenuated vaccines are contraindicated after transplant
Can be administered after transplant	Seasonal influenza vaccine, yearly Pneumococcal polysaccharide vaccine, every 3–5 years Hepatitis B Hepatitis A Polio (inactivated), tetanus, diphtheria, pertussis Human papillomavirus (HPV) vaccine*	Administer vaccines before transplant whenever possible to assure immunogenicity

*No data are currently available for this vaccine on immunogenicity and safety in transplant candidates.

PREVENTIVE STRATEGIES AND VACCINATION IN TRANSPLANTATION

Along with an integrated prescription of an immunosuppressive regimen to avoid rejection, prevention of infection is a cornerstone of the management of organ transplant recipients (see Tables 80.2 and 80.3). Vaccination in kidney transplant recipients should take into consideration two main aspects – namely safety and immunogenicity.[37] Live attenuated vaccines, like inhaled influenza vaccine or varicella vaccine, are generally contraindicated after organ transplantation. Inactivated vaccines can be safely administered after transplantation and studies have failed to show any possible relationship between vaccination and allograft rejection.

However, as immunogenicity may be reduced after organ transplant, a major effort should be undertaken in order to administer vaccines in transplant candidates before kidney transplantation, during the early course of their underlying renal disease, whenever possible. Household members should also be offered vaccination if there is no contraindication. The human papillomavirus virus vaccine deserves further evaluation in transplant candidates.[38]

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Chapter 81

Jonathan Cohen

Vasculitis and other immunologically mediated diseases

INTRODUCTION

This chapter is concerned with a diverse group of conditions that have in common the fact that their cause is thought to be related to disordered immune processes, and that their treatment involves immunosuppressive therapy. In the past these diseases have been called 'autoimmune', 'collagen-vascular' or more frequently 'vasculitis', although as a group I think they are better referred to simply as immunologically mediated diseases (IMDs; Table 81.1). In addition, there is a large group of common disorders (e.g. asthma, eczema or inflammatory bowel disease) in which immunosuppressive therapy may be used, sometimes at high dose. Although not discussed in detail here, they are at risk from the same type of opportunistic infections as other immunosuppressed patients.

EPIDEMIOLOGY

The importance of IMDs to the infectious diseases practitioner lies in the fact that these patients frequently have severe multisystem disease and require high-dose immunosuppressive therapy, so the risk of opportunistic infection is high. Furthermore, they differ from other types of immunosuppressed patients in that they often receive several different modalities of immunosuppression (Table 81.2), and the duration of their treatment is much longer than that for a patient who has leukemia or cancer, in whom the neutropenic period is currently often not much more than 4 weeks (and sometimes much less). Infection is a major cause of morbidity and mortality in patients who have IMD but the incidence of infection in these patients varies considerably depending on the stage of the disease and the intensity of the immunosuppression.

In a study of 75 heavily immunosuppressed patients who had a variety of IMDs, we found a rate of 0.74 infections/patient/week,[1] and in the so-called catastrophic antiphospholipid syndrome, intercurrent infection is one of the major causes of death.[2] In contrast, a study of 200 outpatients with systemic lupus erythematosus (SLE) followed for 2 years found that infections only occurred in a third of cases. Most were single, minor and associated with disease activity.[3] In rheumatoid arthritis, the increased risk of infection is directly associated with the type of anti-inflammatory therapy that is used. Not surprisingly, the greatest risk is associated with glucocorticoids and cyclophosphamide.[4] The recent introduction of infliximab and adalimumab (monoclonal anti-tumor necrosis factor antibodies) into clinical practice for the management of moderate-to-severe

Table 81.1 Immunologically mediated diseases (IMDs)

- Systemic lupus erythematosus
- Polyarteritis nodosa
- Wegener's granulomatosis
 - Lymphomatoid granulomatosis
 - Bronchocentric granulomatosis
- Antiglomerular basement membrane disease (Goodpasture's syndrome)
- Mixed essential cryoglobulinemia
- Rheumatoid arthritis
 - Still's disease
 - Felty's syndrome
- Mixed connective tissue disease
- Progressive systemic sclerosis/scleroderma
- Polymyositis/dermatomyositis
- Relapsing polychondritis
- Behçet's syndrome
- Sjögren's syndrome
- Inflammatory bowel disease
- Churg–Strauss syndrome
- Henoch–Schönlein purpura
- Hemolytic–uremic syndrome/thrombotic thrombocytopenic purpura

High-dose immunosuppression is often used in the above IMDs and major opportunistic infection is a common problem. The list excludes generally less severe diseases such as asthma; although such patients may occasionally need high-dose immunosuppression, it is much less common.

Table 81.2 Immunosuppressive agents and procedures

- Corticosteroids
- Thiopurines
 - 6-Mercaptopurine
 - Azathioprine
- Alkylating agents
- Mycophenolate mofetil
- Cyclophosphamide
- Monoclonal antibodies
 - Basiliximab
 - Daclizumab
 - Rituximab
 - Infliximab
 - Etanercept
 - Adalimumab
 - Natalizumab
- Ciclosporin and related drugs
 - FK506 (tacrolimus)
 - Sirolimus
- Total lymphoid irradiation
- Antilymphocyte globulin
- Intravenous immunoglobulin
- Plasma exchange

This list comprises types of immunosuppression typically used in patients who have IMD. It is common for several of these agents to be used in combination.

rheumatoid arthritis has led to the recognition that this confers an increased risk of infection, in particular due to tuberculosis.[5] Extensions of this approach, either by using different therapeutic targets (e.g. anti-interleukin-6 receptor) or different clinical applications (e.g. Crohn's disease) are in early phase development and will need to be monitored closely for other potential infective complications.

A wide range of opportunistic infections may occur in patients with IMD; indeed, one of the characteristics of this group is that there is a much wider differential diagnosis than typically occurs in bone marrow transplant recipients or other types of immunocompromised host. There are some associations of note: for instance, salmonellosis is a recognized complication of SLE and some other immunocompromised patients, although the mechanism is not at all clear.[6]

PATHOGENESIS AND PATHOLOGY

It is difficult to be certain whether the increased incidence of infection in patients with IMD is attributable to the abnormal immune function of the underlying disease or simply a consequence of the immunosuppressive therapy. Probably both are implicated.[7,8] Interestingly, there is a suggestion that part of the susceptibility to infection seen in patients with SLE is attributable to polymorphisms in the gene for mannose-binding lectin.[7]

In some cases, there may be an etiologic association between an infection and the disease itself. A good example of this is the role of the hepatitis viruses in the pathogenesis of cryoglobulinemia, polyarteritis nodosa and other types of systemic vasculitis,[9] and both Epstein–Barr virus and parvovirus B19 have been associated with various vasculitic illnesses.[10] There have also been intriguing reports suggesting that Wegener's granulomatosis may be caused by an abnormal response to an unknown infection, and that relapses of Wegener's granulomatosis can be prevented by chronic administration of trimethoprim–sulfamethoxazole (TMP–SMX).[11]

Although certain types of treatment are associated with particular defects in immune function, patients who have IMD commonly receive combinations of drugs, and this makes predictions very difficult. Certainly, high-dose corticosteroid therapy is complicated by infections such as *Listeria*, herpesviruses and fungi, whereas patients who develop neutropenia as a consequence of cyclophosphamide, for instance, are susceptible to the same kinds of infection as neutropenic patients. Plasma exchange (plasmapheresis) is a form of immunosuppression used particularly in these patients, and this has its own complications, in particular Gram-positive infections associated with intravenous access.[12,13]

It is not just the longer list of possible infections that makes assessment more difficult in these patients. It is frequently very hard to be sure whether the patient has infection or simply a relapse of the underlying disease. An acute flare-up of SLE involving the central nervous system can be indistinguishable from infective meningitis or encephalitis as a consequence of the immunosuppressive therapy. This leaves the clinician on the horns of an unpleasant dilemma; should the immunosuppression be reduced in order to allow antimicrobial therapy to be more effective, or should it be increased to bring the underlying disease back under control? It is helpful to ask whether the clinical features at this presentation are the same as on previous occasions when it was known to be disease activity. Individual patients tend to be consistent in the form of disease they get when it is active. No single investigation can be relied upon to distinguish infection from relapse. In SLE it is often said that an elevated C-reactive protein (CRP) is only seen in patients with intercurrent infection, but this is not an absolute distinction.[8]

A further complication is the phenomenon of 'infection provoked relapse' whereby in patients who have IMD an intercurrent infection can precipitate a relapse of the underlying disease;[14] conversely, flare-ups of SLE can be associated with dysregulated immune defenses, thereby increasing the risk of infection. The infection and the vasculitis need to be treated simultaneously.

PREVENTION

Patients who have IMD, as with all immunosuppressed patients, are constantly at risk of a very wide range of infections but it is neither practicable nor desirable to try to prevent all of them. Tuberculosis is a particular problem because its presentation may be atypical and extrapulmonary disease is common. Patients who are receiving more than 15 mg/day of prednisolone for prolonged periods and who have a clinical history or radiologic evidence of past tuberculosis should be given prophylaxis with isoniazid 300 mg/day plus pyridoxine 10 mg/day.[15] If the risk is less clear, the complications of isoniazid need to be considered, although my practice is to err on the side of advising prophylaxis.[16] In the case of anti-tumor necrosis factor (TNF) therapy for rheumatoid arthritis, guidelines have been published that identify high-risk groups.[17]

In my opinion, the routine use of TMP–SMX as primary prophylaxis to prevent *Pneumocystis* pneumonia is not warranted in this population, even if they are receiving corticosteroids, because of the low incidence of the infection. Furthermore, in contrast to patients who have AIDS, relapse of *Pneumocystis* pneumonia is most uncommon in this population and secondary prophylaxis is not indicated.

CLINICAL FEATURES AND MANAGEMENT

Immunologically mediated diseases can represent a very complex challenge to the infectious diseases physician. Factors that need to be taken into account in assessing the patient include the nature of the underlying disease and the particular type of immunosuppression used, its duration and its dose (see Practice Point 40). Certain clinical syndromes merit particular attention.

Fever and pulmonary infiltrates

The development of fever and new pulmonary infiltrates is one of the most common and most difficult clinical syndromes that occur in patients who have IMD. The differential diagnosis is extraordinarily wide (Table 81.3), and includes infective and noninfective conditions. Details of specific infections may be found elsewhere in this book; here I consider some general principles that apply to the initial assessment and management of patients who have IMD who develop fever and pneumonia.

- There may be very rapid deterioration, from low-grade fever and cough to severe hypoxia needing mechanical ventilation within 12 hours, particularly if the patient is liable to develop pulmonary hemorrhage.
- Radiologic appearances are very nonspecific. It is rare to be able to 'guess' the diagnosis just on the basis of the chest radiograph, with the possible exception of *Pneumocystis* pneumonia.
- Multiple infections are common. Even if the physician correctly recognizes the clinical and radiologic features of *Pneumocystis* pneumonia, the patient may be co-infected with an additional, equally treatable pathogen such as cytomegalovirus (CMV).
- Sputum microbiology can be confusing. The presence of *Candida* spp., for instance, may indicate nothing more than colonization of the nasopharynx, whereas important pathogens such as *Aspergillus* or *Pneumocystis* often do not appear in the sputum.

Consideration of the nature of the underlying disease, the type of immunosuppression and epidemiologic features in the history are

Table 81.3 Causes of fever and pulmonary infiltrates in patients who have immunologically mediated disease*

Infective	Bacteria	Conventional respiratory pathogens† Mycobacteria *Nocardia*[18] 'Atypical' bacteria (*Mycoplasma* spp., *Coxiella* spp.) *Legionella* spp.
	Fungi	*Aspergillus* spp.[19†] *Candida* spp. *Cryptococcus neoformans* *Zygomycetes* Primary systemic fungi (*Histoplasma* spp., *Blastomyces* spp., etc.) Other systemic fungi (rarely; e.g. *Sporothrix schenckii*) *Pneumocystis*†
	Parasites	*Strongyloides stercoralis* *Toxoplasma*
	Viruses	Cytomegalovirus* Herpes simplex virus Varicella-zoster virus Respiratory syncytial virus Adenovirus Influenza and parainfluenza virus
Noninfective		Edema† Hemorrhage[20†] Infarction Emboli Tumor Radiation Chemotherapy Vasculitis Leukoagglutinin reaction

*The list is long but incomplete and, in practice, any organism isolated in pure or predominant culture from a bronchoalveolar lavage or open lung biopsy should be regarded as a pathogen until proved otherwise. Further details about some of the conditions are provided in the references, where indicated.
†Most common causes.

all helpful in guiding therapy. The single most important factor is gauging the speed of progression of the condition. It cannot be overemphasized that in IMD patients a seemingly trivial community-acquired chest infection can proceed to life-threatening pneumonia and/or pulmonary hemorrhage within a frighteningly short period. Urgent evaluation and investigation is essential. All patients should have simple, basic laboratory investigations performed, including a full blood count, sputum and blood cultures, measurement of arterial oxygen saturation or formal blood gas concentrations and a chest radiograph.

Much has been written about the radiologic features of certain infections. It is perfectly true, for instance, that *Pneumocystis* pneumonia classically produces a bilateral 'ground glass' appearance, but so too does CMV infection and acute pulmonary hemorrhage in a patient who has pulmonary vasculitis. Combining the clinical and radiologic data results in a 'short list' of likely diagnoses, but too great a reliance on this is very hazardous. The main value of the chest radiograph is in indicating the extent and rate of progression of the process, not in guessing the pathogen.

Empiric antibacterial therapy is usually indicated if the clinical and epidemiologic evidence point to a simple community-acquired pneumonia, and when the risk of a major opportunistic pathogen (e.g. invasive fungal infection or *Pneumocystis*) is judged to be low. *Pneumocystis* pneumonia is said to often have a very characteristic presentation, and some advocate empiric therapy. However, this strategy may be unwise in patients who have IMD because of the wide differential diagnosis and the possibility of co-infection with other pathogens.

In the majority of patients further specific investigations should be performed. Bronchoscopy with a bronchoalveolar lavage is valuable; close liaison with the laboratory is essential to ensure a rapid response and the maximum diagnostic yield.[21] High-resolution CT scans will undoubtedly give additional and sometimes useful information but in my experience are rarely diagnostic.[22] Pulmonary function tests can be very valuable in this group of patients, in particular measurement of carbon monoxide uptake (the KCO) to detect intrapulmonary hemorrhage.[23]

When a specific organism is identified the treatment follows conventional guidelines. 'Blind' empiric therapy is rarely advisable because of the wide differential diagnosis.

Acute neurologic problems

A wide differential diagnosis also exists for neurologic problems, and prompt evaluation, investigation and treatment are essential.[24] Knowledge of the nature of the immune deficit can narrow down the list of possibilities. This information can be linked to the clinical presentation to provide useful clues; thus, a patient who has a defect in cellular immunity as a result of high-dose steroid therapy who develops a subacute meningitis is likely to have *Listeria*, cryptococcal or tuberculous meningitis, whereas in a neutropenic patient *Aspergillus* or a pyogenic bacterial infection are more common.[25] These mental exercises are intellectually challenging, but in reality the clinician is faced with a patient in whom even the 'short list' of likely causes all demand quite different treatment. Clearly, it is most important to make the diagnosis as quickly as possible.

The initial assessment should include a detailed clinical and epidemiologic history and a careful neurologic examination. Key areas are:

- exposure to family members or others as a potential source;
- relevant foreign travel (not forgetting malaria);
- previous episodes of neurologic manifestations associated with relapse of underlying disease; and
- speed of progression of the disease.

Immediate investigations include a blood film and full blood count, blood and urine cultures and a chest radiograph. If there are new skin lesions they should be biopsied for immediate smear and culture. If a CT scan is available it is invaluable, and a lumbar puncture should be performed provided there are no contraindications.

Meningitis

Meningitis can be caused by common bacteria (*Streptococcus pneumoniae*, *Neisseria meningitidis*), but in immunosuppressed patients *Listeria monocytogenes* is particularly important. Despite the name, the cerebrospinal fluid (CSF) usually contains neutrophils, not mononuclear cells.

Polymicrobial meningitis (particularly with enteric Gram-negative bacteria) can be a clue to the presence of hyperinfection with strongyloidiasis, a complication of both corticosteroid therapy and also of infection with human T-cell leukemia/lymphoma virus. Enteroviruses are common causes of meningitis in normal hosts; rather curiously, they also occur in patients who have hypogammaglobulinemia.[26] Tuberculosis, cryptococcosis (and much more rarely, *Nocardia*) all manifest with a subacute picture and are recognized causes of meningitis in patients who have IMD.

Meningitis is rarely caused by relapse of the underlying disease, but it can occasionally be caused by drugs used in its treatment, notably nonsteroidal anti-inflammatory drugs (NSAIDs).[27]

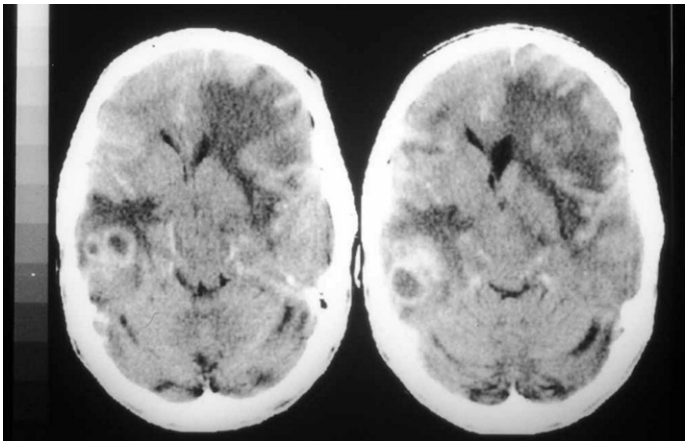


Fig. 81.1 Typical multifocal lesions in the CT scan of a patient with toxoplasmosis.



Fig. 81.2 A large necrotizing lesion caused by herpes simplex type 1 in a patient who has a teratoma. Herpetic stomatitis is common in immunosuppressed patients and is often atypical; any ulcerating lesion in the perioral region should be considered to be herpetic until proved otherwise.

Abscesses

In IMD patients, abscesses are usually nonbacterial. The commonest causes of focal neurologic lesions are fungi (especially *Aspergillus* spp.) and *Toxoplasma* (Fig. 81.1). *Nocardia* infections classically cause multiple focal abnormalities but occur less often. Tuberculoma and cryptococcoma are more common in textbooks than in patients. The diagnosis of single or multiple space-occupying lesions in IMD patients is particularly difficult. Neither the radiologic features nor the CSF findings are pathognomonic; it is rare for the causative organism to be identified from the CSF and, with the exception of the cryptococcal latex agglutination test, serologic tests are unhelpful. Particular care is needed in making a presumptive diagnosis of toxoplasmosis. Whereas in other groups of immunosuppressed patients the appearance of multiple enhancing lesions on the CT scan will often be sufficient grounds to commence empiric therapy, in patients who have IMD the differential diagnosis is much wider and if at all possible a tissue diagnosis should be obtained. Furthermore, *Toxoplasma* infection in SLE can mimic lupus cerebritis.[28]

Encephalitis

Encephalitis can be caused by infective and noninfective processes. Listeriosis and toxoplasmosis can manifest with an encephalitic picture, as can measles. Cerebral vasculitis (typically in SLE) is a particularly important consideration. It can cause a florid and life-threatening illness that can be extremely difficult to distinguish from an opportunist infection. Evaluation is complicated by the fact that high-dose corticosteroid therapy can itself cause neuropsychiatric manifestations. It is helpful if the patient is known to have a past history of cerebral vasculitis, but sometimes the only course of action is to treat with immunosuppression and antimicrobial agents until the picture becomes clearer. The use of monoclonal antibodies (particularly natalizumab to treat multiple sclerosis) has been associated with cases of progressive multifocal leukoencephalopathy (PML) due to JC virus[29] (see Chapter 157).

Two infections merit comment because of their rarity: herpes simplex encephalitis seems to be uncommon, despite the fact that local cutaneous reactivation often occurs, and likewise CMV encephalitis is very unusual in this population.

Gastrointestinal problems

Although immunosuppressed patients are susceptible to a wide range of bacterial, fungal, viral and protozoal infections of the gut,[30] it is largely those that are a feature of high-dose corticosteroid therapy that occur in patients who have IMD. The clinical features of common infections are often modified: herpetic stomatitis can be very severe, for instance (Fig. 81.2).

Extrapulmonary tuberculosis is more common in immunosuppressed patients and even if suspected (e.g. because a patient comes from the Indian subcontinent) can sometimes be hard to prove. The use of polymerase chain reaction to detect mycobacterial DNA in ascitic fluid is invaluable where it is available; meanwhile peritoneal biopsy is often the only diagnostic procedure of use. Not infrequently the only option is empiric therapy. Cytomegalovirus enteritis is perhaps underdiagnosed. It can affect any part of the gut, but particularly the colon.[31] Ganciclovir has been very effective.[32]

Strongyloides stercoralis can be present for many years without causing symptoms, but after corticosteroid therapy can cause subacute obstruction, pulmonary infiltrates and polymicrobial bacteremia or meningitis[33] (Fig. 81.3). Ivermectin is the drug of choice. Vasculitis (especially in SLE) can cause symptoms and signs indistinguishable from acute infection, including diarrhea, obstruction and perforation. Again, there are no diagnostic tests and management must depend on clinical evaluation and, if necessary, a therapeutic trial.

Skin, soft tissue and joints

Many organisms cause skin disease in immunosuppressed patients and the clinical manifestations are protean (Figs 81.4 and 81.5).[34] Many noninfective causes of skin rash need to be remembered, including cutaneous vasculitis and drug eruptions. Soft tissue infections are unusual except that patients who have hypogammaglobulinemia are susceptible to enterovirus polymyositis, although myositis is much more likely to be caused by the underlying disease (dermatomyositis, polymyositis or polyarteritis nodosa).

Acute arthritis is always an indication for aspiration to exclude infection: *Staphylococcus aureus* is the most common isolate. Once again, relapse of the underlying disease is an important part of the differential diagnosis.

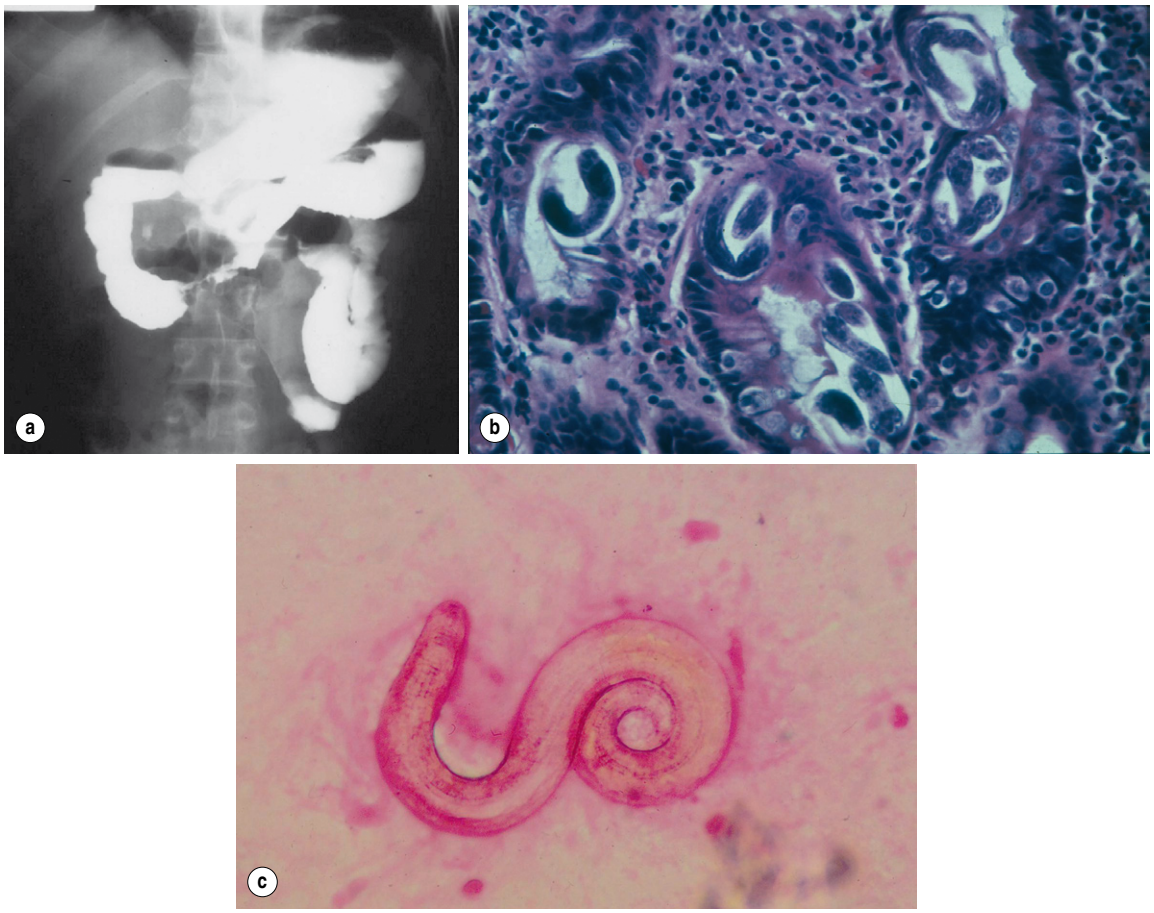


Fig. 81.3 *Strongyloides stercoralis.* (a) Barium meal examination of a patient with impaired cell-mediated immunity who developed a small bowel obstruction due to locally invasive *Strongyloides* infection. (b) Small bowel biopsy showing invasion by *Strongyloides*. (c) In some patients, the worms disseminate widely, leading to so-called hyperinfection syndrome. In this patient, who had an HTLV-1 lymphoma, worms were seen in sputum preparations.



Fig. 81.4 Extensive dermatophyte infection in a bone marrow transplant recipient. Many other infections (and graft-versus-host disease) can give a similar appearance but the diagnosis is quickly established by biopsy and microscopy. This condition is limited to the skin but nevertheless requires systemic antifungal therapy.

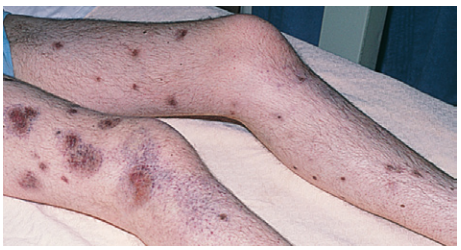


Fig. 81.5 Extensive skin lesions caused by *Mycobacterium chelonae* in a patient who had polyarteritis nodosa. The lesions were palpable but not especially painful.

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References for this chapter can be found online at http://www.expertconsult.com

Chapter 82

Steven M Opal

Splenectomy and splenic dysfunction

INTRODUCTION

The spleen in postnatal life functions primarily as a specialized lymphatic organ. It clears particulate elements from the circulation and promotes a coordinated immune response to systemic antigens. The rapidly progressive and highly lethal syndrome of overwhelming postsplenectomy infection (OPSI) attests the critical importance of the spleen to host defense against disseminated infections in the systemic circulation.

EPIDEMIOLOGY

The incidence of fatal postsplenectomy sepsis has been estimated at approximately 1 per 300 patient-years in children and 1 per 800 patient-years in adults.[1] Serious infectious complications may also occur in patients who have splenic hypofunction found in a broad array of systemic disorders (Table 82.1).[2–7]

Functional asplenia is suggested by the presence of Howell–Jolly bodies (nuclear remnants within red blood cells) and target cells in the peripheral blood smear, and decreased uptake of radioactivity by spleen scan. Splenic hypofunction is more frequent than is commonly appreciated. A recent laboratory-based survey[8] analyzed over 100 000 blood smears per year and revealed that 0.5% of patient samples had Howell–Jolly bodies indicative of hyposplenism. This information was unknown to the majority of these patients and their physicians. The most common cause of functional asplenia is sickle-cell disease which leads to repeated infarction of splenic tissue over the first few years of life. Infants born with congenital asplenia are at particularly high risk of death from systemic infection within the first year of life.[7,9]

PATHOGENESIS AND PATHOLOGY

Structure–function relationships in the spleen

The spleen is organized to provide an optimal environment for particulate antigen clearance and immunologic surveillance within the systemic circulation. Although the spleen is a small structure that accounts for only 0.25% of body weight, it receives 5% of cardiac output and contains up to 25% of the total lymphocyte population within the body.[10] Blood enters the spleen through central arteries, which branch into penicillary arterioles (Fig. 82.1). These vessels are cuffed with T lymphocytes, forming a periarterial lymphocytic sheath. The white pulp of the spleen surrounds arterioles and consists of large populations of T cells with lesser numbers of B cells and natural killer (NK) cells. The marginal zone surrounds the white pulp and principally

Table 82.1 Conditions associated with functional asplenia

Atrophic spleen	Normal-sized or enlarged spleen
Ulcerative colitis	Hemoglobinopathies*
Celiac disease	Sarcoidosis
Graft-versus-host disease following bone marrow transplantation	Amyloidosis
Splenic irradiation	Systemic lupus erythematosus, rheumatoid arthritis
Thyrotoxicosis	Epstein–Barr virus infection
Dermatitis herpetiformis	Vasculitis with antineutrophil cytoplasmic antibodies
Idiopathic thrombocythemia	Liver disease – portal hypertension
Sickle-cell disease	AIDS, immunosuppressive agents

*Other than sickle-cell disease.

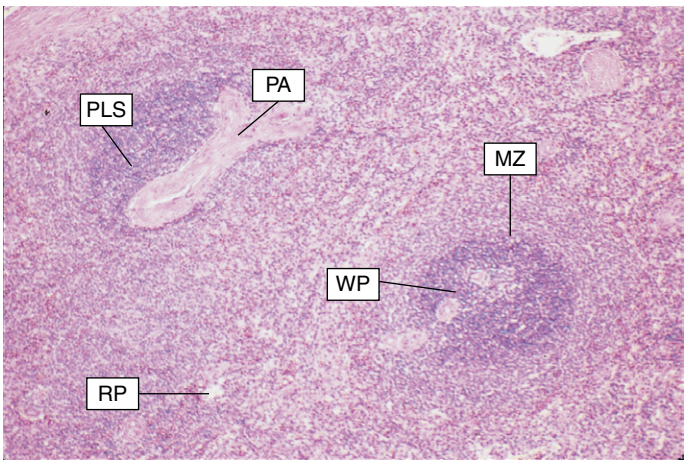


Fig. 82.1 Normal splenic architecture in the adult human. PLS, periarterial lymphatic sheath; PA, penicillary arteriole; MZ, marginal zone (B lymphocytes predominate); WP, white pulp (T cells predominate); RP, red pulp (vascular cords and venous sinuses). Hematoxylin and eosin stain.

consists of large concentrations of B cells with lesser numbers of T cells and antigen-presenting cells. Memory cells of the B-cell lineage are found primarily within the marginal zones of the spleen. The white pulp and marginal zone bring antigen-presenting cells, particulate antigens, T cells and B cells in close proximity. This microenvironment promotes a coordinated immune response to systemic antigens.[1,2,9]

The majority of the spleen consists of red pulp and venous sinuses. Before formed elements within the blood can reach the venous sinuses of the spleen, they must negotiate the red pulp with its tightly compact network of endothelial cells and macrophages (the cords of Billroth). This slow filtration process allows for careful immunologic surveillance and removal of damaged cellular elements and foreign particulate matter. The normal architecture of the spleen is depicted in Figure 82.1.

Immunologic defects and factors predisposing for postsplenectomy sepsis

A number of immunologic defects have been described in the postsplenectomy state (Table 82.2).[1–14] Hosea *et al.*[15] have demonstrated that the principal immunologic defect associated with the postsplenectomy state is an impairment in clearance of poorly opsonized particulate antigens. Invasive, encapsulated, bacterial pathogens possess an outer surface, polysaccharide capsule that impedes opsonization by immunoglobulin or complement upon entry to the systemic circulation. The spleen is much more efficient than the liver in removing poorly opsonized bacterial pathogens. Following splenectomy, decreased clearance of these encapsulated organisms results in disseminated intravascular infection and the OPSI syndrome.

Predisposing factors to postsplenectomy infection

Multiple host factors determine the cumulative risk of OPSI. A number of the most important host determinants of infection following surgical or functional asplenia are listed in Table 82.3. The principal determinant of risk of postsplenectomy sepsis is the age and immunologic experience of the patient before splenectomy.[7,15,16] In a recent review[17] of 19 680 patients followed for an average of 6.9 years after splenectomy, the overall risk of serious infections was found to be 3.2% with a mortality risk of 1.4%. The highest fatality risk was in children with thalassemia major (5.1%) and sickle-cell disease (4.8%). A similar 12-year survey in Scotland calculated the overall incidence of severe postsplenectomy infection to be 7.0 per 100 person-years with an incidence of OPSI at 0.89 per 100 person-years.[18] The report emphasized a previously underappreciated observation that elderly patients are at significantly increased risk of severe infection when compared to young adults following splenectomy.

Table 82.2 Immunologic defects found after splenectomy

Defect	Comment
Decreased clearance of particulate antigens	Hepatic Kupffer cells will partially correct this defect
Diminished clearance of poorly opsonized bacterial antigens	The spleen is the most efficient organ for this purpose
Diminished primary humoral immune response to neoantigens	IgM levels and T-cell-independent antibody responses decrease
Diminished antibody response to polysaccharide antigens	Increased risk from encapsulated bacterial organisms
Decreased tuftsin levels and fibronectin levels	Diminished levels of this tetrapeptide and serum protein reduces nonspecific attachment and phagocytosis
Quantitative and qualitative defects in the alternative complement pathway	Functional defects in the alternative complement pathway interferes with opsonization
Loss of vagally mediated cholinergic anti-inflammatory reflex	Neural control of inflammatory cytokine synthesis in the viscera is primarily mediated by the spleen

Table 82.3 Risk factors for postsplenectomy sepsis

Risk factors	Comment
Patient age: the very young	Lack of immunologic experience (vaccines and naturally acquired infections) prior to splenectomy increases the risk
Patient age: the very old	Poor response to vaccines, immunosenescence, underlying diseases increase the risk in elderly
Absence of spleen at birth	Congenital asplenia results in serious bacterial infections in over 50% of patients in the first year of life if not managed with penicillin prophylaxis
Time interval following splenectomy	Greatest risk of infection is within the first 2–3 years following splenectomy
Traumatic versus other indications for splenectomy	Splenosis (splenic implants within the peritoneum) following trauma offers some protection against infection
Immunocompromised states	Patients with hematologic malignancies and continuing need for immunosuppressive medications increase the risk of postsplenectomy sepsis
Lack of presplenectomy vaccine	Immune responses to polysaccharide antigens better if administered before splenectomy

The spleen is particularly important in the primary immunologic response to polysaccharide antigens. These IgM and IgG_2 antibody responses are largely T-cell-independent B-cell responses that require the spleen for an optimal immune response.[19] This occurs in the marginal zone of the spleen where B cells are abundant for immunologic surveillance in the systemic circulation. This immune response is attenuated after splenectomy. T-cell-dependent B-cell responses to protein antigens and cellular immune responses are reasonably well preserved after splenectomy.[19,20] The end result is that carbohydrate neoantigens are not recognized and processed efficiently in the postsplenectomy state. This results in delayed clearance of virulent microbial pathogens and their toxins[14,15] as they first enter the systemic circulation.

If the growth rate of the pathogen exceeds the clearance rate of the host, overwhelming intravascular infection occurs with potentially lethal consequences. In experimental studies, macrophage synthesis of tumor necrosis factor (TNF) is upregulated after splenectomy. Enhanced TNF synthesis may increase the risk of systemic activation of the proinflammatory cytokines, and this may contribute to postsplenectomy sepsis.[21] Splenic macrophages are hard-wired via the vagal nerve where alpha-7 nicotinic receptors of the parasympathetic nervous system attenuate TNF and high mobility group box 1 (HMGB-1) synthesis. These early-acting (TNF) and late-acting (HMGB-1) cytokines are implicated in the pathogenesis of septic shock.[22,23] Splenectomy removes this cholinergic anti-inflammatory pathway and it is tempting to speculate that the loss of this inhibitory pathway could contribute to the exaggerated inflammatory response seen in OPSI.

PREVENTION OF BABESIOSIS AND MALARIA

Individuals living in areas where *Babesia* spp. is endemic should be advised to avoid areas where ticks are common or, if unavoidable, to check daily for the presence of attached ticks. If the tick is removed within 24–48 hours it appears to protect against transmission of *Babesia* spp. Babesiosis can cause recalcitrant infections in splenectomized adults as well as children and in some cases has required exchange transfusion and prolonged treatment with antiprotozoan therapy (azithromycin–atovaquone or clindamycin and quinine). This infection needs to be prevented in splenectomized people. Chills, fever, anemia, leukopenia and thrombocytopenia can all accompany babesiosis. The diagnosis is usually evident if suspected and sought for on the blood smear. Babesiosis can be confused with *Plasmodium falciparum* malaria. Splenic function is important in clearing malaria parasites. Asplenic patients living or traveling to malaria-endemic areas should be meticulous in following malaria avoidance efforts (mosquito repellent, bed nets, screened windows, antimalarial chemoprophylaxis, etc.).

PREVENTION OF POSTSPLENECTOMY INFECTIONS

Splenic salvage method

The well-recognized risk of OPSI indicates a need to prevent these infections if at all possible. The most direct approach is to minimize the frequency with which splenectomy is performed.[24,25] Recent evidence suggests that the frequency with which splenectomy is undertaken has leveled off or decreased in North America,[8] perhaps with the recognition of the infectious risks associated with splenectomy. Elective splenectomy for congenital hemolytic disorders should be delayed until after the first 5 years of life if possible (Table 82.4). Surgical repair of splenic hematomas, conservative management of splenic trauma without splenectomy, percutaneous drainage of splenic abscesses and a decreased use of splenic irradiation have led to fewer patients at risk for postsplenectomy infectious syndromes.[26,27]

Partial splenectomy and surgical repair of splenic injury have been utilized along with autotransplantation of splenic tissue in an attempt to limit the risk of OPSI.[28,29] Enthusiasm for these splenic salvage maneuvers is tempered by the finding that these methods do not uniformly protect against sepsis in animal models[30] of splenectomy and case reports exist of overwhelming infections despite these maneuvers.[28–30] Preservation of residual splenic tissue, if possible, may still be preferable to total splenectomy in selected patients at high risk for infection.[31] The overall clinical applicability and practical value of splenic salvage techniques remain to be demonstrated in a large patient series.

Table 82.4 Preventive measures in postsplenectomy patients

Method	Comment
Salvage splenic tissue (splenorrhaphy, autotransplants)	Reasonable but unproven benefit
Delay elective splenectomy past early childhood	Greatest risk of OPSI is in early childhood; provide immunizations prior to splenectomy
Immunizations	Pneumococcal, meningococcal and *Haemophilus influenzae* vaccines provide partial protection
Antimicrobial prophylaxis	Indicated for asplenic young children (0–5 years); consider in high-risk adults for 1–3 years postsplenectomy
Early empiric therapy	Rational approach to rapidly progressive syndrome
Medical alert bracelet	Reminder for patient and health-care workers

Immunizations to prevent postsplenectomy sepsis

Immunization with the 23-valent pneumococcal polysaccharide (PPS) vaccine is safe and offers significant protection in children with sickle-cell disease.[32] The efficacy of the pneumococcal vaccine is reduced following splenectomy, yet protective antibody levels are achievable in the majority of patients following splenectomy for a variety of indications.[33–35] To optimize antibody response against T-cell-independent immunogens, it is recommended that the pneumococcal vaccine be administered at least 2 weeks before an elective splenectomy.[33] Postimmunization antibody levels should be measured to assess vaccine response. Nonresponders who do not develop antipneumococcal antibodies with repeated immunizations with PPS should consider other preventive measures against pneumococci (e.g. wear a medical alert bracelet, trial of the pneumococcal conjugate vaccine, carry a preemptive dose of a β-lactam antibiotic to presumptively treat if symptoms develop, seek early medical care for symptoms, etc.).[33,34] Current recommendations of the Task Force on Adult Immunization include repeat pneumococcal immunization every 5–6 years from the initial immunization.[35]

The role of polysaccharide–protein conjugate pneumococcal vaccine (PCV) in asplenic patients has yet to be fully evaluated in large clinical trials but preliminary results appear promising.[19,36] The conjugate protein linker allows for the development of T-cell-dependent B cell responses, with T-helper function amplifying the antibody response. Recent experimental studies[19] have verified that the conjugate vaccine produces peak antibody titers comparable to nonsplenectomized animals, albeit at a slower rate of increase. The secondary antibody response to subsequent doses of the vaccine is well preserved with the PCV formulation. Attempts to mix unconjugated pneumococcal antigens with the protein conjugated antigens were unsuccessful in boosting antibody titers to nonconjugated pneumococcal antigens. The current PCV has only seven serotypes while the standard PPS vaccine contains 23 serotypes. Further studies are warranted to determine if pneumococcal conjugate vaccines will be more efficacious than PPS vaccine in asplenic patients.[36] Current recommendations include the use of both PCV and PPS immunizations (separated by 2–3 months) in children and unimmunized adults with splenic hypofunction.[34]

Haemophilus influenzae type b conjugate vaccine is indicated in all children, including those who have functional or surgical asplenia. Immune responses to the conjugate vaccine are well preserved in the absence of splenic tissue or function.[37] It is unclear whether the *H. influenzae* type b vaccine is useful in adults who have not been vaccinated before splenectomy. The risk–benefit ratio would argue that it is reasonable to immunize children and adults who undergo splenectomy if they have not been previously immunized.[34,37] The same rationale is used to recommend meningococcal vaccine in children and young adults who undergo splenectomy,[38,39] particularly in those patients living or traveling in regions where meningococcal disease is prevalent.[40] The protein conjugate polysaccharide meningococcal vaccine may be a better option in high-risk asplenic patients but clinical data are limited at present.[39]

The duration of protection and vaccine efficacy against *Haemophilus*, pneumococci and meningococci in the asplenic patient have not been fully evaluated as yet. Vaccination is limited by variable antibody responses of uncertain duration and incomplete coverage of important serogroups within *Streptococcus pneumoniae* and *Neisseria meningitidis* (i.e. serogroup B). Vaccine failures are well known in

splenectomized patients and therefore these preventive measures should not be entirely relied upon solely to fully protect asplenic patients. Annual influenza vaccines are now recommended for adults and children after splenectomy to avoid postinfluenza bacterial pneumonia.[39]

The efficacy of bacterial vaccines in the prevention of OPSI has not been adequately tested in large scale, randomized, controlled, clinical trials. The recommendations for these immunizations are largely based upon consensus opinion from expert committees and medical societies. Despite these recommendations, it is clear that the provision of adequate vaccine coverage for the asplenic host is less than perfect. A recent survey in the UK found that only 31% of splenectomized patients had received the pneumococcal vaccine.[41] A more concerted effort will be necessary to provide adequate immunizations to these susceptible patients.

Antimicrobial prophylaxis

The efficacy of long-term penicillin prophylaxis to prevent pneumococcal infections has been studied in detail in the pediatric age group. Sufficient clinical evidence now exists to support the recommendation of penicillin prophylaxis in the first 5 years of life in asplenic children.[34,42] Some authors suggest that prophylaxis be continued indefinitely in immunocompromised patients.[3,15,39,41] Amoxicillin may be preferable to penicillin as it is better tolerated and has activity against most strains of *H. influenzae*.[34] Co-trimoxazole or macrolide antimicrobials are suitable alternatives for patients allergic to β-lactams.

The value of penicillin prophylaxis in adults is more controversial. The uncertain benefits of penicillin prophylaxis in the adult must be weighed against the potential risk of acquisition of penicillin-resistant strains of *S. pneumoniae* and infections by other organisms not susceptible to penicillin.[1,34,39,41,42] This author recommends penicillin prophylaxis for 2 years following splenectomy in adult patients at high risk for OPSI (e.g. hematologic malignancies, severe liver disease, immunocompromised states). If inadequate antipneumococcal antibodies are found following immunizations, indefinite penicillin prophylaxis should be considered.[33,34]

Expectant management with early empiric therapy for symptoms suggestive of OPSI is a logical alternative for non-immunocompromised, asplenic adults. It is essential to educate and motivate patients and their families to administer an initial dose of oral amoxicillin or amoxicillin–clavulanate at the onset of symptoms compatible with systemic infection. Ideally, patients should have blood cultures taken before empiric antimicrobial therapy; however, this may not be feasible in all patients and treatment should not be delayed if symptoms compatible with OPSI exist.

A medical alert bracelet should be provided to patients following splenectomy. This bracelet serves to remind the patient as well as health-care workers that the patient is at risk for OPSI and that urgent management for this potentially devastating syndrome may be life saving. While most postsplenectomy infections occur 1–2 years after splenic excision, and severe infections related to asplenia are uncommon after 10 years, patients have developed severe infections up to six decades after splenectomy.[42] The alert bracelet should provide continued awareness of the risk of infection long after surgical removal of the spleen. A summary of preventative measures in the patient with functional or surgical asplenia is provided in Table 82.4.

CLINICAL FEATURES OF OVERWHELMING POSTSPLENECTOMY INFECTION

A large variety of bacterial, fungal and parasitic pathogens have been reported to cause serious infection in patients who have splenic hypofunction or asplenia (Table 82.5).[1,3,8,34] *Streptococcus pneumoniae* continues to account for the majority of bacterial infection associated with OPSI. *Haemophilus influenzae* and *N. meningitidis* contribute approximately 25% of bacterial infections in the postsplenectomy state. A large number of pathogens are particularly problematic for asplenic patients including babesiosis, salmonellosis and *Capnocytophaga canimorsus* infections.

Table 82.5 Microbial pathogens associated with postsplenectomy infections

Micro-organism	Comment
Streptococcus pneumoniae	Most common, highly lethal, characteristic presentation
Haemophilus influenzae	Increased risk – especially in childhood
Neisseria meningitidis	Possible increased risk – typical features of primary meningococcemia
Salmonella spp.	Increased risk – especially sickle cell disease
Other streptococci, enterococci	Possible increased risk of infection
Capnocytophaga canimorsus	Gram-negative rod may cause infection following dog bites
Babesia microti	Tick-associated or blood transfusion-associated protozoan parasite; may cause severe infection in asplenic patients
Plasmodium spp.	Sporadic reports of activation of latent malaria and fulminant course in non-*falciparum* malaria species
Anaerobes, Gram-negative enteric organisms, *Pseudomonas*, *Burkholderia*, *Plesiomonas*, *Campylobacter* spp., fungal, parasitic and viral pathogens	Case reports; true association is unclear

The syndrome of overwhelming pneumococcal sepsis after splenectomy is one of the most dramatic and rapidly fatal infections in clinical medicine. Symptoms often begin with a vague sense of general malaise, sore throat, myalgia and gastrointestinal symptoms. Fever and true shaking chills are often seen during the prodromal phase of OPSI. While some patients note lower respiratory tract symptoms or symptoms of meningitis, the primary source of origin of the bacteremic infection is not localized in the majority of patients with OPSI.

Within 24–48 hours of onset of symptoms patients rapidly deteriorate with progressive hypotension, diffuse intravascular coagulation, purpuric lesions in the extremities, acute respiratory insufficiency, metabolic acidosis and coma. The rapidly progressive nature of the illness is suggestive of primary meningococcemia. The patient may develop refractory hypotension and die within hours of the onset of symptoms.[1,13,15] Long-term sequelae in survivors include gangrene of the extremities, bilateral adrenal hemorrhage, osteomyelitis from vascular insufficiency, endocarditis, meningitis and neurosensory hearing loss. The pneumococcal polysaccharide capsular serotypes 12, 22 and 23 account for the majority of cases of OPSI from *S. pneumoniae*.[15] All three serotypes are found in the current 23-valent pneumococcal polysaccharide vaccine but not the PCV-7 conjugate vaccine.

DIAGNOSIS OF OVERWHELMING POSTSPLENECTOMY INFECTION

The diagnosis of OPSI is often readily apparent upon clinical examination. Even a remote history of previous splenectomy should raise suspicion of possible OPSI. Supporting laboratory evidence includes consumptive coagulopathy, lactic acidosis, hypoxemia and acute renal

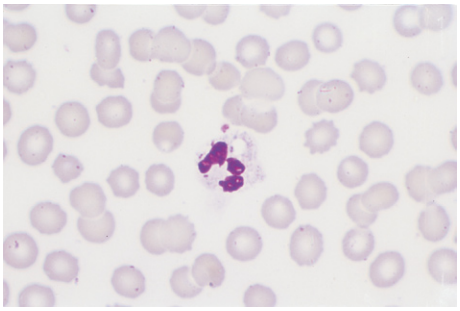


Fig. 82.2 Peripheral blood smear of a patient who has pneumococcal sepsis and meningitis. Note polymorphonuclear leukocyte with several bacterial diplococci in the cytoplasm. Wright stain.

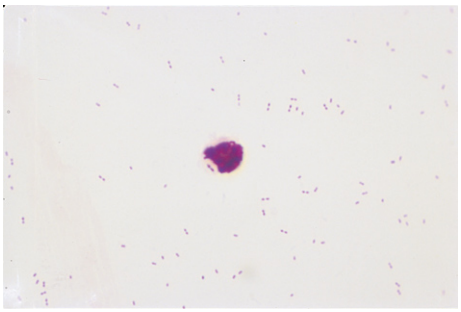


Fig. 82.3 Gram stain of cerebrospinal fluid from a patient who has pneumococcal meningitis. Note the numerous Gram-positive cocci in pairs and a single lymphocyte.

failure. Children are more likely to have concomitant bacterial pneumonia or meningitis than adults.[1,34] As a consequence of high-grade bacteremia, micro-organisms can often be identified in the peripheral blood smear (Fig. 82.2). The blood smear should be reviewed for evidence of parasitemia from *Plasmodium* or *Babesia* spp. A Gram stain of the buffy coat may readily reveal pneumococci as the level of bacteremia may exceed 1 million cfu/ml.

Patients who have concomitant bacterial meningitis may have large numbers of organisms in the cerebrospinal fluid (CSF) with minimal pleocytosis (Fig. 82.3). Blood cultures, bacterial cultures and results of antigen detection measures in the spinal fluid, sputum and urine will confirm the diagnosis.

MANAGEMENT OF OVERWHELMING POSTSPLENECTOMY INFECTION

This syndrome is a true medical emergency requiring immediate therapeutic administration of antimicrobial agents and intensive care support. High-dose intravenous penicillin has been the standard treatment for postsplenectomy pneumococcal sepsis. Vancomycin and ceftriaxone should be used empirically in areas where penicillin-resistant *S. pneumoniae* is prevalent or in patients who have received prolonged penicillin prophylaxis. Moreover, broad-spectrum bactericidal antimicrobial agents such as an extended-spectrum cephalosporin or a carbapenem should be considered in those cases where the suspected micro-organism cannot be identified on Gram strain of the buffy coat or CSF.

Passive immunotherapy with intravenous immunoglobulin has been shown to be of benefit in experimental models of postsplenectomy sepsis as has granulocyte–macrophage-colony stimulating factor (GM-CSF).[8,9] The therapeutic efficacy of passive immunotherapy, GM-CSF or other immunomodulators for human OPSI is worthy of further clinical investigation. Interleukin 18 (IL-18) protects animals from postsplenectomy pneumococcal sepsis by increasing IgM levels.[43] There is no clinical experience with IL-18 in asplenic patients as yet. Fluid resuscitation, vasopressor agents, hematologic support, ventilatory support and expert acid–base and electrolyte management are essential to survival in these critically ill patients.

The mortality rate for OPSI caused by *S. pneumoniae* has been reported to be between 50% and 70%.[1,2,11–15] With early recognition and treatment, combined with skilled supportive care, the mortality rate has decreased to as low as 10% in recent series.[42,44]

During the convalescent period, it may be necessary to surgically excise necrotic tissues that have been irreparably damaged by the prolonged hypotension and intravascular coagulation that often accompanies this syndrome. Late complications such as adrenal insufficiency, osteomyelitis and endocarditis should be sought in patients with persistent fever after an episode of OPSI. Patient education, vaccination and a medical alert bracelet should be offered to survivors to prevent recurrences.

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References for this chapter can be found online at http://www.expertconsult.com



Chapter 83

Bernard P Vaudaux

Vaccination of the immunocompromised patient

INTRODUCTION

Immunosuppression is expected to downmodulate or to suppress responses to vaccination; consequently, immunizing the immunocompromised host is a challenge. Unfortunately, experience with the immunocompromised host is often based on small and composite groups of patients with heterogeneous clinical conditions, different immunosuppressive regimens and diverse immunization backgrounds. This is in contrast to standard vaccinology data generated from large cohorts of healthy young subjects. The available information pertains mostly to immunogenicity, reactogenicity and safety of vaccinations. Data on protection are essentially missing, leaving the clinician wondering if the immunologic surrogates of protection derived from healthy vaccine recipients will apply to the immunocompromised host as well.

The main focus of this chapter is to review the experience with vaccinating patients suffering from different chronic conditions associated with disease- or drug-induced immune depression. These include kidney and liver chronic diseases, long-term inflammatory and autoimmune diseases, malignancies and solid-organ grafts. The intent is to give a glimpse of what can reasonably be expected from immunization in such patients. Revaccination of hematopoietic stem cell transplant recipients and routine vaccination of children with congenital immune deficiency syndromes or HIV infection are also addressed.

BASIC RULES FOR VACCINATING THE IMMUNOCOMPROMISED HOST

1. Live vaccines are contraindicated for fear of uncontrolled disease caused by vaccine strain. Few conditions are sufficiently investigated to allow live vaccines under stringent criteria.
2. Inactivated vaccines can be used with no restrictions at all but the likelihood for them to be of any effectiveness is dependent on the degree of immunosuppression.
3. Immune responses to vaccination should be assessed at completion of vaccination. The common method of assessment is the determination of vaccine-induced antibody levels. Tests for diphtheria, tetanus, influenza and hepatitis B antibodies are available through routine laboratories, whereas tests for pneumococcal serotypes, *Haemophilus influenzae* type b and varicella are only performed in few specialized laboratories. Routine serology tests for measles, rubella, varicella and hepatitis A are commonly available but may be not sensitive enough for detecting vaccine-induced immunity. The ideal time for checking the antibody response to vaccination is 6–8 weeks after the last dose of vaccine.
4. Depending on the underlying disease, the therapeutic regimen and the vaccine's properties, re-establishment of vaccine-induced immunity to preimmunosuppression level may not be achieved by a single recall dose of vaccine and not immediately after cessation of therapy.
5. Hematopoietic stem cell transplant recipients require that the vaccination process be started all over again for all vaccines after engraftment.

IMPACT OF IMMUNOSUPPRESSIVE DRUGS ON THE RESPONSE TO VACCINATION

Corticosteroid therapy

On condition that no other immunosuppressive drug is given concomitantly, the immune response to inactivated vaccines is insignificantly reduced in patients on systemic corticosteroid therapy, irrespective of the daily dose. Adequate antibody responses are reported after recall immunization (tetanus) as well as primary vaccinations (*H. influenzae* type b, influenza, pneumococcus) in both children and adults.

Immune response to live vaccines is less a concern than safety as the control of live antigens may be depressed to the extent that the transient vaccine infection turns into a persistent infection. Live antigens are contraindicated if the daily dose of steroid is equivalent to ≥20 mg of prednisone (or ≥2 mg/kg in children weighing <10 kg) and therapy is applied (or planned) for 2 weeks or longer. Under such circumstances, live vaccine administration should be delayed until 1 month after cessation of treatment. Patients receiving ≥20 mg (or ≥2 mg/kg in children weighing <10 kg) of prednisone for <2 weeks should not be immunized with a live vaccine while on treatment but may be vaccinated at cessation of therapy or preferably 2 weeks later.[1]

Live vaccines are allowed regardless of treatment duration when low-dose steroids (<20 mg of prednisone, or <2 mg/kg in children weighing <10 kg) are applied. Topical treatments (including inhalation and intra-articular injection) and physiologic replacement are not contraindications to live vaccines.[1]

Other immunosuppressive drugs

Most investigations report that the reduction of the immune response is related to the number of immunosuppressive drugs at the time of immunization. Available data pertain mostly to adults with autoimmune diseases or solid-organ transplants receiving a primary protein antigen (inactivated influenza vaccine), a polysaccharide antigen (pneumococcal polysaccharide vaccine) or a recall protein antigen (tetanus vaccine). Table 83.1 lists different immunosuppressive drugs (or drug combinations) and indicates the degree of impairment of

Table 83.1 Comparative impact of immunosuppressive drugs and drug combinations on response to vaccination in patients with organ transplants or autoimmune diseases

	'PRIMARY' PROTEIN ANTIGEN		'RECALL' PROTEIN ANTIGEN		POLYSACCHARIDE ANTIGEN	
Immunosuppressive drugs	**Influenza**		**Tetanus**		**Pneumococcus (23-V)**	
Inhibition of T-cell signaling + prednisone	**Impact**	**Context**	**Impact**	**Context**	**Impact**	**Context**
Sirolimus + prednisone	Significant	Transplantation			Minimal	Transplantation
Ciclosporin A + prednisone	Significant	Transplantation	Minimal	Transplantation	Minimal	Transplantation
Inhibition of DNA synthesis ± prednisone	**Impact**	**Context**			**Impairment**	**Context**
Methotrexate	Minimal	Autoimmunity			Significant	Autoimmunity
Methotrexate + prednisone	Minimal	Autoimmunity			Significant	Autoimmunity
Azathioprine + prednisone	Minimal	Transplantation			Minimal	Transplantation
Mycophenolate + prednisone	Significant	Transplantation				
Inhibition of TNF activity ± prednisone	**Impact**	**Context**			**Impact**	**Context**
Adalimumab	Minimal	Autoimmunity				
Infliximab	Minimal	Autoimmunity				
Etanercept	Minimal	Autoimmunity				
Adalimumab + prednisone	Modest	Autoimmunity			Minimal	Autoimmunity
Infliximab + prednisone	Modest	Autoimmunity			Minimal	Autoimmunity
Etanercept + prednisone	Modest	Autoimmunity			Minimal	Autoimmunity
Inhibition of T-cell signaling and of DNA synthesis	**Impact**	**Context**	**Impact**	**Context**	**Impact**	**Context**
Ciclosporin A + prednisone + azathioprine	Significant	Transplantation			Significant	Transplantation
Ciclosporin A + prednisone + mycophenolate	Significant	Transplantation	Significant	Transplantation	Significant	Transplantation
Tacrolimus + prednisone + mycophenolate					Significant	Transplantation
Inhibition of DNA synthesis and of TNF activity	**Impact**	**Context**			**Impact**	**Context**
Methotrexate + infliximab	Significant	Autoimmunity			Significant	Autoimmunity
Methotrexate + prednisone + infliximab	Significant	Autoimmunity			Significant	Autoimmunity
Methotrexate + etanercept					Significant	Autoimmunity
Methotrexate + prednisone + etanercept	Significant	Autoimmunity			Significant	Autoimmunity
Methotrexate + prednisone + adalimumab	Significant	Autoimmunity			Significant	Autoimmunity
Inhibition of DNA synthesis and depletion of B-cells	**Impact**	**Context**				
Methotrexate + rituximab	Significant	Autoimmunity				

the antibody response to be expected from therapy. Not all double (respectively triple) regimens have an identical impact and some individual drugs exert different immunosuppressive effects depending on the combination.

ACUTE LYMPHOBLASTIC LEUKEMIA

Decline of previously induced vaccine immunity

The main determinant of antibody loss for diphtheria, tetanus and *H. influenzae* type b antibodies is thought to be dependent upon treatment intensity,[2–5] explaining why some investigators report no substantial negative effect,[2,6,7] while others report marginally protective or nonprotective antibody titers.[3,8] Another determinant is likely to be antibody levels at onset of chemotherapy. These are directly influenced by the patient's age and the number of vaccine doses previously received.

An accelerated decline of measles and rubella antibodies is observed in children immunized with a single dose of vaccine prior to the onset of treatment.[9,10] The true clinical significance of the loss of measles antibody is not clear. Indeed, immunoassays used for antibody measurements can be negative in the presence of positive neutralization tests that clearly correlate with protection.

Vaccination during therapy

Diphtheria and tetanus recall vaccination

A single recall dose of either toxoid induces a protective antibody level in all patients.

Haemophilus influenzae *type b vaccination*

Primary vaccination with the conjugate vaccine is associated with a low rate of response ranging from 50% to 60%. The likelihood of an adequate response is inversely related to the duration of therapy and its intensity.

Hepatitis B vaccination

The capacity to respond to hepatitis B vaccination is dependent on the intensity of therapy. Minimal requirements for these patients are double amounts of antigen for age and schedules comprising four to five doses. When these are met, only 35% of patients on intense therapy achieve a protective antibody level compared to 90% of patients with less intense regimens. The vaccination is protective in responders and, interestingly enough, in nonresponders to some extent as well, suggesting an undetected positive effect of cell-mediated immunity. The vaccination is totally ineffective in children receiving induction or consolidation therapy.

Influenza vaccination

The inactivated vaccine is no less immunogenic than in healthy controls but modestly so due to the vaccinees' young age.

Varicella vaccination

The live varicella vaccine was developed specifically for patients with acute lymphoblastic leukemia in remission. Minimal conditions for this vaccine to be used in leukemic children include all of the following:

- disease in remission;
- chemotherapy stopped for 1 week before and 1 week after vaccination;
- corticosteroid treatment stopped for 1 week before and 2 weeks after vaccination.

Overall, 85% of patients develop an immune response after a single dose and the proportion is higher among patients off chemotherapy at the time of vaccination. An additional 10% of children respond to a second dose of vaccine. An interval of 3 months is recommended between doses. The antibody response is accompanied by specific cell-mediated immunity. A sizeable proportion of patients (75%) retain detectable antibody levels for several years. Eighty-five percent of vaccine recipients escape chickenpox despite household exposure and if varicella does occur, symptoms are often mild or moderate. A mild vesicular rash can break out 1–6 weeks after the first vaccine dose. No relapses of leukemia are associated with the vaccination and the incidence of zoster is lower in vaccine recipients than in children experiencing natural infection.

Vaccination after cessation of therapy

A substantial immunologic rebound is usually observed shortly after cessation of therapy. Rarely, the immune depression persists for months affecting antibody or cell-mediated responses.

Diphtheria and tetanus recall vaccination

A single recall dose of either toxoid administered 3–6 months after cessation of therapy elicits protective antibody levels in most children. The immune response is excellent in patients who were at standard or intermediate risk but definitely weaker in high-risk patients. The capacity to respond is clearly correlated with the number of memory B cells left in the bone marrow at the end of treatment. Children who are able to develop a quantitatively adequate response produce fully functional antibodies. The timing of vaccination after cessation of therapy is not a critical factor as no differences are seen whether patients are vaccinated 1 or 6 months after treatment. The strategy for recall diphtheria and tetanus vaccination should be a single dose in standard and intermediate risk patients and a two-dose schedule and antibody measurement in high-risk children.

Haemophilus influenzae *type b recall vaccination*

The same observations and recommendations exist for *H. influenzae* b vaccination as described with diphtheria–tetanus vaccines.

Measles recall vaccination

A single recall dose of live vaccine given at least 3 months after the end of therapy induces a response rate as high as in healthy controls.

Influenza vaccination

Depending on the viral strain considered, between 45% and 88% of patients achieve protective antibody levels after a single dose of inactivated influenza vaccine. Contrary to observations with recall vaccinations, the timing of vaccination appears to be critical and responses rates are far higher and more homogeneous when vaccination is administered at least 6 months after treatment.

MULTIPLE MYELOMA

Vaccination during therapy

Haemophilus influenzae *type b vaccination*

Geometric mean concentrations of antibodies are comparable in multiple myeloma patients and healthy adults following a single dose of conjugate vaccine.

Influenza vaccination

Antibody concentrations are lower in multiple myeloma patients than in healthy controls. Immunization is totally ineffective if given within 7 days from chemotherapy. A delay of 2 weeks between the vaccine injection and the start of the next course of therapy is desirable.

Pneumococcal vaccination

Geometric mean concentrations of pneumococcal antibodies are lower in multiple myeloma patients than in healthy adults and the polysaccharide vaccine is essentially ineffective. A single dose of vaccine induces a serologic response in 50% of patients and despite

vaccination serotype-specific antibody concentrations remain lower than in the unvaccinated age-matched healthy population.

SOLID TUMORS IN CHILDREN

Decline of previously induced vaccine immunity

An accelerated loss of antibody to tetanus, poliomyelitis, hepatitis B, measles, mumps and rubella is observed during therapy of various solid pediatric cancers.[11–13]

Vaccination during therapy

Diphtheria and tetanus vaccination

Primary vaccination of infants on therapy for a neuroblastoma induces protective immunity comparable to historic controls. No special immunization schedule is needed for these children.

Haemophilus influenzae *type b vaccination*

Primary immunization of preschool children with a single dose of conjugate vaccine is significantly less effective than in historic controls. An additional dose of vaccine is indicated in low responders.

Hepatitis B vaccination

Regular schedules are inadequate for primary immunization. The four-dose schedule (0, 1, 2 and 12 months) with 40 µg per dose (regardless of age) induces a protective level in over 70% of patients. The antibody response should be assessed 4 weeks after the third dose in order to detect low responders who will require an extra 40 µg dose (administered at 4 months).

Influenza vaccination

A single dose of inactivated vaccine is clearly insufficient. Two doses are needed and induce significantly lower responses than in healthy children.

Varicella vaccination

The vaccine is as immunogenic, well tolerated and safe in these children as in their healthy siblings on condition that the following criteria be fulfilled (blood lymphocyte count greater than 0.5×10^9/l; no chemotherapy for 2 weeks before and 2 weeks after vaccine administration).

Vaccination after cessation of therapy

Diphtheria, tetanus, poliomyelitis, hepatitis B, measles and rubella recall vaccination

A single dose of vaccine is sufficient to reactivate protective responses to diphtheria, tetanus, poliomyelitis and rubella in vaccine recipients who have lost vaccine-induced immunity during therapy. The impact of a single dose of hepatitis B and measles vaccine is less predictable so a follow up antibody measurement is indicated.

Hepatitis B vaccination

The primary immunization is effective with three or four doses (0, 1 and 6 months/0, 1, 2 and 6 months) on condition that 20 µg of antigen per dose be used regardless of age.

Influenza vaccination

Two doses of inactivated influenza vaccine induce similar responses in patients and age-matched controls.

Varicella vaccination

On condition that minimal conditions be met (at least 3 months after cessation of therapy, lymphocyte count greater than 0.7×10^9/l and normal age-adjusted serum IgG concentration), two doses of live vaccine administered 3 months apart are effective, well tolerated and safe in susceptible children recovering from therapy for a solid tumor.

SOLID TUMORS IN ADULTS

Lymphomas

Vaccination during therapy

Influenza vaccination

Regular immunization with the inactivated vaccine is unreliable in these patients. A single dose induces only a modest response and two doses are suggested to assure significant protective vaccine-induced immunity. It is prudent to maintain a 2-week delay between the vaccine injection and the start of the next course of chemotherapy.

Pneumococcal vaccination

Responses to a single dose of polysaccharide vaccine are clearly reduced and a second dose 1 month later does not increase the rate of response. A minimal interval of 2 weeks, and preferably 4 weeks, should be kept between the vaccine administration and the start of the next chemotherapy course.

Vaccination after cessation of therapy

Haemophilus influenzae *type b vaccination*

Patients recovering from a lymphoma are less likely to have a protective immunity than age-matched healthy controls, indicating a need for them to be immunized after therapy. Overall, a single dose of conjugate vaccine administered 2 years after treatment induces a protective antibody level in 90% of patients. A second dose to nonresponders is ineffective and likely related to the destruction of specific memory T cells during therapy.

Pneumococcal vaccination

The antibody response to a single dose of pneumococcal polysaccharide vaccine administered 2 years after therapy is similar in patients and in healthy age-matched controls. In contrast, the response to the 7-valent conjugate vaccine, in addition to being restricted to seven serotypes, is significantly inferior to that following the plain polysaccharide vaccine, possibly because of the lower antigen content in the former. However, the conjugate vaccine is able to exert a priming effect when administered prior to the polysaccharide vaccine.

OTHER SOLID MALIGNANCIES

Vaccination during therapy

Influenza vaccination

A single dose of inactivated vaccine is less efficient than in healthy controls. Rates of seroconversion are particularly low when vaccination is administered during a course of therapy. A delay of 2 weeks between the vaccine injection and the start of the next course of therapy is sufficient to ensure vaccine responsiveness. Rates of response are inversely correlated to the intensity of the immunosuppressive regimen. Yearly administration of a single dose of vaccine to elderly patients with intestinal cancer is associated with a significant reduction in the incidence of acute influenza pneumonia, a slight decrease in mortality and fewer interruptions of chemotherapy due to febrile episodes.

Pneumococcal vaccination

The effectiveness of a single dose of polysaccharide vaccine is clearly decreased in cancer patients compared to healthy controls

but modestly so in comparison to individuals with various medical conditions not associated with immunosuppression. A minimal interval of 2 weeks, and preferably 4 weeks, should be kept between vaccine administration and chemotherapy courses in order to maintain vaccine efficacy.

SOLID ORGAN TRANSPLANTS IN CHILDREN

Decline of previously induced vaccine immunity

At comparable ages and time points in relation to the latest vaccine dose, the proportion of renal transplant recipients with protective levels of diphtheria, tetanus and poliomyelitis antibodies is lower than in healthy controls.[14–16] In contrast, the immunosuppressive therapy associated with liver transplantation has little impact on immunity to diphtheria and none on tetanus or poliomyelitis.[15]

Vaccination during therapy

Diphtheria and tetanus recall vaccination

Rates of protective response to a single recall dose of diphtheria toxoid range from 65% to 95% and rates to a single dose of tetanus vaccine range between 85% and 100%. In kidney transplant patients, the response to diphtheria, but not tetanus toxoid, is positively correlated to creatinine clearance. It is recommended that antibody levels be checked after recall vaccination in transplant patients and followed up in low responders. Both vaccinations are well tolerated and safe, triggering no episodes of acute rejection and no change in organ function.

Measles and rubella recall vaccination

In children successfully immunized prior to transplantation who become seronegative during treatment, a single dose of live vaccine administered 3 years after engraftment induces seroprotective responses in 85% of patients against measles and in all against rubella. The recall vaccination is safe.

Varicella recall vaccination

A single recall dose of live vaccine given 3 years after engraftment is fully effective and safe in children who were successfully immunized prior to transplantation and turn seronegative during therapy.

Hepatitis B vaccination

The regular three-dose schedule (0, 1 and 6 months) with the regular amount of antigen for age is not as immunogenic in liver transplant recipients as in historic controls. One extra dose of vaccine at 12 months with a double amount of antigen for age is required in nonresponders and brings protection to half of them. Response rates are significantly different in patients on single (100%), double (84%) or triple immunosuppressive therapy (66%).

Influenza vaccination

The influenza vaccination is effective in renal transplant patients but less so in liver transplant recipients, possibly because the latter are much younger. Not only is the antibody response modest in liver recipients but the cell-mediated response is significantly depressed, suggesting that additional measures such as vaccination of household contacts and postexposure chemoprophylaxis are needed to protect liver recipients. The vaccination is safe with no increase in acute graft rejections.

Measles vaccination

Two doses of live vaccine administered 4 years after engraftment induce seroconversion in 65% of susceptible children on single drug therapy and in 40% of patients on a two- or three-drug regimen. Reactogenicity is comparable in transplant recipients and historic controls, and is characterized by minor adverse effects.

Pneumococcal vaccination

The sequential schedule consisting of two doses of 7-valent conjugate vaccine followed with one dose of 23-valent polysaccharide vaccine (all three doses every other month) results in a significant rise in antibody levels to all serotypes in all vaccine recipients. The need for the second dose of conjugate vaccine is questionable.

Varicella vaccination

Two doses of live vaccine administered 3 years after engraftment induce seroconversion in 73% of susceptible children on one-drug immunosuppressive therapy. The reactogenicity is comparable in transplant patients and controls with only minor local adverse effects. Seronegative children with an adequate lymphocyte count (at least 1500 cells/mm^3) can be immunized as early as 6 months after engraftment without modifying the immunosuppressive regimens. The antibody response rate is more modest (66%) in patients on double or triple immunosuppressive therapy but there is evidence of induced cell-mediated immunity. Interestingly, an additional 20% of patients seroconvert in a delayed fashion (6 months after completion of vaccination). Adverse reactions occur at increased frequency (vesicular rashes 55% and febrile episodes 25%) but are self-limited and benign.

SOLID ORGAN TRANSPLANTS IN ADULTS

Vaccination during therapy

Diphtheria, tetanus and poliomyelitis recall vaccination

A single recall dose of diphtheria–tetanus–poliomyelitis vaccine induces a protective response in 90% of patients to diphtheria and in all to tetanus and poliomyelitis. The diphtheria response is short lived compared to the other two. All vaccinations are well tolerated. Considering the possibility that a recall dose might induce a borderline response, it is imperative that the diphtheria antibody level be checked in all vaccine recipients and followed up in low responders.

Haemophilus influenzae *type b vaccination*

A single dose of conjugate vaccine is equally effective in inducing seroconversion among renal transplant recipients and controls but generates a protective level in only 35% of patients (on double or triple immunosuppressive therapy).

Hepatitis A vaccination

Two doses of inactivated vaccine administered 6 months apart are fully immunogenic in liver transplant recipients but less effective in renal transplant patients. In addition, and in contrast to immunocompetent individuals, only a minority of kidney recipients (24–40%) achieve a protective response following the first dose. The full two-dose schedule should then ideally be completed before exposure starts. The immune response is short lived in all patients. Between 60% and 75% of patients with a protective response at completion of vaccination do not maintain a protective antibody level 2 years later, suggesting that the immune memory has not been induced by vaccination.

Hepatitis B vaccination in liver transplant recipients with pre-existent hepatitis B-induced disease

These patients are presumed to receive life-long antiviral chemotherapy or immunotherapy. Hepatitis B vaccination targeting discontinuation of the antiviral therapy is a controversial issue and a complex process. First, the antiviral therapy has to be continued throughout the entire immunization process. Second, vaccines and vaccination schedules under investigation differ in many ways (number of doses

Table 83.2 Hepatitis B vaccination schedules in liver transplant adult recipients with pre-existent hepatitis B infection

Schedule	Concomitant therapy	Results at completion of vaccination (% of patients achieving a given level of anti-HBs antibody)
Conventional antigen and conventional adjuvant		
3 doses of 40 µg	Lamivudine	4–12% achieve level >10 IU/l. Several additional doses do not substantially increase rate of response
6 doses of 40 µg	Lamivudine	8% achieve level >10 IU/l
6 doses of 40 µg	Immunoglobulin	No increase above baseline value
9 doses of 40 µg	Immunoglobulin	8% achieve level >10 IU/l
Novel antigens and conventional adjuvant		
3 doses of 20 µg	Lamivudine	25% achieve level >10 IU/l
6 doses of 20 µg	Lamivudine	50% achieve level >10 IU/l
Conventional antigen and novel adjuvants		
6 doses of 20 µg	Lamivudine	12% achieve level >10 IU/l
5 doses of 40 µg	Immunoglobulin	40% achieve level >500 IU/l
>8 doses of 20 µg	Immunoglobulin	80% achieve level >500 IU/l. One dose of conventional vaccine given 2 years later induces anamnestic response

and/or amount of antigen per dose and/or novel antigen formulation and/or novel adjuvant formulation). The most pertinent features are commented below and summarized in Table 83.2.

- *Schedules based on the conventional HBs antigen*: an antibody level ≥10 IU/l is obtained in a small proportion of patients (<25%), irrespective of the number of doses and the amount of antigen, and there is no evidence that the antibody elevation is associated with a specific T-cell mediated response and correlated with long-term protection.
- *Schedules based on combinations of novel antigen and conventional adjuvant*: an antibody level ≥10 IU/l is induced in a somewhat higher but unpredictable proportion of patients and there is no evidence that the increase in antibody is a T-cell driven response.
- *Schedules based on combinations of novel adjuvant and conventional antigen*: these are likely to trigger a significantly higher antibody response on condition that they include a large number of doses (≥9) or a large amount of antigen per dose (≥40 µg). The priming effect of such schedules is suggested by the observation that a single dose of standard vaccine administered 2 years later is able to induce an anamnestic response.

The vaccination is well tolerated and safe regardless of antigen or adjuvant nature and quantity. Local reactions are occasional with the conventional vaccine and somewhat more frequent with novel adjuvants. No systemic adverse effects, no rejection episodes or alterations in liver enzyme profiles are reported following any of the above schedules.

Influenza vaccination

A single dose of inactivated vaccine has a two- to sixfold lower probability of inducing seroconversion in renal, heart and lung transplant recipients than in healthy controls. In renal patients, the magnitude of the immune response is directly related to the degree of graft function (a creatinine clearance >70 ml/min/1.73 m^2 correlates with high probability of protective response) and may be essentially unaltered in patients with a well-functioning graft. Cell-mediated immunity is commonly maintained in renal graft recipients but severely depressed in lung transplant recipients. Most investigators report no improvement in seroconversion with an additional dose of vaccine. In heart transplant recipients, a three-dose schedule at monthly intervals increases somewhat the probability of achieving seroprotection. The MF59 adjuvanted vaccine formulation is no more immunogenic in transplant patients than the conventional vaccine.

Time elapsed after engraftment is an important determinant of efficacy. In lung transplant recipients, the overall rate of seroconversion is 45% when vaccination is given within 12 months of surgery and 75% after 1 year. Another determinant of efficacy is the intensity of the immunosuppressive therapy and the drug combination. Protection is demonstrated in heart transplant recipients and is similar with the conventional or the MF59 adjuvanted formulation. A single study in heart transplant recipients mentions a temporal association between vaccination and low-level histologic rejection episodes but these are strictly asymptomatic and respond to a single course of therapy. The rejection process appears to be unrelated to the time elapsed from engraftment, to the magnitude of the antibody response and to the number of vaccine doses administered.

Pneumococcal vaccination

Responses to a single dose of polysaccharide vaccine are heterogeneous and modulated by the intensity of the immunosuppressive therapy. Differences involve the proportion of patients responding to vaccination as well as the number and magnitude of serotype-specific responses. Even when nearly as effective as in healthy controls, responses are short-lived (2–3 years) and justify antibody monitoring. The presently available 7-valent conjugate vaccine does not significantly enhance the functional activity, duration or level of the antibody response in transplant recipients.

HEMATOPOIETIC STEM CELL TRANSPLANTS

Decline of previously induced vaccine immunity

The loss of vaccine-induced antibodies is rapid and relentless during the first 2 years after engraftment in both children [17,18] and adults[19–22] and affects all vaccine-induced antibodies that have been investigated (diphtheria, tetanus, poliomyelitis, *H. influenzae* type b, measles, mumps and rubella). Naturally acquired antibodies to pneumococcal serotypes wane in the same fashion.[23,24] Antibody waning may be accelerated by graft-versus-host reactions, depending on the vaccine considered: for instance, graft-versus-host disease (GVHD) has no impact on the decline of diphtheria and tetanus antibodies, but an enhancing effect on the loss of pneumococcal antibodies and poliomyelitis antibodies.

Revaccination

Declining vaccine-induced immunity is a clear indication for revaccinating all patients. Single doses of inactivated vaccines are insufficient to re-establish immunity to preimmunosuppression levels and complete revaccination schedules are needed. It is recommended that immunization be started 12 months after engraftment with inactivated vaccines, and no sooner than 24 months with live vaccines if the patient is deemed immunocompetent and free of GVHD.[25] Influenza immunization with the inactivated vaccine may be administered as early as 6 months after transplantation. A summary of recommendations is given in Table 83.3.

Usual revaccination schedules for inactivated vaccines (diphtheria, tetanus, pertussis in children younger than 7 years, poliomyelitis and hepatitis B) include three doses of vaccine with a '0, 2 and 10 months' timing. Revaccination schedules for live vaccines such as measles, mumps, rubella and varicella include two doses 6 months apart.[25] Interestingly a single dose of measles vaccine to previously unimmunized children induces seroconversion in 70% of patients. In contrast, a single dose to previously vaccinated children induces an antibody response in only 16%. However, protection is likely to be much better in the latter group than it appears because these patients have a strong

Table 83.3 Vaccination of children and adults after stem cell transplants

Vaccine	Age	Number of doses	Post-transplant schedule (months after engraftment)
Diphtheria vaccine	Any age	3 doses	12, 14 and 24 months
Tetanus vaccine	Any age	3 doses	12, 14 and 24 months
Pertussis vaccine	Any age <7 years	3 doses	12, 14 and 24 months
Poliomyelitis inactivated vaccine	Any age	3 doses	12, 14 and 24 months
Haemophilus influenzae type b conjugate vaccine	Any age	3 doses	12, 14 and 24 months
Hepatitis B vaccine	Any age	3 doses	12, 14 and 24 months
Influenza inactivated vaccine	Any age	1 dose (yearly)	6 months (or greater depending on epidemiologic circumstances)
Measles live vaccine	Any age >1 year	2 doses	24 and 30 months
Rubella live vaccine	Any age >1 year	2 dose	24 and 30 months
Varicella live vaccine	Any age >1 year	2 doses	24 and 30 months
Pneumococcus (polysaccharide vaccine schedule)	>5 years	2 doses	12 and 24 months
Pneumococcus (sequential conjugate/ polysaccharide vaccine schedule)	<5 years	3 doses	12, 14 (conjugate vaccine) and 24 months (polysaccharide vaccine)

preferential T-helper 1 (Th1)-mediated activity which depresses antibody response but is capable of controlling wild-type viruses.

For some antigens (diphtheria, *H. influenzae* type b), priming the donor with a single dose of vaccine prior to harvest significantly accelerates the immune response in the recipient. Inactivated vaccines may be started sooner than 12 months following engraftment but, depending on the antigen and the timing of the first dose, the above mentioned three-dose schedule may be suboptimal and one additional dose may be required. Vaccination with the live measles vaccine sooner than 2 years post-transplant has been attempted only once (i.e. as early as 9 months after engraftment) in the setting of a measles outbreak in the community. A single dose was found to induce seroconversion in all seronegative patients and was well tolerated and safe.

Varicella vaccination sooner than 24 months after engraftment would be desirable in children since many cases of chickenpox and zoster occur within that period. If all the following prerequisites are met (no immunosuppressive therapy for past 3 months; lymphocyte count >1000 cells/mm^3; T-cell count >700 cells/mm^3; positive skin test with a recall antigen; total serum IgG level >5 g/l; no administration of gammaglobulin within past 6 weeks) a single dose of varicella vaccine administered 12–23 months after transplantation induces seroconversion in 77% of vaccine recipients and a second dose of vaccine augments it to 88%. The vaccine is well tolerated and appears safe.

CONGENITAL IMMUNE DEFICIENCY SYNDROMES

Principles

Sound theoretic considerations indicate that live vaccines may be insufficiently attenuated for these patients and unfortunate experiences with live products – bacille Calmette–Guérin (BCG), oral poliomyelitis, measles and smallpox vaccines – have confirmed this fear.[26,27] Consequently, patients with a T-cell function defect should not receive any live vaccines at all and patients with granulocyte or macrophage dysfunctions should not be given live-bacterial vaccines but may receive live-virus vaccines. In contrast, inactivated vaccines are never contraindicated in these patients, irrespective of the underlying immune deficiency, but their immunogenic effectiveness is likely to be attenuated or suppressed depending on the degree of B- and T-cell functional capacity.

Avoidance of live vaccines

Avoidance should be strictly abided by, particularly when substitution with inactivated vaccines is possible as is the case for influenza, poliomyelitis and typhoid vaccinations. However, recognition of the potential severity of wild-type virus infections like measles and chickenpox in these patients has led to investigating measles and varicella vaccines in individuals with partial T-cell functional defects such as DiGeorge anomaly. On condition that minimal criteria be met (>400 CD4 cells/mm^3 and 15 months of age or older), these patients can be efficiently and safely immunized with live measles and varicella vaccines.[28] Furthermore, we have learned from inadvertent vaccinations that the immunogenic efficacy, the incidence and the severity of adverse effects are not significantly different in these patients and age-matched healthy controls.[28,29]

By analogy with measles and varicella vaccination in patients with partial T-cell defects, live measles and varicella vaccines are deemed acceptable in patients with a B-cell functional deficiency whose T-cell activity is normal. Two doses of each vaccine may be administered 3 months apart, starting at the age of 15 months.[30] In the setting of an outbreak, the first dose of measles vaccine may be given from 6 months of age and the first dose of varicella vaccine from 12 months.[30] There are no data suggesting that the two vaccines remain effective and safe when administered simultaneously. The measles–mumps–rubella formulation may be used instead of the monovalent measles vaccine.

Interference of concomitant immunoglobulin therapy

Live attenuated antigens, and particularly the further attenuated strain of measles virus, are at risk of neutralization by the immunoglobulin given as replacement therapy to some of these patients. Ideally, an interval of 8 months should be kept between immunoglobulin perfusion and live vaccine administration in order to prevent interference of the former with the latter.[30] When such an extended interval cannot be safely achieved owing to the child's clinical condition, measles or varicella vaccines should still be offered, in spite of probable reduced efficacy, with a minimal interval of 2 weeks between vaccination and the next dose of immunoglobulin.[30] Table 83.4 lists vaccines that are indicated in patients with congenital immune defects.

Table 83.4 Vaccination of infants and children with congenital immune defects

① No restriction
② No restriction (immune response possibly reduced)
③ No restriction (immune response likely to be insufficient or absent)
④ Strict contraindication and no inactivated substitute vaccine available
⑤ Strict contraindication but inactivated substitute vaccine available
⑥ No strict contraindication (immune response possibly reduced)
⑦ No strict contraindication (immune response doubtful in case of concomitant immunoglobulin therapy)
⑧ No strict contraindication but inactivated substitute vaccine prefered

	Diphtheria inactivated	*H. influenzae b inactivated*	*Hepatitis A inactivated*	*Hepatitis B inactivated*	*Human papillomavirus inactivated*	*Influenza inactivated*	*Influenza live*	*Measles live*	*Meningococcal inactivated*	*Mumps live*	*Pertussis inactivated*	*Pneumococcus inactivated*	*Poliomyelitis inactivated (IPV)*	*Poliomyelitis live (OPV)*	*Rabies inactivated*	*Rotavirus live*	*Rubella live*	*Tetanus inactivated*	*Tick-borne encephalitis inactivated*	*Tuberculosis live (BCG)*	*Typhoid inactivated*	*Typhoid live*	*Varicella live*	*Yellow fever live*
Granulocyte defects*	①	①	①	①	①	①	①	①	①	①	①	①	①	①	①	①	①	①	①	④	①	⑤	①	①
Partial antibody deficiency†	②	②	②	②	②	②	⑧	⑥	②	⑥	②	②	②	⑤	②	⑥	⑥	②	②	④	②	⑤	⑥	⑥
Complete antibody deficiency‡	③	③	③	③	③	③	⑤	⑦	③	⑦	③	③	③	⑤	③	④	⑦	③	③	④	③	⑤	⑦	④
Partial T lymphocyte deficiency§	②	②	②	②	②	②	⑤	⑥	②	⑥	②	②	②	⑤	②	④	⑥	②	②	④	②	⑤	⑥	④
Complete T lymphocyte deficiency¶	③	③	③	③	③	③	⑤	④	③	④	③	③	③	⑤	③	④	④	③	③	④	③	⑤	④	④

* e.g. Chronic granulomatous disease, leukocyte adhesion defects
† e.g. Immunoglobulin subclass deficiency, hypogammaglobulinemia, dysgammaglobulinemia
‡ e.g. Agammaglobulinemia
§ e.g. DiGeorge anomaly (without complete T cell absence), Wiskott–Aldrich syndrome
¶ e.g. Severe combined immunodeficiency

Congenital HIV infection

Routine vaccination of HIV-infected infants and toddlers with inactivated vaccines is recommended as per national schedules. Their immunogenic efficacy is proportionate to the patient's immune competence.[31] Asymptomatic and mildly symptomatic patients have normal antibody responses but the capacity to respond to vaccines decreases as the HIV infection advances. Live vaccines are to be avoided because of disastrous experiences in patients with severe immunodepression.[32–35] However, as wild-type measles and varicella infections are particularly dangerous and potentially lethal in patients who have become immunocompromised, routine vaccination with corresponding live vaccines is recommended as long as they have no evidence of severe immunosuppression (age-specific CD4 cell percentage greater than 15%).[30,36] When such criteria are met, vaccination is immunogenic, well tolerated and safe.[30,36] Consequently, both measles and varicella vaccines should be offered to children who are not severely symptomatic and not severely immunosuppressed (i.e. to those fitting into clinical categories N, A or B and immunologic categories 1 or 2) and have a sufficient number of CD4 cells (>750 CD4 cells/mm^3 before 1 year of age; >500 CD4 cells/mm^3 between 1 and 5 years; >200 CD4 cells/mm^3 from 6 years onwards). Two doses of each vaccine should be administered 3 months apart, starting at the age of 15 months.[30]

All above-mentioned precautions for the use of live vaccines in patients with inherited immune deficiencies (earlier vaccination in case of community outbreak and possible negative impact of immunoglobulin therapy) also apply to patients with a congenital HIV infection. Table 83.5 summarizes vaccines allowed or contraindicated in HIV-infected children.

CHRONIC KIDNEY DISEASES IN CHILDREN

Nephrotic syndrome

Pneumococcal vaccination

A single dose of polysaccharide vaccine is immunogenic in children with a steroid-sensitive disease and confers protection against peritonitis despite concomitant administration of low-dose prednisone. However, the decline of antibody is rapid and over half of the patients have antibody levels back to prevaccination values 3 years after vaccination. Responses obtained in children on high-dose prednisone are comparable to those obtained with lower corticosteroid dosage. Vaccination early in the disease process has no deleterious effect on disease activity. The antibody response is significantly lower in patients with a steroid-resistant disease.

Varicella vaccination

A single dose of live vaccine is immunogenic in children in remission (no corticosteroids for at least 6 weeks prior to vaccination), well tolerated and safe (no change in the relapse rate). In patients on uninterrupted low-dose prednisone, two doses of vaccine 4 weeks apart induce a protective response for at least 2 years in all vaccine recipients. Tolerance and safety (rate of relapse) are excellent in spite of the continued steroid treatment.

Chronic peritoneal dialysis

Diphtheria and tetanus vaccination

It is hypothesized that the earlier routine vaccination is started in infancy the better the immune response because of better renal function at the time of vaccination. Response rates to toxoids are slightly lower in infants and toddlers on peritoneal dialysis but modifications of routine schedules are deemed unnecessary.

Haemophilus influenzae *type b vaccination*

The conjugate vaccine induces protection in 90% of patients. However, considering the potential severity of invasive infections in children under 5 years, it is recommended that the antibody titer be checked at completion of vaccination and at regular intervals thereafter. A single extra dose of vaccine is sufficient to reactivate a strong response.

Measles, mumps and rubella vaccination

Responses are clearly suboptimal. A single dose of triple vaccine induces seroconversion to measles, mumps and rubella in 80%, 50% and 80% of patients, respectively, with one-third seroconverting to all three viruses. It is recommended that levels of vaccine-induced antibodies be checked to assure protection.

Chronic hemodialysis

Impact of disease on vaccine-induced immunity

Chronic hemodialysis has little impact on immunity induced by routine vaccinations and booster immunizations. At comparable ages and time points after the last vaccine dose, the same proportion of patients and healthy controls have protective levels of diphtheria and tetanus antibodies. Similarly, pneumococcal vaccine responses are well preserved, yet antibody responses are very short lived and half of patients have inadequate levels 1 year later.

Varicella vaccination

Vaccination is indicated in anticipation of transplantation in view of the potential severity of chickenpox in transplant recipients. Nearly all patients develop a protective antibody level after two doses of vaccine administered 3 months apart. The few children who fail to respond to the initial two doses respond to a third. All patients vaccinated in anticipation of transplantation have a protective antibody level at the time of surgery. Vaccination prior to immunosuppression is protective. The incidence of chickenpox is significantly lower in vaccine recipients and the few breakthrough cases are significantly less severe. In addition, vaccination is clearly correlated with a reduced risk of zoster occurring during immunosuppressive therapy. The vaccination is well tolerated and causes only minor local reactions. Between 60% (patients on triple immunosuppressive therapy) and 100% of transplant recipients vaccinated prior to engraftment retain protective antibody levels 2–3 years after engraftment.

CHRONIC KIDNEY DISEASES IN ADULTS

Predialysis renal failure

Hepatitis B vaccination

Patients in renal failure are low responders to hepatitis B vaccination. At this stage responses are not correlated with the degree of functional insufficiency. The regular schedule (three doses at 0, 1 and 6 months with 20 µg of antigen/dose by the intramuscular route) is inadequate (57% protective responses) so that alternative schedules have been investigated.

- *Alternative 1*: Increasing the amount of antigen to 40 µg per dose provides an insignificant increase in rates of protective responses (67%).
- *Alternative 2*: Increasing the amount of antigen to 40 µg per dose and inserting an extra dose of vaccine at the time point '2 months' within the regular schedule results in a significantly higher rate of protective responses (80%).
- *Alternative 3*: The four-dose schedule (0, 1, 2 and 6 months) based on intradermal injections of a smaller amount of antigen per dose (10 µg) is as effective as the above-mentioned intramuscular schedule, with the advantage of requiring much less antigen altogether, but with the inconvenience of necessitating five concomitant injections of 2 µg each to provide the required amount of antigen intradermally.

Table 83.5 Vaccination of HIV infected infants and children

① No restriction
② No retriction (immune response possibly reduced)
③ No restriction (immune response likely to be insufficient or absent)
④ Strict contraindication and no inactivated substitute vaccine available
⑤ Strict contraindication but inactivated substitute vaccine available
⑥ No strict contraindication (immune response doubtful in case of concomitant immunoglobulin therapy)

	Diphtheria inactivated	*H. influenzae b inactivated*	*Hepatitis A inactivated*	*Hepatitis B inactivated*	*Human papillomavirus inactivated*	*Influenza inactivated*	*Influenza live*	*Measles live*	*Meningococcal inactivated*	*Mumps live*	*Pertussis inactivated*	*Pneumococcus inactivated*	*Poliomyelitis inactivated (IPV)*	*Poliomyelitis live (OPV)*	*Rabies inactivated*	*Rotavirus live*	*Rubella live*	*Tetanus inactivated*	*Tick-borne encephalitis inactivated*	*Tuberculosis live (BCG)*	*Typhoid inactivated*	*Typhoid live*	*Varicella live*	*Yellow fever live*
No immunosuppression*	①	①	①	①	①	①	⑤	①	①	①	①	①	①	⑤	①	①	①	①	①	④	①	⑤	①	④
Moderate immunosuppression†	②	②	②	②	②	②	⑤	⑥	②	⑥	②	②	②	⑤	②	②	⑥	②	②	④	②	⑤	⑥	④
Severe immunosuppression‡	③	③	③	③	③	③	⑤	④	③	④	③	③	③	⑤	③	④	④	③	③	④	③	⑤	④	④

	Age <12 months	Age 1–5 years	Age 6–12 years
* Number of CD4 cells/µl	≥1500	≥1000	≥500
† Number of CD4 cells/µl	750–1499	500–999	200–499
‡ Number of CD4 cells/µl	<750	<500	<200

Influenza vaccination

A single dose of inactivated vaccine is effective and induces a significant increase in antibody levels to all strains. The proportion of patients attaining the protective threshold is slightly lower than in healthy controls but the clinical significance of this finding is undetermined.

Long-term dialysis

Diphtheria and tetanus recall vaccination

Recall immunizations are imperative in these patients. Single recall doses of vaccine have a suboptimal efficacy. Patients do achieve protective antibody levels but responses are of primary type and short lived. One year later, the majority of patients are below the protection threshold for diphtheria and one-third for tetanus. Complete revaccination schedules are no more efficient than single doses, suggesting a failure to induce the immune memory. The suggested approach is to monitor antibody levels and to administer single recall doses of toxoid whenever needed.

Hepatitis B vaccination

Overall, the effectiveness of vaccination is inversely related to the patient's age. When adequate schedules (such as those mentioned below) are used, the probability of obtaining a protective response is close to 100% in patients aged less than 50 years, compared to 75% in those aged 60–74 years and 50% in those over 75 years of age. A functionally efficient hemodialysis is associated with a stronger response. A concomitant asymptomatic hepatitis C infection does not modify the response. Protective response rates are similar in hemodialysis and peritoneal dialysis patients provided that adequate schedules are used.

The regular vaccination schedule (three doses at 0, 1 and 6 months by the intramuscular route with 20 µg of antigen per dose) is grossly inadequate (55–65% protective responses). Repeating an unsuccessful regular schedule is of no benefit in these patients. Consequently, a number of alternative schedules were developed. In peritoneal dialysis patients, increasing the amount of antigen to 40 µg or 80 µg per dose is associated with a significant increase in adequate responses (80%). In hemodialysis patients it is necessary to increase both the number of doses (to four or five) and the amount of antigen per dose (to at least 40 µg) to augment the rate of protective responses to 70–80%. Based on historic comparisons, the five-dose schedule is not significantly more effective than the other.

The intradermal route of vaccination (5 µg of antigen per dose injected on a weekly or bi-weekly basis for several weeks) is as effective as intramuscular schedules on condition that the minimal amount of antigen administered over the entire immunization process is 50 µg in peritoneal dialysis patients and 80 µg in hemodialysis patients. The minimal number of injections required is 10 in peritoneal dialysis patients and may vary from 15 to 25 in hemodialysis patients.

Yet another approach is to give a 5 µg dose of vaccine intradermally on a weekly basis until attainment of the protective level. Intradermal schedules induce significantly earlier responses than intramuscular injections and should be considered when a rapid response is deemed essential. However, peak antibody levels are lower and the longevity of responses is shorter. Protection obtained from intradermal schedules should not be considered as definitive as that obtained after a successful intramuscular schedule. An additional intramuscular dose of 40–80 µg should be given 4–6 months after successful completion of the intradermal injection program and the antibody level should be checked in order to demonstrate that protection relies on the immune memory.

Influenza vaccination

A single dose of inactivated vaccine is associated with seroconversion rates and geometric mean antibody titers significantly lower for all strains than in healthy controls. However, protection is still achieved in a sizeable proportion of patients, varying between 45% and 90% depending on the strain considered. One study suggests that patients on calcitriol therapy at the time of vaccination might have a stronger antibody response.

Pneumococcal vaccination

A single dose of polysaccharide vaccine induces immune responses of lower magnitude and shorter duration than in healthy individuals. These short-lived responses are clinically correlated with frequent breakthrough infections occurring approximately 2 years after vaccination. Vaccination is suspected to induce a phenomenon of immune tolerance to some of the pneumococcal serotypes.

Varicella vaccination

Vaccination of susceptible adults in anticipation of engraftment is indicated in view of the potential severity of chickenpox in these patients. The varicella vaccine is overall less immunogenic in adults than in children but still has a favorable impact. Between 65% and 75% of patients achieve a protective response after two doses administered 6 weeks apart.

CHRONIC LIVER DISEASES IN CHILDREN

End-stage hepatic failure

Diphtheria, tetanus, poliomyelitis, measles, mumps, rubella and varicella vaccination

Antibody responses to inactivated vaccines are not altered in infants and toddlers with chronic liver failure. Live vaccines (measles, mumps, rubella, varicella) are as effective and as safe as in healthy controls. Since geometric mean antibody concentrations generated by live vaccines are inversely related to the severity of liver dysfunction at the time of injection, immunization should be initiated as soon as possible following the first birthday.

Chronic hepatitis B (HBV) or hepatitis C (HCV) disease

Hepatitis A (HAV) vaccination

Immunization is indicated in children with a pre-existent chronic liver disease on the analogy of adults with identical conditions. The regular two-dose schedule (0 and 6 months) of inactivated vaccine is as effective as in healthy controls. Rates of response are independent of the hepatitis serologic status.

CHRONIC LIVER DISEASES IN ADULTS

Chronic hepatitis B (HBV) disease

Hepatitis A (HAV) vaccination

Immunization is indicated in patients with chronic HBV disease. The regular two-dose schedule (0 and 6 months) of inactivated vaccine is equally effective in patients and healthy adults. At 5 years, 88% of vaccine recipients maintain an antibody level above the protection threshold, indicating that the response is based on the stimulation of memory cells.

Chronic hepatitis C (HCV) disease

Hepatitis A (HAV) vaccination

It is recommended that adults with pre-existent HCV disease be vaccinated against HAV because of the increased probability of exposure and severe clinical course. Consequently, hepatitis A vaccination

is considered complementary to hepatitis B immunization, and the combined vaccine is handy. The regular three-dose schedule (0, 1 and 6 months) of Twinrix®720/20 is much less effective in these patients than in healthy controls. At completion of vaccination, 88% of patients have a protective antibody level against HAV and 82% against HBV. Administration of a fourth dose does not significantly enhance the rate of response to either antigen. When exposure to either virus is deemed imminent, the accelerated three-dose schedule (0, 7 and 21 days) of Twinrix®720/20 is effective in providing short-term protection. Nearly 90% of patients develop a protective response against HAV and 80% against HBV. An additional dose administered shortly after the third one is of no benefit. Accelerated schedules are not protective in the long term and should be completed when long-term protection is sought.

Hepatitis B vaccination

Responses to the hepatitis B vaccine are less adequate in these patients than healthy adults so that specific schedules are required. Three intramuscular doses of 40 µg each (at monthly intervals) induce a protective antibody level in 72% of patients. Adding a fourth dose of 80 µg 1 month later enhances the probability of protection to 88%. However, as monthly injections over a rather short period of time do not set suitable conditions for induction of the immune memory, the protection obtained by such schedules is not reliable in the long run and an additional dose of vaccine should be offered 4–6 months later. Patients with a cirrhotic disease respond significantly less adequately to the above four-dose schedule (74% seroprotection). Patients who do not respond to the above four-dose schedule are still reasonably likely (72%) to develop a protective response when they receive monthly doses of 80 µg of antigen up to a maximum of 10 months. Concomitant treatment with pegylated interferon and ribavirin does not affect the immunogenic effectiveness of hepatitis B vaccination.

Chronic alcohol-induced cirrhosis

Hepatitis B vaccination

These patients have a severely impaired response to the hepatitis B vaccine. Three monthly doses of 40 µg administered intramuscularly induce a protective level of antibody in a mere 12% of individuals. Patients who do not respond to the above schedule may achieve a protective response with a monthly dose of 80 µg for a maximum of 10 months. Protection generated by monthly injections over a short period of time is not reliable in the long run and an additional dose of vaccine is required 4–5 months later to obtain a response based on memory cells. Neither of the above schedules is associated with significant adverse effects.

Influenza vaccination

No immunogenicity data are available in these patients. Immunization with the inactivated vaccine is modestly effective in reducing the incidence of influenza-like illness and that of culture-proven influenza infection.

CHRONIC INFLAMMATORY AND AUTOIMMUNE DISEASES IN CHILDREN

Inflammatory bowel diseases

Influenza vaccination

The immune response to a single dose of inactivated vaccine is significantly reduced in patients on immunosuppressive therapy compared to healthy controls. Inadequate responses are particularly likely with drug combinations associating an anti-tumor necrosis factor (TNF) agent and a drug interfering with DNA synthesis. When used as monotherapy (or in combination with prednisone), anti-TNF agents have a minimal impact on the antibody response.

Juvenile idiopathic arthritis

Hepatitis B vaccination

The regular three-dose schedule (0, 1 and 6 months) induces an antibody level ≥10 IU/l in virtually all patients but geometric mean antibody concentrations are significantly lower than in healthy individuals. Consequently, it is essential to check that the antibody level is greater than 100 IU/l at completion of vaccination in order to ensure that long-term protection can indeed be relied upon. The vaccination is very safe with no deterioration of disease activity indices and no flare ups observed during the entire immunization process and beyond.

Influenza vaccination

Yearly immunization is recommended in children on continuous salicylate treatment or on immunosuppressive therapy for a rheumatologic condition. After a single dose of inactivated vaccine, 95% of patients achieve a protective level to all strains, similar to age-matched healthy controls. Safety has been particularly investigated on the suspicion that wild-type influenza viruses might be rheumatogenic and found to be excellent with no increase in disease activity indices following vaccination.

Measles–mumps–rubella (MMR) vaccination

No data are available on the immunogenicity of the MMR vaccine in these patients. However, safety has been particularly investigated in order to relieve the fear that live attenuated viruses might aggravate disease activity. A retrospective study scrutinizing children during a period of 6 months before and after injection of the second dose of vaccine reveals no increase in disease activity or medication use, and no measles-like illness.

Meningococcal conjugate vaccination

A single dose of conjugate vaccine is as effective in these patients as in healthy controls. Furthermore, it is safe with no change in disease activity and no increase in the frequency of relapses over a 6-month observation period.

CHRONIC INFLAMMATORY AND AUTOIMMUNE DISEASES IN ADULTS

Rheumatoid arthritis

Hepatitis B vaccination

The regular three-dose schedule (0, 1 and 6 months) is modestly effective (68% seroconversion) and safe. It is necessary to check the antibody response after completion of immunization and to continue the vaccination process in low responders. A second vaccine cycle or a high-dose vaccine schedule can be used (such as for renal failure patients).

Influenza vaccination

A single dose of inactivated vaccine induces a lower geometric mean concentration of antibodies than in healthy controls. Antibody responses are minimally altered by concomitant treatment of prednisone and methotrexate but significantly depressed by a combination of methotrexate and rituximab. Reactogenicity is similar in patients and healthy controls, and safety is clearly established with no change in disease activity and no increase in autoantibodies or peripheral blood lymphocyte subpopulations.

Pneumococcal vaccination

The polysaccharide vaccine is unreliable in these patients (65% response) but perfectly safe with no deterioration of either clinical or laboratory disease activity indices.

Systemic lupus erythematosus

Tetanus recall vaccination

A single recall dose of toxoid induces a protective antibody level in over 90% of patients. The recall vaccination is no more reactogenic than in historic age-matched controls and has no impact on disease activity.

Hepatitis B vaccination

The regular three-dose schedule is effective and safe in these patients. No change in disease activity indices, or a need to resort to more aggressive therapies, has been reported following any dose of vaccine.

Influenza vaccination

Protection against influenza is desirable in systemic lupus erythematosus (SLE) patients because natural immunity to wild-type viruses is suspected of being weaker than in healthy individuals. A single dose of inactivated vaccine induces a similar response in patients and controls, and reactogenicity is similarly low in both groups. Numerous clinical studies have confirmed the vaccine's safety profile in SLE patients. The number of flares and mean disease activity indices were not modified over observation periods of up to 5 months. Autoantibodies are produced following vaccination (without clinical manifestations) yet antinuclear antibodies are not. It can be concluded that influenza vaccination is immunogenic and sufficiently safe to be recommended to SLE patients.

Pneumococcal vaccination

Recent studies with the 23-valent vaccine indicate that the immune response to pneumococcal vaccine is unreliable in relation to the number of serotype-specific responses. After a single dose, 80% of patients develop an adequate response while others develop a response to just one serotype or to none. The vaccination is well tolerated and safe. No deterioration of either clinical or laboratory disease activity indices and no production of autoantibodies are evidenced.

REFERENCES

References for this chapter can be found online at http://www.expertconsult.com

Practice point **40**

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Immunodeficiencies associated with immunosuppressive agents

INTRODUCTION

This practice point reviews the immunologic effects (i.e. the degree of immunodeficiency) associated with therapeutic interventions of immunosuppressive agents commonly used in transplantation and in the treatment of rheumatic and autoimmune diseases (Fig. PP40.1). Differing mechanisms of action of specific immunosuppressive agents and of their immunologic target(s) reveal qualitative and quantitative differences in the type of immunodeficiency caused by these therapeutic interventions. Many immunosuppressive agents target multiple components of the immune system. Furthermore, the intensity of the immunodeficiency is substantially influenced by the number of agents administered since immunosuppressive agents are mostly used in combination.

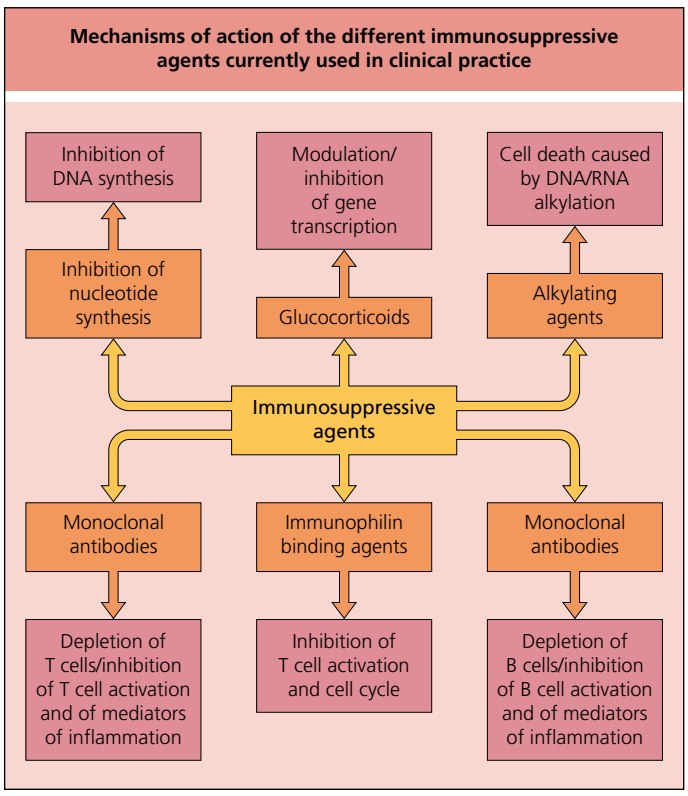


Fig. PP40.1 Mechanisms of action of the different immunosuppressive agents currently used in clinical practice.

HOST DEFENSES – GENERAL CONCEPTS

The immune system represents the primary mechanism of defense against a variety of pathogens (see Chapters 2 and 72). To understand the potential interference of immunosuppressive agents on the immune system, it is important to examine the different components of the immune system and the site of action of these agents. Immunologic dysfunctions can be anticipated depending upon the molecular target of each immunosuppressive treatment.

Anatomic barriers

The natural mechanisms of defense are mediated by anatomic barriers, primarily by the skin and mucosal (respiratory, gastrointestinal and urinary tract) barriers. The ability of these barriers to protect against invading pathogens is dependent upon their intrinsic physical structure as well as a series of factors, including the physical–chemical features of the environment (e.g. the pH and temperature), the local bacterial microflora and soluble local mediators such as secretory IgA and a variety of antimicrobial proteins.

Immunosuppressive treatment may severely compromise the integrity of the mucosal barrier because the particularly high turnover of cell division of the mucosal epithelium is very sensitive to the antiproliferative activity of immunosuppressive agents. This in turn may cause lesions of the epithelium, and the clinical picture may be complicated by the emergence of mucositis of variable severity. This type of complication is relatively frequent in cancer immunosuppressive regimens but is rare in the field of transplantation or immunologic diseases.

Innate immunity

This is an ancient system of defense that acts rapidly and does not require immunologic memory.[1] It is noteworthy that the innate immune system is not entirely nonspecific: the human (and vertebrate) innate immune system is able to recognize micro-organisms through pattern-recognition receptors (PRRs), and then is able to differentiate between self and pathogens. Pattern-recognition receptors recognize components of micro-organisms (known as pathogen-associated molecular patterns, PAMPs),[2] such as viral nucleic acids, components of bacterial or fungal cell walls, flagellar proteins. The first family of PRRs was the Toll-like receptor family. Toll-like receptors are transmembrane proteins that can recognize either extracellular or membrane-encased pathogens. Recently, two other families of PRRs have been described: the NOD-like receptors (NLRs) and the RIG-like helicases (RLHs) are soluble proteins that survey the cytoplasm in order to detect pathogens.

The effector components of innate immunity include soluble and cellular mediators. Amongst the former, the complement (C′) proteins play a critical role in the recruitment and activation of neutrophils (C3a and C5a), in the opsonization of bacteria (C3bi) and in the destruction of certain micro-organisms through the membrane attack complex C5–C9. C-reactive protein (CRP) and lipopolysaccharide (LPS)-binding protein are additional soluble mediators of the innate immunity involved in the opsonization of bacteria or activation of C′.

The cellular effector components of innate immunity include neutrophils, monocytes/macrophages, dendritic cells, natural killer (NK) cells and gamma-delta T cells. These different types of cell, recruited at the site of infection and/or inflammation by the soluble mediators mentioned above, mediate either direct (e.g. phagocytosis and killing of bacteria) or indirect defense mechanisms (e.g. recruitment of other types of immune cell through the secretion of cytokines and chemokines).

Adaptive immunity

Adaptive immunity is very specific; it requires a certain time to become fully functional (the time is necessary for the generation, maturation and expansion of antigen-specific T and/or B cells) and is associated with the generation of immunologic memory following clearance of the pathogen.[3]

T lymphocytes are a critical component of adaptive immunity. The mechanisms of antigen (Ag) recognition by T lymphocytes are extremely specific. Following appropriate processing and presentation by professional antigen-presenting cells (APC), T lymphocytes recognize a complex formed by major histocompatibility complex (MHC) products and the Ag peptide on the surface of APC. Two major types of T lymphocyte (i.e. CD4 and CD8) are involved in the immune response against pathogens.

Based on their ability to produce different cytokines, CD4 T lymphocytes can generally be distinguished into three populations:

- T helper 1 (Th1), which are mostly characterized by the production of interferon gamma (IFN-γ) and interleukin (IL)-2;
- Th2, which produce mostly IL-4, IL-5 and IL-10; and
- Th17, which produce IL-17.

Th1 CD4 T cells are necessary for the maturation, expansion and maintenance of the CD8 T-lymphocyte response, of NK cells and for the activation of macrophages. Th2 CD4 T cells mediate the maturation and activation of pathogen-specific B lymphocytes. Th17 $CD4^+$ T cells – a new T-cell subset secreting IL-17 – activate neutrophils and stimulate release of cytokines and chemokines by different cell types (e.g. macrophages, endothelial and epithelial cells) and probably play a pivotal role in inflammatory processes. CD8 T lymphocytes are the primary effector component of adaptive cell-mediated immunity. They are able to lyse virus-infected cells and tumor cells directly.

B lymphocytes mediate the immune response against pathogens through the production of immunoglobulins (Ig). Immunoglobulins may inhibit the entry of pathogens into target cells by binding to certain regions of the pathogen that are critical for the interaction with specific receptors on the surface of the target cells, a phenomenon known as neutralization. Furthermore, the primary role of Ig is the opsonization of bacteria and the activation of C′ and the different components of innate immunity that may lead to the elimination of the pathogen.

It is therefore clear that immunosuppressive therapy, which is one of the primary causes of secondary immunodeficiency, may significantly impair B-cell or T-cell-mediated immunity and determine increased susceptibility to a variety of pathogens.

IMMUNOLOGIC EFFECTS AND CLINICAL ISSUES ASSOCIATED WITH IMMUNOSUPPRESSIVE THERAPY

In order to evaluate correctly the risk of infectious diseases associated with impairment of immune function(s), it is essential to know the degree of the defect and the component(s) of the immune system targeted by the immunosuppressive agents.

Glucocorticoids

Glucocorticoids (GC) are the cornerstone in the treatment of the majority of inflammatory diseases. Glucocorticoids are highly lipid soluble and thus penetrate easily through the cell membrane. They bind in the cytoplasm to a specific receptor (glucocorticoid receptor, GR) that is present in all nucleated cells.[4] The latter is the reason why it is not possible to dissociate the anti-inflammatory from the metabolic effects of GC.

The clinical picture of patients receiving treatment with GC may be complicated by a variety of infections caused by bacteria, fungi and viruses. The severity of these infectious complications depends upon the degree of immunosuppression resulting from GC treatment (Table PP40.1). Retrospective studies on large cohorts of patients treated with GC have clearly demonstrated that the risk of infection (including lethal infections) in these patients is significantly increased compared to patients not receiving GC.[5] Furthermore, it is important to emphasize that the dosage of GC is strictly linked to the risk of infection. A dosage of prednisone >20 mg/day is associated with a risk of infection twofold greater than that of a control group, while patients receiving a daily dosage of <10 mg/day do not show an increased risk of infection.

Alkylating agents

The alkylation of DNA is responsible for the cytotoxic, mutagenic and chemotherapeutic properties of this class of immunosuppressive agents. Cyclophosphamide (CYC) is effective on both 'resting' and dividing (i.e. activated) lymphocytes. Both T-cell (particularly T-helper) and B-cell functions are severely suppressed by CYC treatment.

The infectious complications associated with CYC treatment (see Table PP40.1) include bacterial, fungal and viral infections. The pathogens responsible for these infections are mostly typical opportunistic pathogens. It is important to emphasize that CYC is generally used in association with GC and therefore the risk for infectious complications is substantially increased, particularly if GC dosage is >0.5 mg/kg/day in patients aged more than 60 years and with a leukocyte nadir $<3 \times 10^9$cells/l. In order to prevent infectious complications, patients treated with CYC require close hematologic and immunologic monitoring and antimicrobial prophylaxis in certain conditions. Anti-*Pneumocystis* prophylaxis with trimethoprim–sulfamethoxazole is recommended in patients:

- undergoing continuous oral administration of CYC; and
- with a CD4 T-cell count of <200 cells/mm^3.

Although no definitive demonstration is available, the general perception is that continuous oral administration of CYC carries an increased risk of infection compared with intravenous pulses.

Immunophilin binding agents

Immunophilins are a large family of broadly expressed proteins that bind to certain immunosuppressive agents, such as ciclosporin A (CsA), tacrolimus and rapamycin. Due to their potent immunosuppressive effects that predominantly target T lymphocytes, these agents are fundamental components of immunosuppressive regimens in both solid organ and bone marrow transplantation.

Physiologically, T-cell receptor (TCR) signaling induces an elevation in the concentration of Ca^{2+} in the cytoplasm and activates the transcription factor AP-1. Ca^{2+} binds to calcineurin that in turn dephosphorylates the cytoplasmic form of the nuclear factor of activated T cells (NFAT). Once NFAT migrates into the nucleus, it forms a complex with AP-1, thus inducing the transcription of genes required for T-cell activation, including IL-2. When CsA is present in the cytoplasm, it forms a complex with cyclophilin (Cyp). The CsA–Cyp complex can bind to calcineurin, blocking its ability to activate NFAT and therefore inhibiting T-cell activation (Fig. PP40.2).

Table PP40.1 Type of infection related to immunosuppressive agents

Type of immunodeficiency	Type of immunosuppressive agent or chemotherapy	Type of infectious risk
Anatomic barriers (mucositis)	High-dose chemotherapy	Bacterial Gram-positive – staphylococci (coagulase ±), streptococci Gram-negative – Enterobacteriaceae, *Pseudomonas* spp. Fungal *Candida* spp. Viral Herpes simplex virus
Innate immunity (quantitative and qualitative defects)	High-dose chemotherapy/ radiotherapy	Bacterial Gram-positive – staphylococci (coagulase ±), streptococci Gram-negative – *Escherichia coli, Klebsiella, Pseudomonas* spp. Fungal *Candida* *Aspergillus* spp.
Adaptive immunity Cellular	High-dose chemotherapy/ radiotherapy Immunosuppressive therapies (GC, CYC, CsA, FK-506, MTX, AZA, rapamycin, antilymphocyte serum, monoclonal antibodies)	Bacterial *Legionella* spp. *Mycobacterium tuberculosis* Atypical mycobacteria *Listeria monocytogenes* *Salmonella* spp. Fungal *Candida* spp. *Cryptococcus neoformans* *Histoplasma capsulatum* *Coccidioides immitis* Viral Cytomegalovirus Varicella zoster virus Herpes simplex virus Epstein-Barr virus Live viral vaccines (measles, mumps, rubella, poliovirus)
Humoral	High-dose GC, CYC, MMF	Bacterial Gram-positive – streptococci (*S. pneumoniae* and other streptococci) Gram-negative – *Haemophilus influenzae, Neisseria meningitidis, Capnocytophaga canimorsus* Viral Enterovirus Parasites *Giardia lamblia*

AZA, azathioprine; CsA, ciclosporin A; CYC, cyclophosphamide; GC, glucocorticoids; MMF, mycophenolate mofetil; MTX, methotrexate; FK-506, tacrolimus.

Inhibitors of nucleotide synthesis

Azathioprine

Activated T lymphocytes are the primary target of azathioprine (AZA), which does not exert any cytotoxic effect on T lymphocytes prior to an antigen challenge, whereas the most potent immunosuppressive activities occur following antigen stimulation (Table PP40.2). On the basis of this mechanism of action, AZA has no effect on memory T lymphocytes. In addition to activated T lymphocytes, AZA exerts potent immunosuppression on B lymphocytes. Due to its selective effect on dividing cells, AZA rarely causes severe lymphopenia and only prolonged treatment may be associated with a substantial reduction in circulating lymphocytes.

Mycophenolate mofetil

Mycophenolate mofetil (MMF) is the prodrug of mycophenolic acid. Mycophenolate mofetil is a highly selective, noncompetitive inhibitor

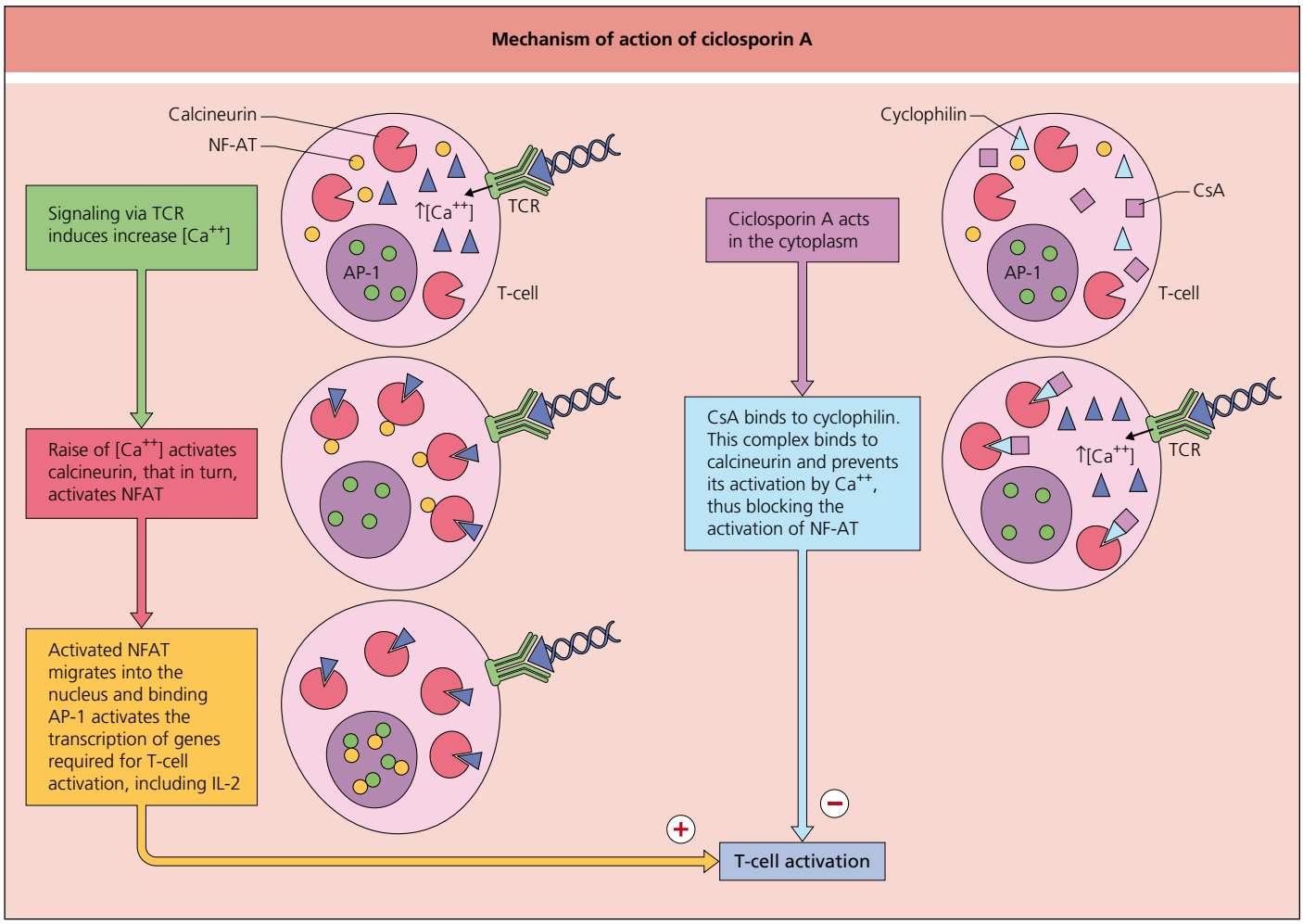


Fig. PP40.2 Mechanism of action of ciclosporin A (CsA). Cyp, cyclophilin; NF-AT, nuclear factor of activated T cells; TCR, T-cell receptor.

of the inosine monophosphate dehydrogenase involved in the de-novo synthesis of guanosine nucleotides (Table PP40.2, Fig. PP40.3). Purine synthesis may occur through two different pathways: de novo and salvage. Blood cells originating from the erythrocyte, megakaryocyte and myeloid lineages may use the salvage pathway if the de-novo pathway is inhibited. In contrast, the salvage pathway is not functional in B and T lymphocytes. From an immunologic standpoint, MMF has no effect on resting B and T lymphocytes but strongly suppresses proliferation of activated T lymphocytes by inducing apoptosis. It is a potent suppressor of primary and secondary T-cell responses. It also mediates potent inhibition of the primary humoral immune response but seems to be less effective in the inhibition of secondary humoral responses. Furthermore, due to the potent suppression of primary humoral immune responses, vaccination generally fails to induce humoral immune responses in patients undergoing MMF treatment.

Methotrexate

Originally developed as an anticancer agent, methotrexate (MTX) is an antifolate with a potent immunosuppressive action. MTX has a structure similar to the folates and uses the same mechanisms of transport to cross the membrane. In particular, two mechanisms have been identified:

- via a low affinity transporter; and
- via a folate-binding-protein (FBP) associated with the cell membrane.

In addition, MTX can also passively cross the membrane.

MTX has numerous enzymatic targets. The inhibition of dihydrofolate reductase (DHFR) is the primary mechanism of suppression of pyrimidine synthesis. Inhibition of pyrimidine synthesis by MTX also occurs through the suppression of another enzyme, thymidylate synthase. Furthermore, MTX inhibits the de-novo synthesis of purines. Therefore, the immunosuppressive effect of MTX is mostly the result of suppression of pyrimidine and purine synthesis. With regard to the anti-inflammatory effect, it has been shown that MTX reduces the chemotaxis of neutrophils, inhibits the synthesis of leukotriene B4 and reduces the synthesis of IL-1. Finally, it has been shown that MTX induces apoptosis of activated but not resting CD4 and CD8 T lymphocytes (see Table PP40.2).

Monoclonal antibodies

Antilymphocyte antibodies are important therapeutic tools in the field of immunosuppression and transplantation. A major limitation of these agents is their lack of specificity and their partial efficacy. Another issue of serious concern is the toxicity profile with an increased risk for opportunistic infections and malignancies. On the basis of these limitations, polyclonal antibodies have been progressively replaced by the development of a series of monoclonal antibodies.

Numerous 'biologicals' are in development or in use in the field of oncology and rheumatology, such as abatacept (cytotoxic T-lymphocyte antigen-4 immunoglobulin) or tocilizumab (anti-IL-6 receptor). It is probably too early to establish the clinical safety of very new agents.[6] Currently, only a few are clinically relevant in transplantation and inflammatory diseases: anti-IL2 antibodies, inhibitors of tumor necrosis factor (TNF), rituximab and alemtuzumab.

Table PP40.2 Immunosuppressive drugs: mechanisms of action, indications and related adverse events

Immunosuppressive agents	Mechanisms of action	Indications for treatment	Infectious adverse events	Other significant adverse events
Glucocorticoids (GC)	Changes in circulating leukocyte populations (lymphocytopenia, monocytopenia, neutrophilia, eosinophilopenia, basophilopenia) Inhibition of synthesis of cytokines and proinflammatory molecules Inhibition of synthesis of the IL-2 receptor Suppression of Ag presentation by causing a reduction of expression of MHC molecules on the surface of APC Inhibition of lymphocyte proliferation by suppression of IL-2 production	Severe allergic reaction, inflammatory diseases, autoimmune diseases (connectivitis and vasculitis)	Gram-positive bacteria (*Staphylococcus aureus, Nocardia* spp., *Listeria monocytogenes*) Gram-negative bacteria (*Legionella pneumophila, Klebsiella pneumoniae, Pseudomonas* spp. *or Salmonella* spp.) Mycobacterial, fungal (*Pneumocystis jirovecii, Aspergillus fumigatus* and different types of *Candida* spp.) and viral infections	Metabolic diseases (osteoporosis, steroid-induced diabetes) Hormonal dysfunction Increase of cardiovascular risk factors (hypertension, dyslipidemia, diabetes) Changes in behavior Cataract Skin atrophy and striae rubrae distensae Gastrointestinal bleeding
Alkylating agents				
Cyclophosphamide (CYC)	Alkylation of purines within the DNA and RNA (the ultimate result is cell death due to a loss of the ability to divide)	Cyclophosphamide is largely used in the treatment of autoimmune and rheumatic diseases (severe manifestations of connectivitis or vasculitis)	Bacterial (*Staph. aureus, K. pneumoniae*), fungal (*Candida albicans*) and viral infections (herpes zoster)	Hematologic toxicity (myelodysplastic syndrome) and oncogenicity (bladder carcinoma) due to its antiproliferative and mutagenic properties Toxicity of CYC is dependent on dosage and duration of treatment (cumulative dose)
Immunophilin binding agents				
Calcineurin inhibitors				
Ciclosporin (CsA)	Lipophilic undecapeptide reversible inhibitor of T-cell activation that acts by interfering with calcineurin and thus IL-2 synthesis and release	Solid organ transplantation	Due to the selective immunosuppressive effects on T lymphocytes, infectious complications associated with immunophilin binding agents are predominantly caused by viral and fungal infections (see Table PP40.1)	Increased risk for cancer (lymphomas) directly related to EBV The incidence of post-transplant lymphoproliferative disease (PTLD) is caused by an EBV primary infection and depends on the intensity of immunosuppressive treatment (spontaneous regression of PTLD following the decrease of the intensity of immunosuppression) Renal toxicity and hypertension (CsA) can be explained on the basis of increased activity of the endothelin-converting enzyme that causes increased production of endothelin-1 (ET-1), a vasopressive and proinflammatory peptide. Neurotoxicity (tacrolimus)
Tacrolimus (FK-506)	Binds to immunophilins and in particular to the FK-506 binding proteins (FKBP) Although CsA and tacrolimus bind to different proteins, their mechanism of action is very similar since tacrolimus also inhibits calcineurin; however, it is 10- to 100-fold more potent than CsA	Occasionally used in the treatment of autoimmune and rheumatic diseases		

mTOR inhibitors				
Rapamycin (sirolimus) Everolimus	Sirolimus binds to the FKBP. However, the sirolimus/FKBP complex does not bind calcineurin but binds to a protein called the mammalian target of sirolimus (mTOR) that regulates the translation of mRNA necessary for cell division A derivative of sirolimus (everolimus) has recently been developed	Solid organ transplantation	Since these agents are often used in combination with other classes of immunosuppressive agent, it is difficult to identify infectious complications specific for a particular class of immunosuppressors Two viral infections, CMV and BK virus, are thought to be strictly related to immunosuppressive treatment with CsA or tacrolimus Some studies have shown a lower incidence of CMV infection using sirolimus compared to other immunosuppressive regimens Sirolimus has *in-vitro* antifungal activity (especially against *Cryptococcus neoformans*)	Although the immunosuppressive potency of sirolimus is very similar to that of CsA and FK-506, the toxicity profile is different Sirolimus and everolimus toxicity includes hyperlipidemia, thrombocytopenia, delayed wound healing, mouth ulcers and pulmonary toxicity (interstitial pneumonitis) Sirolimus and everolimus may have antineoplastic effects
Inhibitors of nucleotide synthesis				
Azathioprine (AZA)	AZA and its metabolite, 6 mercaptopurine (6-MP), belong to the class of thiopurines. The targets of these drugs are the enzymes involved in the *de novo* synthesis of purines. In this way, AZA inhibits DNA replication in dividing cells including lymphocytes	Originally used in the field of transplantation, AZA has been progressively replaced by more potent purine inhibitors such as mycophenolate mofetil (MMF, see below) AZA is particularly effective against the hematologic, pulmonary and skin manifestations of autoimmune and rheumatic diseases	Infectious complications associated with AZA are predominantly caused by viruses (particularly Herpesviridae) and to a lesser extent by fungi and parasites, mostly observed in the field of renal transplantation The most common infectious complication is CMV infection Patients >60 years old represent an important risk factor for infectious complications during immunosuppressive therapy	Major side-effects are bone marrow suppression, hepatitis and infections *Note*: Moderate severity of the toxicity profile of AZA, which rarely requires interruption of treatment, is rapidly reversed by the cessation of treatment Severe bone marrow suppression has been reported in patients with concomitant treatment with allopurinol Safely administered during pregnancy No evidence for increased risk of malignancies
Mycophenolate mofetil (MMF)	Highly selective noncompetitive inhibitor of inosine monophosphate dehydrogenase, an enzyme involved in the *de novo* synthesis of guanosine nucleotides The dual effect on T and B lymphocytes is responsible for the infectious complications associated with MMF therapy	Predominantly used in the field of solid organ transplantation Occasionally used in the treatment of autoimmune and rheumatic diseases (lupus nephritis)	Frequency of CMV infection has been reported to be greater in patients receiving high (>3 g/day) doses of MMF In patients treated with MMF, but receiving aciclovir or ganciclovir anti-CMV prophylaxis, the incidence of CMV infection was not increased MMF has potent *in-vitro* activity against *P. jirovecii*	Hematologic adverse events (thrombocytopenia) and gastrointestinal intolerance have been described

(Continued)

Table PP40.2 Immunosuppressive drugs: mechanisms of action, indications and related adverse events—cont'd

Immunosuppressive agents	Mechanisms of action	Indications for treatment	Infectious adverse events	Other significant adverse events
Methotrexate (MTX)	Antifolate agent with a potent immunosuppressive action MTX inhibits the *de novo* synthesis of purines by blocking the enzymatic activity of the 5-amino-imidazole-4-carboxamide-ribonucleotide transformylase (AICAR) The immunosuppressive effect of MTX is mostly the result of suppression of pyrimidine and purine synthesis The anti-inflammatory properties of MTX reduce the chemotaxis of neutrophils, inhibit the synthesis of leukotriene B4 and reduce the synthesis of IL-1	MTX at higher dosage (100–1000 mg/m^2 per cycle) has been extensively used in the field of oncology At lower doses (5–25 mg/week) it is essentially used as an anti-inflammatory agent and as an immunomodulator in the treatment of autoimmune diseases (rheumatoid arthritis) MTX together with CsA or FK-506 is used in the treatment of graft-versus-host disease	Infectious complications: upper respiratory tract infections, urinary tract infections	Severe liver and lung (interstitial pneumonitis) toxicity has been described in patients treated with MTX In addition, the defect in folate concentrations caused by MTX may be responsible for severe toxic effects such as myelosuppression, hepatotoxicity and diarrhea. These latter toxic effects can in part be prevented or controlled by the administration of folic or folinic acid which competes with the activity of MTX
Leflunomide	Inhibitor of the *de novo* pyrimidine synthesis	Used in the treatment of rheumatoid arthritis Efficacy and toxicity are comparable to those of MTX	Infectious complications affecting the upper respiratory tract; they are generally more frequent during the first year of treatment but only exceptionally cause interruption of therapy Leflunomide has *in-vitro* activity against CMV and BK virus, although its clinical use for CMV infection and BK virus-associated nephropathy has not yet been established	The most commonly reported adverse events are diarrhea, nausea, alopecia, headaches and elevation of LFTs
Monoclonal antibodies				
Anti-IL-2 receptor antibodies				
Basiliximab (BSL) Daclizumab (DCZ)	Antibodies directed against the α chain of IL-2R (anti-CD25 antibodies) BSL is a chimeric antibody that contains less than 10% murine sequences while DCZ is a humanized antibody	The primary goal of using anti-IL-2R antibody in the field of transplantation is to reduce the incidence of acute graft rejection and the dose of the other immunosuppressive agents	No increased risk for viral and bacterial infections has been reported	Therapy with IL-2R antibody is extremely safe and well tolerated

Inhibitors of tumor necrosis factor (TNF)				
Infliximab Etanercept Adalimumab	Infliximab is a chimeric (human/mouse) monoclonal antibody directed against TNF Etanercept is the human recombinant form of the soluble TNF receptor Adalimumab is a recombinant human IgG1 anti-TNF monoclonal antibody	Particularly used in rheumatoid arthritis (all), and in Crohn's disease (infliximab) Very effective in vasculitis like Behçet's syndrome and uveitis (infliximab)	Mycobacterial infections are commonly associated with the blocking of TNF activity especially with infliximab (re-activation of latent tuberculosis) Other infections associated with TNF inhibitor include those caused by *P. jirovecii herpes, Legionella* and *Listeria monocytogenes* There is an increased risk of fungal infections, such as invasive aspergillosis, especially in patients treated with anti-TNF monoclonal antibodies	Systemic (infliximab) or urticarial (etanercept) reactions rarely require interruption of treatment
Rituximab (anti-CD20)	Human/mouse chimeric monoclonal antibody that targets the B-cell CD20 antigen and causes rapid, long-term (2–9 months) specific B-cell depletion	Several reports of efficacy in rheumatoid arthritis, lupus nephritis, cryoglobulinemic vasculitis, pemphigus and Sjögren's syndrome	Uncommon. Bacterial (including sepsis, rarely), fungal and viral infections	Cytokine release syndrome (first administration), hypersensitivity syndrome B-cell depletion Occasionally, serum sickness (arthralgia, malaise, fever)
Alemtuzumab (Campath-1H)	Humanized CD52-specific complement fixing IgG1 monoclonal antibody The human CD52 antigen is found in variable concentrations on peripheral blood mononuclear cells Alemtuzumab especially depletes T cells, but also has a major effect on B cells, natural killer cells, monocytes and dendritic cells In summary, it has a very profound immunosuppressive effect	Initially used in oncology, alemtuzumab is actually not indicated for transplantation. However, it has been assessed as induction therapy in clinical trials in order to prevent acute rejection, and to avoid infectious complications by decreasing dosages of other immunosuppressive therapies (e.g. calcineurin inhibitors and steroids)	In very recently published studies, the most frequent infection was BK virus nephropathy, followed by fungal and CMV infections; mycobacterial infections were also observed. However, these rates were similar to rates reported with other regimens	Reported side-effects include profound thrombocytopenia and lymphopenia

APC, antigen-presenting cells; CMV, cytomegalovirus; EBV, Epstein–Barr virus; MHC, major histocompatibility complex.

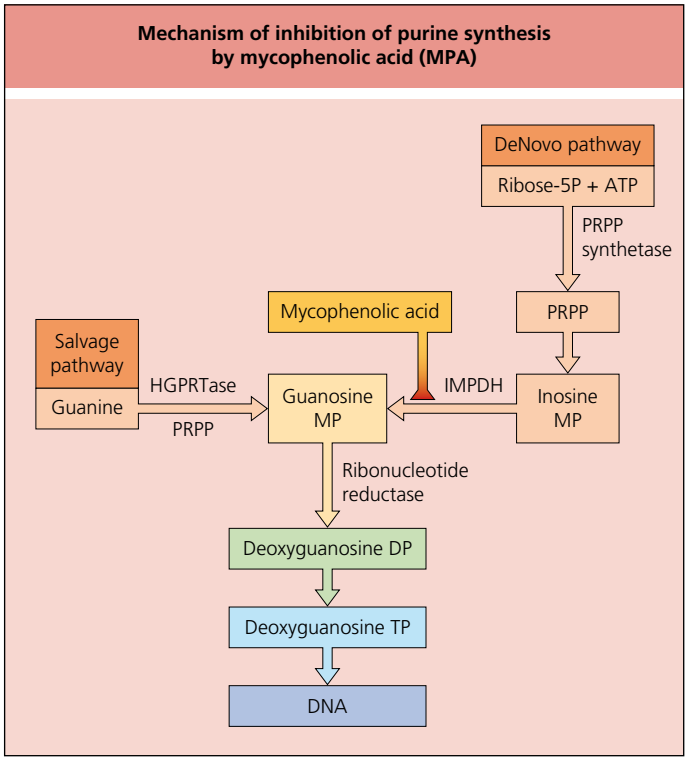


Fig. PP40.3 Mechanism of inhibition of purine synthesis by mycophenolic acid. HGPRTase, hypoxanthine–guanine phosphoribosyltransferase; IMPDH, inosine monophosphate dehydrogenase; PRPP, 5-phosphoribosyl-1(a)-pyrophosphate; ribose-5P, D-ribose-5′-phosphate.

Anti-interleukin-2 receptor antibodies

Interleukin (IL)-2 is a major T-cell growth factor necessary for the expansion of T lymphocytes following antigen-specific stimulation that represents a fundamental step in the generation of the T-cell-mediated immune response. Monoclonal antibodies have been raised against the α chain of the IL-2 receptor (IL-2R) (anti-CD25 antibody) to interfere with the initial expansion of T lymphocytes (see Table PP40.2). Anti-IL-2R monoclonal antibody formulations for clinical use have been developed in order to interfere with the initial expansion of T lymphocytes. These antibodies are directed against the α chain of the IL-2R.

Inhibitors of tumor necrosis factor

Tumor necrosis factor is a potent cytokine and a primary mediator of inflammatory reactions. Tumor necrosis factor plays a central role in many inflammatory diseases including inflammatory bowel and rheumatic diseases.[6] Several studies have demonstrated the fundamental role of TNF in the pathogenesis of rheumatoid arthritis (RA). Transgenic mice with a deregulation of the TNF gene develop a destructive arthritis similar to RA. In addition to RA, TNF is clearly implicated in the pathogenesis of juvenile rheumatoid and psoriatic arthritis and Crohn's disease.

Two specific classes of inhibitors of TNF function have been developed: infliximab and adalimumab, and etanercept. Infliximab is a chimeric (human/mouse) monoclonal antibody directed against TNF. The F_c portion of infliximab is a human IgG_1 while the antigen-binding variable domain that has a high affinity for human TNF is of murine origin. Adalimumab is a recombinant human IgG_1 anti-TNF monoclonal antibody. Etanercept is the human recombinant form of the soluble TNF receptor. It is a dimerized fusion protein formed by an extracellular domain of the TNF type II receptor (p75) and by the F_c portion of a human IgG_1. The mechanism of action of etanercept is the blocking of soluble and membrane-bound TNF and of lymphotoxin α.

Mycobacterial infections are commonly associated with the blocking of TNF activity, especially with infliximab administration. In the majority of cases, re-activation of latent tuberculosis has been observed. On the basis of these complications, the current guidelines for the therapeutic use of TNF inhibitors indicate that a purified protein derivative test or QuantiFERON-TB test should be performed before the initiation of treatment. The QuantiFERON-TB test (which measures the production of IFN-γ after incubation of whole blood with mycobacterial peptides) has recently been approved by the US Food and Drug Administration (FDA). In the case of a positive test without other signs of re-activation of the disease, treatment with isoniazid is advised before and during administration of infliximab. The number of cases of re-activation of tuberculosis seems to be less important following treatment with etanercept.[7]

Rituximab

Initially used in the treatment of non-Hodgkin's lymphoma (NHL), administration of anti-CD20 monoclonal antibodies is currently indicated in numerous rheumatologic (e.g. rheumatoid arthritis) and autoimmune diseases with predominant B-lymphocyte pathogenesis (systemic lupus erythematosus, Sjögren's disease, cryoglobulinemia, pemphigus, etc.). This antibody can cause prolonged depletion of B-cell responses, putting patients at risk for a variety of systemic bacterial and viral infections.[8]

CONCLUSIONS

Major advances have been made in the last two decades in the understanding of fundamental immunoregulatory mechanisms, in the development of novel immunosuppressive agents and in the prevention of infectious complications associated with immunosuppressive therapy. The future goals in the field of immunosuppression should include improvement in the management of infectious complications and the development of novel and more selective agents. The generation of highly selective agents is the only valid strategy to maximize immunosuppressive effects and minimize immunodeficiency.

REFERENCES

References for this chapter can be found online at http://www.expertconsult.com

Practice point | 41 | Kevin Winthrop

Preventing tuberculosis and other serious infections in patients starting anti-tumor necrosis factor therapy

Therapies that inhibit tumor necrosis factor (TNF) have shown remarkable clinical efficacy against rheumatoid arthritis (RA), Crohn's disease, psoriasis, ankylosing spondylitis and other autoimmune diseases. Three anti-TNF compounds in current clinical use are infliximab, etanercept and adalimumab. Their use is now widespread, particularly in RA where over 40% of patients have been treated with such drugs. As a group, these drugs inhibit TNF, a proinflammatory cytokine involved in the pathogenesis of RA and other autoimmune diseases, but also integral to the host's innate immune system.[1–3]

TUMOR NECROSIS FACTOR AND ITS IMMUNE FUNCTION

Tumor necrosis factor is a proinflammatory cytokine expressed by activated immune cells in response to a variety of pathogens. It mediates systemic inflammatory responses via two cellular receptors (p55 and p75). TNF plays a crucial role in the host response against a variety of infections, in particular *Mycobacterium tuberculosis* (TB) and other intracellular pathogens,[1] as it stimulates inflammatory cell recruitment to areas of infection and the formation of granulomas that contain infection. In addition, TNF directly activates macrophages to phagocytose and kill mycobacteria and numerous other pathogens. In animal models, mice deficient in TNF or its p55 signaling pathway are highly susceptible to *Listeria monocytogenes*, *Histoplasma capsulatum* and numerous other pathogens including extracellular bacterial organisms like *Klebsiella pneumoniae* and *Streptococcus pneumoniae*.[1–3]

CURRENT ANTI-TNF DRUGS AND THEIR DIFFERENTIAL PROPERTIES

Infliximab and adalimumab are monoclonal antibodies, while etanercept is a soluble immunofusion construct of the p75 receptor. Infliximab is delivered as an infusion every 4–8 weeks. Adalimumab (given every 2 weeks) and etanercept (given weekly) are delivered by subcutaneous injection.[4] All three agents bind TNF, but important differences exist between these drug types with regard to their modulation of TNF activity. Compared with etanercept, infliximab binds TNF with greater avidity and for a longer duration. The half-life of infliximab is approximately 10.5 days and its biologic effect can persist for up to 2 months. Etanercept's half-life is 3 days and it binds TNF in a reversible manner, with disassociation of nearly 50% after only 10 minutes. These differences, coupled with differences in dosing, suggest that infliximab has greater, more sustained inhibition of TNF activity. Pharmacokinetically, adalimumab behaves similarly to infliximab. Further, infliximab and adalimumab appear to downregulate interferon gamma (IFN-γ) responses more than etanercept. In addition, it is anticipated that two new anti-TNF compounds, golimumab and certolizumab, will receive FDA approval prior to this chapter's publication.

Serious infections associated with anti-TNF agents

Infections from a variety of organisms have been reported in patients using these agents: *Coccidioides*, *Listeria*, *Histoplasma*, *Aspergillus*, *Nocardia* spp., nontuberculous mycobacteria (Fig. PP41.1), streptococci, *Staphylococcal aureus* and others.[2,3] Of serious concern is the development of active tuberculosis (TB) in some patients receiving these drugs. Similar to HIV-infected patients who develop TB, patients using anti-TNF compounds frequently present 'atypically' with extrapulmonary or disseminated disease. Accordingly, TNF antagonists should be used with caution in any person with risk factors for TB, and screening for latent TB infection (LTBI) should be undertaken before starting anti-TNF therapy.[3]

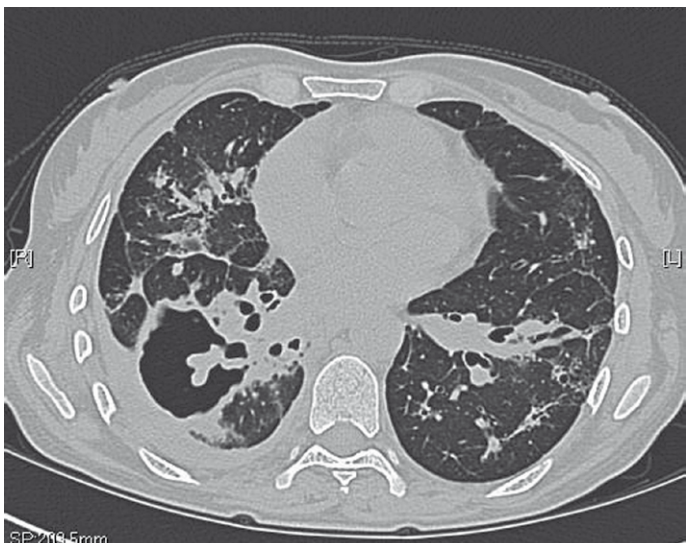


Fig. PP41.1 Pulmonary *Mycobacterium avium* disease in a 57-year-old female with rheumatoid arthritis treated with anti-TNF agents (courtesy of Dr. Michael Iseman). Chest CT shows extensive pulmonary parenchymal destruction and cavitation due to *M. avium* disease, first diagnosed while the patient was receiving infliximab. She subsequently initiated antimycobacterial therapy, briefly received adalimumab without good clinical response, and then continued to use etanercept for 1 year. Despite antimycobacterial therapy, her pulmonary disease continued to progress.

PREVENTION OF TUBERCULOSIS DURING ANTI-TNF THERAPY

Patients with latent TB are at risk for reactivation and progression to disease when exposed to anti-TNF therapy.[4] The risk depends upon the population studied and is greater in patient groups with higher background TB prevalence rates. This complication can be prevented by screening patients for LTBI and initiating preventive TB therapy prior to anti-TNF initiation. The usefulness of this screening, and the methodologies employed, vary by regional differences in TB prevalence and the usage of bacille Calmette–Guérin (BCG) vaccination, which can interfere with the interpretation of the tuberculin skin test (TST). BCG is known to cause false-positive skin tests, and in regions of low TB prevalence is likely responsible for a majority of positive TSTs where BCG is used. This is particularly true when interpreting TST positivity using a lower cut-off (i.e. 5 mm in the setting of immunosuppression).

Recently, new blood-based assays have been developed to detect latent TB infection. These tests, IFN-γ release assays (IGRAs), have a distinct advantage over the TST as they utilize proteins from the RD-1 genome of *M. tuberculosis*, a region that is not contained in BCG. Accordingly, this test is highly specific for *M. tuberculosis* and does not appear to be influenced by BCG vaccination.[5] In areas of high BCG usage, the IGRA reduces the false-positive TST results due to BCG. Some countries have adopted the IGRA as a preferred strategy in screening patients for LTBI in this setting. In other regions positive TST results are further evaluated with an IGRA if the patient has a history of BCG.

The relative sensitivities of TSTs and IGRAs for the diagnosis of LTBI in the anti-TNF setting have yet to be adequately studied. In patients with active TB disease, IGRAs have greater sensitivity, but relative performance is unknown in LTBI where there is no gold standard. Immunocompromised patients are more likely to have false-negative TST results, and similarly might also have false-negative or indeterminate IGRA results. Several small case series of patients receiving anti-TNF therapy suggest that the two methodologies might have at least similar sensitivities, and emerging evidence from other immunocompromised patients suggest that the IGRA might be more sensitive. Presently the US Public Health service recommends using IGRAs in any situation where the TST is employed.

SCREENING FOR LATENT TUBERCULOSIS BEFORE ADMINISTRATING ANTI-TNF THERAPY

Given the regional and national differences in TB prevalence, BCG usage and the potential usefulness of IGRAs in this setting, it is not surprising that screening guidance differs by region. Several points are worth emphasizing in screening[4] (Table PP41.1). The importance of taking a detailed history for TB risk factors cannot be understated. Patients born in or with a history of living in countries of high TB prevalence are at much higher risk for TB in this setting, and the positive predictive value of a TST or IGRA result is accordingly increased. Second, a 5 mm cut-off point should be used to define a positive TST result in these patients, as in other immunosuppressed hosts. Screening patients using both IGRAs and TSTs may be reasonable, with the knowledge that both tests have certain caveats and potential flaws. Chest radiography should be used to screen for active TB in any patient suspected to have LTBI.[5,6]

TREATMENT OF LATENT TUBERCULOSIS INFECTION

For patients with suspected LTBI, the presence of active TB disease should be excluded with appropriate diagnostic studies (i.e. sputum culture or other respiratory sampling). A 9-month course of daily therapy with isoniazid (INH) is the preferred treatment. One approach to treating patients with LTBI is to administer INH for at least 1 month prior to initiating anti-TNF therapy. This is to ensure that patients are taking and tolerating INH. Given the low bacillary load of LTBI, this recommendation seems reasonable and is based on the observational experience of Spanish investigators who adopted such a protocol and

Table PP41.1 Recommendations for screening, diagnosis and treatment of latent tuberculosis infection and tuberculosis in patients administered or scheduled to receive tumor necrosis factor (TNF) antagonists

- Screen patients for risk factors for *Mycobacterium tuberculosis* and test them for infection before initiating immunosuppressive therapies, including TNF antagonists. Risk factors include birth or extended living in a country where TB is prevalent, or history of any of the following:
 - residence in a congregate setting (e.g. jail or prison, homeless shelter or chronic-care facility)
 - a positive tuberculin skin test (TST) result
 - substance abuse (injection or noninjection)
 - health-care employment in settings with TB patients
 - chest radiographic findings consistent with previous TB.
- Utilize diagnostic aids in LTBI screening including the TST and/or interferon gamma release assays (IGRAs).
- Diagnosis and treatment of latent TB infection (LTBI) and TB disease should be in accordance with published guidelines.
- In patients who are immunocompromised (e.g. because of therapy or other medical conditions), interpret a TST induration of ≥5 mm as a positive result and evidence of *M. tuberculosis* infection.
- Interpret a TST induration of <5 mm as a negative result but not an exclusion for *M. tuberculosis* infection.
- Anergy panel testing is not recommended. Results from control-antigen skin testing (e.g. *Candida*) should not alter the interpretation of a negative TST result.
- Test to exclude TB disease before starting treatment for LTBI.
- Start treatment for LTBI before commencing TNF-blocking agents, preferably with 9 months of daily isoniazid.
- Consider treatment for LTBI in patients who have negative TST results but whose epidemiologic and clinical circumstances suggest a probability of LTBI.
- Pursue TB disease as a potential cause of febrile or respiratory illness in all patients receiving TNF-blocking agents.
- Consider postponement of TNF-antagonist therapy until the conclusion of treatment for LTBI or TB disease if possible. If not clinically possible, withhold TNF-antagonist therapy until the patient has demonstrated LTBI treatment adherence and tolerance, or in the case of TB disease, until the patient has shown adherence to appropriate anti-TB drugs to which their organism is known to be sensitive.

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subsequently saw an 85% reduction in TB cases associated with infliximab. A 4-month course of rifampin (rifampicin)-based therapy could be considered an alternative in patients who cannot tolerate 9 months of INH. While INH-related hepatotoxicity is not common, it should be monitored closely in those patients receiving methotrexate or other hepatotoxic drugs. Liver function testing should be performed at baseline and then periodically (e.g. monthly) in all such patients.

ANTI-TNF THERAPY AND OTHER SERIOUS INFECTIONS

Dimorphic fungal infections

Histoplasma capsulatum is an endemic fungus in many areas of the world, and is the second most commonly reported pathogen in association with TNF blockade.[2] Like TB, it can exist in a latent or slowly progressive state, and can progress to active disease, particularly during immunosuppression. The role of routine serologic monitoring for prior evidence of histoplasmosis before starting an anti-TNF inhibitor is unclear and the value of empiric antifungal therapy is unknown. Currently, routine serologic testing for histoplasmosis is not recommended in HIV-infected persons and recommendations about such screening or use of preventive therapy for histoplasmosis await further clinical study.

Coccidioides immitis is endemic to the southwestern US, Central and South America. Like *Histoplasma* spp., it too can exist in a latent or subclinical infectious state after exposure, and can later progress to disease during immunosuppression. Patients in endemic areas could potentially be screened with serology prior to initiation of TNF blockade. However, given that most of the coccidioidomycosis cases reported in association with TNF blockade are cases of acute infection, and not reactivation, the usefulness of pre-treatment serologic screening is unclear.

Bacterial pathogens

Listeria monocytogenes is an opportunistic, intracellular pathogen and fatal cases of listeriosis have occurred in persons taking TNF-blocking agents. Patients under TNF blockade should be advised to avoid raw meat, delicatessen meat and unpasteurized milk products.

Serious infections and death due to extracellular pathogens such as *Staph. aureus* and *Strep. pneumoniae* have also been reported in the anti-TNF setting. There are murine models suggesting that anti-TNF therapy could worsen such infections, and observational studies suggest that serious bacterial infections (e.g. community-acquired pneumonia, skin/soft tissue infection, osteomyelitis) are elevated in patients receiving these therapies. Anti-TNF-treated patients are at increased risk of chronic furunculosis caused by *Staph. aureus*. Screening these patients for colonization and attempting to decolonize them before anti-TNF therapy seems reasonable, yet no controlled studies have as yet clarified the value of this approach. With regard to *Strep. pneumoniae*, it is widely recommended that patients receive 23-valent pneumococcal polysaccharide vaccine prior to initiating these therapies. Vaccine immunogenicity can be diminished by methotrexate; if possible, therefore, patients should be vaccinated while not receiving this drug.

Other pathogens and perioperative infections

Anti-TNF products can reactivate or worsen hepatitis B. Prior to therapy, patients should be screened for hepatitis B and anti-TNF therapy should generally be avoided in infected patients. In contrast, for patients with chronic hepatitis C infection, it is thought that anti-TNF therapy might be used safely, albeit with caution and careful attention to hepatic enzymes. The development of herpes zoster might be slightly more common in patients using anti-TNF therapies, although there are conflicting data in this regard and any elevation in risk is likely small. Several small, retrospective case series reported no increase in perioperative infection risk. Recently, however, two larger retrospective studies suggest a small increase in wound infections after orthopedic procedures and a delay in wound healing in patients who continued anti-TNF drugs. It seems prudent to discontinue anti-TNF therapy several weeks before surgery (depending on the drug's half-life), if possible, until the patient's surgical wounds are healed.[7,8]

Immune reconstitution inflammatory syndrome

The risk of immune reconstitution inflammatory syndrome (IRIS) upon discontinuation of anti-TNF therapy in patients with tuberculosis deserves mention. While it seems prudent to discontinue anti-TNF therapy when a patient is diagnosed with TB, it is unclear whether this is necessary or even beneficial to the patient, presuming the patient is receiving an appropriate multidrug anti-TB therapeutic regimen. There are case reports of IRIS occurring in TB patients in whom anti-TNF therapy was stopped,[7] and there is very limited evidence that concurrent therapy of TB with anti-TNF compounds might be safe. Further, if anti-TNF therapy is stopped, there are no data to suggest when it is safe to resume such therapy. Generally it is appropriate to stop anti-TNF therapy when TB is diagnosed and remain vigilant for IRIS. Anti-TNF therapy can be resumed after the patient has demonstrable clinical improvement during an effective anti-TB regimen.

REFERENCES


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Practice point | 42 |

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Infectious diseases transmitted by grafts

INTRODUCTION

Recipients of transplants face two threats for infection not experienced by other surgical patients. One threat arises from the immunosuppression related to antirejection medications and the patient's underlying organ disease. The other threat arises from the multitude of pathogens that may be transmitted by the donor graft. Table PP42.1 presents a list of organisms that have been transmitted during transplantation.

BACTERIAL PATHOGENS

Donors may have bacterial infections at the time of organ procurement, either latent or symptomatic. Urinary infections in donors can cause graft infections in kidney recipients, particularly if the donor is infected with more virulent organisms such as *Staphylococcus aureus* and Gram-negative rods. Cadaveric donors are intubated and, as a result, nearly half will have evidence of airway or lung colonization. This issue is most pertinent in regards to lung transplant recipients, and tailored antimicrobial therapy should be administered to the lung recipient in the setting of a positive donor sputum or bronchoalveolar lavage (BAL) culture. Most systemic bacterial infections in donors, such as bacteremia or meningitis, are not a contraindication for organ transplantation provided that the donor infection has been brought under control by appropriate antibiotics prior to organ procurement and the recipient is also treated after transplantation.

Transmission of *Mycobacterium tuberculosis* from a donor does occur; in one large series it accounted for 4% of post-transplant *M. tuberculosis* infections. Screening with tuberculin skin testing (TST) is recommended for living donors. As performing TST in cadaveric donors is not feasible, obtaining a history from the donor's relatives regarding previous active infection or exposure to tuberculosis is crucial. Prophylaxis in the recipient is generally recommended if the donor has a positive skin test or had a history of untreated active or latent tuberculosis.

Table PP42.1 Donor-transmitted pathogens

Bacteria	Urinary and pulmonary pathogens (*Staphylococcus aureus* and Gram-negative rods) *Mycobacterium tuberculosis* *Treponema pallidum* (syphilis)
Viruses	Herpesviruses (cytomegalovirus, herpes simplex virus, Epstein–Barr virus, human herpesvirus 6, 8) Hepatitis viruses (hepatitis B, C) Retroviruses (HIV-1, -2, human T-cell lymphotropic virus 1) Rhabdoviruses (rabies virus) Flaviviruses (West Nile virus) Arenaviruses (lymphocytic choriomeningitis virus)
Fungi	*Candida* spp. *Aspergillus* spp. *Histoplasma capsulatum* *Coccidioides immitis*
Parasites	*Toxoplasma gondii* *Trypanosoma cruzi* *Plasmodium* spp. *Strongyloides stercoralis*
Prions	Creutzfeldt–Jakob disease

VIRAL PATHOGENS

Herpesviruses are the most common and significant infectious agents encountered after transplantation. Herpesviruses cause persistent latent infections in their host and are efficiently transmitted during transplantation. Donor-transmitted infections of herpesviruses are most problematic if the recipient is seronegative, because a lack of immunity confers an opportunity for more severe or invasive disease. This phenomenon is true for many of the herpesviruses including cytomegalovirus (CMV), herpes simplex virus (HSV) and Epstein–Barr virus (EBV). Prophylaxis with antivirals in these individuals can dramatically reduce the risk of disease associated with these viruses. Transmission of other herpesviruses such as HHV-6 and -8 has also been described.

The risk of transmission of hepatitis B virus is greatest in donors with clear evidence of active infection, usually identified by either a positive surface antigen (HBsAg) or core IgM antibody (HBcIgM). The risk associated with receiving a graft from a donor who is positive for IgG antibody to core antigen (HBcIgG), but negative for HBsAg and HBcIgM is less clear, but can partially be predicted by the organ that is transplanted and the immune status of the recipient. Roughly half of all liver recipients will acquire hepatitis B infection from an HBcIgG-positive donor, while the transmission rate is low (<3%) for other solid-organ recipients. Pre-existing immunity in the recipient, either by vaccination or previous infection, reduces the risk of transmission. Donors who only have a positive surface antibody (HBsAb) are considered low risk for transmission. Donors are screened for hepatitis C virus (HCV) and organs from seropositive donors are usually rejected because the risk of transmission is high (roughly 50%), even in non-liver recipients. Organs from HCV-positive donors, however, are sometimes utilized in recipients who are severely ill or already seropositive for HCV.

Several cases of transmission of retroviruses, including human T-cell lymphotropic virus 1 (HTLV-1) and HIV, have been documented in

transplant recipients. HTLV-1 can cause adult T-cell lymphoma/leukemia and tropical spastic paraparesis and myelopathy. Both diseases have been described in transplant recipients, who may have acquired their infection during transplantation, or from blood transfusions or hemodialysis. HTLV-1 infections are most prevalent in the Caribbean isles and in Southern Japan; there are also pockets of infection in parts of Africa, Asia and South America. Most reported cases of HIV transmission from organ donation occurred before 1985 when donor screening was first implemented. The transmission of HIV from an HIV-seronegative donor is rare. In reported cases, the donors were likely recently infected and in the early window of infection, usually in the first 2–8 weeks, when HIV antibodies are still undetectable. Most recently in 2007, four organ transplant recipients in Chicago acquired both HIV and hepatitis C from a seronegative donor. Cases like these have sparked increased interest in utilizing molecular methods, such as nucleic acid testing (NAT), to identify donors during the early window of infection when viremia is present, but antibodies have not yet developed. Such methods can potentially reduce the window period in detecting acute donor infections to 1–2 weeks.

In the past few years, cases of donor-transmitted rabies virus and West Nile virus have received attention. In 2004, four organ and tissue recipients with a common donor developed encephalitis within 30 days of transplantation. The donor died from a subarachnoid hemorrhage. All four recipients expired and had Negri bodies seen in brain tissue; three of the four had detectable antibodies against rabies virus. An investigation disclosed that the donor had told acquaintances of a bat bite. A similar episode of rabies transmission from an organ donor occurred in Germany. Rabies transmission has also been described after corneal transplantation. Four transplant recipients acquired West Nile virus infection from their common donor and three subsequently developed encephalitis within weeks of transplantation. The donor had been infected from a blood transfusion. Blood banks now screen for West Nile virus using a nucleic acid test. Routine testing of donors for West Nile virus or rabies virus is not recommended, but organ or tissue transplantation from a donor with an unexplained viral illness and encephalopathy should be avoided.

Lymphocytic choriomeningitis virus (LCMV) is an arenavirus usually acquired from exposure to rodents. Graft-transmitted infection with LCMV was described in two clusters of solid-organ recipients in 2003 and 2005. In all the recipients, symptoms began within 3 weeks of transplantation and included abdominal pain, mental status changes and fever. Peri-incisional erythema and pulmonary infiltrates were also encountered. Interestingly, neither of the two donors involved had laboratory evidence of infection, but prior to her death one donor had recent exposure to a pet hamster, which was infected with a LCMV genetically identical to the recipients' virus. Seven of the eight organ recipients died. One kidney recipient survived with immunosuppression reduction and ribavirin therapy. In 2008, a report from Australia described three organ recipients sharing a common donor, all of whom succumbed to a febrile, encephalopathic illness within 6 weeks of transplantation. The likely causative agent, identified using a high-throughput sequencing method, was an arenavirus closely related to LCMV.

FUNGAL PATHOGENS

Transmission of fungal infections during transplantation is rare. Donor infection with *Candida* spp., including *Candida albicans*, can be transmitted during transplantation, and has been associated with anastomotic and graft infections. Also, anastomotic infections from donor-derived *Aspergillus* colonization have been reported in lung transplantation; isolated cases of suspected *Aspergillus* acquisition from other organs (e.g. kidney) are in the literature, although risks are likely low. Transmission of endemic fungi from donor organs is also uncommon but isolated cases involving transmission of *Histoplasma capsulatum* and *Coccidioides immitis* have been documented. Since there is a high mortality rate associated with donor-transmitted coccidioidomycosis, donors from areas endemic for *C. immitis* should undergo serologic testing, and if positive, antifungal prophylaxis should be given to their organ recipients. Donor screening for *H. capsulatum* is not routinely recommended.

PARASITIC PATHOGENS

Toxoplasma gondii can be transmitted from transplanted organs, especially in heart grafts where cysts can lie dormant in myocardial tissue. Serologic screening is performed on heart and heart–lung donors prior to transplantation and prophylaxis is recommended in seronegative heart recipients who receive their graft from a seropositive donor.

Cases of donor-transmitted malaria infections, from *Plasmodium falciparum* and *Plasmodium vivax*, have been reported in solid-organ and bone marrow transplantation. Infections with *P. falciparum* usually cause earlier symptoms and parasitemia after transplantation (days) than *P. vivax* (weeks to months).

Trypanosoma cruzi is endemic in Latin America and causes Chagas disease. Five cases of donor-transmitted Chagas disease have been described in the United States (two heart, two kidney and one liver recipient). Similar to the cases involving malaria, the donors had either lived in or traveled to an endemic region. All five infected recipients were treated with nifurtimox, but one patient still succumbed to chagasic myocarditis.

PRION DISEASE

Multiple cases of Creutzfeldt–Jakob disease have been associated with cornea- and dura mater-related transplants. Transmission has not been described in solid-organ transplantation.

DONOR SCREENING

Table PP42.2 lists the current laboratory studies performed on all prospective donors, either living or cadaveric, and recommended individual measures to take in the case of a positive test. In addition to the

Table PP42.2 Donor screening

Mandatory tests of all donors	Appropriate actions in event of positive result
Rapid plasma reagin (RPR)	Prophylaxis in recipient
Cytomegalovirus serology	Depending on recipient's serology, consider prophylaxis
Epstein–Barr virus serology	Consider virologic monitoring in seronegative recipient
Hepatitis Bs Ag, core IgM	Consider use in critically ill recipient with prophylaxis
Hepatitis B, core IgG	Consider use in non-liver or immune recipient ± prophylaxis
Hepatitis Bs AB	Considered low risk
Hepatitis C serology	Consider use in critically ill recipient or seropositive recipient
HIV-1/2 serology	Avoid transplant
Human T-cell lymphotropic virus 1 and 2 serology	Avoid transplant
Toxoplasma serology	Prophylaxis in seronegative heart or heart–lung recipient
Blood cultures Urine cultures Bronchial alveolar lavage cultures (lung donor)	Directed antimicrobial therapy against isolated pathogen

laboratory evaluation for infection, a thorough behavioral and social questionnaire plays a crucial part in determining a donor's risk for certain transmissible pathogens. The recommendations, at present, reflect minimal requirements, with laboratory emphasis on serologic screening; currently efforts are underway to define optimal laboratory screening methodology, potentially employing sensitive platforms for nucleic acid testing, with the goal of optimizing and standardizing donor screening.

FURTHER READING



Further reading for this chapter can be found online at http://www.expertconsult.com

Practice point | 43 |

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BK virus replication and disease in transplant patients

INTRODUCTION

The human polyomavirus type I, commonly known as BK virus (BKV), is a nonenveloped icosahedral particle of 40 nm diameter and fairly resistant to environmental inactivation.[1] Serologic studies indicate that BKV infects 60–90% of human populations worldwide during childhood without known symptoms or signs. The mode of natural transmission is unknown, but likely occurs via the oral or respiratory route. Subsequently, BKV establishes latent infection in cells of the urinary tract where the viral genome persists as a double-stranded circular DNA episome of 5 kb.

Reactivation of BKV replication is mediated by the viral noncoding control region which drives expression of the early genes consisting of the large and small T-antigen and the late genes consisting of the capsid proteins VP1, 2, 3 and the agnoprotein. Low-level asymptomatic urinary shedding is found in 5–10% of healthy non-immunosuppressed individuals (<10^5 genome equivalents (geq)/ml). In patients with impaired immune function, urinary shedding is more prevalent, ranging from 20% to >60%, and BKV loads are frequently >10^7 geq/ml.[1]

High-level BK viruria remains asymptomatic in most patients. However, BKV is linked to two major diseases, namely polyomavirus BK-associated nephropathy[2,3] and polyomavirus BK-associated hemorrhagic cystitis.[4,5] Both entities have been encountered sporadically in various types of immunodeficiency, but are consistently found after kidney and hematopoietic stem cell transplantation, respectively, suggesting the presence of specific risk factors linked to either procedure. BKV has been associated with rare cases of hemophagocytic syndrome, ureteric stenosis, pneumonitis, encephalitis, retinitis, multiorgan failure and a presentation reminiscent of progressive multifocal leukoencephalopathy.[1]

POLYOMAVIRUS-BK ASSOCIATED NEPHROPATHY

Polyomavirus-BK associated nephropathy (PVAN) has been diagnosed in 1–10% of kidney transplant recipients with a median time interval of 9 months post-transplantation.[6] Intense immunosuppression is the key risk factor synergizing with determinants of the patient (e.g. age >50 years, male gender, BKV-seronegative recipient), the graft (e.g. BKV-seropositive donor, human leukocyte antigen mismatches, ischemic or immune injury) and the virus (e.g. latent viral load, capsid serotype, rearrangement of control region, replicative fitness). The definitive diagnosis is made by demonstrating BKV replication in renal tubular epithelial cells in allograft tissues.[6] Most commonly, the viral large T-antigen is identified by immunohistochemistry using antibodies cross-reacting with the homologous SV40 protein, but some centers use *in-situ* hybridization to detect the viral genome.

Three different histopathologic presentations have been distinguished:[6,7]

- PVAN A is defined by focal, frequently medullary BKV replication, with tubular epithelial cells with enlarged nuclei, rounding and occasionally sloughing from the basal membrane, and without significant inflammatory infiltrates. At this early stage, allograft function remains unaffected and progression to irreversible graft loss is <10%.
- PVAN B is characterized by extensive BKV replication with widespread cell necrosis, denudation of basal membranes and significant inflammatory cell infiltrates. This presentation is typically associated with impaired graft function and a 50% chance of graft loss.
- PVAN C is characterized by predominance of tubular atrophy, fibrosis and paucity of BKV replication which are associated with poor graft function and return to dialysis in >80% of cases.[6,8]

The histologic diagnosis is viewed as the gold standard for PVAN, but is challenged by:[9]

- false-negative results in 10–30% of cases of PVAN A due to the focality of BKV replication;
- confounding PVAN B and acute interstitial allograft rejection which significantly impacts on treatment and outcome;
- BKV-specific T-cell immune reconstitution after reduced immunosuppression mimicking acute rejection;
- the resemblance of PVAN C with chronic allograft nephropathy; and
- PVAN due to the closely related JC virus which appears to run a more benign course.

These limitations, together with the diverse risk factor profile and variable time of disease onset, have made the quantitative detection of BKV replication in urine and blood the pivotal tests for the management of kidney transplant patients.

As BK viruria and viremia have been demonstrated to precede the histologic PVAN diagnosis in kidney transplants by 12 weeks and 8 weeks, respectively,[2,6,9] it is recommended to screen for BKV replication in urine and plasma at least every 3 months during the first 2 years after transplantation, or if allograft dysfunction occurs prompting an allograft biopsy for a workup of BKV-associated kidney injury. The absence of BK viruria has a high negative predictive value to exclude PVAN. Confirmed BK viruria should trigger testing for BK viremia. Increasing plasma BKV loads persisting at levels greater than 4 log geq/ml for 4 weeks or longer has been used to define presumptive PVAN for which intervention should be considered, even in the absence of

positive histologic findings.[6,9–12] Indeed, in patients with persisting high plasma BKV loads, BKV genomic variants emerge which are associated with higher replicative capacity and more extensive cytopathic damage.[13] Because of earlier manifestations, some centers prefer to screen bi-weekly or monthly during the first 6 months for a pre-emptive intervention strategy.

Intervention is currently based on the timely reduction of immunosuppression which needs to be tailored to the individual's transplant history and risk of rejection. Standard protocol reduction post-transplant probably clears high-level BKV replication in about a third of affected patients. Conversely, more extensive reductions are warranted in patients with persisting high-level viremia (i.e. $>10^4$ geq/ml) and histologically documented PVAN.[11,14] No validated protocols are available, but experts in the field recommend reducing the calcineurin inhibitor and the antiproliferative agent in documented PVAN (e.g. trough levels of tacrolimus <6 ng/ml or ciclosporin <125 µg/ml; daily dose of mycophenolate mofetil <1 g or azathioprine <75 mg).[6,15] Decline of plasma BKV loads can be expected to occur 4–12 weeks after tapering of immunosuppression and coincides with emerging BKV-specific T-cell responses to epitopes of the large T- and VP1 capsid antigens.[11,16] Thus, monitoring plasma BKV load every 2–4 weeks is generally predictive of the course of disease. Urine BKV loads usually follow plasma BKV load decline, with a variable delay of 8–16 weeks.

In cases of coexisting acute rejection, some centers have combined steroid pulse treatment with reducing maintenance immunosuppression in a second step. This presentation is associated with a more protracted course and poor outcome. The adjunct use of low-dose cidofovir[17] or switching from mycophenolate mofetil to leflunomide has been advocated.[18] However, given the long half-life and the risk of nephro-, hepato- and myelotoxicity of these drugs, randomized controlled trials are needed to demonstrate a benefit over timely reduction of maintenance immunosuppression.[19]

Re-transplantation after graft loss due to PVAN has been performed successfully, but recurrence of PVAN should be anticipated by screening for BKV replication. Allograft nephrectomy is required unless pre-emptive re-transplantation is considered. A period of reduced immunosuppression of 8–12 weeks is recommended to allow recovery of BKV-specific immune effectors prior to re-transplantation and can be correlated with clearance of BK viremia.[20]

POLYOMAVIRUS-BK ASSOCIATED HEMORRHAGIC CYSTITIS

Hemorrhagic cystitis causes significant morbidity after hematopoietic stem cell transplantation (HSCT) due to diffuse mucosal bleeding, inflammation, bladder wall thickening, immobilizing pain and progression to outright anemia and obstructive postrenal failure. The incidence of hemorrhagic cystitis ranges from <10% to >50% depending on the case definition, the conditioning regimen and the underlying etiology.[21] To distinguish hemorrhagic cystitis from mere hematuria, the symptoms of cystitis with pain and urge are required together with hematuria. Four grades of hematuria have been proposed:[4] grade I is defined as microscopic hematuria (>100 erythrocytes per high-power field), grade II as macrohematuria, grade III as macrohematuria with clots, and grade IV as macrohematuria with urinary obstruction and postrenal failure. In HSCT patients, early-onset hemorrhagic cystitis occurs pre-engraftment and has been linked to urotoxic conditioning regimens which include cyclophosphamide, ifosfamide, busulfan and/or total body irradiation.[4,5] By contrast, late-onset hemorrhagic cystitis occurs postengraftment and is associated with high-level local replication of viruses such as adenovirus, cytomegalovirus and BKV.[4]

The diagnosis of polyomavirus BK-associated hemorrhagic cystitis (PVHC) is typically made in HSCT patients with postengraftment cystitis, hematuria of grade II or higher, high-level BKV replication (i.e. $>10^7$ geq/ml) and exclusion of other pathogens. Histologic studies may show BKV replication in the uroepithelium, but are rarely performed during the acute stage, since urologic interventions are mostly aimed at hemostasis, clot removal and symptom management. BK viruria is not sufficient to define PVHC, since approximately 50% of HSCT recipients with BKV viruria are asymptomatic.[5] In patients with PVHC, urine BKV loads may be higher (e.g. $>10^9$ geq/ml) than in asymptomatic patients, but the levels frequently overlap.[22–25] Unlike in PVAN, genomic BKV variants seem to have no role in PVHC.[13,26] Recently, urine BKV loads peaking by more than 3 log increase over baseline within 2–3 weeks after onset have been proposed to correlate better with PVHC.[27] BK viremia has been detected in the plasma of one-third of HSCT recipients in the first 100 days after HSCT and sustained plasma BKV loads of 10^4 geq/ml or greater were associated with an increased risk of PVHC.[28–30] Clearly, both of the latter parameters require validation in independent prospective studies, as well as their potential to trigger pre-emptive interventions.

Risk factors for PVHC include allogeneic HSCT, acute graft-versus-host disease (GVHD), unrelated donor, myeloablative conditioning and a higher BKV recipient antibody titer pre-HSCT.[25,27,30] However, statistical significance was not reached for any of these factors in all studies. The pathogenesis of PVHC is not well understood. Late-onset PVHC postengraftment suggests a role of reconstituting immunity where BKV replication might play an important pathogenic role in the sequence of events:[1,5,31]

- damage of the uroepithelial lining by urotoxic conditioning;
- high-level BKV replication in the regenerating uroepithelial cell layer during the immunodeficient period; and
- extensive inflammation upon recovery of immunity postengraftment yielding the characteristics of PVHC.

Local uroepithelial damage and inflammation may also facilitate the leakage of BKV into the circulation, thereby explaining the diagnostic potential of plasma BKV loads.[1,28,29] This model of PVHC as an immune reconstitution syndrome has received widespread attention,[32] but clearly requires further corroborating evidence.

Current treatment of PVHC consists of analgesia, rehydration, forced diuresis and continuous bladder irrigation to prevent clot formation and urinary tract obstruction. Platelets and erythrocytes are administered as needed. PVHC with hematuria grade III–IV may require cystoscopy for cauterization and/or clot evacuation. In case of life-threatening intractable bleeding, selective arterial embolization or cystectomy must be considered. Treatment with steroids may alleviate the inflammatory component of PVHC, but will delay the immune control of BKV replication. Reducing immunosuppression may enhance the risk of acute GVHD. Local treatment with alum, formalin or hyperbaric oxygen has been reported, but these case reports are difficult to distinguish from spontaneous recovery. Intravenous cidofovir as bi-weekly dosing of 5 mg/kg together with probenecid or as a lower dose of 0.25 mg, 0.5 mg or 1 mg/kg given one to three times per week has been reported, but randomized controlled trials demonstrating efficacy are lacking.[19,33] However, in many cases, cidofovir was only applied some weeks after the start of PVHC. Moreover, clearance of clinical symptoms and BKV load decline appeared somewhat delayed and difficult to distinguish from a spontaneous course.

Although most centers would consider the off-label use of cidofovir for this indication, evidence from randomized controlled trials is lacking. Intravesical cidofovir instillation (5 mg/kg in 60–100 ml saline) has been described as a successful therapy option, but was neither well tolerated nor effective in our experience. Ciprofloxacin or levofloxacin may inhibit BKV replication in tissue culture and reduce the peaking of BKV loads in HSCT patients, but significant clinical benefit remains to be demonstrated.[19] In addition, ciprofloxacin is already widely used as antibacterial prophylaxis during neutropenia and seemingly resistant BKV isolates have been reported.[19,34]

CONCLUSION

Significant progress has been made in the diagnosis and timely management of PVAN after kidney transplantation, reducing the impact of this emerging complication. Similar progress seems to be underway for PVHC after HSCT, but diagnostic markers and treatment modalities require validation in randomized clinical studies. Both diseases are characterized by high-level BKV replication in different pathophysiologic settings, but their negative impact is likely to be significantly reduced once more efficacious and better tolerated antiviral therapies become available.

REFERENCES


References for this chapter can be found online at http://www.expertconsult.com

Chapter **84**

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Epidemiology of HIV infection

INTRODUCTION

This chapter describes the distribution and transmission patterns of HIV infections. Although the biology and modes of transmission are broadly the same in the developing world and in high-income countries, there are some large differences in the epidemiology, which are due to a variety of behavioral factors and socioeconomic conditions.

Twenty-five years after the recognition of the first AIDS cases, more than 25 million people have died because of this pandemic, one of the most significant health, social and security challenges facing the global community.[1] At the end of 2007, 33.2 million people (range 30.6–36.1 million) are living with HIV, including 2.5 million children.[2] The HIV epidemics are overwhelmingly affecting developing countries, where more than 90% of all people infected with HIV live, and there is an increasing proportion of adults living with HIV who are women (Fig. 84.1). In sub-Saharan Africa, almost 61% of adults living with HIV in 2007 were women and 42% in the Caribbean, while in the other regions the proportion of women has increased since 1990 as HIV is transmitted to the female partners of men who are likely to have been infected through intravenous drug use, during unprotected paid sex or sex with other men.

Despite a continuing increase in the number of people living with HIV, in a growing world population, in recent years, the adult HIV prevalence percentage has leveled off – 0.8% prevalence since 2001. In addition, the global number of new HIV infections has decreased. There were an estimated 2.5 million new infections in 2007, down from 3.2 million in 2001. The number of people dying of AIDS has declined in the last 2 years to 2.1 million. There are documented reductions in HIV prevalence in seven countries and a reduction in HIV-associated deaths, partly attributable to increased access to antiretroviral treatment. Sub-Saharan Africa is still the most seriously affected region, where AIDS remains the leading cause of death. In the USA and Western Europe, despite the spectacular reduction in numbers of new AIDS cases due to advances in antiretroviral treatment, a constant number of new HIV infections persists every year, with evidence that in some settings high-risk behavior has increased, indicating failure in HIV prevention. The contribution of Asia to the HIV pandemic has considerably diminished.[2] Small variations in estimates of national adult HIV prevalence in India – from 0.9% to 0.4% – and also in China result in large differences in the number of people with HIV because of the population size of these two giant countries.

Trends suggest that HIV prevention efforts are having an impact in several of the most affected countries. Côte d'Ivoire, most East African

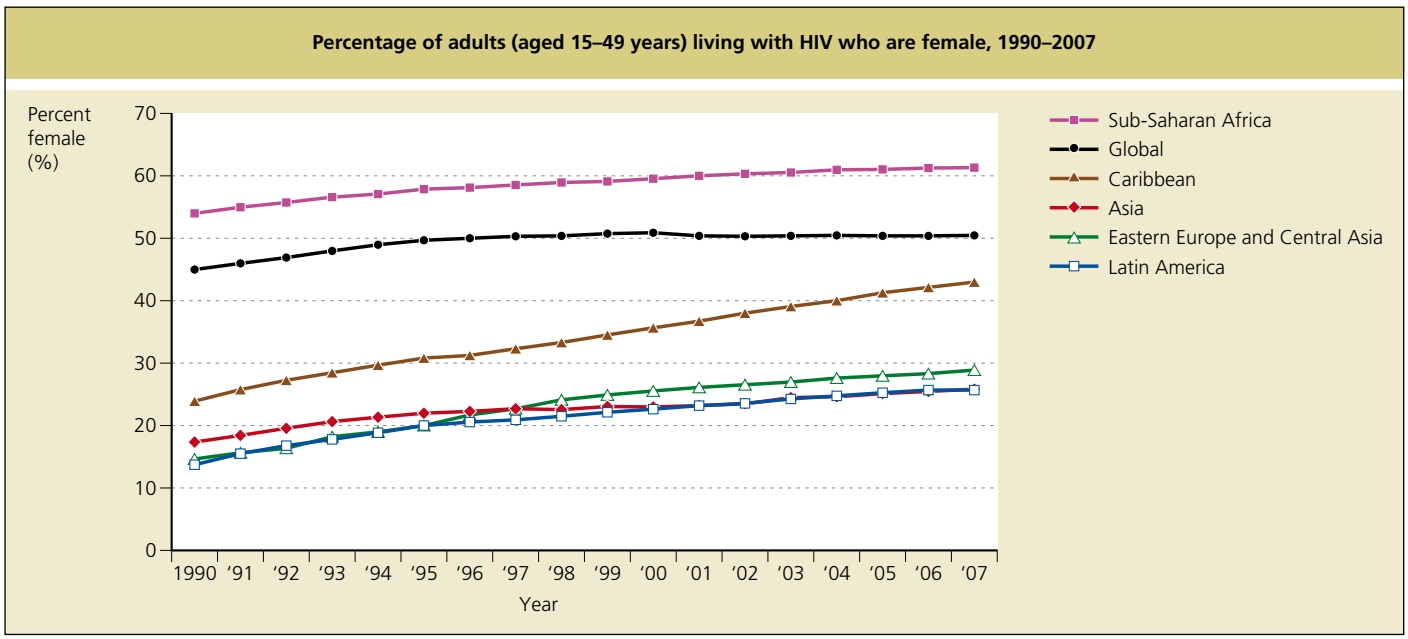


Fig. 84.1 Percentage of adults (aged 15–49 years) living with HIV who are female, 1990–2007. (From WHO: 2007 Aids Epidemic Update.)

countries and several Caribbean countries have all seen downward trends in their national prevalence percentages. HIV prevalence has also declined in South and South East Asia, notably in Cambodia, Myanmar and Southern India.

EXTENT OF HIV INFECTION

Shortly after the first reports of AIDS in the USA in 1981 among homosexual men and intravenous drug users, it became obvious that the disease was also present in Haitians living in North America and in Africans seen in Belgium for medical care at the end of 1983.[3,4] Subsequently, surveys in Haiti and Central Africa confirmed the occurrence of several epidemic foci of HIV in these areas.[5–7]

The identification of another variant, labeled HIV-2, among West African populations further increased the heterogeneity of what quickly emerged as a global pandemic. The routes of transmission and risk factors of HIV-2 and HIV-1 are similar, but it has become increasingly clear that the pathogenic effect of HIV-2 is lower than that of HIV-1.[8] In the rest of this chapter, HIV refers to HIV-1.

The exact magnitude of the HIV epidemic is still difficult to assess.[9] Indeed, despite the recognition that estimates of HIV are more accurate than for most other infectious diseases, the largely silent nature of this infection and the limited HIV surveillance systems in many countries make the tracking of HIV infection difficult. The selection of surveillance populations depends on the type of epidemic. In epidemics with at least 1% infection rates in the adult population, the recommended surveillance population are pregnant women attending antenatal clinics. In epidemics where HIV has not spread into the general population, the primary focus of the surveillance system is among high-risk populations. HIV surveillance systems have improved since 2000. In some countries, improvements have been through an increase of sentinel surveillance sites, both in number and in geographical coverage. For example in India the number of sites increased from 155 in 1998 to 1100 in 2006. In addition, 40 countries, mostly in Africa where heterosexual transmission of HIV is predominant, have conducted national representative population-based household surveys with HIV prevalence measurement. These surveys give a more precise picture of the HIV prevalence in men and women in rural areas, but clearly underestimate the extent of HIV infection among mobile populations and other vulnerable populations. As such, there is a need for several surveillance approaches to achieve the most accurate estimates.

Geographic distribution of HIV infection and AIDS

North America and Australia

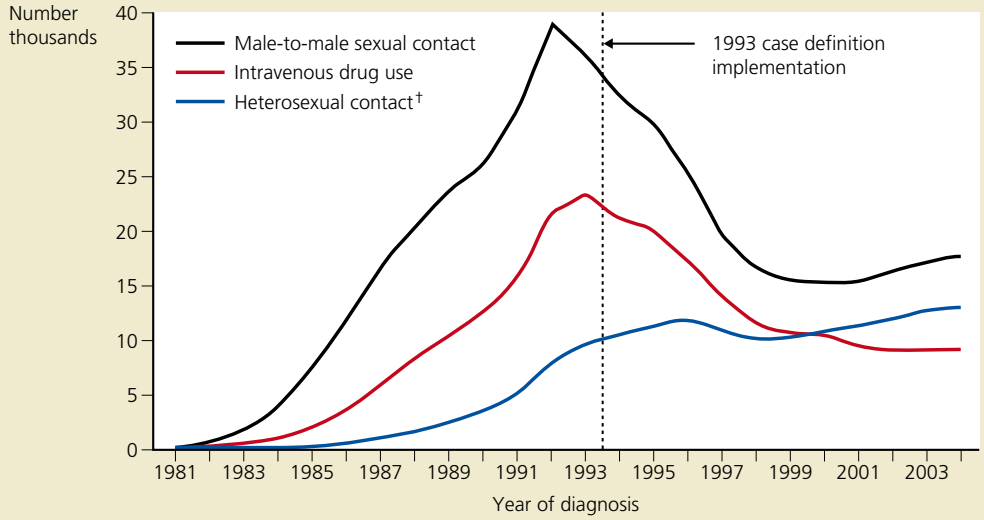
In the USA, AIDS cases increased rapidly in the 1980s and peaked in 1992 with an estimated 78 000 cases diagnosed, before stabilizing in 1998; since then, approximately 40 000 AIDS cases have been diagnosed annually[10] (Fig. 84.2) but reaching around 60 000 in 2007. In 1992, during early prevention and treatment advances, the number of AIDS cases started to decrease in all demographic and transmission categories until around 1998 when the numbers started to increase again.

At the end of 2003, the most recent estimation, approximately 1 100 000 persons in the USA were living with HIV or AIDS, an estimated 25% of whom were unaware of their infection. Based on data from HIV reporting, men accounted for 74% of the HIV or AIDS diagnoses among adults and adolescents. More than half of all newly diagnosed HIV infections in 2005 were among men who have sex with men. Persons exposed to HIV through heterosexual intercourse with a nonregular partner accounted for just under one-third, while about 18% occurred among intravenous drug users.[11]

Racial and ethnic minorities continue to be disproportionately affected by the HIV epidemic. Although African-Americans represent about 13% of the population, they accounted for 51% of new HIV or AIDS diagnoses during 2001–2004. In 2004, estimated HIV and AIDS case rates for blacks and Hispanics were 8.5 and 3.3 times higher, respectively, than rates for whites. Among males and females, case rates among blacks were 7.0 and 21 times higher, respectively, than rates for whites. AIDS was the fourth leading cause of death among African-Americans aged 25–44 years in the United States in 2004.[12]

Between 1981 and 2004, more than half a million deaths among persons with AIDS were reported to the US Centers for Disease Control and Prevention (CDC). The most striking trend has been the decline in AIDS incidence and deaths that have occurred since 1995 when combination antiretroviral therapy first became available (see Fig. 84.2).

Number of reported AIDS cases by major transmission category and year of diagnosis – US, 1981–2004*



* Data adjusted for reporting delays. Cases without and assigned transmission category were redistributed on the basis of historical trends in risk factors.
† Defined as sexual contact with a person at high risk for or infected with HIV.

Fig. 84.2 Number of reported AIDS cases by major transmission category and year of diagnosis: USA, 1981–2004. (Courtesy of UN AIDS 2006 Report on the global AIDS epidemic.)

The proportion of individuals living at 2 years after AIDS diagnosis was 44% for 1981–1992, 64% for 1993–1995, 85% for 1996–2000 and 87% for 2000–2005.

After leveling off in the mid-1990s, the estimated total number of people living with HIV in Canada started to increase again in the late 1990s, mainly because of the life-prolonging effects of antiretroviral treatment to reach around 64 000. Women represented 17% of new HIV infections in 2006, as compared with 8.5% in 1995. The annual number of newly reported HIV infections stayed about the same during that period, at about 2500 per year.[13] Unprotected sex between men continues to account for nearly half of new HIV infections. An estimated 37% of new HIV infections in 2005 were attributed to unprotected heterosexual intercourse, with a substantial proportion among people born in countries with generalized HIV epidemic.

In Australia and New Zealand, just as in several countries of northern Europe and some parts of the USA and Canada, the vast majority of HIV infections have been acquired through sexual contacts between men. The annual number of HIV diagnoses in Australia peaked in 1987. There followed 12 years of decline, after which the rate of diagnoses grew again to reach nearly 1000 in 2006. The proportion of men having sex without a condom nearly doubled to 46% between 1997 and 2001, and the number of men who had unprotected sex with a casual partner climbed 10% to 26% over the same time period. Programs involving community members and allowing easy access to sterile injecting equipment and methadone treatment have permitted the prevalence in intravenous drug users to remain very low (<1%) in several major cities.[14]

Europe and Central Asia

This region had an estimated 2 360 000 persons living with HIV in 2007.[2] In Western Europe, the number of annual reported new HIV diagnoses almost tripled between 1999 and 2005 to reach nearly 20 000 but declined significantly thereafter.[15] Heterosexually acquired HIV infections, most of which were among immigrants and migrants, accounted for 42% of new HIV diagnoses in Western Europe in 2006. Of these newly diagnosed HIV infections, 29% were attributable to unsafe sex between men and only 6% (except in countries such as Spain and Italy) to intravenous drug use. More than half heterosexually transmitted HIV cases originate in countries with high HIV prevalence and, within that group, more than 50% of new HIV diagnoses are in women.[15]

Portugal has the highest HIV prevalence, estimated at around 0.3–0.5%. The annual number of newly diagnosed HIV infections has more than doubled in the UK, reaching about 9000 in 2006. This is mainly due to sustained levels of newly acquired infections among men who have sex with men, an increase in diagnoses among heterosexual men and women who acquired their infection in a high prevalence country, and improved reporting due to expanding HIV testing services. In central Europe, the number of newly diagnosed HIV infections in 2006 surpassed 100 only in Poland, Turkey and Romania. Elsewhere, the epidemics are comparatively small. Intravenous drug use is the most reported mode of HIV transmission in the three Baltic States where the epidemics appear to have stabilized.[16]

In Eastern Europe and Central Asia, the greatest majority of HIV cases in 2006 was from the two countries with the largest populations: the Russian Federation, with an estimated 1 million cases (three times the reported HIV cases) and to a lesser extent Ukraine, with around 380 000 persons living with HIV (four times the estimated number of reported cases) (Fig. 84.3). Nearly two-thirds were attributed to intravenous drug use and 37% were ascribed to unprotected sex. A large number of young and unemployed men inject and share drugs. Syringe exchange programs have recently increased but their coverage is too limited to make an impact on the HIV epidemic curve. As the Russian Federation's epidemics mature, AIDS mortality will contribute significantly to the demographic decline.

In addition to HIV, major epidemics of tuberculosis and syphilis have occurred in Central Asia against a background of political and economic instability, increased mobility, intravenous drug use and degradation of the public health infrastructure. In this region, the future course of the HIV epidemic is unpredictable.

Table 84.1 summarizes the levels of HIV prevalence in selected high-risk populations in Western, Central and Eastern Europe.

Sub-Saharan Africa

Sub-Saharan Africa is more heavily affected by AIDS than any other region of the world. An estimated 22.5 million people were living with HIV in the region in 2007, 61% of them women.[2] The region's epidemics vary significantly in scale, with national adult HIV prevalence ranging from less than 1% in some countries of the Sahel to around 15–20% in most of southern Africa. Southern Africa alone accounted for almost one-third of all new HIV infections and AIDS deaths

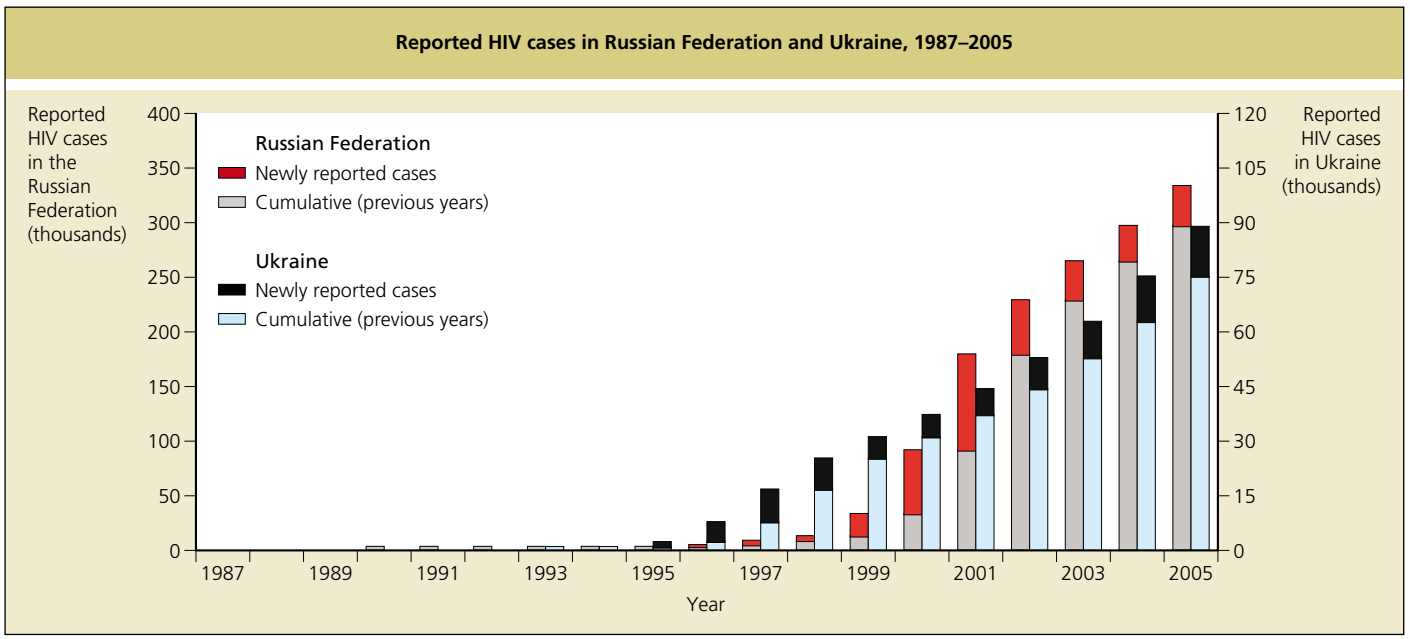


Fig. 84.3 Reported HIV cases in the Russian Federation and Ukraine, 1987–2005.

Table 84.1 HIV prevalence among high-risk populations by European regions

	Western Europe	Central Europe	Eastern Europe
Intravenous drug users	<5% in 11 countries >20% in Mediterranean countries	<2% in all countries except Poland (>20%)	5% in some countries Up to 60% in cities in Ukraine and Russia
Homosexual men	5–15%	3–10%	0–5%
Sex workers	Mostly <1%	<1%	Up to 15%
Patients with sexually transmitted disease	1–2%	<0.1%	0–2%

Adapted from EuroHIV.[15]

globally in 2007. A total of 1.7 million people in sub-Saharan Africa became infected with HIV in the past year, of whom 500 000 were in South Africa, declining from 2.2 million new infections in 2001. AIDS continues to be the single largest cause of mortality in sub-Saharan Africa, before malaria and lower respiratory tract infections. There are an estimated 11.4 million orphans due to AIDS in this region.

HIV prevention efforts are having an impact in some countries[2] (Fig. 84.4). In Kenya, HIV prevalence among young pregnant women declined significantly by more than 25% in both urban and rural areas, while similar declines were observed in urban areas of Malawi and Zimbabwe. Less striking declines in HIV prevalence in young pregnant women have also occurred in numerous sub-Saharan countries. While there is evidence of a significant decline in the national HIV prevalence in Zimbabwe,[17] the epidemics in most of the rest of the South African region have either reached or are approaching a plateau, associated with significant changes in sexual behavior.

HIV-2 is primarily found in West Africa but has also been confirmed in other African countries. The highest prevalence of HIV-2 infection is found in Guinea-Bissau, with prevalence rates as high as 8.0% in 1987–88 but with a decline to 2.5% in 2004. In contrast to the increasing spread of HIV-1, the prevalence of HIV-2 has remained rather stable in West Africa and has declined in Guinea-Bissau and in the Gambia.[18] This is probably the result of the higher transmissibility of HIV-1 compared with that of HIV-2.[8]

Asia and the Pacific

In Asia, national HIV prevalence is highest in South East Asia, with wide variation in epidemic trends between different countries.[19] Overall in Asia, an estimated 4.9 million people were living with HIV in 2007, including the 440 000 people who became newly infected in the past year. Approximately 300 000 died from AIDS-related illnesses in 2007.[2] New, more accurate estimates of HIV indicate that approximately 2.5 million people in India were living with HIV in 2006, with a national adult HIV prevalence of around 0.4%. The epidemic is extremely heterogeneous; for example, in Mumbai, Pune and Tamil Nadu state, HIV prevalences of 24% have been reported in pregnant women, contrasting with Calcutta and Delhi where prevalence has remained below 1%. Intravenous drug use is the predominant mode of transmission in the north-eastern states near Myanmar, where prevalence among drug users exceeds 70%. In Manipur cities, many of the women who tested HIV positive are sexual partners of male drug injectors. HIV prevalence levels among intravenous drug users usually far exceed levels among sex workers (Fig. 84.5).

In China, HIV infection was first noted in Yunnan Province bordering the Golden Triangle where Myanmar, Thailand and Laos meet. Here the prevalence of HIV infection in intravenous drug users climbed rapidly to 70% or more in the early 1990s, and HIV first emerged in sex workers in this region. At least 650 000 persons were living with HIV in China in 2006, around half were intravenous drug users, while

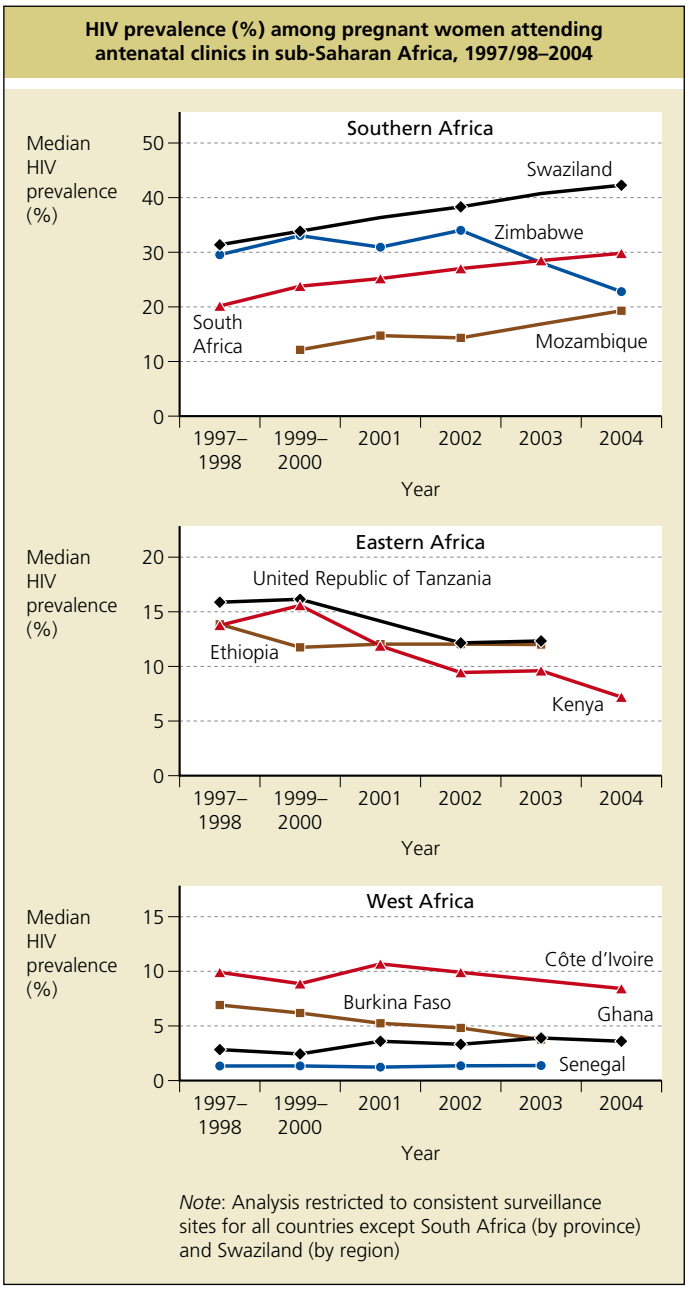


Fig. 84.4 HIV prevalence (%) among pregnant women attending antenatal clinics in sub-Saharan Africa, 1997/98–2004. (Courtesy of UN AIDS 2006 Report on the global AIDS epidemic.)

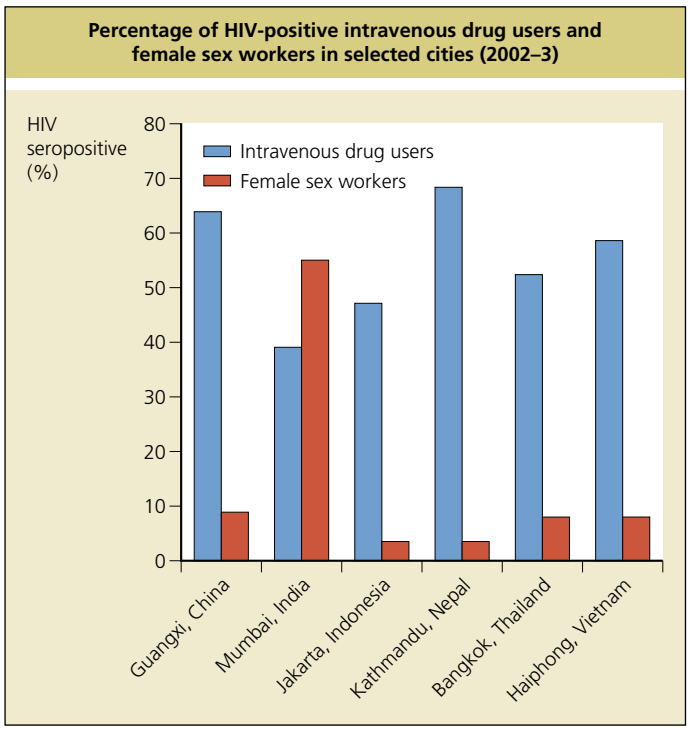


Fig. 84.5 Percentage of HIV-positive intravenous drug users and female sex workers in selected cities (2002–3).

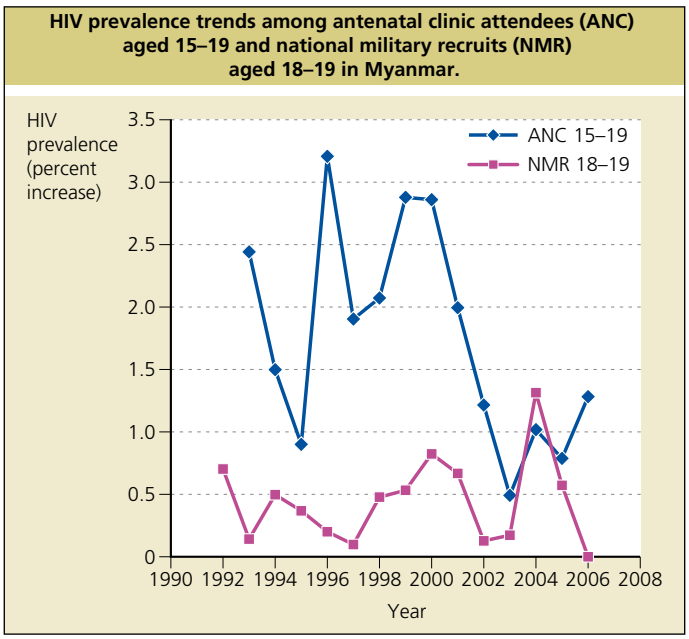


Fig. 84.6 HIV prevalence trends among antenatal clinic (ANC) attendees aged 15–19 and national military recruits (NMR) aged 18–19 in Myanmar.

the others became infected during unprotected paid sex. China has established more than 300 methadone clinics and needle exchange sites. In Sichuan Province these programs reduced the reuse of non-sterile needles by almost half. In 2000, serious HIV epidemics were documented in Henan Province in central China, where many thousands of rural villagers have become infected by selling their blood to illegal collecting centers.

The most heavily affected countries of South East Asia are Cambodia, Thailand and Myanmar, with intravenous drug use and commercial sex driving HIV transmission. In Thailand, the epidemic has spread in overlapping waves through intravenous drug users and sex workers, their clients and the female partners of clients. The prevalence among sex workers rose from 3% in 1989 to 30% in 1996, and that among sexually transmitted disease (STD) clinic patients rose from nearly zero to 9% over the same period before declining in the late 1990s. Risky behaviors were reduced following extensive programs to promote condom use in brothels and to discourage men from visiting them. The number of new annual HIV infections in Thailand continued to decline in 2008, although the decline in HIV prevalence has been slowing in recent years as more people are receiving antiretroviral therapy. HIV prevalence among intravenous drug users has remained high over the past 15 years, ranging between 30% and 50%.[20] Similarly, recent studies show increasing HIV prevalence among men who have sex with men in Bangkok from 17% in 2003 to 28% in 2007.[21]

The epidemic in Myanmar also shows signs of a decline, with HIV prevalence among pregnant women having dropped from 2.2% in 2000 to 1.5% in 2006, as well as in new military recruits (Fig. 84.6). The male to female ratio has changed from almost 8:1 in 1994 to 2.4:1 in 2006, showing a steady increase in the proportion of women being infected. While the epidemics in Cambodia also show declines in HIV prevalence, those in Vietnam and Indonesia (especially in the Papua Province) are growing. An HIV prevalence of more than 1% has been documented in Papua New Guinea, fueled mainly by heterosexual transmission. Risky behavior is widespread, with research showing high rates of unsafe sex with multiple partners and the frequent failure to use condoms.[22]

Recent sero-surveys conducted as part of national HIV surveillance in Asia show new HIV epidemics among men who have sex with men (MSM) in several countries, including India and China. Rates of HIV infection among MSM are as high as 14% in Phnom Penh, Cambodia; 16% in Andhra Pradesh, India, and 28% in Bangkok, Thailand. These data represent an alarming trend, since male–male sexual activity in the region is diverse, often completely hidden, and beyond the reach of current HIV prevention efforts. The increasing sex industry in parts of Asia is of concern, and male clients of sex workers and sex workers themselves are likely to be the most important group to target for prevention programs.[19]

Latin America and the Caribbean

With an estimated 100 000 people living with HIV in 2007, the HIV prevalence in Latin America remain generally stable, but HIV transmission continues to occur among populations at higher risk of exposure, including sex workers, their clients, intravenous drug users and MSM.[2] The epidemic in Latin America was initially similar to that in North America and Europe, with most cases among homosexual men and intravenous drug users. Systematic surveillance in the region is limited and the picture is complex, with MSM transmission predominating in some countries (Mexico, Chile, Ecuador and Peru, as well as in several Central American countries), while in others (Argentina and Brazil), intravenous drug use accounts for about half of all infections. However, in almost all countries there has been a rapid increase in cases attributed to heterosexual transmission, with a corresponding increase in the proportion of infections occurring among women. HIV rates in pregnant women are generally low, although there are estimates of prevalence higher than 1% in Honduras and Guatemala and in urban areas of Brazil and Argentina.

About one-third of all people living with HIV in Latin America reside in Brazil, where in 2005 an estimated 620 000 people were living with HIV. Although initially concentrated primarily among MSM, the epidemic subsequently spread to intravenous drug users and eventually into the general population, with increasing numbers of women becoming infected. It is estimated that a large proportion of infections among women can be attributed to the behavior of their male sexual partners. However, unprotected sex between men remains an important factor and is estimated to account for about half of all sexually

transmitted HIV infections in Brazil. HIV prevalence among intravenous drug users in Brazil has declined in some cities as a result of harm-reduction programs, changing from injecting to inhaling drugs. Mortality among drug users has also declined.[23]

Of the Central American countries, Honduras and Guatemala have been especially widely affected by heterosexual transmission. Overall, however, HIV infection is highest among female sex workers and their clients and among MSM.

In the Caribbean, adult HIV prevalence is estimated at 1.0%. HIV prevalence is highest in the Dominican Republic and Haiti, which together account for nearly three-quarters of the 230 000 people living with HIV in the Caribbean.[2] HIV prevalence shows clear signs of decline in these two countries, but AIDS remains one of the leading causes of death among adults. Declining HIV is also documented in the Bahamas, Barbados and Cuba. The primary mode of HIV transmission in this region is sexual intercourse, with unprotected sex between sex workers and clients a significant factor in the transmission of HIV. Among female sex workers, HIV prevalence of 3.5% has been found in the Dominican Republic, 9% in Jamaica and 31% in Guyana. HIV infection rates remain relatively low in Jamaica but have risen in marginalized groups such as crack cocaine users and migrant laborers.

North Africa and the Middle East

Except for Sudan, the number of HIV infections in these countries is very low.[2] So far, the spread of the virus appears to be limited to paid sex, sex between men and intravenous drug use in large cities. About 75% of the AIDS cases have been reported from Morocco, Sudan, Saudi Arabia, Tunisia and Djibouti. HIV seroprevalence levels among the general population show seropositivity lower than 1%. Djibouti and South Sudan are the hardest hit countries in the region, with prevalence levels of up to 9% in pregnant women in Djibouti and 2–5% in cities in South Sudan. Iran has made needles and syringes available in pharmacies in an effort to cut the spread of HIV infection among an estimated 200 000 intravenous drug users.[24] Libya's epidemic has been growing, with almost 90% of the 6000–10 000 HIV infections in Tripoli attributed to intravenous drug use. Transmission through contaminated injecting equipment has been reported in Libya, Bahrain and Oman. Because of the illegal character of homosexuality, there is concern that HIV may spread undetected among MSM in the region.

Dynamics of the HIV epidemic

Under circumstances that are not yet fully understood, epidemics may suddenly explode, with rates of infection increasing several-fold within only a few years. For example, estimation of HIV seroprevalence among intravenous drug users seeking treatment in Bangkok increased from zero in 1985–6 to 16% in 1988 and 40–60% in 1992.

In Central and Eastern Europe, before 1995, the total number of HIV infections, with over 450 million inhabitants, was less than 30 000. In 2006, Russia estimated at around one million the number of people with HIV and AIDS. Between 1996 and 1998 alone, Russia experienced a 100-fold increase in new HIV infections, demonstrating the rapid onset of the epidemic among intravenous drug users. The epidemic has slowed through better knowledge of the risks of HIV transmission and a saturation effect. Outwith rapid HIV spread among intravenous drug users, contrasting situations have been found in the general population. Cambodia did not record its first diagnosis of HIV infection until 1991. By 1996, HIV prevalence among pregnant women was approaching 5% but was estimated to have declined to 1.6% at the end of 2005 through the implementation of 100% condom use in brothels.

In sub-Saharan Africa, most new HIV infections are occurring among young people and particularly among young girls (Fig. 84.7). There is often a doubling of the HIV rate between the 15–19 age group and the 20–24 age group. The pattern of higher HIV infection among girls than boys in sub-Saharan Africa is consistent regardless of the overall level of HIV prevalence, and regardless of whether the study is conducted in an urban or a rural area.

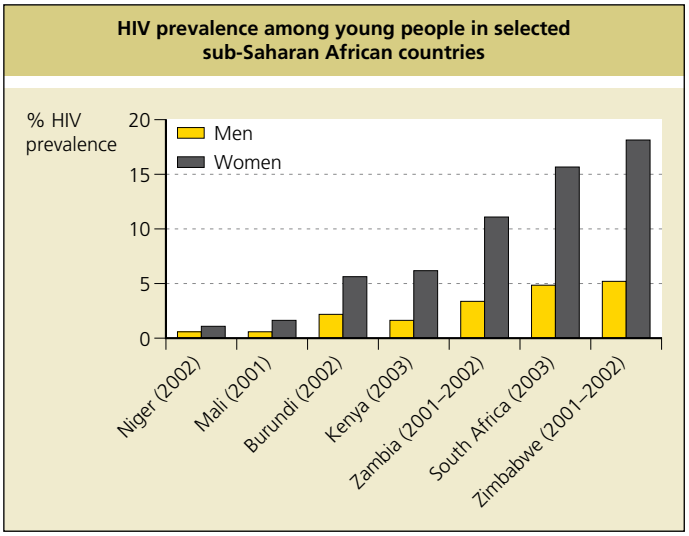


Fig. 84.7 HIV prevalence among 15–24-year-old men and women in selected sub-Saharan African countries, 2001–2003.

Sexual behavior patterns and sexual networks are believed to play a critical role due to an increased biologic susceptibility to HIV/STD associated with early age at first sexual intercourse[25,26] and because of new primary infections associated with a peak in infectiousness.[27] Age at first sex before 15 years is reported by around 15% of young women in selected countries (Fig. 84.8). In only five national surveys from 18 countries for which recent data are available, more men than women had had first sexual relations before age 15. Social factors increasing HIV exposure and susceptibility of young girls include gender inequality, older sexual partner, male sexual violence, standards of masculinity and low levels of access to information and services. The practical effect of these factors is a greater difficulty in refusing sex and/or negotiating protected sexual intercourse. Other factors increasing the efficiency of the transmission of HIV are the lack of male circumcision and the prevalence of other STDs. More attention is given now to the role of herpes simplex virus type 2 (HSV-2) as enhancing susceptibility to HIV.[28]

There is no evidence that different genetic subtypes of HIV-1 are associated with differences in the efficiency of spread of the virus in a population. The observation that some sex workers who had been repeatedly exposed to HIV infections remained HIV-antibody-negative raised the hypothesis that some people have less susceptibility to, or even immunity against, the virus or specific subtypes. Factors such as certain human leukocyte antigen (HLA) haplotypes and polymorphisms of the chemokine receptor gene CCR5, as well as genital HIV-neutralizing IgA and systemic HIV-specific proliferative responses, were associated with HIV nonacquisition.[29,30]

MODES OF TRANSMISSION

Sexual transmission

In contrast to the industrialized world, heterosexual intercourse accounts for more than 85% of cases of HIV infection in developing countries. Early epidemiologic studies showed that risk factors associated with HIV infection were unprotected sexual intercourse with multiple partners or an infected partner and the presence of STDs or a history of STDs.[31,32]. More recent studies have highlighted sex differences in patterns of HIV transmission; for many monogamous women the main risk factor for HIV may be the heterosexual and homosexual behavior of their steady partner.[33] Although the probability of HIV transmission associated with unprotected vaginal sexual intercourse is not constant from one contact to another, estimates per episode – based on discordant couples – ranged from 0.0008 to 0.0015 in a study in Uganda.[34]

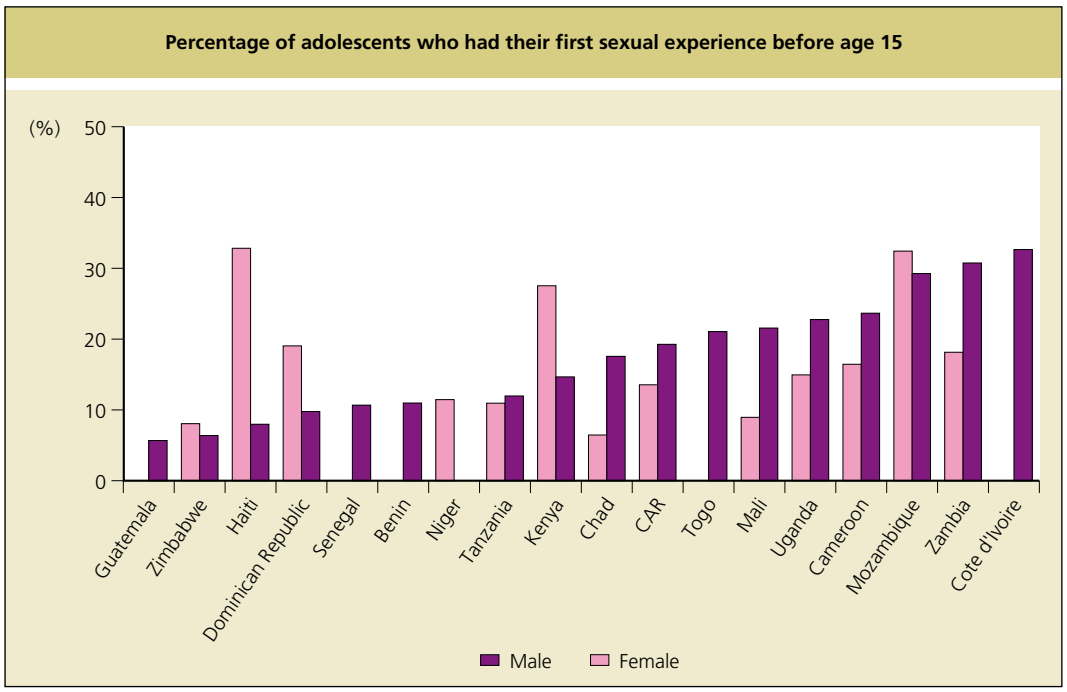


Fig. 84.8 Percentage of adolescents who had their first sexual experience before age 15 years.

A summary of biologic factors influencing the probabilities of HIV transmission from an infected person to a susceptible individual is given in Table 84.2. The presence of STDs suggests a marked risk of concurrent HIV infection, for at least two reasons:

- the modes of transmission of HIV and other STDs are similar; and
- the role of STD-induced genital ulcers, including genital herpes, chancroid and syphilis, as well as nonulcerative STD, facilitates transmission of HIV.

Early studies among female sex workers in Kinshasa and Nairobi found gonorrhea, chlamydial infections and trichomoniasis to be independent risk factors for HIV acquisition, with relative risks of 2.7–3.5.[35] HSV-2 infection may also enhance HIV acquisition and HSV-2 and HIV co-infection may facilitate HIV transmission and perhaps accelerate HIV disease progression.

A meta-analysis of prospective observational cohort studies showed that HSV-2 seropositive male and female patients had a threefold higher risk of acquiring HIV-1 compared with HSV-2 seronegative individuals.[36] More recently, in two cohorts studies in Uganda and Zimbabwe, hazard ratios for HIV-1 acquisition were 2.8% and 4.4% for prevalent HSV-2 infection, respectively, and 4.6% and 8.6% for incident HSV-2 infection, respectively.[37]

Table 84.2 Biologic factors increasing the probability of sexual transmission of HIV

Confirmed	Lack of male circumcision Acute primary HIV infection Advanced clinical stage of HIV Viral load Sexually transmitted diseases Receptive anal intercourse Sex during menstruation
	HIV-1 versus HIV-2
Under study	First sex and traumatic sexual intercourse Cervical ectopy Genital trauma (use of vaginal products)
	Specific HIV-1 clades

HSV-2 is now the leading cause of genital ulcer disease in both developing and developed countries. Several trials have been conducted or are currently in progress to measure the effect of aciclovir suppressive therapy in reducing HIV acquisition among HIV seronegatives, reducing HIV transmission from HIV and HSV-2 dually infected persons, and reducing HIV disease progression. Two recent trials reported no efficacy of HSV-2 suppressive therapy in reducing HIV acquisition, an unexpected outcome considering the extensive and consistent evidence for epidemiologic synergy between the two infections.[38,39]

Early diagnosis and treatment of STDs were shown to reduce the spread of HIV infection in two randomized trials in Mwanza, Tanzania and in Southern Uganda. In the intervention communities, a 42% decline in the rate of newly acquired HIV infections was observed[40,41] but later studies found no effect. Data from a randomized controlled trial undertaken in South Africa[42] and two others in Kenya and Uganda show that male circumcision reduces the risk of heterosexually acquired HIV infection in men by approximately 60%. This evidence supports the correlation between lower HIV prevalence and high rates of male circumcision in some countries in Africa. Lack of male circumcision is associated with the risk of acquiring other STDs, especially genital ulcer.[43]

The impact of male circumcision on HIV transmission to women is under study.

Perinatal transmission

As a result of heterosexual transmission in women, the number of infants born with HIV infection is growing dramatically. It is estimated that globally around 420 000 infants become infected with HIV-1 every year.[2] Nine out of 10 new infections in children occur in sub-Saharan Africa. Mother-to-child transmission of HIV can occur in utero, intrapartum or postnatally via breast-feeding. Out of these annual new pediatric infections, more than 200 000 are the result of breast milk transmission, almost exclusively in developing countries. Breast-feeding by HIV-positive mothers (in the absence of antiretroviral therapy for either mother or baby) can account for up to one-third of HIV infections among babies in sub-Saharan Africa. HIV transmission risk through breast-feeding is enhanced during primary maternal HIV infection acquired after the baby has been put to the breast.[44] The additional risk of postnatal transmission attributable to breast-feeding without a maternal primary HIV infection is estimated

at about 14%. Duration of breast-feeding, maternal plasma and breast milk viral load, cracked nipples, mastitis, breast abscess and infant thrush increase the risk for transmission of HIV-1. Exclusive breast-feeding is associated with a reduced risk of HIV-1 transmission as compared to mixed feeding.[45]

Most countries are making substantial progress towards preventing mother-to-child transmission of HIV, particularly in sub-Saharan Africa. In low- and middle-income countries, the proportion of HIV-positive pregnant women receiving antiretroviral prophylaxis to reduce the risk of transmission increased from 10% in 2004 to more than 30% at the end of 2007 (Fig. 84.9). Of note is the steady progress made in eastern and southern Africa, home to the majority of children who have been newly infected (see Chapters 97 and 98).

Blood products and contaminated equipment

Because of the rational use of blood, systematic HIV screening of blood donors and avoidance of donors self-reporting at higher risk, the risk of transmitting HIV infection through blood transfusion is estimated to be less than 1/100 000 units of blood. Although the situation of contaminated blood is improving and most countries have blood screening policies, implementation is still not universal. In some developing countries, however, a significant proportion of blood donations remain unscreened for HIV and an estimated 5% of HIV infections may still occur through blood transfusion, with women and children at greater risk because of frequent anemia. Clinical indications for appropriate blood transfusions are often not met outside of large cities in low-income countries.

Drug injecting is a strong driver of HIV infection in many regions. In 2003, there were estimated to be around 13 million intravenous drug users worldwide, the majority in Eastern and Central Europe and Asia (Fig. 84.10). Needle sharing with HIV-infected persons is one of the most effective ways of spreading HIV. In Russia, for example, it is estimated that 30–40% of intravenous drug users use nonsterile needles or share needles. In addition, a majority of intravenous drug users have sex not only with their female partners but also with sex workers. In 2007, 34% of countries with a concentrated epidemic implemented harm-reduction programs to reduce risk among intravenous drug users. Regionally, prevention coverage for intravenous drug users is highest in South and South East Asia, at 62%.

In many developing countries disposable needles and syringes are not available and sterilization practices are inadequate. The use of other skin piercing instruments, for instance for scarification and circumcision, also has some potential for HIV transmission. This results in potentially frequent parenteral exposure to HIV in populations where HIV prevalence is high, including among health-care workers.

The mean probability of HIV infection due to puncture by a contaminated needle was recently estimated to be 0.23% (0.00–2.38%).[46] The potential for HIV transmission by unsterilized needles in medical settings is probably weak, but localized outbreaks in the Russian Federation, Romania and Libya among infants and young children have shown that it can occur in special circumstances. In Romania, over 90% of cases have occurred in children living in public institutions as a result of the re-use of contaminated and inadequately sterilized injection equipment or repeated microtransfusions of contaminated blood from one child to another.

In 2001, it became widely known that in a number of Chinese provinces, but mostly in Henan in central China, paid blood donation had caused HIV infections, with estimates ranging from below 100 000 to several hundred thousand. Before 1996, poor rural farmers had been selling blood and plasma to commercial blood processing companies to supplement their small income. Blood from many

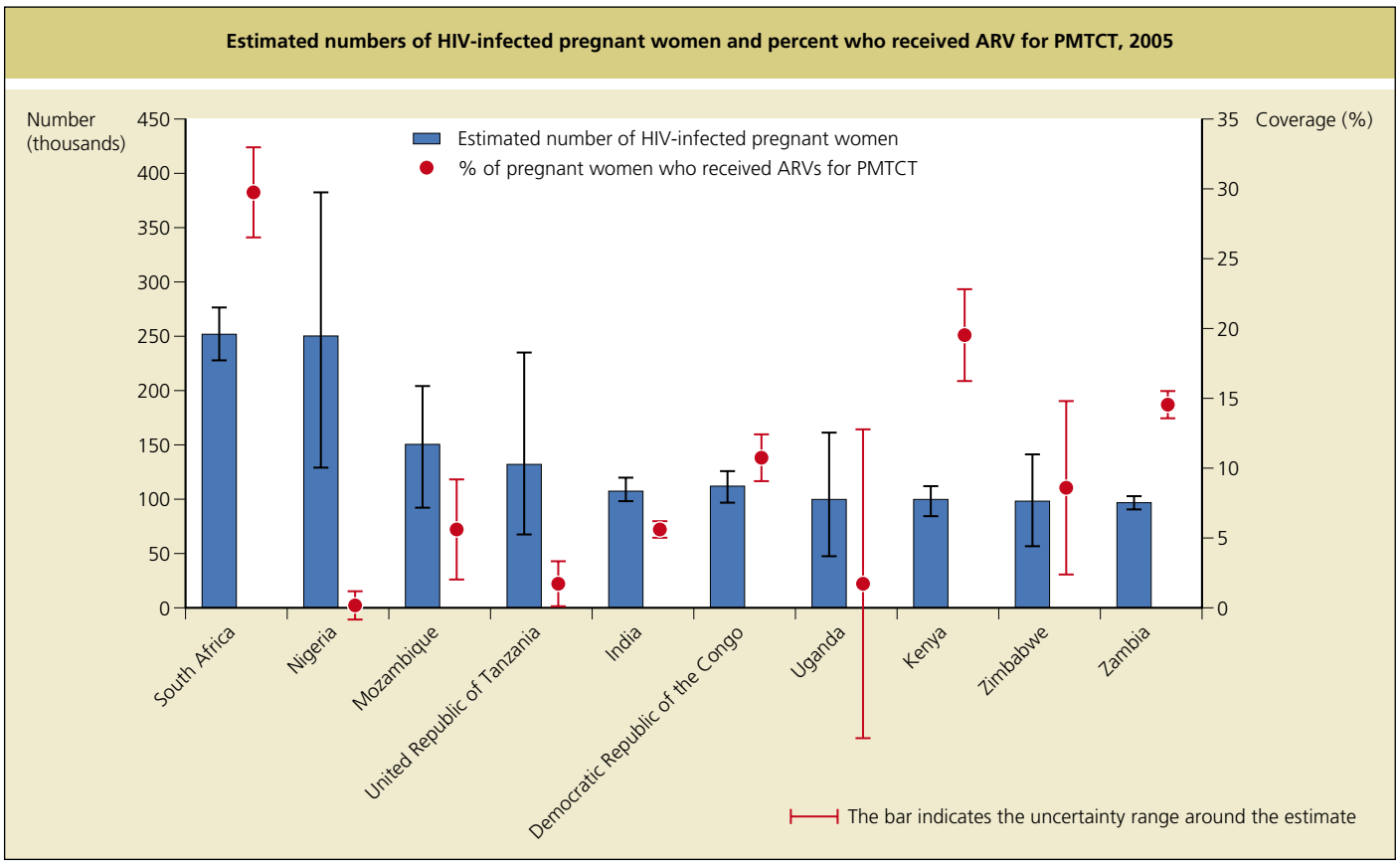


Fig. 84.9 Estimated numbers of HIV-infected pregnant women and percentage who received antiretrovirals (ARVs) for preventing mother-to-child transmission (PMTCT), 2005. Courtesy of WHO.

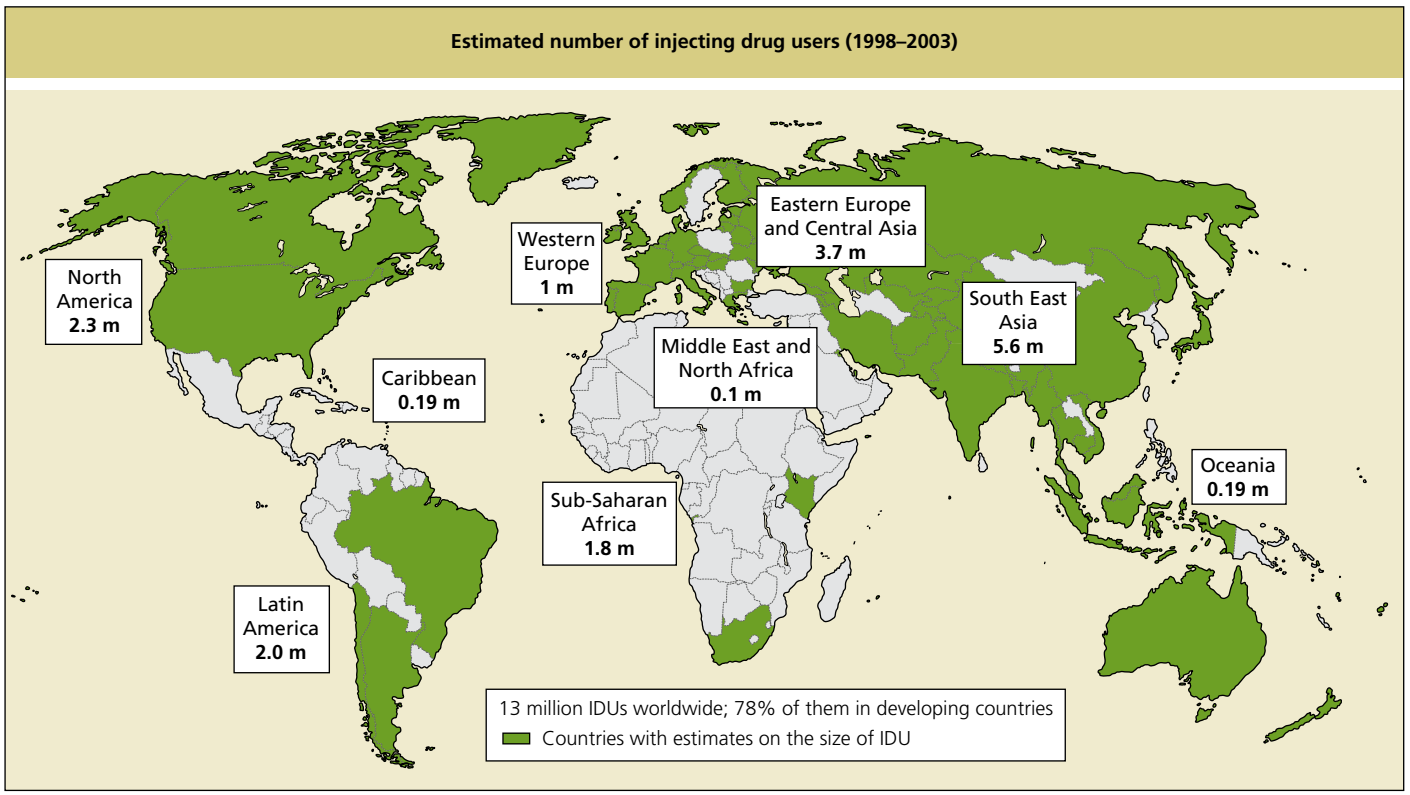


Fig. 84.10 Estimated number of intravenous drug users (IDUs) in 130 countries and territories, 1998–2003.

donors was collected and mixed. The red blood cells were separated from the pooled plasma and re-injected back into donors to reduce anemia. This unacceptable practice has been stopped.

NATURAL HISTORY

The natural history of HIV infection in many developing countries is similar to developed countries. Recently, the UNAIDS reference group estimated that the survival time from seroconversion to death in the absence of antiretroviral therapy (ART) is about 11 years (8 years latent period and 3 years from ART eligibility to death).

It is recognized that HIV-1 subtypes differ in biologic characteristics that affect pathogenicity. A recent study in Uganda found that HIV disease progression is affected by HIV-1 subtype. The median time to AIDS onset was shorter for persons with subtype D (6.5 years), recombinant subtypes (5.6 years) or multiple subtypes (5.8 years) compared with persons with subtype A (8.0 years).[47]

Pneumocystis jirovecii, pneumonia and Kaposi's sarcoma are less frequently associated with AIDS in developing countries than in high-income countries. There is evidence of pathologic interactions between HIV and malaria in dually infected patients. A transient elevation in HIV viral load occurs during febrile malaria episodes; in addition, susceptibility to malaria is enhanced in HIV-infected patients. Co-infection might also have facilitated the geographic expansion of malaria in areas where HIV prevalence is high. Hence, transient and repeated increases in HIV viral load resulting from recurrent co-infection with malaria may be a factor in promoting the spread of HIV in some part of sub-Saharan Africa.[48]

An unanticipated consequence of the AIDS epidemic has been a dramatic rise in the incidence of tuberculosis (TB). TB notification rates in Africa have doubled since the early 1980s, just after the start of the HIV epidemic, with the largest rise in countries most affected by the HIV epidemic. In sub-Saharan Africa, the relative risk of TB in those with, compared to those without, HIV infection has varied from four to more than 20.[49] In addition, among people who have HIV infection, TB is now the most common and deadly opportunistic infection, accounting for 40% of all adult death. In this population, extension of multiple resistance to anti-TB drugs is a major concern. The prevalence and consequences of multidrug-resistant and extensively drug-resistant (XDR) TB was assessed in a rural area in KwaZulu Natal, South Africa.[50] Of the 6% of patients who were diagnosed with XDR TB, all were co-infected with HIV. Of these patients, all except one died, with median survival of 16 days from the time of diagnosis.

This new situation has posed an unprecedented challenge to TB control programs in much of the developing world (see Chapter 93).[51] Only 31% of individuals living with HIV and TB co-infection received both antiretroviral and anti-TB drugs in 2007.

Health and social impact

Approximately 3 million people in low- and middle-income countries were receiving ART as of the end of 2007, compared to about 200 000 receiving ART in 2002. ART programs in Africa retain about 80% of patients 6 months after the initiation of treatment and 60% of patients at 2 years.[52] Antiretroviral therapy coverage has reached around 30% of those in need according to the most recent data but large variations can be seen (Fig. 84.11).

However, despite these achievements, the epidemic continues to outpace the response. With five new infections for every two people on treatment in 2007, it is obvious that HIV prevention efforts must be greatly intensified to stop this epidemic. AIDS remains the leading cause of death in Africa, before malaria and lower respiratory tract infection, with the biggest increase in mortality among adults aged 20–49. Demographic projections suggest that life expectancy in sub-Saharan countries most affected by HIV has been reduced by as much as 15 years when compared with projections without HIV (Fig. 84.12). The estimated impact of AIDS on juvenile mortality in these countries was extremely high between 2002 and 2005 (Fig. 84.13).

Outside of Africa, in regions with lower HIV prevalence, AIDS has slowed rather reversed gains in life expectancy. But for young adults aged between 20 and 35 years, even in Asia for example, AIDS deaths are among the leading cause of death as compared to other diseases (not accidents).[19]

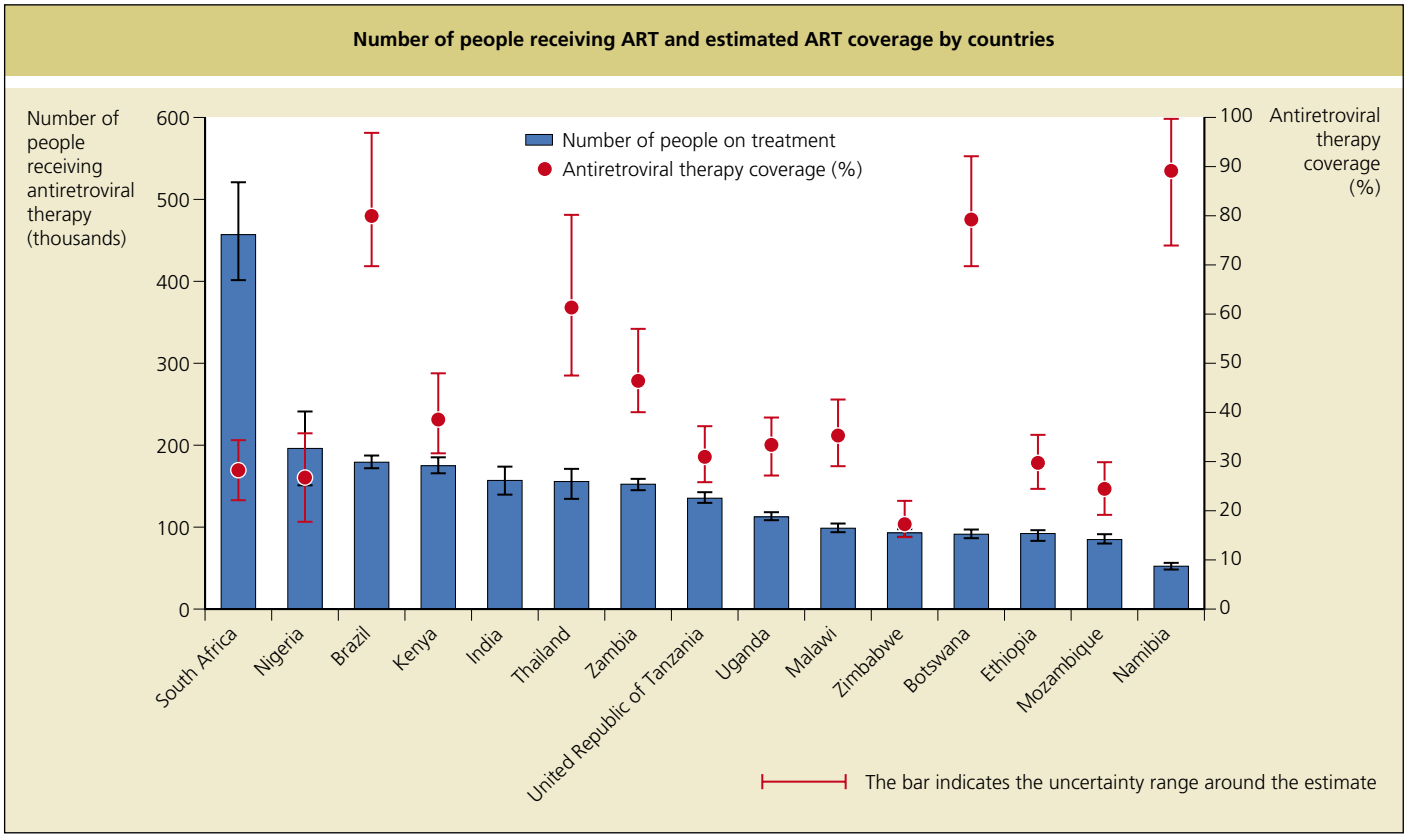


Fig. 84.11 Number of people receiving antiretroviral therapy (ART) and estimated ART coverage by country, 2007. (Courtesy of UN AIDS 2006 Report on the global AIDS epidemic.)

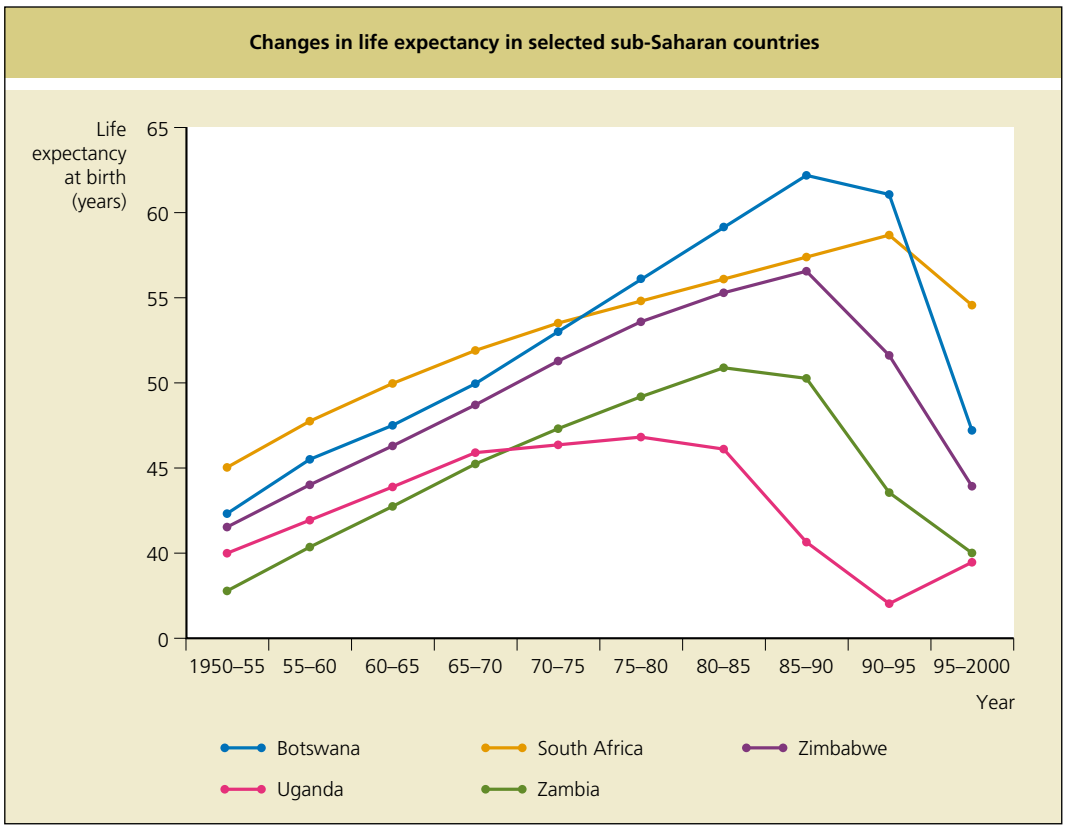


Fig. 84.12 Changes in life expectancy in selected sub-Saharan countries. Projected life expectancy at birth, 1950–2000.

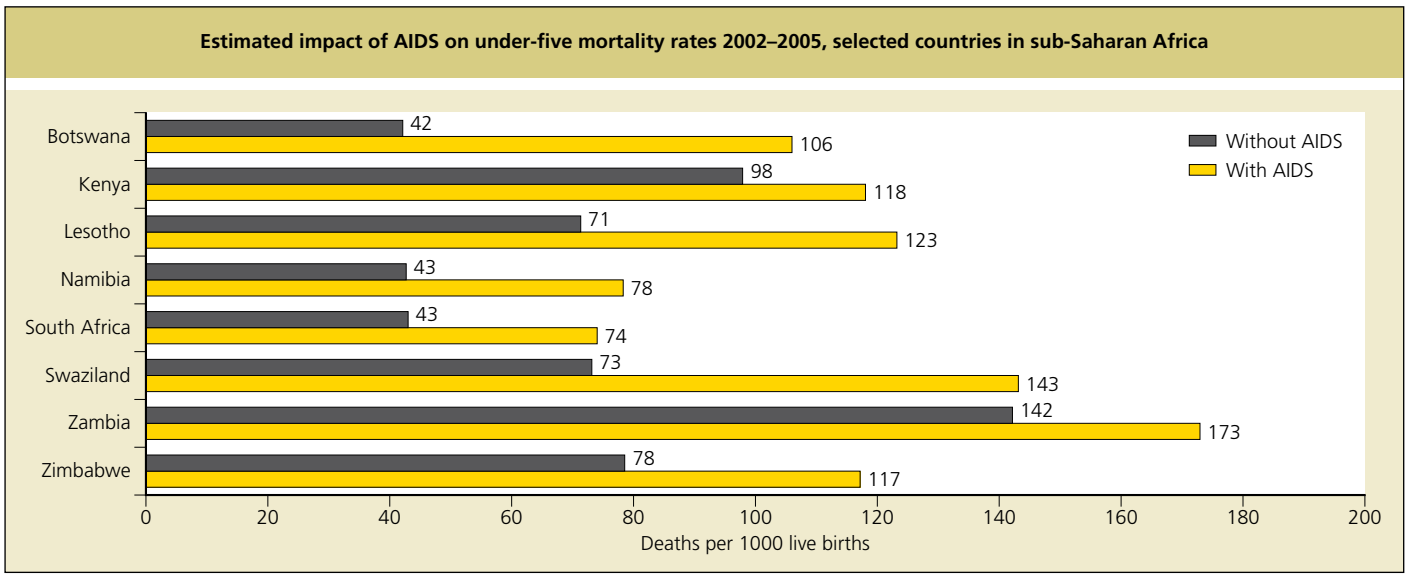


Fig. 84.13 Estimated impact of AIDS on under-five mortality rates, 2002–2005, in selected countries in sub-Saharan Africa. (Courtesy of UN AIDS 2006 Report on the global AIDS epidemic.)

In southern and eastern Africa, the economic impact of AIDS is felt not only on health-care costs but also on skilled labor forces, on certain economic sectors and on households, which in turn has a negative impact on many social indicators such as education and health.

AIDS has profoundly affected the health systems in high-income and developing countries alike. AIDS has illustrated the need to strengthen some of the weakest components of health services, such as access to appropriate care, counseling and prevention activities, and participation of persons living with HIV in treatment and medical decision making. Strong health systems are central to mitigating AIDS impact. The provision of widespread access to ART was central in bringing to light deficiencies in developing world public health systems, most of which are not equipped to manage chronic conditions on a large scale. In addition to severe shortages of basic infrastructure such as facilities and equipment, the countries where HIV is most widespread also face serious shortages of trained health-care providers, including doctors, nurses and support staff. According to the World Health Organization (WHO), 57 countries, mostly in sub-Saharan Africa but also including Bangladesh, India and Indonesia, face health workforce shortages: more than 4 million health workers are needed, including at least 2.4 million doctors, nurses and midwives. The epidemic itself is a leading cause of health worker attrition. Botswana, for example, lost nearly one-fifth of its health workforce to AIDS between 1999 and 2005.[53] Favorable task-shifting approaches, such as including community workers, have been documented by WHO. Most HIV prevention activities besides preventing mother-to-child transmission take place outside health services and many are implemented by nongovernmental organizations.

Recent evidence clearly indicates that at least in Africa, AIDS is a disease of inequality, often associated with economic transition, rather than a disease of poverty in itself.[54] Higher income groups usually living in urban settings have higher HIV prevalence than those living in rural areas. Among urban dwellers, those living in shanty towns and in poor slums also show higher risk behaviors and higher HIV prevalence than those with higher socioeconomic status. Unlike diseases such as TB, HIV is mostly transmitted through sex. This brings into play the economic perspective around reward and dependency, which influences the extent to which individuals are able to make and exercise choices about sexual behavior. Where women's economic and social safety is largely dependent on their partners' occupations and status, they have little choice in determining their own sexual safety. Gender inequality is one of many injustices fuelling the epidemic. The spread of HIV is disproportionately high among many groups that experience discrimination and suffer from a lack of human rights protection. This includes groups that have been marginalized socially, culturally and often economically, such as intravenous drug users, sex workers, migrants, internally displaced persons and MSM.

The HIV pandemic kills adults in their most productive years, when they are responsible for childcare. The epidemic is dramatically increasing the numbers of orphans, particularly in high-prevalence countries. More than 15 million children worldwide have lost their mothers or both parents because of AIDS, over 12 million of them in sub-Saharan Africa, overwhelming the capacity of social networks and traditional patterns of intergenerational dependency. According to recent surveys, an estimated 15% of orphans in 11 high-prevalence countries live in households receiving some form of assistance, including medical care, school assistance, financial support or psychosocial services. As a threat to development of many countries, the AIDS epidemic still requires large public funding, not only for ensuring universal access to HIV prevention, care and support programs, but also for research and development of new prevention tools, such as vaccines and microbicides.

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References for this chapter can be found online at http://www.expertconsult.com

Chapter **85**

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Prevention of HIV transmission through behavioral change and sexual means

HIV TRANSMISSION DYNAMICS

HIV transmission is a high consequence, but low probability event with the majority of relevant exposures *not* resulting in new infections.[1] There are multiple co-factors that may affect HIV transmission, which is why there is a high level of variability around estimates of the relative risk of infection for specific exposures (Table 85.1).[2,3] HIV may be transmitted as cell-free or cell-associated virus, and different factors may affect expression of virus concentrations in different body fluids (i.e. blood, semen or cervicovaginal secretions). Although lower blood concentrations of HIV are associated with lower rates of HIV transmission,[4] antiretroviral drugs do not necessarily make HIV-infected people noninfectious or incapable of transmitting the virus. In fact, the sexual transmission of multidrug-resistant HIV has been well documented,[5,6] underscoring the need for providers to promote safer sex among the patients in their care, including those taking antiretroviral therapy.

BIOLOGIC ISSUES RELATED TO HIV TRANSMISSION

HIV is most often transmitted through intimate sexual contact by rapidly binding to cells that are present in the cervical, vaginal, penile, urethral and rectal mucosa.[7] The male foreskin contains abundant cells that can bind HIV; thus, being uncircumcised confers an additional risk for HIV seroconversion[8,9] (Table 85.2). All of the specific mechanisms responsible for the sexual transmission of HIV in humans are not fully understood, since HIV can be found either as cell-free or cell-associated virus in blood and genital secretions, and can bind multiple cell types.[10,11] The cells that can bind HIV in the genital tract include T-helper lymphocytes, monocyte/macrophage cells and Langerhans cells, as well as follicular dendritic cells. These latter cells may be particularly important because of their mobility, since they can bind HIV on their surface membranes and/or internalize it and migrate via draining lymphatics to distal sites, where propagation in submucosal lymphoid tissue can occur, resulting in subsequent viral dissemination through the bloodstream.

Factors that are associated with increased HIV infectiousness include sexually transmitted infections[12] and noninfectious factors that can result in genital tract inflammation (e.g. douching), recruiting more white blood cells to genital mucosal surfaces.[13] Among the sexually transmitted diseases, ulcerative sexually transmitted infections such as syphilis, chancroid and genital herpes simplex virus infection afford additional portals of entry through mucosal ulcerations, but also recruit inflammatory cells that bind and propagate HIV infection.[14] Other local genital factors that are associated with increased inflammation include douching and traumatic sexual intercourse, particularly after sexual assault.

Different tissues in the genital tract have varying levels of susceptibility to being infected with HIV. The vaginal epithelium is stratified, and contains fewer cells with co-receptors that can bind HIV.[13] Thus, vaginal mucosa is less likely to become HIV infected than the endocervix, which has a thinner layer, is highly vascular and contains a much higher concentration of HIV-binding cells. Any physiologic event that results in ectropion (i.e. increased exposure of the

Table 85.1 Estimates of per-contact risk of HIV infection

Type of contact	Risk
Needle sharing	6/1000 to 3/100
Occupational needlestick	1/300
Receptive anal	8/1000 to 3/100
Receptive vaginal	8/1000 to 2/1000
Insertive anal or vaginal	3/10,000 to 1/1000
Receptive oral	Case reports/no denominator

Table 85.2 Modifiers of the efficiency of HIV transmission

	Infectiousness	Susceptibility
Sexually transmitted diseases	↑	↑
Genital tract inflammation (e.g. traumatic sex, douching)	↑	↑
Circumcision	↓	↓
Cervical ectopy	?	↑
Genetics*	?	↓
HIV subtype†	↑	NA
Monocytotropic strain	↑	NA
Acute infection	↑	NA
Advanced infection	↑	NA
Antiretroviral therapy	↓?	NA

*CCR5 mutation.
†Subtype A or C compared to B.

endocervix), such as the use of hormonal contraceptives or occult *Chlamydia trachomatis* infection, will increase susceptibility to HIV.[15] The penile foreskin contains many cells that can readily bind and express HIV, resulting in increased HIV acquisition or transmission in uncircumcised males.[9] Three randomized trials of HIV-infected men in Africa have shown that adult male circumcision decreased HIV incidence by 50%.[16–18] The oropharynx contains many fewer cells that may bind HIV, which may partially explain the relative inefficiency of oral exposure to HIV as a means of transmission.[19] Moreover, salivary secretions contain several compounds that have been found to inhibit HIV transmission *in vitro*, most notably secretory leukocyte protease inhibitor (SLPI).[20] However, young rhesus macaques have been readily infected with simian immunodeficiency virus after oral challenge, with evidence of viral replication in tonsillar and adenoidal tissues.[21]

EPIDEMIOLOGIC ISSUES RELATED TO HIV TRANSMISSSION

Because of the multiple co-factors that may alter the amount of virus in the blood and genital tract, the calculation of the exact risk for infection for each type of HIV exposure is imprecise (see Tables 85.1, 85.2). Moreover, much of the data that have been obtained to generate per-contact risks have been based on cohort studies in which individuals recollect their level of risk during preceding time intervals (often every 3–6 months). Some of the participants in these studies may be worried that they have become newly infected or their sexual behavior may have been under the influence of drugs or alcohol, affecting precise recall. Thus, although many people want to have a precise calculation of risk associated with specific practices, it is very difficult to determine with any certainty the precise likelihood of transmission for each specific act. It is important when patients ask questions about the likelihood of risk after an exposure to reassure them that a single, one-time exposure to HIV is unlikely to result in transmission, but that the reason why the epidemic has become so widespread is because of individuals engaging in recurrent risk-taking behavior.

Having noted the limitations of how risk calculations have been obtained, certain key principles have emerged. Direct intravenous exposure to HIV (e.g. blood transfusions) is the most efficient way of transmitting the virus, while percutaneous needlestick injuries are much less efficient in transmitting HIV.[22] Individuals who share needles who pull back on the syringe and have substantial blood in the syringe when passing a syringe to their partner are more likely to transmit HIV than in a common health-care setting where an occupational needlestick injury occurs with a solid suture needle.[23] The range of risk for individuals who share intravenous drug paraphernalia ranges from 0.6% to 3% (see Table 85.1). This range overlaps with the level of risk for individuals who engage in unprotected receptive anal or unprotected receptive vaginal intercourse.[24] One study suggested that, on average, receptive anal intercourse was more than seven times as efficient in transmitting HIV as insertive anal intercourse.[25]

For each type of exposure, many contextual variables may alter the risk of transmission. For example, variations in the prevalence of HIV in different communities may mean that the same risk behavior has a different likelihood of resulting in an infection in two different communities. The amount of virus in the infected source plays a role in determining the risk of becoming HIV infected after a relevant contact. Co-factors, like a source with a high concentration of plasma HIV RNA[4,26] or concomitant sexually transmitted infection, can greatly increase the average per-contact risk.[27,28]

In the developed world, the epidemiologic data would suggest that men are more likely to transmit HIV to their female partners. However, in several studies of HIV serodiscordant couples in sub-Saharan Africa, the rates of male-to-female and female-to-male transmission were quite similar.[4] The reasons for the difference in the efficiency of female-to-male transmission in the developing world compared to the developed world may be because of the decreased prevalence of male circumcision in the places where these studies were conducted, as well as the high co-prevalence of other sexually transmitted infections. For anal intercourse, the insertive partner is less likely to acquire HIV from an infected receptive partner than vice versa; however, there are sufficient cells in the distal male urethra and the male foreskin, and such an abundance of HIV-infected cells and mucus secretions containing virus in infected receptive partners, that an insertive partner still is at substantial risk of acquiring HIV from unprotected intercourse.

It is clear that receptive oral intercourse, either fellatio or cunnilingus, is a much less efficient way to acquire HIV, but case reports have been well documented showing that oral exposure to ejaculate may result in HIV transmission. The relative efficiency of oral exposure to HIV is below that of unprotected vaginal intercourse, thus it may be in the realm of less than 1 per 1000 contacts. However, animal studies have demonstrated that HIV can be readily transmitted orally, since there is lymphoid tissue in the oropharynx that may acquire HIV from genital secretions.[21] In counseling patients who have concerns about the risk of acquiring HIV through oral exposure, it is important to indicate that it is less efficient than unprotected anal or vaginal intercourse, but that transmission can still occur. Thus, if an individual wants a zero risk situation, it is preferable for them to avoid any oral exposure to semen or cervicovaginal secretions from a partner who is known to be HIV infected or at risk. However, if oral sex is a substitute for unprotected anal or vaginal sex, the patient can be told that this is less risky. Although HIV has been found in very small concentrations in pre-ejaculatory secretions, there are no reliable case reports of HIV transmission through exposure to pre-ejaculate without semen. Thus, it is important to counsel patients that being able to negotiate with their partners about limiting exposure to semen may be a helpful means for 'harm reduction'.

PREVENTION OF HIV TRANSMISSION

HIV transmission may be decreased by biologic or behavioral means (Fig. 85.1). Treating sexually transmitted infections can decrease genital tract HIV load in co-infected patients and can decrease susceptibility in at-risk persons.[20] Antiretroviral therapy can decrease genital, as well

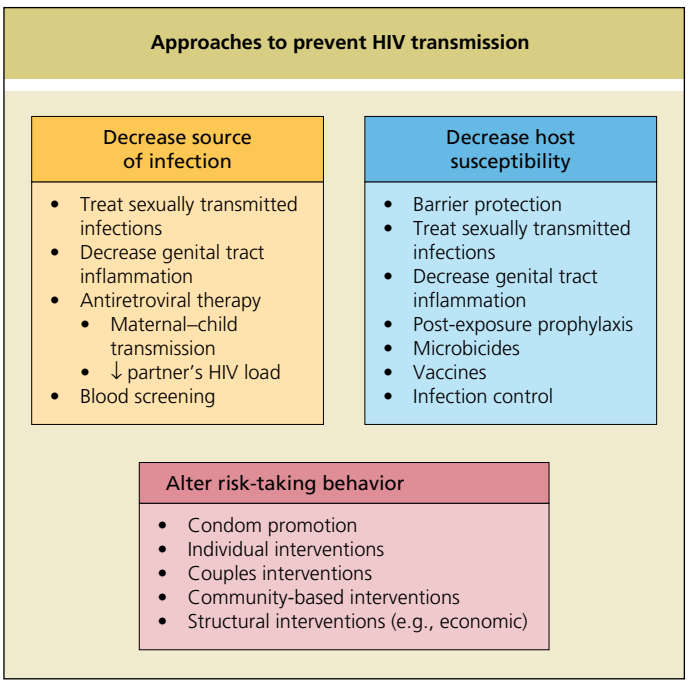


Fig. 85.1 Approaches to preventing HIV transmission.

as plasma viral load, but the transmission of multidrug-resistant HIV suggests that these drugs may only have an impact on wider HIV transmission if coupled with programs that encourage safer sexual behavior and optimal drug therapy adherence. Antiretroviral drugs may be used to decrease the likelihood of mother-to-child transmission, or be used for postexposure prophylaxis. However, for any biologically plausible intervention to work, attention will need to be paid to the behavioral context in which the intervention will be undertaken. For example, a biologically effective microbicide may fail if the at-risk population refrains from lubricated sex. The next section will review interventions designed to decrease HIV through sexually transmitted infection treatment and behavioral interventions.

TREATING SEXUALLY TRANSMITTED INFECTIONS

As noted above, there are several different ways that HIV transmission may be minimized; aggressively diagnosing and treating sexually transmitted infections (STIs) is a biologically plausible way to alter HIV transmission dynamics, and remains cheaper than chronic antiretroviral therapy. There have been several studies conducted in sub-Saharan Africa to test this hypothesis with varying results. In a study in the Mwanza district of Uganda, specific syndromic management of STIs in an area where the epidemic was still in its early stages (i.e. 1% of the adult population were infected at the start of the study) resulted in decreasing HIV incidence in communities where the intervention was undertaken.[29] However, in a study in the Rakai district of Tanzania, periodic mass treatment of at-risk adults for STIs did not result in a decrease in HIV incidence.[27] In the latter study, the HIV epidemic was already much more advanced (i.e. 16% of the adult population were infected at the start of the study) and there was a high background rate of concomitant herpes simplex infection that was not treated in the course of the study.

Herpes simplex virus type 2 (HSV-2) seropositivity has been associated with increased risk for HIV transmission and acquisition.[30] Several studies have attempted to see whether chronic HSV-2 suppression with a thymidine kinase inhibitor (i.e. aciclovir or valaciclovir) could decrease susceptibility to HIV acquisition.[31,32] Unfortunately, these studies did not demonstrate a protective effect when anti-HSV-2 chemoprophylaxis was used.

Thus, the lessons from these studies of STI treatment to prevent HIV transmission are equivocal at best. For STI treatment to affect HIV spread, it should be specifically tailored to individual patients, focusing on aggressively diagnosing diseases that are common in specific communities, as opposed to using general algorithms. In addition, the benefit of STI control will be likely to have the greatest impact in areas of lower HIV prevalence. In communities where the epidemic is already more widespread, the likelihood of encountering a new partner who is HIV infected may be substantial, so the benefit of modifying a modest co-factor like an STI will be more limited.

HIV SCREENING AS A PREVENTION MODALITY

Other approaches to the prevention of HIV in the developed world are so well established that they seem routine (i.e. the routine screening of blood).[33] The use of more sensitive antibody screening and careful donor history have resulted in enhanced safety in the blood supply, such that the transmission of HIV via infected blood in the past two decades is exceedingly rare. In other parts of the world where the relative cost of blood screening is high, blood supplies may not be as safe.

Another routine practice that results in the prevention of HIV transmission is the guideline that pregnant women be universally offered HIV antibody testing before delivery.[34] Although compliance with this guideline is not 100%, the rate of new perinatally transmitted infections in the USA has decreased dramatically, with fewer than 100 new infections annually in recent years.

In the Fall of 2006, the US Centers for Disease Control and Prevention recommended that HIV be routinely screened for in most medical settings for patients aged 13–64 years old because more than a quarter of the more than 1 million HIV-infected Americans were unaware of their serostatus.[35] The net effect on the HIV/AIDS epidemic in the USA remains to be seen.

BEHAVIORAL APPROACHES – OVERVIEW

Despite increasing access to antiretroviral therapy, blood screening and treatment of STIs, the number of new HIV infections in the USA has increased to a plateau of approximately 56 000 new infections per year over most of this past decade.[36] The reason why people engage in HIV risk behavior are complex, and may involve issues related to early life events, such as childhood sexual abuse, low self-esteem, contextual issues in relationships and concomitant substance use, as well as addiction to specific forms of sexual pleasure.[37] Gender-related power dynamics (e.g. the role of women in many societies) may also limit the ability to promote safer sex.[38] Thus, no single behavioral approach will invariably lead to an adaptation of consistent safer sexual or drug-using practices. Much like dieting, regular exercise and smoking cessation, no single program of HIV risk reduction will work for all at-risk individuals. However, several studies have now indicated that the provision of either individual counseling and/or small group sessions can be helpful in assisting at-risk individuals in moderating their risk.[39–43]

Good elements of risk-reduction programs include the ability of the counselor to approach the patient in a nonjudgmental manner to elicit a realistic assessment of the person's pattern of risk-taking behavior. Given the slow progress in the development of cheap, safe and effective vaccines, microbicides and other biologic approaches to the prevention of HIV transmission, the role of the provider in patient education, discussion of risk-taking behavior, initiation of risk-reduction counseling and triage to appropriate prevention services is an essential part of stopping the epidemic's further spread.

PSYCHOSOCIAL MODELS OF RISK BEHAVIOR UNDERLYING PREVENTION INTERVENTIONS

Knowledge is one aspect of HIV prevention but ongoing risk taking is a function of many other complex psychosocial variables. The three most common models that have been employed to explain HIV risk taking are the health belief model, the theory of reasoned action and social cognitive theory (i.e. self-efficacy models).[44] Health beliefs models emphasize the role of perceived benefits and barriers to condom use and perceived severity and vulnerability to getting HIV.[45] In the theory of reasoned action, health behavior (e.g. condom use) is a function of intentions to use condoms and, in turn, intentions to use condoms are a function of variables such as attitudes and norms regarding HIV and condom use.[46] Social cognitive models (i.e. self-efficacy) explain condom use as a function of an individual's knowledge about HIV, expectation about the outcomes of using condoms (i.e. pleasure reduction versus disease prevention) and self-efficacy (i.e. that the individual will be able to use a condom in different sexual situations).[47,48] These psychosocial models of HIV prophylactic behavior have been tested in both cross-sectional and longitudinal studies in a variety of populations and are the basis for many of the behavioral interventions reviewed below.

Intervention models that address information, motivation and behavioral skills[49] typically take into account models of HIV risk prevention. One is the transtheoretical model of change[50] which posits that an intervention needs to be adaptable to an individual's current

readiness to change. For example, people who are currently in a 'precontemplative' level of readiness to change do not see their behavior as problematic and do not see a reason to change; someone in a 'contemplative' level may be ambivalent about changing, and someone at a 'determination or preparation' level is ready to make a commitment. Additional levels of change include 'action', 'maintenance' and 'relapse prevention', each with different suggested strategies to assist an individual in a counseling situation. Interventions based on the transtheoretical model try to move individuals to a more serious, higher level of readiness to change than where they are initially. Accordingly, an individual at an earlier level may benefit most from information and education, whereas someone at a mid-level or higher might benefit from more intensive motivational support and skills training.

Many of the randomized controlled trials of interventions utilize aspects of the transtheoretical model of behavioral change, other information–motivation–behavior skills interventions, and/or variables related to self-efficacy, health beliefs and attitudes in the risk-reduction interventions. HIV prevention studies typically collect sexual risk-taking data on HIV-negative individuals, but STI or HIV incidence may also be used as end points. To address the overarching public health significance of HIV, community randomized designs employ more of a wider scale approach. These interventions are formulated to develop population-based HIV prevention approaches, and compare communities who receive the intervention to other similar communities who do not.

OUTCOME OF LARGE SCALE AND HIGH-IMPACT-FOR-HIV PREVENTION STUDIES IN THE UNITED STATES

Prevention trials delivered primarily to heterosexual individuals in STI and primary care clinics

Two different randomized controlled trials of HIV prevention interventions have examined the efficacy of risk-reduction counseling approaches in individuals at high risk for HIV infection, by studying heterosexual individuals attending STI or primary care clinics. The first study, the US National Institute of Mental Health's Multisite HIV Prevention Trial (Project Light), recruited 3706 individuals from 37 inner-city community-based clinics in seven sites across the USA.[51] The three risk groups were:

- men presenting in an STI clinic;
- women presenting in an STI clinic; and
- women presenting in health service organizations.

At screening, all participants reported engaging in unprotected vaginal or anal sex within 90 days. Approximately one-half were randomized to seven sessions of risk-reduction counseling in a group format, and the other half to watch a 1-hour AIDS video followed by a question-and-answer session. Those in the intervention group reported fewer unprotected sexual acts and were more likely to use condoms consistently over the follow-up period. The intervention group also reported higher overall levels of condom use. With respect to STIs, there were no overall differences in infection rates across intervention and control group; however, of the men who were recruited from STI clinics, there was a decreased incidence of gonorrhea.

The second large-scale study, Project RESPECT, recruited over 5787 heterosexual HIV-negative patients who presented for care at STI clinics across the USA.[52] Participants were randomized to a four-session ('enhanced counseling'), a two-session ('brief counseling') or a noninteractive ('didactic message') condition. Those assigned four-session and two-session interactive interventions had fewer new HIV infections at both 6- and 12-month intervals than those who received the noninteractive counseling. Additionally, self-reported 100% condom use was higher in both interactive counseling groups compared to the didactic message control.

Recently, Crepaz *et al*. evaluated the efficacy of a large number of HIV behavioral interventions in reducing unprotected sex and incident STI among African-American/Black and Latino/Hispanic STI clinic patients.[39] HIV behavioral interventions were found to significantly reduce unprotected sex (OR = 0.77; 95% CI 0.68–0.87; 14 trials; n = 11 590) and incident STIs (OR = 0.85; 95% CI 0.73–0.998; 13 trials; n = 16 172), providing evidence that HIV behavioral interventions provide an efficacious means of HIV/STI prevention for African-American/Black and Latino/Hispanic patients who attend STI clinics.

As HIV risk behavior is a necessary factor for HIV infection, these studies taken together reveal that HIV risk-reduction counseling can both increase condom use and decrease sexually transmitted infections.

Prevention trials for men who have sex with men (MSM)

Individually randomized controlled trials

One of the earlier individually randomized intervention trials[53] compared an integrated cognitive–behavioral intervention to a waiting list control among 104 gay men in a metropolitan area. The integrated intervention included AIDS risk information as well as cognitive–behavioral self-management training, sexual assertion training and strategies for increasing positive social supports/relationship skills. At the post-training assessment, the experimental group had fewer high-risk sexual practices and better behavioral skills for sexual coercive situations than the wait list control.

Project EXPLORE was a large-scale randomized HIV-prevention trial among MSM conducted in six US cities, with a total of 4295 participants.[37,54] Inclusion criteria for the EXPLORE study were men who were HIV uninfected, 16 years or older, had anal sex with another man during the past year, and had not been involved in a mutually monogamous relationship in the past 2 years with a male partner who was HIV uninfected. Men were randomized to receive a behavioral intervention versus standard risk-reduction counseling. Study results suggested a possible modest benefit of the intervention in reducing new HIV infections.[54] Further, the reporting of unprotected receptive anal sex with HIV-positive or unknown-status partners was significantly lower in the intervention group compared with the standard group.

Community randomized controlled trials

One of the first community randomized controlled trials, the Mpowerment Project, studied a peer-outreach program for young gay men (aged 18–29 years).[55] The intervention involved peer outreach (training peers to spread prevention messages and to recruit more individuals to participate), small groups which focused on misperceptions of safer sex, eroticizing safer sex, verbal and nonverbal safer sex strategies, informal outreach and a publicity campaign. To assess outcome, a cohort of 300 individuals from two communities were surveyed. In the intervention community, the proportion of participants who reported unprotected anal intercourse with nonprimary partners decreased, as did the proportion of participants who reported unprotected anal intercourse with their boyfriends. No significant changes occurred in the comparison community.

Another large-scale community randomized trial[56] involved delivering the intervention through opinion leaders (popular individuals) from the gay community in four US states. Each state randomly had both an intervention city and comparison city. In the intervention city, popular gay men were trained to spread behavior-change messages (to change norms), and in the comparison city pamphlets were placed at gay bars. The team identified 'popular' men with the assistance of bartenders in intervention city bars. Participants then completed surveys in the bars. Across all states, 1126 men completed baseline surveys and 1010 completed follow-up surveys. At 1-year follow-up, those in the intervention cities reported a significantly greater reduction in the frequency of unprotected anal sex during the previous 2 months, and

a significantly greater increase in condom use for anal sex compared to comparison cities. Consistent with this finding, more condoms were taken from bars at the intervention cities than the comparison cities.

The community randomized trials among MSM validate the utility of providing HIV prevention, on a larger scale, to communities of MSM. From a public health perspective, raising awareness and changing norms regarding HIV prophylactic behavior can influence transmission rates at the community level. Although recruiting opinion leaders and/or providing an integrated prevention program involving peers, groups, workshops and outreach can be a complex undertaking, such efforts can be useful approaches to curtailing HIV risk taking, which, in turn, would curtail HIV transmission.

Meta-analyses of HIV behavioral intervention studies show that HIV behavioral interventions with adult MSM result in significant reductions in self-reported sexual risk behaviors.[41,42,57] A meta-analytic review by Herbst *et al.* synthesizing evidence and evaluating the effectiveness of HIV behavioral interventions found individual-level, group-level and community-level interventions to be effective in reducing the odds of unprotected anal intercourse (range 27% to 43% decrease) and increasing the odds of condom use for the group-level approach (by 81%).[40] Similarly, Johnson *et al.* found significant reductions in unprotected anal intercourse when comparing the effects of 38 HIV behavioral interventions to minimal or no HIV prevention interventions.[57] HIV behavioral interventions were shown to reduce the overall proportion of MSM reporting unprotected sex by 27% (95% CI 15–37%).[57] Interventions most successful in reducing risky sexual behavior are those based on theoretic models, including interpersonal skills training, incorporating several delivery methods, and being delivered over multiple sessions spanning a minimum of 3 weeks.

Prevention trials for women

Individually randomized controlled trials

Several large scale multisite studies have investigated risk-reduction counseling among low-income and/or minority women. Kelly *et al.* randomized 197 high-risk women from an urban primary health clinic to a cognitive–behavioral risk-reduction intervention or comparison.[58] Three months later, the intervention group evidenced better sexual communication and negotiation skills (assessed by role play and self-report), and less unprotected sexual intercourse. The comparison group had no changes on these measures.

A second study of HIV risk-reduction counseling among women[59] sought to adapt models of behavioral change to social and contextual variables relevant to 128 economically disadvantaged African-American women between the ages of 18 and 29 years. Accordingly, their intervention used the theory of gender and power as a guide, and was social-skills based. At the 3-month follow-up, women in the more intensive intervention showed increased consistent condom use, sexual self-control, sexual communication and sexual assertiveness, and partner's adoption of norms supporting consistent condom use than those in the delayed educational control.

A study of 206 pregnant inner-city women randomized participants to an AIDS prevention group or to one of two controls.[60] The AIDS prevention intervention consisted of four sessions, each delivered in group format, and included videos as well as group activities such as role play or discussions. After the intervention and after 6-month follow-up, the AIDS prevention group had increases in knowledge and safer sex behaviors in comparison with the two control groups.

Due to difficulties with retention and attrition of high-risk and hard-to-reach women in HIV prevention trials, Belcher *et al.* developed and tested the utility of a single session 2-hour one-on-one intervention using motivational interviewing and information–motivation–behavioral skills training.[61] No differences occurred in HIV knowledge, but the group that received skills training and motivational interviewing showed higher levels of HIV protective behaviors than the control group, including higher rates of condom use during vaginal intercourse, demonstrating the efficacy of a brief, minimal intervention.

Two randomized controlled trials of an HIV risk-reduction intervention using information, motivation enhancement and skills training intervention were developed for low-income, primarily African-American women.[62,63] In both studies, the intervention consisted of four 90-minute sessions, which included personalized feedback about their HIV knowledge, risk perceptions and sexual behavior. Women in the first study who received the intervention reported stronger intentions to practice safe sex and to communicate these intentions to partners, and less unprotected intercourse and substance use near the time of sexual activity; these gains were maintained at the 3-month follow-up. In the second study, 102 women comprising a new sample were randomized. Overall, the results of the second study were corroborated.

Taken together, the series of randomized controlled trials of HIV prevention counseling for high-risk women demonstrate both feasibility and efficacy of such approaches. While many of these approaches are useful, some may be difficult to disseminate. These interventions require special training and most require a significant amount of time for both the participants and the counselors. Future study of individually administered interventions should now focus on ways to implement and disseminate interventions to community-based settings.

Community randomized controlled trials

To attempt to address some of the limitations of clinic-based intervention approaches for women, two studies of community-randomized trials have been undertaken. The first used nine low-income housing developments[62] and nine demographically matched control developments. The community-level intervention included workshops and community HIV prevention events implemented by popular opinion leaders within each community. The women in the housing developments (n = 690) were surveyed at baseline and 1 year later. This revealed that women in the intervention communities showed better decreases in unprotected sex (past 2 months) and frequency of unprotected acts.

A second community-based randomized control trial targeted low-income, primarily African-American women in four urban settings.[63] Four communities in metropolitan areas were selected: two public housing communities, a low-income neighborhood and a group of inner-city neighborhoods. The intervention was specifically based on the transtheoretical model of change, attempting to reach women who would be at different levels of readiness to change. The intervention consisted of distribution of HIV prevention materials, developing a peer network of community organizers and businesses, and delivering prevention messages by outreach specialists, both individually and in groups. The intervention communities evidenced increases in talking with main partners about condoms and trying to get main partners to use condoms.

HIV behavioral interventions with women have been shown to be effective in reducing overall HIV risk.[38,64] Neumann *et al.* conducted a meta-analysis of HIV behavioral interventions among heterosexuals and found statistically significant effects in reducing sex-related risk (OR = 0.81, 95% CI 0.53–0.90) and decreased incidence of STIs (OR = 0.74, 95% CI 0.62–0.89). Four of the 10 studies (40%) included in the behavioral analyses were individual interventions and 6 out of 10 (60%) focused exclusively on women.[38]

HIV prevention interventions specific to intravenous drug users

A review of 42 studies between 1989 and 1999 suggested that the majority found that needle-exchange programs prevent HIV risk behavior and seroconversion among intravenous drug users.[65] Most other studies of HIV prevention interventions for drug users are observational or quasi-experimental evaluation studies; however, these studies do show significant within-participant reductions in HIV risk behavior.[66]

Semaan *et al.* examined the efficacy of 33 intervention studies with drug users (94% intravenous drug users, 21% crack users).[67] Relative to no HIV behavioral intervention, drug users in intervention conditions significantly reduced sexual risk behaviors (OR=0.60, 95% CI 0.43–0.85). In other words, when extrapolating results to a population with

a 72% prevalence of risk behaviors, the proportion of drug users who reduced their risk behaviors was 12.6% greater in the intervention groups than in the comparison groups.

Among the studies of prevention interventions for intravenous drug users, methodologies differ and, consequently, so do the results. Sexual behavior, as shown in previous sections, is a difficult and complex behavior to change. When co-morbid with drug dependence or addiction, its complexity grows; intensive multimodal interventions currently show the most utility in this population.

SUMMARY

Behavioral interventions to decrease HIV transmission have been successful in a wide array of settings and with diverse populations. However, in most situations, interventions were needed to sustain behavior change. Moreover, there is limited experience with these interventions in parts of the world where the epidemic is spreading most rapidly and where the social construction of reality (e.g. disempowerment of women, limited health-care infrastructure) may limit the effectiveness of programs developed in resource-rich settings. Clearly, additional work is needed to develop culturally specific behavioral interventions, while the development of more effective biologic prevention modalities (i.e. microbicides and vaccines) is underway.

REFERENCES



References for this chapter can be found online at http://www.expertconsult.com

Chapter 86

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Preventing occupational HIV infection in the health-care environment

This chapter addresses strategies designed to prevent the transmission of HIV in health-care settings.

EPIDEMIOLOGY

Occupational risks, including risks for physical injuries, chemical exposures and infectious diseases, have been well described in health-care settings. The introduction of HIV into the health-care workplace in the 1980s, however, focused the attention of health-care providers, perhaps for the first time, on the issue of occupational acquisition of HIV. Ironically, HIV infection is only one of the blood-borne pathogens that can be occupationally acquired in health-care settings. Hepatitis B virus (HBV), hepatitis C virus (HCV) and HIV can all be transmitted in health-care settings through needlestick injuries and other traumatic blood and body fluid exposures. Blood-borne pathogens have been identified as occupational risks for health-care workers (HCWs) since the epidemiology and routes of transmission of HBV were delineated in the 1960s. For reasons incompletely understood at present, occupational HIV infection remains relatively uncommon.

To date, the US Public Health Service's Centers for Disease Control and Prevention (CDC) had recorded only 57 instances of documented occupational HIV infections and 139 instances of probable or possible occupational HIV infections among HCWs in the USA.[1] For each of the documented 57 cases, the HCW sustained an occupational exposure to HIV, had a baseline serum sample drawn which was negative for HIV antibody and then, during follow-up, developed serologic evidence consistent with HIV infection. Fewer than 50 additional cases of documented occupational infections and approximately 60 instances of 'possible or probable' occupational infections have been reported from outside the USA. For the 139 'possible or probable' occupational infections reported to the CDC, the exposed HCWs were unaware of the occurrence of an occupational exposure, did not report the exposure and/or did not have baseline serologic studies performed to document that they were not HIV-infected prior to the occupational exposure.

A comparison of the demographics of the 'possible/probable' and definite occupational infection cases reveals substantial differences. When one compares the demographics of these categories of infection with those of all HCWs in the USA, the likelihood that some of the 'possible/probable' cases occurred as a result of traditional risk factors occurring outside the workplace (i.e. sexual behavior, intravenous drug use) seems quite high.[2]

A number of both general and specific factors determine an individual practitioner's risk for occupational infection with HIV.

- The prevalence of HIV infection among the population of patients served is a major determinant of the overall risk.
- The type of specialty practice (e.g. medical, emergency room, surgical) is associated with varying levels of risk for occupational exposure to blood-borne pathogens.
- The types and frequencies of procedures performed by the HCW, as well as the conditions under which the procedures are performed (i.e. emergent versus elective), also contribute to the risk equation.
- The extent to which the practitioner adheres to recommended infection control procedures and practices is also likely to be a determinant of risk for exposure and infection.
- The individual HCW's technique and attention to detail also contribute to the risk.

Several specific factors contribute to the risk for occupational HIV infection in the health-care workplace. Tables 86.1 and 86.2 list factors that have been demonstrated[3] or suggested in the literature to contribute to the risk for occupational HIV infection.

Many of the reported occupational HIV cases share several features in common. Most were due to percutaneous exposures and the majority of these were hollow-bore needlestick injuries. All of the clinical occupational HIV cases occurred following exposure to blood or grossly blood-stained bodily fluids from HIV-infected patients. As yet, no case of occupational HIV infection has been documented following a needlestick injury with a solid surgical needle.

Table 86.1 Factors contributing to the risk for occupational HIV infection

Exposure factors
1. Route of exposure (e.g. percutaneous,* mucous membrane, cutaneous)
2. Inoculum size
• Size of the device producing injury
• For needlestick exposures, type of needle (i.e. hollow-bore* vs solid)
• Extent of contamination (e.g. visible blood on device,† whether or not device had been placed in an artery or vein†)
• 'Depth/severity' of exposure*†
• Type of contamination (e.g. blood,* pleural fluid, etc.)
Source/'donor' factors
1. Extent of viremia (e.g. by polymerase chain reaction or branch-chain DNA assay)
2. Stage of illness (as a presumed surrogate for extent of viremia†)
3. Circulating free (as opposed to cell-associated) virus
4. Antiretroviral chemotherapy (presumably reducing level of viremia)

*Features shared by many, if not most, of the occupational infections reported in the literature.
†Features identified as significantly associated with risk for occupational infection in the CDC case-control study.[3]

Table 86.2 Risk factors for occupational HIV infection identified in a retrospective case-control study conducted in the USA, UK and France[3]

The risk for occupational HIV infection was increased when:
- the occupational exposure was deep, as compared with superficial ($p<0.001$)
- blood was visible on the device causing the occupational exposure ($p=0.0014$)
- the device causing the exposure had been placed in a source-patient's vein or artery ($p=0.0028$)
- the source-patient died within 60 days of the exposure ($p=0.0011$)
- the exposed health-care worker did not take zidovudine postexposure chemoprophylaxis ($p=0.0026$).

To attempt to identify specific factors associated with risk for occupational HIV infection, public health authorities from the USA, France and the UK conducted a retrospective case-control study, matching the known anecdotal case reports of occupational HIV infections with 'controls' from the public health surveillance studies of occupational exposures in each of these countries.[3] This study identified five specific risk factors for occupational infection; these five factors and the level of statistical significance assigned to each are listed in Table 86.2. The first four of these factors very likely relate directly to the inoculum effect. That an inoculum effect is present in this setting is supported by several pieces of information:

- transfusion of a unit of blood from an HIV-infected donor has been associated with virtually 100% risk for infection;[4]
- the depth of a percutaneous exposure has been shown to be an independent risk factor for occupational HIV infection;[3]
- the presence of visible blood on the device producing the injury has been independently associated with risk for infection;[3]
- instruments that had been placed in source-patients' vascular channels were more likely to result in occupational infection;[3] and
- the fact that all of the needlestick exposures to blood have been caused by hollow-bore needles (i.e. injection, as compared with suturing needles).

Both in the CDC study, as well as in the majority of the anecdotal case reports, the source-patients for the exposures that resulted in occupational HIV infections had advanced disease. This finding is likely to be a surrogate marker for either the level of the source-patient's viral load or the level of circulating free virus, or both. Finally, most occupational HIV infections have followed parenteral (as compared with mucosal or cutaneous) occupational exposures. These latter routes of exposure are associated with lower risks for occupational HIV infection.

Percutaneous exposure

It is estimated that nearly 400 000 percutaneous injuries occur annually among HCWs in the United States.[5] Several longitudinal studies have attempted to determine the magnitude of risk associated with different types of occupational exposure (summarized by Ippolito *et al*.[6] and Henderson[7]). Combining the data from the available studies, HCWs have sustained more than 6800 percutaneous exposures to sharp devices contaminated with blood or other blood-stained body fluids from patients known to be infected with HIV. Twenty-one occupational HIV infections have been documented in these studies, resulting in a risk of transmission per injury of 0.31% (Table 86.3). Thus, one in 324 parenteral exposures in these studies resulted in occupational HIV infection.

Although such a pooled risk estimate provides the best available data concerning the magnitude of risk for occupational HIV infection, this type of analysis has substantial limitations. For example, the longitudinal studies vary somewhat in experimental design and are therefore not directly comparable. Such an analysis implicitly assumes that all parenteral occupational exposures are associated with equal risk, an assumption that does not make intuitive sense and that has apparently been shown to be flawed by the CDC's case-control study cited above.[3] Similarly, all source-patients are also not likely to present the same level of risk for occupational infection. Patients with advanced disease and high viral loads are more likely to transmit HIV than patients with lower or undetectable viral loads (see Table 86.2). Because of the large number of factors that influence the risk for occupational infection, these summary data cannot address the risk associated with a specific, discrete exposure in an individual HCW.

Table 86.3 Occupational risks for HIV infection

	Percutaneous exposures	Mucous membrane exposures
Number of longitudinal studies	27	21
Number of exposures	6807	2768
Number of documented infections	21	0/1*
Infection rate per exposure	0.31%	0–0.11%†

*See text for discussion.
†Using rule of three.[10]

Mucous membrane exposure

Occupational exposures other than parenteral exposures to blood present a lower level of risk for occupational HIV infection. Anecdotal reports document, in rare circumstances, that mucous membrane or cutaneous exposures may produce occupational HIV infections in HCWs.[8] Some of the longitudinal studies cited above have also addressed occupational risks associated with mucous membrane exposure to blood from HIV-infected patients. To date, with more than 2700 exposures followed prospectively, only one study has reported a seroconversion following a mucous membrane exposure.[9] Thus, as a maximum estimate (using the 'rule of three' as an approximation for a zero numerator),[10] the pooled risk estimate for infection associated with a mucous membrane exposure is 0.11% per exposure (see Table 86.3). Again, such a pooled risk estimate provides only a rough framework for considering the risks associated with a discrete exposure.

Occupational exposures other than percutaneous and mucous membrane exposures

Occupational exposures other than percutaneous and mucous membrane exposures are even less likely to result in infection. Prospective studies of nonpercutaneous and nonmucous membrane exposures, which include hundreds of person-years of follow-up, have not identified a single instance of HIV transmission.[11,12] Whereas exposures to other non-bloody fluids are likely to be associated with some occupational risk, this risk is below currently measurable levels.

When one evaluates the occupational exposures that have produced HIV infection, almost all of them result from exposure to blood from HIV-infected patients. Whereas other body fluids may ultimately be shown to represent a risk for occupational infection, the major risk in the health-care setting has come from occupational exposures to blood from HIV-infected patients.

PATHOGENESIS

Although the retrospective case-control study of risk factors for occupational HIV infection cited above[3] provided some insight into factors associated with risk for occupational HIV infection, the precise pathogenetic mechanisms of the occupational infection event are poorly understood. The major risk for occupational infection is associated with percutaneous injury with a hollow-bore needle or other sharp device that has been used on an HIV-infected patient. The risk is associated primarily with blood exposure. Precisely how the transmission event occurs in the skin, subcutaneous tissue or underlying muscle remains unclear. Current interest in the pathogenesis of HIV infection by this route focuses on the role of host defense and the role of the dendritic cell.

The role of host defense in protection against occupational infection is poorly understood. Scientists working at the National Cancer Institute have demonstrated that 75% of HCWs exposed to blood from HIV-infected patients who do not become infected with HIV develop HIV-specific T-helper activity.[13] In a subsequent study, these investigators demonstrated that 35% of uninfected HCWs who had sustained occupational exposure to blood from HIV-infected patients developed cytotoxic lymphocyte responses to HIV-related envelope antigens.[14] Among HCWs who had sustained occupational exposures to blood from patients who were not infected with HIV, none responded to HIV-associated envelope antigens. Whereas the precise role that cellular immunity plays in host defense against occupational HIV infection remains to be delineated, these data, when combined with results from animal studies, suggest that the role may be an important one. One recent case report also supports a significant role for cellular immunity in the defense against HIV infection.[15] In this case, an HCW who sustained an HIV needlestick exposure had HIV DNA detected by nucleic acid sequence-based amplification during a course of three-drug antiretroviral postexposure chemoprophylaxis. Despite the detection of proviral DNA, this individual ultimately remained uninfected (as assessed by serial nucleic acid tests and antibody determinations). The HCW did, however, develop a robust HIV-specific cellular immune response.

INTERVENTIONS DESIGNED TO DECREASE THE RISK FOR OCCUPATIONAL HIV INFECTION

Interventions designed to limit occupational exposures

Several approaches have been used to attempt to decrease risks for occupational exposure to (and therefore occupational infection with) blood-borne pathogens in the health-care setting (Table 86.4). Such interventions can be considered as primary prevention. Major categories of intervention include:

- education;
- work practice controls (e.g. adherence to infection control procedures designed to limit risk); and
- engineered controls.

Education and use of infection control procedures

Staff should be routinely informed about all occupational risks. Since the major risk for occupational HIV infection (and for infection with other blood-borne pathogens as well) is by parenteral inoculation, some critics of the recommendations to manage all patients as if they were potentially infected with blood-borne pathogens (e.g. universal precautions, body substance isolation, standard precautions; discussed below) have suggested that these precautions will have little impact on the number of occupational infections. However, since these and later guidelines educate staff about the careful handling of needles and sharp objects, recommend against practices associated with needlestick injuries (e.g. needle recapping, needle bending or needle clipping) and stress appropriate disposal of needles and other sharp objects,[16,17] their implementation has been associated with a decreased parenteral exposure rate.

Two centers have documented a significant decrease in such exposures,[18,19] one in temporal association with training in, and implementation of, universal precautions.[18] Up to one-third of parenteral occupational exposures may be preventable by following guidelines designed to minimize occupational exposures.[20] The use of appropriate barriers also reduces occupational risk. The blood inoculum on needles is reduced by as much as 50% when penetrated through a latex glove.[21] Making HCWs aware of the presence of occupational risks also results in occupational behavior modification. In a survey of certified nurse-midwives, both knowledge of the routes of transmission of blood-borne pathogens and perception of risk for occupational infection were statistically associated with the appropriate use of precautions. However, risk perception and use of precautions were more closely linked, suggesting that knowledge, in itself, may be insufficient to produce behavior modification.[22]

Employers in the USA are governed by regulations issued by the Occupational Safety and Health Administration of the US Department of Labor in 1991. One regulation mandates that employers follow certain protocols when managing health-care workers who have sustained occupational exposures to blood-borne pathogens.[23] This 'final rule' requires mandatory education for HCWs at every health-care institution in the USA.

Work practice controls

In 1987, the CDC issued guidelines for the management of patients infected with blood-borne pathogens that have since provided the underpinnings for all subsequent guidelines in the USA.[16] These 'universal precautions' guidelines clearly set out the principle that HCWs should treat blood and blood-stained body fluids from all patients as potentially infectious, in order to prevent the transmission

Table 86.4 Prevention strategies for health-care workers and institutions to decrease risks for occupational HIV infection[2]

1. Use of 'standard precautions' or other isolation procedures designed to place effective barriers between the health-care worker and blood or other body fluids.
2. Educating new staff and retraining existing staff regarding occupational risks for blood-borne pathogen infection in the context of other occupational risks present and prevalent in the health-care workplace; making sure staff are aware of these risks.
3. Including information about all occupational risks (including those associated with caring for patients who have blood-borne pathogen infections) in biomedical training schools' curricula.
4. Evaluating all procedures associated with occupational risk for exposure to blood-borne pathogens (particularly those presenting risks for transcutaneous exposures), with the intent of modifying the aspects of these procedures associated with risks for occupational exposures.
5. Aggressive use of newly developed engineered controls, including careful evaluation of 'safety devices' for safety, efficacy and cost-effectiveness; implementation of those devices that meet these tests.
6. Development of efficient, readily accessible, user-friendly institutional postexposure management systems, including the option for postexposure prophylaxis for documented occupational HIV exposures.

of blood-borne pathogens (in particular HIV) from patients to HCWs. Several years later, the CDC issued revised isolation guidelines, called 'standard precautions', which focus on the bidirectional spread of organisms to and from patients and health-care providers.[17] All of these sets of guidelines and precautions emphasize:

- that blood and other blood-containing body fluids represent risk to HCWs; and
- that HCWs should use barriers and take precautions to prevent occupational exposures to these materials.

Use of these kinds of precautions in health-care settings has resulted in decreased risk of occupational exposure to blood and decreased risk for occupational infections with blood-borne pathogens. Based on self-reports of occupational exposures to blood, Fahey and co-workers at the National Institutes of Health estimated that, in the year prior to training the staff in universal precautions, staff members experienced an average of 36 cutaneous exposures to blood.[24] Eighteen months after the staff were trained in universal precautions, this number decreased to 18. For all categories of employees and information analyzed, exposures to blood were reduced by approximately 50%. These results suggest that changes in behavior occurred between the two surveys, and, although a causal relationship with training cannot be proved, such a relationship can reasonably be inferred.

Initially, compliance with these precautions was problematic. Gerberding and co-workers identified substantial noncompliance at San Francisco General Hospital.[25] Although Fahey identified substantial improvement in her staff's compliance with universal precautions, the reduction in blood exposures was only 50% (implying that 50% of such exposures continued to occur, despite implementation of these precautions).[24]

Although data regarding procedure-specific adverse exposure rates are limited, certain procedures and devices seem intrinsically associated with increased risk for occupational exposures. To the extent that such modifications are possible, inherently risky procedures should be modified. In some instances, risk modification can be achieved by practitioners modifying the way the procedure is performed. In other circumstances, new devices or engineered controls (discussed below) have been developed and implemented. Some work practice interventions have been shown to reduce the risks for blood exposures. For example, the use of 'double-gloving' in surgery reduced the risk for skin exposure to blood significantly.[26,27]

Engineered controls

Whereas work practice controls can eliminate a substantial fraction of occupational exposures to blood-borne pathogens, modifying medical devices that, in their current formats, are intrinsically associated with exposure risks can reduce these risks further. Jagger and co-workers detailed the importance of medical devices designed to prevent occupational exposures to blood-borne pathogens.[28,29]

In the past several years, a number of engineered controls (i.e. 'safer' sharps and safety devices) have been introduced. Of these new safety devices, the following have been identified in at least one published study as being associated with decreased risks for cutaneous and/or percutaneous blood exposures:

- a surgical repair assist device
- blunt surgical needles;
- surgical finger guards and glove liners;
- phlebotomy equipment;
- needleless intravenous administration systems; and
- modified (e.g. self-capping) intravenous catheters.

At one center, the incidence of self-reported percutaneous injuries among HCWs was reduced by 58% after implementation of 'safety-engineered devices' allowing for needle-safe blood drawing, intravenous insertion, etc.[30] Despite implementation of safer devices, some exposures will still occur. Development of a process for the systematic, objective evaluation of these new devices is crucial to reduce the risk of sharps injuries in all health-care settings.[31]

Interventions designed to decrease the risk for occupational infection once exposure to HIV has occurred

Immediate postexposure management

Despite the emphasis on the prevention of occupational exposures, institutions should also develop strategies to effectively manage occupational exposures. Important constituents of a postexposure management program are listed in Table 86.5.

When an exposure occurs, first aid should be administered and the exposure site should be allowed to bleed freely. The wound should be cleansed and decontaminated as soon as patient safety permits. Wounds should be washed with soap and water and then irrigated with sterile saline, a disinfectant or other suitable solution. Mucosal exposures should be decontaminated by vigorously flushing with water. Eyes should be irrigated with clean water, saline or sterile eye irrigants.

HCWs must be educated about the importance of promptly reporting occupational exposures. Institutional reporting procedures should be simple, straightforward and widely publicized. A mechanism

Table 86.5 Components of postexposure management programs for health-care workers exposed to HIV

1. Institutions should develop thorough, thoughtful, aggressive, employee educational campaigns concerning the presence of occupational risks in the health-care setting, including the risks for blood-borne pathogen infection; these educational campaigns should emphasize risk prevalence, risk-reduction strategies, the importance of reporting adverse occupational exposures and postexposure management protocols.
2. Postexposure management systems must include mechanisms to facilitate both exposure reporting and the provision of follow-up care; these systems should be readily accessible, widely publicized, convenient and user friendly; occupational medicine personnel should be instructed to provide immediate 'first aid' for staff sustaining adverse exposures to blood and body fluids.
3. Institutions should develop a system for categorizing occupational exposures that require differing management strategies and different types of follow-up; protocols should address 'source-unknown' and 'source refuses serologic testing' exposures.
4. Postexposure management protocols should include appropriate serologic testing (mindful of state and local laws regarding consent) of both the source-patient as well as the employee sustaining the occupational exposure.
5. Occupational medicine staff should be thoroughly trained in the counseling of staff sustaining occupational exposures to HIV; all exposed staff should be given appropriate counseling regarding risks for infection and prevention of secondary transmission; all staff should have access to further counseling, if needed; all exposed staff should be given access to experts in the areas of occupational risks and HIV infection.
6. Exposed employees should be counseled to return for appropriate clinical and serologic follow-up and should be instructed to return if signs and/or symptoms of acute HIV infection develop.
7. Postexposure management protocols should offer postexposure prophylaxis, with appropriate follow-up, for health-care workers sustaining occupational exposure to HIV.
8. Counseling should include attention to the known/expected toxicities of the selected regimen. Pre-emptive therapy of these symptoms/side-effects (e.g. prescriptions to treat nausea, diarrhea, etc.) may increase regimen adherence.
9. Postexposure management protocols must, at all costs, maintain the exposed health-care worker's medical privacy and confidentiality.

to facilitate reporting and provide follow-up care should be readily accessible. Reporting systems should offer access to expert consultants. Institutional occupational medical systems must protect the confidentiality of the exposed worker.

At the time an exposure is reported, occupational medical personnel should draw baseline serology. For documented occupational exposures to HIV, follow-up should occur at 6 weeks, 3 months, and 6 months postexposure. The follow-up period should be prolonged to 1 year for HCWs who become infected with HCV after exposure to patients with HIV and HCV co-infection. Late seroconversion (i.e. more than 6 months following exposure) has been documented in case reports. However, routinely prolonging the duration of follow-up beyond 6 months for all exposed HCWs is generally not recommended unless a particular situation calls for such action. More aggressive diagnostic evaluation, such as the use of polymerase chain reaction analysis to detect viral or proviral nucleic acids, should be ordered if the HCW develops symptoms suggestive of acute HIV infection. Occupational medicine staff should counsel the exposed HCW about the signs and symptoms of acute HIV infection and should instruct the employee to return for evaluation should any illness consistent with this syndrome occur.

Institutions should have written policies and procedures regarding the testing of source-patients and employees that are consonant with local and state laws. If available, rapid HIV tests should be performed on source-patients as such testing allows faster determination of source-patients' HIV status with sensitivity and specificity that is similar to that of conventional serologic testing.[32,33] This approach has been shown to be cost-effective and can lead to reduced implementation of unnecessary postexposure prophylaxis (PEP), as well as reduce the stress and anxiety often seen with the exposed workers.[34–36]

Postexposure chemoprophylaxis with antiretroviral agents

The use of antiretroviral agents as PEP for occupational exposures was controversial in the mid-1980s.[37] Data accumulated over the past 20 years, however, provide a firmer foundation demonstrating the efficacy of PEP. Animal studies of PEP (most of which use substantially lower viral inocula) have been able to demonstrate efficacy. Another piece of scientific evidence that indirectly supports the use of PEP comes from the success of antiretroviral agents administered in pregnancy to reduce maternal–fetal transmission of HIV.[38,39] One of the factors identified in the collaborative retrospective case-control study of occupational HIV in HCWs as significantly associated with an increased risk for occupational HIV infection was 'not taking zidovudine chemoprophylaxis'.[3] In this study, administering zidovudine chemoprophylaxis to exposed HCWs was associated with an approximately 80% reduction in the risk for occupational infection following percutaneous exposures to HIV.[3]

The US Public Health Service (USPHS) updated its guidelines recommending the use of PEP in 2005.[40] For 'less severe' occupational exposures, the current recommendations (summarized in Table 86.6) advocate the use a basic two-drug regimen consisting of two nucleoside reverse transcriptase inhibitors (NRTIs) or one NRTI plus one nucleotide reverse transcriptase inhibitor (NtRTI). The most frequently used combinations are zidovudine and lamivudine (co-formulated as Combivir) or emtricitabine and tenofovir (co-formulated as Truvada). Other potential combinations include stavudine plus lamivudine or emtricitabine, and tenofovir plus lamivudine or emtricitabine. The combination of didanosine and stavudine is no longer recommended due to potential drug toxicity. Due to the risk of possible serious or life-threatening adverse drug events, abacavir, delavirdine, nevirapine, and zalcitabine are not routinely recommended for PEP.

For 'more severe' exposures, an expanded protease inhibitor-based three-drug (or possibly four-drug) chemoprophylaxis regimen is recommended. The preferred protease inhibitor (PI) is lopinavir–ritonavir (Kaletra), but others may be used as alternatives with certain exceptions (discussed below).

Table 86.6 Current US Public Health Service recommendations for chemoprophylaxis of occupational exposures to HIV[40]

HIV exposures with a recognized transmission risk	Preferred 'basic regimens': • Zidovudine (ZDV) + lamivudine (3TC) • Tenofovir (TDF) + emtricitabine (FTC) • ZDV + FTC • TDF + 3TC
	Alternate 'basic regimens': • 3TC + stavudine (d4T) • FTC + d4T • 3TC + didanosine† (ddI) • FTC + ddI
HIV exposures for which the nature of the exposure suggests an elevated transmission risk*	Basic regimen' plus one of the following agents: • Lopinavir–ritonavir (LPV–RTV) – preferred • Atazanavir (ATV) ± RTV • Fosamprenavir (FOSAPV) ± RTV • Saquinavir (SQV) + RTV • Indinavir† (IDV) ± RTV • Nelfinavir† (NFV) • Efavirenz† (EFV)

*Examples of elevated risk include deep puncture, injury with large-bore hollow needle, visible blood on the device, exposure to 'larger' volume of blood and/or blood from source-patient with high viral load.
†Agents not advisable for use in pregnancy and/or increasingly not recommended in practice.

If the source-patient for an exposure is (or recently has been) receiving antiretroviral therapy, some authorities recommend the use of alternative regimens utilizing agents to which the source-patient's virus has not been exposed. Certain regimens may be inappropriate if the source-patient is known or suspected to harbor a resistant virus. This latter point underscores the importance of obtaining expert guidance from individuals knowledgeable about the use of antiretroviral agents to tailor a PEP regimen for HCWs. Clinicians who are skilled in providing care to HIV-infected patients are perhaps best suited to provide this kind of advice. If you cannot identify a local expert, the US Public Health Service sponsors a postexposure hotline that can provide this expertise (either over the telephone (1-888-448-4911) or at http://www.ucsf.edu/hivcntr/Hotlines/PEPline.html).

Another special circumstance worthy of additional consideration relates to PEP administration to a HCW who is (or thinks she may be) pregnant. Because of the extremely limited experience with the use of these agents in uninfected individuals, the risks associated with administering the drugs to pregnant women are essentially undefined. The only relevant clinical experience in humans comes from the administration of the drugs to HIV-infected pregnant women, a circumstance that is not exactly consonant with the postexposure prophylaxis setting. Based on the limited clinical data, as well as several relevant animal studies, general guidelines for this situation have been developed and are summarized in Table 86.7.[40–42]

Virtually all of the marketed antiretroviral agents have potential for carcinogenicity, teratogenicity and mutagenicity. Since publication of the USPHS guidelines, concern has been raised for potential teratogenicity associated with the use of nelfinavir in animals. Although no definite relationship has been established in humans, the use of this agent in PEP regimens for pregnant women or women of childbearing age is no longer recommended.[42] Efavirenz has been shown to be teratogenic in cynomolgus monkeys at drug levels similar to those used in humans. In addition, administration of the didanosine–stavudine combination in pregnancy has been associated with cases

Table 86.7 General principles for administering antiretroviral chemoprophylaxis to pregnant health-care workers[40–42]

1. A pregnant, exposed health-care worker is the only person who can decide whether to take chemoprophylaxis, and she must be empowered to make this decision. No one should attempt to make this decision for her.
2. The practitioner providing care to a pregnant, HIV-exposed woman must provide up-to-date and accurate information about what is known (and not known) concerning:
 - the magnitude of risk for infection associated with her exposure;
 - the efficacy of postexposure prophylaxis;
 - the safety of the treatment, including the potential for harm to the health-care worker and her fetus; and
 - the risk of the fetus becoming infected (and the possible interventions that could be taken to reduce this risk) should the health-care worker become infected from the exposure.
3. The practitioner should select a regimen appropriate for the exposure; i.e. pregnancy per se should not dictate the regimen, but consideration should be given to agents for which an experience base exists (e.g. zidovudine and lamivudine). Some agents/regimens with known toxicities in pregnancy should be avoided whenever possible (e.g. didanosine plus stavudine; indinavir, efavirenz, nelfinavir).
4. Pregnant workers electing to take postexposure prophylaxis must be followed closely for signs of toxicity (both maternal and fetal); pregnancy represents another circumstance in which expert consultative advice is essential.

of severe pancreatitis and severe lactic acidosis among HIV-infected women, and both maternal and fetal deaths have been recorded.[43] Other issues that may be relevant to the administration of chemoprophylaxis to pregnant HCWs include data from France suggesting a risk for severe mitochondrial toxicity in uninfected infants born to mothers who had taken nucleoside analogues[44] (an experience that has not been detected in the USA), as well as the potential for hepatotoxicity and nephrolithiasis associated with indinavir use.

Monitoring for PEP toxicity

If HCWs are prescribed PEP, chemistry (renal and hepatic function) and complete blood counts should be checked at baseline and 2 weeks into treatment. Additional monitoring may be necessary depending on the specific PEP regimen prescribed. Potential drug toxicity and interactions should be discussed with HCWs, as should the importance of adherence to treatment. Despite the encouraging data concerning the use and efficacy of PEP, legitimate concerns remain about the use of these agents in this setting. Antiretrovirals are not trivial agents in terms of potential toxicity. Virtually all studies of PEP for occupational HIV exposures in HCWs have demonstrated substantial side-effects, often limiting completion of the prescribed regimen.[45] Despite the availability of newer, more tolerable agents, recent data have shown that as many as 20% of HCWs stop PEP prematurely due to adverse events.[46,47] Finally, several cases of zidovudine chemoprophylaxis failure (eight of which have direct clinical relevance) have appeared in the literature.

The US Public Health Service last revised its guidelines concerning postexposure prophylaxis in 2005[40] and intends to revise the guidelines whenever new information becomes available concerning the risks or benefits of postexposure chemoprophylaxis. In spite of these considerations, all institutions need to have a policy on postexposure prophylaxis. Offering chemoprophylaxis is seen as 'empowering' by exposed workers. Workers who have had these frightening occupational exposures appreciate the fact that their institutions are willing to offer these drugs, ongoing support and prevention strategies to improve HCW safety.

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Chapter 87

Barry S Peters

HIV vaccines: past failures and future scientific challenges

INTRODUCTION: WHY WE NEED AN HIV VACCINE?

There are currently around 3 million deaths annually from HIV and AIDS, but this figure is projected to increase much further over the coming decades. By 2030, it is estimated that HIV will be one of the three major causes of death worldwide, alongside heart disease and stroke. Highly active antiretroviral therapy (HAART), which was introduced in the 1990s, will not prevent this outcome, because the majority of people worldwide do not have access to anti-HIV drugs. Even when HAART can be readily obtained, there are major problems associated with its use. These include adverse effects, drug interactions and the development of drug-resistant HIV strains. Measures that reduce or prevent transmission, such as condoms or male circumcision, are needed urgently. Of the various prevention measures, a prophylactic HIV vaccine is the only one likely to have a major impact on the global spread of HIV. There is, therefore, an urgent need for a safe, effective prophylactic HIV vaccine in order to improve global health. Unfortunately, as this chapter discusses, we are far from achieving this goal.

IT WAS NEVER GOING TO BE EASY

When HIV was discovered in 1983, there was initially hope that a vaccine might follow shortly afterwards. Expectations were further raised when experiments in nonhuman primates showed protection against a hybrid between the human and monkey AIDS virus SHIV (simian HIV). As we describe below, this proved to be a false dawn. Phase III efficacy trials in humans that had been predicated on such data failed completely to show any form of protection against HIV. The true magnitude of the challenge in designing an HIV vaccine became apparent as we gained a more thorough understanding of the complex molecular biology of HIV infection.

However, even a basic understanding of HIV and its evolution should enable us to anticipate major difficulties for HIV vaccine development. Ancestral retroviruses have been in existence for many tens of millennia and have had a head start on us in how to evade host immune responses. Combine this with the extraordinarily rapid rate of mutation during HIV replication and it is understandable that HIV has been able to evolve superlatively effective mechanisms against vaccination. These range from the 'hiding' of potential neutralizing domains from neutralizing antibodies to the development of restriction genes whose sole purpose is to defend HIV from the host. The enormity of the scientific challenge is now apparent to all those in the field (Table 87.1).

Table 87.1 Key difficulties in developing an HIV vaccine

- No natural sterilizing immunity to disease
- Huge difficulties in producing neutralizing antibodies
- No good animal models of HIV infection
- A pool of latently infected cells is formed early after infection
- No good correlates of T-cell vaccine efficacy have been identified

WHERE ARE WE WITH CURRENT CLINICAL TRIALS?

Thus far there have been three large efficacy studies, all of which have failed to show any benefit. The latest, the 'STEP trial' of an adenoviral vector HIV vaccine candidate[1] (described in more detail below), was halted prematurely in 2007. This candidate engendered hope as there was a strong T-cell recognition of HIV antigens as measured by enzyme-linked immunosorbent spot (ELISpot) assay responses. The dismal results of this study were a blow to the morale of HIV vaccine researchers.

These failures sent a clear message that we need to re-evaluate our approach to HIV vaccine development. As this chapter emphasizes, a 'quick fix' will not be achievable for HIV vaccines. The opposite is true, and a huge amount of basic scientific work is still required before we will have an HIV vaccine with any realistic chance of success. We will discuss a number of barriers that need to be overcome, including the difficulties in producing neutralizing antibodies to HIV, the problems in measuring and producing effective cytotoxic T-lymphocyte responses and the need to develop other approaches, including those that rely on innate immunity.

HOW WOULD WE DEFINE A 'SUCCESSFUL PROPHYLACTIC HIV VACCINE'?

There has also been a re-evaluation of what might be considered a 'successful prophylactic HIV vaccine' (PHV). An ideal PHV would be one that prevents infection in almost 100% of those vaccinated; it would be cheap, easily (self-) administered, stable in all commonly encountered environmental conditions (including those found in the tropics), be completely safe and have a lifelong effect. However, a PHV would still be valuable if it did not offer protection against infection, but instead mitigated the course of the disease after infection. This would reduce morbidity and mortality from HIV. Similarly, a vaccine that reduced the infectivity of the individual would reduce the spread of the HIV epidemic (Table 87.2).

Table 87.2 Definitions of a successful HIV vaccine

- Provides protection to 70–100% and markedly slows epidemic.
- Only provides protection in 30–70% but still has significant impact on HIV epidemic.
- Does *not* prevent infection at all, but reduces the viral load post infection, leading to:
 - slower disease progression in the individual;
 - reduced infectivity of the individual; and
 - slowing of the epidemic due to reduced transmission.

THE STARTING POINT: APPLYING APPROACHES THAT HAVE BEEN SUCCESSFUL FOR OTHER VACCINES

It is not a prerequisite to understand how the body protects against an infective agent to develop an effective vaccine against that organism. The first successful vaccine against a viral infection was developed by Edward Jenner without any knowledge of the causative infectious agent, the way the agent was transmitted or any knowledge of how the vaccine might mediate disease. Nonetheless, a vaccine against smallpox was developed.[2]

The three main types of vaccine that have been used successfully for the prevention of other viral illnesses are live attenuated, whole inactivated and recombinant proteins (Table 87.3). All of these vaccines protect by means of antibody responses, and hence it seemed only natural in HIV vaccine development to seek vaccines that might produce neutralizing antibodies. This would tie in with our knowledge of the immune response to HIV infection (Fig. 87.1). The choice of vaccine type, however, is limited by safety concerns. Live attenuated AIDS vaccines have protected against infection in the nonhuman primate model but are considered too dangerous for use in humans because of the possible reversion to a more pathogenic type of virus.[3] Whole inactivated viruses seemed to offer protection in nonhuman primate models. However, it transpired that this protection was mediated by neutralizing antibodies (NAbs) against human cellular proteins that had been incorporated into the vaccine during production in human cell lines.[4] Hence, for reasons of safety and efficacy, most HIV vaccine candidates are recombinant/subunit vaccines. The first such vaccine used a recombinant envelope protein of HIV, based on gp120 (Fig. 87.2).

There are many other instances of viral infections for which the 'neutralizing antibody' approach has not yielded a successful vaccine, hepatitis C being one such example. There appear to be several reasons for this, including the rapid multiple mutations that occur, leading to evasion of a neutralizing antibody response. Also, it is not clear if hepatitis C even has a single principal neutralizing domain.

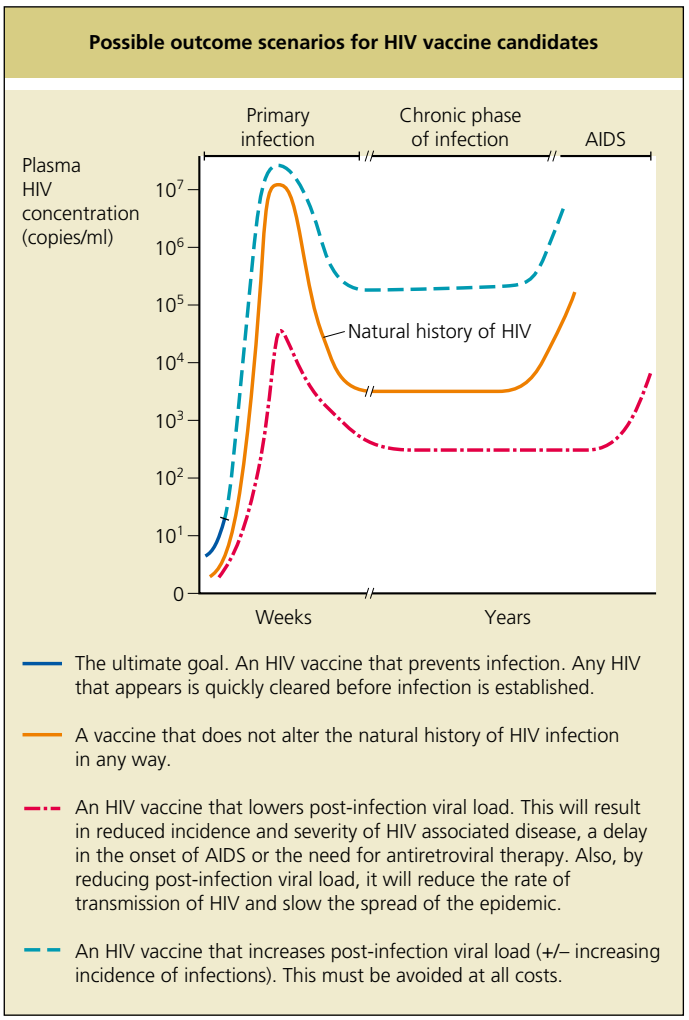


Fig. 87.1 Possible outcome scenarios for HIV vaccine candidates.

Table 87.3 Examples of vaccines used to prevent other viral illnesses

Vaccine type	Diseases	Correlate for protection	Safety issues for HIV vaccines
Live attenuated	Polio (oral) Measles Mumps Rubella Varicella Yellow fever Smallpox	Antibodies	Considered too unsafe to use in HIV because of possible reversion to virulent type
Whole inactivated	Inactivated polio Hepatitis A Influenza	Antibodies	Considered to be relatively safe, although some concerns exist
Recombinant proteins	Hepatitis B Human papillomavirus	Antibodies	Considered to be relatively safe, and is the main current approach, although some viral vectors might increase the risk of infection

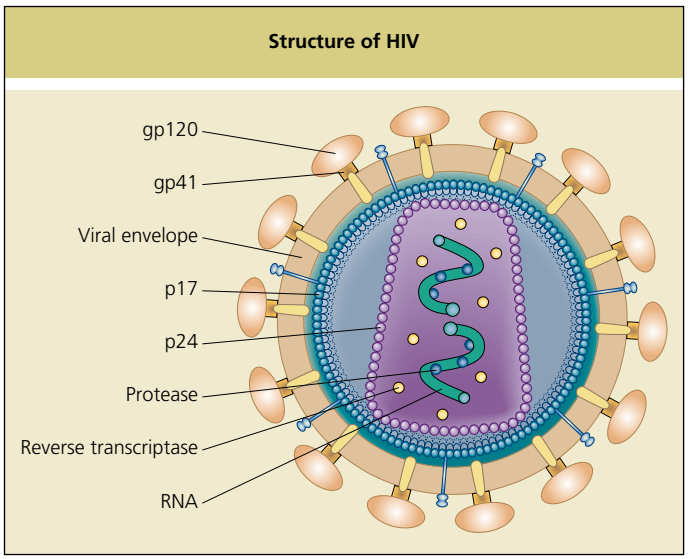


Fig. 87.2 Structure of HIV.

THE NEUTRALIZING ANTIBODY APPROACH FOR HIV

Whereas HIV viruses cultured in the laboratory can be neutralized, primary isolates from HIV patients have so far been resistant to neutralization.[5] HIV does appear to have several neutralizing domains, but none of them is an easy target for potential vaccine-induced antibodies. There are several reasons for this (Table 87.4).

Firstly, the high mutation rate that occurs with HIV replication leads rapidly to escape mutations to most antibodies with neutralizing potential. Therefore, even though neutralizing antibodies against the HIV envelope do occur naturally after infection in humans, they almost immediately select for escape mutants.[6,7] It is sobering to realize that the degree of viral diversity that occurs amongst all influenza viruses in the world in a given year is far exceeded by the diversity of HIV within one newly infected person within the same time period. Hence any antibodies produced by such a vaccine would prove ineffective. Furthermore, 'natural infection' with HIV does not prevent the occurrence of superinfection with other viral clades.

The first phase III efficacy trial of a prophylactic HIV vaccine was based on the gp120 envelope protein and it is not surprising, therefore, that it proved completely ineffective. The study was conceived before the extent of escape mutants was clear, but the results of the study demonstrated the scale of this barrier to vaccine efficacy.

For neutralizing antibodies to be effective in HIV, they need to interfere with fusion of the virus. HIV fuses to target cells by using a trimeric envelope complex of gp120 and gp41 subunits, which attach to $CD4^+$ receptors and a co-receptor (usually CCR5 or CXCR4). Neutralizing antibodies of primary isolates work in one of two ways: either they prevent receptor engagement by binding to the virus surface trimer or they inhibit fusion after viral attachment has occurred.[8]

HIV has evolved a number of mechanisms to evade this neutralization (Fig. 87.3).[9] The HIV envelope is heavily glycosylated. The glycans themselves are poorly immunogenic and they prevent access of antibodies to the peptides beneath them. The trimeric nature of the gp120–gp41 structure also shields the epitopes much more so than for the individual monomeric subunits. The reason for this is not completely clear, but is probably related to conformational changes that occur during trimerization. Hence any sites potentially vulnerable to neutralization are well shielded.

Finally, and partly as a result of the above features, those neutralizing antibodies that have been identified usually need very high levels to prevent infection, and the '2 log' or '100 times' rule has generally been observed to hold. This is where the titer of one of the known neutralizing antibodies required to neutralize the virus *in vivo* is around 100 times the levels required to neutralize the virus in sera.[10,11]

Table 87.4 Difficulties in producing neutralizing antibodies (NAbs)

- Exposed sequences on the envelope are highly variable, which rapidly leads to escape mutants
- Exposed sequences, e.g. gp120, do not lead to NAbs of great breadth or specificity
- The viral envelope exists as a trimer and immunogenic regions on the monomer are shielded (the envelope proteins are shielded by numerous *N*-linked glycans)
- The envelope protein undergoes structural changes when it binds to the CD4 receptor
- High levels of NAbs are probably required to prevent infection

The potential for neutralizing antibodies against primary HIV *in vivo*

Despite all the forgoing reasons that neutralization of HIV *in vivo* is such a difficult prospect, there are reasons to expect progress in this area. Several naturally occurring neutralizing antibodies, with broadly neutralizing activity, have been identified in rare individuals.[12] Primary isolates from different HIV-1 subtypes can be neutralized by different broadly reactive human monoclonal antibodies which have been assigned the following labels: b12, 2G12, 2F5 and 4E10. These sera and monoclonal antibodies have been extensively studied and the way they neutralize HIV might provide some important insights for vaccine development.

It is instructive to look at an example of how one of these antibodies appears to achieve neutralization. B12 was the first broadly neutralizing antibody to be described and it acts by preventing attachment of HIV to the cell. It does this by occluding the CD4 binding site on gp120. There is a recess on the CD4 binding site that forms a contact site for an amino acid, Phe-43, that protrudes from a loop on CD4. This is a key interaction, for without it gp120 binds too weakly for fusion to occur. The b12 antibody acts by means of a long protruding CDR3 loop that 'spears' the center of the CD4 binding site. Although a large number of antibodies have been shown to act in a similar manner, it is only b12 that is able to neutralize primary isolates. The exact qualities of b12 that give it this specificity are yet to be determined, but if this can be established, we can attempt to induce neutralizing antibodies that act in the same way.

The other broadly neutralizing antibodies act in different ways; for example, 2F5 and 4E10 appear to act by inhibiting fusion, and 2G12 prevents virus entry by a different mechanism from b12. The ways that these antibodies neutralize have been the subject of considerable research and their mechanism of action has become much clearer over the past few years.[9]

Interestingly, the '2 log' rule described earlier does not seem to apply to one of the neutralizing antibodies, 2G12, where the relatively low levels required to achieve *in-vitro* neutralization are sufficient to achieve neutralization *in vivo*.[13] It might prove very instructive to find out why 2G12 'punches above its weight'.

Finally, some illuminating clinical studies have been performed in patients, whereby known human monoclonal antibodies have been infused into HIV-infected patients and have markedly reduced HIV viral load.[14] This will never constitute a practical form of therapy, because of the high levels of antibodies required, as well as the requirement for constant infusion. Nonetheless, these studies point to the potential efficacy of these neutralizing antibodies in humans and also provide a means of studying how best to deploy these approaches.

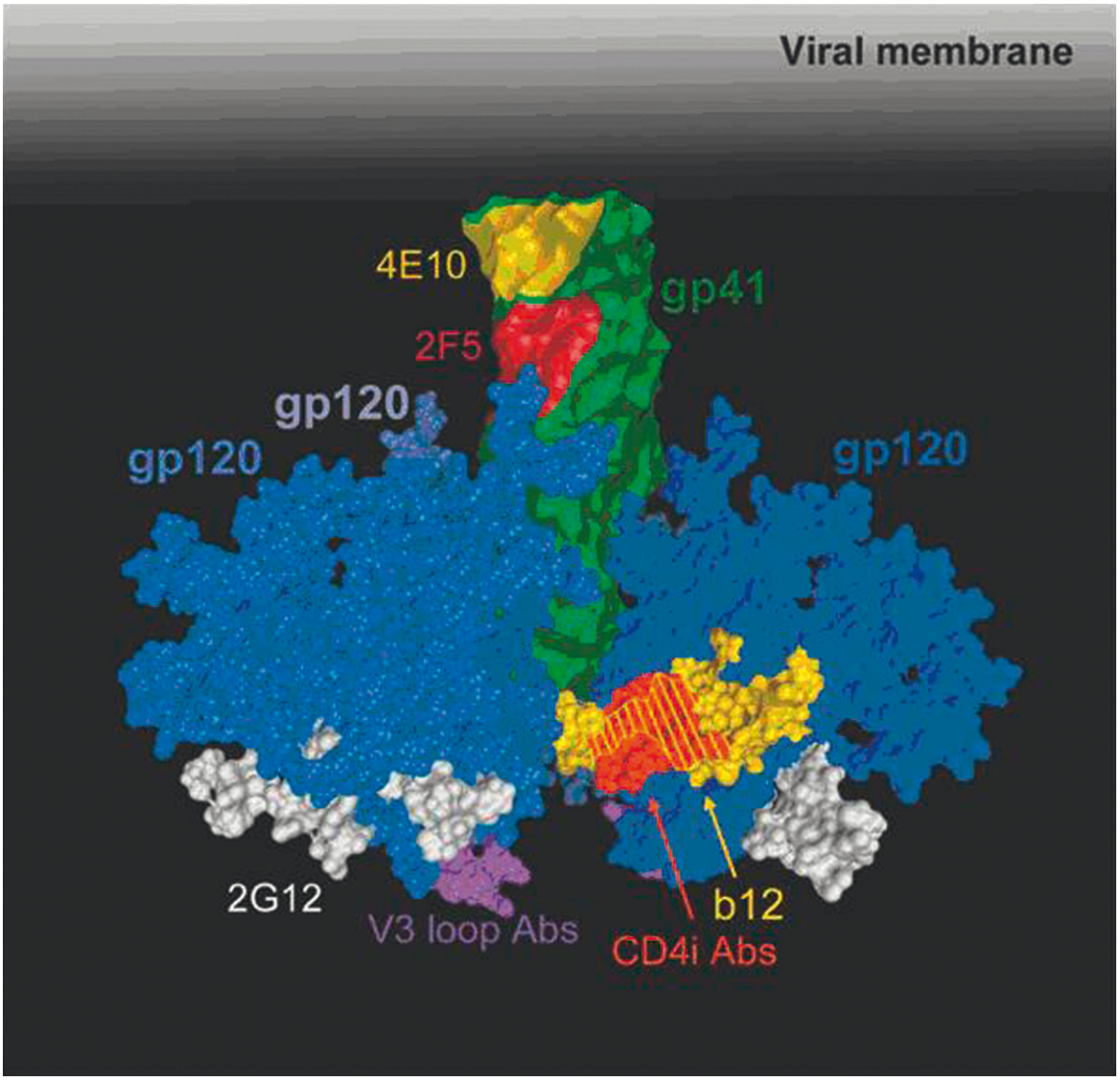


Fig. 87.3 HIV mechanisms of avoiding neutralization. From Burton *et al.*[9]

THE RATIONALE FOR T-CELL VACCINES AGAINST HIV

With the recognition that it will be some time, if ever, that a vaccine will be developed that produces a strong effective neutralizing antibody response, the field has turned to ways to elicit effective T-cell responses against HIV.

The basis of 'T-cell vaccines' is the ability of T cells to recognize peptide sequences of HIV. These sequences can be generated by proteolytic breakdown of the virus, and hence can include the internal, more conserved core proteins of HIV as well as the more variable Env proteins. $CD8^+$ T cells recognize peptides that are 8–11 amino acids in length that are presented on the surface of cells by major histocompatibility complex (MHC) class I antigens; $CD4^+$ T cells recognize peptides 10–18 amino acids in length that are presented on cells by MHC class II antigens. The MHC antigens of infected and of vaccinated individuals can recognize multiple CD4 and CD8 epitopes. This is promising for HIV vaccine development, because by targeting multiple epitopes, there is less likelihood of the vaccine being selected against by escape mutation. This principle is similar to that applied successfully in combination anti-HIV drug therapy.

The disadvantage of T-cell vaccines compared to antibody vaccines is that they can only work once a cell has become infected. Thus T-cell vaccines do not prevent infection but are of potential value in controlling infections. Even with T-cell vaccines, however, there is the possibility of viral escape over time.[15]

There have been some key advances in our knowledge of the immunology of T-cell responses in recent years, and below is a brief summary of those most relevant to T-cell vaccine development.

For a vaccine to generate T cells, the antigens need to be presented by professional 'antigen presenting cells' or APCs. Usually, for an APC to be able to present class I antigens, proteins need to be produced within a cell. These do not have to be produced by the APC, as they could be produced by another cell and then the antigen is taken up by the APC, known as cross-presentation.[16] Class II presentation can occur whether the proteins are produced within the cell or are taken up from outside the cell. APCs need activating in order to initiate an immune response. This occurs by activating signals produced by pattern

recognition receptors (PRRs),[17,18] to which Toll-like receptors belong. The PRRs recognize conserved patterns in the sequences of pathogen molecules and in turn stimulate the APCs to produce activation molecules and inflammatory cytokines. The APCs migrate to the lymph nodes where they present the foreign antigen to naive or memory T cells. The APCs stimulate T cells that recognize presented peptides to expand and differentiate into short-lived acute effector cells, long-lived effector memory cells and long-lived central memory cells.[19]

The vaccine antigen drives the acute effector T-cell response. It is only as the antigen is cleared that effector memory and central memory cells undergo differentiation. The effector memory cells provide an immediate response to a further antigen challenge, whereas the central memory cells traffic through the spleen and lymph nodes looking for their specific antigens. If found, central memory cells rapidly divide and differentiate to provide short-lived effector cells to fight the acute infection. During the clearance of the infection, the central memory cells change their profiles and replenish the effector and central memory cells in order to provide a long-term memory response.

Hence, the key aim of a T-cell vaccine is to present the antigen with the infective agent for which control is desired, such that they elicit both long-lived effector memory and central memory responses.

The problem is how best to generate these T-cell responses. Live attenuated viruses produce not only $CD4^+$ T-lymphocyte responses, but also $CD8^+$ responses, the latter as a result of MHC class processing of proteins that occurs during active replication of the viral vaccine *in vivo*. This approach is used with the current measles vaccine, for instance. Where live attenuated viruses are unsafe or impractical, such as hepatitis A, chemical treatment to remove replicative capacity has been used. However, as neither of these approaches is considered safe enough for HIV, the use of viral proteins that have been expressed by cells lines, such as with hepatitis B, appears to be the safest approach.

An approach that has been used for a recently developed papillomavirus vaccine is the administration of virus-like particles (VLPs). With this method, structural virus proteins are expressed that come together *in vitro* to form nonreplicating, noninfectious particles. These immunogens undergo MHC class II processing and hence are able to express antibodies and $CD4^+$ T-lymphocyte responses. They cannot, however, induce $CD8^+$ T-lymphocyte responses because they do not replicate *in vivo* and so cannot undergo MHC class I processing.

A number of approaches are being used in order to develop T-cell responses within an HIV vaccine. Those undergoing most development at present are the use of recombinant viral vectors, DNA delivery of antigenic material using adjuvant-supported proteins and peptides, and finally a combination of more than one of these approaches. For example, the 'prime-boost' approach uses DNA priming followed by boosting of the response by presenting the antigen by means of a delivery system such as a live viral or bacterial vector. It is beyond the scope of this chapter to give a detailed account of the advantages and disadvantages of DNA vaccination and the use of live viral vectors. Suffice it to say that the 'prime-boost' approach has been extensively developed and used in clinical trials because it appears the most effective means of eliciting good T-cell responses. Until recently, the two most favored boost approaches have used either the DNA priming adenovirus vectors or mammalian poxvirus vectors, with Adenovirus5 (Ad5) and modified vaccinia Ankara (MVA) being the best examples.

The prime boost approach using both of these vectors has delivered some impressive results in animal models, including nonhuman primates. However, none of these successes carried over into trials in humans. A recent MVA candidate vaccine was studied in two large phase II international studies in human volunteers; the results were very disappointing with no ELISpot responses in the majority of vaccines.[20] This has led to a complete halt of the clinical trial program that used this vaccine construct. Similar phase II studies with a construct delivered by Ad5 gave much stronger and sustained ELISpot responses. This gave the rationale for the investigation of this candidate in a much larger efficacy trial, the STEP trial. The results were eagerly awaited, but proved to be a major letdown – not only did the vaccine fail to afford any protection at all, there was also a slightly increased incidence of HIV infections in the vaccine arm compared to the placebo arm.[1] For this reason the use of this vector is being reconsidered.

Another efficacy study, the PAVE 100A trial (www.hivpave.org/about/pave100.html), using a different adenovirus vector and different immunogens, is still going ahead, but will be monitored very carefully for safety. PAVE 100A is designed to enroll several thousand volunteers in two studies focused on distinct populations in the Americas and Africa. The expectations for this study have been readjusted downwards in the light of the previous studies, and some question whether it should be going ahead at all. The rationale to perform such a large study is that we may still have much to learn in terms of breadth of immune responses. The argument against continuing with further studies until we have improved candidate PHV design is discussed later, but centers on the damage it might do to the standing of PHV trials in the future.

There are two key messages that have been reinforced by these two prime boost vaccine candidates:

- there are no good animal models from which results of HIV vaccine trials in humans can be extrapolated; and
- the current measures of immune responsiveness of potential HIV vaccines do not correlate with protection.

With the recognition of this, there are renewed efforts to try to improve these barriers to PHV development. For example, assays are being carried out to determine whether different states of differentiation of T cells can reveal any subset(s) that correlate with protection in nonhuman primates. It appears as if the central memory cells have a better correlation than other cells.

LESSONS FROM THE NATURAL HISTORY OF HIV PROGRESSION

The rates of progression of HIV in the complete absence of treatment, as measured by $CD4^+$ cell count decline, can vary enormously between patients. At one extreme are the rare 'elite nonprogressors', alternatively termed 'HIV controllers', who maintain an undetectable HIV viral load in serum for long periods of time and have no discernable $CD4^+$ count decline during these periods. Such control has been observed for long periods, up to 25 years so far in some patients. These controllers are much more likely to have certain genetic factors, specifically certain HLA class I alleles, and also $CD8^+$ T-cell responses generated through these alleles.[21] They are also more likely to have virus-specific, strong $CD4^+$ T-cell responses. However, no one feature is common to all HIV controllers. Clearly there might be much to learn from these patients with regard to HIV vaccines designed to prevent or treat HIV, and this population is being studied intensively for further clues.

MUCOSAL VACCINES AGAINST HIV

There is an extensive and growing knowledge base concerning mucosal immunity, and this is being applied to the development of HIV vaccine candidates. For successful vaccines for other infections given via the parenteral route, protection appears to be induced by systemic immune responses; the mucosal responses, although poorly studied, appear to be less robust. In nonhuman primate models, high doses of neutralizing antibodies given intravenously can reach mucosal sites and block mucosal transmission of SHIV.[13,22] Unfortunately, neutralizing antibodies of this concentration have not been induced by HIV vaccine candidates thus far.

Most HIV infections are acquired by the mucosal route, vaginally, rectally or, less commonly, orally. Once HIV has gained entry via mucosa, it is transmitted quickly throughout the body. After rhesus macaques were inoculated intravaginally with SIV, the virus was detected in Langerhans cells or dendritic cells under the vaginal epithelium within 4 hours, in the internal iliac lymph nodes within

48 hours and in the blood within 5 days.[23,24] SIV was found in distant lymph nodes and bone marrow at 12 days.[25] Once established in the lymphoid tissue, HIV undergoes massive replication in the largest mucosal-associated lymphoid tissue (MALT), the bowel, and this leads to a marked depletion of $CD4^+$ cells in the intestinal tract.[26] A unique aspect of HIV, and a challenge for vaccine development, is that a pool of latently infected 'resting' $CD4^+$ cells is established early in the course of the primary infection.[27] This pool seems resistant to clearance, even by prolonged antiretroviral therapy.[28]

Therefore, if early HIV infection is to be cleared before it becomes established, then HIV vaccines must induce effective immune responses at the point of entry within the first few days of exposure to the virus.[29] Certainly the effective induction of mucosal immunity is thought to be one of the most important requirements for an HIV vaccine. As such, it seems likely that the best defense against HIV would be vaccines that induce both systemic and mucosal responses against the virus. Mucosal vaccines might offer this more than immunization by other routes[30] because of the potential for enhancement of mucosal immunity, as well as being easier to deliver.

However, not all routes of mucosal vaccination are the same. Both the quality and the quantity of antigen-specific immune responses at a mucosal site appear to be highly dependent on the exact route of mucosal vaccination. As a rule of thumb, oral administration induces antigen–antibody-specific responses in the small intestine, colon, mammary and salivary glands, as well as systemically; however, although antigen–antibody responses in the large intestine and female genital tract are weak,[31–33] they do induce antigen-specific $CD8^+$ cytotoxic T lymphocytes (CTLs) in the rectal mucosa.[34] Rectal administration has been poorly studied in humans, but mice models show that vaccines can induce strong antigen-specific mucosal IgA and CTL responses in the rectum and systemically.[33,35] Nasal administration in humans induces strong antigen-specific antibody responses in salivary glands, the respiratory tract, genital tract and systemically, but not in the intestine, although this route can induce strong antigen-specific IgA and CTLs in the female genital tract.[36,37] Thus, because HIV first infects the majority of people genitally, nasal vaccination is an attractive option for a mucosal HIV vaccine.[33,35] The vaginal and transcutaneous routes would also offer advantages as they have been shown to induce antigen-specific IgA and IgG in the human female genital tract.[33,38]

NOVEL HIV VACCINE STRATEGIES

The word 'novel' is often applied liberally to new HIV vaccine candidates even if they are merely an extension of existing approaches. There is a clear need to explore 'novel' approaches that are substantially, if not radically, different from anything that has preceded them. We are not going to attempt to list those approaches, but instead will briefly give a few examples. None of the examples given below are clear leaders in the race to find the best approach towards an effective HIV vaccine and they are not mutually exclusive. A broad agreement is emerging that a systematic examination of the most promising novel approaches needs to be carefully undertaken, and that those with the most valuable results should be given further accelerated support. This forms the basis of a number of current initiatives, including programs for innovative research from the Gates Foundation.

The following is a highly selective selection of some of the good new ideas that are being evaluated at present.

One example of innovative work is the induction of potent $CD8^+$ T-cell responses by using novel biodegradable nanoparticles carrying: human immunodeficiency virus type 1 (HIV-1) gp120.[39] Another approach that is of promise is the use of passive immunization as a tool to identify protective HIV-1 Env epitopes that might be useful to incorporate in future HIV vaccines.[40] It seems that the adaptive immune response may emerge too slowly to prevent the spread of HIV after the initial inoculation. Hence alternative avenues of research are being directed to innate immune mechanisms that might be incorporated into novel vaccination strategies.

The strongest immune response in humans is the alloimmune response, mediated by presentation of foreign antigens by human leukocyte antigens. There is evidence that this immune response might play a significant role in natural protection against HIV.[41,42] This has encouraged current ongoing work in macaques to determine whether the protective potential of alloimmunity can be incorporated into effective vaccines against SIV and, if so, whether this might lead to studies in humans (T Lehner, personal communication, 2008).

THE FUTURE

It is highly unlikely that the current vaccine approaches that have entered human trials to date will prove successful by themselves. There is a broad consensus amongst the scientific community that we must call a halt to large trials of the efficacy of PHVs in humans unless they are with truly new candidates that offer some reasonable possibility of success. This consensus is not universal, however. The danger of continuing with studies of candidates with little chance of efficacy is the real possibility that volunteers and donors alike would withdraw support from future HIV vaccine trials. We are learning from behavioral studies just what it is that drives volunteers to participate or turn away from PHV trials[43] and more effort is required to keep abreast of this critical aspect of vaccine studies. Major advances are required before we will have a PHV candidate of true merit, one that we can reasonably hope will offer efficacy. At the present time our efforts should be directed towards building the foundations of HIV vaccine science.

So, how should we be directing our energies over the next few years? In the face of one of the greatest public health issues to face mankind, stopping work on HIV vaccines is not an option. The best tactic when facing a seemingly omnipotent 'enemy' is a tactical retreat in order to reconsider tactics.

This work has already begun in coordinating HIV vaccine efforts towards the 'big' questions that include:

- How do we identify and reproduce broadly neutralizing antibody responses in humans?
- How can we identify correlates of immunity in which to screen and select HIV vaccine candidates at an early stage?
- How can we improve animal models of HIV infection?
- How can we best identify those truly original vaccine concepts that deserve further evaluation?
- How can we maintain enthusiasm from volunteers and donors alike in the face of the lack of progress?

To this end a number of consortia are pooling efforts and identifying cohorts of patients to study, such as 'HIV controllers' and immunologic and virologic questions to tackle through groups such as 'neutralizing antibody consortia'.

When the next edition of this textbook is published, we hope that we can report some real progress with some of the 'big questions' of HIV vaccine development.

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Chapter 88

Alexandre Harari
Giuseppe Pantaleo

The immunopathogenesis of HIV-1 infection

This chapter examines the immunologic and virologic mechanisms involved in the pathogenesis of HIV-1 infection and the interaction between the virus and the host. The recent availability of highly active antiretroviral combination therapy (HAART) has significantly influenced the natural history of the infection, delaying the progression to overt AIDS and prolonging survival. At the same time, increasing knowledge of the pathogenic mechanisms and of the limitations of HAART has made it clear that eradication of the virus with the available conventional antiviral drugs is not feasible. Recent advances in our understanding of the correlates of protective immunity have drawn attention to the development of immune-based interventions in order to achieve long-term control of HIV-1 disease.

THE NATURAL HISTORY OF HIV INFECTION

The typical course of HIV-1 infection is defined by different phases that generally occur during a period of between 8 and 12 years. Although the pattern and the course of the infection are highly variable among HIV-1-infected patients, three distinct phases can be identified (Fig. 88.1):[1]

- primary HIV-1 infection;
- chronic asymptomatic phase; and
- overt AIDS.

Primary HIV-1 infection

Primary HIV-1 infection is a transient condition, often accompanied by a symptomatic illness of variable severity in 40–90% of patients, and is invariably accompanied by:

- an initial rapid rise in plasma viremia, often to levels in excess of 1 000 000 RNA copies/ml;
- a decrease in the blood $CD4^+$ T cells and a massive decrease $CD4^+$ T cells in tissues, particularly in the gut and lymph nodes; and
- a large increase in the blood $CD8^+$ T-cell count.

The marked decline of plasma viremia generally coincides with resolution of the clinical syndrome.[2] The decrease in the viral load correlates with the appearance of virus-specific immune responses (see below), particularly HIV-1-specific cytotoxic T lymphocytes (CTLs), indicating that virus-specific immune responses certainly play a crucial role in the initial downregulation of virus replication.[3–6]

The signs and symptoms of primary HIV-1 infection generally appear 2–4 weeks after virus exposure (see Fig. 88.1). The duration of the clinical syndrome ranges between a few days and more than 10 weeks but generally lasts less than 14 days. The clinical presentation of the primary HIV-1 infection may mimic acute mononucleosis (see Chapter 89) as well as many other febrile acute illnesses, emphasizing the nonspecific nature of these symptoms and the difficulty of obtaining an accurate early diagnosis.

Because the acute clinical syndrome associated with primary infection is not specific for HIV-1, the diagnosis is based on laboratory tests. In this regard, it is important to underscore the fact that anti-HIV-1 antibodies are usually negative during the acute phase of illness, as well as the Western blot, which evaluates the generation of specific antibodies against different HIV-1 proteins. The Western blot is considered to be positive when there are at least three specific bands and/or two bands but with antibody reactivity against HIV-1 *env* and *gag*. Early diagnosis, therefore, relies on a history of exposure, a positive p24 antigen enzyme-linked immunosorbent assay (ELISA) or the detection of plasma viral RNA (almost always >50 000 copies/ml of plasma).[2]

The chronic asymptomatic phase

The primary HIV-1 infection is followed by a long phase of clinical latency (median time of 10 years), during which neither signs nor symptoms of illness are present. Relatively stable levels of virus replication and of $CD4^+$ T-cell counts for a variable period of time characterize this phase of infection. This 'stability' of measures of disease activity is apparent in the blood only. Virus replication in the gut and the accumulation of extracellular virus trapped in the follicular dendritic cell network are particularly active in the lymphoid tissue, where a progressive anatomic and functional deterioration occurs, impairing the ability to maintain effective specific immune responses over time.[7,8] This is reflected by the rapid increase in the levels of viremia and by a drop in $CD4^+$ T-cell counts, which can suddenly speed up the transition from this phase to the advanced stage of the disease.

The advanced stage of HIV-1 disease is marked by low $CD4^+$ T-cell counts (<200 cells/ml) and by the appearance of constitutional symptoms. It may be complicated by the development of AIDS-defining opportunistic infections.[1]

Overt AIDS

Overt AIDS defines the end stage of HIV-1 infection. In the absence of antiretroviral therapy, this phase leads to death in 2–3 years. The risk for death and opportunistic infections significantly increases with $CD4^+$ T-cell counts below 50 cells/ml. Fortunately, the recent advent of HAART, including at present as many as six different classes of antiretroviral drugs administered in different combinations, is significantly decreasing the rate of progression, morbidity and mortality of HIV-1 infection (see Chapter 100).

Clinical course of the infection

The clinical course of HIV-1 infection is variable. In the majority (60–70%) of HIV-1-infected patients, the median time between infection and development of AIDS, in the absence of therapy, is

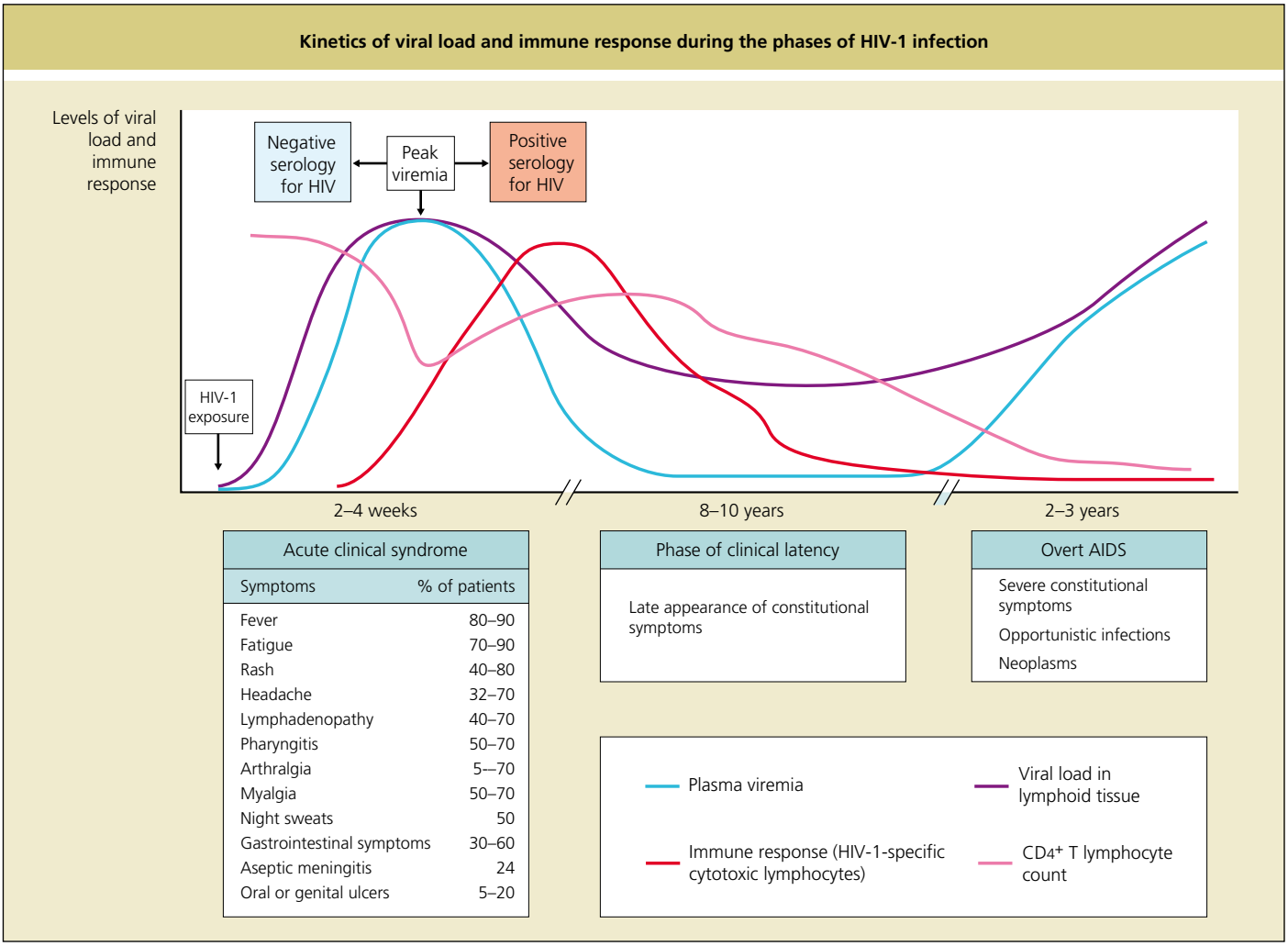


Fig. 88.1 Kinetics of viral load and immune response during the phases of HIV-1 infection. After HIV-1 exposure, initial virus replication and spread occur in the lymphoid organs, and systemic dissemination of HIV-1 is reflected by the peak of plasma viremia. A clinical syndrome of varying severity is associated with this phase of primary HIV-1 infection in up to 70% of HIV-1-infected persons. Downregulation of viremia during the transition from the primary to the early chronic phase coincides with the appearance of HIV-1-specific cytotoxic T cells and with the progressive resolution of the clinical syndrome. The long phase of clinical latency is associated with active virus replication, particularly in the lymphoid tissue. During the clinically latent period, $CD4^+$ T-cell counts slowly decrease, as does the HIV-1-specific immune response. When $CD4^+$ T-cell counts decrease below 200 cells/ml (i.e. when overt AIDS occurs), the clinical picture is characterized by severe constitutional symptoms and by the possible development of opportunistic infections and/or neoplasms.

10–11 years. These HIV-1-infected persons are defined as typical progressors (Fig. 88.2), and the clinical course of the infection that they generally experience is the one described above.

However, about 10–20% of subjects progress rapidly, developing AIDS in less than 5 years of infection, and they are therefore called rapid progressors (see Fig. 88.2). In these patients, after the primary HIV-1 infection, plasma virus levels are often $>10^5$ copies of HIV-1 RNA/ml and, in particular, $CD4^+$ T-cell counts start to decrease much earlier and more rapidly during the chronic asymptomatic phase, leading to the eventual development of AIDS. Furthermore, both humoral and cell-mediated HIV-1-specific immune responses are either never detected or rapidly lost after the transition from the acute to the chronic phase of infection.

At the other extreme, it is estimated that 5–15% of HIV-1-infected people will remain free of AIDS for more than 15 years; these people are termed slow progressors (see Fig. 88.2). In this situation, $CD4^+$ T-cell counts remain stable and they are frequently >500 cells/ml, and plasma virus levels are usually <10 000 HIV-1 RNA copies/ml.

Slow progressors include a further subgroup of HIV-1-infected people, so-called long-term nonprogressors (see Fig. 88.2). About 1% of HIV-1-infected subjects probably fall into this category. The definition of long-term nonprogressors should be limited to those who have had a documented infection for at least 8–10 years, are naive to antiretroviral therapy and have no signs of disease progression (e.g. constant high counts of $CD4^+$ T cells and either low (500–1000 copies of RNA/ml) or very low (<50 copies/ml) plasma virus levels).[1,9] Recently, a subgroup of HIV-1 infected individuals with chronic infection and levels of viremia <50 copies/ml has been termed 'elite controllers'. The definition of elite controllers is independent of the length of the control of viremia.[10]

This wide variability of the natural course of the disease is evidenced by the presence of different driving forces – genetic, immunologic and virologic factors – that determine the evolutionary pattern of HIV-1 infection in the individual patient.[11] It is therefore important, first, to identify the different determinants of the rate of disease progression and, second, to elucidate how these driving forces work together. Furthermore, the potential ability of HAART to restore some determinants of long-term control of the virus must be evaluated in depth. These arguments are discussed in detail below.

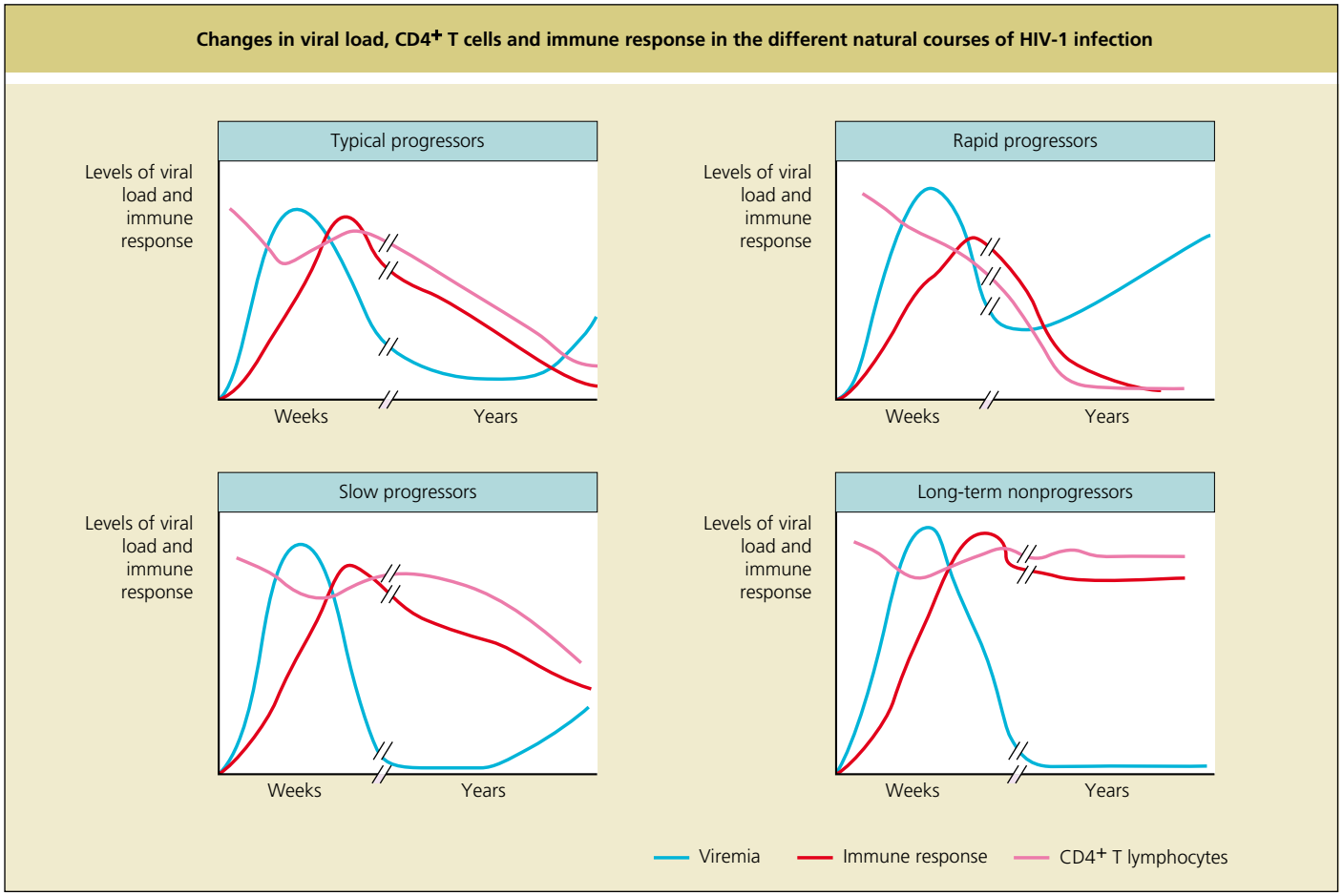


Fig. 88.2 Changes in viral load, CD4+ T cells and immune response in the different natural courses of HIV-1 infection. Typical progressors represent 60–70% of the total HIV-1-infected population, rapid progressors represent 10–20%, slow progressors represent 5–15% and long-term nonprogressors represent 1%.

The variability of the natural course of HIV-1 infection also underlines the need for markers of disease progression that may identify as early as possible the patients who are at risk for a more rapid disease progression. This could warrant either the use of different, perhaps more aggressive, therapeutic strategies or the need to monitor these patients more closely, or both.

SEQUENCE OF PATHOGENIC EVENTS LEADING TO THE ESTABLISHMENT OF HIV-1 INFECTION

HIV-1 can be transmitted by different routes: by sexual contact, through either genital–genital or genital–oral sex; by blood–blood contamination, via either transfusion of blood and infected blood-derived products or needle sharing among intravenous drug users; and by maternal–fetal transmission. The most common route of infection is sexual transmission at the genital mucosa.[12]

Early pathogenic events after entry of HIV-1 into the body

The acute intravaginal infection of rhesus monkeys with the simian immunodeficiency virus (SIV) represents a very useful model for studying the sequence of cellular events that characterize the very early steps of infection after sexual transmission. In this model, tissue dendritic cells (i.e. Langerhans cells that reside in the lamina propria below the vaginal epithelium) are the first potential target cells of HIV-1 (Fig. 88.3).[13]

The dendritic cells constitute a complex and highly developed system of antigen-presenting cells that are able to prime naive T cells. Their potent antigen-presenting ability is associated with the high expression of major histocompatibility complex (MHC) class I and class II molecules and co-stimulatory molecules on the cell surface. The ability of dendritic cells to attract and prime naive T cells can be explained by the expression of a type II membrane protein with an external mannose binding, C-type lectin domain, named DC-SIGN (meaning dendritic cell-specific intercellular adhesion molecule (ICAM)-3 grabbing non-integrin).[14,15] It has been suggested that interaction between DC-SIGN and ICAM-3 is responsible for the initial contact between dendritic cells and resting T cells, which represents a critical step for the initiation of the T-cell immune response. Furthermore, a major contribution to the ability of dendritic cells to initiate T-cell immunity is also provided by the fact that dendritic cells express high levels of specific chemokines that target naive rather than memory T cells.[16] Therefore, dendritic cells play a key role, both in priming the initial virus-specific immune response and in serving as a carrier for the transport of HIV-1 to the nearest lymphoid station.

It is important, however, to mention the differences that exist between the Langerhans cells (also called 'epidermal' or 'epithelial' dendritic cells) and the 'dermal' or 'subepithelial' dendritic cells.[15] Langerhans cells do not express DC-SIGN and, once they have

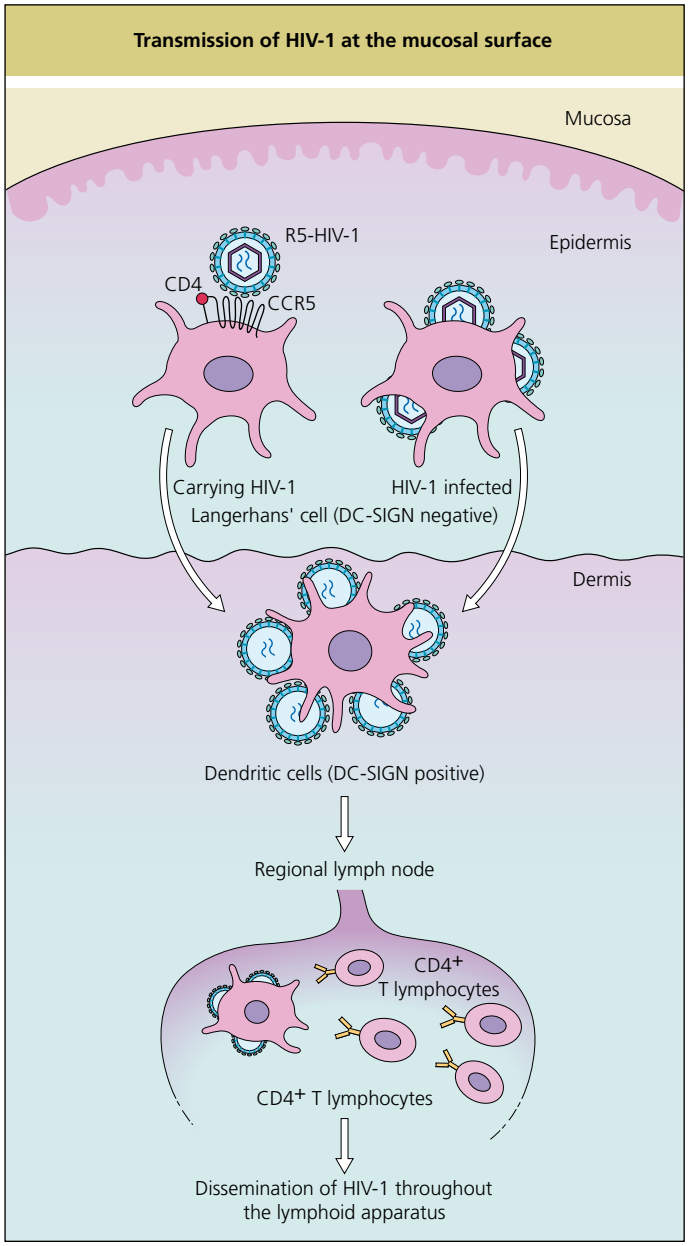


Fig. 88.3 Transmission of HIV-1 at the mucosal surface. After entry at the mucosal epithelium, Langerhans cells, also named epidermal dendritic cells, can either be infected by R5 (macrophage (M)-tropic) strains of HIV-1 or pick up HIV-1 virions. Epidermal, DC-SIGN-negative dendritic cells are thought to select for the M-tropic viruses that are the most frequently transmitted variants. The M-tropic HIV-1 carried by epidermal dendritic cells can bind to additional subepithelial DC-SIGN-positive dendritic cells. DC-SIGN can capture HIV-1 on the cell surface of dendritic cells without allowing viral entry. It is thought that DC-SIGN-positive dendritic cells play the major role in the delivery of virus to T cells, thus greatly amplifying HIV-1 infection.

encountered HIV-1 below the vaginal epithelium, they can either be infected or pick up HIV-1 virions. Epidermal, DC-SIGN-negative dendritic cells are thought to select for the macrophage (M)-tropic viruses that are the most frequently transmitted variants.[17,18] The M-tropic HIV-1 carried by epidermal dendritic cells can bind to additional subepithelial DC-SIGN-positive dendritic cells. DC-SIGN can capture HIV-1 on the cell surface of dendritic cells without allowing viral entry.[19] It is thought that DC-SIGN-positive dendritic cells play the major role in the delivery of virus to T cells, thus greatly amplifying HIV-1 infection.[18] Subsequently, dendritic cells migrate to the internal iliac lymph nodes, where they target the T cell areas and then present the viral antigens to activated virus-specific T cells. Dendritic cells can support viral replication only in the presence of activated T cells.[16]

Recent advances in our understanding of the mechanisms that modulate the infectivity of HIV-1 can explain why 95% of viruses transmitted are M-tropic. For more than 10 years, it has been known that human CD4 is sufficient for binding HIV-1 gp120 to cells, but it is sufficient neither for fusion nor for penetration of the viral envelope into the host cell.[20–22] It is now clear that certain cell-surface receptors for chemokines function as co-receptors by co-operating with the CD4 molecule; moreover, different chemokine receptors, together with CD4, allow entry of HIV-1 strains with distinct cellular tropism. There are two major classes of chemokines: CC, if the first two cysteines are adjacent; and CXC, if they are separated by a single amino acid. The CC-chemokine receptor (CCR)5, a seven-transmembrane G-protein-coupled receptor, is the major co-receptor for M-tropic or R5 strains of HIV-1, whereas CXCR4 is the one for T-cell-tropic or X4 strains of HIV-1. Langerhans cells or epithelial dendritic cells express CCR5 but they may not express CXCR4, giving a reason for the preferential transmission of R5 HIV-1 strains.[23]

To this extent, it is worth noting that subepithelial dendritic cells are able to capture the virus through DC-SIGN,[19] a mechanism that is independent of CD4 and co-receptors. However, although both R5 and X4 strains of HIV-1 are carried by dendritic cells to the nearest lymphoid station, only R5 HIV-1 envelopes have the unique ability to mediate an activation signal into $CD4^+$ T cells and to recruit them by chemotaxis.[24] Therefore, the combination of the two events (i.e. the differential expression of co-receptors on the initial target cells and the unique signaling ability of R5 envelopes of HIV-1) may explain why R5 HIV-1 variants are preferentially transmitted, ensuring the rapid recruitment of a large number of activated $CD4^+$ T cells and spread of HIV-1 in the lymphoid organs. It is important to emphasize that rapid recruitment and spreading of target cells (i.e. activated $CD4^+$ T cells) confers to HIV-1 a major advantage because these events occur before the appearance of effective virus-specific immune responses.

Within 2 days after infection, HIV-1 can be detected in the draining lymphoid tissue, and it rapidly disseminates throughout the lymphoid system. Afterwards, HIV-1 enters the bloodstream, where viral replication can be detected in plasma 5 days after infection (see Fig. 88.3). In humans, the time from mucosal infection and initial plasma viremia varies, ranging between 4 days and 11 days according to available estimates. It is of note that the risk of infection is increased by conditions that decrease the function of mucosal barriers, such as lesions caused by the presence of concomitant inflammatory or infectious diseases (e.g. cervicitis, urethritis, genital ulcers).

These same steps of infection can be described in the case of genital–oral HIV-1 transmission, because nasopharyngeal tonsils and adenoid tissue contain many cells of dendritic origin that can support viral replication more efficiently than Langerhans cells.[2]

The role of the gut and lymph nodes in primary HIV-1 infection

The study of virologic events occurring during primary infection with either HIV-1 or SIV emphasizes the key role of the gastrointestinal (GI) tract and lymphoid tissue in the establishment of infection. With regard to the role of the GI tract, it is important to mention that the majority of T cells, particularly CD4 T cells, are contained within the GI tract. Recent studies have demonstrated that memory CD4 T cells within the GI tract, which express the CCR5 HIV-1 co-receptor, are the earliest targets of HIV-1 during primary infection.[25] The monitoring of CD4 T-cell depletion following SIV infection has shown that initial decrease in CD4 T cells may vary between 60% and 80% in blood and lymph nodes while the loss of CD4 T cells is almost complete in the GI tract.[26] Studies performed in humans have also shown that, in

addition to the greater depletion of CD4 T cells in the gut, the proportion of HIV-1 infected CD4 T cells resident in the gut is 10–100 fold higher compared to blood.[25]

With regard to the role of lymphoid tissues, longitudinal and cross-sectional analyses of HIV-1 or SIV distribution were performed on lymph node biopsies taken from rhesus monkeys or HIV-1-infected patients. In the SIV model of acute infection, virus can be detected in the lymph node as early as 5 days after infection. At this time, as shown by *in-situ* hybridization analysis for the detection of HIV-1/SIV RNA, virus is mostly present in the form of numerous individual cells expressing viral RNA, and the highest number of virus-expressing cells is observed 7 days after SIV inoculation. Interestingly, the occurrence of the peak number of virus-expressing cells in the lymph node occurs at the same time as the peak of plasma viremia, or shortly precedes it. The cross-sectional analysis of lymph nodes obtained from HIV-1-infected patients indicates that the kinetics of virus distribution in lymph nodes is consistent with those in the SIV model of acute infection.

Altogether, these findings indicate that the GI tract is the primary site for CD4 T-cell depletion caused by HIV-1 infection.[27] In addition, both gut and lymph nodes are primary anatomic sites of virus infection, replication and dissemination.[7,28]

During the transition from primary to chronic infection, a switch from individual virus-expressing cells to virus trapped by the follicular dendritic cell network of lymph node germinal centers occurs (Fig. 88.4). The trapped virus becomes the dominant form of virus present in lymph nodes, and this event is associated with a dramatic decrease in the number of individual cells expressing viral RNA.[7,28]

At least in part, this is the result of the emergence of virus-specific CTLs that can be detected very early during primary infection and may mediate the elimination of HIV-1-producing cells. Furthermore, the trapping of HIV-1 virions in the follicular dendritic cell network is itself the result of the HIV-1-specific humoral response. In fact, HIV-1 virions are complexed with immunoglobulins and complement, and the binding of these complexes on the extracellular surface of follicular dendritic cells occurs through complement receptors expressed on follicular dendritic cells. In both SIV and HIV-1 infection, the transition from primary to early chronic infection is marked by a decrease of viral RNA in plasma and the resolution of the acute clinical syndrome. Therefore, it is clear that virus-specific immune responses are not only present very early during primary infection but may also significantly affect virus distribution occurring in the early phase of both SIV and HIV-1 infection.[29]

MECHANISMS RESPONSIBLE FOR VIRUS ESCAPE FROM IMMUNE RESPONSE AND ESTABLISHMENT OF CHRONIC INFECTION

Early in primary HIV-1 infection, vigorous virus-specific immune responses can be detected, and they may contribute to both control of the initial peak of virus replication and the reduction in plasma viremia.[3–6] However, primary HIV-1 infection invariably results in the establishment of chronic disease in the host, inducing a progressive deterioration of the different components of the immune response.

HIV-1-specific immune responses lack the ability to control HIV-1 and to block the progression of the disease. Nevertheless, similar types of immune response are effective against other viruses, such as Epstein–Barr virus (EBV) and cytomegalovirus (CMV). HIV-1 differs from these other viruses by being able to target very early (during the primary infection) a broad spectrum of effector components of the antiviral immune response and in being able to render these antiviral effector mechanisms ineffective or reshape them into self-defense mechanisms.[30]

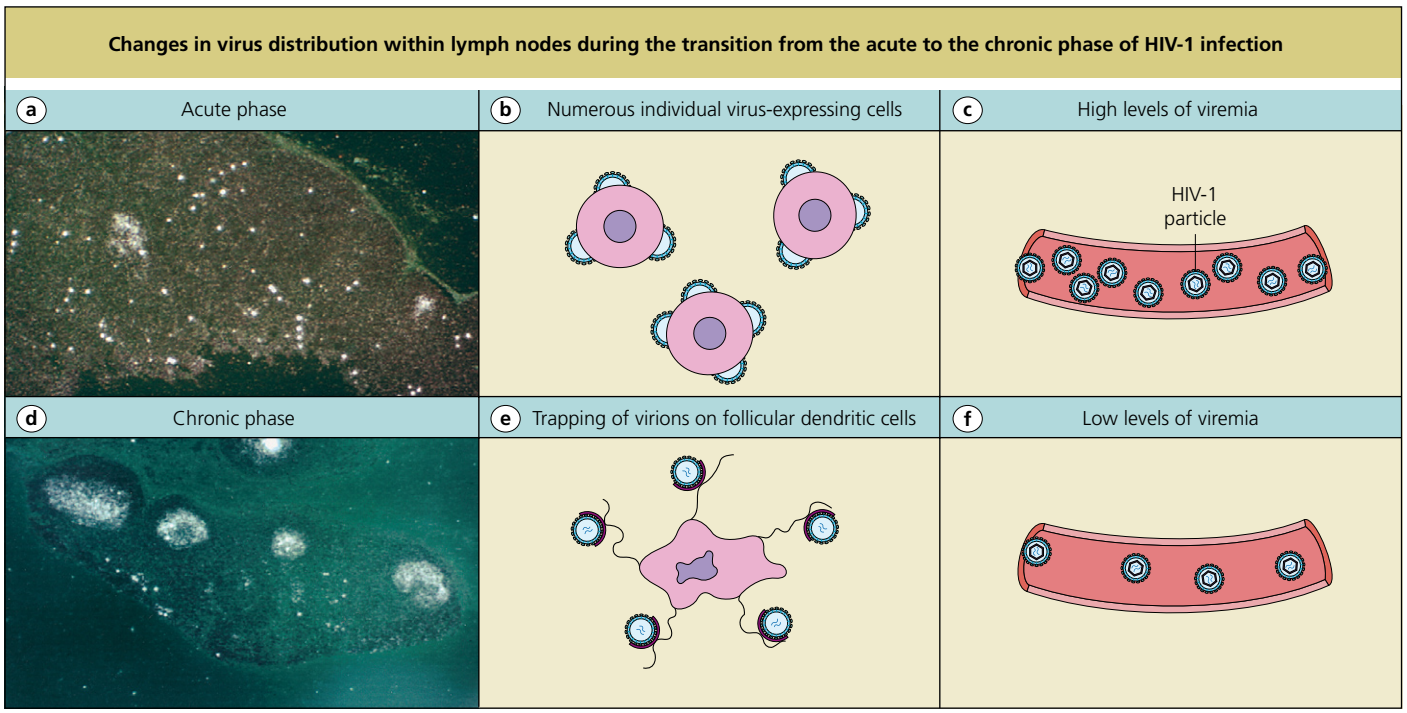


Fig. 88.4 Changes in virus distribution within lymph nodes during the transition from the acute to the chronic phase of HIV-1 infection. (a) During the initial weeks of primary HIV-1 infection, the virus is detected in lymphoid tissue as individual virus-expressing cells. This is shown by *in-situ* hybridization for the detection of HIV-1 RNA (white dots indicate HIV-1 RNA-positive cells). (b) Numerous individual virus-expressing cells seen in the acute phase. (c) Elevated virion levels are found in the circulation in the acute phase. (d) After transition to the chronic phase of the disease, virions trapped in the follicular dendritic cell network become the dominant form of HIV-1, as shown by *in-situ* hybridization for the detection of HIV-1 RNA (diffuse white areas indicate virus trapped within follicular dendritic cells). (e) Binding of virions on the extracellular surface of follicular dendritic cells in the chronic phase. (f) The number of circulating virions is dramatically reduced in the chronic phase.

Virologic mechanisms of HIV-1 escape from the immune response

HIV-1 possesses the ability to put in motion several mechanisms as the result of the interaction with the host. Some of these virologic mechanisms can be identified:

- formation of a stable pool of latently HIV-1-infected CD4+ T cells containing virus that is capable of replicating;[31–35]
- the genetic variability of HIV-1;[36] and
- trapping of infectious virions on the surface of follicular dendritic cells.[37]

The rapid formation of a pool of latently HIV-1-infected CD4+ T cells is a key event in the immunopathogenesis of HIV-1 infection for several reasons. First, this event occurs very early and most probably before the appearance of the host's virus-specific immune responses. Second, the pool of latently infected CD4+ T cells contains replication-competent HIV-1 proviral DNA. The proviral DNA can be detected even in compliant HIV-1-infected persons who have been receiving HAART for a long time (i.e. for more than 2 years); moreover, the virus is wild type with respect to known drug-induced mutations in the genome. This emphasizes the fact that this pool of cells is a stable reservoir in which HIV-1 remains sheltered from the effects of host immune responses and HAART.[31–35] Furthermore, it is worth noting that initiation of HAART very early during primary HIV-1 infection does not appear to have a significant impact on the size of this pool of CD4+ T cells, indicating that this pool is created very rapidly after infection. Third, the decay of this pool of infected CD4+ T lymphocytes is very slow, and the rate of decay is not much influenced by the effective suppression of virus replication obtained by HAART. This clearly represents a major obstacle to the goal of HIV-1 eradication (see below) and long-term control of virus replication.[33–35]

The high genetic variability of the virus is another efficient mechanism by which it escapes the host immune response.[36,38] HIV-1 possesses the intrinsic ability to mutate very rapidly. Both during primary and established chronic infection, rapid mutations in the epitopes recognized by HIV-1-specific CTLs may occur. As a consequence, both humoral and cell-mediated virus-specific immune responses quickly lose their ability to control the virus efficiently.

An additional way by which HIV-1 is able to reshape antiviral mechanisms is the trapping of the virus on the surface of follicular dendritic cells in lymphoid tissue germinal centers. As already described, HIV-1 is trapped by the follicular dendritic cell network during the transition from primary to chronic infection and this becomes the dominant form of virus in lymphoid tissue in the chronic phase. Formation of immune complexes and their attachment to the follicular dendritic cell network are physiologic mechanisms that are generally devoted to the clearance of the pathogen and to the generation and maintenance of effective immune responses. In HIV-1 infection, however, these mechanisms lead to the formation of a stable reservoir of infectious virions, representing a continuous source for the infection of CD4+ T cells, and to a chronic inflammatory reaction that ultimately results in the destruction of the lymphoid tissue.[7,8]

Immunologic mechanisms of HIV-1 escape from the immune response

In addition to the virologic mechanisms described above, through which HIV-1 escapes and reshapes the host immune response, there are some immunologic mechanisms that can be identified:

- deletion of HIV-specific CD4+ T-cell clones;[39]
- deletion/exhaustion of HIV-specific cytotoxic CD8+ T-cell clones;[40–43]
- generation of virus escape mutants mediated by CTLs;[30]
- impairment of the function of antigen-presenting cells;[30] and
- interference with humoral neutralizing response.[30]

In the majority of HIV-1-infected patients who have established chronic infection, HIV-1-specific CD4+ helper T-cell responses, particularly the ability of virus-specific CD4 T cells to proliferate after antigen-specific stimulation, cannot be detected, although it is possible to find evidence of such HIV-1-specific CD4+ T-cell responses in long-term nonprogressors.[39,44] In contrast, HIV-1-specific effector CD4+ T-cell responses, i.e. virus-specific CD4 T cells secreting interferon gamma (IFN-γ) and tumor necrosis factor (TNF), are consistently detected in patients who have chronic infection.[45] The persistence of the CD4+ helper T-cell response may represent one of the determinants of the course of the disease in long-term nonprogressors. In contrast, typical progressors do lose CD4+ helper T-cell responses very early in the natural history of the disease (during the primary HIV-1 infection) as a result of direct or indirect virus-induced cytopathology, particularly within the GI tract and lymph nodes.[39,46] Furthermore, initiation of HAART during chronic infection and even during early HIV-1 infection is associated with the restoration of the HIV-1-specific CD4+ helper T-cell responses only in a subset (about 30–40%) of patients after prolonged treatment.[47] It is, however, possible to preserve HIV-1-specific CD4+ T-cell responses if HAART is initiated at the time of the peak of plasma viral RNA (i.e. very early during primary HIV-1-infection).[39,46]

It is also worth noting that T helper (Th)1 cells, the CD4 T cells mounting the immune responses against HIV-1, preferentially express CCR5 on the membrane surface, suggesting that HIV-1 R5 strains may preferentially and selectively infect Th1 cells during primary HIV infection, when R5 quasispecies predominate. In this regard, a series of studies have demonstrated that the initial expansion of HIV-1-specific CD4+ T cells with effector function is aborted during primary infection.[48] In contrast, the expansion of CMV-specific CD4+ T cells in patients who experienced primary HIV-1 and CMV co-infection was not suppressed. These studies indicate a preferential infection of HIV-1-specific CD4+ T cells. Furthermore, they provide evidence that HIV-1-specific CD4+ T-cell clones may be rapidly deleted very early during primary HIV-1 infection and that the possibility of rescuing these responses is strictly dependent upon the time of initiation of HAART.[39,48]

Furthermore, the lack of HIV-1-specific CD4+ helper T-cell responses has two other important consequences. First, it can significantly affect the induction and persistence of HIV-1-specific CD8+ CTL responses, because the latter require continuous cognate Th function provided by antigen-specific CD4+ T cells. Therefore, generation and maintenance of vigorous virus-specific responses by CTLs can be compromised over time. Second, it can considerably affect the development of HIV-1-specific antibody responses, because the development of humoral responses is strictly dependent on CD4+ antigen-specific Th cells.[30]

A rapid deletion of certain HIV-1-specific cytotoxic CD8+ T-cell clones also occurs during primary HIV-1 infection, in a manner that is analogous to the HIV-1-specific responses mediated by CD4+ helper T cells.[41] These CD8+ T-cell clones undergo massive clonal expansion and may be deleted by a mechanism analogous to the clonal exhaustion that is observed in mice during acute lymphocytic choriomeningitis virus infection. This clonal exhaustion of HIV-1-specific CD8+ CTLs causes the early impairment of the virus-specific responses by CTLs. In this regard, recent studies suggest that during primary infection deletion of CD8 T cells equipped with high avidity T-cell receptors may occur.[49] The extent of this phenomenon may determine the varying ability to control virus replication efficiently and therefore it may significantly affect the rate of disease progression. In fact, it is now clear that the appearance of virus-specific responses by CTLs correlates with the decrease in plasma viremia.[5,50] Furthermore, the higher the relative frequency of HIV-1-specific CTLs, the lower the levels of circulating viral RNA, and higher levels of CTL activity correlate with slower rates of disease progression.[5,50,51] However, the clonal exhaustion of virus-specific CTLs does not necessarily result in a complete loss of HIV-1-specific CTL activity, although it does provide additional evidence of how HIV-1 is able to target and impair host immune responses early in the course of the infection.

The detection of CTL-escape variants is common during the course of chronic HIV-1 infection; however, there is increasing evidence that this phenomenon consistently occurs during primary HIV-1 infection.[52]

This event provides further evidence of the ability of HIV-1 to reshape some antiviral mechanisms of the immune response into self-defense mechanisms. In fact, although HIV-specific CTLs contribute to the control of both plasma viremia and disease progression,[5,50,51] the pressure exerted by CTLs can, at the same time, facilitate the selection of virus mutants that are able to escape the host immune response.[30,41]

In addition to $CD4^+$ and $CD8^+$ HIV-1-specific T cells, HIV-1 may interfere with the function of antigen-presenting cells. These cells play a central role in the generation of an effective host immune response. Cells of the monocyte–macrophage line and dendritic cells can function as specialized antigen-presenting cells and induce both humoral and cell-mediated immune responses. The effect of HIV-1 on these components of the immune system is produced by a quantitative depletion through direct cytopathogenesis or by interference with the formation of MHC-antigenic peptide complexes. To this extent, the expression of MHC class I molecules on antigen-presenting cells can be downregulated by HIV-1 Nef protein, thus affecting both generation of antigen-specific immune responses and recognition of virus-infected target cells by CTLs.[53]

Humoral response against HIV-1 can be detected early during primary HIV-1 infection. This response, however, comprises low-avidity Env-specific IgG that possesses little or no neutralizing activity. Although the reasons for the delay in the appearance of the neutralizing antibody response are poorly understood, such a response is detectable only either after the transition from primary HIV-1 infection to established early chronic HIV-1 infection, or even much later. The virologic and immunologic mechanisms described above significantly affect the global host immune response, within which the $CD4^+$ Th cell function and the interactions between T cells and B cells are profoundly altered. These events may ultimately interfere with circulating titers, avidity maturation and neutralizing activity of HIV-1-specific antibodies.

HOST AND VIROLOGIC FACTORS THAT INFLUENCE THE COURSE OF HIV-1 INFECTION

The events that result in the establishment of chronic HIV-1 infection emphasize the ability of the virus to target the host's antiviral immune response and reshape it into a self-defense mechanism. In this context, the mutual interactions between the virus and the host are major determinants of disease progression. Primary HIV-1 infection is a key phase in the immunopathogenesis of the infection, because all of the events that occur at this stage can determine both the pattern and rate of progression of the disease. In the transition from primary to early chronic HIV-1 infection, levels of plasma viral RNA tend to reach a virologic set-point that is predictive of the rate of disease progression (see below).[54,55] The virologic set-point varies among HIV-1-infected patients and tends to remain stable in the same person during the chronic phase. The virologic set-point that a person attains is determined both by the mechanisms involved in the establishment of chronic infection and by host factors that can modulate the course of HIV-1 disease.

Several factors play an important role in modulating both the antiviral host immune response and the susceptibility to HIV-1, and these factors can thus result in a slower rate of disease progression. These factors are mainly genetic, immunologic and virologic (Table 88.1).[11,30]

Table 88.1 Host factors and virologic factors in HIV-1 infection

Genetic host factors	Immunologic host factors	Virologic factors
HLA class I haplotype	Qualitative differences in the primary immune response	Extent of HIV-1 replication
Mutations in chemokine receptor or ligand genes: Ø32 in CCR5 m303 in CCR5 V641 in CCR2b G801A in SDF	Clonal deletion of HIV-1-specific cytotoxic T cells	Viral phenotype (syncytium-inducing or nonsyncytium-inducing strains of HIV-1)
Levels of β-chemokines (RANTES, MIP-1α and MIP-1β)	Persistence of HIV-1-specific $CD4^+$ T-cell responses	Trapping of virions in follicular dendritic cell network
	Chronic immune activation	Viral latency
		Size of inoculum

Genetic host factors

Patients infected with HIV-1 who experience a nonprogressive disease are more likely to possess certain human leukocyte antigen (HLA) class I haplotypes than other members of the general population. These haplotypes are more efficient than others at binding peptides that correspond to epitopes recognized by virus-specific CTLs. This suggests that persons who have inherited these clusters of haplotypes could generate HIV-1-specific CTL responses against multiple epitopes, thus resulting in a more efficient antiviral response, as a consequence of their HLA genetic background.[51] A panel of HLA class I haplotypes has been clearly shown to be associated with different rates of HIV disease progression.[56] HLA*B5701 and HLA*B5703 are strongly associated with delayed progression of disease.[57–59] Similar protective effects has also been observed with HLA*B27.[56] Other HLA haplotypes, including HLA*B5801, B1503, B13, Bw4 and B51, have also been found to be associated with control of virus replication and disease progression.[60] The interaction between HLA*Bw4 and the corresponding KIR3SD1 receptor expressed on natural killer cells has also been associated with slower disease progression.[61] Finally, recent observations have also shown a beneficial effect of HLA C on disease progression.[56]

The recent identification and study of the function of several α- and β-chemokine receptors that can serve as HIV-1 co-receptors have provided evidence of varying chemokine receptor genetic polymorphisms that can influence susceptibility to HIV-1 infection and are associated with different rates of disease progression. CXC or α-chemokine and CC or β-chemokine receptor molecules are part of the vast and functionally diverse family of seven-transmembrane G protein-coupled receptors. CXCR4, the first identified co-receptor, mediates T-cell-tropic viral fusion and entry (X4 HIV-1 strains), but it does not function as a co-receptor for the M-tropic HIV-1 envelope. The major co-receptor for M-tropic HIV-1 strains is CCR5; other CC chemokine receptors, such as CCR2b, CCR3 and CCR8, can serve as co-receptors for R5 or dual-tropic (i.e. primary HIV-1 isolates) HIV-1 strains.[62–67]

Some HIV-1-negative persons, despite being at high risk of exposure, appear to have an innate ability to 'resist' HIV-1 infection. Mapping of the CCR5 structural gene on the human chromosome 3p21 allowed the identification of a 32 base-pair deletion allele (Δ32CCR5) that encodes a truncated and nonfunctional molecule that fails to reach the cell surface. As a result, cells susceptible to HIV-1 are highly resistant to infection by R5 strains. Available prevalence estimates indicate that between 15% and 20% of Caucasians are heterozygous for the mutation, whereas 1% or fewer are homozygous.[68] The study of large cohorts of HIV-1-negative persons, including those who have had multiple exposures to HIV-1, and HIV-1-infected patients has provided evidence that the homozygous genotype for the mutation (i.e. Δ32CCR5–Δ32CCR5) confers high resistance to infection by HIV-1,[68] although a few HIV-1-infected patients possess the Δ32CCR5–Δ32CCR5 homozygous genotype. However, the heterozygous genotype (CCR5–Δ32CCR5) does not prevent HIV-1 from infecting susceptible cells but is significantly associated with a slower rate of disease progression in HIV-1-infected patients and is found more commonly in long-term nonprogressors.[69] In heterozygotes there is a decreased expression of the CCR5 receptor, which can be explained by a transdominant inhibition of wild-type CCR5 receptor function

owing to the concomitant intracellular presence of both normal and defective gene products withheld in the endoplasmic reticulum.[70] Another mutation (m303) in the CCR5 gene prevents the expression of the receptor on the cell surface by introducing a premature stop codon. This mutation, when in *trans* with the Δ32CCR5 defective gene, confers resistance because no expression of CCR5 receptor occurs.[71] Studies centered on the investigation of the CCR5 promoter have shown a certain degree of CCR5 polymorphism and certain variants are associated with different rates of HIV disease progression.[72–74]

Furthermore, other genetic variants do not prevent infection but rather delay the progression of HIV-1 disease. However, their protective effect has not been confirmed in all studies, emphasizing the need to evaluate their role in larger cohorts. These genetic variants include a mutant CCR2b allele (V64I), which encodes a base mutation that replaces valine with isoleucine at position 64 in the transmembrane domain I of the CCR2b receptor,[75–79] and a guanosine-to-adenosine transition at position 801 (G801A) in the 3′ untranslated region of the reference sequence in the gene of the stromal-derived factor (SDF)-1,[80,81] which is the ligand for CXCR4.

Epidemiologically, the inheritance of the CCR2b mutant is less beneficial than the inheritance of the Δ32CCR5 mutant in terms of reducing the risk of progression to AIDS; however, as in the case of CCR5–Δ32CCR5 heterozygosity, the V64I mutant is more commonly found in long-term nonprogressors.[77] Biologically, it is not clear how the V64I allele could interfere with the co-receptor function, because valine and isoleucine are chemically very similar. It is possible that the V64I allele is in linkage disequilibrium with other mutations,[75,78,79] as it has been described with a point mutation in the CCR5 regulatory region.[75]

As far as the SDF-1 G801A mutant allele is concerned, available data are somewhat puzzling. On the one hand, the status of homozygosity for the mutation has been associated with a lower risk of progression to AIDS.[81] A possible explanation for such a protective effect is that the presence of mutant alleles induces a higher than usual release of SDF-1, which inhibits X4 HIV-1 strains. X4 strains tend to emerge during the late phase of the disease and their appearance is generally associated with a more rapid progression. The SDF-1 at high levels could therefore interfere with the spreading of X4 HIV-1 strains, thus reducing the rate of disease progression. Homozygosity for the SDF-1 mutant has been linked to a higher risk of death, although the potential explanation is unclear.[80] Recent analyses performed on different cohorts highly question the interpretation of the original observations.[82,83]

Different haplotypes of the chemokine receptor CX3CR1, a minor HIV-1 co-receptor, have been associated with different rates of HIV disease progression.[84] The CX3CR1–1249/M280 haplotype with more rapid progression to AIDS and the CX3CR1–1249/T280 haplotype have been more frequently found in long-term nonprogressors.[85] Furthermore, recent results have shown that the CXCR1-Ha haplotype of the CXCR1 chemokine receptor is more frequently found in individuals with slow disease progression and is associated with reduced expression of CD4 and CXCR4.[86] The reduced expression of CD4 and CXCR4 may result in decreased susceptibility of infection of target cells with X4-tropic strains of HIV-1.[86]

The initial studies that discovered the role of chemokine receptors as HIV-1 co-receptors originated from the observation that chemokines can potently modulate HIV-1 infectivity.[87] The β-chemokines that are 'regulated upon activation, normal T expressed and secreted' (RANTES) – macrophage inflammatory protein (MIP)-1α and MIP-1β – have been identified as major HIV-suppressive factors produced by $CD8^+$ T cells *in vitro*. Furthermore, endogenous levels of RANTES, MIP-1α and MIP-1β expression in $CD4^+$ T cells were much elevated in some people who remained uninfected despite multiple sexual exposures to HIV-1-infected partners.[88] The binding of one chemokine to its cognate receptor essentially mediates the downregulation of surface chemokine receptor expression. Therefore, in addition to genetic control, chemokine levels themselves may influence infectivity and disease progression. In particular, there is evidence that very high levels of RANTES, MIP-1α and MIP-1β are detectable in persons bearing the Δ32CCR5–Δ32CCR5 homozygous genotype.[88] Similarly, unusually high levels of β-chemokines have been found in people with hemophilia who have remained uninfected with HIV-1 despite repeated exposure to contaminated blood products before HIV-1 testing became available.[89] Furthermore, levels of MIP-1β (which is the only suppressive CCR5-specific chemokine) in those who have overt AIDS can be significantly lower than those in HIV-1-infected patients who have chronic disease; moreover, higher levels of MIP-1β are associated with a lower risk of disease progression.[90] Recent studies indicate that the different levels of expression of RANTES results from the identification of different genetic variants of RANTES in its promoter region.[91,92] Certain genetic variants of RANTES have been associated with different rates of disease progression but additional studies are needed to support further these preliminary observations.[93]

Along the same line, recent studies have shown the importance of CCL3L1 which is at the same time a potent agonist of CCR5 and a potent inhibitor of R5-HIV-1 strains.[94] It has been shown that CCL3L1 high copy numbers are associated with higher levels of CCL3L1 secretion. Of interest, it was also shown that CCL3L1 low copy numbers were found in infected compared to uninfected individuals.[95]

These observations suggest that suppressive chemokines may play a role in the control of HIV-1. Production of chemokines by effector $CD4^+$ T cells occurs at the site of virus replication, and the chemokines may protect local target cells and activated effector cells by downregulating CCR5 in an autocrine manner. The varying extents to which these mechanisms are put in motion may explain the varying levels of protection against disease progression.

Recent studies have highlighted the importance of endocellular factors and their impact on HIV-1 replication. The host factor APOBEC3G mediates strong antiviral activity and its antiviral activity is suppressed by the Vif HIV-1 protein.[96] Similar antiviral activity has been shown for the host factor TRIM5α.[97] However, contradictory results have been obtained in different *in vitro* systems or patient populations and therefore the antiviral effects of TRIM5α need further confirmation.[98]

In summary, host genetic factors do play a role in modulating the course of HIV-1 infection. Furthermore, the potential role of chemokines in affecting infectivity and disease progression may further widen our therapeutic options along with HAART.

Virologic factors

As discussed above, interaction among different host factors helps to determine a certain virologic set-point in each HIV-1-infected person after the transition from primary to chronic HIV-1 infection.[54,55] This set point represents the level of plasma viral RNA that accurately predicts disease progression (i.e. the level of plasma viremia that corresponds to a risk of progression to either AIDS or death). In the past, many predictors have been identified – clinical, biologic and virologic. The $CD4^+$ T-cell count is historically the most important and widely used predictor. However, the load of plasma RNA is an accurate predictor, especially when used along with the $CD4^+$ T-cell count.[54] Therefore, the higher the plasma viral RNA load, the greater the risk of rapid progression to AIDS and death. It is worth noting that the power of association between levels of plasma HIV-1 RNA and risk of progression does not significantly vary if viremia is measured after seroconversion (i.e. knowing the date and duration of HIV-1 infection) or during the established chronic asymptomatic phase (i.e. with no available information about the duration of the infection, as is often the case in clinical settings).[55] Viral load is therefore a very powerful predictor if measured during the chronic asymptomatic phase, once the virologic set-point has been reached.

However, the level of plasma viral RNA lacks accuracy and reliability as a predictor if measured during primary HIV-1 infection.[99] In this phase, rapid disease progression is predicted by:

- a retroviral syndrome lasting more than 14 days;
- the number and the intensity of clinical signs and symptoms;

- central nervous system involvement; and
- viral phenotype.

The viral phenotype is an important virologic factor that can contribute to the rate of disease progression. R5 HIV-1 strains are non-syncytium-inducing strains, whereas X4 HIV-1 strains are syncytium-inducing strains. Syncytium-inducing strains tend to emerge during the late phase of the disease and a shift in viral phenotype from non-syncytium-inducing strains to syncytium-inducing strains heralds disease progression. The viral phenotype of the non-syncytium-inducing strains is associated with prolonged AIDS-free survival and is more commonly found in long-term nonprogressors.[100]

IMMUNE CORRELATES OF PROTECTION

Functional and phenotypic patterns of HIV-1-specific CD4 T-cell responses

The pattern of the HIV-1-specific CD4 T-cell response during primary infection is typical of an effector response, i.e. IFN-γ/TNF secreting CD4 T-cell response.[101–104] The patterns of HIV-1-specific CD4 T-cell responses in the chronic phase of infection are strictly dependent on a series of factors,[105] including the lack of control of virus replication, the initiation of antiviral therapy during the acute phase and treatment-mediated virus suppression, and the spontaneous control of virus replication as it may occur in a small (1%) percentage of subjects known as long-term nonprogressors (LTNP).[106] In the absence of control of virus replication, the HIV-1-specific CD4 T-cell response during chronic infection remains unchanged compared to the acute infection phase, i.e. typical effector IFN-γ/TNF response.[104,105] Following antiviral therapy-mediated virus suppression and in LTNP, who spontaneously control virus replication with viremia levels <50 HIV-1 RNA copies per ml of plasma (the limit of detection of the standard polymerase chain reaction assay), the HIV-1-specific CD4 T-cell response is composed of single interleukin (IL)-2, IL-2/IFN-γ/TNF and IFN-γ/TNF secreting cell populations similar to the CD4 T-cell response in chronic CMV infection.[104,105,107–109]

With regard to virus-specific proliferation, HIV-1-specific proliferating CD4 T cells are generally not detected during acute infection.[102] The detection of virus-specific proliferative CD4 T-cell responses is consistently associated with the presence of virus-specific IL-2 secreting CD4 T-cell populations during chronic infection.[105] With regard to HIV-1 infection, they are found in LTNP and in a proportion (about 40%) of subjects following antiviral therapy.[110]

The phenotype of HIV-1-specific CD4 T cells during primary infection is typical of effector cells, i.e. $CD45RA^{-}CCR7^{-}$.[105] Therefore, HIV-1-specific CD4 T cells with the $CD45RA^{-}CCR7^{-}$ phenotype belong to the IFN-γ/TNF secreting cells.[104,105] The phenotype of virus-specific CD4 T cells is substantially different during the chronic phase of infection.[104,105] These changes are consistent with the presence of functionally distinct CD4 T-cell populations and of cell populations at different stages of differentiation. The phenotype of HIV-1-specific CD4 T cells remains unchanged compared to primary infection in the absence of control of virus replication, i.e. $CD45RA^{-}CCR7^{-}$, consistent with the presence of effector IFN-γ/TNF secreting cells.[104,105]

Functional and phenotypic patterns of virus-specific CD8 T-cell responses

HIV-1-specific CD8 T cells during primary infection are typical effector cells, i.e. IFN-γ/TNF secreting cells. The patterns of the HIV-1-specific CD8 T-cell response during the chronic phase of infection generally correlate with the extent of virus control. The HIV-1-specific CD8 T-cell response remains predominantly an effector response, i.e. IFN-γ/TNF secreting cells, in association with uncontrolled virus replication. Both IL-2/IFN-γ/TNF and IFN-γ/TNF secreting CD8 T cells are found in LTNP in association with controlled virus replication.[104,105] The presence of both IL-2/IFN-γ/TNF and IFN-γ/TNF secreting HIV-1-specific CD8 T cells was found only in 30–40% of subjects following successful suppression of virus replication by antiviral therapy. Therefore, antiviral therapy-mediated suppression of virus replication is not associated with consistent detection of the IL-2 CD8 T-cell response as is the case for the HIV-1-specific CD4 T-cell response that is constantly associated with the detection of single IL-2 and IL-2/IFN-γ/TNF cells. HIV-1-specific CD8 T-cell proliferation was dependent upon the presence of the IL-2/IFN-γ/TNF secreting cells and thus was consistently observed in the LTNP and in the 30–40% of subjects successfully treated with antiviral therapy.[111]

The phenotype of HIV-1-specific CD8 T cells during primary infection is typical of effector cells, i.e. $CD45RA^{-}CCR7^{-}$. The phenotype of virus-specific CD8 T cells is substantially different during the chronic phase of infection.[111] As in the case of CD4 T cells, the changes were consistent with the presence of functionally distinct CD8 T-cell populations at different stages of differentiation. The phenotype of HIV-1-specific CD8 T cells in chronic infection with uncontrolled virus replication remains unchanged compared to primary infection, i.e. $CD45RA^{-}CCR7^{-}$.[111] The phenotype of HIV-1-specific CD8 T cells in LTNP was mostly composed of two cell populations ($CD45RA^{-}CCR7^{+}$ and $CD45RA^{-}CCR7^{-}$) that contained both IL-2/IFN-γ/TNF cells and IFN-γ/TNF cells.

Protective T-cell response

Recent studies have provided evidence for both functional and phenotypic heterogeneity of Ag-specific CD4 and CD8 T-cell responses observed in different virus infections.[112–122] A number of hypotheses have been proposed such as:

- the virus hypothesis proposing that the heterogeneity of the virus-specific immune response is strictly dependent on each virus;[123]
- the maturation hypothesis proposing that certain viruses such as HIV-1 may skew the maturation of HIV-1-specific T cells as compared to other viruses such as CMV and EBV;[102,124] and
- the senescence hypothesis proposing that the functional loss of HIV-1-specific T cells is due to the rapid senescence of T cells which is selectively induced by HIV-1.[123]

The above hypotheses were originally proposed to explain the functional impairment of CD8 T cells in HIV-1 infection but can also be applied to CD4 T cells. The different observations and hypotheses are not mutually exclusive, and indeed what they reflect is the existence of different functional/phenotypic patterns of T-cell responses that can be explained through a single mechanism. The likely mechanism is that the functional/phenotypic heterogeneity of virus-specific CD4 and CD8 T-cell responses is predominantly dependent on, and modulated by, different levels of Ag load.

In support of this mechanism, different functional patterns of CD4 and CD8 T-cell responses can be observed within the same virus infection.[104,105,107,111] The effector IFN-γ/TNF CD4 and CD8 T-cell response during primary HIV-1 infection associated with high Ag load switches to a memory response composed of single IL-2, IL-2/IFN-γ/TNF and IFN-γ/TNF CD4 T cells and of IL-2/IFN-γ/TNF and IFN-γ/TNF CD8 T cells after the transition to the chronic phase of infection associated with virus control and very low levels (not detectable) of Ag load.[102,104,105,107,108,111] The HIV-1-specific CD4 T-cell response is an effector IFN-γ/TNF response during primary infection and chronic infection with uncontrolled virus replication. It shifts to a memory response composed of single IL-2, IL-2/IFN-γ/TNF and IFN-γ/TNF CD4 T cells either in LTNP or subjects treated with antiviral therapy, i.e. conditions with controlled virus replication and low/undetectable Ag load. The interruption of antiviral therapy causes the loss of control of virus replication, high Ag load and the shift back to the effector IFN-γ/TNF CD4 T-cell response.[104]

The differences in the functional patterns of CD8 T-cell response observed between chronic HIV-1 infection with uncontrolled virus replication and high Ag load and chronic HIV-1 in LTNP associated with virus control/elimination and low/undetectable Ag load support

the hypothesis that HIV-1-specific CD8 T-cell responses are also modulated by Ag load.[111,125] Overall, the functional and the phenotypic patterns of CD4 and CD8 T-cell responses reflect different levels of virus replication and Ag load and different stages of T-cell differentiation, i.e. effector cells and, within memory cells, T_{CM}, T_{EM} and T_{ET}. The functional T-cell patterns seem to follow the rule: high Ag load = effector IFN-γ/TNF T-cell response while low/undetectable Ag load = single IL-2 (only for CD4 T cells) plus IL-2/IFN-γ/TNF plus IFN-γ/TNF T-cell memory response.

The investigation of the functional patterns of virus-specific CD4 and CD8 T-cell responses against the most common human viruses has provided substantial advances in the identification of the T-cell immune responses that may be effective in the control of virus replication and in the prevention of establishment of persistent chronic infection and virus-associated disease.[105] In this regard, it has been instrumental in determining the functional characterization of virus-specific CD4 and CD8 T cells on the basis of their ability to secrete IL-2, TNF and IFN-γ following Ag-specific stimulation for several reasons. These include:[104,105,107,108,111]

- a strong correlation between the presence of virus-specific CD4 and CD8 IL-2 secreting T cells and virus control;
- the presence of CD4 and CD8 IL-2 secreting T cells being always associated with the proliferation capacity; and
- no correlation between the presence of IFN-γ/TNF secreting CD4 and CD8 T cells and also the presence of cytotoxic function in CD8 T cells and virus control.

Therefore, protective CD4 and CD8 T-cell responses are characterized by the presence of multiple cytokine secreting cells, i.e. single IL-2, IL-2/IFN-γ/TNF and IFN-γ/TNF CD4 T cells and IL-2/IFN-γ/TNF and IFN-γ/TNF CD8 T cells, and by the proliferation capacity of these cells.[104,105,111] It is important to mention that the two cytokines secreting CD8 T-cell populations are also cytotoxic.[126]

The ability of CD4 and CD8 T cells to mediate multiple functions identifies functional signatures of CD4 and CD8 T-cell responses (Fig. 88.5). We have termed these responses polyfunctional. The presence of only effector IFN-γ/TNF CD4 and CD8 T-cell responses and of cytotoxic function in CD8 T cells may correspond to an effective response during the effector phase of the response, i.e. during primary infection or virus re-activation in chronic infection, but it is invariably a non-effective immune response in persistent chronic infection with uncontrolled virus replication. The effector IFN-γ/TNF CD4 and CD8 T-cell responses have been termed monofunctional. Although there is no formal demonstration that polyfunctional CD4 and CD8 T-cell responses are directly responsible for control of virus replication and prevention of virus-associated disease, polyfunctional T-cell responses may certainly be used as markers of effective antiviral immunity. The advances in the delineation of antiviral protective T-cell responses may provide important insights in the evaluation of the so-called T-cell vaccines.

The relationship between control of HIV replication and functional profile of virus-specific CD4 and CD8 T-cell responses

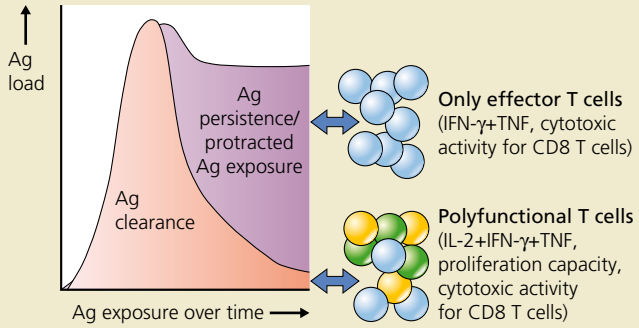


Fig. 88.5 Schematic representation of the relationship between control of HIV replication and functional profile of virus-specific CD4 and CD8 T-cell responses. Only effector T cells secrete only inflammatory cytokines, lack proliferation capacity, are cytotoxic (for CD8 T cells) and are at a more advanced stage of differentiation. Polyfunctional T cells secrete IL-2 in addition to inflammatory cytokines, are endowed with proliferation capacity, are cytotoxic (for CD8 T cells) and also contain cells at an earlier stage of differentiation.

REFERENCES

References for this chapter can be found online at http://www.expertconsult.com

Chapter 89

Bernard Hirschel

Primary HIV infection

INTRODUCTION

Although primary HIV infection (PHI) is a rarely diagnosed, self-limiting disease, it is a topic of considerable interest, since the first encounter of HIV with the immune system sheds light on many aspects of pathogenesis. As the severity of PHI predicts progression to immunodeficiency years later, it is reasonable to hope that treatment of PHI can delay or even prevent progression to AIDS. From a public health perspective, a diagnosis of PHI is important because such patients are highly infectious.[1,2] To miss a diagnosis of PHI is to miss an opportunity both for intervention and for prevention of further transmission.

Experience shows that the diagnosis is not difficult to make; education is the key.

EPIDEMIOLOGY

Primary HIV infection is often asymptomatic but sometimes it presents with dramatic manifestations requiring hospital admission. There is a spectrum between complete absence of symptoms during the time of seroconversion and severe disease; therefore, it is not surprising that opinions vary about the percentage of patients who have symptomatic PHI. A physician's previous experience with PHI and a high index of suspicion greatly increase the rate of recognition of PHI. Retrospective analysis from the US armed forces showed that 33% of patients suffered from an identifiable disease between their last seronegative and first seropositive serum sample. At the other extreme, in Australia,[3] 93% of seroconverting persons reported having been 'sick' compared with 40% of controls; 12% of seroconverting patients were hospitalized as a result of symptomatic PHI.

It is not known what factors determine the severity of PHI. Theoretically, the size of the inoculum, the virulence of the infecting HIV strain (including such factors as cellular tropism and cytopathogenicity) and the patient's immune status could be involved, but evidence as to whether these factors are important is lacking. One series of transfusion-associated cases found that symptomatic PHI was more frequent among those infected by people who had late-stage disease (and presumably high circulating viral loads). There is little evidence that the frequency or severity of PHI differs between transmission categories or between men and women. Symptomatic PHI can occur with HIV-2 infection and in children, although almost all cases have been reported in adults infected with HIV-1. There are theoretic reasons to believe that co-infection with other viruses, particularly from the herpes group, might enhance the proliferation of HIV, and patients who are simultaneously co-infected with cytomegalovirus have had particularly severe symptoms of PHI.

Symptoms typically start 2–4 weeks after infection, with extremes of 5 and more than 90 days. The median duration of symptoms is estimated between 12 and 28 days.[4] Moderate and subjective symptoms such as fatigue may persist for months, although almost all patients eventually enter an asymptomatic phase lasting years.

PATHOGENESIS AND PATHOLOGY

Studies of acute simian immunodeficiency virus (SIV) infection have elucidated aspects of HIV and AIDS. Weeks after inoculation, SIV causes massive loss of memory $CD4^+$ cells in many tissues,[5] but particularly in the intestinal tract.[6] Increased permeability of the gut permits translocation of microbial products, resulting in chronic immune activation which is a hallmark of progressive HIV infection.[7]

Because PHI most often presents as a benign self-limiting disease, pathologic information is only available from easily biopsied tissues. The skin rash is caused by a dermal perivascular lymphohistiocytic infiltrate around vessels of the superficial dermis; the epidermis is normal. The inflammatory cells are predominantly of the $CD4^+$ phenotype, and may represent a T-cell-mediated reaction to HIV and to the p24 antigen, which can be detected in Langerhans cells.

Lymph node biopsies reveal abundant HIV, including the envelope proteins gp120 and gp160 in dendritic reticulum cells, as well as in lymphocytes. The structure of the germinal centers is relatively normal and quite unlike the follicular hyperplasia of established HIV-1 infection, but extrafollicular B lymphocytes are reduced in number and the follicles are infiltrated by $CD8^+$ T cells.[8,9]

Therapy has a pronounced effect on the quantity of HIV detectable in lymph nodes. There is a lag of more than 6 months between disappearance of the virus from plasma and disappearance from lymph nodes. However, even patients who have no detectable viremia for several months while being treated for PHI, and whose lymph node biopsies are apparently free from HIV, relapse after discontinuing medication. These findings emphasize the essential role of a virus reservoir for HIV provided by memory T cells.[10]

CLINICAL FEATURES

During PHI, HIV floods the blood with massive viremia,[11] spreading to the central nervous system (CNS)[12] and the lymphatic system, and invades a number of other tissues. Not surprisingly, PHI is a disease with protean clinical manifestations. Three main presentations have been described.

Cutaneous presentation

This is characterized by a maculopapular rash, 'roseola' and mucosal ulcerations (Figs 89.1–89.4). The rash affects the face, neck and trunk more than the limbs, although the palms and soles may be involved. Individual lesions are usually less than 1 cm in diameter and confluence is rare. Case reports have mentioned pustular eruptions, urticaria, erythema multiforme and, during the healing phase of PHI, alopecia and desquamation. Ulceration may occur on the genital and oral mucosa, including the esophagus, where differentiation from herpetic lesions or esophageal candidiasis is difficult.

Presentation resembling infectious mononucleosis

This is characterized by fever, pharyngitis, arthralgia, myalgia and lymphadenopathy. Although the expression 'mononucleosis-like illness' is firmly established, there are many differences from classic infectious mononucleosis, most notably the lack of prominent tonsillar involvement. In a large series (Table 89.1), only 20% of patients had a fever in combination with sore throat and enlarged cervical lymph nodes, whereas 10% did not have fever, sore throat or cervical lymphadenopathy.



Fig. 89.1 Maculopapular rash during primary HIV infection.



Fig. 89.2 Acneiform lesions during primary HIV infection.



Fig. 89.3 Penile ulcer during primary HIV infection.

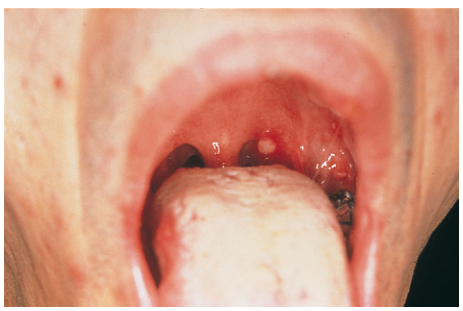


Fig. 89.4 Mucosal ulcerations during primary HIV infection.

Table 89.1 Signs and symptoms of primary HIV infection*

	Symptom/sign	%
Reported by more than 50%	Fever	77
	Lethargy/fatigue	66
	Rash	56
	Myalgia	55
	Headache	51
Reported by 20–50%	Pharyngitis	44
	Cervical adenopathy	39
	Arthralgia	31
	Oral ulcer	29
	Pain on swallowing	28
	Axillary adenopathy	24
	Weight loss	24
	Nausea	24
	Diarrhea	23
	Night sweats	22
	Cough	22
	Anorexia	22
Reported by 5–20%	Abdominal pain	19
	Oral candidiasis	17
	Vomiting	12
	Photophobia	12
	Meningitis	12
	Genital ulcer	7
	Tonsillitis	7
	Depression	7
	Dizziness	6

*These signs and symptoms are reported by at least 5% of patients.[1,2]

Meningoencephalitis

Meningoencephalitis is characterized by photophobia and neck stiffness, headaches and disordered consciousness. The headache is typically retro-orbital and exacerbated by eye movements. Depression and changes in mood are frequent and may reflect underlying encephalitis.

Other symptoms

Table 89.1 shows the frequencies of signs and recorded symptoms in the medical charts of more than 200 patients from Switzerland and Australia. Digestive manifestations have not been well recognized in the past, but they are quite common. In exceptional cases, esophageal candidiasis (an AIDS-defining disease) may occur with a transient decline in $CD4^+$ lymphocyte count.

Unusual manifestations associated with PHI include:

- neurologic syndromes such as radiculopathy, peripheral facial neuropathy and Guillain–Barré syndrome, and severe encephalitis with prolonged coma and seizures; and
- pulmonary involvement, which may be more frequent in intravenous drug users where PHI can be associated with bacterial pneumonia; severe pneumonitis leading to intubation and *Pneumocystis jirovecii* (formerly *P. carinii*) pneumonia[13] (with $CD4^+$ lymphocyte counts of $<100/mm^3$) has been described but is exceptional.

Table 89.2 Important differential diagnoses

Clinical features	**Epstein–Barr virus infection**	**Primary HIV infection**
Onset	Gradual	Abrupt
Tonsil involvement	+++	+
Pharyngeal exudate	+++	–
Rash	Rare except in patients treated with antibiotics	Frequent
Jaundice	10%	Never
Diarrhea	Rare	25%
Clinical features	**Syphilis**	**HIV infection**
Serology	Always positive	At first negative
Chancre: timing	Before generalized rash	At the same time as rash
Chancre: pain	Painless	Painful
Clinical features	**Enterovirus meningitis**	**HIV meningitis**
Population	Young adults	Young adults
Diarrhea	Rare	23%
Season	Summer–autumn	None in particular
Duration	<8 days	Often >20 days
Encephalitis	None	Frequent
Skin lesions	Rare	Frequent
Pain on swallowing	None	Frequent

Differential diagnosis

Important differential diagnoses are listed in Table 89.2; PHI must be distinguished from Epstein–Barr virus infection (infectious mononucleosis) and enterovirus meningitis, and according to the local epidemiologic context, typhoid fever, rickettsial infections and many others. The relatively few primary HIV infections easily get lost among the multitudes who present with fever, rash and pharyngitis. For instance, an estimated 6.4 million patients seeking ambulatory care present with pharyngitis each year in the USA; of these, 8500 (0.13%) have primary HIV infection.[13]

DIAGNOSIS

Seroconversion (i.e. the documented first appearance of HIV antibodies in the serum) occurs days after the beginning of the symptoms of PHI. Therefore, the usual antibody tests for HIV are not entirely reliable; they are expected to be negative during the first few days of PHI (Fig. 89.5). Assays differ in the duration of this 'seronegative period'; with the currently used sensitive tests it is usually less than 1 week after the start of symptoms of PHI.

The p24 antigen is positive when the antibody test is still negative during PHI, and the same is true of HIV viremia. Whereas both tests can be used to screen for PHI, the p24 antigen test is considerably cheaper.[14] Viremia levels reach extremely high values, often in excess of 10^6 viral genomes/ml,[15] and high titers of infectious virus have been isolated from many tissues, including seminal fluid, corroborating the epidemiologic evidence that patients who have PHI are highly infectious. Viremia decreases rapidly – at least 100-fold within days after seroconversion – but remains detectable in more than 95% of patients. Steady-state plasma viremia levels predict progression to advanced immunodeficiency and death.[16] Levels tend to remain higher in those who have symptomatic PHI.[17]

Despite the relatively short duration of PHI, a substantial percentage of newly diagnosed HIV infections are acquired from patients who are themselves recently infected.[17,18]

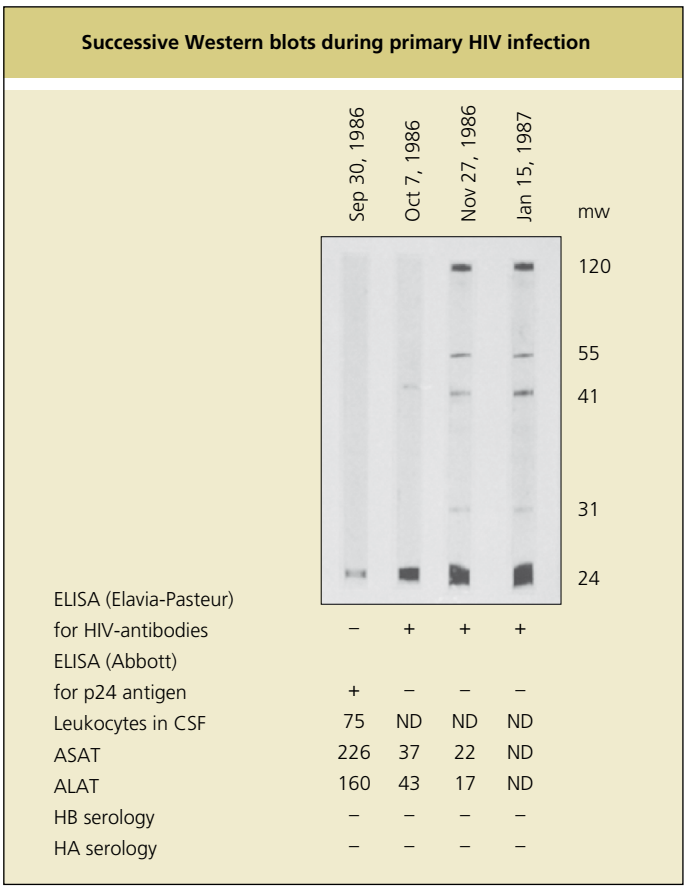


Fig. 89.5 Successive Western blots during primary HIV infection. Note that on September 30, 1986, when the patient presented with fever, rash, meningitis and subclinical hepatitis, the screening enzyme-linked immunosorbent assay (ELISA) test for HIV antibodies was negative, while the Western blot showed only a single weak band corresponding to the p24 antigen. CSF, cerebrospinal fluid; ASAT, aspartate transaminase; ALAT, alanine transaminase; H, hepatitis (A or B); ND, not done.

Like viremia levels, lymphocyte subsets undergo rapid changes during PHI (Fig. 89.6). During the first 5–10 days, lymphopenia characteristically affects both $CD4^+$ and $CD8^+$ lymphocytes, with levels[13] that may be as low as those observed in AIDS. Although opportunistic infections are rare, *in-vitro* tests of both B and T cells show immunosuppression. Within another 2–3 weeks there is a lymphocytosis. The $CD8^+$ lymphocyte count expands more than the $CD4^+$ lymphocyte count, leading to a $CD4^+/CD8^+$ lymphocyte ratio of less than 1. This low ratio persists even in patients whose $CD4^+$ lymphocyte count subsequently rises to normal.

Many other laboratory values may be abnormal during PHI, reflecting the acute inflammatory response (e.g. high erythrocyte sedimentation rate, increase in C-reactive protein) and involvement of the bone marrow (thrombocytopenia), the liver (increase in hepatic transaminases) and the CNS (pleocytosis of the cerebrospinal fluid).

MANAGEMENT

Although PHI can be severe and prolonged, it is most often a self-limiting disease. The symptoms eventually abate and the patient becomes asymptomatic. Years later, immunosuppression may appear and AIDS may develop.

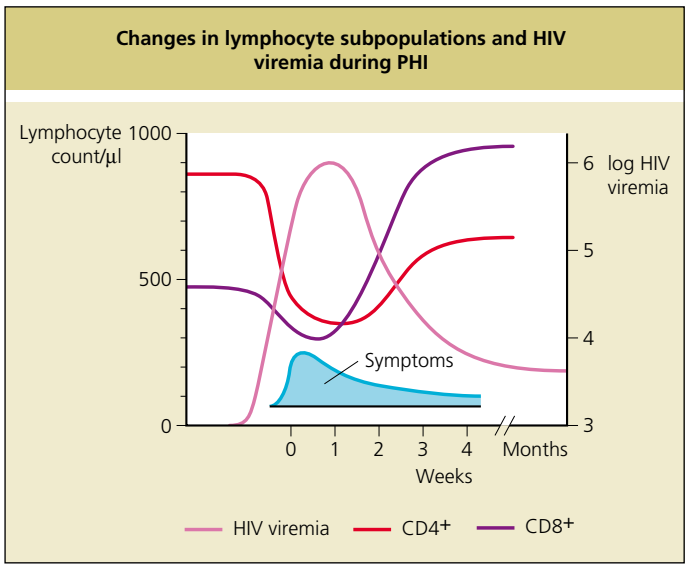


Fig. 89.6 Changes in lymphocyte subpopulations and HIV viremia during primary HIV infection.

Features of primary HIV infection that may predict the subsequent course toward AIDS

A considerable body of evidence suggests that more severe and more prolonged PHI indicates a more unfavorable course toward AIDS.[18,19] For instance, in patients who were followed after seroconversion, 58% of those who had had symptomatic PHI had developed AIDS 7 years later compared with 28% of those who had asymptomatic PHI.[20] Another study suggested that the presence of neurologic signs at the time of PHI predicted accelerated immunosuppression,[19] although there was no specific relation to the neurologic signs of AIDS, such as AIDS-related dementia or opportunistic infections of the CNS.

Does treatment of primary HIV infection improve the long-term outcome?

This is a critically important question that regrettably remains unanswered. Comparative studies between a treated group and an untreated control group face considerable practical obstacles, including the need for a large sample, extremely long follow-up and ethical issues.

Primary HIV infection is a self-limiting disease, usually of only slight or moderate severity. The balance has shifted back and forth between advocates and opponents of treatment.

Arguments in favor are:

- the association of symptomatic PHI with a worse prognosis;
- the limited heterogeneity of the viral population,[21] which should theoretically diminish the probability of emergence of resistance to antiviral drugs;
- the potential impact on infectivity and transmission;[1]
- the generalization of the HIV infection during PHI with invasion of the CNS[12] and lymphoid tissues; and
- the availability of more effective and better tolerated antiviral drugs.[22]

After pilot studies suggested that zidovudine was well tolerated and possibly effective in PHI, a prospective randomized trial in 77 patients compared placebo and zidovudine given for 6 months.[4] An effect on $CD4^+$ lymphocytes and a statistically significant decrease in minor opportunistic infections in patients treated with zidovudine were demonstrated.

Several uncontrolled trials have been conducted in PHI with combinations of antiviral drugs:

- with highly active antiretroviral treatment, including two inhibitors of reverse transcriptase and an inhibitor of the HIV protease, viremia decreases to undetectable levels in practically all compliant patients;[23,24]
- progression to AIDS is less than in historical control groups;[25]
- the $CD4^+/CD8^+$ lymphocyte ratio normalizes; and
- lymph node biopsies of some of the patients who have persistent suppression of viremia show disappearance of virus, and proviral DNA decays faster than in patients who start treatment during chronic HIV infection.[23,26]

However, the hope of viral eradication remains unfulfilled, as discussed below.

Opponents of antiviral treatment for primary HIV infection point to the lack of studies showing clinical benefits, the high incidence of side-effects, possible increased risk of development of resistance to antiretroviral agents, expense and problems with compliance. Some 6 months or so after having started treatment during PHI, patients often have high $CD4^+$ counts and would not otherwise qualify for antiretroviral treatment. Should they stop?

Firm recommendations await the results of properly controlled trials. After years of attempts, the SPARTAC trial in South Africa and the UK has now succeeded in recruiting several hundred patients. The study randomized participants to treatment for 3 or 12 months, or no treatment, with a planned follow-up of several years. Results are not expected before 2011 (see http://www.ctu.mrc.ac.uk/studies/spartac.asp).

REFERENCES

References for this chapter can be found online at http://www.expertconsult.com

Chapter **90**

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Stéphane de Wit

Prevention of opportunistic infections

INTRODUCTION

One of the major clinical advances in the management of patients who have HIV infection has been the implementation in the mid-1980s of antimicrobial prophylaxis for patients with severe immune impairment. Together with the use of antiretroviral drugs, this has led to decreased morbidity from opportunistic infections (OIs) and improved survival in patients with AIDS.

Such prophylaxis requires regular measurements of $CD4^+$ lymphocyte counts and compliance on the part of the patient, who must take many pills each day for the rest of his/her life. There are also issues of tolerance, drug interactions, emergence of resistance and cost.

Since the extensive use in Western countries of highly active antiretroviral therapy (HAART), a further marked decrease in the occurrence of AIDS-related opportunistic infections has been noted and the question of continuing or stopping prophylactic regimens has been raised in patients who have $CD4^+$ counts increasing beyond the critical level of 200 lymphocytes/mm^3.[1] Figure 90.1 summarizes the evolution of the incidence of most opportunistic infections among a cohort of 2000 patients followed at CHU Saint-Pierre hospital in Brussels.

Although prophylactic regimens against most of the opportunistic infections that occur in patients who have HIV infection exist, the decision to use prophylaxis should consider factors such as:

- the incidence and prevalence of specific infections in HIV infected individuals;
- the potential severity of disease in terms of morbidity and mortality;
- the level of immunosuppression at which each disease is likely to occur;

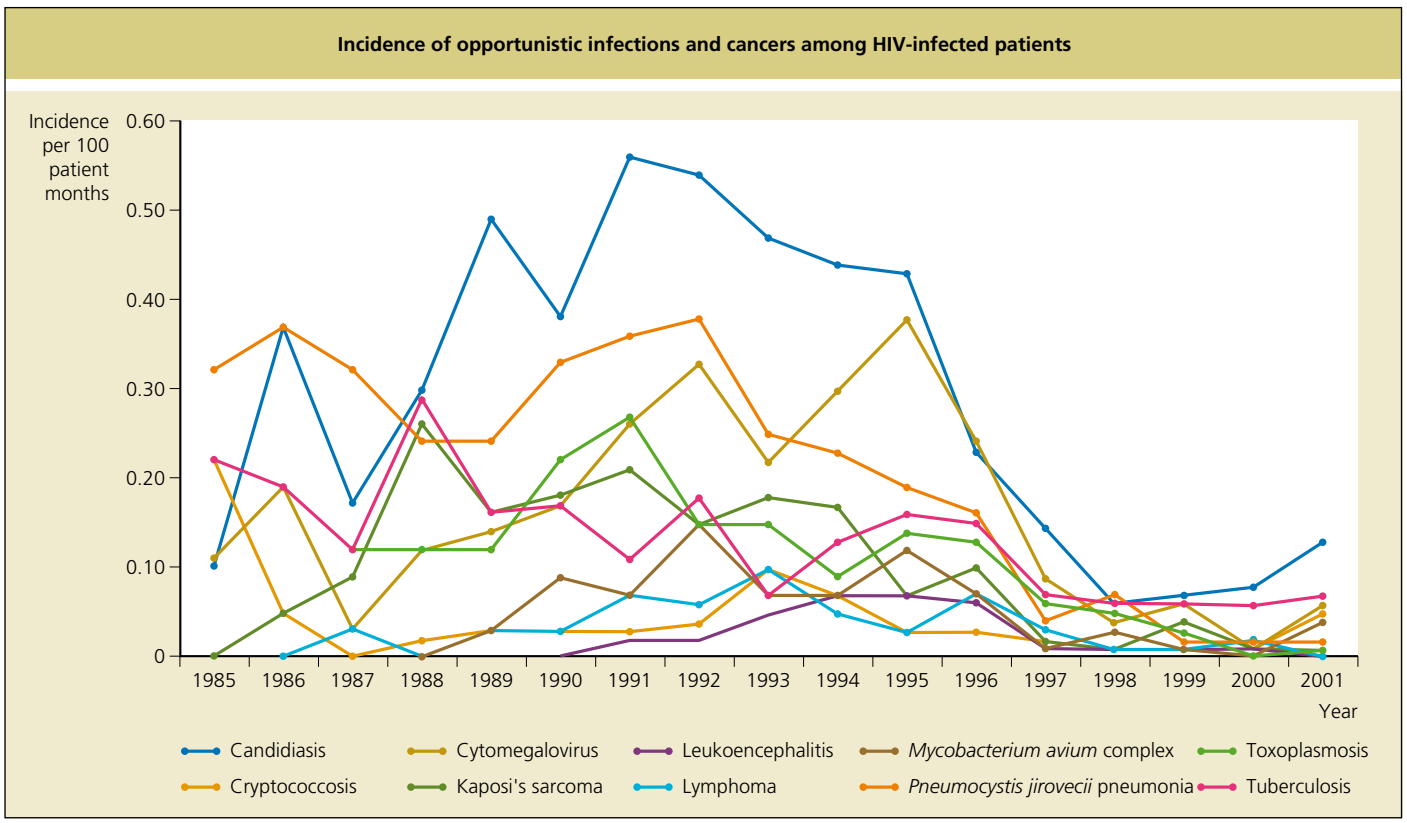


Fig. 90.1 Incidence of opportunistic infections among patients who have HIV infection. CHU Saint-Pierre, 1985–2001.

- the feasibility and efficacy of preventive measures, and their impact on quality of life and survival;
- the potential for emergence of organisms resistant to the agents used for prophylaxis;
- the risk of toxicities and drug interactions with antiretrovirals and other needed medications;
- the issue of compliance; and
- the cost-effectiveness of prophylaxis.

Prevention of infection with hepatitis B virus (HBV), hepatitis C virus (HCV), human papilloma virus (HPV) and herpes viruses through sexual contact should also be part of the long-term management of the patient. The role of newly introduced HPV vaccination in HIV patients remains unknown.

Efforts should also be made to improve early diagnosis of HIV disease, to avoid immunodeficiency or to implement prevention of opportunistic infections.

INCIDENCE AND PREVALENCE OF OPPORTUNISTIC INFECTIONS

There is a wide geographic variability in the epidemiology of opportunistic infections. The probability of developing a given disease depends on the risk for exposure to potential pathogens, the virulence of the pathogens and the level of immunosuppression of the patient.

In the USA and Northern Europe, *Pneumocystis jirovecii* pneumonia (PJP), oropharyngeal or esophageal candidiasis, cytomegalovirus disease and infections caused by *Mycobacterium avium* complex (MAC) are common. The incidence of toxoplasmosis and tuberculosis is higher in central and southern Europe and in the developing countries, depending on the prevalence of latent infection in the general population.

This geographic heterogeneity has important implications for prophylaxis. In the case of low incidence, prophylactic measures should be targeted to high-risk patients such as those who have a positive antitoxoplasma serology, a positive polymerase chain reaction for cytomegalovirus or a positive cutaneous tuberculin test for tuberculosis. In addition, for tuberculosis, epidemiologic assessment of risk should be used for some high-risk populations (intravenous drug users, migrants from an endemic area).

LEVEL OF IMMUNOSUPPRESSION

Blood $CD4^+$ lymphocyte levels is the best marker for immune status. It has been clearly established that the number of circulating $CD4^+$ lymphocytes is closely correlated with the risk of developing several opportunistic infections (Fig. 90.2). Once the $CD4^+$ lymphocyte count falls below 200 cells/µl, the cumulative risk for developing an AIDS-defining opportunistic infection is 33% by year 1 and 58% by 2 years. Therefore, $CD4^+$ lymphocyte counts remain an important parameter for monitoring patients who have HIV infection because of their predictive value for both opportunistic infections and mortality.

EFFICACY OF PROPHYLACTIC MEASURES AND IMPACT ON SURVIVAL

The survival benefit of prophylaxis for opportunistic infections has been demonstrated in a number of studies, particularly in patients who have severe immunosuppression. In the early 1980s, before the widespread use of antiretroviral therapy, it was demonstrated that trimethoprim–sulfamethoxazole (TMP–SMX) use for prevention of PJP significantly prolonged survival. A similar impact has been shown with MAC prophylaxis with clarithromycin or rifabutin.

It is clear that a prophylactic regimen that prolongs survival should be used in preference to one that does not. In this setting, the use of fluconazole for prophylaxis of systemic mycoses and oral ganciclovir for cytomegalovirus disease have failed to demonstrate a clear survival benefit and are not widely recommended.

EMERGENCE OF DRUG RESISTANCE

The development of resistance to the most commonly used agents is one of the major concerns with the use of long-term antimicrobial prophylaxis in patients who have HIV infection.

Resistance or cross-resistance has become increasingly common with prophylaxis for MAC, fungal infections and PJP. The major consequence is that, when the specific drugs are needed to treat acute infections, resistance hinders their use and alternative, less effective, or more toxic agents are the only option.[2]

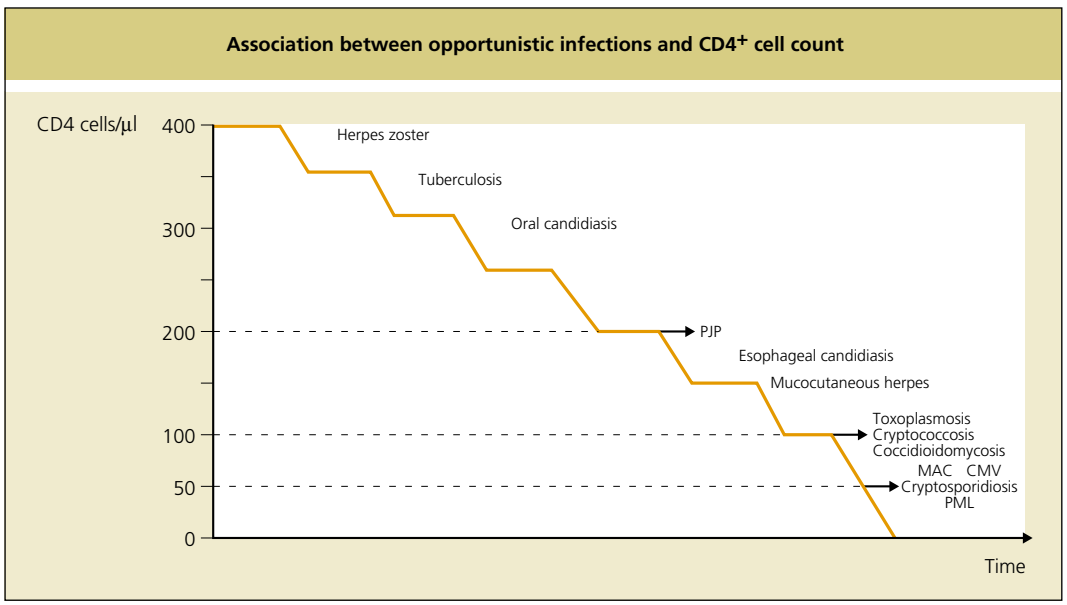


Fig. 90.2 Association between opportunistic infections and $CD4^+$ cell count.

This issue was well illustrated with clarithromycin as prophylaxis for MAC. Despite receiving prophylaxis, 58% of the patients developing MAC had clarithromycin-resistant isolates.[3]

Prophylactic treatment of non-life-threatening infections, such as oral thrush with fluconazole, has probably contributed to the emergence and spreading of azole-resistant fungi.

Prophylactic regimens may also lead to the development of cross-resistance against more common pathogens. For example, rifabutin used for MAC prophylaxis may result in the emergence of rifampin (rifampicin)-resistant strains of *M. tuberculosis*.[4] Likewise, the widespread use of broad-spectrum antibiotics, such as clarithromycin, azithromycin or TMP–SMX, may lead to the development of resistance among organisms such as pneumococci that were not the primary targets of prophylaxis. An increasing prevalence of *Streptococcus pneumoniae* resistant to TMP–SMX, for example, could decrease the effectiveness of this agent in preventing community-acquired pneumonia in advanced HIV patients and limit therapeutic options for treating common outpatient illnesses such as respiratory, skin and soft tissue infections.[2]

DRUG TOXICITY AND DRUG INTERACTIONS

Drug toxicity can be a major factor limiting the usefulness of widely used agents. The incidence of adverse drug reactions in patients who have HIV infection varies with the type of drug and dosages used, the interactions between drugs and the stage of HIV infection.[5]

HIV-related idiosyncratic factors, organ dysfunction in late-stage disease and multiple drug therapy are the primary reasons for the increased risk of drug toxicities in HIV patients.

The issue of drug interactions has become particularly critical in the era of protease inhibitors. However, although the number of potential drug interactions is substantial, few require dosage modifications. Among these, clinicians should be vigilant when using concomitant prophylaxis and treatment of mycobacterial diseases with rifamycin, macrolides and HIV protease inhibitors.

COMPLIANCE ISSUES

The use of combination therapy for prophylaxis against multiple opportunistic pathogens significantly increases the complexity of treatment of HIV patients. In particular, it may increase the number of pills that are necessary in combination antiretroviral regimens. Such regimens may lead to patient noncompliance and inability to tolerate other therapeutic regimens, inducing antiretrovirals.

COST-EFFECTIVENESS

To be most cost-effective, prophylaxis should be directed at the most common infections in the patient population. However, an expensive prophylactic regimen may be cost-effective if it has a positive impact on quality and duration of life or if it reduces other costs. This is particularly true in severely immunocompromised HIV patients, in whom any intervention, even costly prophylaxis, that significantly reduces the incidence of hospitalization will have a favorable impact on the costs of caring. Studies have shown that prophylaxis for PJP and MAC is cost-effective, whereas prophylaxis for fungal disease and cytomegalovirus is not.[6]

DURATION OF PROPHYLAXIS AGAINST OPPORTUNISTIC INFECTIONS

Since HAART was introduced, it has become clear that prophylaxis against opportunistic infections need not necessarily be life long. As mentioned above, susceptibility to opportunistic infections can be assessed accurately by the CD4$^+$ T-cell count. It is thus logical to stop primary or secondary prophylaxis in patients whose immunity has improved as a consequence of HAART.

Data generated until now support this approach and recommendations concerning the safety of stopping primary or secondary prophylaxis can now be made for many pathogens.

By contrast, no data are available regarding the re-initiation of prophylaxis when the CD4$^+$ lymphocyte count decreases again to levels at which the risk for opportunistic infections exists. In particular, it is unknown whether it is better to use the threshold at which prophylaxis was stopped or the threshold below which initial prophylaxis is recommended.[7]

RECOMMENDATIONS FOR PROPHYLAXIS AGAINST OPPORTUNISTIC INFECTIONS

The US Public Health Services and the Infectious Diseases Society of America have established disease-specific recommendations for the prevention of opportunistic infections in individuals who have HIV infection, which were updated in June 2002. These recommendations include guidelines for preventing exposure to pathogens as well as on specific regimens for preventing initial episodes.[8]

Category I regimens are strongly recommended as standard of care. Category II should be strongly considered in eligible patients. Category III regimens are not routinely recommended but may be considered for use in selected patients (Table 90.1). Recommendations on prophylactic regimens to prevent recurrence of opportunistic infections have also been updated (Table 90.2).

PREVENTION OF OPPORTUNISTIC INFECTIONS IN PREGNANT WOMEN

Recommendations are the same as in other groups. Because of concerns related to administering drugs during the first trimester of pregnancy, certain health-care providers might choose to withhold prophylaxis during the first trimester of pregnancy.

DISEASE-SPECIFIC CONSIDERATIONS

Pneumocystis jirovecii

Prophylaxis of PJP has been shown to be highly cost-effective. A meta-analysis of 35 studies of PJP prophylaxis in 6583 patients showed that TMP–SMX was superior to dapsone or aerosolized pentamidine but there was no statistically significant survival advantage for TMP–SMX versus alternative agents. An advantage of TMP–SMX over alternative drugs is a significant reduction in bacterial infections. Side-effects are sufficiently severe to require discontinuation of the drug in 25–50% of TMP–SMX recipients compared to 25–40% of dapsone recipients and 2–4% of recipients of aerosolized pentamidine. There is good evidence that lower doses of TMP–SMX are better tolerated, and many advocate either the use of the lower-dose regimens using one single-strength tablet daily or one double-strength tablet thrice weekly. Patients who have adverse reactions to TMP–SMX usually tolerate dapsone.

Primary and secondary prophylaxis against PJP should be discontinued in patients treated with HAART who show an increase in CD4$^+$ T cells to above 200/mm^3 for at least 3 months. Prophylaxis should be re-introduced if the CD4$^+$ T-cell count decreases to less than 200/mm^3.[9,10]

Toxoplasma gondii

Toxoplasma seropositive patients who have a CD4$^+$ T-cell count below 100/mm^3 or who have had a previous episode of toxoplasmic encephalitis should receive prophylaxis against toxoplasmic encephalitis.

Table 90.1 Prophylaxis of first episode of opportunistic infections in adults and adolescents with HIV infection

Pathogen	Target population	First-choice regimen	Alternative regimens
Category I: recommended as standard of care			
Pneumocystis jirovecii	CD4+ lymphocyte count <200/mm^3 *and/or* oropharyngeal candidiasis	TMP–SMX 1 DS q24h or 1 SS q24h	Dapsone 100 mg q24h or 50 mg q12h Dapsone 50 mg q24h *plus* pyrimethamine 50 mg weekly *plus* leucovorin 25 mg weekly Dapsone 200 mg weekly *plus* pyrimethamine 75 mg weekly *plus* leucovorin 25 mg weekly Aerosolized pentamidine 300 mg monthly by nebulizer Atovaquone 1500 mg q24h TMP–SMX 1 DS three times a week
Toxoplasma gondii	CD4+ lymphocyte count <100/mm^3 *and* positive anti-*Toxoplasma* IgG	TMP–SMX 1 DS q24h	TMP–SMX 1 SS q24h Dapsone 50 mg q24h *plus* pyrimethamine 50 mg weekly *plus* leucovorin 25 mg weekly Dapsone 200 mg weekly *plus* pyrimethamine 75 mg weekly *plus* leucovorin 25 mg weekly
Mycobacterium tuberculosis	Positive PPD (5 mm) *and/or* previous positive PPD without treatment *and/or* contact with active case (regardless of PPD result)	Isoniazid 300 mg q24h *plus* pyridoxine 50 mg q24h for 9 months or isoniazid 900 mg and pyridoxine 50 mg twice weekly with directly observed therapy for 9 months	Rifampin (rifampicin) 600 mg q24h for 4 months *If isoniazid resistance*: rifampin 600 mg q24h for 12 months or rifabutin 600 mg q24h for 12 months *If multidrug resistance*: choice of drugs depends on susceptibility of isolate from source patient
Mycobacterium avium complex	CD4+ lymphocyte count <50 cells/mm^3	Clarithromycin 500 mg q12h *or* azithromycin 1200 mg weekly	Rifabutin 300 mg q24h or azithromycin 1200 mg weekly *plus* rifabutin 300 mg q24h
Varicella-zoster virus (VZV)	Exposure to chickenpox or shingles *and* no history of either or negative serology	Varicella-zoster virus Ig, 5 vials im within 96h of exposure (preferably within 48h)	
Category II: generally recommended			
Streptococcus pneumoniae	CD4+ lymphocyte count >200 cells/mm^3	23-valent polysaccharide vaccine 0.5 ml im (single dose); repeat in 4–5 years time	
Hepatitis B virus	All anti-HBV-negative patients	Hepatitis B vaccine 10 mg im (three doses)	
Influenza virus	All patients	Inactivated trivalent Influenza vaccine 0.5 ml im each year in autumn	Oseltamivir 75 mg q24h (influenza A or B) Amantadine 100 mg q12h Rimantadine 100 mg q12h (influenza A only)
Hepatitis A virus	All anti-HAV-negative patients at increased risk (injection drug users, men who have sex with men, hemophiliacs) or with chronic liver disease, including chronic hepatitis B or C	Hepatitis A vaccine (two doses)	
Category III: not recommended for most patients. To be considered in selected patients only			
Cytomegalovirus	CD4+ lymphocyte count <50 cells/μl *and* positive cytomegalovirus antibodies	Oral ganciclovir 1 g q8h	
Bacteria	Neutropenia	G-CSF 5–10 μg/kg sc q24h for 2–4 weeks *or* GM-CSF 250 μg/m^2 iv q24h for 2–4 weeks	
Cryptococcus neoformans	CD4+ lymphocyte count <50 cells/mm^3	Fluconazole 100–200 mg q24h *or* itraconazole 200 mg q24h	
Histoplasmosis	CD4+ lymphocyte count <100 cells/mm^3 *and* residence in endemic area	Itraconazole 200 mg q24h	

DS, double strength; G-CSF, granulocyte colony-stimulating factor; GM-CSF, granulocyte–macrophage colony-stimulating factor; PPD, purified protein derivatives; SS, single strength; TMP–SMX, trimethoprim–sulfamethoxazole. Adapted from USPHS/IDSA guidelines.[8]

Table 90.2 Prophylaxis to prevent recurrence of opportunistic disease in adults and adolescents with HIV infection

Pathogen	Indication	First-choice regimen	Alternative regimens
Category I: Recommended as standard of care			
Pneumocystis jirovecii	Previous PJP	TMP–SMX 1 SS q24h or 1 DS q24h	Dapsone 50 mg q12h or 100 mg q24h Dapsone 50 mg q24h *plus* pyrimethamine 50 mg weekly *plus* leucovorin 25 mg weekly Dapsone 200 mg *plus* pyrimethamine 75 mg *plus* leucovorin 25 mg weekly Aerosolized pentamidine 300 mg monthly by nebulizer Atovaquone 1500 mg q24h TMP–SMX, 1 DS three times weekly
Toxoplasma gondii	Prior toxoplasmic encephalitis	Sulfadiazine 500–1000 mg q6h *plus* pyrimethamine 25–50 mg q24h *plus* leucovorin 10–25 mg po q24h (confers protection against PJP)	Clindamycin 300–450 mg q6–8h *plus* pyrimethamine 25–50 mg q24h *plus* leucovorin 10–25 mg q24h Atovaquone 750 mg q6–12h *with or without* pyrimethamine 25 mg q24h *plus* leucovorin 10 mg q24h
Mycobacterium avium complex	Documented disseminated disease	Clarithromycin 500 mg q12h *plus* ethambutol 15 mg/kg q24h (*with or without* rifabutin 300 mg q24h)	Azithromycin 500 mg q24h *plus* ethambutol 15 mg/kg q24h (*with or without* rifabutin 300 mg q24h)
Cytomegalovirus	Previous end-organ disease	Ganciclovir 5–6 mg/kg iv q24h 5–7 days/week *or* 1000 mg po q8h *or* foscarnet 90–120 mg/kg q24h *or* (for retinitis) ganciclovir sustained-release implant every 6–9 months *plus* ganciclovir 1.0–1.5 g po q8h	Cidofovir 5 mg/kg every 2 weeks (with probenecid) Fomivirsen 1 vial (330 µg) injected into the vitreous, then repeated every 2–4 weeks Valganciclovir 900 mg q24h
Cryptococcus neoformans	Documented disease	Fluconazole 200 mg q24h	Amphotericin B 0.6–1.0 mg/kg weekly Itraconazole 200 mg q24h
Histoplasma capsulatum	Documented disease	Itraconazole 200 mg q12h	Amphotericin B 1.0 mg/kg weekly
Coccidioides immitis	Documented disease	Fluconazole 400 mg q24h	Amphotericin B 1.0 mg/kg weekly Itraconazole 200 mg q12h
Salmonella spp. (non-*typhi*)	Bacteremia	Ciprofloxacin 500 mg q12h for several months	Antibiotic chemoprophylaxis with another active agent
Category II: Recommended only if subsequent episodes are frequent or severe			
Herpes simplex virus	Frequent/severe recurrences	Aciclovir 200 mg q8h *or* 400 mg q12h *or* Famciclovir 250 mg q12h	Valaciclovir 500 mg q12h
Candida (oropharyngeal, vaginal or esophageal)	Frequent/severe recurrences	Fluconazole 100–200 mg q24h	Itraconazole solution 200 mg q24h

DS, double strength; SS, single strength. Adapted from USPHS/IDSA guidelines.[8]

The double-strength tablet daily dose of TMP–SMX is recommended. Alternative regimens in patients who cannot tolerate TMP–SMX include dapsone–pyrimethamine or atovaquone (with or without pyrimethamine). Prophylactic monotherapy with dapsone, clindamycin, pyrimethamine, azithromycin or clarithromycin is not recommended.

Primary prophylaxis against toxoplasmic encephalitis should be discontinued in patients treated with HAART showing an increase in $CD4^+$ T cells to above 200/mm^3 for at least 3 months. Discontinuation of prophylaxis in patients where $CD4^+$ counts have increased to 100–200 cells/mm^3 has not been carefully evaluated. No firm recommendation can be made regarding discontinuation of secondary prophylaxis, but it appears reasonable to consider discontinuation in patients who have $CD4^+$ T cells above 200/mm^3 for at least 3 months. Prophylaxis should be re-introduced if the $CD4^+$ T-cell count decreases to below 100–200/mm^3.[10,11]

Mycobacterium tuberculosis

Latent tuberculosis infection should be treated in all patients who have HIV infection and a positive tuberculin skin test, with no evidence of active tuberculosis and no history of treatment for active or latent tuberculosis.

Regimens include:

- daily or twice weekly isoniazid (plus pyridoxine) for 9 months; and
- daily rifampin (or rifabutin) for 4 months.

Rifampin (or rifabutin) plus pyrazinamide for 2 months should not be given because severe liver injury has been associated with this combination.

In patients whose initial skin test is negative and whose $CD4^+$ T-cell count has increased to above 200/mm^3 with HAART, a repeat tuberculin skin test should be considered.[12]

Mycobacterium avium complex

Patients who have a CD4+ T-cell count below 50/mm³ should receive clarithromycin (500 mg q12h) or azithromycin (1200 mg weekly). Combination with rifabutin is not recommended. Both macrolides confer protection against respiratory bacterial infections. Rifabutin is an alternative in patients who cannot tolerate macrolides.

Primary prophylaxis should be discontinued in patients treated with HAART who show an increase in CD4+ T cells to above 100/mm³ for at least 3 months and should be re-introduced if the CD4+ T-cell count decreases to less than 50–100/mm³.[13]

Patients who have disseminated MAC should receive maintenance therapy with clarithromycin (or azithromycin) and ethambutol with or without rifabutin. It is reasonable to consider discontinuation of treatment in patients who have completed a course of at least 12 months of therapy, have no symptoms and show a CD4+ T-cell count above 100/mm³ following HAART for at least 6 months.[14]

Streptococcus pneumoniae

Patients who have a CD4+ T-cell count greater than 200/mm³ should receive a single dose of 23-valent polysaccharide pneumococcal vaccine every 4–5 years. If the CD4+ T-cell count is below 200/mm³, vaccination should be considered (with revaccination when the count increases to above 200/mm³).[15]

Cryptococcosis

Primary prophylaxis should not be used routinely. Patients who have had cryptococcosis should receive lifelong suppressive treatment with fluconazole unless they have a sustained (≥6 months) increase in the CD4+ T-cell count following HAART. Suppressive therapy should be reinitiated if the CD4+ T-cell count decreases to less than 100–200/mm³.[16]

Cytomegalovirus

Patients who have had active cytomegalovirus disease should receive maintenance therapy with any of the following regimens: parenteral or oral ganciclovir, parenteral foscarnet, combined parenteral ganciclovir and foscarnet, parenteral cidofovir or oral valganciclovir. Administration of ganciclovir via intraocular implant or repetitive intravitreous injections of fomivirsen may be used in patients who have retinitis only, and are generally combined with oral ganciclovir. Repetitive intravitreous injections of ganciclovir, foscarnet and cidofovir have been shown to be effective in uncontrolled case series.[17,18]

Discontinuation of secondary prophylaxis should be considered in patients who have received HAART and show an increase in CD4+ T cells to above 100–150/mm³ for at least 6 months. All these patients should continue to undergo regular ophthalmologic examination. Secondary prophylaxis should be restarted when the CD4+ T-cell count falls to below 100–150/mm³.[19]

Varicella-zoster virus disease

HIV adults who have no history of chickenpox or are seronegative for varicella-zoster virus (VZV) should receive VZV immunoglobulin as soon as possible but within 96 hours after exposure to a patient who has chickenpox or shingles. The efficacy of aciclovir in this setting is unknown.

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References for this chapter can be found online at http://www.expertconsult.com

Chapter 91

William G Powderly

Opportunistic infections

INTRODUCTION

The major cause of death in patients with AIDS had been the opportunistic infections (OIs) that arose as a consequence of the severe immunodeficiency characteristic of HIV infection.[1] Since the advent of the era of potent antiretroviral therapy (ART), there has been a marked decrease in the occurrence of AIDS-related opportunistic infections and a consequent increase in survival.[2] Nevertheless, there is great variability in access to ART and consequently significant AIDS-associated OIs continue. In the Western world, this is largely a consequence of delayed diagnosis, with patients presenting with an OI as the first recognition of HIV infection; in resource-poor situations, availability of ART is more of a problem. The number of circulating $CD4^+$ lymphocytes is closely correlated with the risk of developing several opportunistic infections (see Fig. 90.2). Once the $CD4^+$ lymphocyte count falls below 200 cells/mm^3, the cumulative risk for developing an AIDS-defining OI is 33% by year 1 and 58% by 2 years. Therefore, $CD4^+$ lymphocyte counts are the critical parameter for monitoring patients who have HIV infection because of their predictive value for both opportunistic infections and mortality. Two patterns of OI development exist – re-emergence of infections acquired early in life (often in childhood) as the $CD4^+$ lymphocyte count falls, and newly acquired infection by organisms that become pathogenic in the setting of progressive immunodeficiency.

The probability of developing a given disease depends on the risk for exposure to potential pathogens, the virulence of the pathogens and the level of immunosuppression of the patient. There is a wide geographic variability in the epidemiology of opportunistic infections. In the USA and Northern Europe, pneumonia caused by *Pneumocystis jirovecii*, oropharyngeal or esophageal candidiasis, cytomegalovirus disease and infections caused by *Mycobacterium avium* complex (MAC) are common. The incidence of toxoplasmosis and tuberculosis is higher in Eastern and Southern Europe and in the developing countries, depending on the prevalence of latent infection in the general population.

Pneumocystis jirovecii Pneumonia

Pneumocystis jirovecii (previously known as *Pneumocystis carinii*, and still often referred to as PCP for *p*neumo*c*ystis *p*neumonia) is a fungal pathogen associated with opportunistic infections, especially pneumonia (PCP) in patients with altered cell-mediated immunity. Historically, the occurrence of PCP in American gay men who had no previously known immune deficiency was the first signal of the AIDS epidemic.[3] Although the implementation of prophylaxis and the advances in ART have markedly decreased its incidence, PCP remains an important OI. Its epidemiology is uncertain; although generally thought to be due to reactivation of latent infection acquired during childhood or adolescence, genetic studies of *P. jirovecii* isolates suggest that recent exposure to environmental strains may be a factor in acquisition of infection. PCP typically occurs when the $CD4^+$ lymphocyte count is less than 200/mm^3. The rate of relapse after a first episode of PCP is high (approximately 60% incidence within 1 year) when neither specific prophylaxis nor highly active antiretroviral therapy (HAART) is initiated.[4]

CLINICAL FEATURES

Pneumocystis jirovecii infection is restricted to the lung in more than 95% of cases. Disseminated infection (bone marrow, spleen, liver, retina and skin) can occur spontaneously or is associated with the use of aerosolized pentamidine. The most common clinical presentation of PCP is a progressive dyspnea with dry cough, fever (often mild) and weight loss. The mean duration of breathlessness is 3–4 weeks at presentation. On examination, fever and tachypnea are common, whereas lung auscultation may be normal or reveal only basal crepitations.

Chest radiography is an important step in the diagnosis procedure of lung diseases in AIDS. The chest radiograph is normal in less than 5% of cases. The most common pattern is a fine bilateral interstitial and then alveolar–interstitial infiltrate progressing from the perihilar to the peripheral regions (Fig. 91.1). Without therapy, or even during the first days of treatment, the alveolar–interstitial pattern worsens.

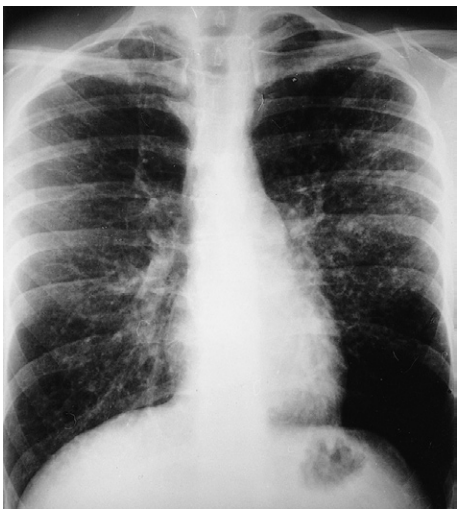


Fig. 91.1 Mild *Pneumocystis jirovecii* pneumonia. There are bilateral micronodular lesions. Courtesy of Pierre-Marie Girard.

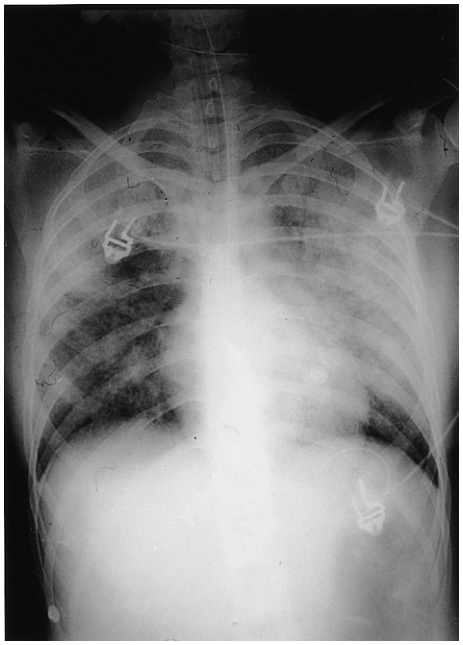


Fig. 91.2 Severe *Pneumocystis jirovecii* pneumonia. This shows an extensive alveolar interstitial infiltrate with consolidation of the left lung and upper right lobe. Courtesy of Pierre-Marie Girard.

In advanced cases, progressive consolidation with air bronchograms and complete opacification of the lungs may develop (Fig. 91.2). Numerous atypical patterns of PCP may occur. They include localized infiltrates, cavitary lesions, solitary lung nodules, spontaneous pneumothoraces and pleural effusions. Hilar or mediastinal lymphadenopathy is very rare.

Establishing a diagnosis of PCP requires the morphologic demonstration of the micro-organism. Arterial blood gases must be measured to assess the severity of the disease, but have no diagnostic value. Sputum examination is the least invasive procedure for collecting lower respiratory specimens. Because patients who have PCP generally do not have a productive cough, an induced sputum can be obtained after aerosolization of hypertonic saline, ideally under the supervision of a physiotherapist. In routine practice, the sensitivity of the test is approximately 50–60%. Fiberoptic bronchoscopy with bronchoalveolar lavage is the reference routine diagnostic procedure because its sensitivity is more than 95% and its specificity is 100%. When the diagnosis cannot be obtained from bronchoalveolar lavage fluid, transbronchial lung biopsy may help, although it is more invasive and is associated with a risk of bleeding and pneumothorax.

MANAGEMENT

The measurement of arterial gas is used routinely to define mild (Pao_2 ≥70 mmHg), moderate (Pao_2 50–70 mmHg) and severe (Pao_2 <50 mmHg) PCP. In mild PCP, outpatient management with oral therapy is often possible providing no other disease is present, there is low risk of drug malabsorption, the management strategy is well understood and the patient is aware of the potential side-effects of therapy.

Hospitalization is required for moderate and severe PCP to ensure careful monitoring of treatment, which should be administered intravenously, at least during the first days.

Trimethoprim–sulfamethoxazole (TMP–SMX) is the drug of choice for PCP whatever its severity, and no other drug or combination of drugs has demonstrated improved efficacy in control trials.[5,6] The drug is given as 15 mg/kg/day trimethoprim with 75 mg/kg/day sulfamethoxazole (in three divided doses). Side-effects include rash, fever, pruritus, digestive disturbances and cytopenia usually after 7–10 days of therapy. Approximately 10–20% of patients will need to be switched to another treatment because of TMP–SMX intolerance. It is important to determine the nature of intolerance to TMP–SMX. In practice, contraindication to reintroduction of TMP–SMX or to the use of a combination with potential cross-intolerance to sulfonamide is based on a history of anaphylaxis or exfoliative dermatitis or other life-threatening toxicity such as severe cytolytic hepatitis. Whenever possible, the first-line therapy for PCP is always TMP–SMX. For cases of mild PCP alternates include dapsone plus trimethoprim, and clindamycin plus primaquine or atovaquone.[6] For more severe PCP, alternates include clindamycin plus primaquine or intravenous pentamidine.[6]

In addition to anti-*P. jirovecii* therapy, corticosteroids are administered as soon as possible in patients who have moderate to severe disease. The use of corticosteroids is recommended in patients whose Pao_2 is less than 65 mmHg and has been shown to improve oxygenation, reduce the risk of fibrosis, decrease the need for mechanical ventilation and reduce the case fatality rate.[7] The recommended corticosteroid regimen is:

- on days 1–5, 40 mg oral prednisone (prednisolone) q12h;
- on days 6–10, 40 mg oral prednisone/day; and
- on days 11–21, 20 mg oral prednisone/day.

In most cases, respiratory symptoms and oxygenation improve after 5–10 days. If a patient has been treated empirally (without defintive diagnosis) failure to improve after 5–7 days of treatment should trigger a re-evaluation, usually with bronchoscopy. Early mild deterioration is not infrequent during the first days of treatment. The duration of treatment is classically 21 days. When TMP–SMX is initiated intravenously, it can be switched to an oral formulation after a few days. Secondary prophylaxis is required after treatment of the attack (see Table 91.2).

PREVENTION (SEE TABLES 91.1 AND 91.2)

Prophylaxis against PCP is indicated for all HIV-infected patients with a $CD4^+$ count less than 200 cells/µl or those with a history of oropharyngeal candidiasis or an AIDS-defining illness.

Viral Infections

Most clinically important viral infections in HIV disease are caused by DNA viruses, the majority belonging to the Herpesviridae family. The role of viral infections such as human herpes virus 8, Epstein–Barr virus and human papillomavirus in HIV-related neoplastic disorders is discussed elsewhere (see Chapter 94).

CYTOMEGALOVIRUS INFECTIONS

Serologic evidence of cytomegalovirus (CMV) infection can be detected in approximately 60% of the adult population in the USA. The prevalence of infection is strongly influenced by socioeconomic status and sexual practices; up to 95% of men who have sex with men (MSM) are seropositive for CMV. In patients who have AIDS, CMV disease usually results from reactivation of latent infection. Progressive loss of cell-mediated immunity in patients abrogates the immunologic suppression of CMV replication (see also Chapter 155). Asymptomatic excretion of CMV in urine can be detected in approximately 50% of patients who have advanced HIV disease, and over half of the patients who have CMV viremia go on to develop clinical CMV disease within 8–12 months. Cytomegalovirus end-organ disease usually occurs when the $CD4^+$ lymphocyte count falls below 50 cells/mm^3.[8]

Cytomegalovirus retinitis

Retinitis is the commonest manifestation of CMV infection in patients who have HIV infection or AIDS, accounting for 85% of CMV disease. The virus causes a relentlessly progressive, necrotizing retinitis. Cytomegalovirus retinitis is usually unilateral in the first instance,

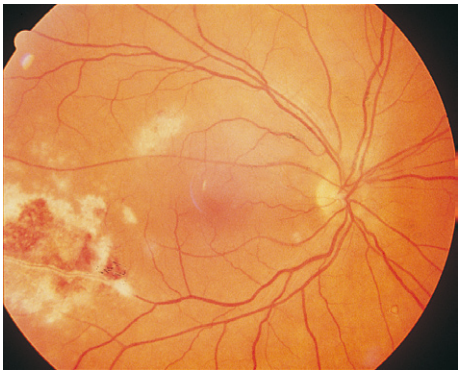


Fig. 91.3 Cytomegalovirus retinitis, with characteristic perivascular hemorrhages and exudates. Courtesy of Maurice E Murphy and Bruce Polsky.

progressing to affect the contralateral eye if untreated. Patients may be initially asymptomatic but may subsequently experience blurring of vision, floaters and painless progressive visual loss.

Diagnosis of CMV retinitis is made by systematic funduscopic examination by direct or indirect ophthalmoscopy. Characteristically, white, fluffy or granular lesions with perivascular white exudates associated with retinal hemorrhages are seen (Fig. 91.3).

Cytomegalovirus lesions may be categorized as occurring in three arbitrarily defined anatomic zones. Retinitis located in the immediate vicinity of the macula or optic disc (zone 1) is sight-threatening and should prompt immediate initiation of treatment. Lesions outside the major vascular vessels (zones 2 and 3), commonly referred to as 'peripheral retinitis', are not immediately sight-threatening but will progress if left untreated.

Gastrointestinal cytomegalovirus disease

In the upper gastrointestinal tract, CMV causes discrete esophageal ulcers, diffuse esophagitis, gastritis, gastric ulcers, duodenal ulcers and enteritis. Esophageal CMV infection usually presents with painful dysphagia; diagnosis is established by endoscopic examination showing inflammation and ulceration, with characteristic pathologic features on tissue biopsy. Cytomegalovirus enterocolitis usually presents with abdominal pain and persistent small-volume diarrhea. Endoscopic examination of the colon typically reveals plaque-like pseudomembranes, multiple erosions and ulcers.

Cytomegalovirus disease of the central nervous system

The two major CMV neurologic syndromes associated with HIV disease are CMV polyradiculopathy and CMV ventriculoencephalitis. Polyradiculopathy is seen in patients who have advanced AIDS. Approximately 50% have an associated myelitis. It is characterized by lower extremity pain, sensory deficits, weakness that can rapidly progress to flaccid paralysis, and bowel and bladder dysfunction. The most marked pathologic changes in CMV polyradiculomyelitis are found in the cauda equina and lumbosacral roots. In the appropriate clinical setting, findings on MRI of diffuse enhancement of the cauda equina and the surface of the conus strongly support the diagnosis. Cerebrospinal fluid (CSF) findings include a polymorphonuclear pleocytosis, raised protein concentration and moderately low glucose concentration. Culture of CSF may be negative but CMV antigen assays and polymerase chain reaction (PCR) for CMV DNA are more sensitive techniques (Chapter 155).

Ventriculoencephalitis usually occurs in the setting of a diagnosis of CMV disease elsewhere. Patients present with fever, lethargy and confusion. Characteristic neurologic findings include nystagmus and cranial nerve palsies. MRI with gadolinium enhancement may demonstrate periventricular enhancement. Cytomegalovirus DNA can often be detected in CSF using PCR.

Pulmonary cytomegalovirus disease

There are no particular distinguishing clinical or radiologic features to differentiate CMV pneumonitis from other causes of pneumonitis in HIV disease. Patients present with shortness of breath, dyspnea on exertion and a dry, nonproductive cough. Chest radiographs show diffuse interstitial infiltrates, and hypoxemia is usually present. Definitive diagnosis of pulmonary CMV disease in patients who have advanced HIV disease is difficult to establish because of a high incidence of asymptomatic CMV shedding – CMV can be isolated in approximately 50% of HIV-infected patients undergoing bronchoscopic examination. However, the true incidence of CMV pneumonitis is less than 10% in patients who have diagnostic bronchoscopy for evaluation of pulmonary infiltrates of unknown origin.

Management of cytomegalovirus infections[6]

Cytomegalovirus retinitis can be managed effectively with either local or systemic therapy; however, local therapy should be accompanied by some form of systemic treatment to control viremia and prevent retinitis in the contralateral eye. Oral valganciclovir, intravenous ganciclovir, intravenous ganciclovir followed by oral valganciclovir, intravenous foscarnet, intravenous cidofovir, and the ganciclovir intraocular implant combined with valganciclovir have all been shown in randomized trials to be effective treatment for CMV retinitis; balance should be maintained between ease of administration, side-effects and need for intravenous therapy. ART should be initiated promptly, and if effective in controlling HIV disease, CMV therapy can usually be discontinued after 6 months.

Intravenous gancliclovir (or oral valganciclovir in less ill patients) is the agent of choice for the treatment of CMV gastrointestinal disease.

HERPES SIMPLEX VIRUS INFECTIONS

Reactivation of herpes simplex virus (HSV) occurs frequently in patients who have advanced HIV disease, particularly in those who have low $CD4^+$ lymphocyte counts (<100 cells/mm^3). The hallmark of herpetic lesions is painful vesicular formation at a mucocutaneous site, progressing rapidly to ulceration with an erythematous base, followed by eventual healing and re-epithelialization.

As patients become more immunosuppressed, infections are characterized by prolonged viral shedding, more frequent episodes, and severe and persistent clinical disease. Recurrent genital and perirectal ulcerative lesions are the most common manifestations of HSV infection in patients who have HIV disease and are usually due to reactivation of HSV-2. Lesions may be atypical and severe in patients who have advanced disease. Prolonged new lesion formation, with continued tissue destruction, persistent viral shedding and severe local pain, is common.

HSV infections can be treated either with episodic therapy when lesions occur or with daily therapy to prevent recurrences – this decision should be individualized based on the frequency and severity of recurrences. Most infections can be treated with oral valaciclovir, famciclovir or aciclovir for 5–14 days. Severe mucocutaneous HSV lesions are best treated initially with intravenous aciclovir.

VARICELLA-ZOSTER VIRUS INFECTIONS

In the USA and Europe, most adults who have HIV disease have previously been infected with varicella-zoster virus (VZV). Owing to impaired cellular immunity, HIV-infected patients who have primary VZV are at risk of prolonged new lesion formation and are at higher risk of life-threatening visceral dissemination.

Herpes zoster may be the first indication of HIV disease and can occur at any stage of HIV infection. It usually appears as a localized or segmental painful erythematous maculopapular eruption along a single dermatome. In HIV-infected patients, zoster lesions may be bullous, hemorrhagic or necrotic. Patients infected with HIV are at risk

of recurrent episodes of herpes zoster. Occasionally, widespread cutaneous and visceral dissemination may occur. Visceral dissemination to the lungs, the liver and the central nervous system (CNS) may cause life-threatening disease.

HIV-infected patients should receive prompt antiviral therapy with oral valaciclovir, or famciclovir, for 7–10 days. More severe disease (extensive skin or visceral involvement) should be treated initially with intravenous aciclovir (10 mg/kg q8h).

PROGRESSIVE MULTIFOCAL LEUKOENCEPHALOPATHY

Progressive multifocal leukoencephalopathy (PML) is an opportunistic demyelinating infection caused by JC virus, a ubiquitous DNA papovavirus for which over 70% of adults are seropositive (see also Chapter 157). PML occurs in patients who have deficient cell-mediated immunity and is estimated to affect up to 4% of patients who have AIDS. Mortality is high, and average reported survival in AIDS patients is 2–4 months. The symptoms and characteristic radiologic findings of PML are due to virus-induced lysis of oligodendrocytes, resulting in microscopic foci of myelin breakdown that coalesce to produce larger white matter lesions (Fig. 91.4). Definitive diagnosis requires tissue from brain biopsy but the identification of JC virus in the CSF by PCR has a high specificity for active disease. There is no definitive antiviral treatment for PML and optimal ART is the treatment of choice.[9] A paradoxical response to ART can be seen in some patients with PML with unexpected neurologic deterioration (see Chapter 92).

Fungal Infections

Fungi are among the most ubiquitous pathogens seen in patients who have HIV disease but are not the most common causes of mortality. Virtually all major fungal pathogens cause disease in patients who have HIV infection.

CANDIDIASIS

Candidal infection in AIDS is almost exclusively mucosal – systemic invasion is a rare and late event. Oropharyngeal candidiasis occurs in about three-quarters of all those who have HIV infection. In about one-third it tends to be recurrent and becomes progressively more severe with increasing immunodeficiency. Esophageal involvement occurs in 20–40% of all AIDS patients, predominantly in patients who have advanced disease and severe depletion of $CD4^+$ lymphocytes. Vulvovaginal candidiasis occurs in about 30–40% of women who have HIV infection; it appears that HIV infection per se is not an important risk factor for vaginal infection, although it may influence the severity and persistence of disease.

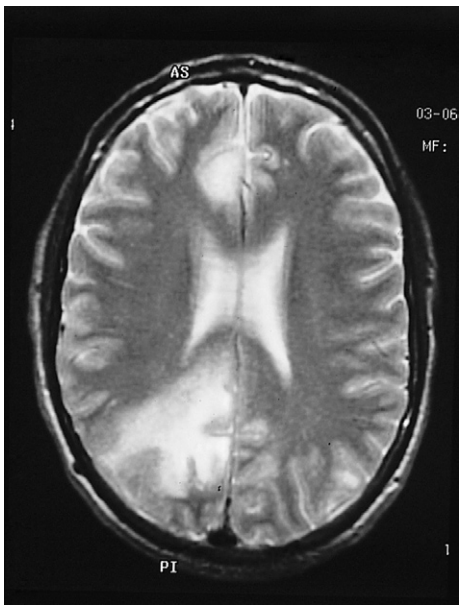


Fig. 91.4 Progressive multifocal leukoencephalopathy. MRI scan showing frontal and occipital white matter lesions. Courtesy of Maurice E Murphy and Bruce Polsky.

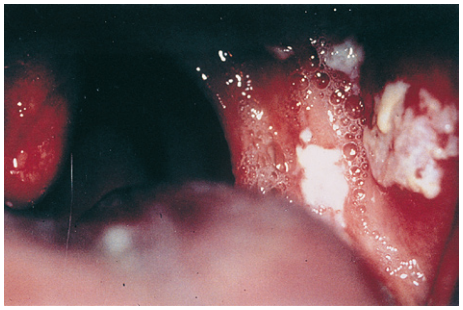


Fig. 91.5 Pseudomembranous oral candidiasis ('thrush').

Most candidal disease, especially initial episodes, is associated with infection by *Candida albicans*. Other species, notably *C. glabrata*, *C. dubliniensis* and *C. parapsilosis*, tend to cause infection in patients who have very advanced disease and have had extensive previous exposure to antifungal agents.

Most patients with oral candidiasis are symptomatic and complain of some oral discomfort. The classic presentation is of creamy-white plaques on an erythematous base – the pseudomembranous form of thrush (Fig. 91.5). Other manifestations include:

- an atrophic form that presents as erythema without plaques (often associated with patchy atrophic glossitis); and
- angular cheilitis, which appears as cracking, fissuring, ulceration or erythema at the corner of the mouth.

Most patients who have vaginal candidiasis present with vaginal itching, burning or pain, and usually complain of a vaginal discharge. Examination of the vaginal cavity usually reveals thrush, similar to that seen in the oropharynx.

Patients who have esophageal candidiasis develop ulcers and erosions of the esophagus and experience odynophagia or dysphagia. The combination of oral candidiasis and esophageal symptoms is both specific and sensitive in predicting esophageal involvement. Patients can be treated empirically with antifungal therapy. Endoscopy is reserved for those patients who fail to respond to evaluate for other diagnoses such as herpetic or cytomegalovirus esophagitis, idiopathic ulceration or resistant candidiasis.

The development of oral candidiasis in an HIV-positive patient should be taken as a sign of progressive immunodeficiency. Patients should have a $CD4^+$ count measured. If they are not currently receiving ART, it should be initiated. If they are on ART, it should be reassessed and, if necessary, changed. A number of options – both local and systemic – are available for the treatment of oral candidiasis. Initially, most patients respond well clinically to any form of antifungal therapy, although mycologic responses are less common. Esophageal disease requires systemic therapy.[6] Fluconazole 200–400 mg q24h orally for 2–3 weeks is probably the therapy of choice; itraconazole 100–200 mg q12h orally is also effective.

Relapses are common if effective ART is not given and at least one-third of patients develop recurrent mucosal candidiasis. One approach to management is to treat each episode as it occurs. However, in many patients, recurrent symptomatic disease may be sufficiently severe to warrant considering chronic suppression. Fluconazole 100–200 mg q24h has proved highly successful in suppressing recurrent oropharyngeal disease and preventing esophagitis,[10] and a dose of 100 mg per week can prevent vaginal candidiasis. The major risk of this approach is the possibility of developing azole-resistant disease. However, resistant infection has become very unusual in the era of potent effective ART.

CRYPTOCOCCOSIS

Virtually all HIV-associated infection is caused by *Cryptococcus neoformans* var. *neoformans*. About 5% of patients who have advanced HIV infection in the Western world develop disseminated cryptococcosis; the disease is more prevalent in sub-Saharan Africa and southern Asia. Most cases of infection occur in patients who have very low $CD4^+$ lymphocyte counts (<50 cells/mm^3).

Cryptococcosis most commonly presents as a subacute meningitis or meningoencephalitis with fever, malaise and headache. Symptoms are usually present for 2–4 weeks before diagnosis. Classic meningeal symptoms and signs (such as neck stiffness or photophobia) occur in about one-third of patients. Some patients may present with encephalopathic symptoms such as lethargy, altered mentation, personality changes and memory loss. Analysis of the CSF usually shows mildly elevated serum protein, normal or slightly low glucose, a few lymphocytes and numerous organisms.

The CSF opening pressure is elevated in 25% of patients, and this has important prognostic and therapeutic implications.

About one-half of patients have evidence of pulmonary involvement with cough or dyspnea and abnormal chest radiographs. Most patients who have cryptococcal meningitis have positive blood cultures. Skin involvement is common and several types of skin lesion have been described; the most common form is that resembling molluscum contagiosum.

Cryptococcal antigen is almost invariably detectable in the CSF at high titer. A positive serum cryptococcal antigen titer of more than 1:8 is presumptive evidence of cryptococcal infection. The serum cryptococcal antigen can therefore be used as a screening test for the presence of cryptococcal infection in febrile patients who have HIV infection. The finding of a positive cryptococcal antigen titer in this setting is sufficient to warrant antifungal therapy.

An algorithm for the treatment of cryptococcal meningitis is shown in Figure 91.6. Untreated, cryptococcal meningitis is fatal. Amphotericin B (0.7 mg/kg intravenously) given for 2 weeks followed by fluconazole 400 mg orally for a further 8 weeks is associated with the best outcome in prospective trials, with a mortality of less than 10% and a mycologic response of approximately 70%.[7] The addition of flucytosine 100 mg/kg q24h with amphotericin B does not improve immediate outcome but may decrease the risk of relapse.[11]

Clinical deterioration may be due to cerebral edema, which may be diagnosed by a raised opening CSF pressure. Elevated opening pressures (>200 mmH_2O) have been linked with an increased risk of early mortality and/or blindness.[12] The opening pressure of all patients who have cryptococcal meningitis should be measured when a lumbar puncture is performed, and strong consideration should be given to reducing such pressure (by repeated lumbar puncture, a lumbar drain or a shunt) if the opening pressure is high (>200 mmH^2O).[11]

All patients who have cryptococcal infection should receive optimal ART. An immune reconstitution syndrome has been described in some patients who initiate potent ART after cryptococcal infection. Cryptococcal meningitis requires long-term suppressive therapy (fluconazole 200 mg daily), unless there is improvement in immune function (undetectable plasma HIV viral RNA and a persistent rise in $CD4^+$ lymphocyte count to at least 200 cells/mm^3).

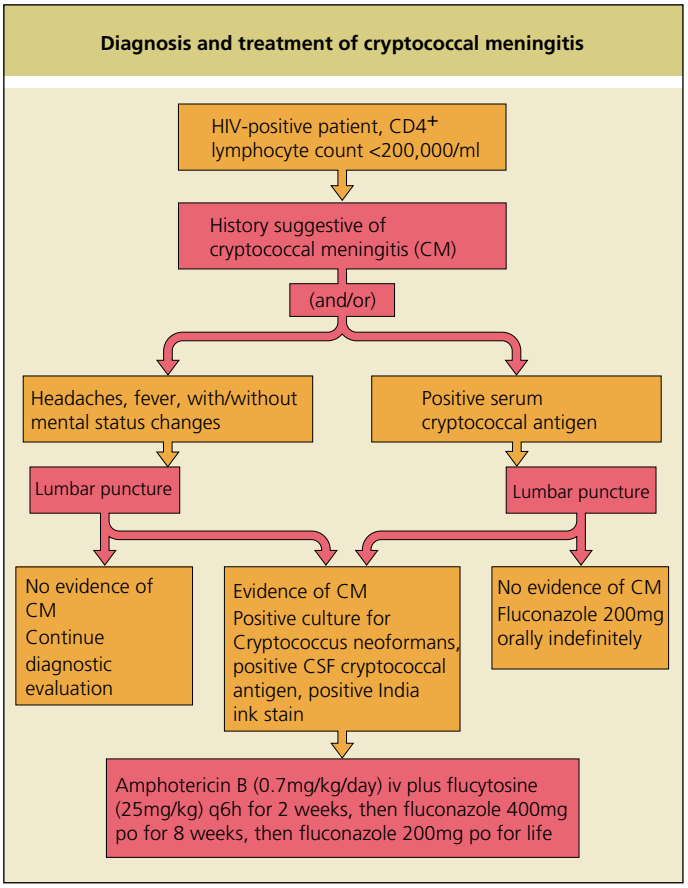


Fig. 91.6 Algorithm for the diagnosis and treatment of cryptococcal meningitis. *Continue unless CD4 T cells recover (>200 cells/mm^3 for >3 months with antiretroviral therapy).

HISTOPLASMOSIS, COCCIDIOIDOMYCOSIS AND PENICILLIOSIS

Histoplasmosis is caused by the dimorphic fungus *Histoplasma capsulatum*, which is endemic in the Mississippi and Ohio river valleys of North America as well as in certain parts of Central and South America, Asia, Africa and the Caribbean. *Coccidioides immitis* is a dimorphic fungus found in the soil in the desert around the south-western USA and northern Mexico, as well as focal areas of Central and South America. Penicilliosis is caused by the dimorphic fungus *Penicillium marneffei*, which is endemic in South East Asia (especially northern Thailand and southern China). These fungi cause disseminated infection in patients who have AIDS in endemic areas, as well as sporadic infection among HIV-positive migrants from and visitors to endemic areas.

The most common presentation is fever and weight loss. Pneumonia is usual in coccidioidomycosis and respiratory symptoms (cough, shortness of breath) occur in about 50% of cases of histoplasmosis and penicilliosis. Local or generalized lymphadenopathy, hepatosplenomegaly, colonic lesions and skin and oral ulcers also occur. Skin involvement is more common in penicilliosis. Involvement of the gastrointestinal tract (usually as ulcers) is a feature of histoplasmosis and may present with abdominal pain or gastrointestinal bleeding. Between 5% and 10% of patients with histoplasmosis have an acute septic-shock-like syndrome that includes hypotension and evidence of disseminated coagulopathy. Meningeal disease, with symptoms of lethargy, fever, headache, nausea, vomiting and/or confusion, occurs in about 10% of patients with coccidioidomycosis. Cerebrospinal fluid analysis typically reveals a lymphocytic pleocytosis with a lymphocyte count greater than 50/mm^3.

Diagnosis is made by culturing the organism from clinical specimens or by demonstrating it on histopathologic examination. In histoplamosis a peripheral blood smear may show intracellular organisms in white cells in many patients, and blood cultures, especially when collected using the lysis centrifugation system, are positive in over 90% of patients. Antigen detection in either urine or serum is an excellent method for diagnosing disseminated histoplasmosis.

For all infections, the management strategy is similar.[6] Patients should receive an initial period of treatment with amphotericin B (for 1–2 weeks) until there is clinical resolution. For histoplasmosis[13] and penicilliosis, this should be followed by itraconazole 200 mg orally

q12h. For coccidioidomycosis, fluconazole, 400–800 mg q24h orally, may be an alternative for patients who have mild disease. Complete eradication is unlikely and chronic suppressive therapy with either fluconazole 200–400 mg q24h or itraconazole 200 mg q12h is needed. Successful treatment with itraconazole or fluconazole has been reported in approximately 80% patients who have *C. immitis* meningitis.

ASPERGILLOSIS

Infection with *Aspergillus* spp. is seen in patients who have advanced HIV disease. Specific risk factors that have been identified include neutropenia, use of corticosteroids and broad-spectrum antibacterial therapy and previous pneumonia, especially *Pneumocystis jirovecii* pneumonia. Typically, patients have extremely low CD4⁺ lymphocyte counts and a history of other AIDS-defining opportunistic infections.

Two major syndromes predominate:

- respiratory tract disease; and
- central nervous system infection.

Patients with pulmonary involvement often present with cough, shortness of breath and fever. Chest pain and hemoptysis are common. Nodular infiltrates, which may be localized or diffuse and commonly cavitate, are seen on chest radiography. Additional respiratory syndromes of pulmonary aspergilloma and localized tracheobronchial aspergillosis have been reported occasionally in patients who have AIDS. Patients who have CNS aspergillosis usually present with symptoms and signs of a mass lesion or with features of a stroke due to invasion of blood vessels. Therefore, seizures, hemiparesis and focal abnormalities are common. CT scan or MRI of the head may show single or multiple lesions, usually nonenhancing, with surrounding edema. Bony invasion is common and, because the disease may have spread from involved sinuses, the sinuses may be abnormal. Patients who have fungal sinusitis usually have the classic features of sinusitis (fever, facial pain and swelling, nasal discharge and headache). CT scan of the sinuses will usually show bony erosion, and penetration into adjacent tissues such as the brain or orbit can occur.

The prognosis of aspergillosis is poor, in part because of the fungal infection itself and in part because it tends to occur in patients who have advanced AIDS and many other complications of end-stage HIV infection. There is a poorer response to therapy in patients who have AIDS than in other immunocompromised patients. Voriconazole is the treatment of choice for invasive aspergillosis[6,14] (see Chapter 149).

Nontuberculous Mycobacterial Infections

Mycobacterium avium complex (MAC) bacteria are ubiquitous in the environment and have been isolated from a variety of sources around the world, including soil, natural water, municipal water systems, food, house dust and domestic and wild animals. These isolates are thought to be the source of most human infections but there is no evidence of MAC transmission from person to person. In untreated HIV-infected patients in the Western world, the incidence of MAC disease increases with falling CD4⁺ lymphocyte counts, with a cumulative probability of disseminated disease due to MAC in subjects who have CD4⁺ lymphocyte counts less than 50 cells/mm³ of 20–30%, and 10–20% in those who have CD4⁺ lymphocyte counts of 50–100 cells/mm³.

CLINICAL FEATURES

MAC infection is acquired through inhalation or ingestion of the organism, with most infections in HIV-infected patients believed to occur through colonization and invasion of the gut mucosa. Disseminated MAC disease is characterized by fever, night sweats and weight loss. The gastrointestinal tract is frequently involved and clinical manifestations include nausea, vomiting, watery diarrhea and abdominal pain. At physical examination hepatomegaly, splenomegaly and lymphadenopathy are very common, and elevations of serum alkaline phosphatase, lactate dehydrogenase and anemia are the most frequent laboratory findings. Other nontuberculous mycobacteria, including *Mycobacterium genavense, M. intracellulare, M. haemophilum, M. simiae, M. xenopi, M. scrofulaceum, M. marinum* and *M. fortuitum*, have also been described as a cause of disseminated infection in HIV-infected patients.

Although MAC can commonly be isolated from sputum, pulmonary disease associated with MAC is rare. Patients should have a repeatedly positive culture in sputum, an infiltrate on chest radiograph, absence of other lung pathogens and preferably biopsy specimens showing acid-fast bacilli in abnormal lung tissue. However the isolation of MAC in the respiratory or gastrointestinal tract in those patients who have CD4⁺ lymphocyte counts less than 50 cells/mm³ represents a high risk for the development of MAC bacteremia. In this case prophylaxis or treatment should be considered.

Diagnosis of disseminated disease caused by MAC requires the isolation of the organism from a sterile site. A single blood culture has a high diagnostic yield, which is 90–95% sensitive. Once sufficient growth is achieved, the diagnosis of MAC can be made in few hours with the use of DNA probes (see Chapter 174). The diagnosis can also be made by identification of MAC from other sterile sites such as bone marrow, liver or lymph node biopsy.

The most frequent nontuberculous mycobacterium isolated from sputum in HIV-infected patients is *Mycobacterium kansasii*. Patients who have *M. kansasii* infection tend to have a low CD4⁺ lymphocyte count (<50 cells/mm³) and the clinical and radiologic manifestations are not different from tuberculosis. The isolation of *M. kansasii* from sputum is always considered diagnostic of pulmonary disease, since colonization is uncommon.

TREATMENT

Therapy for *Mycobacterium avium* complex

Treatment regimens should include a macrolide (clarithromycin or azithromycin) plus ethambutol.[6] The addition of rifabutin should be considered, since triple therapy has been associated with a reduction in relapses and in the emergence of resistant strains.[15] Aminoglycosides and quinolones may be useful in macrolide-resistant cases. If ART is to be used, it is important to recognize potential drug interactions between clarithromycin and both non-nucleoside reverse transcriptase inhibitors (NNRTIs) and protease inhibitors (see Practice Point 45).

In general, as with other opportunistic infections, therapy for disseminated MAC is for life. However, in situations where CD4⁺ T-cell counts remain greater than 100 cells/mm³ after 6–12 months of ART, patients are at low risk of recurrence of MAC and the treatment can be stopped.

Treatment of other nontuberculous mycobacterias

Mycobacterium kansasii is the second most frequent cause of pulmonary and disseminated nontuberculous mycobacterial disease. The treatment consists of a daily regimen of rifampin (rifampicin) or rifabutin, isoniazid and ethambutol for 12–18 months. *Mycobacterium genavense* and *M. haemophilum* are resistant to isoniazid, pyrazinamide and ethambutol. *Mycobacterium genavense* may be treated with a regimen similar to MAC and *M. haemophilum* with a combination of rifampin plus another active antituberculosis drug (ciprofloxacin, doxycycline, clarithromycin or amikacin). *Mycobacterium simiae* and *M. xenopi* are susceptible to isoniazid, rifampin and ethambutol and should be treated for 12 months. *Mycobacterium scrofulaceum* therapy requires surgery and isoniazid plus rifampin for 24 months with amikacin for 2–3 months. *Mycobacterium marinum* is resistant to isoniazid

and pyrazinamide. Minocycline or clarithromycin or rifampin plus ethambutol for 3 months are possible therapies. *Mycobacterium fortuitum* therapy consists of amikacin plus cefoxitin plus ciprofloxacin for 1 month followed by quinolone plus clarithromycin for 3–6 months.

PREVENTION (SEE TABLE 91.1)

Prophylaxis for MAC is recommended for all AIDS patients who have $CD4^+$ lymphocyte counts lower than 50 cells/mm^3. In randomized, placebo-controlled trials, clarithromycin 500 mg/day and 1200 mg azithromycin once a week, were effective in reducing MAC bacteremia.[16,17] Before starting prophylaxis, MAC bacteremia and active tuberculosis should be ruled out. Discontinuation of MAC prophylaxis can occur in patients on ART with sustained viral suppression and a $CD4^+$ T lymphocyte count greater than 100 cells/mm^3.

Parasitic Infections

TOXOPLASMOSIS

Toxoplasmosis, caused by *Toxoplasma gondii*, an obligate intracellular protozoan, is a common OI in patients with AIDS. It has a prevalence ranging from 10% to 40% in Europe, the Caribbean area and Africa, to 5–10% in USA. This is due to the different prevalence of *T. gondii* infection in the general population of these areas. Toxoplasmosis occurs as a result of the reactivation of latent *T. gondii* infection that has persisted in the CNS or extraneural tissues after earlier acute infection. Toxoplasmosis usually occurs late in HIV disease, when the $CD4^+$ lymphocyte count is less than 100/mm^3.

The CNS is by far the most common site of toxoplasmosis and the majority of patients have encephalitis. Retinitis, pneumonitis and myocarditis are less common manifestations. Toxoplasmic encephalitis (TE) commonly manifests as single or multiple intracerebral abscesses, with focal neurologic signs and constitutional symptoms that progress over a few days or weeks. Fever and headaches are present in 40–70% of cases, neurologic dysfunction including confusion and lethargy in 40% of cases, focal CNS deficits in 50–60% of cases, and seizures in 30–40% of cases. The constellation of fever, headaches, mild neurologic deficit or any unexplained neurologic symptoms should suggest the diagnosis of TE (Fig. 91.7), and prompt CT scanning or MRI should be undertaken. MRI is more sensitive than CT scanning. Toxoplasmic abscesses are typically contrast-enhancing lesions surrounded by edema; there may be a mass effect, with displacement of the ventricles. MRI may also reveal hemorrhages, which are highly suggestive of toxoplasmic necrosis.

Most patients will have specific anti-toxoplasmal antibodies, indicating past infection (see Chapter 183). In most of cases of encephalitis, the diagnosis of toxoplasmosis is presumptive, made on clinical–radiologic criteria and confirmed by the therapeutic response. Appropriate treatment is therefore especially important, both because the response to therapy is the main criterion for diagnosis of TE, and because early initiation of therapy gives the best prognosis. Treatment consists of initial acute therapy over 3–6 weeks followed by lifelong maintenance therapy to prevent further reactivation. The combination of pyrimethamine 50–75 mg/day orally and sulfadiazine 4–6 g/day is the first-line acute therapy.[6,18] These drugs act synergistically by blocking the folic acid pathway of tachyzoites, but have no effect on the cyst forms of the parasite. Folinic acid 25 mg/day orally should be given as well to prevent bone marrow toxicity. Clinical improvement occurs within 5–10 days; the diagnosis of TE is confirmed by a decrease in both neuroradiologic and clinical abnormalities. The duration of acute therapy is 3–6 weeks. Suppressive therapy is with pyrimethamine 25 mg/day orally plus sulfadiazine 2 g/day orally lifelong or until a CD4 cell count of greater than 200/mm^3 for more than 3 months is reached with ART. This combination is also effective as primary prophylaxis for *P. jirovecii* pneumonia.

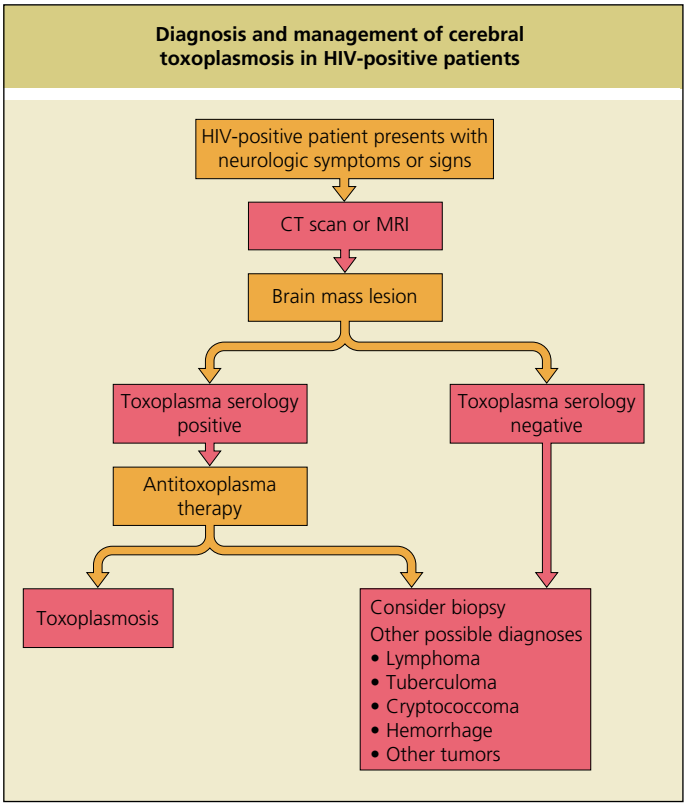


Fig. 91.7 Diagnosis and management of cerebral toxoplasmosis in HIV-positive patients. Courtesy of Christine Katlama.

CRYPTOSPORIDIOSIS

Cryptosporidiosis is a worldwide protozoan infection that is more prevalent in poorly developed countries (see also Chapter 181). It can affect normal hosts but is particularly severe in immunodeficient patients. Human cryptosporidiosis is generally caused by *Cryptosporidium parvum*, which is transmitted primarily by the fecal–oral route through person to person transmission or indirectly; outbreaks from municipal water, person-to-person transmission and animal-to-person transmission have been described. The illness is an acute enteritis. Diarrhea is the main clinical symptom, ranging in severity from mild diarrhea to cholera-like, watery diarrhea. Abdominal cramps, nausea, vomiting and anorexia usually accompany the diarrhea when it is moderate or severe. Fever is uncommon and may be due to other concurrent infections. The severity of the disease is more pronounced in patients with $CD4^+$ lymphocyte counts less than 50/mm^3. Cryptosporidiosis may rarely involve the gallbladder, the biliary tract, and the pancreatic ducts (leading to cholecystitis and pancreatitis). Diagnosis is based on the identification of the parasite in feces, in tissue specimens or in other fluids, using a modified acid-fast stain such as the Ziehl–Neelsen stain (Chapter 181).

Treatment of cryptosporidiosis is essentially symptomatic and the best option for therapy and prevention is the restoration of immune function using potent ART.

Bacterial Infections

Bacterial infections as a group, however, are the most common infectious complication of HIV disease. Patients with HIV infection are at greater risk for infections caused by common bacterial pathogens (e.g. *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Salmonella* spp.) than are HIV-seronegative persons. The introduction of potent ART

has been associated with a decline in the incidence of community-acquired and nosocomial bacterial pneumonia. However, the proportion of hospitalizations due to bacterial infections has increased as rates of PCP and other more opportunistic infections have declined to a greater extent. The increased rate of bacterial pneumonia in HIV-infected patients without AIDS, in comparison to HIV-negative persons, serves to emphasize that pyogenic bacterial infections may occur when immunity is relatively intact owing to the virulence of these organisms compared with other more opportunistic pathogens.

BACTERIAL PNEUMONIAS

The incidence of community-acquired bacterial pneumonia is markedly higher (three- to fivefold) among HIV-infected patients than among HIV-negative persons. Risk factors for the development of bacterial pneumonia include lower $CD4^+$ lymphocyte counts, intravenous drug use and cigarette smoking.

In addition, risk factors for pneumococcal infection specifically include any previous history of pneumonia, low serum albumin and a lack of receipt of the pneumococcal vaccination when the patient had a $CD4^+$ lymphocyte count greater than 200/mm^3.

The clinical presentation of bacterial pneumonia in HIV-infected patients is indistinguishable from that in HIV-negative patients. The history usually reveals an acute onset of symptoms, including fever, productive cough, dyspnea and pleuritic chest pain. Physical examination commonly shows localized pulmonary findings, and laboratory evaluation may show leukocytosis and hypoxemia. Typical chest radiograph findings are of focal infiltrates, although diffuse disease may be seen. Sputum Gram stain will show multiple polymorphonuclear leukocytes, and sputum cultures are usually positive. These findings differ from the classic presentation of PCP, the most common nonbacterial cause of pneumonia in HIV-infected patients, in which the presentation is more often subacute with a nonproductive cough, a paucity of physical findings and diffuse interstitial infiltrates on chest radiograph. Nevertheless, there are frequently cases in which induced sputum examination or bronchoscopy with bronchoalveolar lavage are necessary to obtain a microbiologic diagnosis.

The pathogens most commonly responsible for bacterial pneumonia are *S. pneumoniae*, *H. influenzae* and *Haemophilus* spp. other than *H. influenzae*.[19] Other organisms that may cause disease include *M. catarrhalis*, *Klebsiella pneumoniae* and *Staphylococcus aureus*. Empiric therapy usually includes a second- or third-generation cephalosporin. If there is a lack of clinical response, other bacteria that may not respond to such treatment must be considered as possible causes (e.g. *Chlamydia pneumoniae*, *Nocardia* spp. and *Legionella* spp). In addition, in HIV-infected patients *Pseudomonas aeruginosa* frequently occurs as a community-acquired pneumonia. There are also data that suggest a shift in bacterial pathogens since the introduction of ART. A decline in *P. aeruginosa* infections has been reported, and seems to be associated with both generally higher CD4 lymphocyte counts and a reduction in the use of trimethoprim–sulfamethoxazole prophylaxis.[20]

An unusual organism responsible for bacterial pneumonia in HIV-infected patients is *Rhodococcus equi*. This bacterium is a Gram-positive rod usually associated with infections of domestic animals. In HIV-infected patients, the clinical presentation is often subacute and characterized by fever, cough, pleuritic chest pain, fatigue and weight loss. Chest radiographs often show cavitating lesions. Blood cultures may be more sensitive for diagnosis than sputum cultures – 83% sensitivity compared with 33% in one study. Treatment is usually with vancomycin (intravenously 1 g q12h), but ciprofloxacin (orally 750 mg q12h) or imipenem (intravenously 500 mg q6h) may also be used; duration of therapy is 2–4 weeks.

Bacteremia in the setting of pneumonia occurs more commonly in HIV-infected than in HIV-negative patients – increased rates of bacteremia with pneumococcal and *H. influenzae* pneumonia have been documented. Bacteremia may also occur with other pathogens responsible for pulmonary infections (e.g. *P. aeruginosa*).

SKIN INFECTIONS

Bacterial skin infections in HIV-infected patients may vary from impetigo to folliculitis to cutaneous abscesses; cutaneous abscesses are often associated with intravenous drug use as well. Recurrences may be more frequent than in the HIV-negative population, but physical findings and responsible organisms (*S. aureus* and *Streptococcus* spp.) are similar. A significant increase in subcutaneous and soft tissue abscesses associated with community-acquired methicillin-resistant *S. aureus* (MRSA) has been noted in the last few years in the USA among HIV-infected individuals.[21] Aggressive treatment with surgical drainage and appropriate antimicrobials is necessary.

Bacillary angiomatosis is a distinct skin infection specifically associated with HIV disease. (See Chapter 95.) The condition is caused either by *Bartonella henselae*, the organism also responsible for cat-scratch disease, or by *Bartonella quintana*. The clinical appearance is of very erythematous subcutaneous nodules that may occasionally resemble Kaposi's sarcoma (Fig. 91.8). Lymphadenopathy may be associated with these skin findings. Much less frequently, *B. henselae* has been identified as the cause of more deeply seated infections, including endocarditis, osteomyelitis and hepatic lesions (peliosis hepatis). Diagnosis of the skin infection may be based on the characteristic appearance, but biopsy may be carried out to rule out other causes, and it is necessary to diagnose solid-organ involvement. Histology shows vascular proliferation and mixed inflammatory cells. Warthin–Starry staining reveals the organism. Treatment is with a macrolide (erythromycin orally 250–500 mg q6h) or a tetracycline (doxycycline orally 100 mg q12h) for 1–2 months for skin disease, and longer for other sites of infection.

ENTEROCOLITIS

Among the bacterial pathogens causing diarrhea in HIV-infected patients are nontyphoidal *Salmonella* spp., *Shigella flexneri*, *Campylobacter jejunii* and *Clostridium difficile*. *Shigella* and *Campylobacter* spp. infections manifest similarly – patients note severe diarrhea (which may be bloody) associated with abdominal cramping, nausea and fever. Diagnosis is by stool culture. These infections may both be treated with oral quinolones, although resistance to quinolones is increasing (see Chapter 35).

Nontyphoidal *Salmonella* spp. infections may present either with or without diarrhea and other symptoms of colitis. In patients without gastrointestinal symptoms, the infection may manifest solely as fever without any localizing findings. The occurrence of *Salmonella* bacteremia is more common in patients with HIV infection than in HIV-seronegative groups. In addition, relapses of bacteremia are common. Unlike the recommendations for patients who are not immunosuppressed, even *Salmonella* infections limited to the gastrointestinal tract should be treated in HIV-infected patients. Treatment is usually with ciprofloxacin (500 mg q12h for 2–4 weeks), and suppressive therapy with ciprofloxacin is often used because of the risk of relapse.

Infection due to *C. difficile* may be more severe in HIV-infected patients, and these patients are more likely to have relapses and



Fig. 91.8 Typical appearance of bacillary angiomatosis. Courtesy of Christine Katlama.

chronic symptoms. Antibiotic use and hospitalization are associated with the development of infection, just as they are in HIV-negative patients. *Clostridium difficile* may also occur as a community-acquired infection in HIV-infected patients. Treatment is with oral metronidazole or vancomycin.

BACTEREMIA

As discussed above, bacteremia may frequently occur as a result of *Salmonella* spp. infections and pneumonia. Bacteremia may also be associated with soft tissue infections and urinary infections. Intravenous drug use is associated with the development of *S. aureus* bacteremia and endocarditis. In addition, bacteremia may be a complication of the use of long-term intravenous catheters, which may be needed in the management of HIV-infected patients (e.g. for the intravenous treatment of CMV retinitis). The causative organisms most frequently isolated were *S. aureus* and coagulase-negative staphylococci, although infection with Gram-negative bacilli is also seen. Empiric therapy of line infections while culture results are awaited should therefore involve vancomycin and an aminoglycoside (or vancomycin and a β-lactam with broad Gram-negative coverage). As with other infections, the incidence of HIV-associated bacteremia declined in the era of potent ART, in part because of fewer hospitalizations and a reduction in the use of central venous catheters and in neutropenia.

Use of Antiretroviral Therapy During Management of Acute Opportunistic Infections

Antiretroviral therapy should be initiated as soon as possible in patients with OIs for which treatment is limited or not effective and for whom the overall improvement of immune function would be beneficial. Examples of such conditions include cryptosporidiosis, microsporidiosis, progressive multifocal leukoencephalopathy and HIV-associated dementia.

For patients for whom definitive anti-infective therapy is available, the benefit of immediate initiation of ART in increasing $CD4^+$ cell count and improving the patient's immunologic status must be weighed against the possibility of increased adverse effects, toxicities or drug–drug interactions. A randomized clinical trial demonstrated a clinical and survival benefit of starting ART early, within the first 2 weeks, of initiation of treatment for an acute OI.[22] The majority of OIs in this study were PCP and serious bacterial infections, and some caution must be taken extending this recommendation to OIs (such as cryptococcosis and mycobacterial infection) where the risk of immune reconstitution inflammatory syndrome (IRIS) may be higher (see Chapter 92).

The choice of regimen should follow relevant guidelines; however, in the acute setting, results of resistance testing may not be available and a boosted protease inhibitor regimen may be preferable.

Paradoxical reactions can occur in patients with active (or unrecognized) infection in whom ART can lead to an improved immune response to the underlying infective organism. This can, in turn, lead to an inflammatory response with adverse, sometimes severe, clinical consequences. These consequences of immune recovery are discussed in detail in Chapter 92.

PROPHYLAXIS OF OPPORTUNISTIC INFECTIONS

Preventive therapy for OIs was the mainstay of treatment in HIV prior to the introduction of effective ART and remains important for patients with severe immunodeficiency, even when ART-associated immune recovery can be expected. Guidelines for the primary prevention of OIs are given in Table 91.1 and recommendations for secondary prophylaxis (prevention of recurrence of OIs) are given in Table 91.2.

Table 91.1 Prophylaxis of first episode of opportunistic infections in adults and adolescents with HIV infection

Pathogen	Target population	First-choice regimen	Alternative regimens
Category I: recommended as standard of care			
Pneumocystis jirovecii	$CD4^+$ lymphocyte count <200/mm^3 and/or oropharyngeal candidiasis	TMP–SMX 1 DS q24h or 1 SS q24h	Dapsone 100 mg q24h or 50 mg q12h Dapsone 50 mg q24h plus pyrimethamine 50 mg weekly plus leucovorin 25 mg weekly Dapsone 200 mg weekly plus pyrimethamine 75 mg weekly plus leucovorin 25 mg weekly Aerosolized pentamidine 300 mg monthly by nebulizer Atovaquone 1500 mg q24h TMP–SMX 1 DS three times a week
Toxoplasma gondii	$CD4^+$ lymphocyte count <100/mm^3 and positive anti-*Toxoplasma* IgG	TMP–SMX 1 DS q24h	TMP–SMX 1 SS q24h Dapsone 50 mg q24h plus pyrimethamine 50 mg weekly plus leucovorin 25 mg weekly Dapsone 200 mg weekly plus pyrimethamine 75 mg weekly plus leucovorin 25 mg weekly Atovaquone 1500 mg q24h with or without pyrimethamine 25 mg and leucovorin 10 mg q24h

Table 91.1 Prophylaxis of first episode of opportunistic infections in adults and adolescents with HIV infection—cont'd

Pathogen	Target population	First-choice regimen	Alternative regimens
Mycobacterium tuberculosis	Positive PPD (5 mm) and/or previous positive PPD without treatment and/or contact with active case (regardless of PPD result)	Isoniazid 300 mg q24h or isoniazid 900 mg twice weekly for 9 months either plus pyridoxine 50 mg q24h *If resistance*: choice of drugs depends on susceptibility of isolate from source patient; consult with public health authorities	Rifampin (rifampicin) 300 mg q24h for 4 months Rifabutin (dose based on antiretroviral therapy) for 4 months
Mycobacterium avium complex	CD4⁺ lymphocyte count <50 cells/mm³ (need to rule out active mycobacterial infection before starting prophylaxis)	Clarithromycin 500 mg q12h or azithromycin 1200 mg weekly	Rifabutin 300 mg q24h
Varicella-zoster virus (VZV)	Exposure to chickenpox or shingles and no history of either infection, vaccination or negative serology	Varicella-zoster virus 1 g, 5 vials im within 96 hours of exposure (preferably within 48 h)	
Category II: generally recommended			
Streptococcus pneumoniae	CD4⁺ lymphocyte count >200 cells/mm³	223-valent polysaccharide vaccine 0.5 ml im (single dose); repeat in 5 years	
Hepatitis B virus	All anti-HBV-negative patients	Hepatitis B vaccine 10 mg im (three doses)	Anti-HBs should be obtained 1 month after completion of the vaccine series Nonresponders should be revaccinated with a second vaccine series
Influenza virus	All patients	Inactivated trivalent Influenza vaccine 0.5 ml im each year in autumn	
Hepatitis A virus	All anti-HAV-negative patients at increased risk (intravenous drug users, men who have sex with men, hemophiliacs) or with chronic liver disease, including chronic hepatitis B or C	Hepatitis A vaccine (two doses)	
Category III: not recommended for most patients. To be considered in selected patients only			
Cytomegalovirus (CMV)	CD4⁺ lymphocyte count <50 cells/ml and positive CMV viremia	Oral valganciclovir 900 mg q24h	
Cryptococcus neoformans	CD4⁺ lymphocyte count <50 cells/mm³	Fluconazole 100–200 mg q24h	
Histoplasmosis	CD4⁺ lymphocyte count <100 cells/mm³ and residence in endemic area	Itraconazole 200 mg q24h	

DS, double strength; PPD, purified protein derivatives; SS, single strength; TMP–SMX, trimethoprim–sulfamethoxazole.
Adapted from USPHS/IDSA guidelines.[6]

Certain principles underlie the decision to use prophylaxis for different pathogens:

- the incidence and prevalence of specific infections in HIV-infected individuals;
- the potential severity of disease in terms of morbidity and mortality;
- the level of immunosuppression at which each disease is likely to occur;
- the feasibility and efficacy of preventive measures, and in particular their impact on quality of life and survival;
- the potential for emergence of organisms resistant to the agents used for prophylaxis;
- the risk of toxicities and drug interactions with antiretrovirals and other drugs used by HIV-infected patients;
- the issue of compliance; and
- the cost-effectiveness of prophylaxis.

As an example, PCP is very common, causes significant mortality and can be easily prevented; as a result routine prophylaxis is recommended for all HIV-positive patients with CD4⁺ T-cell counts less than 200 cells/mm³. In contrast, candidiasis is also very common, but rarely causes mortality, is easily treated and prophylactic antifungals are associated with a significant risk of resistance, so prophylaxis is rarely given. Cryptococcal meningitis is associated with significant mortality and is preventable using fluconazole; however, prevention is not regarded as cost-effective in the West because it is relatively rare; in contrast primary prophylaxis is justified in areas such as sub-Saharan Africa and South Asia.

Table 91.2 Prophylaxis to prevent recurrence of opportunistic disease in adults and adolescents with HIV infection

Pathogen	Indication	First-choice regimen	Alternative regimens
Category I: Recommended as standard of care			
Pneumocystis jirovecii (PCP)	Previous PCP	TMP–SMX 1 SS q24h or 1 DS q24h	Dapsone 50 mg q12h or 100 mg q24h Dapsone 50 mg q24h plus pyrimethamine 50 mg weekly plus leucovorin 25 mg weekly Dapsone 200 mg plus pyrimethamine 75 mg plus leucovorin 25 mg weekly Aerosolized pentamidine 300 mg monthly by nebulizer Atovaquone 1500 mg q24h TMP–SMX, 1 DS three times weekly
Toxoplasma gondii	Prior toxoplasmic encephalitis	Sulfadiazine 500–1000 mg q6h plus pyrimethamine 25–50 mg q24h plus leucovorin 10–25 mg po q24h (confers protection against PCP)	Clindamycin 300–450 mg q6–8h plus pyrimethamine 25–50 mg q24h plus leucovorin 10–25 mg q24h Atovaquone 750 mg q6–12h with or without pyrimethamine 25 mg q24h plus leucovorin 10 mg q24h
Mycobacterium avium complex	Documented disseminated disease	Clarithromycin 500 mg q12h plus ethambutol 15 mg/kg q24h (with or without rifabutin 300 mg q24h)	Azithromycin 500–600 mg q24h plus ethambutol 15 mg/kg q24h (with or without rifabutin 300 mg q24h)
Cytomegalovirus	Previous end-organ disease	Valganciclovir 900 mg q24h Ganciclovir sustained-release implant every 6–9 months plus valganciclovir 900 mg q24h	Ganciclovir 5 mg/kg/q24h iv 5–7 days/week or foscarnet 90–120 mg/kg iv q24h or cidofovir 5 mg/kg iv every 2 weeks (with saline hydration probenecid)
Cryptococcus neoformans	Documented disease	Fluconazole 200 mg q24h	Itraconazole 200 mg q24h
Histoplasma capsulatum	Documented disease	Itraconazole 200 mg q12h	Amphotericin B 1.0 mg/kg weekly
Coccidioides immitis	Documented disease	Fluconazole 400 mg q24h	Itraconazole 200 mg q12h
Salmonella spp. (non-*typhi*)	Bacteremia	Ciprofloxacin 500 mg q12h for several months	Antibiotic chemoprophylaxis with another active agent
Category II: Recommended only if subsequent episodes are frequent or severe			
Herpes simplex virus	Frequent/severe recurrences	Valaciclovir 500 mg q12h or aciclovir 400 mg q12h or famciclovir 500 mg q12h	
Candida (oropharyngeal, vaginal or esophageal)	Frequent/severe recurrences	Fluconazole 100–200 mg q24h	

DS, double strength; SS, single strength.
Adapted from USPHS/IDSA guidelines.[6]

DURATION OF PROPHYLAXIS AGAINST OPPORTUNISTIC INFECTIONS

Susceptibility to opportunistic infections can be accurately assessed by the CD4⁺ T-cell count. It is therefore possible to stop primary or secondary prophylaxis in patients whose immunity has improved as a consequence of HAART and whose T cells have risen above the threshold of increased susceptibity. This is particulary true for patients with complete viral suppression, as ongoing improvements in immune function can be expected.

By contrast, no data are available regarding the re-initiation of prophylaxis when the CD4⁺ lymphocyte count decreases again to levels at which the risk for opportunistic infections exists. In particular, it is unknown whether it is better to use the threshold at which prophylaxis was stopped or the threshold below which initial prophylaxis is recommended.

REFERENCES

References for this chapter can be found online at http://www.expertconsult.com

Chapter **92**

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Disorders of immune reconstitution in patients with HIV infection

INTRODUCTION

Suppression of HIV infection by combination antiretroviral therapy (ART) usually results in the reversal of HIV-induced immune defects, leading to at least partial reconstitution of the immune system and the prevention or regression of immunodeficiency disease. However, those patients who were very immunodeficient prior to commencing ART are susceptible to disorders of immune reconstitution that may cause disease.[1] These disorders most often present clinically as inflammatory and/or autoimmune immune disease and are often referred to collectively as the immune reconstitution inflammatory syndrome (IRIS).[2] This syndrome consists of more than one disorder, which may have different pathogenic mechanisms. These are:

- immune restoration disease (IRD) resulting from the restoration of pathogen-specific immune responses that cause inflammatory or cellular proliferative disease;
- immune-mediated inflammatory disease (IMID), in which immune mechanisms are implicated but the triggers are poorly understood; and
- autoimmune disease, predominantly Graves' disease.

In addition, persistent deficiency and/or dysfunction of T and B cells are other disorders of immune reconstitution that may increase the risk of illness in patients receiving ART. As increased numbers of HIV-infected individuals throughout the world receive ART, many patients will experience disorders of immune reconstitution and physicians managing patients with HIV infection should become familiar with their presentation, assessment and management.

IMMUNE RESTORATION DISEASE

History and immunopathogenesis

When zidovudine was introduced as the first antiretroviral drug in the late 1980s, it was observed that *Mycobacterium avium* complex (MAC) disease sometimes occurred during the first weeks to months of treatment. This had an atypical clinical presentation for MAC disease complicating AIDS because it was usually localized in distribution and associated with more inflammation than usually occurred in MAC disease complicating AIDS. Furthermore, it was associated with the restoration, rather than absence, of a cellular immune response to mycobacterial antigens. This type of MAC disease therefore appeared to reflect the restoration of a cellular immune response against subclinical MAC infection that resulted in immunopathology.[3] Similar observations were made when ART was introduced in the latter half of the 1990s[4,5] but the range of pathogens that could provoke an inflammatory response was much larger. Indeed, virtually any pathogen that can cause an opportunistic infection can provoke IRD when pathogen-specific immune responses are restored by ART (Table 92.1). As can be seen from Table 92.1, the nomenclature used for these disorders often reflects the clinical perspective from which they were described. Immune restoration disease can be differentiated from immunodeficiency disease based on clinical, histopathologic and immunologic characteristics (Table 92.2).

The anatomic site and clinicopathologic characteristics of the inflammation in IRD are largely determined by the provoking pathogen.[6] For example, *Mycobacterium tuberculosis* IRD mainly affects lymph nodes and lung, cryptococcal IRD usually affects the meninges and JC polyomavirus IRD (inflammatory progressive multifocal leukoencephalopathy after ART) affects the brain. Histopathology of affected tissues often reveals granulomatous inflammation when IRD is associated with an infection by mycobacteria, fungi or protozoa, and a $CD8^+$ T-cell response when associated with infection by viruses, such as JC polyomavirus.

Mycobacterial IRD is associated with an increase in circulating T cells reacting with mycobacterial antigens, which has been demonstrated by skin testing, lymphoproliferation assays and interferon gamma (IFN-γ) release assays (IGRAs).[6,7] Restoration of mycobacteria-specific immune responses may not be associated with an increase in total circulating $CD4^+$ T cells. For example, about 10% of patients with IRD provoked by nontuberculous mycobacteria (NTM) do not experience an increased blood $CD4^+$ T-cell count[8] and several studies have not shown an association between *M. tuberculosis* IRD and the magnitude of the $CD4^+$ T-cell count increase.[9] The role that restoration of pathogen-specific immune responses plays in IRD related to other pathogens is less clear. However, what evidence there is, along with the clinical features and timing of disease onset, suggest that pathogenic mechanisms are similar.[1,6]

Most cases of IRD present during the first 3 months of ART and usually during the first few weeks (early IRD). Some present later and up to 2 years after commencing ART (late IRD). The infection by the provoking pathogen may not be apparent before ART is commenced and is 'unmasked' by the immune response against it. Viable pathogens are usually isolated from the affected tissues of this 'unmasking' type of IRD but isolation from blood cultures is unusual. In other patients, IRD is provoked by an infection for which the patient is on treatment or that has recently been treated. Pathogens are usually not cultured, or are very difficult to culture, from specimens taken from the site of the IRD. It appears that there is an immune response against the antigens of nonviable pathogens. In this situation, there often appears to be a paradoxical worsening of the opportunistic infection when ART is commenced and, therefore, the term 'paradoxical IRD' is often used. It is important to recognize that this does not represent failure of treatment for the opportunistic infection, but rather an immunopathologic reaction to residual antigens of the pathogen. As there are currently no confirmatory diagnostic tests for IRD, it is necessary to use diagnostic criteria and exclude differential diagnoses that may account for the clinical deterioration before making the diagnosis of paradoxical IRD (Table 92.3).

Table 92.1 Pathogens that may provoke IRD when pathogen-specific immune responses are restored by antiretroviral therapy (ART)

Pathogen	Typical disease presentation/ nomenclature
Mycobacteria	
M. avium	MAC IRD
M. tuberculosis	*Mtb* IRD or TB-IRIS
M. leprae	Leprosy reversal reaction after commencing ART
Bacille Calmette–Guérin	BCG lymphadenitis after ART or BCG IRIS
Other nontuberculous mycobacteria	
Fungi and yeasts	
Cryptococcus spp.	Cryptococcal IRIS or IRD (mainly meningitis)
Histoplasma spp.	
Malasezia furfur	Inflammatory seborrheic dermatitis after ART
Pityrosporon spp.	Folliculitis after ART
Pneumocystis jirovecii	'Relapse' of pneumonitis after ART
Candida	
Aspergillus	
Penicillium	
Dermatophytes	Inflammatory tinea corporis after ART
Protozoans	
Toxoplasma gondii	Exacerbation or presentation of cerebral toxoplasmosis after ART
Leishmania spp.	
Microsporidia	
Cryptosporidium parvum	
Viruses	
Cytomegalovirus (CMV)	CMV immune recovery uveitis
Varicella-zoster virus	Cutaneous zoster or CNS disease
Herpes simplex virus 1 and 2	Mucocutaneous herpes (which may be necrotizing or hemorrhagic) or CNS disease
Human herpesvirus 8	Exacerbation or presentation of Kaposi's sarcoma
Molluscum contagiosum virus	Inflammatory molluscum contagiosum
JC polyomavirus	Inflammatory progressive multifocal leukoencephalopathy
BK polyomavirus	
Hepatitis B virus	ART-associated hepatotoxicity
Hepatitis C virus	ART-associated hepatotoxicity
Parvovirus B19	Exacerbation of red cell aplasia, encephalitis
Human papillomavirus	Wart enlargement or inflammation
HIV	Exacerbation of HIV encephalitis after ART
Helminths	
Schistosoma	
Strongyloides	
Bacteria	
Bartonella	

IRD, immune restoration disease; IRIS, immune reconstitution inflammatory syndrome; MAC, *Mycobacterium avium* complex.

Table 92.2 Comparison of immune restoration disease and immunodeficiency disease in patients with HIV infection

Immune restoration disease	Immunodeficiency disease
Restoration of a pathogen-specific 'protective' immune response that causes immunopathology*	Failure of 'protective' immune responses to control pathogen replication
Always associated with a decline in the plasma HIV RNA level	Usually associated with a high plasma HIV RNA level†
Usually associated with an increased circulating $CD4^+$ T cell count‡	Associated with a low circulating $CD4^+$ T cell count
Inflammation usually greater than in immunodeficiency disease, e.g. painful tissue lesions	Tissue inflammation may be absent
Tissue lesions exhibit changes of an immune response, e.g. scarcity of pathogens, and of immunopathology, e.g. granulomatous reaction, suppuration or necrosis	Histopathology of tissue lesions demonstrates changes of an impaired immune response, e.g. abundance of pathogens, poorly formed granulomata in mycobacterial disease

*Immune restoration disease (IRD) provoked by infections with nontuberculous mycobacteria and *M. tuberculosis* has clearly been associated with restoration of cellular immune responses to mycobacterial antigens. The evidence for other pathogens is circumstantial or unclear.
†Persistent immune defects in patients with long-term optimal control of HIV replication may rarely be complicated by opportunistic infections.
‡The circulating $CD4^+$ T cell count may not be increased in some patients with IRD, e.g. about 10% of patients with *Mycobacterium avium* complex IRD do not have an increased count.[8]

Table 92.3 Key diagnostic features of immune restoration disease

- Proven HIV infection
- Temporal relationship to antiretroviral therapy initiation
- Evidence or inference of immune recovery, for example:
 - Increase of the circulating $CD4^+$ T cell count
 - Decrease of plasma HIV RNA level
 - Evidence of increased pathogen-specific immune response, e.g. tuberculin skin test conversion
 - Evidence of an immunopathologic 'protective' pathogenic-specific immune response in tissues affected by immune restoration disease
- Presentation characterized by prominent inflammatory features clinically or radiologically
- Exclusion of other causes for clinical deterioration, which may include:
 - Resistance of opportunistic pathogens to antimicrobial drugs
 - Another infection or malignancy
 - Adverse drug reactions
 - Poor compliance with or malabsorption of treatment for the opportunistic infection

The major risk factors for the development of paradoxical IRD are a very low $CD4^+$ T-cell count, disseminated infection by the provoking pathogen and a short time of therapy for the opportunistic infection before ART is commenced.[4,9,10] All of these risk factors are probably markers of a high pathogen load. A low $CD4^+$ T-cell count may also be a marker of an increased susceptibility to immune dysregulation during immune reconstitution.

The incidence of IRD amongst cohorts of patients initiating ART has been reported to be between 10% and 45% for those with a documented underlying opportunistic infection and between 10% and 25% amongst unselected patients in retrospective studies. A prospective study from South Africa reported an incidence of 10.4% in an unselected cohort of 423 patients during the first 6 months of ART.[11] In this study, *M. tuberculosis* IRD (which is commonly known as TB-IRIS) was the commonest type of IRD. This presents in many ways (Table 92.4) and is the most significant type of IRD in areas where co-infection with HIV and *M. tuberculosis* is common.[12,13]

Management

The important principles in managing IRD are to ensure that the patient is on appropriate antimicrobial therapy to suppress replication of the provoking pathogen and reduce pathogen load, continue ART unless disease is life threatening, and consider the use of anti-inflammatory therapy to control inflammation and provide symptom relief. Symptom relief might also require other medication (e.g. analgesics) or drainage of inflammatory fluid collections.

Several characteristics of IRD are relevant to making decisions about treatment: most cases are self-limiting, mortality is uncommon and corticosteroid therapy (the most frequently used anti-inflammatory therapy) has important adverse effects. In a minority of cases, IRD may present with severe and potentially life-threatening manifestations. This is especially the case when there is central nervous system (CNS) involvement. Systemic corticosteroids have been used in patients with IRD related to infection with *M. tuberculosis*, MAC and *Cryptococcus neoformans*, with several reports of a favorable response. Nonsteroidal anti-inflammatory drugs (NSAIDs) have also been used to treat IRD. However, no prospective controlled clinical trials regarding the treatment of IRD have been published so far and treatment guidelines are therefore informed by case series.

Table 92.4 Common manifestations of *M. tuberculosis* IRD (TB-IRIS)

- Worsening or recurrence of TB symptoms
- Weight loss
- Fever and/or systemic inflammatory syndrome
- Lymph node enlargement
- Cold abscess formation
- Respiratory manifestations
 - Progressive pulmonary infiltrates on chest radiography (several patterns described, including miliary infiltrates and alveolitis)
 - Pleural effusions
 - Lymph node compression of airways
- Abdominal manifestations
 - Hepatic enlargement and abscesses
 - Splenic enlargement, rupture and abscesses
 - Intestinal involvement, e.g. ileocecal perforation
 - Peritonitis
 - Ascites
 - Psoas and intra-abdominal abscesses
- Central nervous system manifestations
 - Tuberculoma enlargement
 - Meningitis
 - Myeloradiculitis
- Genitourinary tract manifestations
 - Renal involvement with acute renal failure
 - Ureteric compression
 - Epididymo-orchitis
- Other system involvement
 - Arthritis and osteitis
 - Pericardial effusion
 - Bone marrow involvement

ART is continued in most settings, but some authorities have suggested interrupting ART if IRD is life threatening or unresponsive to corticosteroids. However, interruption of ART places the patient at risk of other opportunistic infections and the development of antiretroviral resistance, and IRD may still recur when ART is re-initiated.

Corticosteroid therapy for *M. tuberculosis* IRD

Corticosteroids have been used to treat *M. tuberculosis* IRD more than any other type of IRD.[13,14] However, the risks and benefits of corticosteroid therapy are unclear and need to be carefully weighed up. Because of the immunosuppressive effect of corticosteroid therapy, its use has been associated with an excess of Kaposi's sarcoma and herpes virus reactivations in HIV-infected patients. Also, if the diagnosis of IRD is incorrect and the cause for deterioration is drug resistance, or an additional unrecognized infection, corticosteroids could compound the diagnostic error.

Given that most cases of *M. tuberculosis* IRD are self-limiting, corticosteroids are usually reserved for patients with more severe manifestations and in whom the diagnosis is certain. Such patients include those with CNS involvement, airway compression due to enlarging lymphadenopathy, refractory or debilitating lymphadenitis, or respiratory distress. The duration of corticosteroid therapy required is variable. Some patients only require a few weeks whereas a subgroup of patients with *M. tuberculosis* IRD may require prolonged courses to control symptoms. Prednisone is the most frequently used corticosteroid and has been used at doses varying from 10 to 120 mg daily.

Clinical presentation

The great majority of pathogens that cause opportunistic infections (OIs) in patients with HIV infection may also provoke IRD. Consequently, the clinical presentation of IRD can vary greatly.

Central nervous system disease

IRD involving the CNS is potentially life threatening and can result in permanent neurologic disability. Manifestations include new or recurrent meningitis and inflammation involving the brain parenchyma, spinal cord (myelitis) or nerve roots (radiculitis). A number of pathogens have been associated with IRD of the CNS and, like other forms of IRD, disease may present as an unmasking of a previously untreated OI or paradoxical deterioration or recurrent manifestations of a treated infection. The interruption of ART should be seriously considered in these patients, particularly those who have a depressed level of consciousness and those who do not respond to other measures such as corticosteroid therapy.

Meningitis

Patients with cryptococcal meningitis who have been appropriately treated with antifungal therapy may experience a recurrence of meningitis after commencing ART.[15–17] This occurs in up to 30% of patients and typically manifests with recurrent headaches and features of raised intracranial pressure. Cerebrospinal fluid (CSF) fungal culture is usually negative, but the Indian ink and cryptococcal antigen tests are usually positive, reflecting persistence of nonviable organisms. The meningitis usually occurs around 4 weeks after ART is started but cases presenting months to years after ART initiation are described. The reported mortality rate has varied from 9% to 66%. Risk factors identified are disseminated disease, higher cryptococcal antigen titer at diagnosis of cryptococcal disease and a shorter time between starting antifungal therapy and ART. Treatment should include analgesia, therapeutic lumbar punctures to reduce raised intracranial pressure and corticosteroid therapy in refractory cases. ART interruption is seldom necessary.

Similarly, patients receiving treatment for tuberculosis (TB) may present with TB meningitis (TBM) after ART initiation. In some patients this will be recurrent TBM, but others will have had TB at an extraneural site and present with new TBM as an IRD phenomenon. Treatment is with corticosteroids, for which there is evidence of efficacy in TBM.[18]

Inflammation of the brain parenchyma

Inflammation of the brain parenchyma due to IRD may manifest as focal mass lesions (granulomas, such as tuberculomas, or brain abscesses) or a diffuse process (encephalitis). Both processes may be complicated by cerebral edema. Mass lesions tend to present with focal neurologic deficits or seizures. *M. tuberculosis* IRD may present with the emergence or enlargement of cerebral tuberculomas (Fig. 92.1). This may occur in patients already on treatment for TB. Cases of cerebral toxoplasmosis, cryptococcomas and abscesses associated with infection by NTM emerging or enlarging due to IRD are also described. Treatment of these conditions involves appropriate treatment of the infection and corticosteroid therapy, particularly when there is significant cerebral edema and mass effect.

Patients with encephalitis caused by IRD may present with multiple focal deficits or a disturbance of higher cerebral functions. JC polyomavirus infection of oligodendrocytes typically results in progressive multifocal leukoencephalopathy (PML) in patients with severe HIV-induced immunodeficiency. In patients not receiving ART, this is a subacute illness with progressive neurologic deterioration. Brain imaging typically does not demonstrate significant inflammation. In patients who develop IRD related to known or previously undiagnosed JC polyomavirus infection, the clinical progression is typically accelerated and imaging demonstrates prominent inflammation. Histopathology of brain biopsies in such patients shows a perivascular mononuclear cell infiltrate consisting predominantly of $CD8^+$ T cells.[19,20]

Rare cases of encephalitis resulting from IRD associated with infections by varicella-zoster virus (VZV), herpes simplex virus (HSV), cytomegalovirus (CMV), parvovirus B19, BK virus and HIV itself have been described. In HIV-associated encephalitis, patients have experienced rapid deterioration of HIV dementia, which has been ascribed to an inflammatory response against HIV itself in brain parenchyma. In the few cases reported to date, histopathology on brain samples obtained by biopsy or at post-mortem showed infiltration with mononuclear cells (particularly $CD8^+$ T cells), which in some instances was demonstrated to be associated with HIV DNA.[21]

Myelitis and radiculitis

Myelitis caused by IRD has been described in association with VZV and HSV infection. The pathogen associated with the IRD may be identified by examination of CSF for DNA by polymerase chain reaction (PCR). Spinal cord intramedullary abscess due to cryptococcal IRD has also been reported.

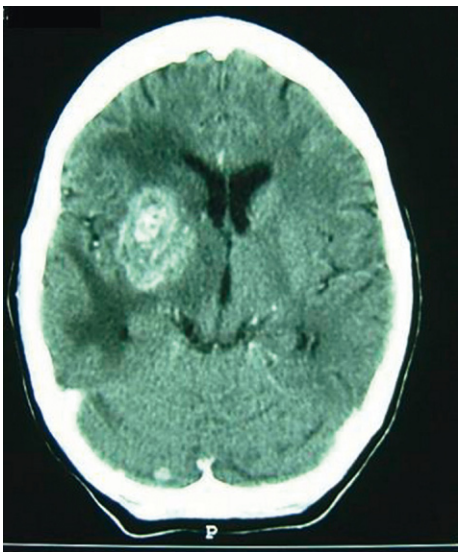


Fig. 92.1 Tuberculoma with surrounding edema in *M. tuberculosis* IRD (TB-IRIS).

Myeloradiculitis may rarely be a complication of meningitis in *M. tuberculosis* IRD. Inflammation in the meninges results in nerve root inflammation causing neurologic fall-out in the nerve roots affected, most frequently lumbosacral nerve roots.

Eye disease

CMV eye disease typically manifests as necrotizing retinitis without significant inflammation in patients with advanced HIV infection who are not receiving ART. After commencing ART, retinitis may result from the unmasking of subclinical CMV infection of the eye[22] or present as a paradoxical 'relapse' of retinitis in patients with treated CMV retinitis.[4,6] This is usually associated with an increased circulating $CD4^+$ T-cell count. Frequently, uveitis characterized by prominent inflammation also occurs in association with the retinitis.

Uveitis related to CMV may also present in patients with healed retinitis and is the most common form of ocular IRD. There may be intense inflammation in the absence of active CMV infection and it appears that the uveitis results from an immune response against retained CMV antigens. This condition has therefore been referred to as immune recovery uveitis (IRU).[23] It may result in vitritis, papillitis, macular edema and epiretinal membranes, and can be sight threatening. Furthermore, retinal neovascularization may be a late complication and occur over 4 years after cessation of anti-CMV therapy.[24] The incidence of IRU is highest in patients with the largest areas of previous retinitis and may be reduced by giving therapy for CMV retinitis prior to commencing ART,[25] suggesting that the amount of residual antigen is a determinant of disease onset. Many cases are self-limiting, but orbital floor corticosteroid injections may be necessary, especially if there is declining visual acuity. The condition should be managed by an ophthalmologist.

Uveitis may also result from IRD associated with other pathogens including mycobacteria, *Histoplasma* spp. or *Leishmania major*. Anterior stromal keratitis resulting from IRD associated with VZV infection has also been described.

Lymphadenitis and soft tissue abscesses

Lymphadenitis resulting from IRD may develop in any region of the body and is often painful (Fig. 92.2). Multiple nodes that suppurate and coalesce may form inflammatory masses or cold abscesses, common sites being in the omentum and retroperitoneal space. The latter may form a psoas abscess. Cold abscesses (e.g. subcutaneous cold abscesses) may also form *de novo* in soft tissues, unrelated to lymph nodes.

In developing countries, *M. tuberculosis* is the most frequent underlying infection and lymphadenitis is the most frequent clinical manifestation of *M. tuberculosis* IRD, occurring in up to 70% of patients.[12] Patients are usually receiving treatment for TB and present with recurrent, new or worsening node enlargement after starting ART (paradoxical IRD). This is most frequently in the cervical region. Nodal masses can enlarge to greater than 10 cm in diameter. Often the lymphadenitis has prominent acute inflammatory features with tenderness and red



Fig. 92.2 Lymphadenitis in *M. tuberculosis* IRD (TB-IRIS).

discoloration of the overlying skin and suppuration. These features would be unusual for TB lymphadenitis in a patient not receiving ART. Large amounts of pus may be aspirated from these nodes and draining sinuses may form. The pus is typically negative for *M. tuberculosis* cultures, but may be smear-positive, reflecting the presence of non-viable mycobacteria.

Lymphadenitis is also a frequent manifestation of IRD associated with infection by NTM, such as MAC and *M. scrofulaceum*. Lymphadenitis due to MAC IRD may persist for months or even years in some patients despite treatment for the MAC infection and corticosteroid therapy.[8] IRD related to bacille Calmette-Guérin (BCG) infection following BCG vaccination of children with HIV infection has also been described.[26] This condition manifests as an inflammatory reaction at the vaccination site as well as lymphadenitis involving the regional draining lymph nodes.

Lymphadenitis and subcutaneous abscesses have also been reported in IRD associated with infection by fungi, specifically cryptococci and *Histoplasma* spp.[27] and the protozoan pathogen *Leishmania* spp.

Potential complications of IRD lymphadenitis are airway compression, venous compression resulting in deep vein thrombosis, ureteric compression and chronic draining sinuses. Compression of airways or other vital structures is usually treated with corticosteroids.

Mucocutaneous disease

A wide variety of skin conditions have been reported to present or worsen after ART is commenced.[28] Most of these conditions cause minor morbidity and usually respond to specific therapy for the associated infection. An important clinical issue is the differentiation of IRD manifestations such as acne, papular pruritic eruption of AIDS and eosinophilic folliculitis from a drug hypersensitivity reaction in the skin in order to avoid unnecessary interruption of that drug.

Both herpes simplex and herpes zoster have been reported to occur with increased frequency during early ART compared to HIV-infected patients not receiving ART. In one study, herpes zoster was found to occur five times more frequently from weeks 4 to 16 after ART initiation.[29] Herpes zoster usually manifests as typical monodermatomal lesions that respond to antiviral therapy, but disseminated cases are reported. Herpes simplex manifests with either nasolabial or anogenital ulceration, which may be necrotizing and hemorrhagic. It typically responds to appropriate systemic antiviral therapy, although refractory cases are described.

Exacerbations of Kaposi's sarcoma (KS) due to IRD occur in a minority of patients starting ART (7% in one study[30]) but may result in tumor enlargement, new lesions, inflammation and edema. Although most cases involve the skin, rapid and fatal progression of visceral KS due to IRD has been described. It is probable that the restoration of an immune response against antigens of human herpesvirus 8 (the causative agent of KS) or possibly tumor antigens provokes this IRD. Resolution without treatment may occur but severe cases may require treatment with systemic chemotherapy.

Mycobacterium leprae infection of the skin is recognized in the setting of HIV disease and has been documented to present as unmasking IRD. The reported cases have manifested with inflammatory reversal reactions involving skin lesions or neuritis.[31]

Liver disease

Hepatotoxicity or a hepatitis flare presenting as an increase in the serum transaminase levels is a well-recognized adverse effect of ART. Many studies have examined risk factors for hepatotoxicity and demonstrated that drug hypersensitivity reactions and drug-induced mitochondrial toxicity are implicated in some cases. Hepatitis C virus (HCV) and hepatitis B virus (HBV) co-infection are also important risk factors.[32] However, the pathogenesis of the liver disease is debated. The timing of hepatotoxicity after ART in patients with HIV and HCV or HBV co-infection is similar to other cases of IRD, suggesting that it might be a manifestation of IRD in the liver. Supportive evidence is provided by reports of the exacerbation of hepatitis on liver biopsy and changes in serologic immune markers in HIV/HCV co-infected patients[33,34] and case reports of clearance of the hepatitis virus infection after the hepatitis flare for both co-infections. However, definitive studies of the immunopathogenesis have not yet been reported.

Infection of the liver by mycobacteria, fungi and protozoa may also provoke IRD after ART is commenced. The inflammation may cause right upper quadrant pain, fever and an inflammatory infiltrate in the liver that is characterized by granulomas or abscess formation. *M. tuberculosis* is the most common pathogen[35] but MAC, *Leishmania* spp., *Histoplasma* spp. and *Cryptococcus neoformans* have also been implicated.

Abdominal and renal disease

Abdominal pain is a common manifestation of intra-abdominal lymphadenitis resulting from IRD, which is usually associated with mycobacterial infection. Pain may also result from hepatitis caused by IRD (see above). It may also be a manifestation of IRD associated with infections by *Histoplasma* spp. resulting in infarction of the spleen or intestinal inflammation.[36] Peritonitis due to *M. tuberculosis* IRD may also result in abdominal pain and even mimic a 'surgical abdomen'.

Acute renal failure may result directly from granulomatous inflammation in the kidneys caused by *M. tuberculosis* IRD.

Lung disease

Paradoxical *M. tuberculosis* IRD frequently presents with a recurrence of cough and other respiratory symptoms after patients on treatment for *M. tuberculosis* infection start ART. This may be associated with new or worsening radiographic features of TB, including expanding pulmonary infiltrates, progressive cavitation, enlarging thoracic lymph nodes and enlarging pleural effusions.

In addition, patients with undiagnosed *M. tuberculosis* infection prior to ART may present with pulmonary disease soon after starting ART. This often presents in an accelerated way with prominent inflammatory features and appears to result from *M. tuberculosis* infection being unmasked by restoration of *M. tuberculosis*-specific immune responses. This presentation may mimic that of a community-acquired bacterial pneumonia and may be associated with a systemic inflammatory syndrome and respiratory failure.

MAC IRD may manifest with thoracic involvement including pulmonary infiltrates, endobronchial lesions and thoracic node enlargement. Other NTM such as *M. celatum*, *M. xenopi* and *M. kansasii* have also been associated with focal pulmonary infiltrates due to IRD.

Pneumonitis associated with *Pneumocystis jirovecii* infection has also been reported as a manifestation of IRD. Patients who have been treated for *P. jirovecii* pneumonitis (PJP) have developed a self-limiting or corticosteroid-responsive pneumonitis days to weeks after starting ART. Presentation has been with recurrent fever, dyspnea and cough, and chest radiography demonstrating alveolar infiltrates. Bronchoscopy at the time of IRD typically demonstrates an unusually vigorous inflammatory response but few or no *Pneumocystis* organisms, suggesting that this is an inflammatory response to residual *P. jirovecii* antigens.[37] Treatment is with appropriate antibiotics for PJP, usually TMP–SMX, and corticosteroid therapy.

Cryptococcal IRD has also been reported to present as necrotizing pneumonitis and thoracic lymphadenitis.[38]

Serous effusions

M. tuberculosis IRD may manifest as new or enlarging serous effusions (pleural effusions, pericardial effusions and ascites). Pleural effusions may cause respiratory distress due to compression of the underlying lung and pericardial effusions may result in cardiac tamponade, necessitating needle aspiration. Peritonitis and chylous ascites due to IRD associated with infection by NTM have also been described.

IMMUNE-MEDIATED INFLAMMATORY DISEASE IN HIV PATIENTS RESPONDING TO ANTIRETROVIRAL THERAPY

Immune-mediated inflammatory disease (IMID), in which inflammation is the result of immune responses that are apparently not precipitated by infections, may also present in HIV patients responding to ART. There are many reports of sarcoidosis presenting in this context[39] and Reiter's syndrome, lymphoid interstitial pneumonitis, Peyronie's disease, foreign body reactions and photodermatitis have also been described. Sarcoidosis may present up to 3 years after commencement of ART. Most cases present with thoracic disease but extrathoracic disease also occurs in the skin, liver, spleen, kidneys, peripheral lymph nodes, eyes, muscle and salivary glands. In some cases, the use of interleukin (IL)-2 or interferon alpha (IFN-α) therapy appears to have been a precipitating factor.[39]

The immunopathogenesis of sarcoidosis in HIV patients receiving ART is unclear but it is most likely to reflect an increased susceptibility to dysregulation of granulomatous inflammatory responses in the reconstituted immune system, which can be increased further by cytokine therapy that enhances Th1 immune responses, such as IL-2 and IFN-α.

ART-associated sarcoidosis may resolve without treatment but corticosteroid therapy is sometimes necessary. It is therefore important to differentiate sarcoidosis from IRD associated with infections that has resulted in granulomatous inflammation, such as mycobacterial IRD. Measurement of the cutaneous delayed-type hypersensitivity (DTH) response to tuberculin may be informative in this situation because it is usually absent in ART-associated sarcoidosis[39] but often present in mycobacterial IRD.[6]

AUTOIMMUNE DISEASE IN HIV PATIENTS RECEIVING ANTIRETROVIRAL THERAPY

Several types of autoimmune disease have been reported in HIV patients who have experienced immune reconstitution on ART. However, most are single case reports and it is unclear whether their occurrence during ART is merely a coincidence. In contrast, there is compelling evidence that Graves' disease is truly a disorder of immune reconstitution in HIV patients. Thus, there are over 40 cases reported in the literature, including cases in different racial groups.[40,41] It occurs predominantly in patients who had a very low CD4⁺ T-cell count at the time of commencing ART and who experience a substantial increase of the count on ART. Unlike IRD, ART-associated Graves' disease presents at a median time of almost 2 years after commencing ART. The immunopathogenesis therefore appears to be different from IRD and is most likely to be the result of an acquired defect of immune tolerance.

Systemic or cutaneous lupus has also been reported to present in HIV patients on ART.[42] Those cases presenting during the first 3 months of ART have had serologic evidence of lupus before treatment and it appears that the immunologic changes immediately after ART precipitate presentation with clinical disease. The other cases have presented after 9 months of ART and the lupus appears to have been acquired.

The management of autoimmune disease in HIV patients receiving ART is similar to non-HIV patients but caution has to be exercised if corticosteroid and immunosuppressant therapy are used in patients with HIV infection.

PERSISTENT DEFICIENCY AND/OR DYSFUNCTION OF T CELLS AND B CELLS

Contemporary ART is both effective and tolerable for long periods of time. It is therefore possible to suppress HIV replication for many years and realistic to aim for full reconstitution of the immune system. The majority of HIV patients will experience a continuing increase in the circulating CD4⁺ T-cell count over the first 5 years of ART, which generally plateaus thereafter.[43,44] Patients who achieve a CD4⁺ T-cell count of >500/μl after 6 years of ART may have a life expectancy that is similar to the general population.[45] However, despite long-term control of HIV replication, some patients do not achieve a normal CD4⁺ T-cell count (approximately >500/μl). Data from the Swiss HIV Cohort Study indicated that 36% of patients had a CD4⁺ T-cell count of <500/μl after 5 years of effective ART.[46] CD4⁺ T-cell counts below 500/μl were associated with a low CD4⁺ T-cell count and older age at the time of starting ART. Patients with a CD4⁺ T-cell count of <350/μl on ART continue to have an increased risk of AIDS-related disease and also non-AIDS related disease.[47]

CD4⁺ T-cell deficiency in HIV patients receiving effective ART has several possible causes. The most common causes are a low number of circulating naive CD4⁺ T cells, which in part is a consequence of failure to regenerate the thymus,[48] and ongoing immune activation, which results in CD4⁺ T-cell apoptosis and senescence.[49] The immune activation may be a consequence of HIV replication in reservoirs, such as gut-associated lymphoid tissue.[50]

Persistent CD4⁺ T-cell deficiency on ART can substantially be avoided by starting ART before the onset of severe immunodeficiency. The CD4⁺ T-cell count that is achieved after 4–7 years of ART is related to the CD4⁺ T-cell count at the time of starting ART.[43–46] In one study, the median CD4⁺ T-cell count after 7 years of continuous ART was 870/μl in patients with a baseline count of >500/μl and 73% of patients with a baseline count of >350/μl achieved a count of ≥800/μl.[44] The findings of this study provide support for recommendations that ART should be started before the CD4⁺ T-cell count drops below 350/μl.

Some patients with very low CD4⁺ T-cell counts may achieve substantial increases in the count on ART. In the ATHENA study, the median CD4⁺ T-cell count after 7 years of therapy in patients with a baseline count of <50/μl was 410/μl.[44] Furthermore, 20% of patients with a baseline CD4⁺ T-cell count of <50/μl achieved a count of ≥800/μl after 7 years. Therefore, even patients who are severely immunodeficient when ART is commenced have the potential to achieve a normal CD4⁺ T-cell count. Failure to do this is more common in patients who have periods of HIV replication and who are older than 50 years of age when ART is commenced. It is therefore important to maintain long-term control of HIV replication and reasonable to consider starting ART earlier than usually would be done in patients over the age of 50.

For those patients who do not experience an increase in the CD4⁺ T-cell count to above 350/μl, several therapeutic strategies to increase CD4⁺ T-cell counts are under investigation. These are recombinant IL-2 to increase counts by enhancing cell proliferation, recombinant human growth hormone to increase thymus size and naïve T cell production, and intensification of ART to reduce immune activation and CD4⁺ T-cell loss. At present, none can be recommended for routine use.

In patients who had a very low CD4⁺ T-cell count before commencing ART and achieve a normal or near normal CD4⁺ T-cell count on ART, CD4⁺ T-cell dysfunction may persist and occasionally be associated with opportunistic infections.[51] Persistent depletion and/or dysfunction of CD4⁺ T cells and memory B cells in HIV patients receiving effective ART may result in impaired antibody responses to vaccines in some patients.

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Chapter 93

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Tuberculosis in HIV

EPIDEMIOLOGY

HIV and TB risk

HIV infection is a potent risk factor for the development of active tuberculosis (TB). This may result from:

- high risk of reactivation of latent *Mycobacterium tuberculosis* infection;
- increased risk of exposure to TB;
- greater risk of infection with *M. tuberculosis* following exposure to an infectious source; and
- progression of *M. tuberculosis* infection to primary active TB.

HIV-seronegative individuals who have latent infection with *M. tuberculosis* have a lifetime risk of developing active TB of approximately 10–15%. Following HIV seroconversion, however, TB risk doubles and progressively increases with advancing immunodeficiency. The overall risk of developing active TB in HIV-infected individuals with latent *M. tuberculosis* infection is approximately 10% per year. However, the risk among patients with AIDS living in communities with high TB burden in sub-Saharan Africa may exceed 30% per year.[1]

HIV-infected patients may be at increased likelihood of exposure to TB at health-care settings,[2] for example, or through social mixing with high-risk groups such as intravenous drug users. However, it is not clear whether for a given exposure their susceptibility to *M. tuberculosis* infection is also increased. Although prospective studies of intravenous drug users in New York, USA, suggested that this was not the case, these data were confounded by the insensitivity of tuberculin skin tests (TSTs) in HIV-infected people.

HIV infection not only increases the risk of reactivation of latent *M. tuberculosis* infection, but also increases the risk of progressive primary disease following exogenous infection. Outbreaks in health-care settings in high-income countries have been characterized by attack rates of 30–40% among HIV-infected patients exposed to TB, with many developing disease within 1–2 months.

HIV and the global burden of TB

Outbreaks of HIV-associated TB – often with multidrug-resistant (MDR) disease – in New York, USA, and other cities in developed countries in the early 1990s heralded the growing recognition that TB was resurgent worldwide. In 1993, the World Health Organization (WHO) declared that TB was a global emergency, with HIV infection being identified as one of the key underlying factors that subsequently fueled a 1% annual increase in global TB incidence between 1990 and 2003.[3]

India is the country with the largest number of new TB cases each year and approximately 5% of these are estimated to be HIV co-infected. However, it is the countries of sub-Saharan Africa that have borne the brunt of the dual TB/HIV epidemics (Fig. 93.1).[4] Here the HIV epidemic is generalized within the population and high HIV prevalence rates have intersected with poor existing TB control. Worldwide it is estimated that one in three people have latent TB infection and yet the proportion in the highest burden communities in sub-Saharan Africa exceeds 60%.

Since 1990, annual TB notification rates have increased two- to threefold in many countries in sub-Saharan Africa. Although Africa contains just 10% of the world's population, it accounts for over a quarter of the global burden of TB and a majority of the world's cases of HIV-associated TB. In 2000, 31% of all adult TB cases in the African region were estimated to be attributable to HIV infection. Countries in the southern African region have been hardest hit and here HIV prevalence among new TB cases exceeds 50% (Fig. 93.2).[5] In some impoverished communities in South Africa HIV prevalence rates among antenatal women are approximately 30% and annual TB notification rates as high as 1500/100 000 population have been recorded.[6]

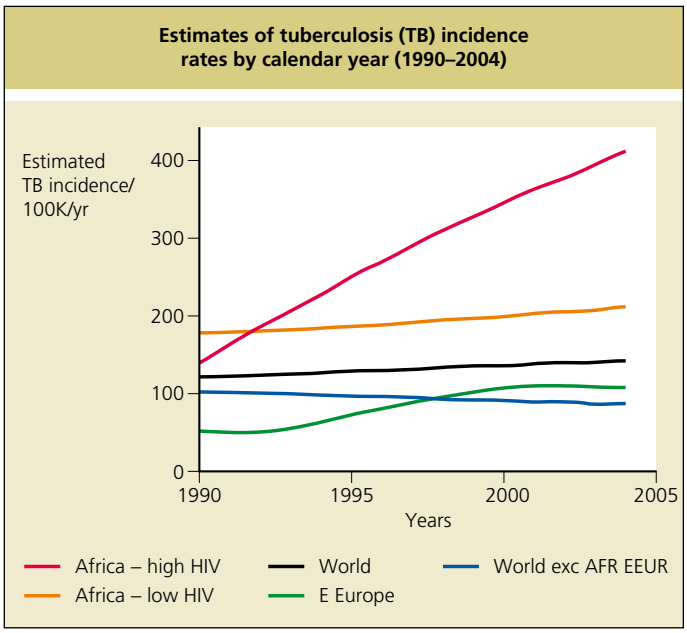


Fig. 93.1 Estimates of tuberculosis (TB) incidence rates (cases/100 000 population) by calendar year between 1990 and 2004. 'Africa – high HIV' refers to all countries in sub-Saharan Africa where HIV prevalence in adults aged 15–49 years was <4% and 'Africa – low HIV' refers to those countries where this rate was >4%. 'World exc AFR EEUR' refers to the world excluding the countries of Africa and Eastern Europe. Reproduced with permission from the World Health Organization.[5]

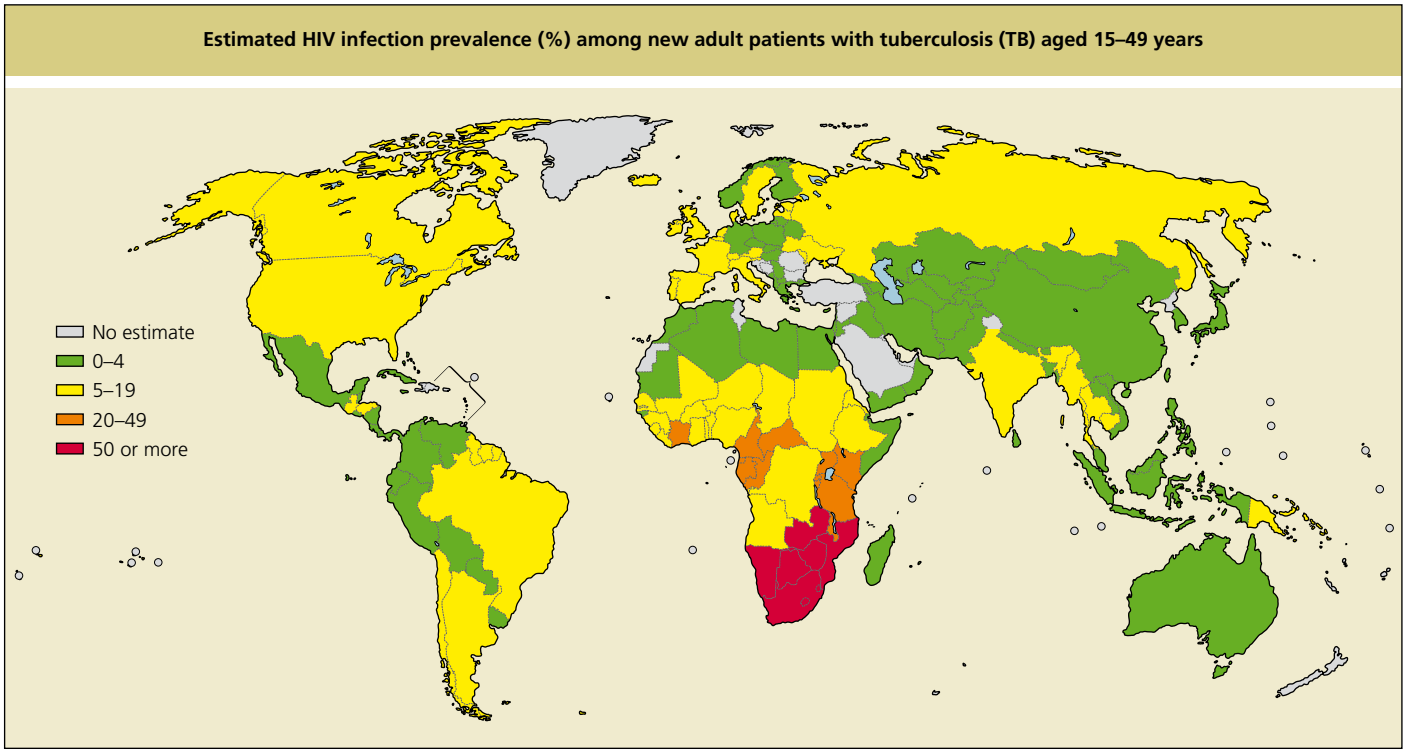


Fig. 93.2 Estimated HIV infection prevalence (%) among new adult patients with tuberculosis (TB) aged 15–49 years. Reproduced with permission from the World Health Organization.[5]

Mortality and TB transmission risk

Patients with HIV-associated TB have high mortality risk. In sub-Saharan Africa in the pre-antiretroviral therapy (ART) era, such patients had case fatality rates that were approximately fivefold higher than HIV-seronegative patients during the course of TB treatment (16–35% versus 4–9%).[7] Data from a randomized placebo-controlled trial in West Africa, however, found that mortality among patients with HIV-associated TB could be halved by trimethoprim–sulfamethoxazole (TMP–SMX) prophylaxis.[8] This suggests that many deaths result from other HIV-associated opportunistic bacterial infections and co-morbidities associated with immunodeficiency. Since TB is more difficult to recognize and diagnose in HIV-infected patients, much of the overall burden of disease may remain unascertained. In a hospital-based post-mortem study of patients in West Africa dying with AIDS, over 50% of patients were found to have previously unrecognized TB, which was often disseminated.[9]

Surprisingly, HIV-infected patients with TB are, on average, actually less infectious than those without HIV co-infection. This is due to the fact that HIV-infected patients are more likely to have either noninfectious extrapulmonary disease or noncavitating pulmonary disease with low concentrations of bacilli in the sputum (Fig. 93.3). In addition, debilitated patients with advanced HIV may have poor cough strength and therefore generate infectious aerosols less efficiently. Nevertheless, TB/HIV patients collectively are an important source of TB transmission at the community level and within health-care settings.[2]

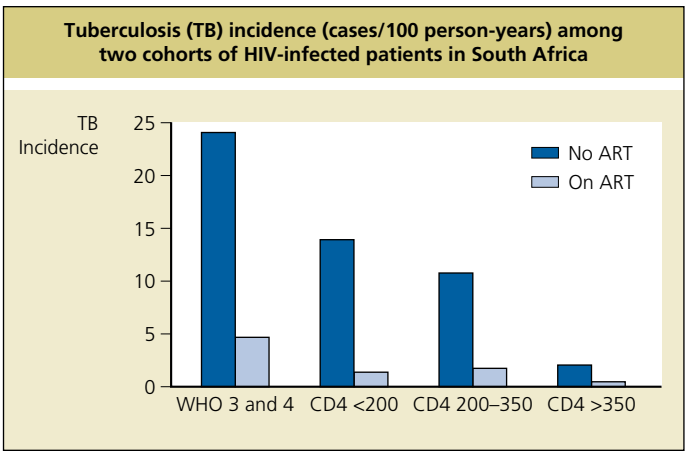


Fig. 93.3 Tuberculosis (TB) incidence (cases/100 person-years) among two cohorts of HIV-infected patients in South Africa who were or were not receiving antiretroviral treatment (ART). Patients are stratified according to baseline CD4 cell count (cells/μl) and World Health Organization clinical stage. Those receiving ART had incidence rates that were approximately 80% lower than the untreated group. Adapted from Badri *et al.*[18]

HIV and multidrug-resistant TB

Multidrug-resistant TB (MDRTB) is caused by strains of *M. tuberculosis* that are resistant to at least isoniazid and rifampin (rifampicin) and has emerged as a global epidemic, with over 400 000 cases developing each year.[10] While this has largely resulted from deficiencies in TB case management and TB control programs, important synergies with the HIV epidemic are emerging. Institutional outbreaks of MDRTB have primarily affected HIV-infected patients with high attack rates and mortality. These outbreaks have been linked to poor infectious control practices in hospitals and prisons where patients with unrecognized TB and those with HIV-infection were mixed and where diagnoses of MDRTB were often delayed.

In Eastern European countries of the former Soviet Union, the epidemic of HIV-associated TB has been associated with very high rates of MDRTB and has been particularly concentrated among intravenous drug users within prisons.[10] In 2005, a large outbreak of MDRTB and extensively drug-resistant (XDR) TB cases was identified among

HIV-infected patients in rural Kwazulu, Natal Province, South Africa.[11] Again, nosocomial transmission played a key role in this outbreak (see Chapter 143 for a detailed description of therapy for MDRTB).

Although many reported TB outbreaks have been associated with the Beijing genotype of *M. tuberculosis*, drug-resistant strains do not appear to be inherently more likely to cause infection and disease in HIV-infected persons.[10] It is possible that TB drug malabsorption in HIV-infected individuals might provide a mechanism favoring the development of drug resistance. In addition, TB services in some areas of sub-Saharan Africa have been overwhelmed by the escalating case load caused by HIV, thereby undermining TB treatment completion rates and cure rates. In the South African outbreak of MDR- and XDRTB, laboratory data suggest that, in the absence of routine drug susceptibility testing, drug pressure from inappropriate treatment regimens led to sequential acquisition of resistance. Emerging data suggest an association between HIV and MDRTB at the population level, but overall most evidence suggests the association is closely related to environmental factors such as transmission in congregate settings rather than biologic factors.[10]

PATHOGENESIS

The immune response to *M. tuberculosis* is multifaceted, engaging several arms of the immune system (see also Chapters 30 and 174). CD4 T-helper cell function is central to the orchestration of cell-mediated responses to *M. tuberculosis*, culminating in the formation of epithelioid granulomas, which restrict growth of the organism. The role of CD4 cells within these processes is fundamentally undermined by HIV-1 infection.[12] In addition, the host response to *M. tuberculosis* at the site of disease provides an immunologic environment that promotes HIV replication and pathogenesis. As a result, the interaction between these diseases is bi-directional.

Impaired immune responses to *Mycobacterium tuberculosis*

Formation of immunologically competent granulomas is essential to the initial containment of *M. tuberculosis* infection and the later prevention of reactivation of latent infection. HIV-1 infection impairs a series of key elements needed to generate this immune response.[12] Antigen presentation, cytokine secretion and intracellular killing of mycobacteria by mononuclear phagocytes may be impaired. Most importantly, HIV infection also leads to numeric depletion and functional impairment of CD4 cells. In particular, HIV inhibits interleukin (IL)-2 signaling, causing impairment of T cell proliferation and activation. In addition, there is loss of secretion of type 1 cytokines such as interferon gamma (IFN-γ) and IL-12 and failure of mononuclear cell recruitment to sites of *M. tuberculosis* infection. Rates of CD4 cell loss are also greatly increased due to virus-mediated cytolysis and activation-induced apoptosis. Impaired chemotaxis, cytokine dysregulation, T-cell depletion and mononuclear cell dysfunction culminate in failure of granuloma formation and the host response to *M. tuberculosis* is thereby undermined.[12]

Impact of tuberculosis on HIV-1 pathogenesis

A substantial body of literature including *in vitro* and *ex vivo* laboratory studies and epidemiologic data collectively suggest that TB may serve as a co-factor that accelerates the rate of immunologic decline in co-infected patients.[13] While the laboratory data are clear, however, the population level impact is debated.[14] Any co-factor effect is thought to be primarily mediated by the impact of TB on HIV replication. *In vitro*, *M. tuberculosis* increases HIV production from chronically infected cell lines, enhances the infectivity of mononuclear cells for HIV and promotes HIV transmission between cells.[15]

Increases in viral load have been documented in patients upon development of TB, especially among those with relatively well preserved CD4 cell counts. Compared to the systemic circulation, HIV load is specifically increased at sites of active TB such as in the pleural space.[13] Development of TB also leads to genotypic diversification of HIV-1, both in the systemic virus pool and at the site of disease. Expression of new or more virulent HIV-1 quasi-species has the potential to permit immune escape, thereby accelerating HIV-1 disease progression.[13]

The effects of TB on HIV progression can be understood in the context of the HIV-1 life cycle, which is intimately related to the activation state of the host cell.[15] Mononuclear cell activation may upregulate expression of chemokine co-receptors such as CCR5 that enhance viral uptake into the cell and may also promote reverse transcription of HIV-1 RNA. Perhaps most importantly, the rate of transcription of proviral DNA within the host cell genome is regulated by sequences within the long terminal repeat (LTR) at the 5′′ end of the HIV-1 genome. Proinflammatory cytokines such as tumor necrosis factor (TNF) lead to activation of receptors in the LTR, causing massive upregulation of HIV-1 transcription.[15,15]

Sites of TB disease and granulomas provide a favorable environment for HIV-1 replication. The host response to *M. tuberculosis* involves the recruitment and activation of large numbers of mononuclear cells and the secretion of TNF and other proinflammatory cytokines. These create a highly permissive environment for HIV, thereby maximizing the deleterious impact of HIV at the interface between *M. tuberculosis* and the host.

PREVENTION

Isoniazid preventive therapy

Many studies from around the world have shown that isoniazid preventive therapy (IPT) is an effective intervention that can substantially reduce risk of TB in HIV-infected patients.[16] A large meta-analysis that included studies with a variety of preventive regimens found an overall risk reduction of 36% in comparison with placebo.[17] The greatest reduction in TB incidence (62%) was observed among patients with a positive TST whereas the reduction among TST-negative individuals (17%) was not statistically significant. Isoniazid alone given for 6–12 months reduced TB incidence by 33% overall and by 63% in those with positive TST results. Multidrug regimens given for 2–3 months were as effective as isoniazid alone for 6–12 months. IPT has also been shown to be effective in reducing high TB recurrence rates following completion of TB treatment (secondary prophylaxis) in high TB prevalence settings where rates of exogenous re-infection are likely to be high.[16]

The durability of the benefits of IPT is unclear but is likely to be strongly dependent upon the risk of re-infection with *M. tuberculosis* following completion of therapy and may also be affected by the rate of HIV progression. The benefits are likely to be shorter lived in high TB prevalence settings; data from Zambia found a sustained benefit for approximately 2.5 years.

Overall, a strong trend towards a reduction in mortality has been observed with the use of preventive therapy (RR = 0.8, 95% CI 0.63–1.02). Significant reductions in mortality have been observed in a study of HIV-infected children in South Africa and in studies of combined isoniazid and rifampin in adults.[16] Rates of hepatotoxicity are overall very low but are higher for regimens using a combination of rifampin and pyrazinamide.

Although IPT is an effective intervention, it has not been widely implemented in resource-limited settings for several reasons:

- limited health-care capacity to deliver this intervention;
- difficulties in excluding active TB prior to initiating IPT;
- associated concerns about development of isoniazid resistance; and
- limited patient adherence under program conditions.

Although patients with advanced immunodeficiency potentially have the most to gain from IPT, they also present a challenge as they are the patients in whom it is most difficult to exclude active TB prior to initiation of IPT. Ongoing research is needed to overcome these hurdles so that the benefits of this intervention can be realized. In addition, randomized controlled trials are needed to determine whether IPT used concurrently with ART reduces TB rates more than ART alone.

Antiretroviral therapy

Cohort studies in both high-income and resource-limited settings have shown reduction in TB incidence of approximately 80–90% with ART in the short term (Fig. 93.4);[18,19] further small reductions may accrue during the first few years of ART. ART is therefore the most effective available intervention for reducing TB risk in HIV-infected patients. However, during long-term therapy, TB risk does not decrease to background rates observed in HIV-negative individuals living in the same communities; in South Africa, rates remained approximately fivefold higher despite excellent long-term virologic responses.[20]

It is as yet unclear whether incident TB results from a persisting high rate of latent *M. tuberculosis* infection, high rates of nosocomial exposure or frequent exogenous re-infection in the community. However, since incident TB developing during long-term ART often occurs in the context of rising CD4 cell counts, recent exogenous exposure is perhaps a more likely infection source than reactivation disease. Ongoing risk is largely determined by a patient's absolute CD4 cell count during ART.[20] Some evidence also suggests that underlying specific defects in immune responses to *M. tuberculosis* may persist during long-term ART.[19] Such defects may be greater for those patients with the lowest pre-ART nadir CD4 cell counts, potentially highlighting the importance of earlier initiation of ART.

Infection control

The dangers of TB transmission within health facilities have long been recognized.[2] In 1928, for example, 95% of 220 student nurses in Norway acquired TB infection by their graduation as shown by TST conversion and 22% developed TB disease. Transmission risk decreased dramatically in the era of TB chemotherapy administered on an ambulatory basis rather than in hospitals. However, in the 1990s a global resurgence of TB was heralded by many reports of nosocomial outbreaks of TB (mostly MDRTB) in HIV care settings in the USA and elsewhere. In more recent years, rapid expansion of ART in resource-limited countries with high TB burden has paradoxically created unprecedented opportunities for HIV-infected patients and health-care personnel to be exposed to TB in health-care settings.[2] This was most vividly highlighted by an outbreak of XDRTB at a hospital in rural South Africa first reported in 2005.[11] By 2008, over 200 patients had died, including some hospital staff.

Infection control within health-care settings requires a tiered approach of:

- administrative controls, leading to prompt identification and separation of individuals with potentially infectious TB;
- environmental controls, such as adequate natural or mechanical ventilation or ultraviolet light germicidal irradiation; and
- respiratory protection using a face mask or respirator.[2]

How to implement infection control cost-effectively within health-care facilities in resource-limited settings is an urgent research priority.

TB control in settings with high TB/HIV prevalence

The WHO DOTS (directly observed therapy, short course) strategy is regarded as having contributed to decreases in TB notification rates in many areas of the world. However, in sub-Saharan Africa where HIV prevalence rates are high, DOTS has failed to control the TB epidemic, even in those countries such as Malawi and Tanzania which have long-standing, well-functioning, national TB control programs.

The DOTS strategy targets those with sputum smear-positive pulmonary disease who are likely to transmit TB most efficiently. DOTS, however, does not reduce the number of individuals who remain highly susceptible to TB in the community and herein lies a

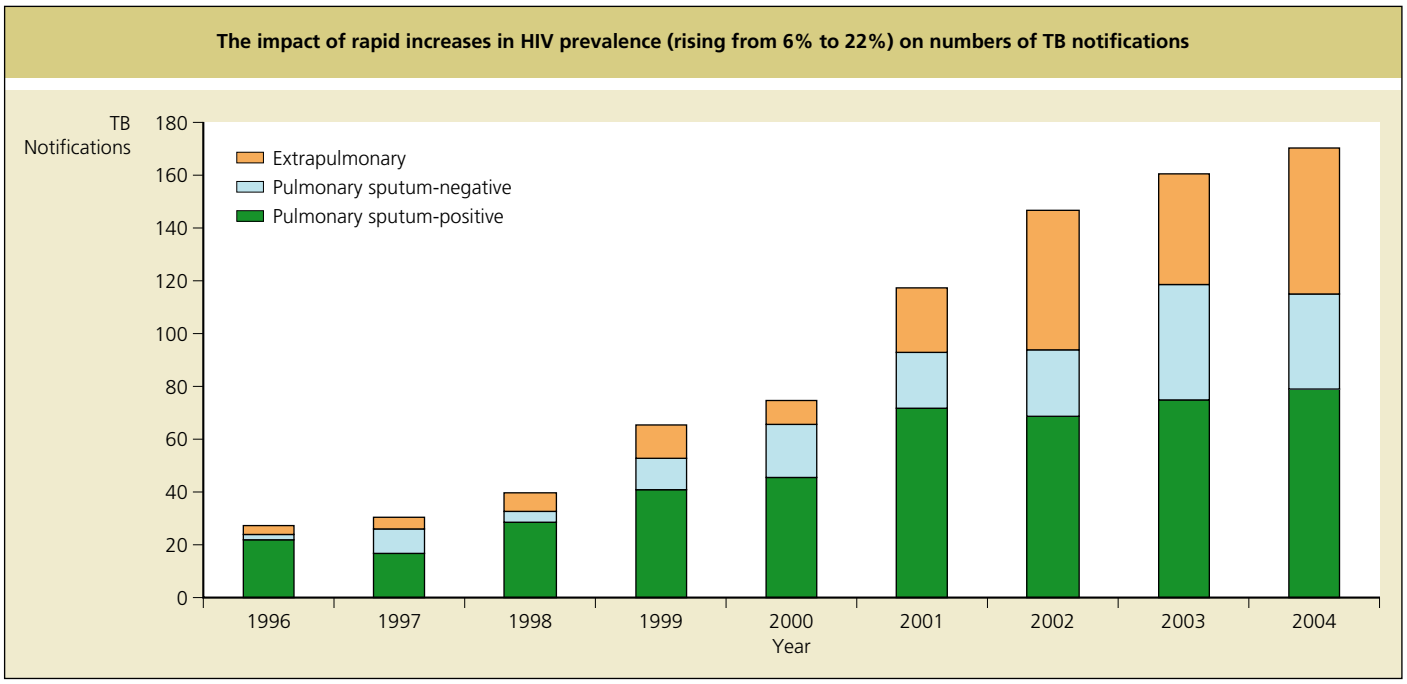


Fig. 93.4 The impact of rapid increases in HIV prevalence (rising from 6% to 22%) on numbers of TB notifications among adults in a South African community of 13 000 people between 1996 and 2004. Case numbers increased steeply as the HIV epidemic evolved, with disproportionate increases in sputum smear-negative pulmonary TB and extrapulmonary TB. Adapted from Lawn *et al.*[6]

key underlying reason for the failure of DOTS as a single intervention to stem this epidemic in sub-Saharan Africa.[21] DOTS programs must clearly be strengthened and optimized and yet this strategy must also be accompanied by other adjunctive interventions. These might include intensified TB case-finding in the community, more widespread use of HIV testing, targeted active case-finding among HIV-infected individuals, implementation of isoniazid prophylaxis and expansion of ART access. However, how these interventions can be implemented programmatically on a large scale is not clear and optimal models need to be developed.

The potential impact of scale-up of ART in sub-Saharan Africa on TB control has yet to be determined. However, since ART greatly prolongs patient survival but the long-term risk of ART remains chronically elevated (more than fivefold greater than that of HIV-seronegative people), ART is unlikely to contribute substantially to TB control.[20,22] It is therefore inevitable that combinations of interventions will be required.

CLINICAL FEATURES

M. tuberculosis causes disease across the full spectrum of HIV-associated immunodeficiency and the impact of HIV on the clinical manifestations of TB is directly related to the degree of immunodeficiency.[23] The median CD4 cell count at presentation appears to vary greatly between published series and may depend on the specific patient population and the maturity of the HIV epidemic. In a report from San Francisco in 1990, for example, the median CD4 cell count at TB presentation was 326 cells/μl, compared to <200 cells/μl in countries of southern Africa in more recent times. TB is commonly the first clinical manifestation of HIV infection, highlighting the great importance of HIV testing of all TB patients.

The spectrum of clinicopathologic manifestations of TB directly relate to blood CD4 cell counts.[23] Features of TB in individuals with well-preserved CD4 cell counts are indistinguishable from those of HIV-seronegative individuals with TB. Progressive immunodeficiency is associated with an increasing frequency of anergic TST responses, atypical presentations of pulmonary TB and sputum smear-negative disease, and an increased frequency of extrapulmonary, miliary and disseminated disease (see Fig. 93.4).

Irrespective of the site of TB, a majority of patients have systemic manifestations with fever, night sweats or weight loss. Unexplained systemic symptoms such as these in the absence of organ-specific symptoms are a not uncommon presentation of TB. In studies in Asia and sub-Saharan Africa, in which HIV-infected patient populations have been actively investigated for TB, substantial rates of subclinical TB have been documented. Although cough, for example, is strongly predictive of TB among selected TB suspects, used as a single screening symptom in active screening fails to identify approximately 20–30% of cases of pulmonary TB in HIV-infected patients.

Pulmonary disease is the major manifestation of TB in both HIV-infected and noninfected patients. Symptoms and clinical findings, however, generally have low sensitivity and specificity for diagnosis of pulmonary TB in the HIV-infected patient. Radiographic appearances of pulmonary TB with HIV co-infection reveal a lower frequency of upper lobe disease, cavitation, parenchymal opacification and fibrosis, but an increased frequency of mediastinal lymphadenopathy, small pleural effusions and miliary disease.[24] Normal radiographs may be observed in a proportion (0–30%) of patients with sputum culture-positive disease. The spectrum of appearances correlates strongly with the degree of immunodeficiency; those with high CD4 cell counts usually have a typical pattern of postprimary disease whereas those with low CD4 cell counts have a higher proportion with a primary disease pattern.

Hematogenous and lymphatic dissemination of mycobacteria in HIV-infected patients increases the risk of extrapulmonary TB. This is strongly related to the degree of immunodeficiency, with the risk particularly increasing as the CD4 cell count falls below 200 cells/μl. The relative frequency of reported extrapulmonary disease also relates to the intensity of the available diagnostic workup. Autopsy specimens from patients who died of TB and AIDS show a high frequency of extrapulmonary and disseminated disease with multiorgan involvement. Moreover, occult disseminated TB is a frequent finding in postmortem studies of people from sub-Saharan Africa who died with AIDS or 'slim disease'.[9]

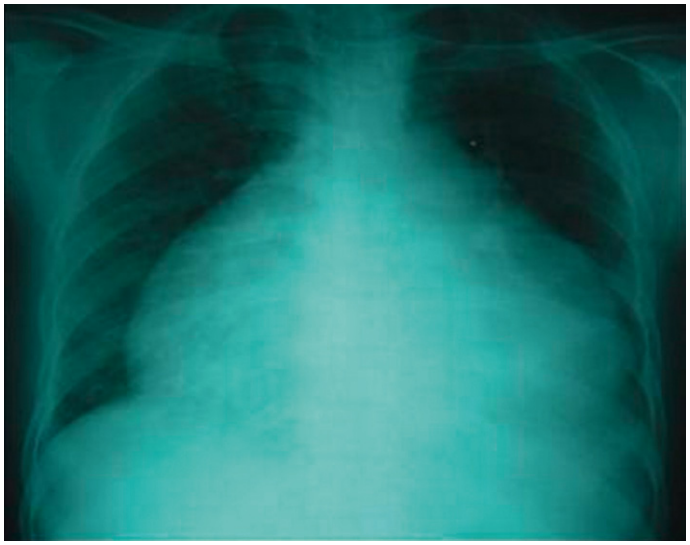


Fig. 93.5 Chest radiograph of a South African patient with HIV infection and a large tuberculous pericardial effusion. Image kindly supplied by Mpiko Ntsekhe, Division of Cardiology, Department of Medicine, Groote Schuur Hospital, University of Cape Town.

Lymphadenitis is the most common form of extrapulmonary TB; cervical nodes are most frequently involved, but disease also often occurs in inguinal, mesenteric and mediastinal nodes. Miliary radiographic shadowing has been reported in up to 38% of AIDS patients with extrapulmonary TB and typically presents with marked constitutional symptoms. HIV infection increases the risk of TB meningitis several-fold, but does not substantially alter the clinical and laboratory manifestations.

Pleural effusions were detected radiographically in one-fifth of a series of consecutive TB/HIV patients in South Africa and were more common than disseminated or miliary TB in patients with higher CD4 cell counts. Pericardial TB is strongly associated with HIV co-infection (Fig. 93.5) and is associated with a pleural effusion in up to approximately one-third of cases.

TB may involve any part of the gastrointestinal tract due to either ingestion of infected sputum or hematogenous dissemination. Whereas mediastinal adenopathy is frequently detected by thoracic radiologic imaging, mesenteric TB lymphadenitis may remain undiagnosed until initiation of ART triggers the clinical presentation as TB immune restoration disease (see also Chapter 92). Development of abscesses in the liver, spleen and other intra-abdominal organs is also a frequent finding.

DIAGNOSIS

The increased likelihood of atypical clinical presentations and subclinical TB requires a high clinical index of suspicion for TB in the HIV-infected patient. All current diagnostic tests for TB, however, have limited performance characteristics when applied to HIV-infected patients.[25] As described above, the frequency of atypical radiologic appearances of pulmonary TB increases with declining immune function.[24] Histopathologic examination of tissue specimens is similarly less likely to show granulomatous inflammation typical of TB.[26] Diagnosis is highly dependent upon obtaining multiple good quality clinical samples for laboratory analyses, which must be interpreted in the light of their known limited sensitivity.

Detection of acid-fast bacilli by light microscopy has been the cornerstone of TB diagnosis worldwide since Koch's discovery of *M. tuberculosis* in 1882. However, the average sensitivity of sputum microscopy in research settings is <60% in immunocompetent patients with pulmonary TB and is reduced to 20–50% in HIV-infected patients due to lower concentrations of bacilli in the sputum. This may be improved to a degree by use of fluorescence staining. Whereas positive sputum smears have low specificity in industrialized countries, they have good specificity in settings such as Africa where TB is far more common than nontuberculous mycobacterial disease.

Mycobacterial culture in selective media remains the most sensitive means of detecting *M. tuberculosis* bacilli in clinical specimens. This is the gold standard for diagnosis but is not available in most resource-limited regions of the world.[25] Although patients with advanced immunodeficiency may have high mycobacterial loads, bacillary numbers in sputum nevertheless tend to be low. As a result, the high sensitivity of culture diagnosis is particularly important for analysis of sputum specimens and, furthermore, culture of other specimens such as urine, blood or bone marrow may also have a useful diagnostic yield. Blood cultures have been found to be positive in up to one-third of patients with advanced immunodeficiency and extrapulmonary TB.

At present there are no serologic or antigen detection assays that offer a means of improving the diagnosis of HIV-associated TB. However, nucleic acid amplification tests (NAATs) for *M. tuberculosis* have provided a significant advance and offer the prospect of far more rapid diagnosis and rapid genotypic assessment of drug susceptibility.[25] These tests are increasingly used in high-income settings and countries with centralized laboratory services (see Chapter 174). However, their cost and complexity are currently beyond the scope of most resource-limited countries. NAATs have extremely high specificity (98–100%) and their sensitivity in sputum smear-positive specimens is also extremely high (>95%), permitting rapid diagnosis. However, their sensitivity does not yet match that of liquid culture in smear-negative specimens (60–70%), although new generations of tests are improving.

TSTs have limited utility in the investigation of the HIV-infected patient. Cutaneous anergy to purified protein derivative (PPD) has been observed in more than 50% of North American and African patients with TB and advanced immunodeficiency. In high TB prevalence settings, TSTs have some role in TB diagnosis in children but not in adults due to poor specificity for active disease. In low TB prevalence settings, use of TSTs is increasingly being superseded by the use of *in vitro* IFN-γ release assays (IGRAs). Responses assessed by the enzyme-linked immunospot (ELISpot) assay in particular appear to be preserved with HIV and advanced immunodeficiency. Positive IGRA responses support a diagnosis of either latent *M. tuberculosis* infection or active TB. However, the utility of these assays in clinical practice has yet to emerge fully.

MANAGEMENT

Patients with HIV-associated TB require optimized antituberculosis treatment and TMP–SMX prophylaxis, and should be considered for antiretroviral therapy.[27]

TB treatment

The key tenets of TB treatment in HIV-infected patients are:

- the need for a rifampin (or alternative rifamycin)-containing intensive phase administered on a daily basis to optimize smear and culture conversion rates;
- the use of rifampin throughout the continuation phase wherever possible to minimize treatment failure and risk of relapse; and
- administration of the continuation phase at least thrice weekly to reduce the risks of development of rifampin resistance and relapse.[27,28]

Early in the HIV epidemic, it was thought that patients with HIV-associated TB had similar responses to antituberculosis treatment. However, TB recurrence rates are often higher than the rate of <5% typically observed in HIV-seronegative TB patients.[29] This may be due to either incomplete clearance of the infection and relapse or new disease resulting from high rates of exogenous re-infection. Recurrence rates may primarily be reduced by optimizing the TB treatment regimen and using ART. Additional strategies might include extending the duration of TB treatment (some guidelines suggest increasing the duration of treatment from 6 to 9 months in patients who have evidence of slow response to treatment) or the use of secondary isoniazid prophylaxis after completion of TB treatment in settings where risk of exogenous re-infection is high.[30]

Trimethoprim–sulfamethoxazole prophylaxis

TMP–SMX has been widely used in developed countries for prophylaxis of HIV-infected patients against *Pneumocystis jirovecii* pneumonia, *Toxoplasma gondii* encephalitis and bacterial sepsis. However, this was little used in resource-limited settings until a randomized placebo-controlled trial in Cote D'Ivoire in 1999 showed a 46% lower mortality rate among HIV-infected TB patients receiving TMP–SMX.[8] Further studies demonstrated that prophylaxis in TB/HIV co-infected patients increased survival by 60% and that this survival benefit continues during ART. Despite WHO recommendations, routine TMP–SMX prophylaxis has remained at a low level in sub-Saharan Africa and other resource-limited settings. Concerns over emerging resistance to this drug are not well founded since survival benefit has been demonstrated in countries even with high rates of drug resistance.[27]

Antiretroviral therapy

Benefits of ART

Development of combination ART in the mid-1990s has transformed HIV/AIDS from a fatal infectious disease into one that is potentially chronic and manageable. Early reports of combining TB treatment with ART in industrialized countries reported high rates of adverse effects and treatment switching. However, as experience has grown and the range of antiretroviral agents has expanded, good outcomes of concurrent treatment for TB and HIV are now achievable.[30]

The benefits of ART for TB/HIV patients are great. Mortality risk is substantially reduced, with a 20-fold lower adjusted hazard risk of death being reported among Thai TB patients with CD4 cell counts around 50 cells/μl at baseline. Observational data from the USA suggest that mean times to conversion of sputum smears and cultures are shortened. Since ART also decreases the incidence of TB by around 80–90%, it is very likely that ART will also greatly reduce TB recurrence rates. The full benefits of ART, however, have yet to be realized fully as a number of issues concerning concurrent administration of TB treatment with ART are still to be resolved and optimal drug regimens are lacking in resource-limited regions of the world.[30]

Pharmacokinetic interactions

Pharmacokinetic interactions between rifamycins (rifampin, rifapentine and rifabutin), non-nucleoside reverse transcriptase inhibitors (NNRTIs) and protease inhibitors (PIs) are mediated by the complex effects of these drug classes on the hepatic cytochrome P450 (CYP450) enzyme system. NNRTIs and PIs are substrates for these enzymes but also act as inducers or inhibitors.[31] In contrast, the rifamycins act only as potent inducers of this enzyme system, with rifampin having the greatest effect and rifabutin the least. They therefore cause reductions in plasma concentrations of the NNRTIs nevirapine and efavirenz by 30–40% and 20–25%, respectively.[31] In addition, with the exception of ritonavir, plasma concentrations of PIs are reduced by approximately 80% due to enhanced hepatic metabolism and increased activity of the efflux multidrug transporter P-glycoprotein.[31]

Various TB and ART regimens can be successfully combined (Table 93.1). Despite reductions in concentrations of NNRTIs, excellent

Table 93.1 Recommended regimens for the concurrent treatment of tuberculosis and HIV infection

Combined regimen for treatment of HIV andtuberculosis	PK effect of rifamycin	Tolerability/toxicity	Antiviral activity when used with rifampin (rifampicin)	Recommendation (comments)
Efavirenz-based antiretroviral therapy* with rifampin-based TB treatment	Well-characterized, modest effect	Low rates of discontinuation	Excellent	Preferred (efavirenz should not be used during the first trimester of pregnancy)
Protease inhibitor (PI)-based antiretroviral therapy* with rifabutin-based TB treatment	Little effect of rifabutin on PI concentrations, but marked increases in rifabutin concentrations	Low rates of discontinuation (if rifabutin is appropriately dose-reduced)	Favorable, though published clinical experience is not extensive	Preferred for patients unable to take efavirenz†
Nevirapine-based antiretroviral therapy with rifampin-based TB treatment	Moderate effect	Concern about hepatotoxicity when used with isoniazid, rifampin and pyrazinamide	Favorable	Alternative for patients who cannot take efavirenz and if rifabutin not available
Zidovudine/lamivudine/abacavir/tenofovir with rifampin-based TB treatment	50% decrease in zidovudine, possible effect on abacavir not evaluated	Anemia	No published clinical experience	Alternative for patients who cannot take efavirenz and if rifabutin not available
Zidovudine/lamivudine/tenofovir with rifampin-based TB treatment	50% decrease in zidovudine, no other effects predicted	Anemia	Favorable, but not evaluated in a randomized trial	Alternative for patients who cannot take efavirenz and if rifabutin not available
Zidovudine/lamivudine/abacavir with rifampin-based TB treatment	50% decrease in zidovudine, possible effect on abacavir not evaluated	Anemia	Early favorable experience, but this combination is less effective than efavirenz-based regimens in persons not taking rifampin	Alternative for patients who cannot take efavirenz and if rifabutin not available
Super-boosted lopinavir-based antiretroviral therapy with rifampin-based TB treatment	Little effect	Hepatitis among healthy adults, but favorable experience, among young children (<3 years)	Good among young children (<3 years)	Alternative if rifabutin not available; preferred for young children when rifabutin not available

* With two nucleoside analogues.
† Includes patients with non-nucleoside reverse transcriptase inhibitor-resistant HIV, those unable to tolerate efavirenz, women during the first or second trimester of pregnancy.
Reproduced with permission from Centers for Disease Control and Prevention.[31]

virologic outcomes have been reported using the standard 600 mg dose of efavirenz in combination with rifampin, and this is the first-choice recommendation in international guidelines.[32] Outcomes of nevirapine therapy may be undermined if the usual 200 mg per day lead-in dose is used in patients already receiving rifampin; preinduction of hepatic enzymes may reduce drug concentrations to subtherapeutic levels. Increased doses of nevirapine, however, are associated with greater toxicity and current WHO recommendations are to use NNRTIs at standard doses when combined with rifampin.[32] A useful alternative strategy is to use rifabutin which has less impact on the metabolism of NNRTIs.[31] This agent, however, is not widely available in resource-limited settings.

Protease inhibitors do not achieve adequate plasma concentrations when combined with rifampin except when lopinavir or saquinavir is used in combination with ritonavir. However, this frequently results in hepatotoxicity and there is substantial interpatient variability in plasma concentrations, requiring therapeutic drug monitoring wherever possible.[31] A much better alternative is to use rifabutin, which is an effective alternative to rifampin that can be combined with most protease inhibitors.

Immune restoration disease

TB treatment in HIV-negative patients may be associated with a transient worsening of clinical disease (paradoxical reactions) within the first weeks of initiation of therapy. However, the frequency of these reactions is greatly increased by concurrent use of ART, and these demonstrate a strong temporal relationship with ART initiation. These immune restoration disease (IRD) events are believed to be due to the restoration of cell-mediated immunity in response to mycobacterial antigens.[33] IRD has been reported to occur in up to one-third of TB patients initiating ART in high-income settings, and most commonly presents with fever, worsening respiratory manifestations and lymphadenopathy (Fig. 93.6).[33] Initial reports from resource-poor countries to date have found lower rates.

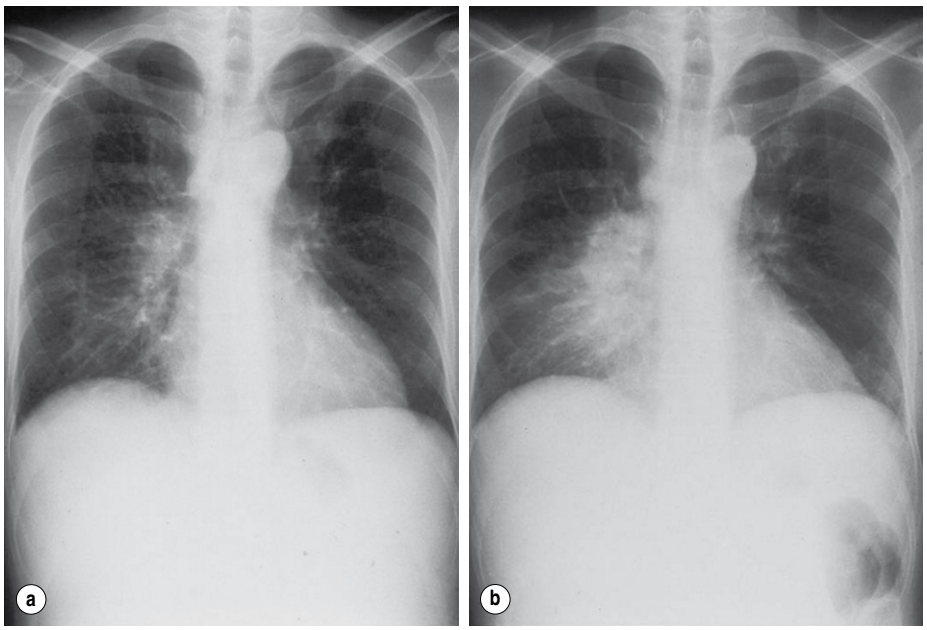


Fig. 93.6 Chest radiographs illustrating tuberculosis (TB) immune restoration disease (IRD). This HIV-infected patient with advanced immunodeficiency had culture-confirmed drug-sensitive TB involving the right middle lobe of the lung and right hilar lymph nodes. This responded well to TB treatment with substantial resolution of the radiographic appearance over the first few weeks of treatment (a). Subsequent initiation of antiretroviral therapy resulted in a clinical and radiologic deterioration (b) and the tuberculin skin test converted from negative to strongly positive. Reproduced with permission from Lawn & French.[35]

In a study from South Africa, the timing of ART initiation was the overriding determinant of risk for TB-IRD; patients starting in the first month of anti-TB treatment had a 70-fold greater adjusted risk of developing IRD compared with patients starting ART beyond 3 months of treatment. Other risk factors for IRD include low baseline CD4-cell count, disseminated/extrapulmonary TB and rapid immunologic and virologic responses to ART.[33] Although some patients with TB-IRD die, the background mortality risk of these patients is also high as they typically have very advanced immunodeficiency and disseminated TB. No data have yet shown that development of TB-IRD is associated with a substantially increased mortality risk.

Results from randomized controlled trials are needed to determine the optimal management of TB-IRD. Symptoms and signs of TB-IRD can usually be controlled by nonsteroidal anti-inflammatory drugs or by use of corticosteroids in more severe cases. Doses of corticosteroid must be adjusted for rifampin interaction and tapered according to response. ART should be continued unless severe or life-threatening IRD develops because of the ongoing risk of immunodeficiency-associated mortality.

Optimal timing of ART initiation

The optimal time to initiate ART among patients with HIV-associated TB is an important but complicated issue. Expert opinion and various national and international guidelines differ in their recommendations, and randomized controlled trials are needed to provide definitive data. Many factors favor either early or delayed initiation of ART (Table 93.2). In resource-limited settings, the mortality associated with delays in ART initiation appears to be substantially greater than in high-income countries. As a result, the need for early initiation of ART may be greater in resource-limited settings. WHO currently recommends that TB patients with CD4 cell counts <200 cells/μl should start ART between 2 and 8 weeks of TB treatment.[32] Observational data from South Africa suggest that those with CD4 cell counts <100 cells/μl or stage 4 disease should commence ART as early as possible within this time frame.

Drug-resistant tuberculosis

MDRTB requires the use of a combination of a minimum of four effective drugs and XDRTB requires the use of as many active drugs as can be achieved.[34] Mortality rates in HIV-infected and MDR- or XDRTB patients are very high, even in specialized management programs. ART should improve the prognosis of such patients, although there are no data to confirm this. Due to slow clearance of mycobacterial antigen load, such patients may be at greater risk of developing TB-IRD compared to those with drug-sensitive TB. Second line anti-TB drugs are associated with considerable adverse effects that overlap with those of ART drugs. However, the absence of rifampin from the regimens does simplify the issue to some extent. In view of the high mortality risk, delays in initiation of ART should probably be minimized in those with MDRTB.

Table 93.2 Factors favoring either early initiation of antiretroviral therapy (ART) (during intensive phase of TB therapy) or delayed initiation (during continuation phase)

Favors early initiation of ART	Favors delayed initiation of ART
• Risk of further morbidity associated with advanced immunodeficiency • Risk of mortality associated with advanced immunodeficiency • Immune recovery may enhance mycobacterial clearance with the potential to shorten sputum conversion times and reduce risk of relapse	• Avoids high pill burden during intensive phase of TB therapy, potentially improving tolerability and adherence • Easier to diagnose and manage drug toxicities and risk of toxicity may be decreased • Avoids pharmacokinetic drug interactions during intensive phase of TB therapy • Lower risk of TB immune restoration disease

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Chapter 94

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Neoplastic disease

INTRODUCTION

Widespread access to highly active antiretroviral therapy (HAART) has significantly changed the spectrum of HIV-related malignancies in the industrialized countries. Among AIDS-defining cancers, Kaposi's sarcoma (KS) and non-Hodgkin's lymphoma (NHL) incidences have significantly decreased in the HAART era. The risk of invasive cervical cancer, however, has remained stable over time. The overall risk of certain non-AIDS defining cancers in HIV-infected persons is two to three times that in the general population, with no beneficial effect from HAART. Hodgkin's lymphoma is the most common non-AIDS defining cancer, with standardized incidence ratios ranging from 8 to 16, followed by cancer of the anus, lung, liver and skin.[1–5]

Malignant disease has now become a major cause of death among HIV-infected patients. Whereas KS and NHL are associated with advanced immunosuppression, Burkitt's lymphomas, Hodgkin's disease (HD) and solid tumors can occur in patients with controlled HIV infection.[6–8]

In this chapter, we will describe the epidemiology, pathology, clinical features and treatment of the two most common malignant tumors in patients with HIV infection: KS and NHL.

Cervical cancer which is related to human papillomavirus (HPV) infection (see Chapter 59) should be anticipated because of the high prevalence of HPV infection and persistence among HIV-infected women. Cervical cancer screening should be considered a must in the HAART era.

Kaposi's Sarcoma

EPIDEMIOLOGY

In industrialized countries, decline in KS incidence began in the early 1990s, but risk fell sharply in 1996 after the introduction of HAART. Among adults with AIDS, the risk for KS has currently decreased by approximately 90% compared with the pre-HAART era.[1–4] Among patients receiving HAART, there continues to be an increased risk for homosexual men and for patients with low CD4 cell counts.[8] Immune restoration induced by protease inhibitor (PI)-based and non-nucleoside reverse transcriptase inhibitor (NNRTI)-based HAART regimens appears to be equally effective in preventing KS.[9,10] However, patients with AIDS remain at significant risk compared with the general population.[1–4] The standardized incidence ratio (SIR) ranges from 3600 in the United States to 1749 in Europe.[1–4,10] The geographic distribution of KS closely reflects the prevalence of sexually acquired HIV infection worldwide. KS tends to be much higher in countries where most individuals with AIDS are homosexual than, for instance, in Italy and Spain where intravenous drug users account for the majority of cases. In contrast, in Africa, where HAART is not readily available, KS has reached epidemic proportions.

PATHOLOGY AND PATHOGENESIS

KS is a multifocal angioproliferative disease characterized histologically by neoangiogenesis and proliferating spindle-shaped cells, admixed with a variable chronic inflammatory infiltrate.[11] Spindle tumor cells express many endothelial markers and human herpesvirus 8 (HHV-8) can infect endothelial cells and induce KS-like spindle cell morphology *in vitro*. It has recently been shown that circulating endothelial precursor cells in patients with KS have evidence of HHV-8 infection. HHV-8, a member of the transforming family of gamma herpes viruses, has been found in all forms of KS and in two lymphoproliferative diseases: primary effusion lymphoma (PEL) and multicentric Castleman's disease. The virus encodes a number of genes homologous to human genes involved in cell proliferation, anti-apoptosis, angiogenesis and cytokine action. Multiple signal transduction pathways are activated by HHV-8 and both latent and lytic viral replicative cycles contribute significantly – but differently – to KS development (Table 94.1).

Extensive evidence indicates that HIV trans-activating protein Tat plays an oncogenic role in the development of KS. Tat promotes tumorigenesis of endothelial cells via stimulation of vascular endothelial growth factors, anti-apoptotic activity and HHV-8 replication.[11,12] A model of KS pathogenesis involves exposure to HHV-8, alteration in cytokine expression and response to cytokine, modulation of growth by HIV Tat and, finally, malignant transformation.

CLINICAL FEATURES

KS ranges from an indolent to an aggressive disease with significant morbidity and mortality. However, KS exhibits a less aggressive presentation in patients already receiving HAART compared with patients who are naive to HAART at KS diagnosis.[13]

Typically, the disease presents with disseminated skin lesions, often with lymph node and visceral involvement such as the gastrointestinal tract and lungs. Skin lesions arise as macular or papular eruptions, which progress to nodular plaques or lesions (Fig. 94.1); any area of the skin may be involved. Nodular KS does not usually cause necrosis of overlying skin and rarely invades underlying bone structure.

Lymphedema, particularly of the face, genitalia and lower extremities, may be out of proportion to the cutaneous disease and may be related to lymphatic obstruction and the expression of cytokines involved in the pathogenesis of KS. Lymphadenopathic KS primarily affects peripheral

Table 94.1 Major HHV-8 genes associated with tumorigenesis

Genes	Major functions
Latent genes	
LANA	Maintenance viral latency, interaction with p53 and pRB
V-Cyclin	Cell cycle progression
vFLIP	Inhibition of Fas-induced apoptosis Activation of nuclear factor kappa B (NF-κB) pathways
$VIRF_1$	Inhibition of interferon-induced gene expression Activation of myc Interaction with p53
Kaposin (K12)	Interaction with MAPK signaling pathway
Lytic genes	
VGPCR	Homologue of cellular interleukin (IL)-8 receptor Stimulation of MAPK pathway and VEGF secretion
vIL6	Homologue of cellular IL-6 Cell cycle progression Anti-apoptotic action
K4, 4.1, 6	Homologue of cellular chemokines (i.e. RANTES, MIP-1) Activation angiogenesis

K, genes unique to HHV-8; LANA, viral latency-associated nuclear antigen; MAPK, mitogen-activated protein kinase; VEGF, vascular endothelial growth factor; vFLIP, viral Fas-associated death domain-like interleukin 1β-converting enzyme; VGPCR, viral G protein-coupled receptor; VIRF, viral interferon regulatory factor.



Fig. 94.1 Kaposi's sarcoma. There are large confluent hyperpigmented patch-stage lesions with lymphedema.

lymph nodes, sometimes causing massive nodal enlargement, and it may be present in the absence of mucocutaneous disease.

Oral cavity KS occurs in approximately 35% of patients and is the initial site of disease in about 15%. Intraoral lesions most commonly affect the palate and gingiva, and may interfere with nutrition and speech.

Over 50% of patients with skin disease have gastrointestinal lesions. Any segment of gastrointestinal tract may be involved, although the stomach and duodenum are most commonly affected. Gastrointestinal KS is seldom symptomatic, but may cause bowel malabsorption or obstruction, and, rarely, bleeding.

Pulmonary involvement is also quite common and may be life threatening. In approximately 20% of cases it may occur in the absence of skin lesions. The symptoms, including shortness of breath, fever, cough, hemoptysis and chest pain, and radiologic appearance of pulmonary KS are indistinguishable from those of the more common opportunistic infections. Radiographic findings vary greatly and can include nodular, interstitial and alveolar infiltrates, pleural effusion, hilar and mediastinal adenopathy, and even an isolated pulmonary nodule. The pleural effusions of KS are typically serosanguinous in nature and are associated with KS lesions on the visceral pleura.

DIAGNOSIS

Although a presumptive diagnosis of KS can often be made readily by a trained observer, a simple skin biopsy can confirm the diagnosis. It is especially important to biopsy lesions that are less typical of KS because other conditions, such as bacillary angiomatosis, may be confused with KS.

Occult blood testing is an excellent screening method for gastrointestinal tract lesions and endoscopy should be reserved for patients with positive test and/or gastrointestinal symptoms. Lesions in the gastrointestinal tract are often recognized easily on endoscopy; however, because the lesions tend to be submucosal, biopsies may not demonstrate KS.

Bronchoscopy is the procedure of choice for pulmonary KS, but gallium–thallium scanning may also be helpful in evaluating an abnormal radiograph. Kaposi's sarcoma is usually thallium avid and gallium negative, whereas infections are usually gallium avid and thallium negative.

The AIDS Clinical Trial Group (ACTG) classification groups patients according to the extent of the tumor, immune status and severity of systemic illness[14] (Table 94.2). A re-evaluation of the TIS staging system during the HAART era suggested that only the high tumor burden (T_1) and the poor systemic disease status (S_1) could identify patients with a poor prognosis.[15]

MANAGEMENT

Treatment of KS has changed substantially in the HAART era. The degree of immunocompetence, the extent of tumor burden (T) and its rate of progression, HIV comorbidity and the patient's performance status (PS) dictate the choice of treatment. However,

Table 94.2 Staging system for HIV-associated Kaposi's sarcoma

	Good risk	Poor risk
Tumor	Confined to skin and/or lymph nodes and/or minimal oral disease (confined to palate)	Tumor-associated edema or ulceration; extensive oral Kaposi's sarcoma; gastrointestinal Kaposi's sarcoma; Kaposi's sarcoma in visceral organs
Immune system	$CD4^+$ lymphocytes ≥150/μl	$CD4^+$ lymphocytes <150/μl
Systemic illness	No history of opportunistic infection or thrush; no systemic B symptoms; Karnofsky performance status	History of opportunistic infection or thrush; systemic B symptoms; Karnofsky performance status <70%; other HIV-related illness

optimal antiretroviral therapy is a key component of KS management. Regression of KS with HAART has already been documented in many studies and HAART has been associated with prolonged time-to-treatment failure and longer survival among KS patients who have received chemotherapy. The available evidence suggests that HAART leads to regression of limited KS. Thus, HAART may be the only antineoplastic therapy in the early stage of disease (T_0) and/or for slowly proliferating disease, when tumor growth is consistent with the long time interval to the development of HAART anti-KS activity (median 8–12 months).[16–19]

KS may have a recrudescence during the first 2–3 months of HAART, as an immune reconstitution inflammatory syndrome (IRIS). The potential additive effects of IRIS with steroid use in KS contraindicate its use as an anti-inflammatory therapy.

There are several mechanisms by which HAART may be active in KS, including the increase of CD4 cell count, the suppression of HIV replication and the induction of an anti-angiogenic effect, which is mediated by PIs.[12,20]

In patients with T_1 and/or rapidly proliferating disease, the first-line treatment is chemotherapy plus HAART, followed by maintenance therapy with HAART. Although several chemotherapeutic agents (i.e. bleomycin, vinblastine, vincristine, vinorelbine, doxorubicin and etoposide) were noted to be efficacious against KS in the past, current systemic cytotoxic therapy comprises liposomal anthracyclines – pegylated liposomal doxorubicin (PLD) and liposomal daunorubicin – and taxanes. Liposomal encapsulation alters drugs kinetics, resulting in a prolonged half-life. In two pre-HAART randomized studies PLD (20 mg/m^2 intravenously every 2 weeks) had activity superior to combination of ABV (doxorubicin, bleomycin, vincristine) or BV (bleomycin, vincristine), with an overall response rate ranging from 46% to 59% and a better safety profile. Liposomal daunorubicin at an intravenous dose of 60 mg/m^2 every 2 weeks in patients with pulmonary KS has resulted in clinical benefit and objective response in 59% and 32% of cases, respectively.

Myelosuppression remains the most important dose-limiting toxicity of these drugs. Peripheral neuropathy and palmar–plantar erythrodysesthesias occur infrequently and cardiotoxicity is rare. Currently, these liposomal drugs are considered the best first-line chemotherapy for the majority of advanced KS patients.[19,21,22] Preliminary evidence indicates that the combination of liposomal anthracyclines and HAART is safe and effective.

Paclitaxel, a microtubule-stabilizing drug known to inhibit Bcl-2 anti-apoptotic activity, is effective even in patients with anthracycline resistant-KS. The drug (100 mg/m^2 intravenously every 2 weeks) results in an overall response rate of 56–59%, with a median duration response of 10.4 months. The major side-effects include myalgias, arthralgias and myelosuppression. Based on these data, paclitaxel is now used after failure of first-line systemic chemotherapy. Since the metabolism of paclitaxel involves the cytochrome P450 pathway, as does PIs and NNRTIs, caution is necessary when the drug is co-administered with HAART.

The growing knowledge of KS biology provides multiple opportunities for rational targeted therapies. However, preliminary results of these clinical trials and the complexity of KS pathogenesis suggest that effective treatment strategies will require a combination of agents targeting multiple pathogenetic pathways.[22]

PROGNOSIS

Although KS sometimes has a rapid course with extensive visceral involvement in some patients, in others it is an indolent disease for many years.

In the pre-HAART era, the ACTG TIS classification predicted survival in KS patients, and CD4 count (I stage) and T stage provided the most predictive information.[14] In the HAART era, only T and S stages maintained their correlation with survival. Two different risk categories are identified: a poor risk (T_1S_1) and a good risk (T_0S_0, T_1S_0, T_0S_1), with a 3-year survival rate of 53% versus 80–88%, respectively.

Furthermore, pulmonary involvement predicts survival better than tumor extension, independent of the S stage, and identifies the poorest category.[15]

Non-Hodgkin's Lymphoma

EPIDEMIOLOGY

The incidence of NHL in HIV-infected individuals is over 100 times the incidence among the general population. NHL accounts for AIDS-defining illness in about 3% of patients in the USA and 3–6% in Europe. In the HAART era, the incidence of NHL has decreased by approximately 50%; the decline has been marked for primary central nervous system lymphoma (PCNSL) and immunoblastic lymphoma, but not for Burkitt's lymphoma (BL). The risk factors for the majority of NHLs are severe immunodeficiency, prolonged HIV infection and older age.[1–4,8]

NHL occurs among all population groups at risk for HIV infection, in all age groups and in different countries, with similar epidemiologic and clinical pathologic features.

Systemic NHL constitutes 80% of all cases; the remaining 20% are PCNSL and PEL (<5%).

PATHOLOGY AND PATHOGENESIS

According the World Health Organization, NHLs are divided into three categories:

- lymphomas occurring in immunocompetent patients, such as BL and diffuse large B-cell lymphomas (DLBCL), including centroblastic, immunoblastic and anaplastic variants;
- lymphomas occurring more specifically in HIV-infected patients, such as PEL and plasmoblastic lymphoma of the oral cavity; and
- lymphomas also occurring in other immunodeficiency states, such as polymorphic or post-transplant lymphoproliferative disorders.[23]

The pathologic heterogeneity of HIV-associated NHL reflects the heterogeneity of their associated molecular lesions. In BL, the genetic lesions involve activation of c-*myc*, inactivation of p53 and infection with Epstein–Barr virus (EBV) in approximately 30% of cases. Immunoblastic lymphoma, infected by EBV in 90% of cases, is characterized by frequent expression of latent membrane protein-1 (LMP-1), an EBV oncoprotein. PEL, typically growing as lymphomatous effusions, is consistently associated with HHV-8 infection. Morphologically, PEL shows features bridging immunoblastic and anaplastic large-cell lymphomas and frequently displays a certain degree of plasma cell differentiation. Recently, HHV-8 has also been detected in solid extracavitary-based lymphomas. Plasmoblastic lymphoma, a rare (~3%) distinct type of DLBCL occurring often in the oral cavity or jaw, is associated with EBV in 50–100% of cases, but usually lacks expression of LMP-1 and EBV nuclear antigen-2. Several studies have recently shown that NHLs derive from two distinct cellular pathways: lymphomas that derive from germinal center (GC) B cells are unlikely to be EBV associated, whereas those from post-GC B cells are more likely to show immunoblastic histology and to be EBV associated.[24]

CLINICAL FEATURES

One of the distinguishing features of NHL is the widespread extent of disease at initial presentation and the frequency of systemic 'B' symptoms, including fever, night sweats and weight loss of more than 10% of the normal body weight. At the time of diagnosis approximately 75% of patients have advanced disease with frequent involvement

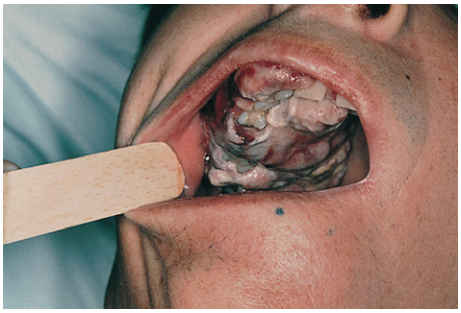


Fig. 94.2 Non-Hodgkin's lymphoma. Bulky disease in the gingiva.

of extranodal sites, the most common being the CNS, bone marrow, gastrointestinal tract and liver. Any site of the body, however, may be affected (Fig. 94.2).

Leptomeningeal disease, identified during routine lumbar puncture as part of the initial staging evaluation, remains asymptomatic in approximately 20% of patients. Gastrointestinal tract involvement, sometimes at multiple sites, develops in 10–40% of cases. Bulky disease can be observed in the anorectal region, particularly in homosexual men.

The PCNSL (i.e. intracranial parenchymal lymphoma limited to the CNS) is a manifestation of very advanced HIV disease. Usually the $CD4^+$ lymphocyte count at diagnosis is less than 50/μl. The lymphoma develops as single or multiple lesions in the deep regions of white matter, in the basal ganglia and in the cerebellum. The clinical presentation of PCNSL is not specific and approximately 50% of patients present with lethargy, confusion and personality change, whereas many others lack lateralizing neurologic signs.

The PEL grows exclusively or mainly within pleural, pericardial or peritoneal cavities as lymphomatous effusions, usually in the absence of contiguous tumor mass. It usually remains strictly localized to the body cavity of origin and only infrequently spreads to local lymph nodes or distant sites.

In the setting of HIV disease, NHL can be difficult to diagnose because of its variable presentation. It can mask many conditions of both HIV disease itself and its associated opportunistic infections. For instance, systemic 'B' symptoms are frequently associated with both advanced HIV infection and opportunistic infections. These symptoms mandate a careful evaluation to exclude other causes, including the presence of *Mycobacterium avium-intracellulare*, cytomegalovirus or tuberculosis infection.

The PCNSL may be radiographically indistinguishable from cerebral toxoplasmosis or other CNS infections. It has been shown that detection of cerebrospinal fluid (CSF) EBV-DNA by polymerase chain reaction (PCR) in HIV-infected patients is reliably associated with PCNSL. By combining CSF EBV-DNA detection by PCR with ^{201}Tl single photon emission computed tomography, the presence of increased uptake and positive EBV-DNA had 100% sensitivity and 100% negative predictive value. Thus in patients with hyperactive lesions and positive EBV-DNA, brain biopsy may be avoided and patients could promptly undergo definitive therapy.

DIAGNOSIS

A diagnosis of NHL should be made by histologic examination of the tissue obtained by incisional or excisional biopsy. It may be possible, however, to make an adequate diagnosis from needle aspiration cytology, and this may be required if the patient's clinical condition is critical or deteriorating rapidly.

In the absence of clinical suspicion for toxoplasmosis, EBV in the CSF in the presence of a brain lesion inaccessible to biopsy remains the gold standard for diagnosing PCNSL.

MANAGEMENT

The poor prognosis of NHL and the palliative approach to treatment are no longer the rule since the introduction of HAART. Patients with good performance status and without severe immunosuppression should be treated with curative intent in a manner comparable to immunocompetent patients, but with greater vigilance for the development of opportunistic infections. Long-term complete remission can be now observed with a possible cure of lymphoma.[25–28]

Although different chemotherapy regimens have been tested, there is still disagreement regarding the optimal chemotherapy even in the HAART era. A large European randomized trial, designed to evaluate different dose-intensive treatments according to HIV score, showed that HAART use, HIV score and age-adjusted international prognostic index (aa-IPI) were the strongest predictors for survival, but not the intensity of cyclophosphamide–doxorubicin–vincristine–prednisone (CHOP)-based chemotherapy.[26] Infusional chemotherapy is thought to overcome some drug resistance and possibly high tumor proliferative indices. Cyclophosphamide–doxorubicin–etoposide (CDE), as a 96-hour continuous infusion combined with rituximab, has yielded a complete response of 70% with an estimated 2-year overall survival of 62%.[27] A National Cancer Institute study demonstrated 74% complete remission and 2-year overall survival of 60% with infusional etoposide–prednisone–vincristine–cyclophosphamide–doxorubicin (EPOCH).[28] Preliminary results with rituximab-EPOCH show a complete remission of 65% and, compared with historical controls, improved survival from 16% to 57% after a median follow-up of 19 months. These survival outcomes reflect a dramatic improvement compared with pre-HAART trials.

Rituximab is a humanized anti-CD20 antibody that has revolutionized the treatment of HIV-negative NHL patients; however, it might increase the risk of life-threatening infections. Treatment-related infectious deaths occurred in 14% of patients treated with concurrent rituximab-CHOP followed by maintenance rituximab, but such infections were not increased in other studies (Table 94.3). Severe neutropenia is a common complication of rituximab chemotherapy, whereas hypogammaglobulinemia has rarely been reported.[27,29,30] Antiviral therapy with lamivudine has been shown to reduce viral reactivation in patients with chronic hepatitis B infection receiving chemotherapy and is recommended for patients treated with rituximab.

Preliminary studies show that high-dose therapy with autologous stem cell transplantation plus HAART is feasible and effective in patients with relapsed or refractory NHL or HD. Adequate stem cell collection is obtained in approximately 80% of cases with prompt engraftment in all cases and absence of toxic deaths. The disease-free survival and the overall survival rates are 55% and 60%, respectively, at a median of 36 months of follow-up.

Available data suggest that first-generation HAART regimens could be co-administered safely with chemotherapy, with the exception of zidovudine because of its myelosuppressive effects.[25] Additional studies are, however, warranted with the new boosted PI-based HAART, because of the high risk of pharmacokinetic interactions.

PROGNOSIS

In HIV NHLs the conventional prognostic factors influencing survival in lymphoma patients in the general population – age, disease stage, extranodal involvement, performance status, lactate dehydrogenase (LDH) levels or international prognostic index (IPI) score – have been used together with HIV-specific factors. In the pre-HAART era, the main unfavorable prognostic factors were presence of a severe immune deficit ($CD4^+$ <100/μl), prior AIDS diagnosis and

Table 94.3 Rituximab in non-Hodgkin's lymphoma HIV: major series in the literature

Regimen	No. patients (%)	aa-IPI ≥2 (%)	Median CD4/μl	CR (%)	Febrile neutropenia (%)	Death due to progression
Rituximab + cyclophosphamide, doxorubicin and etoposide (R-CDE)	74	52	161	70	23	5
Rituximab + cyclophosphamide, doxorubicin, vincristine and prednisone (R-CHOP)	52	46	180	77	17	4
Rituximab + cyclophosphamide, doxorubicin, vincristine and prednisone + maintenance rituximab (R-CHOP-mR)	80	58	128	57	30	14
Rituximab + etoposide, prednisone, vincristine, cyclophosphamide and doxorubicin (R-EPOCH)				65		

aa-IPI, age-adjusted international prognostic index; CR, complete remission

poor general health.[26] The advent of HAART into clinical practice has changed outcome significantly: conventional survival-influencing lymphoma-related factors actually have a stronger impact on survival than factors associated with the underlying HIV infection.[25,26] Particularly in the most extensive surveys the age-adjusted IPI score (disease stage, performance status, LDH levels) is the most discriminating negative prognostic factor in patients with HIV NHLs, together with Burkitt subtype, and failure to obtain complete remission after first-line chemotherapy.[25–30]

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Chapter 95

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Dermatologic manifestations of HIV infection

INTRODUCTION

The skin is commonly affected in patients who have HIV infection and AIDS.[1] The prevalence of cutaneous involvement approaches 100% and in many cases is the motivating force that causes the patient to seek medical care.

Dermatologic manifestations of HIV infection may provide the initial diagnostic clues, not only of the presence of the infection per se but also in some cases the stage of involvement.[2] Early-stage disease ($CD4^+$ count 200–500 cells/mm^3) can be associated with seborrheic dermatitis, oral hairy leukoplakia (OHL), Kaposi's sarcoma (KS), oropharyngeal or recurrent vulvovaginal candidiasis, herpes zoster or recurrent herpes simplex virus (HSV) infection. With a $CD4^+$ count of 50–200 cells/mm^3, bacillary angiomatosis, esophageal candidiasis, disseminated deep fungal infections, molluscum contagiosum, chronic herpetic ulcers and eosinophilic folliculitis become more prominent. With advanced HIV disease ($CD4^+$ <50 cells/mm^3), profound immunosuppression may lead to the occurrence of several coexisting infections and neoplasms. In general, skin infections and neoplastic conditions in patients who have AIDS are aggressive and difficult to treat. Early recognition of the different skin diseases will facilitate the use of an appropriate and timely therapeutic approach.

With the introduction of highly active antiretroviral therapy (HAART) in the mid 1990s, changes in the dermatologic manifestations paralleled the stabilization and improvement in $CD4^+$ counts.[3,4] As $CD4^+$ counts normalize with HAART therapy, many of the skin disorders affecting HIV-infected patients become similar to those seen in immunocompetent patients. Significant decreases in the incidence of KS, OHL, oral candidiasis, bacterial folliculitis, recurrent HSV and, to a lesser degree, seborrheic dermatitis, psoriasis, drug eruptions, dry skin and pruritus were noted. Conversely, conditions such as warts, scabies, photosensitivity and eosinophilic folliculitis did not abate significantly and, in some cases, increased in incidence. This increase in certain dermatologic diseases, mostly within the first 3 months following HAART initiation, is known as immune reconstitution disease (IRD) (see Chapter 92). Some entities, such as molluscum contagiosum, became less prevalent in some series and more in others, reflecting varying influences such as immune restoration, altered cytokine patterns, improved lifestyle and increased exposure. Effects of drug reactions can also be exacerbated or mitigated at this time.

This chapter describes the most common dermatologic manifestations of HIV infection, including skin changes present early in infection and in advanced disease, such as neoplastic, infectious and noninfectious conditions, hair, nail and oral changes and drug reactions.

EARLY DERMATOLOGIC MANIFESTATIONS OF HIV INFECTION: ACUTE EXANTHEM OF HIV INFECTION

The acute exanthem, which is symptomatic in about 80% of patients, begins 1–6 weeks after exposure to the virus and is associated with prodromal symptoms such as lymphadenopathy, fatigue, fever, headache, nausea, diarrhea and night sweats. The skin eruption consists of erythematous, round to oval macules and papules affecting the trunk, chest, back and upper back (Fig. 95.1). Less commonly, hives and pruritic papules and pustules can occur on the extremities. The syndrome generally lasts for 4–5 days and resolves with complete recovery.[5] The prognosis for patients who have a more severe form of acute HIV infection is poorer than for those who are asymptomatic or have mild symptoms (see Chapter 89).[5]



Fig. 95.1 Acute exanthem of HIV infection. There is morbilliform eruption involving the trunk and extremities. The eruption is similar to a morbilliform drug eruption and to other viral exanthemata.

NON-NEOPLASTIC INFECTIOUS DERMATOLOGIC CONDITIONS

Viral infections

Herpes simplex virus

Infections with HSV types 1 and 2 result in recurrent, severe painful grouped vesicles with an erythematous base localized mainly on the lips and genital and perianal areas. If left untreated, these lesions may enlarge and become confluent ulcerations that may persist for over 1 month, displaying slow healing and often becoming secondarily infected with bacteria. Ulcerations can occur without the patient noticing pre-existing, well-defined vesicles.[6] It is essential to establish the specific diagnosis by means of polymerase chain reaction (PCR), direct fluorescent antibody (DFA) testing, biopsies and/or viral cultures (see Chapter 155).

While the immune system is intact, the course of the disease is similar to that in noninfected individuals. Once immunosuppression sets in, the lesions can become persistent. Ulcers can expand, reach large size and even become necrotic. Nonhealing ulcers that persist for more than 1 month are one of the AIDS-defining conditions.[7] An aggressive therapeutic approach should be used with these patients, as they may be poorly responsive to standard treatment and HSV infection increases the probability of HIV transmission and shedding[8] (for therapy, see Chapter 91).

Varicella-zoster virus

The development of recrudescent varicella-zoster virus (VZV) infection in a patient at risk of HIV infection may be a sign of the presence of HIV and should alert the clinician to screen the patient.[9] It usually occurs early in the course of the disease. Varicella-zoster virus exists in a dormant state in dorsal root ganglion after the primary vesicular infection commonly known as chickenpox. With reactivation, the virus progresses downward through the nerve tracts of a solitary dermatome, leading to the characteristic zosteriform distribution of painful tense vesicles of skin (Fig. 95.2). In HIV-infected individuals, VZV infection may be recurrent and severe, with more than one dermatome involved, and may run a protracted course associated with residual postherpetic neuralgia and scarring. Disseminated herpes-zoster may occur. Atypical presentations of VZV include verrucous zoster, follicular zoster and ecthymatous or crusted zoster. Chronic lesions can be verrucous or ecthymatous and can be a presenting sign of HIV disease.[10]

Primary varicella infection can develop in previously unexposed individuals with HIV infection. The infection can be more severe, cause extracutaneous disease and be fatal.

Occurrences of VZV are common after initiation of HAART as part of IRD (Chapter 92). Diagnosis of VZV is largely clinical; however, DFA testing is a rapid and effective way to diagnose atypical presentations (see also Chapter 155; for therapy, see Chapter 91).

Cytomegalovirus

Cytomegalovirus (CMV) is the most common cause of serious opportunistic viral infection in patients who have AIDS.[11] However, cutaneous involvement is rare. In the skin, CMV has different clinical manifestations, including ulcerations, keratotic verrucous lesions, hyperpigmented plaques, morbilliform eruptions and palpable purpuric papules. Because the mucocutaneous lesions caused by CMV do not have specific features, tissue biopsy of the lesions, as well as direct viral diagnostic approaches (Chapter 155) are required to define the etiologic cause. The treatment of choice for CMV infection is intravenous ganciclovir 5 mg/kg every 12 hours. Foscarnet should be used if ganciclovir resistance is suspected (for further discussion of therapy, see Chapter 91).

Epstein–Barr virus

The majority of adults harbor latent Epstein–Barr virus (EBV) within B lymphocytes. With advanced immunodeficiency seen in HIV-infected patients, EBV replication occurs, leading to OHL, Burkitt's lymphoma or EBV-associated large cell lymphoma. Oral hairy leukoplakia manifests as single or multiple white plaques on the lateral margins of the tongue, with a verrucous surface. The presence of OHL correlates with moderate to advanced immunodeficiency.[12] Oral hairy leukoplakia responds well to systemically administered aciclovir and valaciclovir, although there is prompt recurrence after treatment.[13,14] Good response to topical application of podophyllin resin has also been reported, although recurrence is probable and podophyllin can be toxic in large doses.[15] Many clinicians elect not to treat because the lesions are asymptomatic. In some patients, OHL may regress with antiretroviral therapy alone.

Human papillomavirus

Different types of human papillomavirus (HPV) tend to cause different clinical lesions, although there is significant overlap. Infections of the skin and mucous membranes by HPV can cause widespread warts in patients who have AIDS. Types of warts observed include filiform, flat and plantar. Warts can develop in unusual locations, such as on the lips, tongue and oral mucosa (Fig. 95.3). These lesions are often treated with cryotherapy, electrocauterization or topical treatment with caustic agents such as podophyllin, imiquimod, trichloroacetic acid and 5-fluorouracil. However, the majority of these lesions are resistant to treatment and require repeated therapy.

In men, HPV affects the penis, urethra, scrotum, perianal, anal and rectal mucosa in the form of condylomata acuminata that are usually recognized as soft sessile lesions with finger-like projections. In women, the spectrum of clinical disease induced by HPV is broad, with vulvar, vaginal and cervical condylomata being observed. Cervical, vulvar and anal intraepithelial dysplasia may manifest as flat condyloma with viral cytopathic effect seen on histology but without raised warty lesions.



Fig. 95.2 Herpes zoster. A painful linear-zosteriform eruption of vesicles on an erythematous base is characteristic of herpes zoster. The eruption may be persistent and verrucous lesions are not uncommon.



Fig. 95.3 Human papillomavirus infection. Human papillomavirus infections are common in HIV-infected patients. They may have unusual features, as demonstrated here, and may be refractory to therapy.

HIV is associated with increased incidence of high-grade dysplasia and increased risk of progression to invasive squamous cell carcinoma.[16] Human papillomavirus has been detected in more than 95% of cervical cancers and in more than 50% of anal cancers.[17] Certain subtypes of HPV (16, 18, 31, 33, 35, 39, 45 and 51) are considered to be high risk for development of anogenital intraepithelial neoplasia and associated squamous cell carcinoma. A solution of 2–5% acetic acid is helpful in delineating HPV lesions. Colposcopy or anoscopy may also reveal atypical vascular patterns in areas of intraepithelial dysplasia. As with zoster, whenever extensive warts develop in otherwise healthy patients known to be at risk of HIV infection, the patient should be screened for HIV.

Poxvirus

The most common disease caused by poxviruses is molluscum contagiosum, which develops in 10–20% of patients who have AIDS. Molluscum contagiosum is transmitted by direct skin-to-skin contact to produce cutaneous and, rarely, mucosal lesions. In adults it is most commonly transmitted by sexual contact. The lesions are characterized by dome-shaped umbilicated translucent 2–4 mm papules that develop in any part of the body but especially on the face and genital areas (Fig. 95.4). In AIDS patients these lesions are widespread, may attain immense size and can be disfiguring.[18] Most patients who have extensive molluscum contagiosum associated with HIV infection have $CD4^+$ counts well below 250 cells/mm^3. In immunosuppressed patients the diagnosis of molluscum contagiosum should be confirmed by histologic examination in any case that is questionable because it may resemble more serious infections such as cutaneous pneumocystosis, histoplasmosis and *Penicillium marneffei* infection and cryptococcosis. Toluidine or Giemsa staining can be used for cytologic examination of the white material expressed from the lesion to search for the typical large cytoplasmic inclusion bodies. Molluscum contagiosum is treated with cryotherapy, electrodessication, curettage or topical application of podophyllin, tretinoin or imiquimod cream.

Other viral infections

Several viral infections have been reported to develop with increased frequency in patients who have HIV infection. Parvovirus B19, which causes erythema infectiosum, has been reported to produce an exanthem and polyarthralgia in HIV-positive patients. Coxsackie virus and enterovirus may also lead to morbilliform or vesicular eruptions.

Bacterial infections

Folliculitis

Bacterial folliculitis is common in HIV-infected patients, appearing as widely distributed acneiform papules and pustules. Lesions may be pruritic and become excoriated. Recurrent bacterial folliculitis may serve as a clue to screen a patient for possible HIV infection. Most cases are caused by *Staphylococcus aureus*,[19] but other organisms such as *S. epidermidis* and *Pseudomonas aeruginosa* may also cause folliculitis. Nosocomial and community-acquired methicillin-resistant *S. aureus* (MRSA) should be considered when confronting folliculitis in HIV-infected individuals. Additional risk factors for MRSA exposure include intravenous drug use, men who have sex with men, recent hospitalization or incarceration.[20] Bacterial folliculitis in HIV-infected patients is often resistant to standard treatment and prolonged use of systemic antibiotics may be required.[21]



Fig. 95.4 Molluscum contagiosum. These lesions are characteristically translucent, waxy papules with central umbilication.

Impetigo, abscesses, cellulitis, lymphadenitis and necrotizing fasciitis

Impetigo, usually caused by *S. aureus*, is seen most commonly on the face, shoulders, axillary and inguinal areas. The infection begins with painful red macules that become vesicles and pustules and contain purulent fluid; these soon rupture and give rise to the characteristic honey-colored crust. Soft tissue and deep-seated bacterial infections such as cellulitis, abscesses and necrotizing fasciitis may also develop in HIV-infected patients and are often due to MRSA.[20] These manifest as diffuse, red, warm, tender areas in the skin, associated with severe toxemia. Streptococcal axillary lymphadenitis is a diffuse, painful swelling of lymph nodes in the axilla that is usually bilateral.

Aggressive antibiotic treatment is recommended for these processes. Excision and drainage of pus-containing abscesses has been reported as an effective treatment in community-acquired MRSA soft tissue lesions, even in the absence of antibiotic treatment.

Mycobacterial infections

Mycobacteria may produce a wide variety of skin lesions in HIV-infected individuals and infection by these organisms usually signifies severe disseminated systemic infection. Since the presenting lesions of cutaneous infections have nonspecific morphology, tissue biopsy and culture are requisite for diagnosis.

Active infection is caused primarily by *Mycobacterium avium-intracellulare* and *M. tuberculosis* and less commonly by other mycobacteria. The infection can manifest as small papules and pustules resembling folliculitis, atopic dermatitis-like eruptions, cutaneous abscesses, lymphadenitis and ulcerations.

Infections with *M. tuberculosis* may be the consequence of either primary infection at the site of broken skin that results in a local verrucous lesion, or systemic disseminated infection. Cutaneous tuberculosis is treated with the same regimen as pulmonary or extrapulmonary disease (see Chapter 93).

Bacillary angiomatosis

Bacillary angiomatosis (BA) is a pseudoneoplastic, infectious cutaneous vascular disorder[22] caused by bacteria of the genus *Bartonella*, including *Bartonella quintana* and *Bartonella henselae*.[23] There are a number of clinical manifestations of BA. The earliest and most common lesion appears as discrete pinpoint red–purple papules similar to pyogenic granulomata. These lesions may ulcerate and become crusted (Fig. 95.5). Another variant consists of subcutaneous nodules that may extend into the underlying skeletal muscle and bone. Patients who have BA may have systemic signs and symptoms, including fever, chills, night sweats and weight loss. In advanced cases, the liver and spleen may be involved. Bacillary angiomatosis occurs primarily in the context of the advanced stage of HIV infection, but may occur in patients who have other forms of immunosuppression or in a healthy host. Because the clinical presentation of this infection can easily be confused with pyogenic granuloma, biopsy should be performed. A 2- to 3-month course of erythromycin (2 g/d) or doxycycline (100 mg q12h) is effective against BA.[24,25] Recurrence is common.

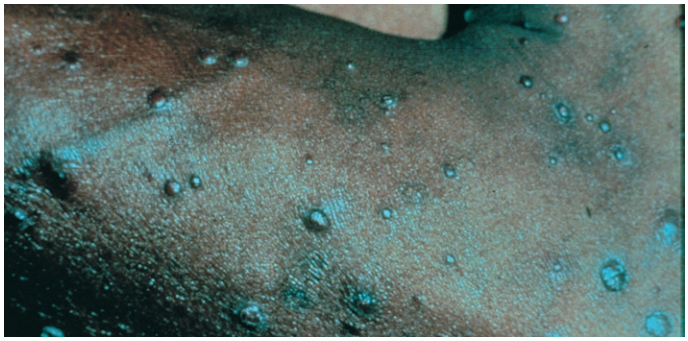


Fig. 95.5 Bacillary angiomatosis. There are elevated vascular papules of the glabrous skin. When incised, these lesions bleed profusely.

Sexually transmitted disease

The accurate diagnosis of sexually transmitted diseases (STDs) is of exceptional importance in individuals at high risk of HIV infection, because their presence increases the risk of transmitting and acquiring HIV infection.[26]

Syphilis

A high prevalence of syphilis, active as well as latent, has been found among HIV-positive patients in the USA.[26] Primary infection with *Treponema pallidum* does not show major variations, beginning with a single chancre at the site of inoculation. Chancres are 1–2 cm, painless, round to oval ulcers with raised indurated borders. Recent studies have reported multiple chancres that can be aggressive and slower to heal.[27,28] Secondary syphilis may occur in a number of forms in patients who have HIV infection, ranging from the classic papulosquamous form with involvement of palms, soles and mucous membranes to unusual forms such as verrucous plaques, extensive oral ulcerations, alopecia, keratoderma, deep cutaneous nodules and widespread gummata.

Syphilis may progress faster from secondary to tertiary disease in HIV-seropositive patients than in HIV-seronegative individuals. Those with $CD4^+$ counts <350 cells/mm^3 are at a fourfold higher risk for neurosyphilis. Early central nervous system (CNS) relapse and unresponsiveness to treatment can also be more common in HIV-infected individuals. Syphilis is a strong indicator that an individual may be infected with HIV and all patients should undergo HIV serotesting. Serologic negativity may not rule out secondary syphilis in HIV-infected individuals as true negative serologic studies can be seen with both the fluorescent treponemal antibody absorption (FTA-ABS) and VDRL tests. Skin biopsy for demonstration of spirochetes by special stains or immunohistochemical techniques may also establish the diagnosis. Current treatment involves a single dose of long-acting benzathine penicillin or ceftriaxone (1 g daily for 8–10 days).[29]

Other sexually transmitted diseases (see also Chapter 60)

Lymphogranuloma venereum (LGV) is uncommon in HIV-infected patients in the developed world, though its incidence is rising. It manifests as generalized lymphadenopathy with vulvar or penile edema with ulcerations and erosions.[30] The causative organism is one of three specific serotypes (L1, L2 and L3) of *Chlamydia trachomatis*. Diagnosis is presumptive based on clinical findings. Those thought to be infected with LGV should be started on a course of 200 mg of doxycycline daily for 21 days.[29]

Fungal infections

Candidiasis

Recurrent or persistent oral candidiasis may be the initial sign of HIV infection in many individuals and has been shown to be a predictor of progression from HIV infection to AIDS.[30] The clinical presentation is that of a whitish exudate present on the tongue or buccal mucosa that can easily be scraped away. *Candida* is also implicated in causing angular cheilitis, chronic paronychia, onychodystrophy, distal urethritis and persistent intertriginous infections. *Candida* infections are discussed in detail in Chapter 91.

Dermatophytosis

Cutaneous dermatophytosis is more common in patients with HIV than in the general population and can be extensive and severe. In any individual with extensive tinea corporis, the possibility of underlying HIV infection should be considered. Tinea corporis, often caused by *Trichophyton rubrum*, can present as a widespread dermal infection with multiple fluctuant ethematous ulcerative nodules seen mostly on the extremities.

Systemic fungal infections

Cryptococcus

The most common opportunistic fungal infection to affect the skin in HIV-seropositive patients is cryptococcosis. Umbilicated papules similar to molluscum contagiosum, nodules, pustules, ulcers and erythematous papules are all common manifestations of cutaneous cryptococcosis. If cutaneous cryptococcosis is diagnosed, a thorough investigation for extracutaneous disease should be undertaken (see Chapter 91).

Histoplasmosis

A variety of lesions are seen including nodules, plaques, vesicles, erythematous scaly plaques, necrotizing lesions simulating pyoderma gangrenosum, purpura, petechiae and pustules, with or without ulceration. Diagnosis is made through biopsy (see Chapter 91).

Coccidioidomycosis

Morbilliform eruptions, violaceous or ulcerating plaques, papules and pustules are common cutaneous lesions. If a patient has cutaneous disease, a search for other systemic manifestations should be initiated (see Chapter 91).

Other systemic fungal infections

Blastomycosis and sporotrichosis are rarely observed in HIV-seropositive patients. Mucocutaneous lesions associated with systemic fungal infections consist of pustules, ulcers, papules and nodules (Fig. 95.6). Mucocutaneous fungal infections in general may mimic other disorders such as HSV infection, cellulitis or molluscum

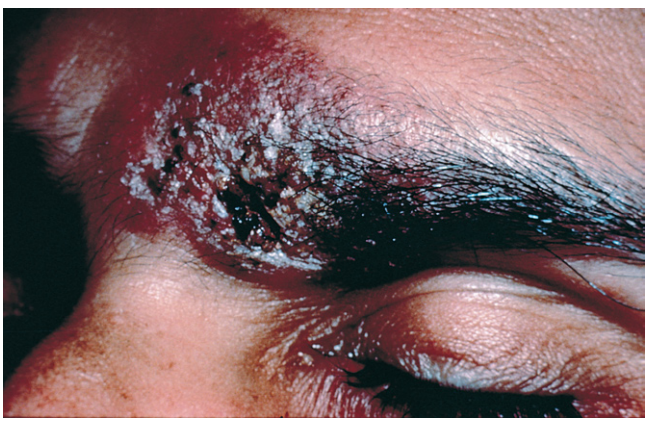


Fig. 95.6 Cutaneous histoplasmosis. These lesions are characteristically quite nondescript and may simulate other infectious disorders and verrucous neoplastic conditions.

contagiosum infection. For this reason, when the clinical diagnosis of possible fungal infection is considered, a tissue biopsy of the lesion should be performed for histologic evaluation and microbiologic cultures (see Chapter 91).[31]

Parasitic and ectoparasitic infections

HIV-seropositive individuals may present with a wide variety of parasitic and ectoparasitic infections, including scabies, demodicidosis, acanthamebiasis, leishmaniasis and toxoplasmosis. This group of infections manifests either as localized conditions or disseminated disease. The clinical presentation can be unusual and the use of cultures and skin biopsies is essential to render accurate diagnosis.[32]

Scabies

The causative agent of scabies is the mite *Sarcoptes scabei*. Scabies is one of the most frequent conditions encountered in HIV infection and is the most common ectoparasitic infection.[21] The clinical presentation can vary from discrete scattered pruritic papules and slight scale to a widespread papulosquamous eruption that resembles atopic dermatitis. A common clinical presentation is that of hyperkeratotic plaques present on the palms, soles, trunk and extremities. Patients complain of intense pruritus that is worse at night. Contacts are almost always infected. Severe scabies can lead to bacteremia and even death due to sepsis. In HIV-infected patients who have $CD4^+$ counts <150 cells/mm^3, or in patients who have advanced peripheral neuropathy and diminished sensation, scabies infection may be severe and have a much greater number of mites than normal. This presents as crusted or 'Norwegian' scabies. Microscopic examination of scrapings is used to diagnose mites or ova. Topical treatments include permethrin and benzyl benzoate. Ivermectin can be given orally.

Demodicidosis

The causative agent of demodicidosis is the mite *Demodex*. Demodicidosis has been reported only sporadically in patients who have AIDS. The clinical presentation is that of a persistent pruritic follicular eruption of the face, trunk and extremities. Demodicidosis has also been associated with the pathogenesis of rosacea-like dermatoses, perioral dermatoses and blepharitis. Diagnosis is by clinical pathologic correlation, since *Demodex* spp. are part of the normal skin microbiotica.

Leishmaniasis

Leishmaniasis has three classifications: cutaneous, mucocutaneous and visceral. The skin lesions of kala-azar with scaly lichenified depigmented plaques resemble those of lichen simplex chronicus. Nonulcerated nodules on the extensor surfaces of the limbs overlying the joints have also been described. Mucocutaneous disease begins with single or multiple lesions that heal and eventually lead to mucosal lesions. These lesions are painful and disfiguring. Leishmaniasis is more difficult to treat when associated with HIV infection. Amastigotes persist for years in macrophages and actually accelerate compromised immunity in hosts by increasing HIV viral replication.[33]

NONINFECTIOUS DERMATOSES

The noninfectious dermatoses associated with HIV infection are numerous and may occur in all stages of the disease. Although none of the disorders reported in this group has been linked directly to an infectious agent, these conditions may be in part caused by an abnormal host response to infectious agents. Noninfectious dermatoses in these patients may have atypical clinical presentations, greater severity and may fail to respond to routine treatment.[34] Because these dermatoses often have atypical presentations in these patients, biopsies and cultures are often required for diagnosis.

Acquired xerosis and ichthyosis

HIV-infected individuals commonly complain of increased dryness of skin. Typically, xerosis is most prominent on the anterior lower legs, manifesting as diffuse dryness with focal crusting and hyperpigmented scales. In the winter it is more severe and may be associated with inflammation. Patients who have advanced AIDS may present with ichthyosis – dry, thick skin with plate-like scales. The severity of the ichthyosis correlates with the degree of wasting. Treatment includes mid-potency steroid ointments or emollients containing salicylic acid, urea or lactic acid.

Seborrheic dermatitis

This is perhaps the best known dermatosis associated with HIV infection and is seen in up to 85% of all these individuals at some point during the course of the illness.[35] The etiology of seborrheic dermatitis is poorly understood but is thought to be multifactorial. Clinically, the disease is characterized by slightly indurated, diffuse or confluent pinkish-red plaques with yellowish greasy scales and crusting in typical locations, including malar and retro-auricular areas, nasolabial folds, eyebrows and scalp (Fig. 95.7). In severe cases, which are also more common in HIV-infected patients, it extends to the chest, neck and other parts of the body. Seborrheic dermatitis in patients who have HIV infection is often resistant to treatment, which should serve as a clue that a patient might be infected. With HAART therapy, the incidence of refractory cases appears to be diminishing. For mild cases, anti-dandruff shampoo may be used. For moderate to severe disease, ketoconazole shampoo, topical steroids, coal tar, sulfur and UVB light have been reported as effective.

Psoriasis and reactive arthritis

Psoriasis develops in up to 5% of individuals with HIV infection[36] and may have a number of different manifestations. It may resemble the classic form found in immunocompetent hosts, which consists of reddish plaques with superficial gray to silver scales on the extensor surfaces and nail changes of onycholysis, pitting and subungual hyperkeratosis. Guttate psoriasis may also be seen, with or without classic psoriasis vulgaris. Severe forms, such as erythrodermic psoriasis, may be encountered. Treatment of psoriasis in these patients may be difficult, although fortunately it is one of the inflammatory conditions that responds well to therapy, especially with systemic retinoids, either isotretinoin or etretinate. Psoriasis may undergo partial remission in response to zidovudine therapy, although recurrences are common.

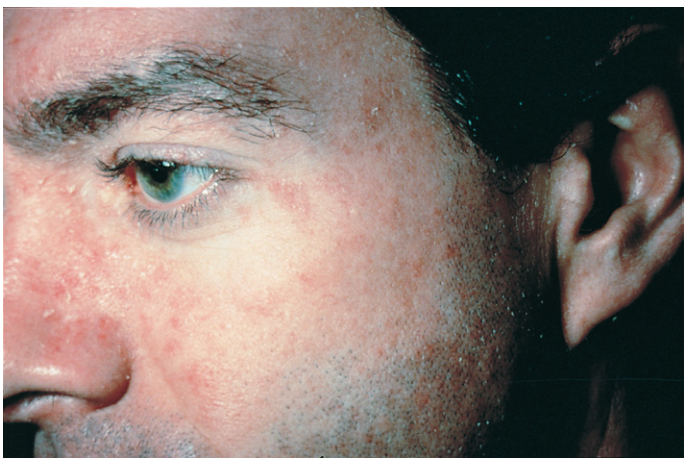


Fig. 95.7 Seborrheic dermatitis. Note the characteristic greasy scale and the erythematous plaques involving the face, especially the nasolabial folds and the eyebrows.

Other treatments that may be beneficial include systemic acitretin, topical keratolytic agents, systemic methotrexate and phototherapy.

Reactive arthritis (formerly known as Reiter's syndrome) is characterized by a triad of polyarthritis, conjunctivitis and urethritis.[37] Cutaneous manifestations include a palmoplantar pustular dermatosis that can be associated with nail dystrophy, hyperkeratosis and periungual erythema. Initially, the lesions present as erythematous macules that evolve into waxy, hyperkeratotic papules surrounded by an erythematous halo. The lesions may coalesce to form thickened plaques that are usually distributed on the palms, soles and trunk. Histologically, reactive arthritis looks identical to pustular psoriasis and relies on a clinical diagnosis. Treatments mirror those used for psoriasis in HIV-infected individuals.

Eosinophilic folliculitis

Eosinophilic folliculitis (EF), also known as eosinophilic pustular folliculitis or Ofuji's disease, is an inflammatory process of the hair follicles, the etiology of which remains undetermined. Mites, Gram-negative bacteria and fungi have all been implicated as possible etiologies. The condition is more common in men and has a peak incidence in the third decade of life. Affected patients may develop marked eosinophilia and elevated levels of IgE. Virtually all patients who have EF have $CD4^+$ counts <200 cells/mm^3; thus, it is an important cutaneous marker of advanced HIV infection.[38]

Clinically, patients develop folliculocentric pruritic urticarial papules measuring 1–4 mm in diameter on the upper trunk, face, neck and proximal extremities (Fig. 95.8). There may be coalescence of papules to form plaques. Virtually all cases are associated with lichenification secondary to chronic rubbing and scratching. In dark-skinned individuals, postinflammatory hyperpigmentation can cause considerable disfigurement.

Due to EF's clinical similarities to other pruritic papular eruptions, a biopsy should be taken of a new folliculocentric lesion to correctly diagnose EF. Therapy of EF includes exposure to ultraviolet B and natural sunlight, oral metronidazole, itraconazole, erythromycin and isotretinoin.

Papular dermatitis of AIDS

This is a nonspecific chronic papular eruption that has been reported in HIV-seropositive patients.[39] Most recently it has been termed pruritic papular eruption (PPE). The nosology of this condition has been the subject of debate and many experts consider this to represent a form of eosinophilic folliculitis. Clinically, skin-colored papules are present on the head, neck and upper trunk, many of which may be folliculocentric. Lesions are often excoriated, do not form confluent plaques and neglect mucous membranes. The diagnosis is made only by exclusion; thus other eruptions with similar clinical presentation should be excluded, including EF, scabies, papular mucinosis, secondary syphilis, viral exanthem and drug eruption. Treatments are anecdotal for PPE, but may include topical corticosteroids, oral antihistamines, antipruritics, UVB light and pentoxifylline given in 400 mg dosages every 8 hours for 8 weeks.[40]

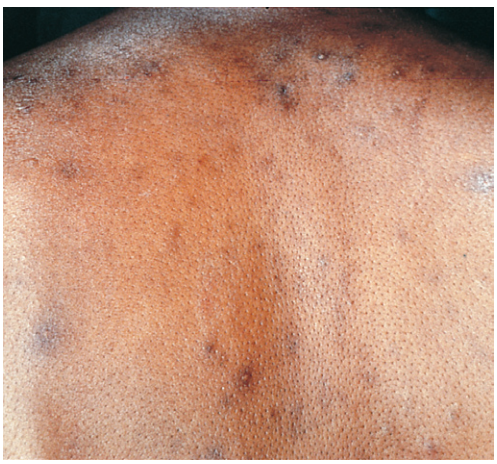


Fig. 95.8 Eosinophilic folliculitis. There are follicular papules, many of which have been excoriated, involving the upper trunk and the face. Histologically, numerous eosinophils are present within the follicular ostia.

Prurigo nodularis

Prurigo nodularis refers to thickened, verrucous papules and nodules and is a reaction pattern of the skin that is associated with a number of pruritic conditions. Patients who have itchy skin chronically rub and scratch, resulting in thickening with formation of typical lesions of prurigo nodularis. Although many different disorders may lead to the condition, in many patients the preceding event has long since resolved and the condition is perpetuated by the so-called itch–scratch–itch cycle.

Treatment of the disorder is based on correcting the underlying pruritus by the use of systemic and topical agents to lessen itching, such as topical corticosteroids, topical menthol- and phenol-containing lotions, topical antihistamines and systemic antipruritic agents.

Atopic dermatitis

Atopic dermatitis is seen somewhat more commonly in children who have HIV infection, having been reported in up to 20%.[41] Patients who have atopic dermatitis antedating HIV infection experience increased severity and persistence of their disease. The pathogenesis of atopic dermatitis in these patients has been associated with elevated circulating IgE antibodies to HIV and *S. aureus*. The clinical presentation is similar to that in immunocompetent hosts, with erythematous patches and plaques with fine papulovesicles associated with scaling and crusting. Treatment includes emollients, topical steroids and oral antihistamines.

Granuloma annulare

There have been a number of sporadic case reports of granuloma annulare (GA) in HIV-seropositive patients.[42] A unique feature of GA in these patients is a tendency toward widespread distribution and photoexacerbation. The clinical presentation is that of violaceous, firm papules with annular arrangement distributed on the hands, feet, arms, legs and trunk. In some patients, there may be similarity to KS. The reason for the development of GA in these patients remains unknown.

Leukocytoclastic vasculitis

Patients who have HIV disease may develop leukocytoclastic vasculitis.[43] This condition is a manifestation of immune complex-mediated disease. The clinical presentation progresses through several stages, beginning as urticarial papules or small petechiae. In most cases, characteristic palpable purpuric papules develop. Lesions are distributed on the extremities, although any body site can be affected. In HIV-infected individuals, they are more numerous and more florid than in immunocompetent hosts. The treatment of leukocytoclastic vasculitis consists primarily of identifying the underlying cause and correcting the associated abnormalities. Administration of nonsteroidal anti-inflammatory agents may be beneficial, although colchicine, dapsone or systemic corticosteroids may be required in severe cases.

NEOPLASMS

A number of different neoplastic disorders may develop in HIV-positive patients. Although lymphoreticular and vascular neoplasms are commonly observed, HIV-associated epithelial, mesenchymal and

melanocytic neoplasms have also been described. The development of malignant neoplasms is of great significance because they are often sources of morbidity and mortality.[16]

Kaposi's sarcoma

Kaposi's sarcoma is a viral-associated vascular tumor that is divided into classic and epidemic forms. The classic form is an uncommon disorder that was first described in 1827 and is seen mainly in elderly men of Mediterranean origin, black equatorial Africans and patients who have lymphoma or with primary or iatrogenically induced immune deficiencies. The epidemic form of KS is associated with HIV infection.[44] Epidemic KS is associated with infection by human herpesvirus type 8 (KS-associated herpesvirus) (see Chapter 155).[45] The incidence of KS has decreased dramatically since the development of effective antiretroviral agents.

The clinical presentation may be that of single or multiple skin lesions and there may be mucosal, visceral and/or lymphatic involvement, particularly in the gastrointestinal tract and pulmonary parenchyma. Clinically, KS has three stages, including macule or patch, plaque and nodule (Fig. 95.9). Regression of KS has been seen in patients treated with effective antiretroviral combinations.

Macule or patch

These lesions are faint pink macules or patches oriented along skin cleavage lines. Initially lesions may be innocuous in appearance and are easily overlooked, being mistaken for bruises, purpura or nevi. Correct recognition of early lesions can be accomplished by performing biopsies for histopathologic examination.

Plaque

In time lesions darken and develop into raised firm indurated plaques. The color is purple to brownish because of the presence of abundant blood vessels, extravasated erythrocytes and siderophages. In some cases, lesions may ulcerate.

Nodule

Nodular lesions are dome-shaped, elevated lesions that are usually purple. On palpation they are firm and may be ulcerated. They may simulate bacillary angiomatosis and pyogenic granulomata.

Treatment

All patients should be started on effective antiretroviral therapy and skin lesions can resolve completely with HAART. For localized cutaneous disease, additional treatment of uncomplicated KS can be performed for cosmetic purposes. Local destructive measures are generally the most effective. Liquid nitrogen cryotherapy, radiation, topical alitretinoin gel and intralesional injections of vinblastine sulfate 2–4 mg/ml and interferon-α-2b have all been used successfully. Pigmentary changes may occur and relapse is common.[46] For disseminated disease, antiretrovirals and chemotherapy is recommended (see also Chapter 94).[47]

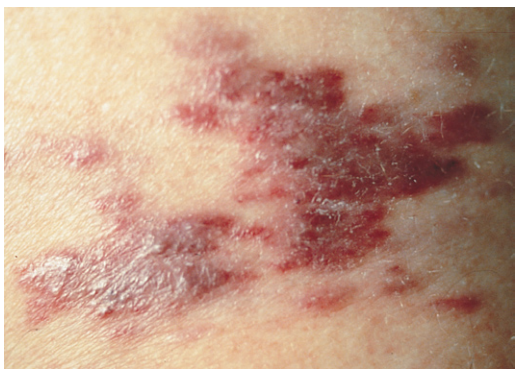


Fig. 95.9 Kaposi's sarcoma, plaque stage. There is an erythematous plaque, which is linear in shape, arranged along skin cleavage lines.

Lymphomas and lymphoreticular neoplasms

A number of different lymphoreticular malignancies of both B and T cells may develop in HIV-positive patients. The majority of these are based in the lymph nodes and reticuloendothelial system, although the skin may be involved primarily or secondarily. Most are advanced at the time of diagnosis and are associated with a short median survival time (see Chapter 94).

Non-Hodgkin's lymphoma with skin manifestations in patients who have AIDS is most commonly of B-lymphocyte origin and mostly high and intermediate grade. Cutaneous T-lymphocyte lymphoma (CTCL), Hodgkin's disease, lymphomatoid granulomatosis and adult T-lymphocyte leukemia/lymphoma have also been reported. The pathogenesis of lymphoreticular neoplasms in these patients is controversial. Chromosomal abnormalities have been encountered and a possible viral etiology has also been suggested.[48] Most cases involve visceral sites and, when the skin is affected, these are usually pink–purple papules or nodules with necrosis. Deep-seated soft tissue involvement may expand superficially, forming dome-shaped nodules that often ulcerate. Hodgkin's lymphoma appears similar to non-Hodgkin's lymphoma, either as diffuse nodular lesions or as a panniculitis. CTCL may have the clinical appearance of mycosis fungoides with widespread erythematous scaly lesions distributed usually on the trunk and resembling 'eczema'. Lesions become red–brown ulcerated plaques and tumors during the more advanced stages of the disease.

The diagnosis of lymphoreticular neoplasms should be based on histopathologic examination of tissue biopsies. In many cases the use of gene rearrangement studies, flow cytometry and DNA analysis is necessary to characterize these neoplasms. Treatment consists of the usual therapy for systemic lymphoma plus antiretrovirals (see Chapter 94).

Other cancers

Several reports in the literature have described an increased incidence of intraepithelial and invasive carcinoma of the anus in patients who have AIDS-associated human papillomavirus (HPV) infection.[17] HIV is associated with increased incidence of high-grade dysplasia and increased risk of progression to invasive squamous cell carcinoma. HPV has been detected in more than 95% of cervical cancers and in more than 50% of anal cancers.

Hair and nail changes

A number of abnormalities of hair and nails may be encountered in HIV-positive patients.

Chronic inflammatory and noninflammatory alopecia has been observed. Alopecia universalis may also develop and is associated with decreased $CD4^+$ counts.[49] Other hair changes that have been observed include premature graying, thinning and diffuse hair loss. Hypertrichosis has also been associated with HIV infection; however, the cause is unknown but is often associated with antiretroviral use.

Nail changes, including yellow discoloration, hyperpigmentation, transverse and longitudinal ridging and decreased size or loss of the lunulae, have all been reported. Longitudinal hyperpigmentation is observed in association with treatment with zidovudine.

Recurrent aphthous ulcers and other oral manifestations

Recurrent aphthous ulcers are usually idiopathic. Occurrence may represent simply as self-limited pinpoint lesions or can be progressively

enlarging destructive lesions with extensive hemorrhage and necrosis. They are frequently recurrent. Pain can be quite severe and esophageal ulceration is well described. Lesions should be cultured for HSV and a biopsy may be needed to rule out malignancy (e.g. lymphoma) or infection. Smaller ulcers may be treated with a topical corticosteroid preparation. Thalidomide, 200 mg/day orally, given for 4 weeks, has been shown to be effective for larger oral and esophageal lesions.[50] It is vital that precautions are taken to avoid exposure to thalidomide during pregnancy.

Oral hairy leukoplakia and intraoral KS may be the first clue to HIV infection.

Intraoral KS may appear alone or be associated with skin or disseminated lesions. It may be flat, raised, solitary or multiple and is red–blue or purple. A biopsy may be required to distinguish it from other vascular or pigmented lesions.

Salivary gland involvement with lymphoepithelial cyst formation can lead to xerostomia and salivary gland enlargement in HIV patients. Labial salivary glands may demonstrate lymphocytic infiltrates similar to Sjögren's syndrome although the infiltrate is composed predominantly of $CD8^+$ T cells.

Complications of antiretroviral agents

With the advent of more effective antiretroviral agents, many of the cutaneous manifestations of HIV discussed previously in this chapter occur less frequently. However, a new subset of dermatologic conditions has arisen related to the use of these drugs, namely cutaneous side-effects of antiretroviral agents. Currently there are five classes of medication used for the treatment of HIV: protease inhibitors (PIs), nucleoside analogue reverse transcriptase inhibitors (NRTIs), non-nucleoside reverse transcriptase inhibitors (NNRTIs), integrase inhibitors and entry inhibitors. Common patterns of drug eruptions include morbilliform exanthem, hyperpigmentation, urticarial eruptions, erythema muliforme minor and, rarely, Stevens–Johnson syndrome.

Morbilliform exanthems are the most common HIV-related cutaneous drug eruptions. They are characterized as a widespread, maculopapular rash that is usually found on the trunk and extremities beginning 2–10 days after drug initiation. They usually resolve on discontinuation of the offending agent. Hyperpigmentation is often related to photosensitivity and/or antiretroviral usage. Manifestations include hyperpigmentation of the palms and soles, as well as pigmented bands in the nails of the fingers and toes. Urticarial eruptions presents as wheals distributed over the face, trunk and extremities. The peak reaction is seen 1–2 days after the drug is initiated and resolves within a week after drug termination.

Erythema multiforme is an acute inflammatory reaction with characteristic patches, papules, macules and plaques that evolve into bull's eye lesions. Stevens–Johnson syndrome is a severe form of erythema multiforme. It is characterized by coalescent skin lesions and involvement of mucosal surfaces. Cutaneous manifestations include variable violaceous macules and patches originating on the face and trunk. Mucosal erosions are seen and the epidermis may peel off in large sheets.

Protease inhibitor-associated skin effects

Fixed drug eruptions have occurred as a result of therapy with saquinavir.[51] Indinavir has been associated with paronychia, alopecia, cheilitis and dry skin. Atazanavir is associated most notably with jaundice since it competitively inhibits an enzyme involved in bilirubin metabolism.[52] Fosamprenavir, darunavir, atazanavir and liponavir/ritonavir have all been associated with mild to moderate morbilliform eruptions lasting for 7–13 days.



Fig. 95.10 Morbilliform drug eruption. There is a diffuse eruption of fine pink macules and papules, which have coalesced, involving the trunk and the extremities. The most common cause of these eruptions is trimethoprim–sulfamethoxazole.

Nucleoside reverse transcriptase inhibitor-associated skin effects

Morbilliform eruptions have been reported with all NRTIs but are uncommon (Fig. 95.10). Two well-known side-effects of zidovudine are nail pigmentation and hypertrichosis. Nail pigmentation may occur on several[53] or all nails of the hands and feet.[54] Emtricitabine is associated with hyperpigmentation of the palms and soles.[55]

Hypersensitivity reactions have been reported with nevirapine. These reactions are associated with fever, malaise, nausea and a mild rash that may progress to more severe cutaneous involvement, and severe fatal Stevens–Johnson reaction may occur.[56] Nevirapine hypersensitivity is more common in women and in patients with $CD4^+$ counts >400 cells/mm^3, when given as initial therapy.

Abacavir can cause a severe hypersensitivity reaction which may even be life threatening, especially if treatment is continued despite the reaction or if the patient is rechallenged with abacavir after resolution of the initial reaction.[57] Abacavir hypersensitivity has been associated with a specific human leukocyte antigen (HLA) haplotype (HLA-B*5701). Avoidance of abacavir in individuals positive for HLA-B*5701 can prevent severe hypersensitivity.

Entry and integrase inhibitor drug eruptions

Enfuvirtide is associated with drug site reactions consisting of erythema, ecchymosis, induration, nodules, cysts, pruritus, tenderness, and/or pain.[78] Rotating injection sites may reduce the severity of the reactions. Skin rash and pruritus have been rarely reported in trials of maraviroc and raltegravir.

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Chapter **96**

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HIV/AIDS-related problems in developing countries

INTRODUCTION AND EPIDEMIOLOGY

As of December 2007, an estimated 33.2 million people (30.8 million adults and 2.5 million children) were infected with HIV.[1] In 2007 alone, 2.5 million new HIV infections occurred worldwide and 2.1 million people died of AIDS. In the developing world, the majority of incident cases occur in young adults. People aged 15–24 years comprise about one-third of those living with HIV/AIDS. In 2007, illnesses associated with HIV/AIDS caused the deaths of approximately 2.1 million people, including an estimated 330 000 children younger than 15 years. More than 95% of these infections and deaths occurred in developing countries. Accompanying the morbidity and mortality borne by those infected with HIV is the dramatic alteration of the social structure attributable to the HIV epidemic. Because of the premature death of HIV-infected parents, 13 million children have been orphaned. The number of orphaned children is forecast to more than double by 2010.[2]

The contrast between the impact of HIV/AIDS in the developed world and in the developing world is striking. Figure 96.1 illustrates the discrepancy between the HIV prevalence in sub-Saharan Africa versus the rest of the world. Whereas AIDS-related mortality is declining in the USA, western Europe and Australia, it has continued to rise rapidly in sub-Saharan Africa, South East Asia and Latin America with only recent reports of optimism resulting from the scale up of antiretroviral therapy (ART).[3–6] One in 200 adults aged 15–49 years is infected with HIV in the USA and Europe, but 10–40% of pregnant women in some parts of sub-Saharan Africa are infected with HIV. Although antiretroviral therapy is given to pregnant women and their newborn infants in developed countries to prevent transmission, most pregnant women who have HIV infection in developing countries receive little or no antiretroviral therapy, creating an enormous discrepancy in perinatal transmission of HIV in these two regions of the world. In addition, recent surveys have demonstrated that nearly 75% of people with HIV infection are aware of their serostatus in the USA and Europe, whereas in a recent national serobehavioral survey in Uganda, only 21% were aware of their own status and 9% knew their partners' status, with the majority of individuals either never being tested or being unaware of their HIV status.[7]

Since the initial recognition of the AIDS epidemic, much has been done to respond to HIV infection by way of prevention and behavioral

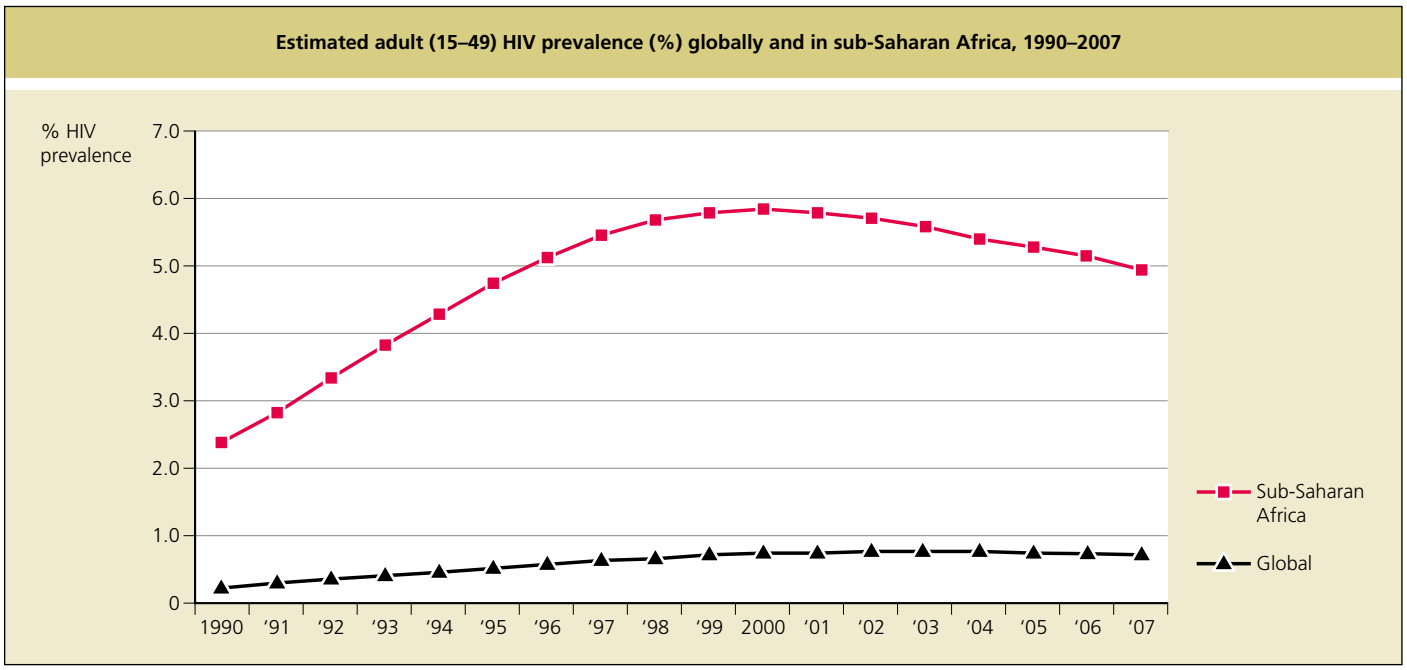


Fig. 96.1 Estimated adult (15–49 years) HIV prevalence (%) globally and in sub-Saharan Africa, 1990–2007. Data from UNAIDS Report 2007.[1]

modification. Government-supported efforts in Thailand, including HIV and AIDS surveillance systems and programs such as the '100% condom use' campaign for commercial sex, have contributed to decreased rates of HIV and sexually transmitted diseases in military recruits.[8] Similarly, in Cambodia the prevalence of HIV among pregnant women declined by almost a third between 1997 and 2000, to 2.3%. Widespread health education, increased use of condoms and voluntary HIV counseling and testing appear to have lowered the prevalence of HIV infection in some areas of sub-Saharan Africa. Recent declines in HIV prevalence among populations in Kenya, Uganda, Zimbabwe and Haiti which appear to be attributable to behavior change offer some encouraging support for the ongoing HIV prevention efforts.[9,10]

However, in the setting of this mature epidemic, declines in HIV seroprevalence have been seen in the presence of stable and high incidence.[10] Death due to HIV disease, rather than a true decrease in the incidence of HIV infection, appears to have contributed most to the reduced HIV prevalence measured in Rakai, Uganda.[11] Figure 96.2 models the lifetime risk of AIDS death for 15-year-old boys based on current HIV prevalence in adults aged 15–49 years. The upper line, based on current HIV prevalence, is compared with the lower line, which assumes that the risk for new HIV infection at each age decreases by half over the next 15 years. This indicates that, even with successful prevention campaigns, without access to treatment the proportion of young people who will die of AIDS is very high.[12]

Despite ongoing prevention efforts, the overwhelming burden of HIV disease is borne by the developing world. The factors responsible for the discrepancies between the developed and developing world in terms of the diagnosis and care of people with HIV infection and the magnitude of the HIV pandemic are multifactorial. Limited access to care, lack of diagnostic equipment and insufficient money to support either prevention or treatment programs are primarily responsible for the continuing rise in morbidity and mortality associated with HIV infection in developing countries. Underlying high prevalence rates of HIV infection and a combination of high-risk behavior and the widespread prevalence of sexually transmitted diseases act synergistically to propel the epidemic further in many areas of the developing world. Furthermore, the coexistence of other endemic diseases that are widely prevalent in developing countries, such as tuberculosis and gastrointestinal infections, complicate the care of people with HIV infection and pose additional problems for the medical personnel caring for them. Recent increases in donor resources for HIV prevention, care and treatment have dramatically changed the previously gloomy picture in developing countries. The rapid scale up of basic care and antiretroviral therapy for people infected with HIV is changing the landscape and providing optimism where previously there was little hope. This chapter reviews some of these aspects as they relate to the care of people with HIV infection living in developing countries.

CLINICAL FEATURES

The clinical manifestations of HIV infection in developing countries are diverse and reflect the wide variety of other endemic diseases within each region.[13] More than 100 pathogens, including viruses, bacteria, fungi, protozoa, helminths and arthropods, have been identified as having caused opportunistic disease in persons with HIV infection. A relatively small number of these pathogens cause a majority of the infections, yet their impact on the health of persons with HIV infection is enormous. Although the reasons for the differences between the spectrum of opportunistic infections observed in developing countries

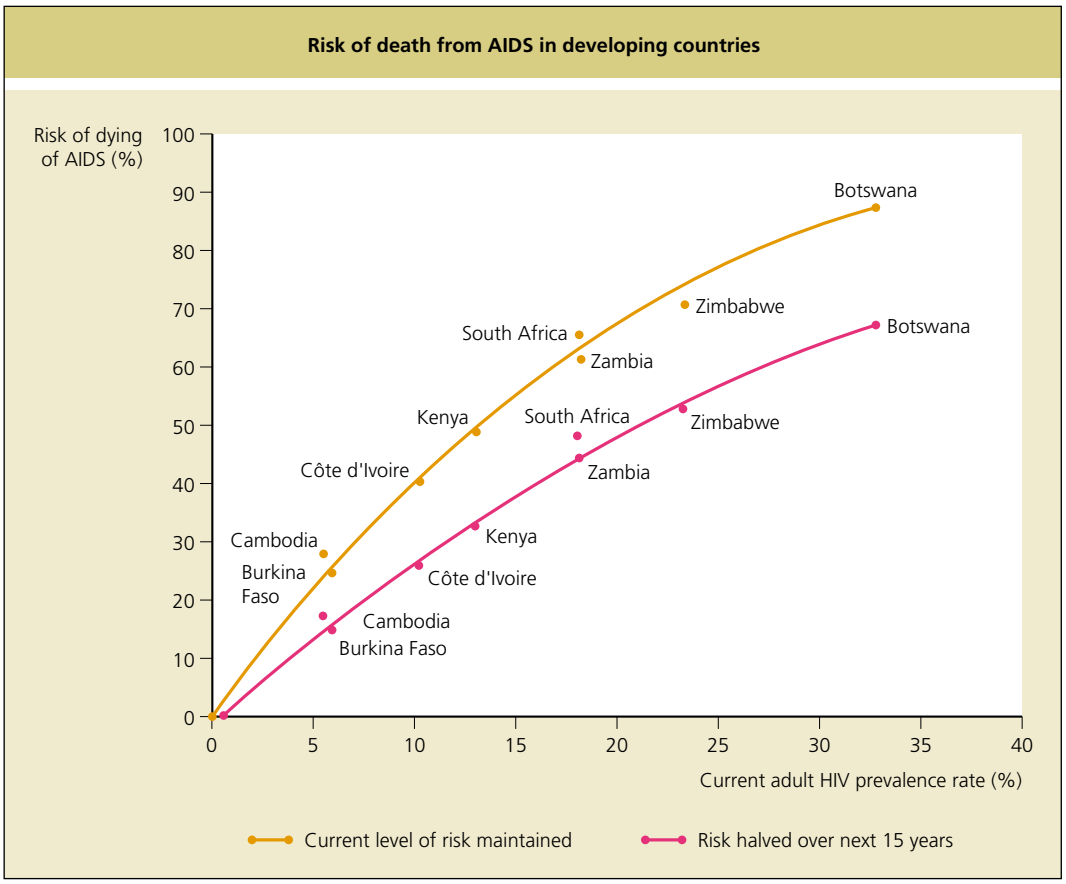


Fig. 96.2 Risk of death from AIDS in developing countries. Lifetime risk of AIDS death among 15-year-old boys, assuming unchanged or halved risk of becoming infected with HIV, in selected countries.

and in developed countries are not completely understood, they are likely to include factors such as the prevalence of pathogens in the environment, social behaviors, ecologic factors that result in exposure to these pathogens, and other undefined factors.

Determining the spectrum of opportunistic infections in a given region requires surveillance systems and diagnostic services that may not be available in many developing countries. For example, opportunistic infections that can be diagnosed with reasonable accuracy by physical examination (e.g. oral candidiasis) or by inexpensive laboratory techniques (e.g. India ink stain of cerebrospinal fluid for *Cryptococcus neoformans*) may be documented more frequently than opportunistic infections requiring more expensive diagnostic technologies (e.g. *Pneumocystis jirovecii* pneumonia, disseminated *Mycobacterium avium* complex (MAC) and cytomegalovirus disease). Because most clinical studies that document opportunistic infections are conducted in urban hospitals, those infected outside of urban centers are often not included in disease surveillance efforts. Furthermore, longitudinal cohort studies are costly to maintain in resource-poor settings. Biases in diagnosing and reporting opportunistic infections may be especially important among socially disadvantaged groups with limited access to diagnostic and health-care services. Finally, differences in clinical definitions may make comparisons between published reports difficult. For these reasons and for others, much less is known about the frequency of different opportunistic infections in the developing world than in industrialized countries.

Although it is clear that HIV infection has a definite impact on a wide variety of microbial agents in developing countries, less is known about the impact of these diseases on HIV infection. Although it has been shown that HIV disease progresses more rapidly in developing countries, recent studies report similar rates of HIV disease progression when compared with the epidemiology of HIV disease in developed countries prior to the introduction of highly active antiretroviral therapy (HAART).[14] Data quantifying the median time from seroconversion to AIDS is limited. Early studies suggested that time to AIDS was much shorter in sub-Saharan Africa and South East Asia. Obstacles included pinpointing the time of seroconversion and reconciling different definitions of AIDS. Similarly, time from seroconversion to AIDS and death has been compared with that seen in the developed world early in the HIV epidemic. In a population-based study in Uganda, the median survival from AIDS to death was 9.3 months but varied according to AIDS-defining illness.[15] Median survival associated with wasting syndrome, Kaposi's sarcoma and esophageal candidiasis was less than 3.5 months, compared with survival longer than 20 months associated with cryptosporidial diarrhea, chronic herpes simplex virus (HSV) infection and extrapulmonary tuberculosis. The median $CD4^+$ lymphocyte count at the time of AIDS onset was 150 cells/mm^3. Notably, the most common opportunistic infections in resource-poor settings, including tuberculosis and endemic bacterial infections, develop at $CD4^+$ lymphocyte counts higher than 150 cells/mm^3. A recent observational study from Uganda revealed that the median time from seroconversion to death was 9 years with a median time to $CD4^+$ lymphocyte count of 200 was 6.3 years.[16] Evidence from Uganda also suggests that the subtype of HIV affects disease progression with subtype D progressing to AIDS more rapidly than subtype A (6.5 versus 8.0 years).[17] The recent scale up of ART access and basic HIV care including trimethoprim–sulfamethoxazole prophylaxis should ultimately have a major impact on survival in countries currently suffering high AIDS-related morbidity and death.

In addition to the clinical spectrum of disease defined by opportunistic infection, co-infection with endemic diseases and resulting immune activation may increase susceptibility to HIV infection. A person whose immune system is activated at the time of exposure to HIV may be more susceptible to infection with HIV. In this regard, the success of an aggressive sexually transmitted disease (STD) treatment program in Tanzania in decreasing the incidence of new HIV infections may be due in part to the removal of immune-activating factors. A similar community-based study conducted in Uganda and designed to evaluate the effect of STD treatment on the development of incident HIV infection found no difference.[18] The lack of a positive effect may be related to the effect of anthelmintic therapy provided to the control group. The eradication of helminths may have had an effect similar to the treatment of STDs. Furthermore, the trial did not treat for genital ulcerative disease due to HSV infection. Unfortunately, a recent clinical trial treating HSV infection among high risk women did not show any efficacy in preventing HIV acquisition. Current clinical trials are evaluating the effect of treating genital HSV infection on HIV infection and transmission as well as HIV disease progression. Chronic immune activation and infections prevalent in the developing world appear to enhance the pathogenesis of HIV.[19] Thus, both the rate of spread of the HIV epidemic as well as the clinical course in the developing world may be greatly influenced by the underlying state of heightened immune activation that exists in many people in these countries.

Tropical diseases may directly lead to an increased risk of infection with HIV. Treatment of severe anemia induced by malaria has led to HIV infection by transfusion. Female genital schistosomiasis, like other genital inflammatory conditions, may increase the efficiency of HIV transmission. Several drugs used in the treatment and prophylaxis of tropical diseases have an immunosuppressive effect and may thereby influence susceptibility to HIV. Specific interactions between HIV and infectious diseases in developing countries are listed briefly below.

Mycobacterial infections

Tuberculosis (see also Chapter 93)

Tuberculosis is the most important severe opportunistic infection observed among patients with HIV infection in developing countries because it occurs frequently, is transmissible to both people with HIV infection and uninfected persons, can be readily treated and prevented. Globally, tuberculosis is the leading cause of death among people with HIV infection, accounting for a third of deaths due to AIDS. The World Health Organization (WHO) estimates that the number of people infected with the tubercle bacillus is one-third of the world population; throughout the world a new tuberculosis infection occurs every second.[20] The greatest number of tuberculosis infections occur in people living in Asia, sub-Saharan Africa and Latin America, where about half of the adult population is infected. These are the same areas in which HIV continues to increase steadily. The WHO considers HIV as the single most important factor driving the increase in tuberculosis incidence in Africa over the past 10 years.

The increase in tuberculosis attributable to HIV has resulted in increased demand for already overburdened tuberculosis programs. During the initial stages of the HIV epidemic, when incidence rates increased dramatically, tuberculosis rates increased as well. In developing countries where young adults have high rates of infection with both HIV and tuberculosis, the risk of co-infection is correspondingly high. Worldwide, an estimated 640 000 incident cases of tuberculosis (8%) occur in the setting of HIV infection. Although the largest group of co-infected individuals lives in India, the burden of HIV infection per capita is highest in sub-Saharan Africa, with 32% of tuberculosis cases co-infected with HIV.

Infection with HIV and tuberculosis has become a particular problem in many African countries. The rates of incident tuberculosis continue to rise in Africa because of the HIV epidemic while rates are falling elsewhere because of the HIV epidemic.[21] In the central African countries of Zimbabwe and Malawi, the annual incidence of tuberculosis doubled in the 5 years 1985–90 and 20–44% of AIDS patients developed tuberculosis during the course of their infection. In contrast, only 4% of AIDS patients in the USA have tuberculosis. In a study of tuberculosis treatment in Uganda, a high prevalence (49%) of *Mycobacterium africanum* isolates was identified in patients with HIV infection who had pulmonary tuberculosis.[22] In Uganda, the prevalence of HIV infection among tuberculosis patients was 5.9 times greater than that among patients who had inactive tuberculosis. In Lusaka, Zambia, as many as 37% of hospitalized children who had tuberculosis were infected with HIV, compared with 11% of nontuberculosis controls. Cohort studies have also shown that

the risk of developing active tuberculosis among persons infected with *Mycobacterium tuberculosis* is much higher in persons with HIV infection than in HIV-seronegative persons. For someone who is co-infected with HIV and tuberculosis, the annual risk of developing active tuberculosis ranges from 5% to 15%, whereas the lifetime risk among people who have tuberculosis in the absence of HIV infection is only 5–10%.

During the period 1997–2004, the estimated number of incident cases of tuberculosis increased from 8.0 million to 8.9 million, with an estimated 1.7 million deaths occurring in 2004 due to tuberculosis.[23] The progressive increase is largely due to the HIV/AIDS pandemic. In patients dying with HIV disease in Abidjan, Côte d'Ivoire, tuberculosis was present in more than half of those who had AIDS and was responsible for one-third of the deaths. The importance of tuberculosis was demonstrated in an autopsy study among patients dying of pulmonary disease in Abidjan; 40% of the HIV-positive patients died of tuberculosis, compared with only 4% of the HIV-negative patients. In Mexico, disseminated tuberculosis was found at autopsy in 25% of patients who had AIDS; this compares with 6% of patients in the USA and 5% of patients in Italy. This observation is consistent with the higher incidence of pulmonary tuberculosis in Latin America than in the USA, as well as with the higher incidence of tuberculosis in the USA among foreign-born people and people of Latin American or Asian descent.

A prospective cohort study conducted in South Africa demonstrated that the increased mortality associated with tuberculosis was observed only in patients with a $CD4^+$ lymphocyte count greater than 200 cells/mm^3 and in those without AIDS at baseline.[24] The authors proposed that the immune activation due to tuberculosis increased HIV replication and accelerated HIV disease progression.

Diagnosis, treatment and prophylaxis of HIV-associated tuberculosis are discussed in detail in Chapter 93. Meta-analyses of trials of isoniazid prophylaxis of HIV-positive populations with a positive tuberculin skin test showed a definite reduction in the development of active tuberculosis in the treated group compared with the placebo group during a period of 2–3 years.[25,26] No significant protection could be demonstrated in the groups with skin test anergy who were included in the same trials. Thus, the benefit of prophylaxis for populations that are already anergic as a result of immunosuppression is more difficult to evaluate because they indicate either no previous exposure to tuberculosis or previous infection with loss of immunologic reactivity. As with many other diseases, the cost of medication needed to carry out effective treatment of tuberculosis is lacking in many developing countries where the problem is greatest. Furthermore, patient compliance may be a limiting factor in treatment regimens of multiple drugs for periods of several months. Research priorities include the determination of the best method for detection of active tuberculosis in resource-poor settings, and optimum duration of preventive therapy.

Resistance to antituberculosis drugs has emerged as a major public health problem, particularly in settings with high HIV/tuberculosis co-infection rates. Extensively drug-resistant tuberculosis (XDR), defined by resistance to at least isoniazid and rifampicin in addition to any quinolone and at least one second-line injectable agent (capreomycin, amikacin, kanamycin). In a recent outbreak of tuberculosis among HIV-infected patients in South Africa, XDR cases comprised 24% of all multidrug-resistant isolates. These cases were virtually untreatable and 52 of 53 cases died in a median of only 16 days.[27] The strain of tuberculosis identified among these patients had the same genetic background material, suggesting that recent, possibly nosocomial transmission may have contributed to this outbreak. XDR tuberculosis has now been identified in several countries and poses a major public health threat to the fight against HIV/AIDS and tuberculosis, highlighting the need for improved diagnostic strategies, infection control measures and newer antituberculosis drugs.

In order to prevent active tuberculosis, up to 70% of the world's children are vaccinated with bacille Calmette–Guérin (BCG). Unfortunately, the efficacy of BCG in HIV-infected populations is unclear. One study found no benefit of vaccination in HIV-seropositive children; another study found a benefit of childhood vaccination in HIV-seropositive adults and protection from disease caused by *M. tuberculosis*. Recent data suggesting a beneficial effect of early BCG vaccination on mortality from all causes in children not infected with HIV indicate that measures of benefit in HIV-seropositive people need to be broader than mere prevention of tuberculosis. The WHO recommends that BCG should be given to people who have asymptomatic HIV infection in areas with a high risk of tuberculosis infection. In areas where the risk of tuberculosis is minimal, BCG is not recommended, particularly for those who are infected with HIV. Recombinant BCG vector-based vaccines are currently being evaluated in animal models for potential use in vaccination against HIV.

Mycobacterium avium complex

Although MAC is very common in advanced HIV infection in the USA and Europe, it has rarely been documented in developing countries. In one study in Uganda, none of 95 blood cultures from severely ill patients who had advanced AIDS was positive for *M. avium*; neither were any of 165 mycobacterial sputum cultures from HIV-seropositive and HIV-seronegative patients at the same hospital. In Côte d'Ivoire, none of 202 blood cultures from HIV-positive adult inpatients was positive for *M. avium*, whereas 4% grew *M. tuberculosis*. Intestinal biopsies from 98 Ugandan, Zairian and Zambian patients who had chronic HIV-related enteropathy yielded histology suggestive of *M. avium* infection in only one case. Similarly, autopsies on 78 HIV-seropositive children in Côte d'Ivoire revealed no evidence of *M. avium* infection. In Kenya and Mexico, *M. avium* has been documented in 6% of patients hospitalized with late-stage HIV disease; in Brazil it has been documented in 18% of 125 hospitalized patients. A recent study conducted in South Africa demonstrated a 10% prevalence of disseminated MAC in 10% of hospitalized patients with a $CD4^+$ lymphocyte count of less than 100 cells/mm^3.[28]

The reasons for the low frequency of disseminated disease caused by MAC in the developing world are unclear, but there are many possibilities, including less exposure to MAC, exposure to different variants of MAC, differences in host susceptibility, greater acquired immunity to mycobacteria, earlier death from infection with more virulent pathogens, and diagnostic difficulties. There may be greater acquired immunity to mycobacterial disease through BCG vaccine or previous infection with *M. tuberculosis*, but the reported BCG coverage (50%) and purified protein derivative reactivity (82%) in Uganda seem unlikely to explain the lack of any disseminated MAC.

Mycobacterium leprae

To date, there is little evidence that HIV infection has a profound effect on the frequency of *M. leprae* infection (see Chapter 103). Several studies have examined serology for HIV in newly diagnosed leprosy. In one study in Uganda of 189 new cases of leprosy matched for age, sex and district of residence, no significant difference in overall rates of HIV seropositivity was found between the patients who had leprosy (12% HIV seropositive) and the controls (18% HIV seropositive). Interestingly, HIV seropositivity was more frequent among the multibacillary cases than the paucibacillary cases. An association between HIV and leprosy was seen in studies conducted in Zambia and Nigeria. Studies in Kenya and Nigeria both support the hypothesis that HIV infection favors the multibacillary form of leprosy. A different clinical association was noted in Zambian leprosy patients who had active neuritis. The study suggested that HIV-positive patients have poorer recovery of nerve function than controls after treatment with corticosteroids.

Thus, there appears to be no striking evidence that HIV infection has an adverse effect on the course of leprosy. Multibacillary disease could possibly develop under the influence of HIV infection but, because leprosy is chronic and slow in progression, it is difficult to discern the influence of HIV on leprosy directly.

Nonmycobacterial pulmonary infections

Bacterial pneumonia

Investigations of patients with HIV who have pulmonary disease demonstrate that bacterial pneumonia caused by *Streptococcus pneumoniae* and *Haemophilus influenzae* occur as frequently in developing countries as they do in the developed world. In a study in Nairobi, Kenya, 79 episodes of invasive pneumococcal disease were seen in 587 HIV-positive women, whereas there was only one episode in 132 HIV-seronegative women.[29] Serotyping revealed that most recurrent events were related to re-infection. A wide spectrum of HIV-related pneumococcal disease was seen; 56% of cases were pneumonia, 30% were sinusitis and 11% were occult bacteremia. The mean $CD4^+$ lymphocyte count was 302 cells/mm^3 at first presentation and 171 cells/mm^3 for recurrent episodes. In this study *S. pneumoniae* caused more disease at an earlier stage of HIV immunosuppression than did *M. tuberculosis*.

The importance of pneumococcal disease in the host with HIV infection cannot be underestimated. Invasive pneumococcal disease has been shown to cause significant morbidity and mortality. The use of the pneumococcal polysaccharide vaccine to prevent infection in people with HIV infection has been evaluated and generally recommended by the Centers for Disease Control and Prevention. In light of the increased prevalence of antibiotic resistance and ease of administration, this vaccine would appear to be a prudent use of resources. However, studies have shown that the polysaccharide vaccine is not beneficial in individuals with HIV infection in African populations. Conjugate pneumococcal vaccines have proven to be more immunogenic in both HIV-positive and HIV-negative children in the Gambia and South Africa and hold greater promise to reduce the incidence of invasive pneumococcal disease.

Pneumocystis jirovecii pneumonia

Several opportunistic infections that are common in developed countries are rarely identified in developing countries. For example, in Abidjan, *P. jirovecii* pneumonia accounts for only 8% of deaths from HIV-associated pulmonary disease and only 2% of all HIV-associated deaths. A recent report from Zimbabwe demonstrated that even after selecting patients who had abnormal chest radiographs that were consistent with *P. jirovecii* pneumonia, tuberculosis was still a more frequent diagnosis than *P. jirovecii* pneumonia.

Although *P. jirovecii* pneumonia appears to be infrequent in Africa and Asia, it is relatively common in Latin America and Caribbean countries, with rates similar to those documented in the USA. A retrospective case series in a clinic in London, UK, found a significantly higher rate of *P. jirovecii* pneumonia as a presenting diagnosis in non-Africans (34%) than in Africans (17%). Prevalence rates are also low in Haiti, with a case series incidence of less than 10%. Other reported rates include 7–25% in Thailand, 20–24% in Mexico and 32–45% in Brazil.

The reasons for lower rates of *P. jirovecii* pneumonia in parts of the developing world are unclear. Possible explanations include less environmental exposure to *P. jirovecii*, exposure to different strains of *P. jirovecii*, differences in host susceptibility, earlier deaths in patients in the tropics who have AIDS owing to exposure to more virulent organisms, and diagnostic difficulties. When it does occur, the clinical presentation of *P. jirovecii* pneumonia in developing countries appears to be similar to that in industrialized countries. Frequent co-infection with tuberculosis may obscure the diagnosis. The diagnosis and treatment of *P. jirovecii* pneumonia are discussed in Chapter 91.

Diarrheal disease

In addition to tuberculosis, one of the more common clinical syndromes seen in persons with HIV infection in developing countries is a diarrhea-wasting syndrome, referred to as 'slim disease'. Diarrhea lasting longer than 1 month occurs in up to 50% of patients in Africa who have AIDS, a rate that is more frequent than that observed in persons with HIV infection in industrialized countries. The diarrhea is usually intermittent and not associated with blood or mucus, and it is only rarely secretory in nature. In one-third to two-thirds of patients who have diarrhea in Uganda, Zaire and Zambia, no cause was found despite detailed examinations. In other studies, pathogens (including cryptosporidia, microsporidia, *Shigella* spp., *Salmonella* spp. and *Campylobacter* spp.) have been identified with frequencies of 7–48%.

Other diagnoses are also common among patients who have such profound wasting. In an autopsy study in Côte d'Ivoire, 44% of patients dying with HIV wasting syndrome had disseminated tuberculosis, compared with 25% without the syndrome. A chronic fever syndrome is also frequently associated with tuberculosis and nontyphoid salmonellosis. Often there is very little that can be done for patients who have this syndrome except to provide nutritional support.

Protozoan infections

Toxoplasmosis

Toxoplasma gondii is a common opportunistic infection in both developed and developing countries, and the incidence is proportional to the prevalence of latent infection in the population at risk for HIV. A higher prevalence of cerebral toxoplasmosis has been documented among Latin American patients than in the USA, which is consistent with the higher underlying prevalence of toxoplasmosis in Latin America. Although there is a suggestion that up to 50% of seropositive AIDS patients in some parts of the world may develop toxoplasmal encephalitis, its frequency in developing countries is unclear because of limited diagnostic capabilities. Autopsy series that have included examination of the brain have suggested disease prevalence rates in late-stage AIDS patients of 15% in Abidjan, 25% in Mexico City and 36% in Kampala. For more detailed information on toxoplasmosis, see Chapters 91 and 106.

Visceral leishmaniasis

The overlap of visceral leishmaniasis and AIDS is increasing because of the spread of the AIDS pandemic to rural areas and the spread of visceral leishmaniasis to suburban areas. Consequently, cases of co-infection with *Leishmania* spp. and HIV are becoming more frequent, with important clinical, diagnostic, chemotherapeutic, epidemiologic and economic implications. Co-infection with *Leishmania* spp. and HIV is now considered an 'emerging disease', especially in southern Europe, where 25–75% of adult visceral leishmaniasis cases are related to HIV infection and 1.5–9% of patients who have AIDS suffer from newly acquired or reactivated visceral leishmaniasis. The WHO reviewed 692 retrospective cases that occurred between 1985 and 1996 in southern Europe and eastern Africa.[30] Of the cases of co-infection, 90% were observed in patients with $CD4^+$ lymphocyte counts of less than 200 cells/mm^3. Bone marrow aspiration was the most frequently used technique for parasitologic diagnosis. In two-thirds of these cases, the diagnosis of HIV was made before the diagnosis of leishmaniasis.

Multiple visceral locations outside of the reticuloendothelial system are frequently involved during co-infection; these locations include the blood, skin, gastrointestinal tract, lungs and central nervous system. Because of the high frequency of leishmaniasis in the peripheral blood of these patients, transmission via blood or needles, particularly among intravenous drug users, is a major problem.

The *Leishmania* spp. frequently involved in HIV infection are those that cause visceral disease, such as *Leishmania donovani* and *L. infantum* in Asia, southern Europe and Africa, and *L. chagasi* in Latin America. Cutaneous leishmaniasis has a much wider geographic distribution than visceral disease but it is only rarely involved as a complication of HIV infection. Classic visceral leishmaniasis documented in patients

with HIV infection is probably caused by reactivation of a latent infection, owing to increasing immunosuppression. In one study, the CD4+ lymphocyte count was less than 200 cells/mm³ in more than 75% of the patients with HIV infection in whom visceral leishmaniasis was diagnosed, and fewer than 5% of patients had counts of 500 cells/mm³ or greater.

The clinical presentations of visceral leishmaniasis in HIV are quite similar to that described in HIV-negative patients; clinical features include weight loss, fever, pancytopenia and hepatosplenomegaly. Diagnosis is based on a high index of suspicion in a person who has resided in or traveled to an endemic area, and treatment is similar to that employed in patients without HIV infection who have leishmaniasis (see Chapter 117). In Ethiopia, a recent clinical trial evaluated the treatment of visceral leishmaniasis in patients with and without HIV infection. Those co-infected had a greater mortality (33.3% vs 3.6%) and relapse rate (16.7% vs 1.2%). The authors expressed the concern that HIV-positive patients with relapsing visceral leishmaniasis could serve as a reservoir of resistant organisms. Treatment guidelines support the use of two drug combination therapy.

Trypanosomiasis

Trypanosoma cruzi, the cause of Chagas disease, infects millions of people in Latin America. Reports from Argentina, Brazil and Chile have described clinical and laboratory findings in about two dozen patients co-infected with HIV and *T. cruzi*.[31] These reports suggest that Chagas disease may result from reactivation of latent *T. cruzi* infection and that clinical manifestations such as meningoencephalitis may be more frequent and more severe in those infected with HIV. Activation of *T. cruzi* infection by HIV or AIDS usually presents with central nervous system manifestations, but in one review trypanosomes were demonstrated in the blood of five of six cases in whom the examination was done. In one pathologic study of 23 cases, acute myocarditis was frequently noted in those cases that were autopsied, but no information was presented as to whether clinical evidence of myocarditis was present during life.

Malaria

Initial studies examining the effect of HIV on the course of malaria found no changes in the severity, incidence or successful treatment of malaria (see Chapter 111). This may have been due to the complex immune response to malaria, which is not easily perturbed by a predominantly T-cell immunodeficiency. In one study of patients in Burkina Faso, investigators demonstrated a preservation of some components of the malaria-specific immune response in AIDS patients who were co-infected with *Plasmodium falciparum*.

Recently, a complex interplay has been found between malaria and HIV, challenging the apparent lack of a significant interaction. In Uganda, a hospital-based case-control study, a rural population-based cohort study and an urban clinic-based cohort study all showed that HIV infection was associated with increased frequency and severity of clinical episodes of malaria parasitemia.[32–34] The effect increased with declining immunosuppression as indicated by decreasing CD4+ lymphocyte counts. The interplay between malaria and HIV appears to vary with the dynamics of malaria transmission. In regions with unstable malaria transmission, HIV infection is a risk factor for severe malaria in both young children and adults.[35,36] In regions of holoendemnicity, however, HIV infection appears to have only a modest effect on the risk of parasitemia and clinical malaria among semi-immune adults. Two recent studies have shown that infant mortality is higher in babies born to mothers who are co-infected with placental malaria and HIV. It was also shown that HIV infection impairs the pregnant woman's ability to control *P. falciparum* infection. Thus, there is an interaction between HIV and malaria in the placenta. In addition, malaria can contribute to the increased spread of HIV, owing to the need for more frequent transfusions to treat the anemia of malaria.

Enteric parasitic infections

Enteric parasitic infections such as isosporiasis and cryptosporidiosis may be reported in as many as 5–10% of patients who have AIDS in the tropics, compared with 0.2% of patients who have AIDS in the USA. Reports have also shown that the risk of isosporiasis among residents of the USA with AIDS is higher among those born in Latin America and Haiti than among those born in the USA. In Zambia, evaluation of persistent diarrhea in patients with AIDS revealed cryptosporidiosis (7%), microsporidiosis (16%), isosporiasis (37%) and no etiology (40%); treatment with albendazole resulted in a complete or partial response in 60% of those shown to have enteric parasitic infection.[37]

Penicillium marneffei

A common fungal pathogen in AIDS patients in South East Asia is *Penicillium marneffei*.[38] It causes the third most common opportunistic infection in HIV disease in northern Thailand, after extrapulmonary tuberculosis and cryptococcal meningitis. *P. marneffei* was first isolated in 1956 and infection was a rare event before the arrival of the AIDS pandemic in South East Asia. Since then hundreds of cases have been diagnosed, mainly in southern China, northern Thailand, Hong Kong, Vietnam, Singapore, Indonesia and Myanmar. The environmental reservoir of *P. marneffei* is unknown, but the organism has been isolated from the organs, feces and burrows of three species of bamboo rat. Exposure to soil appears to be a key factor in transmission.

Disseminated *P. marneffei* infection is characterized by fever, anemia, weight loss and papular skin lesions. Other frequent signs and symptoms include cough, generalized lymphadenopathy, hepatomegaly and diarrhea. The most common cutaneous manifestation is a generalized papular rash with a central umbilication that resembles the lesions of molluscum contagiosum. These are predominantly found on the face, scalp and upper extremities, but occur throughout the body. Chest radiographs are frequently abnormal, with diffused reticulonodular or localized alveolar infiltrates.

The mean duration of illness before presentation is 4 weeks. The incubation period of disseminated disease is unclear and the disease may be a result of reactivation of latent infection as opposed to new infection or re-infection. However, the development of clinically active disease within weeks of exposure in endemic areas, and the reports of children who have vertically transmitted HIV infection developing disease in the first months and years of life, demonstrate that primary infection can quickly lead to disseminated disease. Finally, the pronounced seasonal variation in disease incidence implies an important role for exogenous re-infection and the expression of disease with *P. marneffei* in AIDS patients in endemic areas. In addition to endemic areas, travelers from regions where *P. marneffei* is not endemic have become infected with this pathogen while traveling in South East Asia. Diagnosis and treatment are discussed in Chapter 91.

Other opportunistic infections

Other opportunistic infections, which may be similar in all areas of the world, include oral and esophageal candidiasis, cryptococcosis, cytomegalovirus infection and Kaposi's sarcoma. Herpes simplex virus infection, herpes zoster and cerebral toxoplasmosis also appear to be relatively common in most areas where diagnostic equipment is readily available. It should be noted that regional variations in frequencies of these diseases do exist within developing countries. Cryptococcosis accounted for only 2% of AIDS deaths in Abidjan but is more common in central and southern Africa.

Mycobacterial infections apart from tuberculosis, such as *Mycobacterium kansasii*, have been longstanding health problems among miners in South Africa and may now be emerging as HIV-associated infections in those miners with HIV infection.

Endemic Kaposi's sarcoma has a striking geographic distribution, being most common in central Africa. Kaposi's sarcoma associated

with HIV is likely to have a similar heterogeneous disease frequency, although the incidence of Kaposi's sarcoma has increased in all countries in which HIV disease occurs. The epidemiology of the new human herpes virus, HHV-8, the viral agent causing Kaposi's sarcoma, has been the subject of intensive investigation since its discovery in 1994. Serologic studies have revealed a worldwide geographic distribution of HHV-8 with the highest seroprevalence (40–60%) in sub-Saharan Africa (see Chapter 155).

HIV TESTING

Currently, HIV testing in the developing world is done for surveillance, prevention and identification of those in need of basic care and ART. Despite improvements in the access to testing, most people still do not know their HIV status. In a recent nationwide sero-behavioral survey in Uganda, only 27% of individuals knew their HIV status. The recent efforts to scale up voluntary counseling and testing in conjunction with the expansion of antiretroviral treatment programs has greatly increased the access to HIV testing in the developing world. Unfortunately, most people still do not know their HIV status. In a recent nationwide serobehavioral survey in Uganda, only 21% of individuals knew their HIV status.[7] Rapid test formats for HIV detection of antibody have also helped improve access to HIV testing by removing the sophisticated laboratory requirements, allowing same-day provision of test results and eliminating the need for subsequent follow-up.

Despite improvements in the access to testing, the majority of people with HIV infection in the developing world do not know their HIV status. There are important reasons for knowing one's HIV status. The earlier people know they are infected, the greater the opportunity for them to access treatment, some forms of which are not expensive, and to apply pressure on communities and governments for improved access to care. The earlier people are aware of their infection, the better able they are to make informed and responsible decisions about child-bearing and avoiding transmission to spouses or partners, and to make plans for family welfare before they become ill or die. Furthermore, the one important benefit of self-knowledge of HIV status is that it helps unmask the invisible epidemic and permits a genuine community response. If people become aware of their infection early on, while they are still relatively healthy, this gives them time and energy to support one another as well as to alert the community to the epidemic.

However, these benefits to individuals, families and communities are realistically achievable only where people feel safe in finding out whether they are infected. Efforts by governments and civil society to combat rejection and discrimination directed at people who have HIV are vital (UNAIDS).

In 1998, WHO and UNAIDS issued revised guidelines for the selection and use of HIV antibody tests applicable for use in developing countries.[39] The three main objectives for HIV antibody testing are:

- screening of blood and blood products;
- unlinked and anonymous testing for the purpose of monitoring prevalence and trends of HIV infection; and
- diagnosis of HIV infection among asymptomatic people and those with clinical signs and symptoms suggestive of HIV infection.

There are three strategies recommended by the WHO. Strategy 1 applies to blood safety and surveillance in settings where HIV prevalence exceeds 10%; all serum and plasma is tested with one enzyme-linked immunosorbent assay (ELISA) or rapid assay. Serum that is reactive is considered HIV antibody positive. This strategy can be used for screening blood donors to protect the blood supply but it should not be used for notification of the blood donor unless strategy 2 or 3 is implemented.

Strategy 2 includes testing serum by one ELISA or rapid assay and, if it is reactive on the first assay, it is then retested with a second ELISA or rapid assay based on a different antigen preparation or a different test principle. Concordant results after repeat testing will indicate a positive or negative result. If the results of the two assays remain discordant, the specimen is considered indeterminate. Strategy 2 is recommended for surveillance, particularly when testing populations with a low prevalence of HIV, and for diagnosis where the prevalence of HIV among asymptomatic populations exceeds 10%. The additional test in strategy 2 compared with strategy 1 is necessary in order not to overestimate the HIV prevalence of such regions. Strategy 2 is also recommended for notification of the blood donor, particularly in developing countries, where strategy 2 is more cost-effective than strategy 3.

Strategy 3 is similar to strategy 2, except that it requires a third test if the serum is found to be reactive on the second assay. The three tests in this strategy should be based on different antigen preparations or different test principles. A specimen is considered to be antibody positive if it is reactive on all three assays. If the serum is discordant on any of the three assays, it is considered to be indeterminate. This strategy is recommended for diagnosis in settings where HIV prevalence is less than 10%.

In the selection of HIV antibody tests for use in strategies 2 and 3, the first test should have the highest sensitivity, whereas the second and third tests should have higher specificity than the first. The number of initial discordant or indeterminate results should not exceed 5%.

An HIV test kit bulk purchase program has been established by the WHO in collaboration with UNAIDS in order to provide national AIDS control programs that use tests giving the most accurate results at the lowest possible cost.

PREVENTION OF HIV INFECTION

Although the epidemic continues to spread throughout the developing world, it is also likely that increased preventive efforts could effectively limit the magnitude of the epidemic. For example, in those countries where the epidemic is still in an expansion phase but is not yet fully visible, the public health response is likely to have a decisive influence on its course. There is every reason to believe that the course of the pandemic could still be altered profoundly by the introduction of HIV prevention strategies that are within the technical reach of all countries. Several successes in slowing the HIV pandemic have provided encouragement and further stimulus for improved programs. Aggressive treatment of STDs coupled with a program of condom distribution has already been shown to be effective in decreasing HIV incidence rates in populations at high risk for HIV in sub-Saharan Africa and Asia. Needle-exchange programs have helped to stabilize and in some cases reduce HIV incidence among intravenous drug users in selected countries. HIV incidence rates can be reduced by an estimated 50% with adequately supported prevention programs. In Asia alone this could mean the prevention of several million AIDS deaths among young adults in their most productive years.

While we await the availability of a vaccine and the effect of therapeutic interventions, a primary prevention strategy must be focused on educational efforts to influence social, cultural and behavioral factors. To control the AIDS epidemic, countries will need not only to promote individual behavior change but also to address the related problems of social disruption associated with mounting unemployment, accelerated urbanization, commercial sex, rapid decline in health services and drug abuse. Fundamental social changes, such as improving the social status of women, will be required if AIDS control efforts are to succeed. In view of the rapid pace of HIV transmission in sub-Saharan Africa and Asia, implementation of these principles of AIDS prevention and care is needed urgently.

Male circumcision has emerged as a new, highly effective HIV prevention measure. Clinical trials from Uganda, Kenya and South Africa have all revealed the protective efficacy of male circumcision for HIV prevention, reducing the risk of HIV acquisition by about 60%. Access to safe male circumcision services has been endorsed by the WHO, UNAIDS and donor communities as an important component in the fight against the spread of HIV/AIDS. Several countries including Uganda, Rwanda and Kenya have incorporated male circumcision into their HIV prevention efforts and scale up of male circumcision services is currently underway.

PREVENTION OF OPPORTUNISTIC INFECTIONS

Those opportunistic infections associated with the greatest degree of morbidity and mortality, as well as available preventive and therapeutic options, include tuberculosis and bacteremia due to non-*typhi Salmonella* spp. and *S. pneumoniae*.[40] Tuberculosis and other endemic bacterial infections regularly occur in patients with HIV infection but without profound immunosuppression. Primary preventive therapy against tuberculosis provides an important first line of defense against the development of disease in earlier stages of HIV infection.

Recent studies have evaluated the role of trimethoprim–sulfamethoxazole (TMP–SMX) prophylaxis in reduction of infections other than *P. jirovecii* pneumonia in patients with HIV infection. A randomized trial conducted in Côte d'Ivoire showed that events leading to hospitalization or death were 43% lower in African adults with early symptomatic HIV disease treated with TMP–SMX than with placebo. The beneficial effect was due to activity of TMP–SMX against bacterial infections, malaria and isosporosis. A recent prospective cohort study conducted by the Centers for Disease Control in Uganda illustrated that TMP–SMX prophylaxis to HIV-positive individuals resulted in a 46% reduction in mortality with lower rates of malaria, diarrhea and hospital admission. Beneficial effects were also seen on the rate of $CD4^+$ T-cell count decline and HIV viral load.[41] These findings have resulted in widespread introduction of TMP–SMX as part of basic HIV care in developing countries. Concerns cited as limitations to the use of TMP–SMX include an anticipated increase in antimicrobial resistance in pathogens such as nontyphoidal salmonellas and the pneumococcus. Of additional concern is the potential for cross-resistance between pyrimethamine and trimethoprim, as sulfadoxine–pyrimethamine is one of the most widely used treatments against *P. falciparum*.

HIV TREATMENT IN DEVELOPING COUNTRIES

On April 22, 2002, the WHO announced the first treatment guidelines for children and adults infected with HIV in the developing world.[42] This action set the stage for a tremendous increase in access to care; WHO estimated that at least 1 330 000 people needing treatment had access to antiretroviral medicines at the end of 2005. Although far below the target of 3 million, the momentum has continued, with some countries greatly expanding the numbers on treatment and hopefully able to achieve the revised WHO goal of universal access by 2010. The treatment guidelines were accompanied by the expansion of the Essential Medicines List to include antiretrovirals in addition to nevirapine and zidovudine, which were previously listed for the prevention of mother-to-child HIV transmission. The WHO public health approach to antiretroviral therapy was updated in 2006 and presents a standardization and simplification of ART. However, the complexity of providing treatment regimens to millions of people in resource-poor areas extends well beyond access to medications.

In order to ensure the safe and appropriate use of antiretrovirals, resources for evaluating patients prior to the initiation of therapy and monitoring for response to therapy, as well as for the development of potential side-effects, must also be available. The WHO recommends initiation of therapy based on clinical staging and $CD4^+$ lymphocyte count or total lymphocyte count if $CD4^+$ lymphocyte count is not available:

- WHO stage I includes those individuals with HIV infection who are asymptomatic or manifest persistent generalized lymphadenopathy;
- stage II includes those with weight loss (<10% body weight), minor mucocutaneous manifestations, herpes zoster and recurrent upper respiratory tract infections;
- stage III includes those with weight loss (>10% body weight), unexplained chronic diarrhea, unexplained prolonged fever, thrush, oral hairy leukoplakia, pulmonary tuberculosis, severe bacterial infections and bedridden for less than 50% of the day during the past month; and
- stage IV includes those with clinical syndromes consistent with AIDS and/or bedridden for more than 50% of the day during the past month.

Table 96.1 World Health Organization recommendations for starting HIV/AIDS therapy. Recommendations for initiating antiretroviral therapy in adults and adolescents with documented HIV infection

If CD4 testing is available	WHO stage IV disease irrespective of $CD4^+$ lymphocyte count WHO stage I, II, III with $CD4^+$ lymphocyte counts <200 cells/mm^3
If CD4 testing unavailable	WHO stage IV disease irrespective of total lymphocyte count WHO stage II or III disease with a total lymphocyte count <1200 cells/mm^3

The guidelines, shown in Table 96.1, recommend starting ART in those who have WHO stage IV disease irrespective of $CD4^+$ lymphocyte count and WHO stage I, II or III with $CD4^+$ lymphocyte count below 200 cells/mm^3 in areas where $CD4^+$ lymphocyte count is available. If $CD4^+$ lymphocyte count is unavailable, ART is recommended in those with WHO stage II or III with total lymphocyte count below 1200 cells/mm^3. Assessment of HIV viral load is not considered essential for determining the need for therapy.

In addition to the assessment of immunologic function as indicated by symptoms and lymphocyte count, further testing for the safe and effective use of ART is divided into four categories as listed in Table 96.2: absolute minimum tests, basic tests, desirable tests and optional tests. Absolute minimum testing includes an HIV antibody test and hemoglobin or hematocrit level if starting a zidovudine-based regimen. Other monitoring is recommended based on the antiretroviral regimen chosen and guided by symptoms. Testing for HIV viral load is deemed to be optional.

The guidelines are based on rigorous evaluation conducted almost exclusively in developed countries. A matter for concern is whether guidelines created for the populations of developed nations are adaptable to HIV-infected populations worldwide. Specifics regarding the presence of different HIV subtypes, endemic infections such as tuberculosis, genetic determinants and environmental factors such as nutritional status may introduce factors that alter response to treatment.

Several developing countries have successfully scaled up ART including Brazil, Thailand, Senegal, Uganda and South Africa to name only a few. Numerous publications from these settings have shown that high levels of adherence and positive treatment outcomes are achievable through carefully implemented programs. With initiation of ART on a population-wide scale, continuous surveillance of drug-resistant viruses will be needed to inform treatment guidelines. Of great concern is that antiviral drug resistance due to suboptimal therapies could limit the potency of available treatments. In parallel with promulgation of the guidelines, the WHO, in collaboration with the International AIDS Society, is developing a global HIV drug resistance surveillance network.

Multiple studies conducted in developed nations have proved the tremendous benefit available to people with HIV infection by the initiation of ART. Consequently, morbidity and mortality due to HIV have declined dramatically. In developed or developing nations, ART provides the only hope of survival for those with HIV infection who are able to adhere to daily lifelong therapy. Moreover, the availability of ART can reinforce prevention activities by offering an incentive to seek HIV testing, preventing mother-to-child transmission and decreasing the risk of sexual transmission.

Table 96.2 World Health Organization guidelines for laboratory monitoring of antiretroviral (ARV) drug use

Diagnosis and monitoring laboratory tests	Pre-ART (at entry into care)	At initiation of first-line or second-line ARV regimen	Every 6 months	As required (depending on symptoms)
HIV diagnostic testing	Yes			
Hemoglobin		For AZT regimens		Yes
WBC and differential		Yes		Yes
CD4 cell count	Yes	Yes	Yes	
Pregnancy test		For efavirenz regimens		Yes
Chemistry (ALT, other liver enzymes, renal function, glucose, lipids, amylase, lipase, lactate and serum electrolytes)				Yes
Viral load measurement				Yes

ART, antiretroviral therapy.

IMPACT OF HIV AND AIDS ON HEALTH-CARE SYSTEMS

As the number of people with HIV infection in developing countries continues to increase, HIV/AIDS will continue to make increasing demands on the health-care system at all levels. The allocation of funds for prevention versus care and support varies according to region, with 66% and 32% of estimated expenditure needed for care and support in sub-Saharan Africa and Asia, respectively. AIDS prevention activities include teacher training and peer education, condom promotion and distribution, treatment of STDs, voluntary testing and counseling, transfusion screening and prevention of mother-to-child HIV transmission. Included in care and support activities are diagnostic testing, palliative care, opportunistic infection treatment, drug costs and monitoring for ART, as well as orphanage care and living assistance. Mobilization of such tremendous resources will require a considerable commitment from both domestic and donor sources.

Unfortunately, these demands come at a time of great financial vulnerability for health systems and at a stage, particularly in developing countries, when a great deal of work remains to be done to increase primary health care. Primary care is intended to be the interface of contact between communities and the national health-care system, bringing health care as close as possible to where people live and work. Diagnosis and treatment to relieve symptoms and to prevent and treat opportunistic infections can ease suffering and prolong the productive lives of people who have HIV, sometimes at a low cost. As the patient's immune system collapses, however, available treatments become increasingly more expensive. An analysis by the World Bank of alternative treatment and care options concludes that community-initiated care provided at home, although often shifting costs from the national taxpayer to the local community, also greatly reduces the cost of care and thereby offers hope of affordability in improving the quality of the last years of life of people dying with AIDS.

The epidemic will undoubtedly increase demand for medical care and reduce its supply at a given quality and price. As the number of people who have HIV infection increases, access to medical care will become more difficult and more expensive for everyone, including people not infected with HIV, and total health expenditure per capita will rise. Governments are under pressure to increase their share of health-care spending and to provide special subsidies for the treatment of HIV infection. Unfortunately, because of the scarcity of resources and the inability or unwillingness of governments to increase public health spending enough to offset these pressures, either of these policies may exacerbate the impact of the epidemic on the health-care sector.

Governments should ensure that patients who have HIV infection benefit from the same access to care as other patients who have comparable illnesses and similar ability to pay. Because of discrimination, people who have HIV are frequently denied treatment or face barriers to care that others do not encounter. Governments should also provide information about the efficacy of treatments for opportunistic illnesses, HIV infection and AIDS; subsidize the treatment of STDs and contagious opportunistic infections; subsidize the screening of the blood supply; and ensure access to health care for the poorest, regardless of HIV infection status.

REFERENCES


References for this chapter can be found online at http://www.expertconsult.com

Practice point **44**

Yves Benhamou

Hepatitis B in the HIV co-infected patient

RECIPROCAL INFLUENCE BETWEEN HBV AND HIV

Extended follow-up of highly active antiretroviral therapy (HAART)-treated patients suggests that hepatitis B virus (HBV) co-infection and serum hepatitis B surface antigen (HBsAg) does not substantially affect HIV progression and HIV-related mortality.[1]

On the contrary, co-infection with HIV and treatment with HAART each modifies the natural history of HBV infection. Most of the liver damage associated with HBV infection stems from the immune system response to HBV. HIV infection can dampen this immunologic response. HAART leads to immune system reconstitution, which in HBV-infected patients can be advantageous or deleterious. These phenomena may explain some of the effects of HIV co-infection and HAART on HBV which are:[2]

- a higher risk of chronicity after acute HBV infection;
- a higher level of HBV replication and a higher rate of reactivation compared with persons without HIV co-infection;
- increased liver injury (so-called immune-reconstitution hepatitis) and liver disease progression in case of antiretroviral therapy; and
- a higher risk of liver-related death.

A lamivudine-containing HAART regimen is independently associated with a decreased risk of liver decompensation.[3] Early use of HAART containing dual-activity agents is generally positive for preventing severe immune dysfunction, controlling HBV replication, slowing liver disease progression and preventing immune-reconstitution hepatitis.

TREATMENT OF HBV IN HIV CO-INFECTED PATIENTS

Goals of therapy

The principal goals of anti-HBV treatment are to stop or decrease liver disease progression, and to prevent cirrhosis and hepatocellular carcinoma. HIV co-infected patients infrequently seroconvert to anti-HBe and anti-HBs status. Seroconversion remains an objective, but a more realistic virologic goal is prolonged suppression of HBV replication. Attaining this milestone leads to histologic improvement, significant decrease or normalization of aminotransferases and prevention of progression to cirrhosis and end-stage liver disease.

Sustained viral control requires long-term maintenance therapy. Treatment discontinuation, especially of lamivudine, has been associated with HBV reactivation, alanine aminotransferase (ALT) flares and, in rare cases, hepatic decompensation. The drawback of long-term therapy is the possibility of resistance (around 20% per year for lamivudine). Therefore, most co-infected patients require combination therapy for anti-HBV treatment.

Anti-HBV therapy recommendations

Guidelines consider two situations based on the need for anti-HIV therapy or not. The threshold of serum HBV DNA level for anti-HBV initiation is >2000 IU/ml and liver histologic evaluation may be needed in patients with serum HBV DNA >2000 IU/ml and persistently normal ALT in HIV-infected patients who do not need anti-HIV therapy (Figs PP44.1, PP44.2).[4,5]

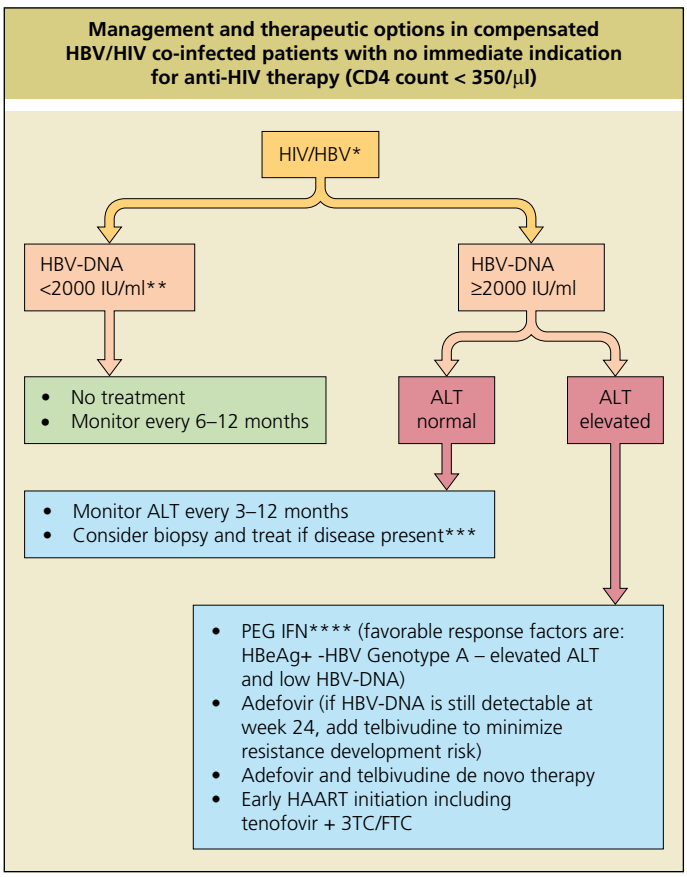


Fig. PP44.1 Management and therapeutic options in compensated HBV/HIV co-infected patients with no immediate indication for anti-HIV therapy (CD4 count >350/μl). ALT, alanine aminotransferase; 3TC, lamivudine; FTC, emtricitabine; HAART, highly active antiretroviral therapy; PEG IFN, pegylated interferon.

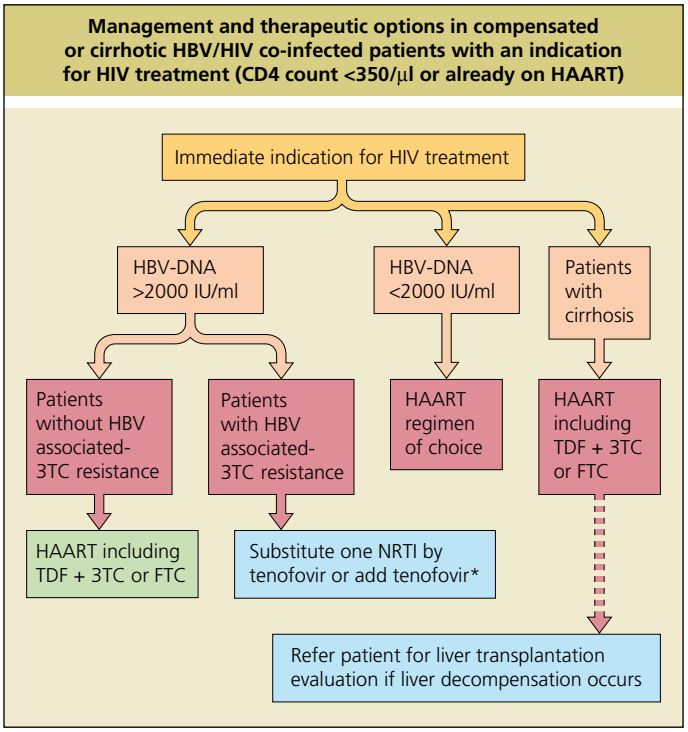


Fig. PP44.2 Management and therapeutic options in compensated or cirrhotic HBV/HIV co-infected patients with an indication for HIV treatment (CD4 count <350/μl or already on HAART). 3TC, lamivudine; FTC, emtricitabine; NRTI, nucleoside reverse transcriptase inhibitor; TDF, tenofovir disoproxil fumarate.

Patients who do not meet the criteria for anti-HIV treatment (Fig. PP44.1)

These patients should not receive anti-HBV agents with dual anti-HIV activity (see Table PP44.1). Using anti-HIV therapy at this juncture raises the risk of early HIV resistance, with consequent limitations of HIV therapeutic options. In hepatitis B e antigen (HBeAg)-positive patients, pegylated interferon α-2a, adefovir dipivoxil or telbivudine should be considered. Typically, pegylated interferon is not preferred for the treatment of HBeAg-negative patients as the opportunity to achieve HBsAg seroconversion and to maintain HBV DNA suppression off therapy is low. Although not demonstrated, combination therapy of a nucleoside and a nucleotide (adefovir and telbivudine) for such patients rather than monotherapy or pegylated interferon should be considered.

Patients who meet the criteria for anti-HIV treatment (Fig. PP44.2)

HAART, including two dual-acting drugs, constitutes the preferred option for these patients. The best choice is to combine a nucleoside and a nucleotide analogue in order to prevent long-term resistance (i.e. tenofovir plus either lamivudine or emtricitabine).

When patients change anti-HIV treatments because of intolerance or lack of efficacy, the anti-HBV component should be continued even if it is not part of the subsequent anti-HIV regimen; stopping anti-HBV therapy has been associated with reactivation of HBV infection and ALT flares.

Patients with cirrhosis

Sustained control of HBV replication in patients with cirrhosis is critical to prevent liver decompensation, hepatocellular carcinoma, and death. Preventing resistance and ensuring compliance are paramount considerations. Therefore, cirrhotic patients should receive combination anti-HBV therapy and should be monitored closely during the first 12–24 weeks of therapy, due to risk of ALT flare and immune-reconstitution hepatitis. Serum HBV DNA should be assessed every 12 weeks, especially for those with CD4 counts <200/mm^3. Patients with liver decompensation should be considered for liver transplantation.

Anti-HBV therapy

Six therapeutic agents are currently approved for the treatment of chronic HBV in the USA and the European Union (Table PP44.1).

Interferon alpha

Historically, standard interferon alpha (5 MU daily or 9–10 MU three times weekly) has been the first-line therapy for HBV monoinfection. Data conflict about the value of interferon alpha in HIV-positive patients with chronic hepatitis B. The optimal dose and duration of pegylated interferon remains unknown in co-infected patients. However, pegylated interferon may be used in HBeAg patients who do not need anti-HIV therapy.

Lamivudine

This nucleoside analogue offers both anti-HIV and anti-HBV activity. It inhibits HBV replication in up to 87% of HIV/HBV co-infected patients. Rates of anti-HBe seroconversion are highly variable, ranging up to 11% of patients in some studies. HBV resistance is a major problem with lamivudine (47% of patients at 2 years and 90% at 4 years of treatment).

Table PP44.1 Responses to anti-HBV agents tested in HIV/HBV co-infected patients

	IFN	LAM	ETV	FTC	TDF	ADV*
Duration (weeks)	12–24	48	24	48	24–48	48–192
Anti-HBV activity tested in HIV patients	wt	wt	LAM-R	wt	wt, LAM-R	LAM-R
HBV DNA decline (log_{10} copies/ml)	26%†	2.7	3.6	–	4.4	4.7–6*
HBe seroconversion (%)	9	11	–	–	4	7
ALT normalization (%)	12–20	30–50	49	–	–	35–66*
Histologic improvement	–	–	–	–	–	33–50*

IFN, standard interferon; LAM, lamivudine; ETV, entecavir; FTC, emtricitabine; TDF, tenofovir disoproxil fumarate; ADV, adefovir dipivoxil; wt, wild type; LAM-R, lamivudine HBV-resistant strain.
*Results at 48–192 weeks of ADV.
†Proportion of patients with serum HBV DNA <6 log_{10} copies/ml.

Adefovir dipivoxil

This once-daily nucleotide analogue reverse transcriptase inhibitor is active against wild-type and lamivudine-resistant HBV. In a small series of HIV/HBV lamivudine-resistant co-infected patients,[6] adding once-daily adefovir 10 mg reduced serum HBV DNA by a median of 5.9 $\log_{10}$ copies/ml at week 144. No adefovir-associated HBV and HIV mutations surfaced during 144 weeks of follow-up. Co-infected patients generally tolerate adefovir well during short- and long-term therapy. Rare cases of renal toxicity have been reported.

HIV agents with HBV activity

Entecavir

Entecavir is a purine-derived nucleoside analogue which reduced HBV DNA by nearly 7 $\log_{10}$ copies/ml with no observed resistance over 48 weeks in HBV monoinfected, lamivudine-naive patients.

In HIV co-infected, lamivudine-resistant patients, adding entecavir reduced HBV DNA by a mean of 3.66 $\log_{10}$ copies/ml by 24 weeks of therapy. It also inhibited HIV replication with an IC_{50} lower than the plasma concentrations achieved *in vivo* at doses given for anti-HBV therapy. After 6 months of entecavir therapy, 96% of isolates from the co-infected patient contained the M184V lamivudine mutation. This variant confers high-level resistance to entecavir. Therefore, entecavir should be considered as a drug with anti-HIV activity and should be avoided in patients who do not need anti-HIV therapy.

Emtricitabine

This newer nucleoside reverse transcriptase inhibitor is a 5-fluorinated derivative of lamivudine. Emtricitabine (200 mg/day) reduces serum HBV DNA by up to 3.13 $\log_{10}$ copies/ml in HIV/HBV co-infected patients. In treatment-naive HIV/HBV co-infected patients, the incidence of mutations in the viral polymerase following 1 year of therapy with emtricitabine is around 10%. Emtricitabine is generally well tolerated.

Tenofovir disoproxil fumarate

This agent, an acyclic nucleotide reverse transcriptase inhibitor, is effective against HIV, and against wild-type and lamivudine-resistant HBV.

In HIV co-infected patients the mean $\log_{10}$ decline in HBV DNA after 48 weeks of tenofovir therapy was −4.7 with tenofovir plus lamivudine compared with −3.0 with lamivudine alone. Tenofovir anti-HBV activity was similar for patients with wild-type (−5.3 $\log_{10}$) and lamivudine-resistant HBV (−4.6 $\log_{10}$).[7]

Among HBeAg-positive patients, serum HBV DNA became undetectable in 29.6%, 7.4% lost HBeAg and 3.7% seroconverted to anti-HBe (median follow-up, 12 months).[7]

When compared to adefovir, mean $\log_{10}$ time-weighted average change from baseline to week 48 is 1 log higher with tenofovir when compared with adefovir.

No HBV resistance to tenofovir has been so far reported in HIV/HBV co-infected patients. However, almost all the HIV/HBV co-infected patients in tenofovir studies were receiving concomitant lamivudine therapy as a part of HAART. This add-on strategy may reduce the risk of HBV resistance to tenofovir.

Tenofovir use may be limited by renal toxicity (tubulopathy) that can occur even years after initiation. Creatinine and potassium levels should be monitored every 3 or 4 months.

CONCLUSIONS

The interactions of HIV, HBV and HAART complicate the disease course and management of patients co-infected with HIV and HBV. HBV co-infection does not substantially affect the course of HIV disease, but HIV co-infection does significantly alter the course of HBV disease. On the whole, early use of HAART is beneficial in co-infected patients who meet the criteria for antiretroviral therapy. Availability of agents with dual activity against HIV and HBV facilitates management of co-infected patients. It also mandates careful monitoring of treatment effects on both infections to detect and to avoid triggering resistance or relapse.

REFERENCES

References for this chapter can be found online at http://www.expertconsult.com

Chapter 97

Stéphane Blanche

HIV infection in children

Children become infected with HIV almost exclusively by the mother-to-child transmission route.[1] However, the risk of horizontal contamination, which has been little studied, has not completely disappeared in highly endemic, resource-poor countries.[2] Horizontal contamination may occur through unsafe blood transfusions or the use of nonsterile equipment for medical or traditional purposes. Child rape is also a significant risk. The progression of pediatric epidemics largely parallels that for infection in adults. However, the extremely effective prevention of mother-to-child transmission has greatly increased the gap between rich and poor countries. The World Health Organization (WHO) estimated that about two million children were infected with HIV at the end of 2007, with 420 000 new infections, leading to 330 000 deaths (Table 97.1). The proportion of pregnant women who are seropositive for human immunodeficiency virus type 1 (HIV-1) is in the range 3–8% in western and central Africa, and 15–30% in southern Africa.[3]

MOTHER-TO-CHILD TRANSMISSION

In the absence of prophylactic treatment, about 15–20% of infected mothers transmit HIV-1 to their child pre- or peripartum, with an additional risk of 10–15% associated with breast-feeding.[4] Although never strictly compared in a same cohort, the risk of mother-to-child transmission appears to be similar for the different subtypes of HIV-1 (groups A, B, C and recombinant strains), although it has been suggested that there is a higher risk associated with group D viruses.[5] HIV-2 is phylogenetically different from HIV-1 with a much lower replicative potential in humans; it also has a much lower mother-to-child transmission rate than HIV-1. Even without treatment, the transmission rate of HIV-2 is only 1–2% and mostly involves women with primary infection during pregnancy or at an advanced stage of the disease.[6]

There are several pathophysiologic mechanisms underlying transmission. In-utero contamination is possible, but occurs in only a minority of cases, despite possible HIV-1 replication in placental monocytes and CD4 cells.[4] The risk of in-utero contamination is higher in cases of chorioamnionitis and in women with very high levels of viral replication, as is observed in primary infection or in advanced stages of disease. Most infected children, however, are contaminated during labor, probably through mother-to-fetus microtransfusion.[7] The level of maternal viral replication is again a major risk factor for contamination at this point, although no lower threshold for transmission in the absence of antiretroviral prophylaxis has been established. The conditions of birth, particularly the duration of amniotic membrane rupture, can also increase the risk of transmission. Passage through the birth canal probably only makes a small contribution to the risk of infection: delivery by Caesarian section reduces the risk of transmission only if it is performed before labor begins. Premature birth is strongly associated with infection of the child, although it is unclear whether prematurity and its co-factors are a cause or consequence of infection[8] (Table 97.2). In clinical practice, viral replication detected in newborns during the first few days of life indicates in-utero replication. By contrast, peripartum infection is detected later, generally before the age of 3 months. In-utero infection is more frequently associated with a worse prognosis in children.[9,10]

Table 97.1 Global summary of the AIDS epidemic – December 2007

Number of people living with HIV in 2007	
Total	33.2 million (30.6–36.1 million)
Adults	30.8 million (28.2–33.6 million)
Women	15.4 million (13.9–16.6 million)
Children under 15 years	2.5 million (2.2–2.6 million)
People newly infected with HIV in 2007	
Total	2.5 million (1.8–4.1 million)
Adults	2.1 million (1.4–3.6 million)
Children under 15 years	420 000 (350 000–540 000)
AIDS deaths in 2007	
Total	2.1 million (1.9–2.4 million)
Adults	1.7 million (1.6–2.1 million)
Children under 15 years	330 000 (310 000–380 000)

The ranges around the estimates in this table define the boundaries within which the actual numbers lie, based on the best available information.

ANTIRETROVIRAL PROPHYLAXIS DURING PREGNANCY

The use of antiretroviral drugs to prevent transmission has proved very effective: in optimal conditions the risk of transmission is almost zero.[1,4,11] In a recent nationwide analysis of residual transmission despite

Table 97.2 Main risk factors for mother-to-child HIV-1 transmission

- Maternal viral load
- Low CD4+ cell count
- Prolonged delivery
- Chorioamnionitis
- Prematurity
- Breast-feeding

Table 97.3 Mother-to-child transmission in women receiving antiretroviral therapy

Maternal HIV-1 viral load at delivery (copies/ml)	n	% infected children
>10 000	440	6.8
1000–9999	938	1.5
400–999	440	0.7
<400	3256	0.6

From ANRS French perinatal cohort.[11]

highly active antiretroviral treatment during pregnancy and undetectable viral load at delivery, only 0.6% of children born at term were found to be infected[12] (Table 97.3). Risk factors for this residual contamination despite the full suppression of viral replication in the plasma at delivery remain to be identified. The duration of treatment and initial viral load may play a role but obstetric factors are no longer significant.[8]

The first step in prophylactic treatment is the screening of pregnant women for HIV infection at a sufficiently early stage of pregnancy. This is well managed in industrialized countries, but remains problematic in low-income countries, both for practical reasons (availability and performance of the test, collection of test results, delivery of results, the taking of appropriate therapeutic decisions in light of the results) and because such testing is not always accepted by women.[12] Potential stigmatization and the personal, familial and social consequences of testing for women are still major issues in many parts of the world.[13] Optimal prevention of transmission during pregnancy is currently based on antiretroviral therapy[14,15] (Table 97.4). Antiretroviral therapy is generally initiated at the start of the third trimester of pregnancy and continued until birth, with the aim of ensuring that maternal viral replication rates are kept below the detection threshold during the critical period for transmission to the child. If the mother is already receiving treatment or needs to be treated earlier during pregnancy, maternal treatment is continued or started without delay. During labor and until delivery, treatment is replaced by intravenous zidovudine infusion. Recent observational data have suggested that this peripartum phase of treatment may be omitted if viral replication in the mother is fully suppressed for at least a few weeks before delivery.

Alternative 'simplified' strategies have been developed for cases in which the reference treatment is not available or cannot be distributed in regions with limited access to health care.[15] These strategies – of shorter duration and inhibiting maternal viral replication less strongly – nonetheless appear to be relatively effective. Thus, the 2006 WHO recommendations for 'simplified' treatment involved zidovudine monotherapy combined with a single dose of nevirapine at birth. The transmission rate recorded with this therapy (not including the breast-feeding period) is of the order of 3–4%, versus around 20% without treatment. A single dose of nevirapine during labor alone halves the risk of transmission and remains widely used. This simple solution should, however, only be used when other options are not possible. In addition to being only moderately effective, treatment with a single dose of nevirapine favors the selection of maternal viruses resistant to this class of drug.[16] The co-administration of antiretroviral dual therapy (zidovudine–lamivudine or, more recently, tenofovir–emtricitabine) for several days after labor reduces this risk of resistance. There is no consensus on the optimal prophylactic treatment for HIV-2. An aggressive approach is certainly justified in both primary infection during pregnancy and advanced disease, both associated with a significant risk of transmission. Non-nucleoside inhibitors, such as nevirapine, have proved ineffective against HIV-2.

The dynamic nature of clinical research in this field leads to the frequent updating of these recommendations. Concerned readers are strongly encouraged to visit several dedicated sites easily accessible via the Internet.

Table 97.4 General principles for the prophylaxis of mother-to-child transmission (may vary according to national guidelines)

Prepartum

HIV-1-infected pregnant women currently receiving antiretroviral treatment

Continue the treatment if effective (plasma viral load undetectable).
Avoid efavirenz if possible during the first trimester
Optimize the treatment if ineffective according to viral resistance genotype*

HIV-1-infected women who have never received antiretroviral drugs ('antiretroviral naive')

If criteria for antiretroviral (ARV) treatment initiation according to adult guidelines (clinical, immunologic, virologic) are met:
- Start without delay, as recommended for nonpregnant adults
- Select specific drug combination for pregnancy†

If no criteria for immediate treatment initiation, consider prophylaxis alone:
- Treatment with a combination of three drugs is preferred‡
- Start no later than 28 weeks

Peripartum

Zidovudine intravenous infusion during labor, until delivery‡
Elective Caesarian section only if uncontrolled viral replication in the plasma

Postpartum in children

Zidovudine for 4–6 weeks‡
Reinforced postnatal antiretroviral prophylaxis if maternal viral load uncontrolled or maternal treatment suboptimal
Bottle feeding whenever possible§

*If available.
†Main drugs selected for pregnancy (July 2008): **Nucleoside analogue**: zidovudine, lamivudine; **Protease inhibitor**: ritonavir-boosted lopinavir; **Non-nucleoside reverse transcriptase inhibitor**: nevirapine if CD4 cell count <250/mm^3.
‡When impossible, simplified regimens are proposed (see[15]).
§When replacement feeding is acceptable, feasible, affordable, sustainable and safe. In other cases, consider extended ARV prophylaxis program during breast-feeding (either maternal ARV or postexposure prophylaxis in the infant).

THE RISK OF TRANSMISSION ASSOCIATED WITH BREAST-FEEDING

Eliminating the risk associated with breast-feeding is currently a major issue (see Mofenson[17] for a review). In industrialized countries, bottle feeding is advised, but the risk of nutritional infection associated with this type of feeding poses a major problem for most, but not all, children living in poor countries. According to WHO recommendations, bottle feeding should be suggested only where it can be offered at a reasonable price, for a feasible duration, with reasonable acceptance rates and with sufficient food safety and precautionary measures against infection. In all other cases, maternal breast-feeding is encouraged.

Several approaches to improving the safety of maternal breast-feeding are currently being implemented. The first involves the promotion of exclusive breast-feeding, given that mixed feeding methods increase the risk of child contamination.[18] Another approach exploits the efficacy of antiretroviral drugs, either for maternal antiretroviral treatment during breast-feeding, or for 'postexposure prophylactic' treatment of the child for the duration of breast-feeding. The preliminary findings

for these two strategies are very encouraging, suggesting that it should be possible to manage the risk of transmission associated with breast-feeding effectively in the near future. The feasibility of, and tolerance of children to, this postnatal prophylactic treatment – whether given directly to the child or introduced through maternal milk – will nevertheless need to be carefully examined.

TOLERANCE OF ANTIRETROVIRAL PROPHYLAXIS IN FETUSES AND NEONATES

Fetuses and neonates generally have satisfactory tolerance to antiretroviral drugs. No evidence of teratogenesis has been found in humans.[19] Efavirenz causes neural tube defects in primates and its use in humans is contraindicated, except in cases for which no other feasible options are available. There is still some debate about the risk of prematurity associated with protease inhibitors.[20] Most recent analyses suggest that neither prematurity nor hypotrophy is linked to protease inhibitor use during pregnancy. However, current analyses are never stratified by drug and specific toxicity cannot be excluded.

The genotoxic potential of nucleoside analogues – including zidovudine, which is known to interact with both mitochondrial and nuclear DNA in humans[21,22] – merits particular attention. The main toxic effect of this molecule is reversible anemia, but the persistent inhibition of other hematopoietic lines until the age of 2 years has been observed in a number of cohorts. An impact of zidovudine on the mitochondria of treated children has been clearly demonstrated through transient biochemical abnormalities, concerning lactate concentrations, for example. A low risk of encephalopathy with persistent mitochondrial dysfunction has also been reported. A large study on cancer incidence after in-utero treatment with zidovudine alone or with lamivudine revealed no significant difference from expectations for the general population within 5 years of follow-up.[23] Tolerance to other nucleoside combinations must be evaluated. Fetal tolerance to other molecules of this class remains largely unknown, as does tolerance to new classes of drug (integrase inhibitors, fusion inhibitors).

INITIAL FOLLOW-UP OF NEWBORNS

Most children born to HIV-infected mothers are not infected by HIV, but careful management is required to optimize prevention and reduce the risk of infection further. Furthermore, the child and mother are often in a situation of social, psychological and administrative vulnerability, and may be in need of appropriate support.

All usual childcare activities can be carried out as normal. The potential benefit of an antiseptic bath with a virucidal solution has not been determined; any such act should be carried out very carefully because of the potential deleterious effects of breaks in the skin or mucous membranes in the presence of virus on these surfaces (including gastric membranes).

The continuation of prophylactic treatment must be considered, if possible, from the end of pregnancy, as the choice of treatment depends partly on the treatment already received by the mother. Treatment continued in the form of zidovudine alone is considered to be optimal in the case of maternal prophylactic treatment. Recommendations in industrialized countries suggest that this should be maintained for 4–6 weeks, but it is offered for 1 week in countries in which the full course of treatment cannot be administered. More aggressive treatment is required if the mother's treatment is considered insufficient because its duration is too short or because compliance is poor, or if there is a detectable viral load (where the test is available) before birth. There is currently no consensus recommendation concerning such intensification of treatment; it often involves triple antiretroviral therapy when such treated can be implemented, or is simplified to zidovudine for 4 weeks combined with a single dose of nevirapine when other options are unavailable. The use of single-dose nevirapine, as for the mother, is associated with an increased risk of acquiring mutations conferring resistance to the molecule if prophylaxis fails, and this problem must be taken into account when selecting treatment.[15,16]

DIAGNOSIS IN CHILDREN AND USUAL POSTNATAL CARE

Maternal IgG antibodies cross the placenta, so the early diagnosis of infection requires a polymerase chain reaction (PCR)-based virus isolation test. PCR-based tests for DNA and those for RNA are both used and seem to give equivalent rates of diagnostic sensitivity and specificity when used for young children. These tests are ideally performed immediately after birth, but the number of infected children identified in the perinatal period is small, because most cases of contamination occur at the moment of delivery. Several weeks of viral replication are required before replication can be detected by standard methods, so screening for the virus is generally repeated at 1 and 3 months, and even at 6 months at certain centers. The sensitivity of PCR at 3 months in non-breast-fed children is close to 100%.[24] Negative serologic results at 18 months are often considered the end point in the testing process, although some centers stop follow-up after a negative PCR result at 6 months.

In cases of limited access to health care, the first PCR test is often carried out at 6 weeks, as a compromise between reducing costs and maintaining test sensitivity. In cases of breast-feeding, the risk of infection remains until weaning, and a diagnostic test (PCR before 18 months or serologic test after 18 months) must be carried out at least 4–6 after the end of breast-feeding for the valid interpretation of test results.[25] Any positive PCR result should lead to retesting without delay to confirm infection. Symptoms observed in the child may also contribute to diagnosis. Axillary adenopathy, splenomegaly and oral thrush in the first few months are highly suggestive of infection, although clearly not disease specific. A persistent cough, recurrent ENT (ear, nose and throat), skin and digestive infections, a state of malnutrition, neurologic hypertonia or hypotonia are indicative of already advanced disease. In areas in which access to PCR is restricted, a presumptive clinical diagnosis may be sufficient for the initiation of antiretroviral treatment in cases of suspected infection whilst awaiting confirmation by PCR-based diagnostic tests.

Vaccine schedules and the expanded immunization program (EIP) can be followed in each country as normal. The only vaccine posing a potential problem is bacille Calmette–Guérin (BCG), which persists in the long term, such that a subsequent vaccine disease is possible. Indeed, BCG vaccination is contraindicated for HIV-infected children in industrialized countries with a low incidence of tuberculosis. Elsewhere BCG vaccination is generally carried out in all HIV-exposed children, but recent WHO recommendations also advise against such vaccination in southern hemisphere countries in which infected children are diagnosed early.[26] Other live vaccines, such as measles–mumps–rubella (MMR) and yellow fever, do not pose the same problem of persistence and can be given normally, except to children at an advanced stage of HIV infection.

Early prevention of *Pneumocystis jirovecii* infection is offered from 1 month until 12–18 months to children exposed to HIV, while waiting for confirmation of noninfection (trimethoprim–sulfamethoxazole; 30 mg/kg sulfamethoxazole). When diagnostic procedures can easily be completed and PCR results obtained in real time, this systematic prophylaxis is offered to infected children only.

CHILDREN INFECTED WITH HIV

In the absence of antiretroviral treatment, disease progression differs between children in industrialized countries and those in countries with limited access to health care. Between 15% and 20% of infected children in Europe and North America have a risk of

early and severe opportunistic infection and encephalopathy in the first 12–18 months of life.[27,28] Predictive factors for this rapid form of disease progression include the detection of in-utero viral contamination at birth, an advanced stage of illness in the mother during pregnancy and cytomegalovirus co-infection. In low-income countries, morbidity and early death rates, mostly due to infection, are much higher. In certain child cohorts from sub-Saharan Africa, the mortality rate is 50% in the first 2 years of life.[29–31] After this, disease progression tends to be slower, close to that observed in adults, although this has not been clearly demonstrated for African children.

The combination of symptoms observed in adults has also been described in children and detailed symptomatology in the WHO classification[28] (Table 97.5A). In addition to the specific risk of encephalopathy, two other complications are more frequently observed in children than in adults. The first is lymphoid interstitial pneumonia, leading to recurrent bacterial superinfections, the major clinical symptoms thus being those of the infections caused.[32] Unusual radiologic symptoms are also observed, reflecting an interstitial syndrome of variable intensity associated with mediastinal adenopathy. This can lead to the differential diagnosis of miliary tuberculosis (especially in cases of bacterial superinfection); however, the contrast between the major abnormalities observed on radiologic images and the mild symptoms can be used to exclude this diagnosis. The underlying pathophysiology remains unknown, but is often associated with bilateral parotitis, and the biologic signs include substantial CD8 hyperlymphocytosis. Co-infection may play a role and Epstein–Barr virus (EBV) has also been implicated. Bronchoalveolar lavage, when possible, reveals hyperlymphocytosis.

Another specific feature observed in children, contrasting with adults, is a higher frequency of leiomyoma or of leiomyosarcoma tumors in cases of severe immunodeficiency.[33] All organs may be affected. Here again, opportunistic EBV infection has been implicated in the pathophysiology. The proportion of children with non-progressive disease in the long term (between 10 and 15 years) has been determined in industrialized countries and found to be similar to that observed for infected adults. Predictive criteria for disease progression, in children as in adults, are CD4 levels and the viral load determined by quantitative PCR amplification of DNA. CD4 levels indicate the degree of immune deficiency and, thus, the risk of related short-term or mid-term complications.[30,34,35] The viral load indicates the rate of viral replication and probable rate of subsequent changes to the immune system. The interpretation of CD4 levels is more difficult than in adults, because of the physiologic hyperlymphocytosis observed in early life; normal adult levels are not reached until the age of 5–6 years. Counts expressed as the percentage of lymphocytes are less subject to variation and thus often preferred by pediatricians (Table 97.5B). CD4 counts are nevertheless poorly predictive during the first year of life, particularly for the prediction of early-onset severe forms of disease.

TREATMENT OF INFECTED CHILDREN

The prophylactic treatment of opportunistic or bacterial infections initially made a major contribution to reducing mortality rates in infected children. The preventive antibacterial efficacy of systematic administration of trimethoprim–sulfamethoxazole (TMP–SMX) has been demonstrated for all ages, starting as early as 1 month of age, leading to a significant decrease in mortality. It is recommended for all infected children in countries in which access to health care is limited.[36,37] Elsewhere, its prescription is more restricted: it is used either following recurrent bacterial infections despite a seemingly normal CD4 level or, more typically, following detection of CD4 lymphopenia. In countries with a high incidence of tuberculosis, the systematic prescription of isoniazid also reduces the mortality rate.[38] However, no recommendations have been established for this prophylactic treatment.

Table 97.5A WHO classification for HIV-1-infected children – clinical

Clinical stage 1
Asymptomatic
Persistent generalized lymphadenopathy
Clinical stage 2*
Unexplained persistent hepatosplenomegaly
Papular pruritic eruptions
Extensive wart virus infection
Extensive molluscum contagiosum
Recurrent oral ulcerations
Unexplained persistent parotid enlargement
Lineal gingival erythema
Herpes zoster
Recurrent or chronic upper respiratory tract infections (otitis media, otorrhea, sinusitis, tonsillitis)
Fungal nail infections
Clinical stage 3*
Unexplained moderate malnutrition not adequately responding to standard therapy
Unexplained persistent diarrhea (≥14 days)
Unexplained persistent fever (above 100°F/37.5°C, intermittent or constant, for >1 month)
Persistent oral candidiasis (after first 6 weeks of life)
Oral hairy leukoplakia
Acute necrotizing ulcerative gingivitis/periodontitis
Lymph node tuberculosis
Pulmonary tuberculosis
Severe recurrent bacterial pneumonia
Symptomatic lymphoid interstitial pneumonitis
Chronic HIV-associated lung disease including bronchiectasis
Unexplained anemia (<8.0 g/dl), neutropenia (<0.5 × 10^9/l^3) or chronic thrombocytopenia (<50 × 10^9/l^3)
Clinical stage 4*,†
Unexplained severe wasting, stunting or severe malnutrition not responding to standard therapy
Pneumocystis jirovecii pneumonia
Recurrent severe bacterial infections (e.g. empyema, pyomyositis, bone or joint infection, meningitis, but excluding pneumonia)
Chronic herpes simplex infection (orolabial or cutaneous for >1 month, or visceral at any site)
Extrapulmonary tuberculosis
Kaposi's sarcoma
Esophageal candidiasis (or *Candida* infection of trachea, bronchi or lungs)
Central nervous system toxoplasmosis (after the neonatal period)
HIV encephalopathy
Cytomegalovirus (CMV) infection, retinitis or CMV infection affecting another organ, with onset at age >1 month
Extrapulmonary cryptococcosis (including meningitis)
Disseminated endemic mycosis (extrapulmonary histoplasmosis, coccidioidomycosis)
Chronic cryptosporidiosis (with diarrhea)
Chronic isosporiasis
Disseminated non tuberculous mycobacterial infection
Cerebral or B-cell non-Hodgkin's lymphoma
Progressive multifocal leukoencephalopathy
HIV-associated cardiomyopathy or nephropathy

*Unexplained indicates that the condition is not explained by other causes.
†Some additional specific conditions may be induced in regional classifications (e.g. penicilliosis in Asia, HIV-associated rectovaginal fistula in Africa).

Table 97.5B WHO classification for HIV-1-infected children – immunologic

	AGE-RELATED CD4 VALUES			
Classification of HIV-associated immunodeficiency	**≤11 months (%)**	**12–35 months (%)**	**36–59 months (%)**	**≥5 years (cells/mm³ and %)**
Not significant	>35	>30	>25	>500
Mild	30–35	25–30	20–25	350–499
Advanced	25–29	20–24	15–19	200–349
Severe	<25	<20	<15	<200 or <15%

It is antiretroviral treatments, where available, that have changed the outlook for children infected with HIV by greatly decreasing morbidity and mortality rates.[35,39] The yearly mortality in an HIV-infected cohort of children in Europe and North America decreased from 7–10 per 100 patient-years in 1994 to less than 0.5 in 2006. Antiretroviral treatment leads to chronic, stable disease in these children, compatible with balanced and satisfactory development. The toxicity profile is globally similar to that observed in adults, but with a lower drug-induced morbidity rate, probably through lower co-morbidity (alcohol, tobacco, aging).[40,41] Compliance and adherence to the treatment regimen is critical and may require specifically tailored interventions, such as individual or group support.[42,43] The risk of AIDS has now been virtually eliminated in antiretroviral-treated children, at least in the medium term, with 10 years follow-up from the introduction of antiretroviral combination therapy. The main issues now concern the long-term prognosis for quality of life, particularly relating to the potentially deleterious effects of the virus and/or treatment on various organs, including the vascular endothelial and central nervous systems.

The general principles underlying antiretroviral treatment for children are the same as those for adults (Table 97.6; see also Chapter 100). In particular, viral replication should be decreased such that levels are stable and below the detection threshold – the only guarantee of long-term efficacy and prevention of the selection of resistance mutations. In infants over the age of 1 year, indications for treatment are based on CD4 levels. Before this age, most recommendations now suggest systematic treatment, given the difficulty of predicting early and severe forms of disease. However, it is still not possible to determine how long this early treatment should last. Recommendations concerning the choice of molecules and the threshold for starting treatment often change, and, therefore, the WHO guidelines and those of the health authorities in a number of countries should be consulted regularly. The first findings from poor countries are very encouraging, also showing marked decreases in mortality rates similar to those observed in more affluent countries. The main issue for the future, given the considerable number of children needing treatment, is the feasibility of implementing decentralized and long-term treatment programs on a wider scale in poor countries.[44,45]

Table 97.6 General principles of antiretroviral treatment for HIV-1 infected children

	AGE-SPECIFIC RECOMMENDATIONS	
A: WHO classification (see Table 97.5A)		
Clinical stage	**<12 months**	**>12 months**
1	Treat all	CD4 guided*
2	Treat all	CD4 guided*
3	Treat all	Treat all
4	Treat all	Treat all

B: Age-specific CD4 threshold to initiate antiretroviral treatment for children >12 months			
CD4	**12–35 months**	**36–59 months**	**>5 years**
Percentage	<20%	<20%	<15%
Absolute number cells/mm³	<750	<350	<200

*See part B.
Adapted from WHO guidelines 2006, revised 2008. May vary according national guidelines.

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References for this chapter can be found online at http://www.expertconsult.com

Chapter 98

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Special problems in women who have HIV disease

EPIDEMIOLOGY

The number and proportion of women who have HIV-1 infection in the USA have been gradually increasing. Of 39 002 persons reported to the Centers for Disease Control and Prevention (CDC) who had AIDS in 2006, 10 591 (27.2%) were women.[1] This contrasts with 1985 statistics, when 7% (534/8153) of people reported to the CDC who had AIDS were women. In 2004, HIV-1 infection was the fifth leading cause of death for white women aged 35–44 years, the leading cause of death for African-American women aged 25–34 years, and the fourth leading cause of death for Hispanic women aged 35–44 years. The racial distribution of women who were diagnosed with HIV/AIDS in 2006 was 65.2% African-American (56.2/100 000), 18% white (2.9/100 000) and 15.1% Hispanic (15.1/100 000).

Heterosexual transmission has surpassed intravenous drug use as the primary route for women acquiring HIV-1 infection in the USA. Current estimates are that 40 000 new infections occur annually in the USA, of which 30% are in women. Of newly infected women, estimates are that more than 80% acquired HIV-1 through heterosexual sex and less than 20% through intravenous drug use.

Worldwide 2007 UNAIDS estimates are that, of the 30.8 million adults living with HIV-1/AIDS, 15.4 million (50%) are women.[2] Of these, 68% live in sub-Saharan Africa. Nearly one (0.8%) in every 100 adults worldwide aged 15–49 years is HIV-1 infected. In sub-Saharan Africa, 5% of all adults in this age group are HIV-1 infected. More than 80% of all adults worldwide acquired HIV-1 infection via heterosexual intercourse.

TRANSMISSION

Factors that are important in male-to-female transmission of HIV-1 infection include:

- advanced disease in the infected source partner;
- plasma HIV-1 RNA level (and virus shedding in genital secretions);
- anal receptive intercourse;
- presence of genital ulcers;
- absence of condom use; and
- absence of zidovudine use.[3–6]

Factors associated with heterosexual female-to-male transmission of HIV-1 infection include:

- advanced disease in the infected source partner;
- plasma HIV-1 RNA level (and virus shedding in genital secretions);
- presence of genital ulcers;
- sexual intercourse during menses; and
- absence of condom use.

In developing countries, observational studies have also suggested an association of nonulcerative sexually transmitted diseases with increased rates of HIV-1 transmission. For women, *Candida* vaginitis, bacterial vaginosis and use of depot medroxyprogesterone acetate have been associated with an increased incidence of HIV-1 infection. The efficiency of male-to-female transmission of HIV-1 has been estimated to be twice that of female-to-male transmission, although some studies have found equal rates.[4,5] Suppression of herpes simplex virus (HSV)-2 infections in HIV-negative women did not decrease the risk of HIV acquisition.[8] Cellulose sulfate as a vaginal microbicide was ineffective, but women who applied 0.5% PRO 2000/5 Gel vaginally every day had a 30% lower rate of HIV acquisition ($p = 0.06$).[8A]

CLINICAL FEATURES

Disease manifestations and progression

Overall, there are few differences in the incidence of nongynecologic opportunistic diseases between men and women who have HIV-1 infection, except for a higher incidence of esophageal candidiasis as an AIDS-defining condition and a lower incidence of Kaposi's sarcoma in women.[9] It is speculated that the higher incidence of candidiasis in women might be due to vaginal colonization with yeast or perhaps hormonal influences.

Several studies have now demonstrated that women have lower plasma HIV-1 RNA levels than do men after controlling for age, the interval from seroconversion and the $CD4^+$ lymphocyte count.[10] Another study found that this sex difference in plasma HIV-1 RNA levels disappeared 5–6 years after seroconversion. Although women may progress to AIDS with lower viral loads, the time to progression to AIDS appears to be similar for men and women. Similarly, for HIV-1-infected men and women who have adequate access to health care and treatment, treatment response and survival appears to be equivalent.[11,12]

Gynecologic manifestations

Infection

Gynecologic disorders are common in women who have HIV-1 infection.[13] Up to 50% of HIV-1-infected women develop recurrent *Candida* vaginitis, which often precedes the development of oral or esophageal candidiasis. Several longitudinal cohorts have reported that 14–18% of women who have HIV-1 infection have or develop recurrent genital HSV infection, which can be more severe or refractory to treatment than among HIV-1 seronegative women.[12]

The relationship between HIV-1 and pelvic inflammatory disease (PID) in women is less clear. Several retrospective studies suggest that women presenting with PID have a high rate of co-infection

with HIV-1 and that women who have HIV-1 infection with PID require surgical intervention more frequently because of abscess formation.[14] Prospective studies will be necessary to define this relationship and determine whether there are differences in response to therapy, microbiologic etiology and risk of recurrence or long-term sequelae.

Genital dysplasia

Cervicovaginal dysplasia is common in women who have HIV-1 infection.[13,15] Abnormal Papanicolaou smears were found in 38.3% of 1713 HIV-1-infected women and 16.2% of 482 women uninfected with HIV-1 but at risk, in the Women's Interagency HIV-1 Study cohort.[15] In multivariate analyses, risk factors for abnormal cytology included HIV-1 infection, low $CD4^+$ lymphocyte counts, high plasma HIV-1 RNA levels and detection of human papillomavirus (HPV). In follow-up of this cohort, HPV detection, $CD4^+$ lymphocyte count and plasma HIV-1 RNA levels predicted regression.[16] Rates of incidence, progression and regression of abnormal cytology did not differ between the HIV-1-uninfected controls and HIV-1-infected women with $CD4^+$ lymphocyte counts >200 cells/mm^3 and plasma HIV-1 RNA levels below 4000 copies/ml. In another study, 20% (80/398) of HIV-1-seropositive women compared with 4% (15/357) of HIV-1-seronegative women had cervical intraepithelial neoplasia (CIN) confirmed by colposcopy.[17] The presence of CIN was found to be independently associated with:

- HPV infection (odds ratio 9.8);
- HIV-1 infection (odds ratio 3.5);
- $CD4^+$ lymphocyte count <200/mm^3 (odds ratio 2.7); and
- age greater than 34 years (odds ratio 2.0).

The rate of CIN in women who have HIV-1 infection with <200 $CD4^+$ lymphocytes/mm^3 was 28% (27/95) compared with 19% (45/236) for those with higher $CD4^+$ lymphocyte counts.

Conflicting data exist regarding whether CIN and invasive cervical cancer have a more aggressive course or a less favorable response to therapy among women who have HIV-1 infection. In one study prior to the availability of highly active antiretroviral therapy (HAART), 62% of 127 women who had HIV-1 infection with CIN developed recurrent CIN within 36 months of treatment compared with 18% of HIV-1-seronegative controls.[18] During the 36-month follow-up period, progression to higher grade dysplasia, including one invasive cancer, occurred in 25% of women who had HIV-1 infection compared with 2% of controls. Recently, HIV-1-infected women in New York were reported to be at higher risk than HIV-1-seronegative women for other HPV-associated malignancies, including vulvar and anal cancers. HAART that reverses immunosuppression is likely to impact favorably on the rate of HPV detection and prevalence of genital tract and anal dysplasia. Indeed, more recent studies have found a lower prevalence of CIN II and III and HPV DNA positivity among women treated with HAART.[19,20] A new quadrivalent HPV vaccine approved for HIV-uninfected females 9–26 years of age has been shown to reduce the risk of high-grade dysplasia, CIN and cervical cancer. Whether the same degree of protection will be observed among HIV-1-infected women has yet to be determined.

The 2006 Consensus Guidelines for the management of women who have cervical cytologic abnormalities are detailed in Table 98.1.[21] These guidelines now state that immunosuppressed women should be managed as per guidelines for the general population of women. These Consensus Guidelines reflect updated terminology for reporting cervical cytology results, recent availability of HPV DNA testing and further follow-up data on the natural history of atypical squamous cells of undetermined significance/low-grade squamous intraepithelial lesions.[22]

Because *Candida* vaginitis, genital HSV disease, PID and cervical dysplasia are common in women who have HIV-1 infection, these conditions, along with other common sexually transmitted diseases such as gonorrhea, chlamydial infection and syphilis, should prompt a determination of HIV-1 risk factors and appropriate HIV-1 screening as part of routine care for women.

Table 98.1 Guidelines* for cervical cancer screening of women with HIV-1 infection

Initial gynecologic examination with Papanicolaou smear	If normal, repeat in 6 months If atypical squamous cells of uncertain significance (ASCUS), three options exist for women >20 years: • repeat Pap smear at 6 and 12 months; if abnormal proceed to colposcopy • colposcopy • HPV (high-risk types) DNA testing; if positive proceed to colposcopy If atypical squamous cells cannot rule out high-grade SIL (ASC-H), proceed to colposcopy If low-grade SIL, proceed to colposcopy If high-grade SIL, immediate loop electrosurgical excision or proceed to colposcopy If atypical glandular cells (AGC), proceed to colposcopy
If initial two Papanicolaou smears are normal	Repeat annually as long as Pap smears remain normal

*Based on the 2006 Consensus Guidelines and the United States Public Health Service Guidelines.[21,22]
SIL, squamous intraepithelial lesion.

Menstrual cycle and menopause

The impact of HIV-1 infection on the menstrual cycle appears to be limited. Reports of increased rates of dysmenorrhea, oligomenorrhea, amenorrhea or menorrhagia exist, but these have been discounted by other studies.[23] A combined analysis from the Women's Interagency HIV Study (WIHS) and the Heart and Estrogen/progestin Replacement Study (HERS) with 802 HIV-infected women and 273 high-risk seronegative women found little relationship between HIV status and amenorrhea, menstural cycle length or variability.[24]

Limited data exist on the effect of HIV infection on menopause. Some studies have suggested a slightly earlier onset of menopause among HIV-infected women (46 years vs 47 years) but this may be more related to other factors known to predict earlier onset of menopause such as substance use, tobacco smoking, low relative body weight, low socioeconomic status, depression and African-American ethnicity.

PREVENTION

Contraception

Condoms are important for preventing the transmission of HIV-1 and other sexually transmitted diseases. However, alone they may not be adequate to prevent pregnancy. Reported breakage rates for male condoms are less than 2% for vaginal and anal intercourse. An evaluation of the effectiveness of the female condom at preventing pregnancy in 147 women over a 6-month period demonstrated an annual failure rate of 26%. Among 86 women who reported using the condom consistently and correctly, the annual failure rate was still 11%.[25]

Hormonal contraceptives should be considered in addition to condoms for women who have HIV-1 infection, are sexually active and wish to avoid pregnancy. In view of the wide array of potential drugs available for antiretroviral therapy and prevention and treatment

Table 98.2 Drug interactions between antiretrovirals and oral contraceptives and recommended adjustments

Agent	Effect on oral contraceptive	RECOMMENDATION		
		No dose adjustment	No data	Use alternative agent or second method
Indinavir	Norethindrone levels ↑26% Ethinylestradiol levels ↑24%	X		
Ritonavir	Ethinylestradiol levels ↓40%			X
Saquinavir			X	
Nelfinavir	Norethindrone levels ↓18% Ethinylestradiol levels ↓47%			X
Fosamprenavir	↑ in ethinylestradiol with APV ↑ in norethindrone with APV APV levels ↓20%			X
Lopinavir	Ethinylestradiol levels ↓42%			X
Atazanavir	Ethinylestradiol AUC ↑48% Norethindrone AUC ↑110%			Use lowest dose
Tipranavir	Ethinylestradiol C_{max} and AUC ↓50%			X
Darunavir	Potential for interaction from ritonavir			X
Nevirapine	Ethinylestradiol levels ↓20%			X
Delavirdine	Ethinylestradiol levels may ↑		X	
Efavirenz	Ethinylestradiol levels ↑37% No data on norethindrone levels			X
Etravirine	Ethinylestradiol AUC ↑22%	X		
Maraviroc	No significant interaction	X		
Raltegravir	No significant interaction	X		

APV, amprenavir; AUC, area under the curve.
Data from Panel on Antiretroviral Guidelines for Adult and Adolescents.[26]

of opportunistic infections, the medications of women receiving hormonal agents should be critically reviewed to avoid drug–drug interactions such as:

- those that might lead to a reduction in the efficacy of hormonal agents; and
- those that may reduce the efficacy or increase the toxicity of other drugs in the presence of hormonal agents.

In particular, protease inhibitors and non-nucleoside reverse transcriptase inhibitors (NNRTIs) can affect the levels of hormonal agents. Table 98.2 details these interactions and recommendations for their concomitant use.[26]

Pregnancy

In the USA, in 1993, the rate of HIV-1 infection among women of child-bearing age was 1.7 per 1000 and there were between 1000 and 2000 perinatally infected infants born to 6000–7000 HIV-1-infected women. UNAIDS estimates for 2007 are that, worldwide, 330 000 children under 15 years of age died in 2007, and another 2.5 million children are currently living with HIV-1/AIDS, of whom 420 000 were infected in 2007.[2]

Perinatal transmission can occur in utero and during labor and delivery, as well as postpartum, primarily through breast-feeding.[27,28] Excluding postpartum transmission through breast-feeding, data suggest that 80% of maternal–infant transmission occurs late in gestation or during labor and delivery.[29] Several studies have found that breast-feeding increases the rate of transmission by 16% over 2 years and thus breast-feeding is not recommended when safe alternatives are available.[28] The majority of infections transmitted through breast milk occur during the first few weeks or months of life.[30] Extending prophylaxis with nevirapine ± zidovudine for the first 14 weeks of life was shown to significantly reduce postnatal HIV-1 infection in 9-month-old infants.[30A] Risk factors associated with transmission via breast-feeding include level of virus in the breast milk, mastitis, breast abscesses, maternal seroconversion during lactation, duration of feeding and mixed feeding.

A number of maternal and delivery factors appear to influence the risk of transmission and are listed in Table 98.3.[27,31] In addition, the rate of maternal–infant transmission has been reported to be increased when maternal virus exhibits rapid or high titer replication in human peripheral blood mononuclear cells, T-cell line tropism or resistance to neutralization by maternal serum. Interventions currently recommended to reduce transmission include:

- avoidance of invasive monitoring whenever possible;
- avoidance of breast-feeding;
- the use of zidovudine with or without other antiretroviral therapy during pregnancy for the mother and in the peripartum and postpartum period for the infant;
- treatment of sexually transmitted disease or vaginitis; and
- elective Caesarian section if plasma HIV-1 RNA level remains above 1000 copies/ml near term.

Table 98.3 Maternal, labor and delivery factors that increase the risk of maternal–infant transmission

Maternal factors	Labor and delivery factors
Advanced maternal HIV disease Maternal p24 antigenemia Low maternal CD4+ lymphocyte counts High maternal plasma HIV-1 RNA levels Acute maternal HIV infection during pregnancy Genital inflammation or maternal sexually transmitted disease at the time of delivery Detectable genital tract HIV-1 RNA near delivery Lack of antiretroviral therapy Breastfeeding	Chorioamnionitis Prolonged rupture of membranes (>4 hours) Premature delivery before 34 weeks' gestation Use of fetal scalp electrodes Episiotomy with severe lacerations Non-elective Caesarian section delivery

Antiretroviral therapy to prevent perinatal transmission

The landmark AIDS Clinical Trials Group (ACTG) 076 trial demonstrated a 66% reduction in maternal–infant HIV-1 transmission with zidovudine use in the mother during pregnancy, labor and delivery, and in the baby for the first 6 weeks of life (7.6% zidovudine vs 22.6% placebo). Since that study was reported, advances have occurred in our understanding of the pathogenesis of HIV-1 disease and its treatment and management.[32] The precise mechanisms by which zidovudine diminishes maternal–infant transmission of HIV-1 remain unclear but probably include:

- decreased maternal plasma HIV-1 RNA levels;
- decreased exposure of the fetus to the virus in utero; and/or
- decreased HIV exposure of the infant at delivery or in the neonatal period.

The end result is prevention of infection from becoming established in the fetus or infant. Additional analyses have now shown that transmission of HIV-1 occurs at all levels of CD4+ lymphocyte counts and even in some women who have undetectable plasma HIV-1 RNA levels, although overall the risk of transmission is greater in women who have lower CD4+ lymphocyte counts and higher plasma HIV-1 RNA levels, particularly at the time of delivery.[33] Detectable virus in the female genital tract at 38 weeks gestation has also been independently associated with maternal–infant transmission of HIV-1.[34]

It is important to stress that even intervening late in pregnancy (including initiating therapy in the peripartum period) can diminish the risk of transmission.[35–37] In developing countries, a two-dose nevirapine regimen (oral nevirapine, 200 mg as a single dose given to the mother at the onset of labor and oral nevirapine 2 mg/kg given to the infant within 72 hours of birth) is widely used based on a randomized comparison that demonstrated the two-dose nevirapine regimen reduced the risk of maternal–infant transmission by 47% compared with a truncated zidovudine regimen.[38] Follow-up data from this study, however, documented the occurrence of K103N mutations in virus recovered from up to 15% of women who were randomized to nevirapine, a mutation conferring cross-class resistance to NNRTIs (with the exception of etravirine).[39] Fortunately, later data have shown that this resistance has not affected subsequent perinatal transmission rates when nevirapine is again used as the mode for prevention. The predominant circulating strain of virus generally reverts to wild-type once nevirapine is cleared. More concerning is that women randomized to receive nevirapine + tenofovir–emtricitabine for treatment of their HIV 6 or more months after receiving single-dose nevirapine for prevention of maternal–infant HIV transmission did worse than women randomized to lopinavir–ritonavir + tenofovir–emtricitabine.[39A] After a median follow-up of 73 weeks, 26% of women receiving nevirapine developed virologic failure or death compared with 8% in the lopinavir arm. Similarly, response to nevirapine-based treatment regimens have not been compromised if initiated 6–12 months postexposure to single-dose nevirapine, although the durability of the response longer term compared with other regimens that do not contain NNRTIs remains to be established in ongoing clinical trials.[40]

All pregnant women should be offered HIV-1 testing. Repeat HIV antibody testing is now recommended in the third trimester (preferably before 36 weeks gestation) for women at high-risk of acquiring HIV infection who have negative tests earlier in their pregnancy. Women who present to labor and delivery without prior HIV testing should be offered a rapid HIV test to guide decisions. In general, current recommendations are to approach the treatment of the HIV-1-infected pregnant woman as if she were not pregnant and strive for maximal suppression of viral replication. Nevertheless, knowledge that limited data exist regarding toxicity of the 23 Food and Drug Administration (FDA)-approved antiretroviral agents to the developing fetus and the infant must also be taken into account. Tables 98.4–98.7 summarize the current information known about reverse transcriptase inhibitors, protease inhibitors, entry inhibitors and integrase inhibitors with regard to FDA approval status, placental transfer and carcinogenicity data.

To date, neither pre-term delivery nor birth defects have been clearly associated with any antiretroviral agent; however, data are limited, particularly for infants exposed to combination therapy. Only efavirenz is listed as a class D drug based on four restrospective case reports of neural tube defects (n = 3) and a case of Dandy–Walker malformation (n=1). As of 1/31/07, the prospective Antiretroviral Pregnancy Registry reported seven birth defects in 281 women with first trimester efavirenz exposure.[41] This 2.5% prevalence rate falls within the expected rate of 3.0/100 live births and no neural tube defects were reported. Currently, efavirenz should be avoided in the first trimester, because of evidence of teratogenicity in rhesus macaques at human doses.

From September 2007 through March 2008, nelfinavir was not recommended for pregnant women or women who might become pregnant due to the presence of low levels of ethyl methane sulfonate (EMS) in nelfinavir. EMS is a process-related impurity that is known to be teratogenic, mutagenic and carcinogenic in animals. The manufacturing process has been modified and nelfinavir is again safe to use in women and children. The Women's and Infants Transmission Study (WITS) found an increased rate of hypospadias only in first trimester zidovudine-exposed boys but not in the rate of hypospadias among zidovudine-exposed boys overall. A French group reported 12 cases of mitochondrial dysfunction among 2644 uninfected infants who received zidovudine or the combination of zidovudine and lamivudine in utero and/or after birth. Two of these children developed progressive neurologic dysfunction and died. This syndrome has not been detected in over 20 000 children born to HIV-1-infected women in the USA.

On the basis of this information, the US Public Health Service (USPHS) has drafted the following guidelines for the treatment of HIV-1-infected pregnant women.[42]

If the woman has had no prior antiretroviral therapy, treatment should be based on standard indications. However, at a minimum, the three-part zidovudine regimen as outlined in ACTG 076 should be administered. In a meta-analysis, antenatal antiretroviral prophylaxis primarily with zidovudine alone was shown to reduce transmission for women who have plasma HIV-1 RNA levels below 1000 copies/ml at or near delivery compared with no antenatal therapy (1% vs 9.8%, respectively).[43] Most experts feel comfortable substituting zidovudine 300 mg q12 h orally for the maternal component. Combination therapy is recommended for women who have a plasma HIV-1 RNA level over 1000 copies/ml. Consideration can be given to delaying the initiation of therapy until after 10–12 weeks gestation, which is thought to be the critical time for fetal organogenesis.

If the HIV-1-infected pregnant woman is already receiving antiretroviral therapy when pregnancy is diagnosed, then therapy is generally continued, even in the first trimester. Although not recommended

Table 98.4 Reverse transcriptase inhibitors

Agent	Studies showing transmission to fetus prevented	FDA APPROVED		FDA pregnancy category*	Placental transfer (%)
		Neonates	Children		
Zidovudine	Yes	Yes	Yes	C	80
Didanosine	No	Yes	Yes	B	50
Lamivudine	Yes	Yes	≥3 months	C	100
Stavudine	No	Yes	≥1 month	C	80 (rhesus monkeys)
Abacavir	No	No	≥3 months	C	Yes (rats)
Emtricitabine	Yes	No	≥3 months	B	40 (mice)
Tenofovir	No	No	≥18 years	B	95
Nevirapine	Yes	Yes	≥2 months	C	100
Delavirdine	No	No	No	C	?
Efavirenz	No	No	≥3 years	C	100 (rhesus monkeys)
Etravirine	Yes	No	≥18 years	B	?

*FDA pregnancy categories:
A Adequate and well-controlled studies of pregnant women fail to demonstrate a risk to the fetus during the first trimester and no evidence of risk during later trimesters.
B Animal studies fail to demonstrate a risk to the fetus but no adequate studies exist in pregnant women.
C Animal studies demonstrate risk or have not been conducted and safety in human pregnancy has not yet been determined. However, the benefit of the drug may still outweigh the risk.
D Positive evidence of human fetal risk exists based on adverse reaction data but benefits may still outweigh the risks.
Data from Panel on Antiretroviral Guidelines for Adult and Adolescents[26] and from Guidelines for the Use of Antiviral Agents in Pediatric HIV Infection.[26A]

Table 98.5 Protease inhibitors

Agent	Studies showing transmission to fetus prevented	FDA APPROVED		FDA pregnancy category*	Placental transfer
		Neonates	Children		
Nelfinavir	No	No	≥2 years	B	Minimal
Indinavir	No	No	≥18 years	C	Minimal
Ritonavir	No	No	>1 month	B	Minimal
Saquinavir	No	No	≥16 years	B	Minimal
Fosamprenavir	No	No	≥2 years	C	?
Lopinavir/ritonavir	No	≥14 days	Yes	C	20%
Atazanavir	No	No	≥6 years	C	10%
Tipranavir	No	No	≥2 years	C	?
Darunavir	No	No	≥3 years	B	?

*FDA pregnancy categories – see Table 98.4.
Data from Panel on Antiretroviral Guidelines for Adult and Adolescents[26]and from Guidelines for the Use of Antiviral Agents in Pediatric HIV Infection.[26A]

Table 98.6 Entry inhibitors (FDA approved)

Agent	Studies showing transmission to fetus prevented	Neonates	Children	FDA pregnancy category*	Placental transfer
Enfuvirtide	No	No	≥6 years	B	None
Maraviroc	No	No	≥16 years	B	?

*FDA pregnancy categories – see Table 98.4.
Data from Panel on Antiretroviral Guidelines for Adult and Adolescents[26]and from Guidelines for the Use of Antiviral Agents in Pediatric HIV Infection.[26A]

Table 98.7 Integrase inhibitors (FDA approved)

Agent	Studies showing transmission to fetus prevented	Neonates	Children	FDA pregnancy category*	Placental transfer
Raltegravir	No	No	≥16 years	C	Y (rats)

*FDA pregnancy categories – see Table 98.4.
Data from Panel on Antiretroviral Guidelines for Adult and Adolescents[26] and from Guidelines for the Use of Antiviral Agents in Pediatric HIV Infection.[26A]

(particularly for women with CD4+ lymphocyte counts <200 cells/μl), if the decision is made to stop therapy during the first trimester owing to concerns about teratogenicity, all drugs should be stopped and then restarted simultaneously in order to minimize the development of resistance. Women on efavirenz should stop their antiretroviral therapy until past the first trimester and then restart a new regimen.

Zidovudine should be incorporated into the regimen whenever feasible; however, if resistance or intolerance precludes its use, zidovudine should still be administered intravenously intrapartum and to the baby as outlined above. Additionally, zidovudine and stavudine should not be co-administered. Some experts would also recommend zidovudine in combination with other antiretroviral agents for infants born to mothers who have known or suspected zidovudine resistance.

For women receiving standard antenatal antiretroviral therapy, the addition of the two-dose nevirapine regimen did not further reduce transmission rates and, as previously discussed, resulted in the development of mutations conferring resistance to NNRTIs.[44,45] Thus, for women who do not achieve adequate viral suppression near delivery, Caesarian section is recommended without the addition of the two-dose nevirapine regimen.

If an HIV-1-infected woman presents in labor and no prior antiretroviral therapy has been given, two antiretroviral treatment options are recommended:

- intrapartum intravenous zidovudine until delivery, and zidovudine to the newborn for 6 weeks; or
- the two-dose nevirapine regimen combined with intrapartum intravenous zidovudine and 6 weeks of zidovudine for the newborn.

If nevirapine is administered, consider adding lamivudine to zidovudine intrapartum or soon therafter for 7 days to limit the development of resistance to nevirapine. This is the preferred option at our institutions. Results from a recent study completed in pregnant women in South Africa indicated that this strategy reduced the frequency of nevirapine resistance compared with the two-dose nevirapine regimen without lamivudine and zidovudine.[46] Another study added a single dose of tenofovir and emtricitabine at delivery to nevirapine and found that this approach decreased resistance to nevirapine by 53% 6 weeks after delivery.[47]

Infants born to HIV-1-infected mothers who have received no antiretroviral therapy during pregnancy or intrapartum should still receive zidovudine for the first 6 weeks of life. Consideration may also be given to treating the infant with additional antiretroviral medications. The mother's therapy should be re-evaluated after delivery in both these situations.

Resistance testing is available to guide antiretroviral therapy choices. The International AIDS Society–USA Panel and EuroGuidelines Group for HIV-1 Resistance recommend that all pregnant women who have detectable viremia have resistance testing performed prior to initiating or changing antiretroviral therapy. The USPHS recommends resistance testing for HIV-1-infected pregnant women prior to initiating therapy and for those entering pregnancy with a detectable HIV-1 RNA level while on therapy. While underlying resistance can affect the ability to achieve maximal viral suppression, it is not clear that the presence of mutations increases the likelihood of transmission to the infant. Resistant virus has been transmitted to infants; however, women who have zidovudine resistance mutations have not consistently transmitted infection to their infants at higher rates and, in some cases where transmission has occurred, wild-type virus was transmitted. When less than potent antiretroviral regimens (zidovudine monotherapy or dual nucleoside analogues) are administered to pregnant HIV-1-infected women because antiretroviral therapy is not indicated for the women themselves or women choose to minimize exposure to antiretroviral therapy during pregnancy, development of resistance can occur and can potentially limit future treatment options.

A meta-analysis of 15 prospective cohort studies, conducted in an era when pregnant HIV-1-infected women received no antiretroviral therapy or zidovudine monotherapy, showed that elective Caesarian section decreased the risk of maternal–infant transmission of HIV-1 compared with other modes of delivery.[48] For women on zidovudine, transmission was 2% with elective Caesarian section and 7.3% with other modes of delivery. Because transmission rates are expected to be below 2% for women on potent antiretroviral therapy with controlled viremia, the American College of Obstetricians and Gynecologists' Committee on Obstetric Practice recommends Caesarian section before the onset of labor for women who have plasma HIV-1 RNA levels above 1000 copies/ml near term. The Caesarian section should be performed at 38 weeks gestation without amniocentesis to assess for fetal lung maturity. For a scheduled Caesarian section, intravenous zidovudine should be started 3 hours before surgery. Caesarian section has greater morbidity than vaginal delivery but current data suggest that HIV-1-infected women have similar complications to those in uninfected women except for those with advanced disease where the complication rates are higher.

Duration of ruptured membranes is also associated with an increased risk of transmission. A meta-analysis of the same 15 studies used to assess the benefit of Caesarian section showed, for the first 24 hours after membrane rupture, a 2% increase in transmission for every additional hour of membrane rupture.[49] Cleansing the birth canal with a 0.25% chlorhexidine solution every 4 hours until delivery did not reduce transmission in one study, except when membranes were ruptured more than 4 hours before delivery.[50] Whether or not nonelective Caesarian section to shorten the duration of ruptured membranes or labor will further reduce transmission rates is currently unknown.

Safety monitoring guided by the pregnant woman's specific antiretroviral therapy should be performed. Routine hematologic and hepatic enzyme monitoring is recommended for women on zidovudine. Liver function should be monitored in all women receiving antiretroviral therapy. Women receiving nucleoside reverse transcriptase inhibitors (NRTIs) should also be assessed for development of lactic acidosis and hepatic steatosis with frequent liver enzyme and electrolyte monitoring in the third trimester. Cases of fatal lactic acidosis have been reported in pregnant women receiving prolonged courses of didanosine and stavudine. When other alternatives are available, this combination should be avoided during pregnancy. Women initiating nevirapine should have frequent monitoring of transaminase levels due to the risk of hepatotoxicty that can occur within the first 18 weeks. Standard glucose tolerance testing is recommended at 24–28 weeks gestation. Some experts recommend earlier testing for women on chronic protease inhibitor therapy begun prior to pregnancy. In general, the plasma HIV-1 RNA level should be monitored 2–6 weeks after initiating or changing antiretroviral therapy, monthly until undetectable and then every 2 months until delivery. The CD4+ lymphocyte count should be monitored every 3 months. A plasma HIV-1 RNA level should be obtained at 34–36 weeks gestation to guide decisions on mode of delivery. A first trimester ultrasound is recommended to guide dates. A second trimester, level II ultrasound is recommended to assess the fetus for women on combination antiretroviral therapy.

In the future, ongoing or planned studies will certainly provide more information regarding the use of the newer reverse transcriptase inhibitors, protease inhibitors, entry inhibitors, integrase inhibitors and combination regimens for the treatment of pregnant women and the prevention of transmission to the fetus/infant. In the meantime, HIV-1-infected pregnant women should be referred for participation in clinical trials whenever possible and be reported to the appropriate agencies that collect safety and teratogenicity data. In the USA, the Antiretroviral Pregnancy Registry can be reached at http://www.apregistry.com.

In general, pregnant women who have HIV-1 infection should receive prophylaxis for opportunistic infections appropriate for their stage of disease.[22] For *Pneumocystis jirovecii* pneumonia prophylaxis, some experts recommend avoiding trimethoprim–sulfamethoxazole and dapsone in the first trimester, and trimethoprim–sulfamethoxazole close to term to reduce the risk of kernicterus in the infant. Aerosolized pentamidine can be substituted during these time periods. While rifabutin and the macrolides have not been studied in pregnant women, those at high risk of disseminated *Mycobacterium avium* complex disease may benefit from the use of these medications after the first trimester. Azithromycin is favored owing to its safety profile

because clarithromycin is teratogenic in animals. Pregnant women who have a positive purified protein derivative skin test for tuberculosis should also receive isoniazid prophylaxis after the first trimester. Pregnant women should also avoid eating raw or undercooked meat and avoid contact with cat feces to diminish the risk of toxoplasmosis. Good handwashing techniques should be employed for prevention of cytomegalovirus (CMV) disease, particularly in women who are health-care workers or who have children in day-care settings.[22] For CMV-seronegative women who require blood transfusions, only CMV-seronegative blood products should be used.[22]

Several studies have now demonstrated no adverse effect of pregnancy on the progression of HIV-1 disease for women who have $CD4^+$ lymphocyte counts >200 cells/μl.[51] Unfortunately, women who have more advanced HIV-1 disease may not tolerate pregnancy as well and may have a higher rate of spontaneous abortion, prematurity, low birth weight infants and other complications of pregnancy.[52]

REFERENCES



References for this chapter can be found online at http://www.expertconsult.com

Chapter 99

Mark W Hull
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Principles of management of HIV in the developed world

INTRODUCTION

The management of HIV infection continues to evolve as a result of a better understanding of HIV pathogenesis and with the emergence of new drugs, treatment strategies and laboratory monitoring tools. Antiretroviral therapy has advanced considerably since the first introduction of highly active antiretroviral therapy (HAART).[1,2] Regimens have become simpler, better tolerated and safer.[3] HAART-related immune reconstitution reliably prevents AIDS-related morbidity and mortality and furthermore allows discontinuation of primary and secondary prophylaxis for AIDS-related opportunistic infections in some settings.[4,5] Life expectancy of HIV-infected individuals in the modern HAART era is now measured in decades.[6,7] Improved longevity has been associated with the emergence of new challenges, including newly recognized adverse effects of long-term exposure to antiretroviral drugs. These include disorders of fat accumulation and fat wasting (lipodystrophy), dyslipidemias and glucose intolerance, hypertension, osteoporosis and increased risk of myocardial infarction.[8,9] Treatment interruptions, once considered a possible means of mitigating adverse events, have now been shown to be associated with increased morbidity and mortality.[10]

Management strategies now focus on preparing patients for initiation of antiretroviral therapy, and for long-term use of HAART with its possible concomitant metabolic effects. Patients who do not yet meet criteria for starting antiretroviral therapy must be protected from other infectious diseases by vaccination or prophylaxis when appropriate, and must be followed closely from a laboratory perspective to determine the optimal time of intervention with antiretroviral therapy.

Traditionally, the $CD4^+$ lymphocyte (CD4 cell) count has been regarded as the key surrogate marker for prognostic staging and therapeutic monitoring of HIV-infected individuals. $CD4^+$ lymphocyte loss is linked to the rate of HIV viral turnover, and the interaction between the quantifiable plasma HIV RNA level (i.e. the HIV viral load) and $CD4^+$ lymphocyte production will ultimately determine CD4 cell loss.[11,12]

BASELINE EVALUATION OF THE HIV-INFECTED INDIVIDUAL

History and physical examination

The history should elicit the date of diagnosis and, if available, information regarding prior negative tests in order to estimate the length of time since infection. Symptoms related to progression of HIV infection and manifestations of opportunistic infections should be explored. For patients who had previously received antiretroviral therapy, detailed information regarding CD4 cell nadir, prior drug regimens, adherence, CD4 and viral load response and adverse effects should be documented. In addition, details regarding co-morbidities and concomitant medication should be well documented. A detailed history of drug and environmental allergies should be elicited.

Personal lifestyle, history of prior sexually transmitted diseases, safer sex, harm-reduction practices and reproductive history and expectations should be discussed in detail. Prior immunizations should be documented.

The physical examination should focus on clinical manifestations of HIV disease and those of AIDS-related opportunistic infections or malignancies. The oropharynx and dermis should be closely examined, and in patients with more advanced disease (CD4 cell count <50 cells/mm^3) it may be necessary to arrange proper ophthalmologic assessment with a slit-lamp examination to rule out cytomegalovirus (CMV) retinitis. In female patients, Papanicolaou (Pap) testing should be performed to assess for cervical dysplasia.

General laboratory tests are also very important, particularly before the start of a treatment program. These should include those listed in Table 99.1. Abnormalities of the complete blood count are common, and updated serologic testing for co-infections such as hepatitis B and C and toxoplasmosis is required.

Prognostic laboratory markers

The CD4 cell count and plasma HIV viral load are the key prognostic markers for disease progression to AIDS or death among untreated HIV-infected individuals. These remain the essential components of laboratory monitoring. The initiation of antiretroviral therapy depends primarily on the CD4 cell count and to a lesser extent on the plasma HIV viral load among asymptomatic HIV-infected individuals.

CD4 cell counts are reported in absolute numbers and as the percentage (fraction) of total lymphocytes that are CD4 positive. The CD4 fraction is directly measured, while the absolute count is calculated as the product of the absolute lymphocyte count and the CD4 percentage. The CD4 cell count reflects the level of immune suppression. In adults, the CD4 cell count is normally between 400 and 1400 cells/mm^3. As the CD4 cell count falls below normal, and particularly once thresholds of 200 cells/mm^3 or a CD4 percentage of 15% are reached, the risk of opportunistic infection rises.[13,14]

There is substantial diurnal variation in the CD4 cell count; it is lowest in the morning and highest in the evening. As a result, clinical specimens are best collected consistently in the morning. The CD4 cell count can also be influenced by exercise, acute illness, co-morbidities or drugs such as systemic corticosteroids. In some patients, the CD4 percentage may serve as a better prognostic marker for progression to AIDS or death than the absolute count; this would include patients with splenectomy and pregnant women.[15,16] Short-term CD4 fluctuations of up to 30% have been shown to occur in HIV-infected individuals who are clinically stable.[17,18] As a result, it is important to monitor

Table 99.1 Laboratory tests for baseline evaluation of HIV-infected individuals

Test	Comment
HIV-specific tests	
Plasma HIV RNA (viral load)	
$CD4^+$ lymphocyte count (absolute and percentage)	
General laboratory profile	
Complete blood count	Evaluates baseline abnormalities such as anemia or HIV-related thrombocytopenias
Electrolytes, creatinine, urinalysis	Baseline renal abnormalities may require further investigation and influence choice of antiretrovirals
Liver profile (alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, total bilirubin), amylase	Abnormalities may require further hepatic investigations. Elevated baseline liver profile may affect antiretroviral choice (avoidance of agents associated with higher rate of hepatotoxicity)
Fasting lipid profile, fasting blood sugar	Future risk of cardiac-related morbidity may require early interventions and influence the choice of antiretrovirals
Co-infection/opportunistic diseases assessment	
Urine, urethral or cervical screens for sexually transmitted infections as indicated by history and examination	
Serologic testing for syphilis	Rapid plasma reagin (RPR)
Serologic testing for hepatitis A, B and C	If hepatitis B serology is in keeping with chronic infection or resolved infection, consider hepatitis B DNA screen. Vaccinate nonimmune individuals
Toxoplasma and cytomegalovirus serology	
Tuberculin skin test (± sputum smears and cultures for mycobacteria, as indicated by history and physical examination)	If >5 mm and no signs of active infection, consider isoniazid prophylaxis
Chest radiography	
Cervical Papanicolaou smear	
Pregnancy test	

trends in the CD4 cell count over time rather than placing too much emphasis on a single reading. Similarly, two CD4 cell counts, separated by at least 1–4 weeks should be obtained at baseline, prior to initiating antiretroviral therapy.

Plasma viral load has been shown to be an independent predictor of disease progression and death in untreated HIV-infected individuals.[11,19,20] Plasma viral load is also a factor in deciding when to initiate antiretroviral therapy.[3,21] From a practical standpoint it is useful to consider the CD4 cell count as indicative of the level of immunologic suppression or 'damage that has already occurred' and the viral load as indicative of the level of disease activity or the 'damage that is about to occur'. Three assays are currently available to quantify plasma HIV-1 viral load. These are the HIV RNA polymerase chain reaction (PCR), the branched-chain DNA and the nucleic acid sequence-based amplification assays. Ultrasensitive assays have lower limits of detection of 50 copies/ml. The variability of the assay is approximately 0.3 $\log_{10}$ copies/ml and, as a result, a 0.5 $\log_{10}$ change in HIV RNA level is regarded as a significant viral load change in the context of antiretroviral therapy. Among patients on HAART, the plasma viral load is the primary laboratory marker of treatment efficacy. A treatment-induced 10-fold (1 $\log_{10}$) reduction in plasma HIV-1 RNA is associated with a decrease of approximately 50% in the relative risk of death.[1,22]

Resistance testing

A baseline genotypic resistance test should be performed in patients with recent infection (i.e. less than 3 years) to evaluate for transmission of drug-resistant virus.[23] Determination of resistance at this stage will help to guide future therapy, and resistance mutations should be taken into account even if later testing reveals wild-type virus. Baseline resistance testing is also recommended in all individuals before selection of the first HAART regimen. Baseline resistance testing has been shown to be cost-effective once prevalence of primary resistance reaches 5%.[23–25] In addition, baseline resistance testing is particularly important if the initial regimen will contain a non-nucleoside reverse transcriptase inhibitor.

FOLLOW-UP ASSESSMENT

Asymptomatic, untreated patients with CD4 cell counts >500 cells/mm^3 should be monitored every 3–4 months both clinically and with CD4 cell counts and plasma viral load.[21,25] In patients with CD4 cell counts between 350 and 500 cells/mm^3, similar monitoring every 2 months is warranted.[26] Once the CD4 cell count is <350 cells/mm^3 (or below 15%), patients should be offered initiation of therapy. If this is deferred for any reason, monthly monitoring should be recommended. In addition, among patients with high CD4 cell counts (>500 cells/mm^3), and CD4 declines in excess of 100 cells/year or plasma HIV RNA >100 000 copies/ml, more frequent (i.e. monthly) monitoring is also warranted.

Asymptomatic individuals with CD4 cell counts within the normal range are considered to have adequate immune function; opportunistic infections are therefore rare in this setting, except for tuberculosis, Kaposi's sarcoma or lymphomas. However, common infections, in particular respiratory and dermatologic conditions, are frequent in

this setting. Management of nonopportunistic common infections in otherwise healthy HIV-infected individuals should follow the same protocols as in uninfected individuals. Pap testing, screening for sexually transmitted infections and tuberculin skin tests should be performed annually in at-risk patients.

Metabolic testing

Lipid abnormalities are common in HIV infection, with perturbations in triglyceride and high-density lipoprotein (HDL) values.[27] Antiretroviral therapy can lead to further abnormalities with increases in total cholesterol and triglyceride levels.[28,29] Fasting lipid profiles should be obtained at baseline, in conjunction with an assessment of overall cardiovascular risk using the Framingham risk assessment score.[30] Furthermore, lipid values should be monitored every 2–3 months after initiating antiretroviral therapy. Patients with abnormal lipid profiles should be managed according to the underlying targets set by the updated National Cholesterol Education Program guidelines. In this setting, revision of the HAART regimen in favor of a similarly active but more lipid-friendly option should be considered. Alternatively, lipid-lowering strategies recommended for HIV-infected individuals with dyslipidemia should be pursued[31–33] (see Practice Point 47).

HIV-infected patients may be at increased risk for osteopenia and osteoporosis.[34] This may again reflect underlying HIV effects, although antiretroviral drugs have also been implicated.[35,36] Patients with other risk factors for bone loss should be screened using dual-energy X-ray absorptiometry (DEXA). Some would advise that this test be repeated every few years, particularly in high-risk patients; however, routine use of DEXA scans remains controversial.[25,37]

Counseling

The need for risk reduction counseling should be assessed at all encounters. This should include assessment of higher-risk sexual activities, substance use and other factors that may influence adherence to antiretrovirals such as psychiatric disorders and depression. When issues are identified, patients should receive targeted counseling or referral to multidisciplinary care teams. Patients should have the opportunity to discuss the most recent trends in management and antiretroviral therapy.

PROPHYLACTIC MANAGEMENT

Tuberculin skin test

A tuberculin skin test should be performed at baseline and annually in patients at risk for exposure. Induration >5 mm is considered positive, and should prompt evaluation for possible active tuberculosis prior to consideration of treatment for latent tuberculosis infection (tuberculosis chemoprophylaxis). Chemoprophylaxis is also recommended for patients with recent exposure to a documented case of active tuberculosis, chest radiography supportive of previous tuberculosis or a history of untreated or inadequately treated previous tuberculosis. A 9-month course of isoniazid in conjunction with pyridoxine to reduce the risk of isoniazid toxicity is recommended.[38,39]

Vaccinations

While antigen recognition is still intact, it is essential to boost humoral immunity against common pathogens. Standard vaccines such as the tetanus–diphtheria toxoid vaccine should be updated as necessary. Influenza vaccine has been shown to be safe and immunogenic in the setting of HIV, and should be administered annually at the beginning of the winter season.[40,41] The polyvalent polysaccharide *Streptococcus pneumoniae* vaccine (Pneumovax) should be administered at least once, and repeated after 5 years.[42] Hepatitis A and B vaccines should be encouraged in susceptible patients, and in particular in at-risk individuals such as intravenous drug users, those with multiple sexual partners, men who have sex with men and individuals co-infected with hepatitis C.[43,44]

Prophylaxis (see also Chapter 91)

As seen in Table 99.2, as HIV disease progresses, prophylaxis for opportunistic infections becomes increasingly important. Once the CD4 cell count decreases below 200 cells/mm^3 the risk for *Pneumocystis jirovecii* (previously *P. carinii*) pneumonia rises, and more than 80% of individuals with untreated HIV will experience an episode of *Pneumocystis* pneumonia over their lifetime.[13] Prophylaxis is indicated if the CD4 cell count is <200 cells/mm^3, the CD4 percentage is below 15% and in patients with recurrent oral thrush.[39]

Patients with other AIDS-defining illnesses should also be offered primary prophylaxis, and patients experiencing *Pneumocystis* pneumonia should be placed on secondary prophylaxis after completion of acute treatment. The preferred regimen consists of one double-strength (DS) tablet of trimethoprim–sulfamethoxazole (TMP–SMX) daily. The use of intermittent dosing (3 days each week) for the DS tablet, or the use of one single-strength tablet of TMP–SMX daily, have been shown to be less effective than a regimen of 1 DS tablet daily.[45] Alternatives include dapsone 100 mg or atovaquone 1500 mg daily or inhaled pentamidine 300 mg monthly.

Prophylaxis for toxoplasmosis is indicated for those individuals who have positive serum IgG for toxoplasmosis and CD4 cell counts <100 cells/mm^3.[39] Trimethoprim–sulfamethoxazole remains the first-line regimen for primary prophylaxis. Dapsone with pyrimethamine, and folinic acid or single agent atovaquone are possible alternatives. As HIV disease progresses, prophylaxis for *Mycobacterium avium* complex (MAC) with weekly azithromycin should be initiated once CD4 cell counts

Table 99.2 Prophylaxis and treatment for opportunistic infections

CD4$^+$ lymphocyte cell count	Management strategy
Any	If needed, update standard diphtheria–pertussis–tetanus and inactivated polio vaccines Annual influenza vaccine 23-Valent pneumococcal polysaccharide vaccine. Responses may be better if CD4 >200 cells/mm^3. Revaccination should be considered after 5 years Hepatitis A and B vaccines Tuberculin skin test and 9 months isoniazid prophylaxis if ≥5 mm induration
<500	Monitor CD4 cell count and viral load every 3–4 months
<350	Consider monitoring CD4 cell count and viral load every 1–2 months, and discuss antiretroviral initiation
<200	Initiate *Pneumocystis jirovecii* prophylaxis. Trimethoprim–sulfamethoxazole 160 mg/800 mg (1 double-strength tablet) q24 h is first-line agent
<100	Start prophylaxis for toxoplasmosis if seropositive, if not on trimethoprim–sulfamethoxazole
<50	*Mycobacterium avium* complex prophylaxis with azithromycin 1200 mg po weekly Ophthalmologic screening for cytomegalovirus retinitis (repeat at 6-monthly intervals)

drop to <50 cells/mm³ but only after active MAC has been excluded.[39] Routine ophthalmologic assessment for CMV retinitis is indicated at 3- to 6-month intervals while the CD4 cell count is <50 cells/mm³.

Both primary and secondary prophylaxis for these common opportunistic infections can be discontinued in patients who experience HAART-related consistent increases in their CD4 cell counts to levels >100 cells/mm³ for MAC, and >200 cells/mm³ for toxoplasmosis and *Pneumocystis jirovecii*.[4,46,47]

INITIATION OF ANTIRETROVIRAL THERAPY

The objective of antiretroviral therapy is to prevent disease progression and prolong survival while maintaining quality of life. Long-term non-progression will be achieved by reducing plasma viral load to below 50 copies/ml on a sustained basis. The use of combinations of antiretroviral agents with no overlapping toxicity and demonstrated additive to synergistic antiviral effect is recommended to maximize the duration of the virologic response.[3,21] Since 1996, triple drug combination antiretroviral therapy has been shown to decrease morbidity and mortality dramatically in symptomatic and asymptomatic HIV-1-infected individuals.[1,2,48–52]

The Department of Health and Human Services and the International AIDS Society–USA have produced comprehensive consensus guidelines for the management of HIV, including issues around the initiation of therapy.[3,21] These are generally consistent with guidelines published by a number of national societies.[53,54] An approach to antiretroviral therapy is suggested in Figure 99.1.

WHEN TO START THERAPY

General principles

Antiretroviral therapy should be commenced in any patient with HIV-related symptoms or signs, opportunistic infections or cancers.[3,21,53] For asymptomatic patients, recommendations for the initiation of therapy are based primarily on thresholds of CD4⁺ lymphocyte counts and to a lesser extent on plasma HIV-1 RNA; these have been identified as key prognostic markers of the risk for disease progression in natural history and observational studies as well as randomized clinical trials.[11,55–57] Cohort studies have characterized rates of disease progression to AIDS or death based on CD4⁺ and plasma viral load thresholds in treated patients, and have long demonstrated that 200 cells/mm³ represents a critical threshold. CD4 cell levels below 200 cells/mm³ identify patients who are at high short-term risk of developing AIDS-defining illnesses and death, and for whom the effectiveness of antiretroviral therapy is partially compromised.[58–60] Recent data from large cohort studies and observations from clinical trials suggest that HIV-related morbidity and mortality may occur more commonly at higher CD4 strata than previously recognized, and that HAART may also reduce non-AIDS-related morbidity and mortality at CD4 cell counts above 200 cells/mm³.[61-63] Thus, current treatment guidelines consistently recommend that all individuals with CD4 cell counts <350 cells/mm³ should be encouraged to start therapy.[3,21]

Of note, among individuals with CD4 cell counts of 200 to 350/mm³, a CD4 cell count fraction ≤15% of the total lymphocyte count has been shown to be an independent predictor of short-term risk of developing an AIDS-defining illnesses or death, and decreased effectiveness of antiretroviral therapy.[64]

If a decision is reached to defer initiation of antiretroviral therapy in this setting, this decision should be re-evaluated frequently (i.e. monthly) with clinical and laboratory evaluation.

There is growing recognition of the deleterious consequences of chronic inflammatory states due to uncontrolled HIV replication. As such, although currently it is generally recommended that initiation of therapy be deferred among asymptomatic patients with CD4 cell counts >350 cells/mm³,[3,21] guidelines do recognize the need for earlier initiation of antiretroviral therapy in the presence of conditions that may be exacerbated by chronic HIV-related inflammatory states or ongoing uncontrolled viral replication. These conditions include underlying cardiovascular disease, hepatitis B or C co-infection, and HIV-associated nephropathy. In these cases, consideration should be given to initiation of HAART regardless of CD4 cell count.[3] Other features that may lead to consideration of HAART therapy at CD4 cell counts >350 cells/mm³ include an HIV-1-RNA level that is consistently above 100 000 copies/ml or rapid CD4 cell loss (≥100 cells per year). However, the issue of when to start HAART remains a matter of some debate; ongoing cohort analyses suggests that there may be benefit to initiating therapy at CD4 cell counts in excess of 500 cells/mm³.[65] Other theoretical benefits of earlier HAART initiation include greater immunological preservation or restoration, and potential for reduced HIV transmission.[66,67] New simpler, safer and better-tolerated regimens make earlier treatment a more attractive proposition today. However, the potential benefits of earlier treatment initiation need to be weighed against the risks associated with longer duration of drug exposure, such as drug toxicities, emergence of resistance, and cost. This strategy needs to be tested in controlled clinical trials before being widely adopted in clinical practice.

The effectiveness of antiretroviral therapy is also critically dependent on maintaining a nearly perfect level of adherence to therapy. Incomplete adherence is a key determinant of the emergence of drug-resistant HIV,[68,69] increased risk for CD4⁺ cell decline, progression to AIDS, and death.[70–72] HIV drug resistance is typically associated with cross-resistance to other members of the same class,[73,74] limiting future treatment options. Thus, it is critical that patients have access to optimal adherence support before initiating antiretroviral therapy.

Special considerations

Gender

Study results are conflicting as to whether there are gender-specific differences in the natural history of HIV disease.[75,76] However, the effectiveness of antiretroviral therapy as measured by the risk of progression to AIDS or death is independent of gender.[51,58,59,77] There is general agreement among guidelines that timing of HAART initiation should be the same in men and women.

Intravenous drug users

With regard to the critical role of adherence in treatment success, physician perception of a patient's potential ability to adhere to HAART does not justify withholding HAART when medically indicated, even in patients with relatively chaotic lifestyles or active intravenous drug use.[78] Rather, adherence should be optimized in all patients starting HAART, through counseling, education and addressing relevant co-morbidities, including psychosocial issues (e.g. addictions, depression) that might pose barriers to adherence. Whenever possible, selection of compact once-daily regimens is to be encouraged, as long as it is deemed clinically appropriate. Assisted therapy programs, such as directly observed therapy or programs that link daily HAART and methadone dispensing, have proven of great value in some settings.[79,80]

Co-infection with viral hepatitis

Co-infection with hepatitis C and/or B virus is common among HIV-infected individuals. Co-infection with HIV may accelerate the progression of liver disease due to viral hepatitis[81,82] and conversely may enhance the risk of hepatotoxicity of some antiretroviral agents.[83] An additional consideration is the risk for exacerbation of hepatitis, and potentially hepatic failure, due to immune reconstitution syndrome occurring early after starting antiretroviral therapy. Nonetheless, control of HIV replication by initiating antiretroviral therapy has been shown to decrease mortality due to liver disease in the setting of hepatitis C co-infection.[84,85]

Hepatitis C treatment, while potentially curative, can be associated with substantial adverse effects and tolerability issues, particularly

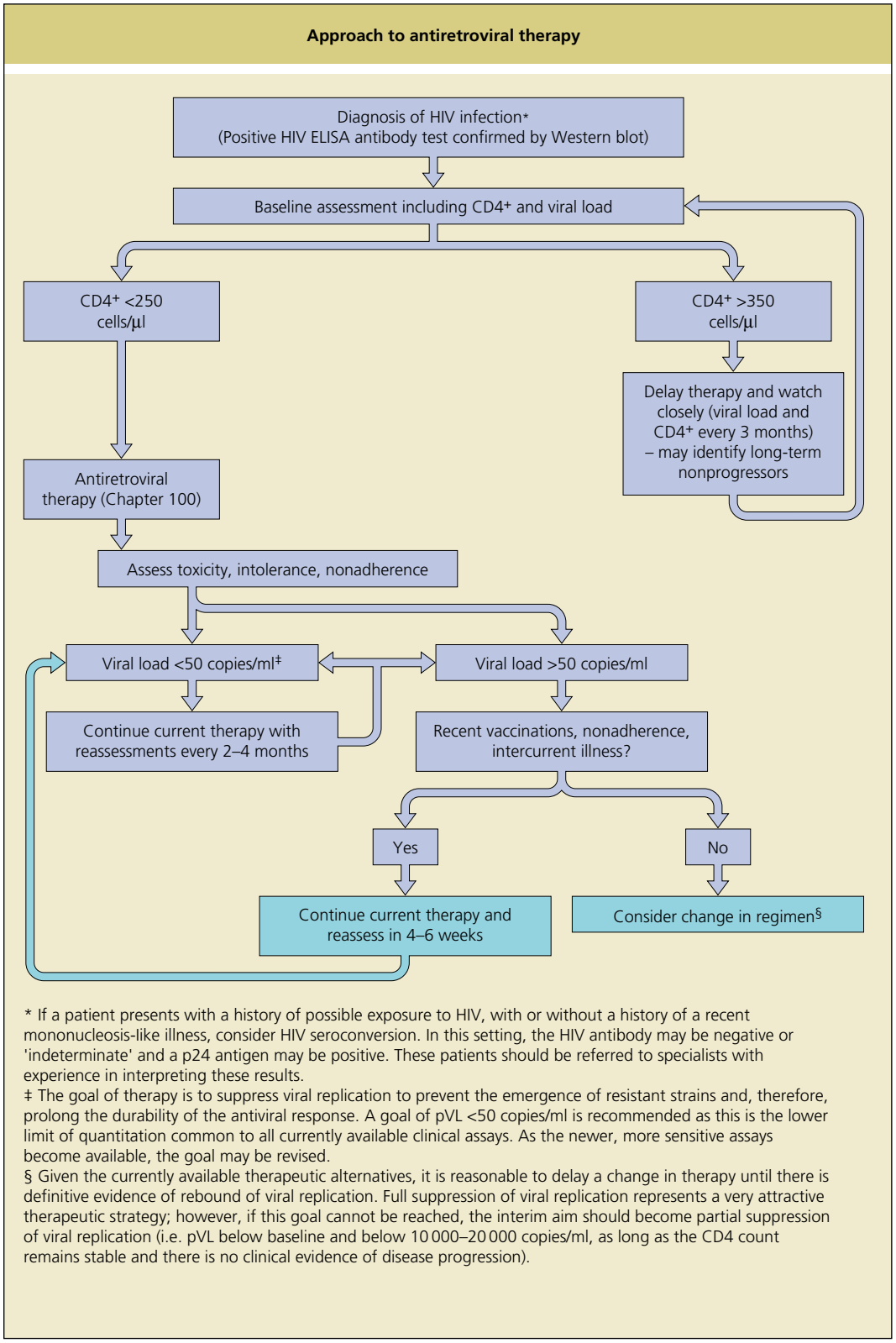


Fig. 99.1 An approach to the initiation of antiretroviral therapy. pVL, plasma viral load.

when given in combination with HIV treatment. Thus when the CD4 cell count is >350 cells/mm^3, it is preferable to initiate (and, if possible complete) therapy for hepatitis C first, prior to initiating HAART. On the other hand, a co-infected patient with a CD4 count <350 cells/mm^3 should be stabilized on HAART before therapy for hepatitis C is undertaken.[83–85] Patients with CD4 cell count >350 cells/mm^3 who are unable to receive hepatitis C therapy should be evaluated for early initiation of HAART according to current guidelines due to recognition of improved liver-related mortality. (See Practice Point 46.)

In the setting of co-infection with HIV and hepatitis B, it is noteworthy that some antiretroviral agents have activity against both HIV

and hepatitis B virus (HBV), notably tenofovir, adefovir, lamivudine and emtricitabine. As a result, HAART regimens containing tenofovir in combination with either lamivudine or emtricitabine are expected to suppress serum hepatitis B DNA levels in a durable fashion, with associated biochemical and clinical improvements, even in patients with advanced liver disease.[21] Use of lamivudine, emtricitabine or tenofovir as the only active hepatitis B agent should be avoided due to risks of development of hepatitis B resistance.[21] Significant rebound in hepatitis B viral load and even clinical hepatitis can occur if one or both of these agents is discontinued without replacement by another anti-HBV agent.[21]

More recently, the anti-HBV drug entecavir was also found to have some (still poorly characterized) anti-HIV activity, resulting in emergence of nucleoside-associated resistance mutations among patients who were not on suppressive antiretroviral therapy for HIV.[86] For co-infected patients requiring treatment for hepatitis B but not immediately for HIV, agents without crossover anti-HIV effect (such as interferon) are preferable, to avert the emergence of drug-resistant HIV.[21] While adefovir has modest anti-HIV activity, it does not appear to select for significant drug-resistant HIV mutations at the doses used for treatment of hepatitis B[87,88] (see Practice Point 44).

CHOICE OF INITIAL ANTIRETROVIRAL REGIMEN

Current guidelines advise that regimens consist of a backbone of two nucleoside or nucleotide reverse transcriptase inhibitors (NRTIs) plus either a non-nucleoside reverse transcriptase inhibitor (NNRTI) or a ritonavir-boosted protease inhibitor (PI).[3,21] The choice of specific regimens and agents should be individualized, taking into account results of testing for primary resistance, patient preferences and lifestyle, co-infections (e.g. viral hepatitis), co-morbidities (e.g. diabetes, kidney disease), cardiovascular risk factors and concomitant medications. Considerations for selecting individual agents are outside the scope of this chapter. Guideline recommendations for specific preferred agents are revised regularly based on new information and the availability of new agents and fixed-dose formulations which enhance convenience and compliance. Specific antiretroviral agents are described in detail in Chapter 100.

MONITORING PATIENTS ON INITIAL ANTIRETROVIRAL THERAPY

After starting a new antiretroviral regimen, the patient should have the plasma HIV RNA level checked every 4–8 weeks until a target of <50 copies/ml is achieved, a threshold typically reached within 24 weeks. Viral load monitoring can then be decreased to approximately 3-monthly intervals, along with CD4 cell counts in stable patients. Adherence, drug intolerance, drug toxicities and clinical disease status should also be re-evaluated at each visit.

CHANGING ANTIRETROVIRAL THERAPY

When to change

Treatment failure

From a practical standpoint, treatment failure is defined in virologic terms as either the failure to achieve a viral load <50 copies/ml or the occurrence of a confirmed viral load rebound above 50 copies/ml in the absence of other obvious explanation (e.g. treatment interruption, intercurrent illness or immunizations).

It is critically important to understand and correct the determinants of treatment failure in any given individual. This must be done before a change in therapy is implemented. If the plasma viral load rebounds despite ongoing therapy, consider imperfect adherence to the antiretroviral regimen as the most likely cause.[69] The 'forgiveness' of a regimen to breakdowns in adherence is dependent to some degree on the pharmacokinetics and barrier to resistance posed by the regimen. However, in many cases, even relatively minor degrees of nonadherence can lead to viral breakthrough and ultimately emergence of resistance.[89] If suboptimal adherence is identified, the pattern of and reasons for missed doses should be evaluated. Alternative dosing regimens can often be accommodated to better suit an individual's lifestyle. Intolerance to medications may be mitigated by altering the time of dosing, administering with food and, if possible, altering dosing interval. Other issues such as depression or substance use, which can negatively impact adherence, may need to be addressed.

Another major cause of treatment failure is reduced susceptibility of the virus to one or more antiretroviral drugs in the regimen. Patients can be initially infected with drug-resistant strains of HIV.[24] Resistant strains can also emerge when the virus is allowed to replicate in the presence of drug, as in the case of incomplete adherence, insufficiently potent regimens or suboptimal drug levels. Considerable cross-resistance exists within antiretroviral drug classes, so patients may harbor virus resistant to an agent as a result of exposure to another agent within the same drug class.[23] Resistance to multiple classes of antiretrovirals severely limits the chances of success with future regimens. Resistance testing should be performed as soon as virologic failure is established, and the results used to design an alternative fully suppressive regimen to avoid further development of resistance. Additional discussion of HIV drug resistance appears in Practice Point 48.

Treatment failure may also be due to inadequate drug levels. Most antiretroviral agents are prescribed without regard to individual patient characteristics such as weight; however, for many of these agents, notably the protease inhibitors, there is considerable interindividual variation in pharmacokinetics. This can only be assessed by therapeutic drug monitoring (TDM), i.e. directly measuring plasma drug levels. However, optimal target levels for antiretroviral drugs are not always well defined. Another common cause of inadequate antiretroviral drug levels is unrecognized drug–drug interactions, either with other antiretrovirals within the regimen or with concomitant medications taken for other indications.[90] Dose adjustments may overcome some of these interactions; however, in the case of multiple drug regimens with complicated potential interactions, TDM will be necessary to establish the direction of the interaction and make appropriate adjustments.

A change in therapy in the setting of failure of previous treatment should only be carried out after careful evaluation of prior drug exposure, prior response to therapies, prior tolerability and toxicity issues, as well as the results of resistance testing done on a real-time basis and on relevant stored samples, if available. Failure to address these issues effectively will invariably compromise the chances of success with the new regimen. Whenever possible, pharmacokinetic issues (past and present) should also be evaluated. Multiple variables must be considered when changing regimens in the context of virologic failure in a given clinical case. As such, guidelines cannot replace expert advice in this setting. It is critical, therefore, that the decision of when to change and what to change to be arrived at under the guidance of an experienced practitioner.

Toxicity

Tolerability issues are a frequent reason for modification of HAART regimens. When the causal agent responsible for a given toxicity is known, replacing that single agent is relatively simple and usually effective. The issue is more challenging if the link between the responsible agent and the emerging toxicity is not clear. At times it is difficult to differentiate specific antiretroviral drug toxicity from a co-morbid condition or toxicity due to a concomitant medication, for example in the case of hepatic transaminase elevations. In such instances, other

potential causes should be evaluated and managed if possible, or ruled out prior to implementing changes in the antiretroviral regimen.

Significant toxicities may also develop due to drug–drug interactions, particularly if the exposure to one agent is substantially increased in the presence of another, for example rhabdomyolysis due to increased levels of certain statins in the presence of ritonavir-boosted protease inhibitors.[91] Management of patients in a multidisciplinary setting with access to specialized pharmacists with a thorough knowledge of potential drug interactions can alleviate these problems.

ALTERATION OF ANTIRETROVIRAL REGIMENS

The composition of a new regimen will depend on the underlying reasons for changing therapy. If a patient experiences drug toxicity, brief cessation of all medications is recommended. Decreasing dosages or discontinuing a single medication should be discouraged, as this will compromise the potency of the regimen and eventually promote the development of resistance. In general, as long as the antiviral potency of the regimen is preserved, exchanging an individual component of the regimen to deal with a toxicity problem is acceptable. The closer the agents are in terms of their potency and resistance profile, the easier and safer the change will be. Drug substitutions become more complicated when the patient presents with a history of prior drug exposure and/or failure of multiple regimens. In such cases, changes in therapy should only be performed under the guidance of an experienced physician.

When a decision is made to change therapy for confirmed virologic failure, the new regimen should be one with the highest probable effectiveness, as predicted by the patient's complete drug history and the resistance test results, as well as the highest likelihood of tolerability and adherence. New regimens should contain at least two (and if possible three) drugs deemed to be fully active.[3,21] It is important to emphasize that HIV isolated particularly early on during virologic treatment failure is often not resistant to all of the drugs in the failing regimen. In addition, latently infected lymphocytes may harbor archived viruses resistant to drugs used in the past which are not detected by routine resistance testing in the plasma.

Cross-resistance among drugs in the same class may be extensive and this often limits the number of fully active alternative regimens. With the currently approved first generation NNRTIs, the risk of cross-resistance is high after an NNRTI-containing regimen fails.[23] With protease inhibitors, intraclass cross-resistance is less predictable. Depending on the pattern of resistance, an alternative protease inhibitor can often be selected. With NRTIs, the extent of class cross-resistance varies from drug to drug and is poorly reflected by genotype or phenotype testing results. In rare circumstances, multidrug resistance to NRTIs may develop through a unique mutational pathway.[23] Maximal activity would be expected from drugs belonging to a new class to which the patient's virus has not previously been exposed and would not be expected to have any mutations conferring resistance. Cross-resistance is also known to occur within newer classes of drugs, including fusion inhibitors, CCR5 receptor antagonists and integrase inhibitors.

Monitoring patients after regimen change

As with a first regimen, the patient should have the plasma HIV RNA level checked 4–8 weeks after starting a new regimen to ensure the desired early response, and followed closely (i.e. monthly) until the viral load is below 50 copies/ml. Adherence, drug tolerance and emerging toxicities with the new regimen will also need to be monitored as well as any changes in clinical disease status. Such follow-up is best undertaken in close collaboration with an experienced physician.

TREATMENT INTERRUPTIONS

CD4 cell count-guided treatment interruptions have been considered as a means of sparing patients from the adverse effects of long-term exposure to antiretrovirals. However, a recent large controlled clinical trial has shown conclusively that structured treatment interruptions are associated with AIDS-related events and significant drops in CD4 cell counts, as well as AIDS- and non-AIDS-related mortality.[10] Currently structured treatment interruptions are not recommended except in cases of treatment-limiting intolerance or toxicity, and then under close expert medical supervision.

REFERENCES


References for this chapter can be found online at http://www.expertconsult.com

Chapter **100**

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Antiviral therapy

INTRODUCTION

HIV infection is a chronic disease in which, if left untreated, high-level viral replication occurs continuously for years, even during the clinically latent phase. Without optimal antiviral therapy opportunistic infections or malignancies characteristic of AIDS typically start occurring 10–12 years after HIV infection, although may occur much sooner. Viral replication drives the progression of HIV disease and leads to worsening immunodeficiency as measured by decreasing $CD4^+$ lymphocyte cell counts which are highly predictive of the subsequent risk of disease progression and death. Suppression of HIV replication to levels in plasma below the limit of detection is the primary objective of antiretroviral (ARV) treatment as this is associated with durable clinical, virologic and immunologic responses. Residual viral replication due to incomplete suppression in the presence of drugs is responsible for the development of viral resistance to antiretroviral drugs, thereby contributing to treatment failure and disease progression. Inability to maintain full virologic suppression is most commonly related to suboptimal adherence but may be due to viral mutations leading to decreased drug susceptibility and resistance.

Thus virologic failure is defined as either treatment being unable to reduce plasma HIV viral load to below the limit of detection or the development of virologic rebound in someone who previously was fully suppressed. With virologic failure, resistance mutations may be selected which usually decrease the susceptibility of virus to the relevant drug(s) and often will also result in decreased susceptibility to other drugs within the same class (cross-class resistance). The impact of individual mutations will depend on the mutation and the drug and class affected. For example, the mutation Y181C is selected very early in patients failing nevirapine and results in loss of susceptibility to all available members of the non-nucleoside reverse transcriptase inhibitor class.

Most individuals commencing ARV treatment will be expected to have successful, durable responses to treatment. In clinical trials after long periods of follow-up virologic undetectable rates at 3–5 years of 60–70% have been reported. However, for individuals who subsequently develop virologic failure on these initial treatments and for those who were previously treated with suboptimal therapies, the availability of new drugs with activity against resistant viruses is important in order to construct effective regimens. Also critical is the development of new drugs with better or different tolerability/toxicity profiles and with better, more patient-friendly formulations, as regimen switches are commonly related to drug intolerance or toxicity.

There are now 22 licensed drugs available for the treatment of HIV infection (Table 100.1), including six different classes acting at different sites of HIV cell entry and replication (Fig. 100.1). (See Chapter 145 for detailed discussion of antiretroviral drugs.) The availability of an increasing number of drugs and the rapidly evolving scientific information have made HIV treatment an extremely complex field. Treatment should be supervised by experts, and several national and international guidelines have been developed in order to help in the clinical management of HIV-infected adults and adolescents, pregnant women, infants and children, those with hepatitis and tuberculosis co-infections and uninfected individuals who have occupational exposure to HIV.

Table 100.1 Currently licensed antiretroviral drugs by class

NRTIs	NNRTIs	Protease inhibitors	Entry inhibitors	Integrase inhibitors
Zidovudine (ZDV) Stavudine Lamivudine (3TC) Emtricitabine (FTC) Abacavir (ABC) Zalcitabine* Didanosine Tenofovir (TDF)	Nevirapine Efavirenz (EFV) Delavirdine* Etravirine	Saquinavir Indinavir Nelfinavir Fosamprenavir Amprenavir* Lopinavir (LPV) Ritonavir (RTV) Atazanavir Tipranavir Darunavir	Enfuvirtide Maraviroc	Raltegravir

Six fixed-dose combinations are approved: ZDV + 3TC; ZDV + 3TC + ABC; ABC + 3TC; FTC + TDF; LPV + RTV; TDF + FTC + EFV.
*No longer commercially available.

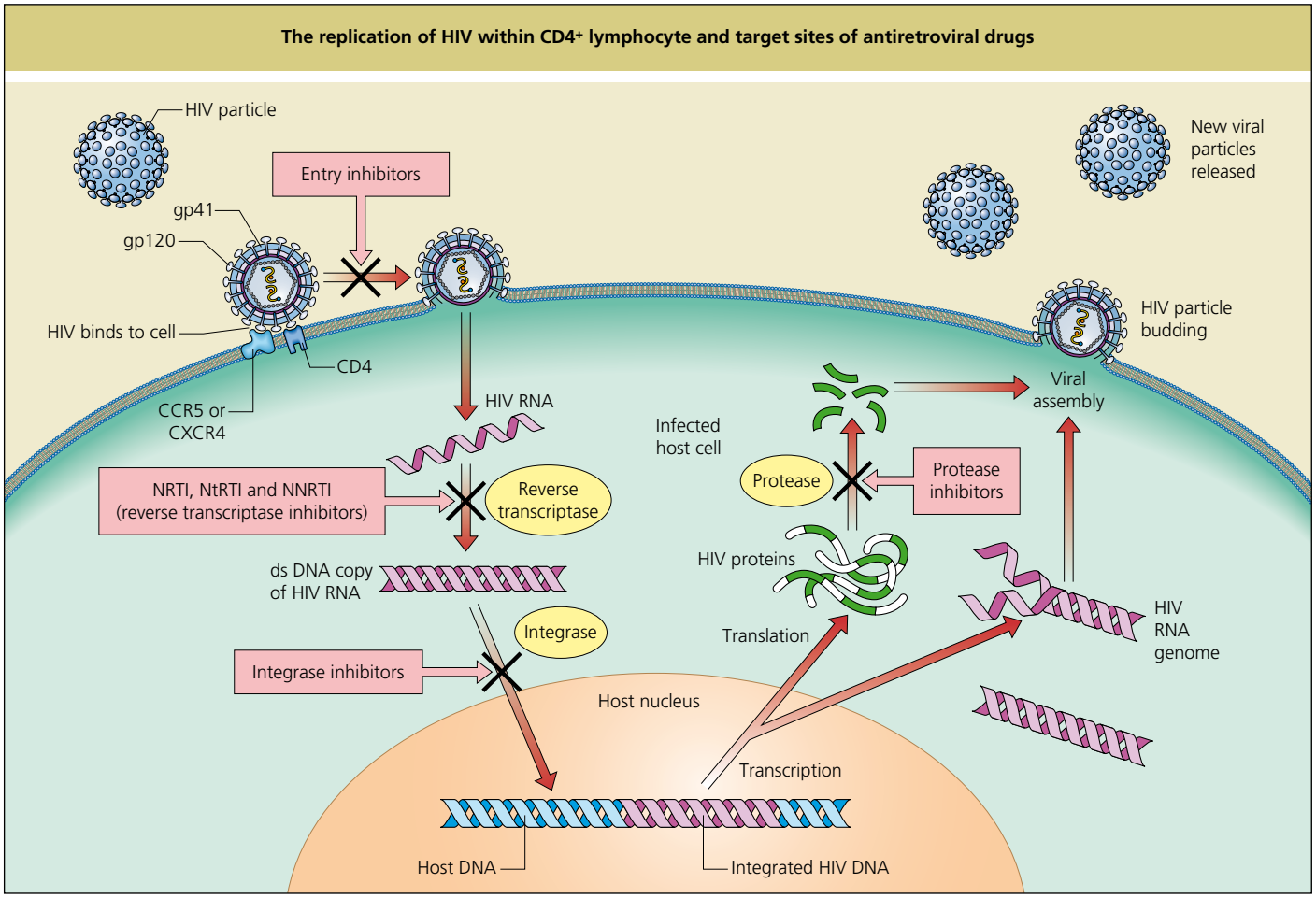


Fig. 100.1 Diagram of HIV life cycle and place of action of different drugs. With permission from Bolognia JL, Jorizzo JL, Rapini RP, *et al*., eds. Dermatology, 2nd ed. London: Mosby; 2008.

WHEN SHOULD ANTIRETROVIRAL DRUGS BE STARTED?

Established HIV infection

Until recently, most guidelines suggested commencing treatment if individuals had symptomatic disease or if asymptomatic when the CD4 count declined to between 200 and 350 cells/mm^3. In practice many started with CD4 counts of approximately 200 cells/mm^3 or lower (particularly in those presenting late with advanced immunosuppression). More recently, guidelines have been recommending earlier treatment (Table 100.2). For example the European AIDS Clinical Society (EACS) recommend treating all individuals with a CD4 between 200 and 350 cells/mm^3 but also to consider treatment in those with CD4 counts up to 500 cells/mm^3 in presence of any of the following:

- a high viral load (greater than 100 000 copies/ml);
- a rapidly declining CD4;
- older age; or
- hepatitis C co-infection.

The trend to recommend earlier treatment has been informed by several studies which indicate that the risk of clinical disease at higher CD4 counts is greater than previously thought. This may be driving the finding that even HIV-positive individuals with high CD4 counts have raised standardized mortality ratios compared with the general population. In the SMART study of treatment interruption, individuals with CD4 counts >350 cells/mm^3, including those not on treatment and those randomized to interrupt treatment, experienced more clinical disease than those who remained on treatment throughout.[2] Surprisingly, this excess of clinical disease comprised not only conditions considered to be HIV related but also such diverse conditions as cardiovascular disease, renal and hepatic disease. The US Department of Health and Human Sciences (DHHS) guidelines now specify that if HIV-related renal disease is present, early commencement of ARVs should be recommended. The International AIDS Society (IAS) USA guidelines suggest that therapy should be considered and decision individualized if an individual's CD4 count is >350 cells/mm^3 and there is the presence of, or high risk for, cardiovascular disease, active hepatitis B virus (HBV) or hepatitis C virus (HCV) co-infection or HIV-associated nephropathy. There are studies planned to further examine even earlier commencement of ARVs.

Acute HIV infection

Overall there are no convincing data that treating patients during acute HIV infection has led to any substantial long-term benefits in terms of their immune system recovery. However, there are data suggesting that if patients were treated in the very early stages of acute HIV infection, they may preserve immune function, especially in the gut, and may also prevent dissemination of virus to sanctuary sites such as the brain. Treatment for acute HIV infection should be undertaken within a clinical trial or perhaps considered in an individual with moderate to severe symptoms, those who have a precipitous fall in CD4 count to <200 cells/mm^3 or those with neurologic involvement (see also Chapter 89).

Table 100.2 Guidelines for when to start antiretroviral treatment

Guidelines	Symptomatic	Asymptomatic	Other
European AIDS Clinical Society[1]	CDC stage B	CD4 <200: start without delay CD4 201–350: treatment recommended CD4 350–500: may be offered	Consider at higher CD4 if: • viral load >100 000/ml • rapid CD4 decline • age >55 years
US Department of Health and Human Sciences	Symptomatic	CD4 <350 cells/mm^3: treatment recommended CD4 >350 cells/mm^3	Patient scenarios and comorbidities should be taken into consideration
International AIDS Society, USA	Symptomatic	CD4 <350 cells/mm^3 CD4 >350 cells/mm^3	Co-morbidities Patient willingness Rapid decline CD4 (>100 cells/year) Plasma viral load >100 000 copies Therapy should be considered and decision individualized

WHICH ANTIRETROVIRAL DRUGS TO START?

Individuals commencing ARV therapy for the first time will usually start with a combination of two nucleoside reverse transcriptase inhibitors (NRTIs) and either a non-nucleoside reverse transcriptase inhibitor (NNRTI) or a protease inhibitor (PI), boosted with low-dose ritonavir (Table 100.3). Before selecting a combination, it is important to take account of several pieces of clinical and laboratory information.

Baseline resistance testing

Transmission of drug-resistant virus is well documented, with up to 15% of ARV-naive individuals in some studies being found to harbor HIV with at least one important drug-related mutation.[3] Studies demonstrate poorer virologic outcomes for individuals commencing combinations where transmitted resistance is present. It is widely recommended that resistance testing, where available, be performed prior to commencement of first-line therapy. With time, evidence of transmitted drug resistance may be lost due to outgrowth of wild-type virus which is more fit, resulting in 'archiving' of resistant virus. It is recommended, therefore, that resistance testing is done on the earliest available sample following the diagnosis of HIV infection.

Table 100.3 Which antiretroviral drugs to start

Guidelines	A	B
EACS, recommended	Efavirenz Nevirapine Fosamprenavir–ritonavir Lopinavir–ritonavir Saquinavir–ritonavir Atazanavir–ritonavir	Abacavir–lamivudine Tenofovir–emtricitabine
EACS, alternative	Darunavir–ritonavir	Zidovudine–lamivudine Didanosine–lamivudine or didanosine–emtricitabine
IAS-USA	Efavirenz Atazanavir–ritonavir Lopinavir–ritonavir Darunavir–ritonavir Fosamprenavir–ritonavir Saquinavir–ritonavir	Tenofovir–emtricitabine Abacavir–lamivudine
DHHS preferred	Efavirenz Atazanavir–ritonavir Darunavir–ritonavir Fosamprenavir–ritonavir Lopinavir–ritonavir	Tenofovir–emtricitabine

For EACS, IAS and DHHS guidelines, one drug (or ritonavir-boosted antiretroviral) from column A should be prescribed with a combination of drugs from column B.

Co-infections

It is important to establish whether an individual is co-infected with HBV or HCV before commencing ARV treatment. Tenofovir, lamivudine and emtricitabine are also active against hepatitis B. When starting ARVs, it is recommended that HBV/HIV co-infected individuals should be treated with a combination that includes two agents active against HBV.[4] The usual combination is either lamivudine or emtricitabine with tenofovir. In individuals with HCV infection an assessment of whether specific HCV treatment is required should be made. Zidovudine, didanosine and stavudine should be avoided due to increased toxicity in combination with ribavirin. There is also evidence of lower sustained virologic response rates in those on abacavir treated with interferon–ribavirin.

Co-morbidities

Comorbidities should also be considered. For example some ritonavir-boosted protease inhibitors may increase insulin resistance and therefore make control of diabetes more difficult. Protease inhibitors are also associated with increases in total cholesterol and triglycerides and in cohort studies with an increased risk of myocardial infarction. Efavirenz is associated with depression and other neuropsychiatric side-effects. In a small percentage of individuals, tenofovir leads to a decline in glomerular filtration rate (GFR). Agents other than tenofovir should be considered if individuals have severe renal impairment and caution taken in patients taking other drugs known to cause renal dysfunction.

Drug interactions

It is important in assessing co-morbid conditions to also consider co-prescribed medications. NNRTIs and PIs in particular interact with numerous other medications. Interactions may lead to lower or higher levels of ARVs, risking either treatment failure or toxicity. Furthermore, as both NNRTIs and PIs can act as inducers or inhibitors of cytochrome P450, the levels of other drugs may be affected.

Lifestyle

Finally, it must always be borne in mind that HIV infection is a long-term disease, often affecting young people. Therapy for HIV (as for other chronic diseases necessitating continuous treatment), tolerability, compliance and accessibility are important factors that must carefully be considered in the design of treatment to ensure compliance and efficacy.

Most combinations of ARVs have shown broadly similar activity and efficacy in the short to medium term. Considerations in the selection of the most appropriate regimen for the individual patient may include potency, side-effect profile, patient's predicted adherence, quality of life issues and pill burden, the potential for maintenance of future options in terms of drug class sparing and cross-resistance profile, the presence of co-morbid conditions and medications. Finally, in certain clinical circumstances and geographic areas, the potential for primary acquisition of resistant viral strains needs to be considered.

SPECIFIC ANTIRETROVIRAL DRUGS

Nucleos(t)ide reverse transcriptase inhibitors

Reverse transcriptase inhibitors act through one of two mechanisms. First, as 'chain terminators', they block the elongation of the DNA chain through blockage of further nucleosides. This mechanism is characteristic of the nucleoside and nucleotide analogues and depends on the intracellular phosphorylation of the drugs into the corresponding triphosphate. Second, they act by competition/binding of the reverse transcriptase in functionally essential sites. NNRTIs act only through this mechanism and not as 'chain terminators'. In general, nucleoside analogues have good oral bioavailability, are only minimally bound to plasma proteins, do not interfere with cytochrome P450 enzyme systems and are excreted through the kidneys. Because of these metabolic characteristics, they have relatively few interactions with other drugs as compared with PIs and NNRTIs. They are generally active against HIV-1 and HIV-2.

The most widely studied and recommended nucleoside/nucleotide backbones for initial ARV treatment are zidovudine–lamivudine, abacavir–lamivudine, or tenofovir with either lamivudine or emtricitabine.

Zidovudine and stavudine

Zidovudine is a thymidine analogue and was the first ARV agent available for use. For many years it was a recommended component of first-line combination therapy. Its relegation in guidelines to that of an alternative agent has been driven by its association with lipoatrophy. This toxicity starts to manifest after 12 months on treatment and may affect up to 50–60% of those taking zidovudine in the medium to long term. In individuals with lipoatrophy, switching away from zidovudine may result in partial reversal of the subcutaneous fat loss but this is usually slow and probably incomplete. Zidovudine remains a recommended agent for use in pregnant women to prevent mother-to-child HIV transmission.

Viruses resistant to currently recommended first-line NRTIs (tenofovir, abacavir, lamivudine and emtricitabine) will usually be susceptible to zidovudine and so it is also often useful as a second-line agent in those individuals who have virologically failed their initial treatment.

Stavudine is the other thymidine analogue in use. It is associated with a higher incidence of lipoatrophy, the onset of which is more rapid. Stavudine can also cause peripheral neuropathy, hyperlactatemia and lactic acidosis. The latter is a problem particularly in resource-poor regions as it is more commonly reported in women and the diagnosis may be delayed due to lack of available testing facilities. Stavudine has been dropped from most guidelines in developed countries and its widespread use in resource-poor countries, often for reasons of cost, is being widely questioned.

Abacavir and tenofovir

Abacavir–lamivudine or tenofovir with either lamivudine or emtricitabine have become the most commonly used NRTI backbones in developed countries. The ZODIAC study (CNA 30024) demonstrated abacavir–lamivudine (given twice daily) with efavirenz was not inferior to zidovudine–lamivudine and efavirenz.[5] Although both abacavir and lamivudine have plasma half-lives that do not appear to support once-daily dosing, intracellular concentrations of the active metabolites (both drugs need to be phosphorylated intracellularly) mean once-daily dosing is feasible. The CNA 30021 study demonstrated that abacavir and lamivudine given once daily (same daily dose) was not inferior to the standard twice-daily regimen. Again this was given in combination with efavirenz.[6]

The most important adverse effect of abacavir is a hypersensitivity reaction (HSR). This syndrome consists of multisystem presentation, usually 1–4 weeks after starting abacavir. Symptoms include fever, rash, abdominal pain, cough, shortness of breath and hypotension. When they start, symptoms are often mild but escalate in severity with each dose. The most serious problems, including shock and death, occur in those who continue treatment despite the development of hypersensitivity, or in those who stop drug and then are rechallenged with abacavir.

The prevalence of HSR in different populations varies widely, being seen most commonly in those of white race. Retrospective analyses have identified HLA-B*5701 carriage as the dominant risk factor for abacavir HSR. In the Predict study subjects starting treatment with an abacavir-containing regimen were randomized to have prospective HLA-B*5701 screening. Screening reduced the incidence of HSR to zero. The negative predictive value was 100% and the positive predictive value 50%.[7] HLA-B*5701 screening prior to using abacavir has become routine in clinical practice.

Two other important issues affect the use of abacavir in current clinical practice. The first is its association seen in cohort data with worsening cardiovascular risk. This appears to be most significant in those patients who already have a high cardiovascular risk that comprises at least five risk factors. The second is in one large clinical trial (ACTG 5202) of poorer efficacy seen in those ARV-naive patients who were randomized to receive abacavir and whose baseline viral load was over 100 000 copies/ml. More data are required to understand these issues and current guidelines suggest caution in using abacavir in these situations.

Tenofovir is an NRTI that also has activity against HBV. In the GS-934 study, tenofovir, lamivudine and efavirenz were compared over 144 weeks to zidovudine, lamivudine and efavirenz. In the primary time to loss of virologic response (TLOVR) analysis the tenofovir arm performed better. Much of the difference between the arms was related to early discontinuation in the zidovudine arm due to anemia and poorer tolerability.

Tenofovir and renal function

Numerous studies, including clinical trials, observational cohorts and expanded access data, have identified serious renal toxicity in approximately 0.5% of patients on tenofovir, a rate no different from that observed with comparator NRTIs. However, other studies have demonstrated a small but significant reduction in renal function over time when compared to other nucleosides. Tenofovir should be used cautiously in patients who have, or are at risk of developing, renal disease. This includes those who are co-prescribed other potentially nephrotoxic agents.

Lamivudine and emtricitabine

Lamivudine is a cytidine analogue and an important component of several recommended first-line combinations. It is very well tolerated and can be given either 150 mg twice daily or 300 mg once daily. It is also

available as a component of the fixed-dose combinations Combivir (zidovudine, lamivudine), Trizivir (zidovudine, abacavir, lamivudine) and Kivexa (abacavir, lamivudine). Lamivudine has a low barrier to resistance. Viruses which contain the M184V mutation are, however, less 'fit' than wild-type viruses. Previous studies have demonstrated that patients with the M184V mutation who fail have plasma viral loads that do not return to pretreatment levels, remaining approximately 0.5 log below baseline. Lamivudine is also active against HBV.

Emtricitabine is a cytidine analogue similar to lamivudine. These drugs should not be used together. Emtricitabine is also active against HBV. This drug has been tested in randomized controlled trials in combination with tenofovir for the treatment of ARV-naive individuals and is available as part of a fixed-dose combination with tenofovir (Truvada) and with both tenofovir and efavirenz (Atripla).

Didanosine

Didanosine is an alternative agent for treating ARV-naive individuals and is usually given in combination with lamivudine and either an NNRTI or a protease inhibitor. Its use has been relatively limited in this context because of its tolerability and toxicity profile. While reformulation has improved tolerability, it is associated with pancreatitis, lactic acidosis and peripheral neuropathy. Recent reports have linked hepatic fibrosis and portal vein thrombosis with this drug. While these adverse effects are relative rare, their potential severity limits its use.

As it has activity against some viruses with resistance it is mostly used in second and subsequent lines of treatment. Due to pharmacokinetic interactions the dose of didanosine should be reduced if used in combination with tenofovir; however, due to concerns over efficacy and toxicity this combination should, where possible, be avoided.

Choosing either an NNRTI- or PI-based combination for first-line therapy

All currently recommended first-line therapies include a drug from the NNRTI or PI class. NNRTIs nevirapine and efavirenz have the benefit of low pill burden, potential for once-daily administration (although for nevirapine this is not a licensed schedule) and good gastrointestinal profile. However, they have a low barrier to resistance and a single mutation results in high-level resistance that impacts drugs within the same class. Both drugs but particularly nevirapine are associated with rare but potentially serious hepatic and/or cutaneous toxicity.

In contrast, ritonavir-boosted PIs are relatively forgiving in the face of suboptimal adherence, with high-level resistance usually requiring more than one mutation. Sequencing of PIs, even if resistance is present, may be possible. However PI-based combinations have a higher pill burden, sometimes have food requirements and the ritonavir used for pharmacokinetic boosting requires refrigeration. As a class they are more frequently associated with abdominal bloating and diarrhea. They are also implicated in aspects of the lipodystrophy syndrome including lipohypertrophy, insulin resistance and hyperlipidemia. Hypercholesterolemia can also occur with the NNRTI efavirenz.

One large strategic study (ACTG 5142) compared outcomes in those starting a two NRTI–efavirenz combination, a two NRTI (lopinavir–ritonavir) and an NRTI-sparing (efavirenz–lopinavir–ritonavir) combination. This study found that at 96 weeks the two NRTI–efavirenz group was associated with fewest virologic failures.[8] The NRTI-sparing arm also performed better than the PI arm but was the arm in which most resistance was seen in those who failed.

NNRTIs: Selecting nevirapine or efavirenz

The 2NN study compared nevirapine with efavirenz. Both drugs were given in combination with stavudine and lamivudine in treatment-naive individuals.[9] At 48 weeks 43.7% of the nevirapine twice-daily group and 37.8% of the efavirenz group failed. This difference of 5.9% (95% CI, 0.9–12.8) just failed to reach significance and hence the combinations were non-inferior to one another. Two patients taking nevirapine died, one from hepatic toxicity and one from sepsis related to Stevens–Johnson syndrome.

These toxicities are well-recognized side-effects of nevirapine. To reduce the risk of these toxicities nevirapine should be started at a dose of 200 mg once daily. Patients should be reviewed at 2 weeks and the dose increased to 200 mg twice daily if there is no evidence of toxicity. The risks of these toxicities are substantially higher in those with higher CD4 counts and in women, hence it is recommended that nevirapine is not used in men with CD4 counts >400 cells/mm^3 and in women with CD4 counts >250 cells/mm^3.

While efavirenz may also be associated with both cutaneous and hepatic toxicity, the risk of serious problems is lower. However, efavirenz is associated with neuropsychiatric effects including dizziness, abnormal dreams, insomnia, hallucination and euphoria. These side-effects are more pronounced in the first 1–2 weeks of treatment. Occasionally patients report disabling side-effects which persist beyond this induction period, necessitating switching off efavirenz.

Congenital abnormalities have been observed in cynomolgus monkeys whose mothers were treated with efavirenz during pregnancy. While prospective studies of its use in pregnant women have failed to demonstrate an excess risk of congenital abnormalities, it remains the recommendation that an alternative agent is used in women trying to conceive or in those at higher risk of unplanned pregnancy.

Protease inhibitors

Protease inhibitors act on the binding to the catalytic site of the HIV aspartic protease. This enzyme is critical in the post-translational processing of the polyprotein products of *gag* and *gag-pol* genes into the functional core proteins and viral enzymes, respectively. Its inhibition leads to the release of immature, noninfectious viral particles.

Protease inhibitors are generally dependent on the cytochrome P450 3A4 hepatic isoenzyme for metabolism and can compete with other substrates of this enzyme. When the metabolism of other drugs that are dependent on the same enzyme is inhibited, the blood levels of these drugs can increase dramatically and toxic interactions may occur. This phenomenon has been evaluated for many of the compounds that are frequently used in patients who have HIV disease, and it has been shown that the combination of nucleoside analogues and PIs does not generally lead to untoward effects, although important pharmacokinetic interactions are possible for rifabutin, ketoconazole, rifampin (rifampicin), astemizole, terfenadine, cisapride and other drugs that are dependent on the same cytochrome enzyme.

Metabolic complications have emerged in patients treated with PIs. These include glucose metabolism abnormalities (hyperglycemia or diabetes), hyperlipidemia (mainly hypertriglyceridemia, with or without associated hypercholesterolemia), lipodystrophy or abnormal fat distribution (accumulation in the posterior neck, upper back and central abdomen). The pathogenesis of these abnormalities is still unclear and further studies are needed to define the role played by HIV infection, individual predisposing factors and treatment with specific drugs in the development of these complications.

Protease inhibitors are mostly co-prescribed with ritonavir. Ritonavir even at low dose is a potent inhibitor of cytochrome P450 3A4. This results in higher drug levels of the substrate PI over the dosing period, and is associated with a lower risk of virologic failure and a lower incidence of drug resistance in those individuals who fail treatment. The strategy to use ritonavir to boost PI levels and raise the barrier to resistance is one of the principal reasons, alongside availability of new drug classes, that 'salvage' of individuals with extensive drug resistance has been possible.

Saquinavir–ritonavir

Saquinavir has had three different formulations, the latest being that of a tablet. The recommended dose is saquinavir 1000 mg with ritonavir 100 mg twice daily. If once-daily treatment is desired in

ARV-naive patients, then saquinavir can be given at a dose of 1500–2000 mg with ritonavir 100 mg once daily. In the treatment of ARV-naive individuals, boosted saquinavir was found to be non-inferior to boosted lopinavir at 48 weeks both in combination with tenofovir and emtricitabine. There were more failures in the saquinavir arm (with drug resistance demonstrated by genotyping) than in the lopinavir arm.[10] Smaller increases in triglycerides were seen in the saquinavir arm.

Lopinavir–ritonavir

Lopinavir is a potent inhibitor of HIV-1 protease. Its bioavailability is greatly enhanced by co-administration of ritonavir and is therefore co-formulated both in a soft-gel capsule and the more recently developed, heat-stable tablet formulation. Available data indicate that the drug has good efficacy in previously untreated patients. Lopinavir is principally metabolized by the liver, and shares many of the drug interactions and contraindications common to other PIs. Two recent randomized controlled trials have shown better efficacy with atazanavir–ritonavir or darunavir–ritonavir when compared to lopinavir–ritonavir, partly driven by poorer tolerability of the lopinavir–ritonavir combination.

Fosamprenavir–ritonavir

Fosamprenavir is a prodrug of amprenavir. Metabolism of fosamprenavir increases the duration that amprenavir is available, making fosamprenavir a slow-release version of amprenavir and thus reducing the number of pills required versus standard amprenavir. A head-to-head study with lopinavir–ritonavir showed the two drugs to have comparable potency.[11] Principal side-effects include nausea, vomiting, abdominal bloating and diarrhea, along with rash, hypercholesterolemia and hypertriglyceridemia.

Atazanavir–ritonavir

Atazanavir was the first licensed once-a-day protease inhibitor to become available for use. While it can be given to ARV-naive individuals without ritonavir boosting (atazanavir 400 mg once daily) it is more commonly given with ritonavir (atazanavir 300 mg/ritonavir 100 mg once daily). Studies have demonstrated that patients failing unboosted atazanavir are more likely to develop mutations not only to protease inhibitors but also to the other components of the regimen.

The most common atazanavir-related side-effect is unconjugated hyperbilirubinemia due to atazanavir inhibiting glucuronosyltransferase. Elevated plasma bilirubin is not harmful in adults; however, individuals might find this unacceptable if they are noticeably jaundiced, particularly if scleral icterus is present. Atazanavir is less likely to cause both gastrointestinal side-effects and lipid abnormalities than other PIs. It is unknown whether fat redistribution, particularly the fat accumulation seen with other PIs, is also less likely with atazanavir. In the Castle study of ARV-naive patients, atazanavir 300 mg/ritonavir 100 mg with tenofovir and emtricitabine once daily was found to be non-inferior (in fact the data showed superiority) to lopinavir–ritonavir in combination at 96 weeks.[12]

Atazanavir has several important drug interactions:

- As it requires an acid gastric environment for optimal absorption, proton pump inhibitors such as omeprazole are contraindicated as they lead to lower plasma atazanavir levels. Care must also be taken with less potent reducers of gastric acidity such as ranitidine.
- Efavirenz induces the metabolism of atazanavir and, if co-prescribed, atazanavir should be given boosted with ritonavir and at a higher dose (atazanavir 400 mg/ritonavir 100 mg).
- Tenofovir leads to reduced atazanavir levels and, if co-prescribed, atazanavir should be given with ritonavir.

Darunavir–ritonavir

This is the most recently licensed protease inhibitor. In the Artemis study of ARV-naive patients, darunavir 800 mg/ritonavir 100 mg with tenofovir and emtricitabine once daily was found to be superior to lopinavir–ritonavir in combination at 96 weeks.[13] Ritonavir-boosted darunavir also has substantial activity against viruses with drug-resistance mutations and therefore is an important component of salvage regimens. In treatment-experienced individuals the recommended dose is darunavir 600 mg q12h/ritonavir 100 mg q12h.

MONITORING FIRST-LINE ANTIRETROVIRAL TREATMENT

Virologic and immunologic responses

The primary goal of first-line ARV treatment is to reduce the plasma viral load to undetectable levels. This has been shown to minimize the risk of selecting resistant viruses and is, as a result, associated with durability of treatment response. After initiating first-line ARV treatment it can take up to 24–32 weeks to reach undetectability on an ultrasensitive viral load assay (limit of detection 40–50 copies/ml). The viral load decline after treatment initiation occurs in three phases. The first phase representing the switching off of replication in productively infected cells is dramatic, with plasma viral loads often declining by 2–3 log in 2 weeks. Most individuals who will fully suppress have a viral load <1000 copies/ml by 8 weeks. Individuals started on ARVs should be reviewed in the first 2–4 weeks to assess for side-effects, toxicity and suboptimal adherence. If available, a viral load should be checked after 1 month on treatment.

CD4 responses to treatment are less predictable. In clinical trials, ARV-naive individuals given virologically effective treatment will have average CD4 increases of 150–200 cells in 48 weeks. However inter-subject variation is large, with some having only small increases in CD4 count (so-called discordant responders) and these patients may be older or have had prolonged HIV infection prior to treatment.

Adherence

Good adherence is critical to the success of ARV treatment. However, the minimal level of adherence necessary for success will depend on several factors, including the genetic barrier of the treatment regimen and the presence of drug resistance. Also important is the timing of nonadherence in relation to starting therapy. Poor adherence during the first few weeks when there is abundant replicating virus present is likely to be more risky than similar nonadherence when patients are fully suppressed. Self-report provides a quick, inexpensive way of assessing adherence although in some individuals probably overestimates compliance. The use of a combination of questions such as 7-day recall or a 30-day visual analogue scale has been recommended.

NEW STRATEGIES

To limit toxicity and/or cost some novel strategies using protease inhibitor monotherapy have been used both in individuals commencing treatment and in those on fully suppressive regimens. The data thus far show that this is relatively successful. However, in naive patients started on PI monotherapy a substantial proportion do not fully suppress, having viral loads between 50 and 500 cells/mm^3. It is not known what proportion of these patients will go on to evolve resistance and eventual virologic failure.

Another concern about this regimen is that it may not penetrate into sanctuary sites such as the brain, genital fluids, etc. This may leave virus replicating in these areas causing local disease, transmission or the development of resistance.

Newer drugs such as the integrase inhibitor raltegravir have been used successfully in ARV-naive patients and may have potential for first-line treatment in the future. The role of CCR5 inhibitors such as maraviroc is debatable.

WHEN TO CHANGE TREATMENT

In patients with fully suppressed virus

In these patients switching of ARVs is usually done to improve the patient's quality of life, improve adherence, and to avoid or prevent worsening of long-term toxicities. It is absolutely essential that any switches do not compromise antiviral efficacy. Drugs that have been previously used and have or may have been associated with prior antiviral failure and actual or potential resistance should be avoided.

There are some data supporting the concept that in HIV-infected persons regimens with reduced dosing frequency and low pill burdens have higher levels of adherence. Switching to a newer agent, co-formulated drugs or a formulation with a lower pill burden and dosing frequency, or one that would be less likely to cause toxicity, are strategies used to both simplify therapy and avoid toxicity. For example, changing from zidovudine or stavudine to tenofovir or abacavir may allow a regimen with a lower dosing frequency (e.g. once daily) that is co-formulated and can avoid or prevent worsening of long-term toxicities such as lipoatrophy, dyslipidemia, peripheral neuropathy, etc.[14] Switching from nevirapine to efavirenz can reduce dosing frequency and co-formulated agents will reduce the pill burden.

Switching from one PI to another, or even to the same PI at a lower dosing frequency (e.g. once a day), can reduce dosing frequency, pill count, drug–drug or drug–food interactions and dyslipidemia, or can take advantage of co-formulation. In some cases, atazanavir can be given without ritonavir if there is ritonavir intolerance or toxicity, but not if the patient is taking tenofovir because of its lowering effect on atazanavir plasma concentrations.

Switching from one class of drug to another is performed for all the above reasons. The most common substitutions for regimen simplification involve a change from a PI-based to an NNRTI-based regimen. Newer agents such as raltegravir and maraviroc might also be used in switching strategies.

Virologic failure

Virologic failure is usually defined as an inability to attain an undetectable viral load within 24–32 weeks after commencing therapy or confirmed rebound from undetectable levels. If an individual has developed resistance, the initial rebound viral load is usually low, mostly less than 1000 copies/ml. In time further mutations may ensue, resulting in higher levels of replicating virus. If the initial viral load rebound is high, this is usually due to periods of poor adherence. In this setting resistance mutations may also be identified on viral genotype. Significant falls in CD4 count and clinical disease are not usually seen with low-level virologic failure.

Resistance

It has been estimated that 10^9 to 10^{10} virions of HIV are produced per day. Mutations in the HIV genome arise spontaneously during the replication process on average once each time a viral genome is replicated. In uncontrolled HIV infection, the high HIV replication rate coupled with the mutation rate generates every possible mutation in the HIV genome each day. Thus a large pool of 'quasispecies' is created. These are genetically related but distinct HIV strains, any of which has the potential to be dominant. In most patients the dominant strain prior to any drug therapy can replicate rapidly and is termed 'wild type' based on its sequence. This wild type is usually fully susceptible to antiviral drugs. A potent ARV regimen will significantly inhibit HIV replication but an ineffective regimen or inadequate adherence by the patient will result in suboptimal inhibition of viral replication, and generation and selection of resistant mutations. It has been calculated – and borne out in clinical practice – that the selection out of any quasispecies is highly unlikely in the presence of three drugs to which the virus is susceptible. If only one or two drugs to which the virus is sensitive are used, the selection of resistant mutants will occur.

Mutations that provide a growth advantage in the presence of ARVs will allow a quasispecies to outcompete the others and become the dominant viral strain in the population, and the patient will have a 'resistant' virus.

Mutations cause resistance by structural alteration of the target molecule that prevents or reduces drug binding (in the case of PIs, NRTIs, NNRTIs and entry inhibitors), or by affecting viral enzyme function in ways that reduce the effectiveness of the drugs (in the case of NRTIs or integrase). They decrease the antiviral activity of the drugs used and cause lack of virologic response.

Most mutations also reduce the viral replication rate compared with that of wild-type virus and may take a long time to emerge as the major quasispecies. Resistance to PIs usually requires the accumulation of several mutations and this may take months or even years to occur.

Mutations associated with resistance to NNRTIs (e.g. Y181C or K103N) do not appear to affect the viral replication rate and so virus can appear as the dominant quasispecies in weeks after starting nevirapine- or efavirenz-based regimens.

Without continuous pressure on the virus to maintain the dominant strain (e.g. when the drugs are stopped), the wild-type strain will re-emerge as this is usually the most efficient at replicating. The mutated strain, however, does not disappear completely but will become a minority quasispecies, only to become dominant again if the drug that selected it is restarted.

Acquired resistance develops through the mechanism described above but primary drug resistance is through transmission of a resistant strain from one individual to another sexually, vertically or through infected blood. Rates of primary drug resistance vary with the methodology used, class of drug, risk behavior, geographic distribution and over time. Many centers are reporting declining rates or stable rates of primary drug resistance. Primary resistance occurs frequently enough in high-income countries to recommend baseline resistance testing prior to starting antiviral therapy for the first time.

Resistance by drug class

There are six licensed antiretroviral classes with 22 drugs in current use. The impact of various mutations alone and in combination is continuing to evolve as new drugs and new classes of these compounds are being developed.[15] There are now more than 200 mutations associated with antiretroviral resistance drugs.[16,17]

Nucleos(t)ide reverse transcriptase inhibitors

In the NRTI class there are 50 mutations associated with resistance to thymidine analogue- and nonthymidine analogue-containing regimens, as well as multinucleoside resistance mutations and accessory mutations.

M184V, nonthymidine analogue-associated mutations such as K65R and L74V, and the multinucleoside resistance mutation Q151M act by decreasing NRTI incorporation.

By promoting a phosphorolytic reaction, thymidine analogue mutations, the triple 69 insertions associated with multinucleoside resistance and many of the accessory mutations facilitate primer unblocking and enhanced removal of incorporated NRTI.

Thymidine analogue mutations associated with zidovudine and stavudine accumulate in two distinct but overlapping patterns:

- type I pattern mutations M41L, D67N, L210W and T215Y; and
- type II mutations D67N, K70R, T215F and K219Q/E.

The type I pattern causes higher levels of phenotypic and clinical resistance to the thymidine analogues than the type II pattern and cross-resistance to abacavir, didanosine and tenofovir.

Non-nucleoside reverse transcriptase inhibitors

Non-nucleoside reverse transcriptase inhibitors have more than 40 reverse transcriptase mutations associated with resistance, some of which, when occurring as single mutations, can confer resistance and cross-resistance to at least three drugs in this class. The primary NNRTI resistance mutations are K103N/S, V106A/M, Y181C/I/V, Y188L/C/H and G190A/S/E. Any one of these will cause high-level resistance to nevirapine and variable levels of resistance to efavirenz. The newer NNRTI etravirine usually requires multiple mutations for resistance to be significant. It appears to retain good activity against common single mutations (e.g. K103N.)

Protease inhibitors

Protease inhibitor resistance is complex and there are more than 60 mutations associated with reducing susceptibility to these drugs. These include major protease, accessory protease and protease cleavage site mutations.

More mutations are selected by PIs than by any other ARV class. There are 17 primary resistance mutations and usually a combination of several of these is required to develop high-level resistance. The effect of these PI resistance mutations on any individual PI can be difficult to predict when many mutations are present in the same virus isolate or when mutations occur in unusual patterns. Gag cleavage site mutations and accessory compensatory mutations can all impact on the susceptibility of the virus to these drugs.

Integrase inhibitors

There is already much known about resistance to the recently developed integrase inhibitors raltegravir and elvitegravir, with more than 30 integrase mutations found to be associated with these agents. Common mutations include N155H and Q148H/R/K.

Entry inhibitors

At least 15 gp41 mutations are associated with resistance to the fusion inhibitor enfuvirtide, the commonest occurring between positions 36 and 45. Resistance to the entry inhibitors targeting the CCR5 receptor (e.g. maraviroc) is unusual and more complex, resulting from mutations that promote gp120 binding of the virus to the CCR5 receptor which is bound to the drug. The majority of virologic failures with this drug, however, are the outgrowth of minority species that can use the alternative CXCR4 receptor for entry.

Measuring resistance

Most resistance tests use genomic sequencing techniques ('genotyping') that are sufficiently sensitive to pick up quasispecies which comprise at least 20% of the viral swarm as long as the viral load is >1000 copies/ml. The sequences are then analyzed to produce a mutation list and this is related to whether a particular drug is predicted to be active or not. Phenotypic techniques are also available but are expensive and time consuming, and add little extra information above genotyping. More sensitive tests looking at minority quasispecies are in development.

Rates of resistance

Currently, approximately half of treated patients undergoing testing show evidence of drug resistance and around 11% have evidence of resistance mutations affecting at least three classes of antiretroviral drug. Primary resistance is found in around 5% of patients but varies from <1% to 15% depending on geography and how long ARV therapy has been available.

Use of resistance tests

The use of resistance tests allows optimal drug combinations to be selected by the physician. Most guidelines suggest that a baseline resistance test be performed at first presentation in case there has been primary transmitted resistance.

The prevalence of drug resistance has declined among treatment-experienced patients in many developed countries as a result of improved management of HIV antiviral treatment with more and better drugs being available. One important consequence has been the improved management of treatment failure.

Resistance testing is recommended in all patients experiencing virologic failure while on treatment and changes in therapy should be guided by the results of resistance testing in these patients. Other factors in choosing therapy are also important, especially drug history as some mutations are 'lost' over time. This is especially true of the M184V mutation which cannot usually be found by routine genotyping once these drugs have been discontinued. It is inferred to be present in patients who have had virologic failure on these drugs but the resistance test was performed some time after the drug was discontinued. This phenomenon is why resistance testing should be performed at the time of virologic failure when the patient is still on the drug combination.

Viral load blips

Transient rises in viral load levels to detectable from undetectable can occur frequently. They may represent 'noise' on the viral load assay or events related to viral replication such as intercurrent systemic infection or vaccination. If low-level detectable viremia represents early virologic failure, persistently detectable viral loads are usually seen, although these may remain low level for long periods despite significant development of resistance. If this is a true 'blip', a single detectable viral load is followed by a return to the undetectable state. It is controversial as to whether blips are associated with a future risk of virologic failure but most are probably bursts of replication of wild-type virus from latently infected cells.

PRINCIPLES OF SECOND- AND THIRD-LINE TREATMENTS

Individuals with virologic failure should be changed to a regimen of antiretroviral drugs based on treatment history and resistance testing, which provides two to three fully active drugs. This may result in drugs being recycled in subsequent lines of therapy if no significant resistance is present, sequencing onto new drugs in the same class if there is nonoverlapping resistance or incorporating new classes of drug. In switching for reasons of side-effects or toxicity, care needs to be taken where a particular toxicity may be a class effect. With the number of new drugs available now most patients can have three or four lines of therapy constructed in this manner.

Other drugs available to use in second and subsequent lines of therapy

Non-nucleoside reverse transcriptase inhibitors

The problems related to this class include:

- a single mutation causing high-level cross-class resistance;
- toxicity (rash and hepatotoxicity for nevirapine) and neuropsychiatric and lipid effects (efavirenz); and
- teratogenicity (CNS malformations were seen in monkey studies on efavirenz).

New generation NNRTIs have to overcome these obstacles before being acceptable and becoming drugs of choice for highly active antiretroviral therapy (HAART) regimens. Etravirine was licensed for use in 2008.

Protease inhibitors

Tipranavir is a nonpeptide protease inhibitor which is administered with ritonavir. Tipranavir has activity against viruses that are resistant to other protease inhibitors and is recommended for patients who are resistant to other treatments. Resistance to tipranavir itself seems to require multiple mutations. However, side-effects of tipranavir can be more severe than other antiretrovirals. It needs to be co-administered with ritonavir 200 mg twice daily and this exposure is tolerated poorly by a significant proportion of patients. It is associated with higher rates of liver toxicity and gastrointestinal intolerance than other PIs. There are also significant pharmacokinetic interactions with other antiretrovirals, including agents used in salvage therapy such as etravirine.

CCR5 inhibitors

Maraviroc

Maraviroc is the first CCR5 receptor antagonist licensed for the treatment of HIV infection. Co-receptor tropism should be determined prior to using maraviroc as it is not effective against CXCR4-tropic or mixed- or dual-tropic viruses. Maraviroc is a substrate of cytochrome P450 (CYP3A) and *p*-glycoprotein, and has clinically significant interactions with other drugs including efavirenz and rifampin.

In the MOTIVATE 1 and 2 studies that compared maraviroc with placebo, each given in combination with an optimized background regimen to patients with advanced HIV disease and CCR5-tropic HIV-1, at 48 weeks the rates of virologic suppression to <50 copies/ml were not significant, although the mean increase in CD4 cell count was greater in the maraviroc groups.[19] Virologic failure is usually associated with the emergence of CXCR4 virus as the development of resistance mutations is rare.

Maraviroc did not reach nonsignificance when compared to efavirenz, both combined with zidovudine and lamivudine in HIV treatment-naive patients. The result was influenced by the relatively low sensitivity of the tropism assay used.

Fusion inhibitors

Enfuvirtide blocks HIV-1 entry by inhibiting fusion. It is expensive and needs to be given by subcutaneous injection twice daily. These factors have limited its widespread use. Individuals frequently develop painful injection site reactions, the impact of which can to some degree be limited by careful attention to injection technique. The effectiveness of this novel agent has been demonstrated as salvage treatment of individuals with extensive, often triple class resistance. The use of enfuvirtide is largely being replaced with newer oral agents such as raltegravir, maraviroc and etravirine.

Integrase inhibitors

Integrase inhibitors target the viral integrase enzyme, which plays an important role in the viral life cycle. The integrase inhibitors the furthest in development are raltegravir (formerly MK-0518), which has been approved by the FDA, and EMEA for use in treatment-experienced patients. It is given twice daily.

OTHER AGENTS IN ADVANCED DEVELOPMENT

The need for new drugs?

Currently licensed drugs have short- and long-term toxicities. Convenience and ease of adherence are important in constructing regimens that are easy to tolerate. Once-a-day dosing and a low pill burden are important factors in achieving these aims. Moreover, patients who have developed virologic failure, often with multiple resistant mutations, need new drugs that are active against these drug-resistant strains. New drugs that penetrate into sanctuary sites that may act as a reservoir for HIV replication such as the CNS and genital tract are much in need. Finally, new ARV drugs known to be safe in pregnancy would be a valuable addition to the currently available treatment options for HIV infection.

DRUG–DRUG INTERACTIONS

Most drug interactions with ARVs are mediated through inhibition or induction of hepatic drug metabolism. For example, all PIs and NNRTIs are metabolized in the liver by the cytochrome P450 system, particularly by the CYP3A4 isoenzyme. Some PIs may also be inducers or inhibitors of other CYP isoenzymes and of *p*-glycoprotein or other transporters. There is an extensive and continuously expanding list of drugs that may have significant interactions not only with PIs or NNRTIs but also with new drug classes. Some examples include medications that are commonly prescribed for non-HIV medical conditions, such as statins, benzodiazepines, calcium channel blockers, ciclosporin and tacrolimus, anticonvulsants, rifamycins, sildenafil, ergot derivatives, azole antifungals, macrolides, oral contraceptives and methadone. St John's wort, a herbal product, can cause a decrease in PI plasma levels.

The inhibitory effect of ritonavir is used to advantage in boosting concentrations of the other PIs but may cause abnormally high levels of some drugs such as simvastatin. The NNRTIs CYP3A4-inducing effect can be an issue when giving atazanavir or lopinavir–ritonavir. Efavirenz can cause PI concentration to fall.

These drug interactions are not always predictable. As an example, tenofovir interacts with didanosine, increasing didanosine intracellular levels, but will decrease atazanavir plasma concentrations.

There are web-based and other resources to help identify drug–drug interactions between antiretrovirals and between other commonly prescribed drugs. A good source is the 2008 DHHS guidelines and interactive web sites http://www.hiv-druginteractions.org and http://www.hivpharmacology.com.

HAART AND ANTITUBERCULOUS THERAPY

There are many difficulties when it comes to managing tuberculosis (TB) and HIV co-infection. When to start antiretroviral treatment in a patient on antituberculous therapy has been an ongoing issue. Studies suggest that delaying HAART can lead to a worse prognosis with progression to AIDS and death. The fear of poor adherence, drug interactions, toxicity and development of immune reconstitution inflammatory syndrome (IRIS) might be outweighed by this risk of progression and death if the HIV remains untreated in the severely immunocompromised with CD4 counts <200 cells/mm^3. Randomized controlled trials are ongoing to try to answer this question.

Drug interactions with the essential component of TB treatment – the rifamycins – are a problem.[25,26] Rifamycins are potent inducers of drug efflux *p*-glycoprotein and of the cytochrome P450 enzyme system, especially 3A4 which is responsible for the metabolism of PIs, NNRTIs and the CCR5 inhibitor maraviroc. Rifampin also induces metabolism of the integrase inhibitor raltegravir.

This limits the drugs that can be used with rifampin, leaving four nucleoside regimens thus far of unproven efficacy in HIV, or using efavirenz (600–800 mg/day) or nevirapine plus nucleosides. Efavirenz is preferred because of the better interaction profile and better efficacy seen in a cohort study from South Africa. Efavirenz doses are adjusted by some physicians to 800 mg if the patient is over 60 kg and the use of drug level monitoring can be useful in this situation. Protease inhibitors boosted with ritonavir can lead to significant decreases in plasma levels and a high rate of hepatotoxicity with rifampin. If they have to be used, it is better to switch to rifabutin.

There are many overlapping toxicities of HAART and antituberculous therapy. Stavudine in particular should not be used as it leads to increased rates of peripheral neuropathy and overlaps with isoniazid in terms of this side-effect. Hepatitis and rash are other common toxicities and this is another reason why nevirapine should be used with caution.

CHRONIC HEPATITIS

Co-infection of chronic HBV or HCV with HIV increases the rate of progression to cirrhosis and liver cancer by four- to fivefold compared with hepatitis monoinfected individuals. The mortality rate of untreated HIV/HBV or HIV/HCV co-infected patients is approximately 10 times higher than that of patients with either infection alone.[26,27]

Antiretroviral therapy may reduce the rate of progression to cirrhosis and death in these patients and in those with HCV infection.

There is a chance of cure with specific therapy. Zidovudine, didanosine and abacavir interact with this therapy and should be avoided.

Tenofovir, lamivudine and emtricitabine are agents that work against HIV; however, as they also suppress hepatitis B viral replication, they should be included in regimens given to patients co-infected with HIV and HBV. The treatment of these co-infections is discussed in Practice Points 44 and 46.

PREGNANCY

In pregnant women, prevention of mother-to-child transmission (PMTCT) is paramount. Maximal continual viral suppression is needed to reduce the risk of transmission of HIV to the fetus and newborn (see Chapter 98).

The choice of an antiretroviral combination in pregnant women should be based on the safety, efficacy and pharmacokinetic data of drugs used during pregnancy. Results of a baseline resistance test are important in this situation.

Efavirenz should be avoided in a pregnant woman during the first trimester and many women switch away from this to a boosted PI prior to becoming pregnant. Boosted PIs such as lopinavir–ritonavir and saquinavir–ritonavir have been used safely and effectively in pregnancy. A more detailed discussion of this issue can be found in Chapter 98 and guidelines for the management of pregnancy and HIV can be found at US Department of Health and Human Services Public Health Service Task Force *Recommendations for Use of Antiretroviral Drugs in Pregnant HIV-Infected Women for Maternal Health* and *Interventions to Reduce Perinatal HIV Transmission in the United States* (http://aidsinfo.nih.gov/ContentFiles/PerinatalGL.pdf), the British HIV Association pregnancy guidelines (http://www.bhiva.org/cms1191540.asp) and the European AIDS Clinical Society antiviral guidelines (http://www.europeanaidsclinicalsociety.org/guidelines.asp).

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References for this chapter can be found online at http://www.expertconsult.com

Practice point

45

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Drug interactions in HIV and AIDS

INTRODUCTION

The recognition and management of pharmacokinetic and pharmacodynamic drug interactions is a major factor in choosing drugs and designing dosage regimens for the HIV-infected patient. Complex multidrug regimens have become the standard of care and clinicians must be aware of the potential need for dosage alterations with combination therapy. Many of the antiretrovirals in current use can alter concentrations of concomitantly administered drugs or have their own concentrations altered by other agents. These interactions can be detrimental or beneficial to the patient.

NUCLEOSIDE REVERSE TRANSCRIPTASE INHIBITORS

The nucleoside reverse transcriptase inhibitors (NRTIs) are primarily eliminated by renal mechanisms and thus they are not generally involved in clinically significant pharmacokinetic interactions. These agents can be used with protease inhibitors, CCR5 antagonist (maraviroc), integrase inhibitor (raltegravir), fusion inhibitor (enfuvirtide) and non-nucleoside reverse transcriptase inhibitors (NNRTIs) without the need for dosage alterations. Two NRTIs – zidovudine and abacavir – are eliminated in part by hepatic glucuronidation. Inducers of glucuronyl transferase activity can modestly lower plasma concentrations of these agents, although the clinical significance of this is uncertain since the active metabolites are intracellular nucleoside triphosphates.

Nucleoside reverse transcriptase inhibitors may interact pharmacodynamically with agents that have similar side-effect profiles. Increased bone marrow toxicity may be observed when zidovudine is used with other drugs capable of bone marrow suppression, such as ganciclovir, pyrimethamine, sulfamethoxazole and chemotherapy. Many NRTI-induced toxicities have been attributed to inhibition of mitochondrial DNA (mtDNA) polymerase, which results in depletion of mtDNA and consequently depletion of components of the electron transport chain encoded by mtDNA. Common examples of mitochondrial toxicity include peripheral neuropathy and lipoatrophy. Lactic acidosis, which may be accompanied by hepatosteatosis, can be fatal. Although there is a US Food and Drug Administration (FDA) black box warning for all NRTIs, zidovudine, didanosine and stavudine have been the most commonly implicated agents. The combination of didanosine plus stavudine and/or hydroxyurea has been particularly associated with pancreatitis, peripheral neuropathy and lactic acidosis, and should be avoided.

The NRTI tenofovir increases the plasma concentrations of concomitantly administered didanosine and may increase the toxicity of didanosine if standard doses are used. A dose reduction to didanosine 250 mg/day for patients >60 kg or 200 mg daily for patients <60 kg can be considered, but the co-administration of didanosine, tenofovir and an NNRTI is generally not recommended due to the high rate of virologic failure.

NON-NUCLEOSIDE REVERSE TRANSCRIPTASE INHIBITORS

Etravirine (ETR) is a CYP3A4, 2C19, and 2C9 substrate and also undergoes glucuronidation *in vitro*. Unlike efavirenz and nevirapine, etravirine does not induce its own metabolism. ETR is a mild inducer of CYP3A4, 2B6, and glucuronidation *in vitro*, but also inhibits 2C9 and 2C19. Due to ETR induction properties, co-administration of ETR with unboosted PIs is not recommended. Although co-administration with boosted darunavir resulted in a 37% reduction in the ETR AUC, the combination has performed well in clinical trials and can be co-administered in treatment-experienced patients. Potent enzyme inducers such as rifampin, rifapentine, phenobarbital, carbamazepine, phenytoin, and St. John's wort should be avoided with ETR. In a cocktail study with warfarin, a 2C9 substrate, warfarin concentrations were significantly increased; therefore, close INR monitoring with ETR co-administration is critical.

PROTEASE INHIBITORS

Etravirine (ETR) is a CYP3A4, 2C19, and 2C9 substrate and also undergoes glucuronidation *in vitro*. Unlike efavirenz and nevirapine, etravirine does not induce its own metabolism. ETR is a mild inducer of CYP3A4, 2B6, and glucuronidation *in vitro*, but also inhibits 2C9 and 2C19. Due to ETR induction properties, co-administration of ETR with unboosted PIs is not recommended. Although co-administration with boosted darunavir resulted in a 37% reduction in the ETR AUC, the combination has performed well in clinical trials and can be co-administered in treatment-experienced patients. Potent enzyme inducers such as rifampin, rifapentine, phenobarbital, carbamazepine, phenytoin, and St. John's wort should be avoided with ETR. In a cocktail study with warfarin, a 2C9 substrate, warfarin concentrations were significantly increased; therefore, close INR monitoring with ETR co-administration is critical.

The potent inhibition of CYP3A4 by ritonavir is routinely used to optimize the AUC of all protease inhibitors except nelfinavir. For example, concomitant administration with ritonavir results in a 20-fold increase in steady-state saquinavir concentrations, allowing for a dosage and frequency reduction of saquinavir, while maintaining high blood concentrations. With ritonavir co-administration, once-daily dosing of atazanavir, fosamprenavir, saquinavir and lopinavir is possible. These combinations successfully suppress HIV replication in clinical trials in protease inhibitor-naive subjects.

In addition to enzyme inhibition, ritonavir and nelfinavir possess moderate enzyme-inducing properties and decrease concentrations of a number of co-administered agents. Ritonavir, lopinavir/ritonavir and nelfinavir increase glucuronosyl transferase activity. Ethinyl estradiol and progesterone concentrations can be decreased by concomitant administration of these protease inhibitors, necessitating alternative forms of birth control.

Similar to other protease inhibitors, tipranavir (TPV) is a substrate and inhibitor of CYP3A4; however, TPV is also a substrate and potent inducer of the p-glycoprotein drug transporter, P-gp. When co-administered with ritonavir, TPV/ritonavir produces a net inhibition of CYP3A4 which results in an increased plasma concentration of CYP3A4 substrate (i.e. atorvastatin). However, for drugs that are dual substrates of CYP3A4 and P-gp, a net induction results. For example, an AUC decrease of 45%, 49% and 70% was observed with amprenavir (APV), lopinavir/ritonavir (LPV/r), and saquinavir (SQV) co-administration, respectively.

Certain enzyme-inducing agents can profoundly decrease the concentrations of protease inhibitors. For example, rifampin has been shown to decrease the saquinavir AUC by 80% and the indinavir AUC by 65%. The resulting low blood concentrations have the potential to promote drug resistance and treatment failure, so all protease inhibitors (with the possible exception of LPV/r 400/100 mg plus RTV 300 mg q12h) should be avoided in patients receiving rifampin. Rifabutin can be considered as an alternative to rifampin, but the rifabutin dose must be decreased to 150 mg three times a week with boosted protease inhibitor co-administration. Other potent enzyme inducers, such as phenytoin, phenobarbital and carbamazepine, are likely to produce similar reductions in PI concentrations.

CCR5 ANTAGONIST

Maraviroc, the first FDA-approved CCR5 antagonist, does not inhibit or induce CYP3A4 or glucuronyl transferase activity. Unlike protease inhibitor or non-nucleoside reverse transcriptase inhibitors, maraviroc does not affect the pharmacokinetics of oral contraceptives or midazolam. As maraviroc is a substrate for CYP3A4 and P-gp, the dose of maraviroc should be halved when co-administered with CYP3A4 and/or P-gp inhibitors. When maraviroc is co-administered with HIV-protease inhibitors (with the exception of tipranavir/ritonavir) a two- to eightfold elevation of maraviroc's AUC was observed. On the other hand, efavirenz and rifampin, CYP3A4 inducers, significantly decrease maraviroc's serum concentrations. Maraviroc's dose should be adjusted based on the CYP3A4 inhibitors and/or inducers with which it is combined (Table PP45.1).

Table PP45.1 Consequences of selected CYP450-mediated drug interactions

	Agents affected	Recommendations
CYP3A4 inhibitors		
All protease inhibitors (PI)* Cimetidine Clarithromycin Delavirdine Erythromycin Itraconazole Ketoconazole Nefazodone Telithromycin	*Increased concentration of co-administered drug:* Antiarrhythmics (amiodarone, disopyramide, dofetilide, flecainide, lidocaine, mexiletine, propafenone, and quinidine); calcium channel antagonists, fentanyl, maraviroc, midazolam, simvastatin, lovastatin, triazolam, pimozide, rifabutin, terfenadine, astemizole, cisapride, ergotamine, erectile dysfunction agents	Decrease maraviroc dose to 150 mg q12h Use calcium channel antagonist with close monitoring Reduce rifabutin dose to 150 mg three times a week with boosted PI co-administration Avoid midazolam, triazolam, fentanyl, terfendadine, astemizole, cisapride, ergotamine, pimozide, simvastatin, and lovastatin co-administration
CYP3A4 inducers		
Efavirenz (EFV) Nevirapine (NVP) Anticonvulsants (i.e. phenytoin, carbamazepine, phenobarbital) Rifampin (rifampicin) Rifapentine, St John's wort	*Decreased concentration of co-administered drug:* All PIs, methadone, maraviroc	With EFV or NVP co-administration, administer all PIs with ritonavir Maraviroc dose should be increased to 600 mg twice daily Methadone dose may need to be increased with EFV or NVP co-administration Consider valproic acid or levetiracetam with CYP3A4 inducers Avoid rifampin and St John's wort with PIs and methadone co-administration
P-gp inducer		
Tipranavir/ritonavir (TPV/r)	All PIs	Avoid PI co-administration with TPV/r
UGT-glucuronosyl transferase enzyme inducers		
Lopinavir/ritonavir Ritonavir Nelfinavir Rifampin (rifampicin) Phenobarbital Phenytoin	*Decreased concentration of co-administered drug:* Oral contraceptive (ethinyl estradiol and progesterone) and raltegravir	Consider alternative barrier form of contraception or medroxyprogesterone Low-dose ritonavir (100 mg q12h) can be administered with raltegravir, but rifampin, phenobarbital and phenytoin should be avoided
Acid blockers		
H_2-blockers Proton pump inhibitors (PPIs) Antacids	*Decreased concentrations of co-administered drug:* Atazanavir, nelfinavir and delavirdine	Avoid co-administration with PPIs H_2-blockers or antacids may be administered 2 hours after atazanavir, nelfinavir and delavirdine administration

*Atazanavir, darunavir, fosamprenavir, lopinavir, ritonavir, saquinavir and tipranavir.

INTEGRASE INHIBITOR

Raltegravir, the first FDA-approved integrase inhibitor, is mainly metabolized by glucuronidation (like zidovudine) and thus should be less susceptible to drug interactions. Its metabolism is partially blocked by atazanavir, a selective inhibitor of the UGT-glucuronosyl transferase enzyme UGT1A1; this resulted in a 41% increase in the raltegravir AUC, but no dose adjustment is needed. Potent inducers of UGT-glucuronosyl transferase enzymes (i.e. rifampin, phenobarbital and phenytoin) can significantly decrease raltegravir concentrations and should be avoided.

MANAGEMENT OF DRUG INTERACTIONS

A careful review of patient medication profiles is essential to managing drug interactions in the HIV-infected patient. This should include complementary and alternative medicines, since agents such as St John's wort induce CYP3A4 and can reduce concentrations of indinavir and presumably other protease inhibitors. Clinicians need to have a general understanding of certain 'red flag' drugs that are potent inhibitors and inducers of CYP450 (i.e. rifampin, ritonavir, efavirenz, etc.). In this era of complex regimens, computerized pharmacy systems can detect many drug–drug interactions and decrease the number of inappropriate prescriptions; however, a limitation of such systems is their inability to update information beyond the FDA-approved labeling. Therefore, clinicians are encouraged to use computerized and internet-based databases that are updated more regularly. In addition, clinicians should be vigilant about the potential for drug interactions.

Patient counseling is critically important when complex antiretroviral regimens are prescribed. Patients must be instructed on how to take their medications with regard to timing and content of meals. In some cases, a written daily calendar of medications and times of administration may be useful to the patient and may improve adherence. Proper separation of dosing of certain drugs (i.e. didanosine without food and atazanavir with food) must be explained and can also be documented on a daily dosage planner.

The HIV clinician can use a variety of interventions to manage drug interactions. Selection of a drug with fewer interactions should be considered if warranted by the clinical situation. For example, azithromycin is not metabolized by CYP450 and has far fewer drug interactions compared with other macrolides. Also, drugs that can be administered once or twice daily may be useful to lessen food-related interactions or dosage separation problems. Finally, clinicians should be aware of and try to avoid drugs with overlapping toxicities. However, such combinations are sometimes unavoidable and patients who receive these agents must be closely monitored for the development of toxicity.

FURTHER READING


Further reading for this chapter can be found online at http://www.expertconsult.com

Practice point | 46 |

Stanislas Pol
Anaïs Vallet-Pichard
Vincent Mallet

How to manage the hepatitis C virus co-infected HIV patient

While hepatitis C virus (HCV) infection has no clear impact on HIV infection, HIV infection clearly has a deleterious impact on the natural history of HCV infection, especially since the introduction of highly active antiretroviral therapy (HAART). The higher hepatitis C viral load leads to higher contagiosity, increased liver-related morbidity and mortality. Since the improvement in treating HCV infection paralleled the progress in treating HIV, the question now is how to manage HIV–HCV co-infected patients.

WHY EVALUATE HIV–HEPATITIS C VIRUS CO-INFECTION?

Anti-HCV antibodies are detected in around 10–30% of HIV-infected patients; they reflect active infection as assessed by detectable hepatitis C viremia in most. The impact of HIV on HCV is characterized:

- by increasing levels of hepatitis C viremia;
- by significantly increasing the risk of mother-to-child or sexual transmission (from a mean of 6–20% and from 0% to 3%, respectively); and
- by increasing (two- to fivefold) and accelerating the risk of cirrhosis and of occurrence of cirrhosis complications; the progression of fibrosis depends on the CD4 cell count level (<200 cells/mm^3), chronic alcohol consumption and steatosis.

Thus, HCV infection is, since HAART, one of the leading causes of morbidity and mortality of HIV-1-infected patients which underlines the need for early diagnostic procedures and therapeutic strategies.

The pathobiology of the severity of liver disease in co-infected patients is multifactorial, including:

- rarely immune restoration;
- direct viral cytotoxicity in immunocompromised patients;
- nonalcoholic steatohepatitis (NASH) related mainly to the metabolic syndrome which may be induced by HAART;
- all the classic causes of liver toxicity (alcohol but also vascular diseases such as nodular regenerative hyperplasia); and
- drug-related hepatic toxicity and all antiviral drugs having potential toxicity (Fig. PP46.1).

The rate of HAART withdrawal in relation to hepatotoxicity is around 2% without significant difference regarding the type of antiretroviral treatment. Nucleoside reverse transcriptase inhibitors cause rare but severe hepatitis, which is due to a mitochondrial cytopathy with severe microsteatosis and lethal lactic acidosis. Non-nucleoside reverse transcriptase inhibitors are responsible for clinical hepatitis in 2–5% of cases, which are sometimes severe (0.1% of cases) and are associated with signs of hypersensitivity in two-thirds of cases. Drug-related hepatitis in association with protease inhibitors is observed in 2–8.5% of treated patients, with a predilection for patients co-infected with HCV or HBV. In most cases of drug-related hepatitis, liver biochemical abnormalities resolve after discontinuation of the drug.

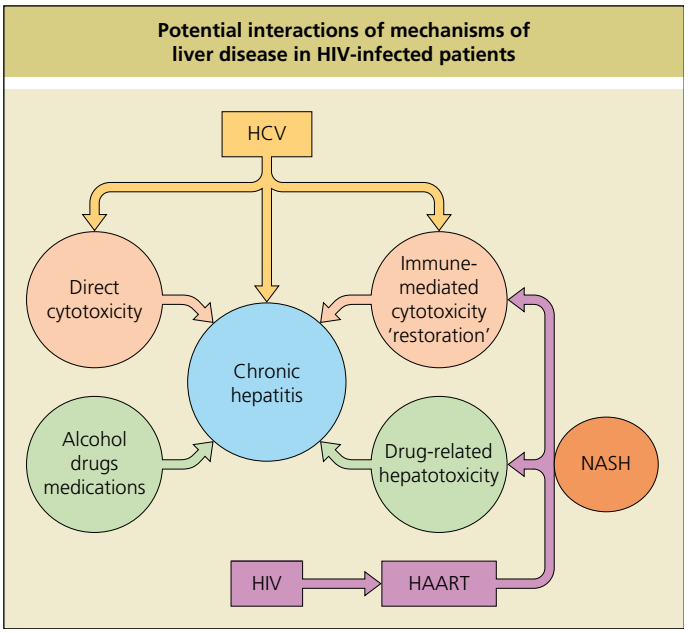


Fig. PP46.1 Potential interactions of mechanisms of liver disease in HIV-infected patients

THERAPEUTIC IMPLICATIONS

The deleterious impact of HIV on HCV infection underlines the need for reliable anti-HCV antiviral therapies. All anti-HCV-positive, HIV-co-infected patients have to be carefully evaluated. HCV RNA detection will delineate patients with complete recovery (HCV RNA-negative) from those with active infection (HCV RNA-positive). These latter patients will undergo evaluation of the liver impact of HCV infection either by a liver biopsy or by noninvasive procedures (biochemical or morphologic by evaluation of liver stiffness).

Antiviral treatment is preceded by exclusion of nucleoside analogues which may:

- enhance ribavirin-associated anemia or interferon-induced neutropenia (azidothymidine);
- interfere with ribavirin metabolism with an enhanced risk of mitochondrial toxicity (didanosine); or
- exacerbate decompensation of liver disease (didanosine, stavudine and nevirapine). The negative influence of abacavir is questioned.

In five randomized clinical trials, 27–55% of co-infected patients who received the pegylated interferon (α2a: 180 μg or α2b: 1.5 μg/kg subcutaneously weekly) plus ribavirin (800–1200 mg/day according to the weight) combination, the recommended anti-HCV therapy, attained sustained virologic response. Absence of early virologic response (a decrease in serum HCV RNA of ≥2 $\log_{10}$ from baseline or undetectable HCV RNA at week 12) has an accurate negative predictive value (98–100% of absence of sustained virologic response, SVR); on the other hand, rapid virologic response (undetectable HCV RNA at week 4 of therapy) has a high positive predictive value of approximately 90% of SVR. Because of this accuracy, the European treatment guidelines adopted the 12-week stopping rule in co-infected patients without early virologic response.

There is no deleterious impact of the interferon–ribavirin combination on immune status with a reduction of the absolute CD4 T-cell counts during treatment (the mean decrease ranging from 130 cells/mm³ to 331 cells/mm³) but the percentage of the CD4 T-cell count either increased or remained stable: CD4 T-cell counts generally returned to baseline levels after completion of therapy.

PRAGMATIC MANAGEMENT OF HEPATITIS C VIRUS CO-INFECTED PATIENTS

Pre-existing biochemical abnormalities do not contraindicate the use of antiretroviral drugs. In some difficult situations (e.g. cirrhosis, liver transplantation), the measurement of the plasma levels of antiretroviral drugs may be valuable in order to avoid any overdoses that may lead to hepatotoxicity. In HCV–HIV co-infected patients, monthly biochemical follow-up is warranted in the first 3 months after introduction of any new antiretroviral drug and then every 3 months in order to identify potential drug-related toxicity. An increase in alanine aminotransferase levels (five times the upper normal value, but the rate of increase is more important than the absolute values) should lead to a switch of the protease inhibitor or to the introduction of a non-nucleoside reverse transcriptase inhibitor. Biochemical liver abnormalities occurring with a non-nucleoside reverse transcriptase inhibitor should lead to treatment withdrawal, especially if cutaneous signs of hypersensitivity are associated. However, the interpretation of such biochemical liver abnormalities is difficult, raising not only the question of drug-related hepatitis but also of other toxic causes such as alcohol, cocaine or metamphetamine.

An unsolved question is the respective place of anti-HCV and anti-HIV therapies. In clinical practice, most HIV patients are referred to the hepatologist while receiving HAART and the question is not relevant. In the future, physicians will have to consider the priority of each treatment according to liver histology and immune status. Given the chance of complete eradication of HCV-associated chronic hepatitis (SVR), anti-HCV therapy should be considered a priority and be treated first if the CD4 lymphocyte count is not less than 300 cells/mm³. Marked necroinflammatory activity or fibrosis (A2-A3 or F3-F4) indicates a need for anti-HCV treatment in order to avoid deleterious evolution; in contrast, low inflammatory activity or fibrosis (A0-A1 or F0-F1) does not necessarily indicate anti-HCV treatment but does indicate liver follow-up by biopsy every 3 years or noninvasive biochemical or morphologic evaluation of fibrosis each year. In intermediate situations (A2-F2), the choice to treat or not to treat should be based mainly on predictive factors, including genotype and quantitative viremia (Fig. PP46.2).

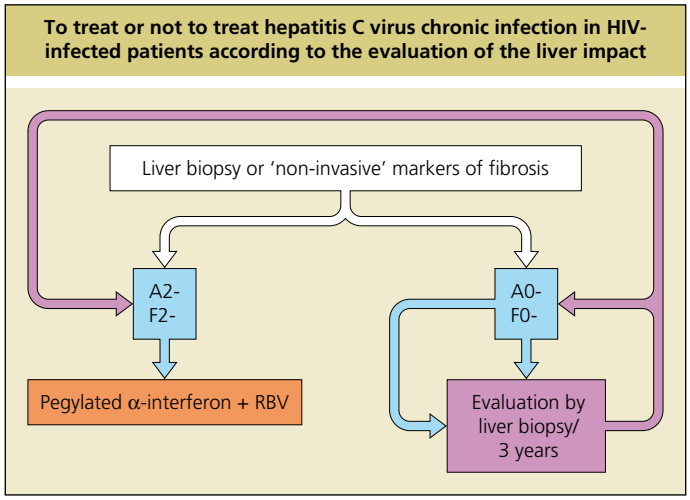


Fig. PP46.2 Algorithm to treat or not to treat hepatitis C virus chronic infection in HIV-infected patients according to the evaluation of the liver impact. RBV, ribavirin.

The newest recommendations from the 1st European Consensus Conference on the Treatment of Chronic Hepatitis B and C in HIV Co-infected Patients deal with three circumstances during the evolution of the disease:

1. during the acute phase of HCV infection (treatment outcomes are improved if treatment is initiated early);
2. during the chronic stage of hepatitis C, in which SVR rates can restrict progression to cirrhosis and hepatocellular carcinoma; and
3. finally, at the stage of cirrhosis, treatment is indicated to decrease the risk for hepatocellular carcinoma or other complications (e.g. portal hypertension and liver failure) that may necessitate liver transplantation.

In conclusion, physicians should be aware of the potential risk of:

- symptomatic liver disease in HCV–HIV co-infected patients in the era of antiretroviral therapy;
- liver deterioration paralleling immune function restoration, steatosis and immune deficiency;
- lack of clear impact of active antiretroviral therapy on HCV load; and
- potential drug-related hepatitis that may modify the natural history of HCV-related liver disease.

Liver evaluation of HCV infection should be performed regularly to identify patients with severe liver disease who require early anti-HCV therapy (see Fig. PP46.2) under close monitoring of their immune status.

FURTHER READING

Further reading for this chapter can be found online at http://www.expertconsult.com

Practice point 47

Judith Currier

How to manage hyperlipidemia in the HIV patient

CASE HISTORY

A 48-year-old man with HIV infection presents for routine follow-up. He was diagnosed with HIV in 2000 when he developed *Pneumocystis* pneumonia. He has maintained an undetectable HIV RNA on his current regimen of zidovudine, lamivudine and efavirenz. He has no family history of diabetes or heart disease. He smokes one pack of cigarettes per day and drinks a six pack of beer 2 nights per week. His physical examination is normal and his blood pressure is 142/80 mmHg. Fasting lipid values include triglycerides of 420 mg/dl (4.75 mmol/l), total cholesterol 260 mg/dl (6.73 mmol/l) and HDL cholesterol 28 mg/dl (0.73 mmol/l). Lipid values prior to starting treatment are not available. Does this patient have a clinically significant dyslipidemia and, if so, what are the options for management?

Dyslipidemia in HIV infection

The dyslipidemia associated with HIV infection may be a consequence of HIV infection per se, a direct effect of the antiretroviral agents used to treat HIV infection, secondary to central adiposity and insulin resistance that can occur during treatment or it may be unrelated to HIV treatment or disease. Currently marketed protease inhibitors (PIs) differ slightly in their ability to cause dyslipidemia, whereas in general non-PI-containing regimens have been found to have less of an effect on lipids. Exceptions to this include the modest triglyceride increases that have been observed with efavirenz and the increases in triglycerides associated with stavudine and zidovudine. In a recent randomized clinical trial in treatment-naive patients, increases in total cholesterol, HDL cholesterol and triglycerides were similar in patients receiving either efavirenz or lopinavir/ritonavir combined with two nucleosides. The magnitude of the effect on lipids may vary for different PI combinations. More modest increases in triglycerides have been reported with the following ritonavir-boosted protease inhibitors: atazanavir, saquinavir and fos-amprenavir. Patients with untreated advanced HIV infection have been noted to have a pattern of low HDL cholesterol and increased triglycerides.

Estimating cardiovascular risk

The long-term significance of dyslipidemia in HIV-infected individuals is not completely known; however, it has been suggested that HIV treatment-related lipid increases contribute to an increased risk for myocardial infarction associated with protease inhibitor exposure in the Data Collection on Adverse Events of Anti-HIV Drugs (DAD) study. The patterns of lipid abnormalities most commonly observed in patients with HIV infection might be expected to result in increased cardiovascular morbidity. In many cases both antiretroviral therapies and HIV infection per se are thought to contribute. Recommendations for the management of dyslipidemia in HIV-infected individuals have been developed.

These guidelines first consider the overall risk of cardiovascular disease (CVD) and consider the following as important risk factors: current cigarette smoking, diabetes, hypertension, family history of coronary heart disease and HDL cholesterol. The first step in evaluating cardiovascular risk is to determine which risk factors are present. If so, then an estimate of the 10-year cardiovascular risk using the Framingham scoring system is suggested (see http://www.nhlbi.nih.gov/guidelines/cholesterol/index.htm). In this example, the patient has several classic risk factors for CVD: he is a smoker with untreated hypertension who also has low HDL cholesterol and elevated total cholesterol and elevated triglycerides. Using the online risk calculator available from the National Heart, Lung, and Blood Institute (NHLBI) we discover that his estimated 10-year cardiovascular risk is over 30%. This is considered a high risk, similar to that of someone with established coronary heart disease.

The reliance on LDL cholesterol complicates the use of NCEP guidelines in the management of patients with HIV infection. When the value for triglycerides exceeds 400 mg/dl (4.52 mmol/l), calculation of LDL cholesterol from the total cholesterol and HDL is not accurate. Direct measurement of LDL is not available in many settings and hence it may be reasonable to base decisions for intervention on the values of non-HDL cholesterol. The value for non-HDL cholesterol is the total cholesterol minus HDL cholesterol. In this example, the value for non-HDL cholesterol would be 232 mg/dl (6.0 mmol/l). The NCEP ATP III guidelines include goals for non-HDL cholesterol in addition to LDL cholesterol and these are included in Table PP47.1.

MANAGEMENT

Now that we have established that the patient in this example requires intervention for his dyslipidemia (and his overall cardiovascular risk), we can consider the options for management. The first steps would include discussing his diet and lifestyle. The importance of smoking cessation and treatment of his hypertension should be aggressively addressed as these factors are clearly contributing to the patient's high level of cardiovascular risk. These modifiable risk factors should be addressed prior to considering altering his currently successful antiretroviral regimen. His diet should be reviewed and he should be given information on a low-fat (with reduced saturated fat), low-cholesterol diet. It is possible that his intake of alcohol, two six packs of beer each night during the weekend, could be contributing to his hypertriglyceridemia. Given his calculated level of cardiovascular risk, additional measures to lower his non-HDL cholesterol and his elevated triglycerides are likely to be needed.

Table PP47.1 Lipid targets based on NCEP risk categories

Category	LDL-C goal	Non-HDL-C (if TG >200 mg/dl)
High risk	<100 mg/dl	<130 mg/dl
10-year risk >20% or coronary heart disease	<70 mg/dl*	<100 mg/dl*
>2 Risk factors and 10-year risk ≤20%	<130 mg/dl	<160 mg/dl
0–1 Risk factor	<160 mg/dl	<190 mg/dl

*Grundy SM, Cleeman JI, Merz CN, *et al*. Implications of recent clinical trials for the National Cholesterol Education Program Adult Treatment Panel III Guidelines. Circulation 2004;110:227–39. This paper includes updated targets for very high risk patients such as those who have had a recent heart attack, or those who have cardiovascular disease combined with either diabetes or severe or poorly controlled risk factors (such as continued smoking) or metabolic syndrome. Adapted from Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults. Executive summary of the third report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). JAMA 2001;285:2486–97.

The additional measures for management of this patient's dyslipidemia include alteration in his antiretroviral regimen and the use of lipid-lowering agents. There are limited data at present to guide clinicians in this regard. The patient is currently receiving a non-nucleoside-based regimen that contains the thymidine nucleoside zidovudine; both of these drugs may be contributing to his elevated cholesterol and triglycerides. Several studies have suggested a benefit of substituting a nonthymidine nucleoside analogue such as tenofovir or abacavir as a reasonable option for patients who are well suppressed on a thymidine-based regimen with dyslipidemia. There are scant data on the impact of switching of efavirenz to either unboosted atazanavir or to nevirapine in this setting. One potential caution is the risk of a hypersensitivity reaction if nevirapine is used when CD4 cell counts are >400 cells/mm^3 in men (or >200 cells/mm^3 in women). If the patient is reluctant to change his therapy, and even if he does make these changes, the use of lipid-lowering agents should be considered given his high level of risk.

The HMG-CoA reductase inhibitors (statins) are generally the first-line intervention for lipid disorders in the general population, especially when the estimated coronary heart disease risk is as high as that of the patient in this example. Important drug interactions between the statin agents and the PIs have been reported and it is becoming more difficult to generalize recommendations. Simvastatin and lovastatin should not be used with protease inhibitors, pravastatin appears to be the least likely to interact with PIs with the exception of darunavir and recently, rosuvastatin was found to increase significantly when co-administered with lopinavir/ritonavir. Drug interactions are unlikely to occur with the use of the fibric acid derivatives gemfibrozil and fenofibrate. In the case above, with elevations in both triglycerides and non-HDL cholesterol and an estimated 10-year risk of 30%, therapy with a statin agent should be strongly considered. Pravastatin at a dose of 20–40 mg/day or atorvastatin at a dose of 10–20 mg/day are reasonable choices; the latter may be more beneficial for the patient's elevated level of triglycerides. In addition, consideration should be given to changing the zidovudine to either abacavir or tenofovir. If the patient does not respond to these interventions, higher doses of statin therapy can be considered. In addition, daily low-dose aspirin therapy should be considered in this middle-aged man with an elevated cardiovascular risk.

As the treatment of HIV infection has evolved into the management of a chronic disease, more emphasis is being placed on preventing long-term complications from both the disease and the therapies used to treat it. It is important for clinicians to be aware of these issues and to screen patients for lipid disorders prior to starting antiretroviral therapy and during treatment. Attention to modifiable cardiac risk factors such as smoking, diet and level of physical activity is critical and should not be overlooked. In settings where the underlying cardiovascular risk appears to be high, further interventions with lipid-lowering agents or modification of the antiretroviral regimen may be warranted.

FURTHER READING

Further reading for this chapter can be found online at http://www.expertconsult.com

Practice point | 48 |

Pablo Tebas

Multidrug-resistant HIV

FREQUENCY OF THE PROBLEM

Unfortunately, due to difficulty with adherence to antiretroviral treatment or the sequential use of partially suppressive regimens when therapy was initiated, a very significant proportion of patients with HIV infection develop multidrug-resistant HIV. In a survey of the ongoing HIV Costs and Service Utilization Study (HCSUS), approximately 63% of the patients under care had a detectable viral load at the time of the last determination, 78% of those had resistance to at least one drug and more than 50% were resistant to two. These data suggest that more than 100 000 patients in the USA alone (or half of the total number of patients in care) are infected with viruses that are resistant to antiretroviral drugs. HIV disease is similar to other infectious diseases such as tuberculosis and malaria, in which the development of resistance is a frequent clinical problem.

The problem of drug resistance in HIV is more common among subjects receiving therapy and who are in care than among patients who have never received treatment. Primary resistance to HIV therapies is rising alarmingly among new and chronically infected individuals in the developed world, and in many areas of the USA more than 15% of new HIV infection diagnoses harbor some resistance. Unfortunately, this problem will also affect the developing world when antiretroviral therapy becomes more widely available. Primary resistance is more frequently seen with nucleoside reverse transcriptase inhibitors since these are the drugs that have been used for the longest period of time, but it is increasing among non-nucleoside reverse transcriptase inhibitors and protease inhibitors. The goal is to prevent this problem by the intelligent use of antiretroviral medications and resistance testing. However, despite these efforts, the use of antiretroviral therapy will always be associated with the development of resistance, and clinicians will frequently face patients for whom no therapeutic options are readily available.

THERAPEUTIC OPTIONS FOR THE SUBJECT WITH MULTIDRUG-RESISTANT HIV

Thanks to the development of new classes of drug such as the CCR5 inhibitors (maraviroc approved in 2007), integrase inhibitors (raltegravir, 2007) the development of new agents in existing classes such as the protease inhibitors tipranavir (2005) and darunavir (2006) and the new non-nucleoside reverse transcriptase inhibitor etravirine (2008), the goal of treatment when the clinician faces a patient with multidrug-resistant virus is to resuppress HIV RNA maximally to prevent the ongoing development of viral resistance. The approach is to select at least two new active drugs in the regimen, preferably from new classes of agents. In the setting of clinical trials up to 70–80% of the participants with extensive treatment experience may be able to reach resuppression of their HIV-RNA levels.

However it might be difficult to achieve these goals in some patients. Several considerations are important in this situation. First, virologic failure is not equivalent to immunologic and clinical failure. Deeks and colleagues have shown that the median period of time between virologic and immunologic failure is 36.4 months (3 years). The delay between virologic and immunologic failure seems to be associated with the decreased replicative fitness of viruses that harbor protease inhibitor and nucleoside-associated resistance mutations. Viruses with multiple mutations are less cytopathic *in vitro* and *in vivo* when compared to wild-type isolates. Second, discontinuing therapy in the patient with multidrug-resistant virus is associated with the overgrowth of wild-type virus after a period of approximately 12 weeks. This switch to wild type as the predominant quasispecies in the patient is associated with a significant decay in the $CD4^+$ T-cell count, rapid progression of HIV disease and, in some cases, serious opportunistic infections. Third, there is growing evidence that the rates of clinical progression are low in patients that continue antiretroviral therapy in the presence of virologic failure, and that these subjects maintain their CD4 cell numbers, especially if the viral load is maintained below the natural set-point of the patient (the HIV RNA load before the initiation of antiretroviral therapy).

Based on the above, most experienced clinicians would maintain a partially suppressive regimen in a patient with limited therapeutic options, especially if the disease is in an advanced stage. The objective of therapy in this situation shifts from complete suppression of viral replication to partial suppression to prevent immunologic and clinical decline, and to wait until two new active therapeutic agents become available to try to achieve the goal of full viral suppression again.

Many clinicians also maintain lamivudine in these patients because the presence of the M184V mutation (usually associated with the use of lamivudine) may enhance susceptibility to zidovudine or tenofovir, drugs frequently used individually as part of combination regimens in the patient with multidrug resistance. The presence of this mutation also stabilizes the reverse transcriptase of the virus and makes it less prone to make errors, which are associated with the development of resistance. This effect may be overcome by an accumulation of nucleoside-associated mutations (also known as NAMs): M41L, E44D, D67N, K70R, V118I, L210W, T215Y/F and K219Q/E.

The development of new classes of drug has made the use of multidrug combination regimens (six or more drugs), also called 'mega-HAART regimens', very unusual and probably unnecessary.

Although some authors suggested that transient discontinuation of antiretroviral treatment might 'resensitize' the virus and make it susceptible to previously used antiretroviral agents, this phenomenon is short lived because of 'archived' resistance of the integrated HIV in the T-cell memory reservoirs. This approach was tested in several

randomized trials and proved to be deleterious for the participants because of the high risk of significant clinical deterioration in the patient with advanced disease.

In the patient with multidrug-resistant HIV it is also critically important to maintain all prophylactic regimens that the subject should be taking, at the usually recommended $CD4^+$ T-cell count thresholds. Thus, it is important to maintain *Pneumocystis jirovecii*, *Toxoplasma gondii* and *Mycobacterium avium intracellulare* prophylaxis if indicated. It is also a good idea to increase the frequency of the clinical visits to once every month or two, so that opportunistic infections can be readily identified and treated.

NEW DRUG AVAILABILITY

Before the US Food and Drug Administration (FDA) grants full approval, new drugs become available as part of compassionate use and expanded access programs. The clinician frequently is pressed and tempted to add this 'single' new drug to the regimen that the patient is taking, hoping to obtain at least a partial virologic and clinical benefit in the patient with very limited options. However, with the development of new classes of drug it has become clearly apparent that the use of at least two new agents should be tried in patients with evidence of resistance. Unless the patient is clinically deteriorating rapidly, the soundest approach might be to wait until the development of two or more new active drugs. The decision to add a single drug to a failing regimen should be based on the clinical situation of the patient and the types of drug available.

For some classes of drug (e.g. non-nucleoside reverse transcriptase inhibitors and fusion inhibitors), resistance develops very quickly if these drugs are not used as part of fully suppressive regimens. With other classes of drug (e.g. protease inhibitors or nucleoside/nucleotide analogues), resistance takes longer to develop and durable virologic and potentially clinical benefits might be obtained. So for compounds where the threshold to develop resistance is low it might be reasonable to wait until more drugs became available and a combination regimen with a good chance of response can be developed. For drugs with a higher threshold for resistance immediate use might be advisable, especially in the patient who is clinically deteriorating. For example, enfuvirtide (also known as T-20) is an injectable peptide fusion inhibitor that binds to the HR-1 domain of gp41. Its use has been associated with significant decreases in viral load when used as monotherapy and to improve the viral load response of otherwise optimized rescue regimens. HIV becomes very quickly resistant to this drug if it is not used as part of a fully suppressive antiretroviral regimen. Resistance mutations in the gp41 envelope gene have been identified primarily at positions 36–45 of the first heptad repeat (HR-1) region. On the other hand, tenofovir, a nucleotide, was evaluated as an 'intensification' (the addition of a single drug to a failing antiretroviral regimen) in Gilead study 907. The virologic benefits (approximately half a log decrease in HIV viral load) were still present after 48 weeks of treatment.

If the patient is clinically failing antiretroviral therapy, or the addition of a single drug is being considered, it is important to maximize the benefits of the background regimen that the patient is taking. Phenotypic resistance testing might have an edge over genotypic or 'virtual phenotype' testing (a genotype test with an automatic interpretation linked to a large database of geno-phenotypes) in this very complicated situation. It might identify better which drugs maintain a better residual activity *in vitro* in a patient with limited therapeutic options. It also might provide a measurement of 'replicative capacity' of the patient's virus.

THE USE OF THE REPLICATION CAPACITY ASSAY

Replicative fitness is the ability of a species or strain of virus to compete against others in a defined environment – for example, a wild-type virus is more fit than a virus with multidrug resistance in an environment where there is no drug, but the reverse is true in the presence of antiretroviral therapy. To evaluate replicative fitness, it is necessary to conduct very cumbersome assays where the two different strains to be tested compete against each other. Monogram Inc. modified their phenotypic assay and developed a 'replication capacity assay' which is provided as part of the phenotypic assay. In this assay patient-derived HIV reverse transcriptase or protease undergoes a single round of replication. The vector contains a luciferase gene that permits quantitation of replication that is then compared to the level of replication of a wild-type HIV reference strain. The result is provided as the ratio of the patient strain replication to the wild-type (reference) strain replication. Normally multidrug-resistant viruses have replication capacity values smaller than 1. Theoretically the lower the number, the less fit the virus is.

As the availability of new therapeutic agents has increased over the last years, the interest of using replication capacity as a tool for guiding therapy in the patient with multidrug resistance has decreased. A few years ago some clinicians used this assay to guide therapy in the patient with multidrug resistance, selecting combination regimens that lower the replication capacity of the predominant quasispecies of the patient. However, clinical validation of the utility of this assay is still lacking, and this approach might lead to unnecessary changes of otherwise well-tolerated regimens. Assuring the viral load is maintained as low as possible, preferably below the natural set-point of the patient, might be another approach to handle the patient who has limited therapeutic options.

FURTHER READING


Further reading for this chapter can be found online at http://www.expertconsult.com

Chapter 101

Mary E Wilson

Geography of infectious diseases

INTRODUCTION

Infectious diseases vary by geographic region and population, and they change over time. Increasingly, humans are moving from one region to another, thereby becoming exposed to a variety of potential pathogens and also serving as part of the global dispersal process.[1] Microbes picked up at one time and in one place may manifest in disease far away in time and place. Because many microbes have the capacity of persisting in the human host for months, years or even decades, the relevant time frame for study of exposures becomes a lifetime. Furthermore, microbes also move and change and reach humans via multiple channels.

Caring for patients in today's world requires an understanding of the basic factors that underlie the geography of human diseases and events that cause shifts in the distribution and burden of specific diseases. Current technology contributes to massive population movements and rapid shifts in diseases and their distributions, but it also provides communication channels that can aid clinicians who care for patients with unfamiliar medical problems. This chapter reviews the factors that shape the global distribution of infectious diseases and the forces that are expected to shift distributions in the future. Several examples are used to illustrate the broad range of factors that affect the distribution and expression of infectious diseases.[2]

Many authors have traced the origins and spread of specific infectious diseases through human history. A century and a half ago, John Snow noted that epidemics of cholera followed major routes of commerce and appeared first at seaports when entering a new region. *Yersinia pestis*, the cause of plague, accompanied trade caravans and moved across oceans with rats on ships. Exploration of the New World by Europeans introduced a range of human pathogens that killed one-third or more of the local populations in some areas of the Americas. The plants and animals introduced as a result of this exploration have also had profound and long-lasting consequences for the ecology and economics of the new environment.[3] The speed, reach and volume of today's travel are unprecedented in human history and offer multiple potential routes to move biologic species around the globe. Pathogens of animals and plants are being transported as well, and this can affect global food security.[4] Establishment of arthopod vectors, such as mosquitoes that are competent to transmit human pathogens, in new geographic areas can expand the regions that are vulnerable to outbreaks of some vector-borne infections. This chapter focuses only on pathogens that directly affect human health and on their sources (Table 101.1). When thinking about geography of human infections, it is useful to consider both the origin of the organism and the conveyor or immediate source for the human (Fig. 101.1).

Table 101.1 Origins and conveyors of human pathogens*

Origin or carrier	Conveyor or immediate source	Examples of disease
Humans	Humans	HIV, syphilis, hepatitis B
Humans	Humans (air-borne pathogen)	Measles, tuberculosis
Soil	Soil, air-borne	Coccidioidomycosis
Soil	Food	Botulism
Animals	Water	Leptospirosis
Humans	Mosquitoes	Malaria, dengue
Humans	Soil	Hookworm, strongyloidiasis
Animals	Ticks	Lyme disease
Animals, humans	Sand flies	Leishmaniasis
Animals	Animals	Rabies
Rodents	Rodent excreta	Hantaviruses
Humans	Water, marine life	Cholera
Humans or animals (with snails as essential intermediate host)	Water	Schistosomiasis
Humans	Food, water	Typhoid fever
Animals	Water	Cryptosporidiosis, giardiasis

*Some pathogens have multiple potential sources.

This chapter addresses three key issues:

- factors influencing geographic distribution: why are some infectious diseases found only in focal geographic regions or in isolated populations?
- factors influencing the burden of disease: why does the impact from widely distributed infections vary markedly from one region or one population to another? and
- factors influencing emergence of disease: what allows or facilitates the introduction, persistence and spread of an infection in a new region and what makes a region or population resistant to the introduction of an infection?

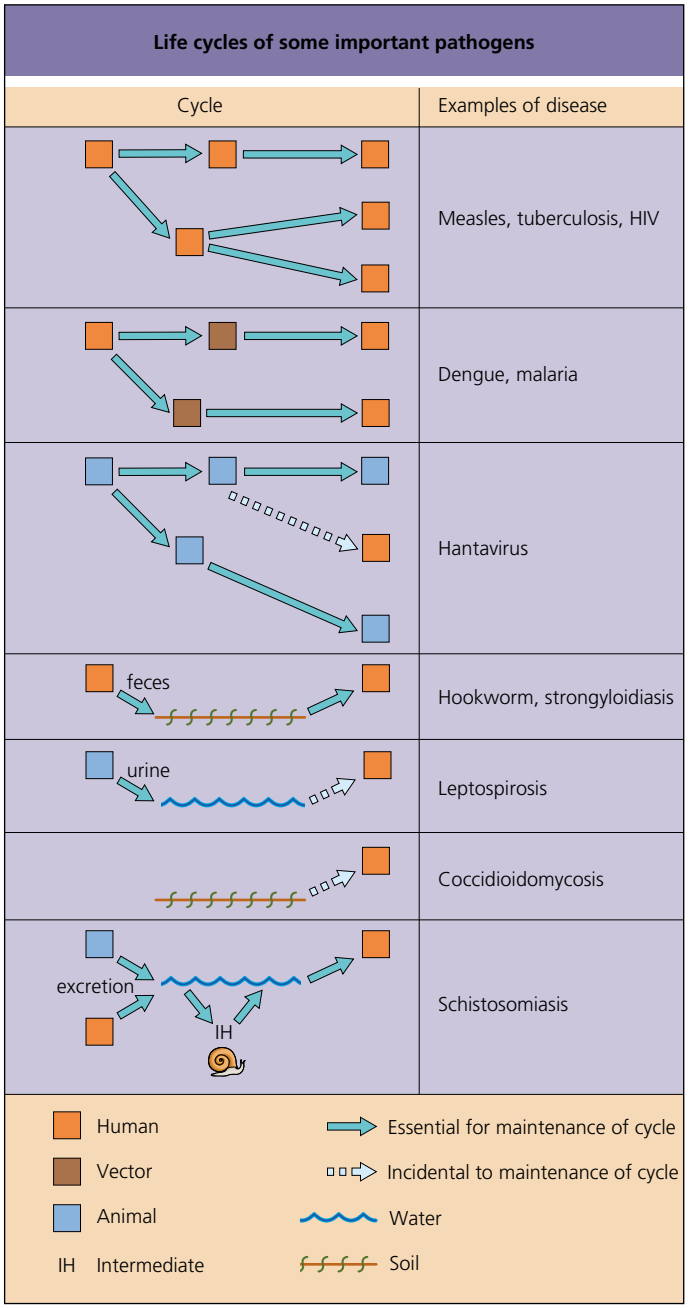


Fig. 101.1 Life cycles of some important pathogens.

FACTORS INFLUENCING GEOGRAPHIC DISTRIBUTION

In past centuries, lack of interaction with the outside world could allow an infection to remain geographically isolated. Today, most infections that are found only in focal areas have biologic or geoclimatic constraints that prevent them from being introduced into other geographic regions. For example, the fungus *Coccidioides immitis*, which causes coccidioidomycosis, thrives in surface soil in arid and semiarid areas with alkaline soil, hot summers and short, moist winters; it is endemic in parts of south-western USA, Mexico and Central and South America. People become infected when they inhale arthroconidia from soil. An unusual wind storm in 1977 lifted soil from the endemic region and deposited it in northern California, outside the usual endemic region.[5] In general, infection is associated with residence in or travel through the endemic region. However, because the fungus can persist in the human host for years, even decades, after initial infection (which may be mild and unrecognized), disease may be diagnosed far from the endemic regions. Although it is a 'place' disease, coccidioidomycosis has increased in the southwestern USA in recent years, in part attributable to a large influx of susceptible humans into the endemic zone and construction and other activities that disturb the soil. Outbreaks are also linked to climatic and environmental changes.[6]

Vectors

Many microbes require a specific arthropod vector for transmission or an animal reservoir host and hence inhabit circumscribed regions and may be unable to survive in other habitats. Malaria is a vector-borne infection that cannot become established in a region unless a competent vector is present. The presence of a competent vector is a necessary but not sufficient condition for human infection. The mosquito must have a source of malarial parasites (gametocytemic human or rarely other species), appropriate bioclimatic conditions and access to other humans. The ambient temperature influences the human biting rate of the mosquito, the incubation period for the parasite in the mosquito and the daily survival rate of the mosquito. Prevailing temperature and humidity must allow the mosquito to survive long enough for the malarial parasite to undergo maturation to reach an infective state for humans. Competent vectors exist in many areas without malaria transmission, because the other conditions are not met. These areas are at risk of the introduction of malaria, as illustrated by several recent examples in the USA and elsewhere.[7,8]

An estimated 77% of the world population lived in areas with malaria transmission in 1900. Today about 48% live in at-risk areas, but because of population growth and migration the total global population exposed to malaria today has increased by 2 billion since 1900.[9] Malaria was endemic in many parts of the USA into the 20th century (Fig. 101.2), with estimates of more than 600 000 cases in 1914. Even before extensive mosquito control programs were instituted, transmission declined. Demographic factors (population shifts from rural to urban areas), improved housing with screened doors and windows, and the availability of treatment were among the factors that contributed to this decrease.

The distribution of onchocerciasis in Africa is notable for its association with rivers.[10] The reason becomes clear by understanding that the vector of this filarial parasite, the black fly (genus *Simulium*), lays her eggs on vegetation and rocks of rapidly flowing rivers and usually

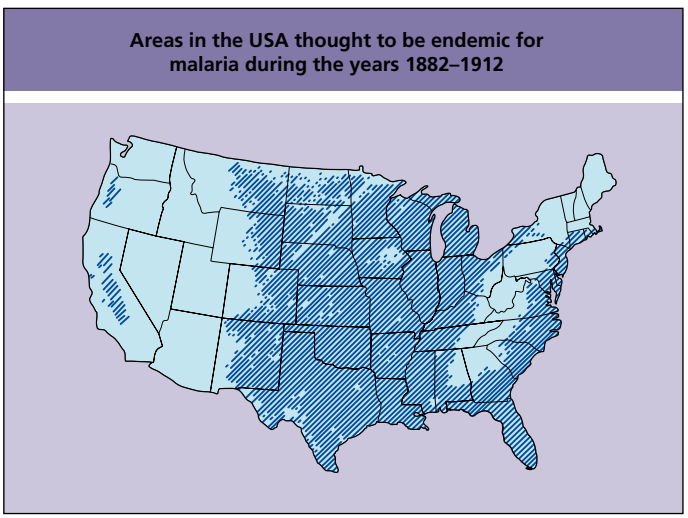


Fig. 101.2 Areas of the USA thought to be endemic for malaria during the years 1882–1912.

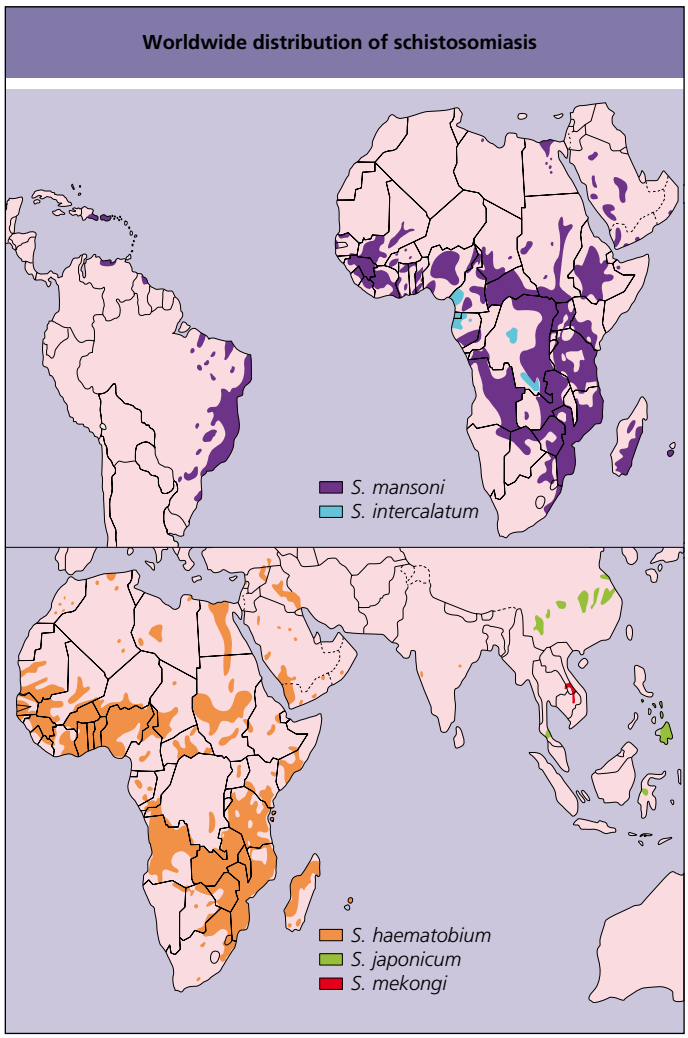


Fig. 101.3 Worldwide distribution of schistosomiasis.

inhabits a region within 5–10 km on either side of a river. Another name for onchocerciasis, river blindness, describes the epidemiology as well as one consequence of infection.

Some pathogens have a complex cycle of development that requires one or more intermediate hosts. Distribution may remain relatively fixed, even when infected humans travel widely, if other regions do not supply the right combination and geographic proximity of hosts (Fig. 101.3). Although persons with schistosomiasis visit many regions of the world, the parasite cannot be introduced into a new region unless an appropriate snail host is present, excreted eggs (in urine or feces) are released into water where they reach the snail hosts and humans subsequently have contact with the untreated water.[11] Local ecologic changes and climate change, however, can be associated with expansion of transmission in endemic areas or increased intensity of transmission, as has been projected as a possible consequence of warming temperatures in China.[12]

Many hantaviruses exist worldwide with distributions that are still being defined. Each hantavirus seems to have its specific rodent reservoir with which it has evolved. As with many zoonoses, humans are incidental to the survival of the virus in rodents, yet humans can develop severe and sometimes fatal disease if they enter an environment where they are exposed to the virus. Undoubtedly, other rodent-associated viruses and other pathogens (as well as pathogens associated with other animals or insects) with the capacity to infect humans will be identified as humans enter unexplored environments in the future.

Ebola and Marburg viruses are other viruses that have focal distributions but have caused dramatic human outbreaks with high mortality.

Table 101.2 Biologic attributes of organisms that influence their epidemiology

- Host range
- Duration of survival in host
- Route of exit from host
- Route of entry into human
- Inoculum need to establish infection
- Virulence
- Capacity to survive outside host
- Resistance to antimicrobials and chemicals

They also infect nonhuman primates and threaten the survival of great apes.[13] Recent studies suggest that bats may be the reservoir hosts.[14,15] Because these infections can be spread from person to person, secondary household and nosocomial spread in several instances has amplified what began as an isolated event. Lack of adequate resources in hospitals in many developing regions contributes to the spread of infections within hospitals and to persons receiving outpatient care, such as those receiving injections.

Cultural practices can lead to unusual infections in isolated areas. Residents of the highlands of Papua New Guinea developed kuru after ingestion (or percutaneous inoculation) of human tissue during the preparation of the tissues of dead relatives.

Thus, the presence of a pathogen in a region may reflect the biologic properties of the organism, its need for a certain physicochemical environment or its dependence on specific arthropods, plants or animals to provide the milieu where it can sustain its life cycle (Table 101.2). The presence of a pathogen in a region does not necessarily equate with human disease, because mechanisms must exist for the pathogen to reach a susceptible human host for human disease to occur. Sometimes it is only with exploration of new regions or changes in land use that humans place themselves in an environment where they come into contact with microbes that were previously unidentified or unrecognized as human pathogens. Preferences for specific foods, certain preparation techniques or cultural traditions may place one population at a unique risk for infection.

FACTORS INFLUENCING THE BURDEN OF DISEASE

Among the infectious diseases that impose the greatest burden of death globally, most are widely distributed: respiratory tract infections (e.g. influenza, *Streptococcus pneumoniae* and others), diarrheal infections, tuberculosis, measles, AIDS and hepatitis B.[16] Most of these infections are spread from person to person. The World Health Organization estimated that about 65% of infectious diseases deaths globally in 1995 were due to infections transmitted from person to person (Table 101.3).[16]

Table 101.3 Modes of transmission for major global infectious diseases

Mode of transmission	% of total*
Person-to-person	65
Food-borne, water-borne or soil-borne	22
Insect-borne	13
Animal-borne	0.3

*The figures are based on an estimated 17.3 million deaths due to infectious diseases in 1995, as reported by the World Health Organization.[16]

Burden from these diseases is unevenly distributed across populations and among different countries. Poor sanitation, lack of clean water, crowded living conditions and lack of vaccination contribute to the disproportionate burden from many of these infections in developing regions of the world. In industrialized countries, pockets of high risk persist. Disadvantaged populations have higher rates of tuberculosis, HIV and many other infectious and noninfectious diseases. Rates of reported cases of tuberculosis vary widely by region (Table 101.4).[17] Variation also exists within countries. Figure 101.4 shows the effect of crowded living conditions on rates of tuberculosis in England and Wales in 1992.[18] Among welfare applicants and recipients addicted to drugs or alcohol in New York City, the rate of tuberculosis was 744 per 100 000 person years or more than 70 times the overall rate for the USA.[19] The impact of an infection derives not only from the risk of exposure but also from the access to effective therapy. For example, treatment of a patient with active tuberculosis can cure the individual and eliminate a source of infection for others in the community.

Diphtheria, controlled in many parts of the world through the use of immunization, resurged in new independent states of the former Soviet Union in the 1990s, a reminder of the tenuous control over many infectious diseases. Populations in other countries also felt the impact as cases related to exposures in the Russian Federation were reported in Poland, Finland, Germany and the USA. Serologic studies in America and Europe suggest that up to 60% of adults may be susceptible to diphtheria.

Table 101.4 Rates of reported cases of tuberculosis worldwide by region, 1990 and 2006

Region	INCIDENCE PER 100 000 POPULATION	
	1990	2006
Africa	162	363
Americas	65	37
Eastern Mediterranean	111	105
Europe	37	49
South East Asia	200	180
Western Pacific	127	109

Data from World Health Organization.[17]

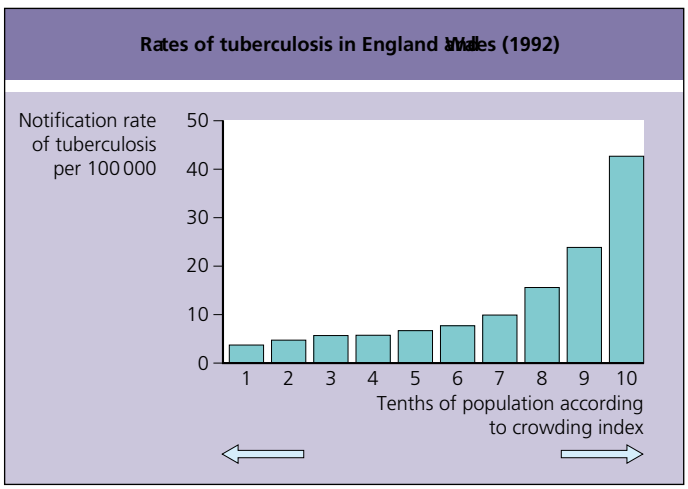


Fig. 101.4 Rates of tuberculosis in England and Wales by crowding index (1992). Adapted from Bhatti *et al.*[18]

Table 101.5 Factors that influence the types and abundance of microbes in a community

- Biogeoclimatic conditions
- Socioeconomic conditions
- Public health infrastructure
- Urban versus rural environment
- Density and mobility of population
- Season of the year
- Animal populations

Table 101.6 Factors that influence the probability of exposure to pathogens

- Living accommodation
- Level of sanitation
- Occupational and recreational activities
- Food preparation and preferences
- Sexual activities and other behavior
- Contact with pets, other animals, vectors
- Time spent in the area

Travelers to tropical and developing regions of the world can pick up geographically focal, often vector- or animal-associated infections (such as malaria and dengue),[20] but travelers most often acquire infections with a worldwide distribution that are especially common in areas lacking good sanitation.[21] Food- and water-borne infections are common and lead to travelers' diarrhea, which is caused by multiple agents (including *Escherichia coli*, *Salmonella*, *Shigella* and *Campylobacter* spp., and others), typhoid fever and hepatitis A. Respiratory tract infections may be acquired from other travelers from all over the globe during the crowding that occurs in travel (e.g. in buses, airplanes, terminals and on cruise ships) as well as from persons in the local environment. Tables 101.5 and 101.6 note factors that influence the types and abundance of microbes in a community and the probability of exposure to pathogens.

Hepatitis A virus remains a common cause of infection in developing regions of the world although it is not considered a major cause of morbidity or mortality in those regions where most persons are infected at a young age and become immune for life. The presence and severity of symptoms are related to the age at which a person becomes infected. Infection in young children is typically mild or inapparent. Persons living in areas of high transmission may be unaware of the presence of high levels of transmission, although nonimmune, older people (such as travelers) who enter the environment may develop severe, and occasionally fatal, infection. Some countries with an improving standard of living have noted a paradoxic increase in the incidence of disease from hepatitis A virus as the likelihood of exposure at a young age decreases, shifting upward the age of infection to a time when jaundice and other symptoms are more likely to occur.

Travelers may also contribute to the spread of infectious diseases and influence the global burden of these diseases.[22,23] *Neisseria meningitidis*, a global pathogen, occurs in seasonal epidemics in parts of Africa – the so-called meningitis belt (Fig. 101.5).[24] Irritation of the throat by the dry, dusty air probably contributes to invasion by colonizing bacteria. Pilgrims carried an epidemic strain of group A *N. meningitidis* from southern Asia to Mecca in 1987. Other pilgrims who became colonized with the epidemic strain introduced it into sub-Saharan Africa, where it caused a wave of epidemics in 1988 and 1989. Using molecular markers, investigators were able to trace the spread of the epidemic clone to several other countries.[25] In 1996 in Africa, major outbreaks of meningococcal meningitis occurred

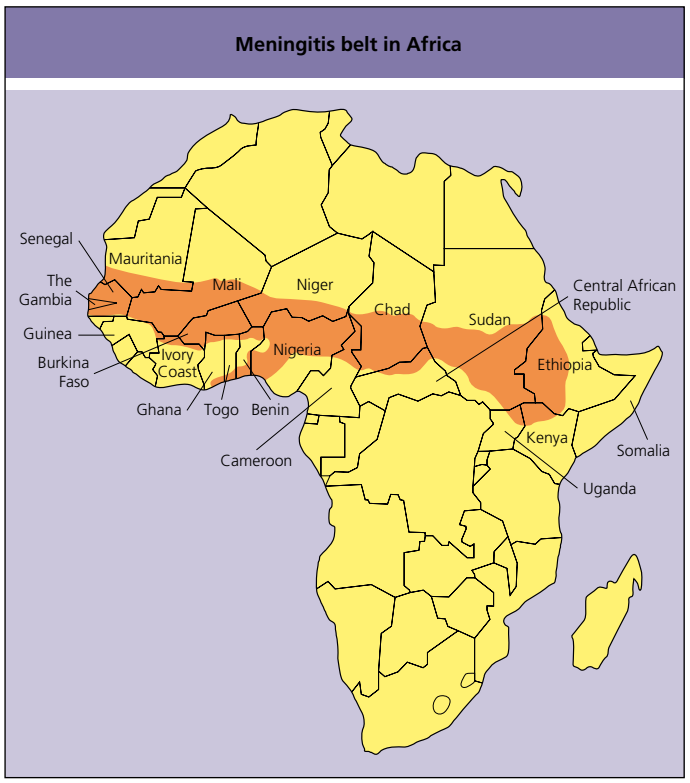


Fig. 101.5 Meningitis belt in Africa.

(>185 000 reported cases with a case fatality rate of ~10%) caused by *N. meningitidis* serogroup A, clone III-1.[26] A virulent group C, ET-15 strain of *N. meningitidis* spread in Canada and was associated with an increased case fatality rate and a higher proportion of cases in persons over the age of 5 years.[27] In these examples, the virulence of the microbe and travel and trade acted synergistically to change the epidemiology and burden of disease. In the spring of 2000 serogroup W135 *N. meningitidis* caused an outbreak of infection in pilgrims to the Hajj and subsequently spread to their contacts and others around the world. Studies using serotyping, multilocus sequence typing, multilocus DNA fingerprints and other techniques found identical W135 isolates in multiple countries. Pilgrims are required to receive a meningococcal vaccine but before this outbreak, pilgrims from many countries received a vaccine that protected against serotype A but not W135. The vaccine reduces risk of disease but does not prevent oropharyngeal carriage of *N. meningitidis*.[28]

FACTORS INFLUENCING EMERGENCE OF DISEASE

Regular, rapid movement of persons from tropical regions to major urban areas throughout the world raises concerns that unusual infections could be introduced into an environment where they could spread to large populations. In order to assess the potential for a pathogen to be introduced into a new population, information is required about the biologic properties of the organism, the region and population being considered and the mechanisms of transmission (see Table 101.1). A key factor that determines whether a pathogen can persist and spread in a new population is its basic reproductive rate, which is the number of secondary infections produced in a susceptible population by a typical infectious individual. To become established in a new host population, a pathogen must have a basic reproductive rate that exceeds one. The concept is simple but invasion and persistence are affected by a range of biologic, social and environmental factors. Also critical in determining how easily an infection can be controlled is the proportion of transmission that occurs before onset of symptoms or during asymptomatic infection.[29]

Table 101.7 Factors that restrict the introduction and spread of infections

- Geoclimatic factors that cannot support vector or intermediate host
- Genetics of human population, making it genetically resistant or relatively resistant
- Immunity of human population, making it not susceptible because of past infection with same or related microbe or via vaccination
- Demographic factors (e.g. size and density of population will not support sustained transmission of diseases such as measles)
- Social and behavioral factors (e.g. absence of activities such as iv drug use and unprotected sex with multiple partners)
- Food preparation habits and local traditions (e.g. certain dishes not eaten, food always well cooked)
- High-quality housing, sanitation, public health infrastructure, good surveillance
- High standard of living, good nutrition, lack of crowding, access to good medical care
- Biologic characteristics of the microbe

Certain factors restrict the introduction and spread or persistence of infection in a region (Table 101.7); many of these are discussed above. Nutrition is also important in determining susceptibility to and severity of many infections. A substantial proportion of disease burden in developing countries can be attributed to childhood and maternal underweight and micronutrient deficiences.[30] Before measles vaccine was introduced, the epidemiology of measles exhibited marked periodicity in large populations, with peaks typically occurring every 2–3 years.[31] In general, a community size of about 250 000 is necessary to provide a sufficient number of susceptible people to sustain the virus. In small island communities (or other isolated populations), outbreaks typically occur only after periodic introductions from outside. Size and density of a population thus influence the epidemiology of some infections. It has been suggested that measles as it has been known in the 20th century could not have established itself much before 3000 BC because before that time human populations had not achieved sufficient size to sustain the virus. Measles could not have persisted in nomadic, hunting communities.

Examples of emerging pathogens

It is instructive to look at examples of infections that have recently undergone major shifts in distribution and to review the key factors that have influenced their geographic spread. They are a reminder of the complexity of the interactions among host, microbe and environment. A recurring theme is the movement of humans who introduce pathogens into a new region (see also Chapter 4) and human alteration of the landscape or ecology that permits contact with previously unrecognized microbes, often through interaction with animals or animal products. Many infections in humans, in the past and in recent years, have domestic or wild animals as their sources.[32]

Human immunodeficiency virus and other pathogens carried by humans

Organisms that survive primarily or entirely in the human host and are spread from person to person (e.g. by sexual or other close contact or by droplet nuclei) can be carried to any part of the world. The spread of HIV in the past three decades to all parts of the world is a reminder of the rapid and broad reach of travel networks. Although the infection has also spread via blood and shared needles, it has been the human host engaging in sex and reproduction who has been the origin for the majority of the infections worldwide. Person-to-person

spread accounted for the rapid worldwide distribution of severe acute respiratory syndrome (SARS), a coronavirus infection, in the spring of 2003, after the virus emerged from an animal reservoir, most likely bats.[33]

Drugs or vaccines injected by reused inadequately sterilized needles and syringes have been and continue to be an important means of spread of blood-borne infections, such as hepatitis C, hepatitis B and HIV, in some parts of the world.[34]

Multidrug-resistant (MDR) tuberculosis has continued to increase, with World Health Organization estimates of 500 000 new cases of MDR annually (now about 5% of all new TB cases each year).[35] Extensively drug-resistant tuberculosis, which is virtually untreatable, had been reported in 45 countries by 2007. It is not only the pathogens carried by humans that are relevant. Humans also carry resistance and virulence factors that can be transferred to and exchanged with other microbes.[36]

Dengue fever

Dengue fever is a mosquito-borne viral infection that has now spread to most tropical and subtropical regions of the world and continues to increase in incidence and severity. Viremic humans regularly enter regions infested with *Aedes aegypti*, the principal vector of dengue, transporting the virus for new outbreaks. Infection can spread rapidly and outbreaks are sometimes massive, involving >30% of the population. Because four serotypes of dengue virus exist and infection with one serotype does not confer lasting immunity against other serotypes, a person can be infected more than once. The risk of developing severe dengue (e.g. dengue shock syndrome or dengue hemorrhagic fever, DSS or DHF) after repeat infection is 82–103 times greater than after primary infection.[37] In an outbreak in Cuba, 98.5% of cases of DSS or DHF were in persons with a prior dengue infection. The rate of DSS or DHF was 4.2% in persons with prior dengue infection who became infected with a new serotype.[38]

Geographic regions where multiple serotypes are circulating have continued to expand, setting the stage for more severe consequences of infection. Factors that have aided the spread of dengue include increasing (and rapid) travel to and from tropical regions; expansion of the regions infested with *Aedes aegypti*; increasing urbanization, especially in tropical areas, which has provided large, dense populations; the use of nonbiodegradable and other containers that make ideal breeding sites for the mosquito; and lack of support for vector control programs.

Most of the world population growth globally is in urban/peri-urban areas in tropical and developing regions. The expectation is that more urban areas in tropical regions will reach the critical population size, perhaps somewhere between 150 000 and 1 million people, to permit sustained transmission of dengue, and to increase the risk of the severe forms of infection, dengue hemorrhagic fever and dengue shock syndrome.

In 2001 the vector that was implicated in an outbreak of dengue in Hawaii[39] was *Aedes albopictus*, a mosquito species that has been newly introduced into many new regions in recent decades, probably primarily by shipping.[40] On phylogenic analysis the virus responsible for the Hawaii outbreak was similar to dengue, isolates from Tahiti, suggesting that viremic travelers introduced the virus from the South Pacific.

It is instructive to ask not only where dengue occurs but also where it does not. Although large dengue epidemics occurred in the USA in the 20th century, only a handful of cases have been acquired in the USA in recent years, despite the presence of epidemic disease in adjacent areas of Mexico and the presence of a competent vector (*Aedes aegypti*) in south-eastern USA (Fig. 101.6).[24] It is possible that the presence of screened dwellings and air conditioning may make an area relatively resistant to the introduction of infection, even if a competent vector infests a region, though serologic studies have also documented that unrecognized dengue infections are occurring in Texas.[41]

Chikungunya virus

Chikungunya, a mosquito-borne alphavirus originally isolated in Tanzania in 1953, has spread from Africa, causing massive outbreaks in the Indian Ocean islands and India since 2005. Although it has typically been considered an infection of tropical regions, in the summer of 2007 an outbreak caused hundreds of cases (175 laboratory confirmed) in north-eastern Italy. The index case was a visitor from India. Of note, the vector implicated was *Aedes albopictus*, which had been first documented in Italy in 1990 and was postulated to have been introduced via used tires.[42] Mutations in the virus may have enabled it to replicate more efficiently in the mosquito vector. Chikungunya virus can be transmitted by *Aedes aegypti* and *Aedes albopictus* mosquitoes, which are now both widely distributed, so many regions of the world are vulnerable to introduction of this virus by viremic travelers.

Cholera

Cholera illustrates the complex interactions between microbe, environment and host.[43] Epidemics are seasonal in endemic regions. *Vibrio cholerae* lives in close association with marine life, binding to chitin in crustacean shells and colonizing surfaces of algae, phytoplankton, zooplankton and water plants. *V. cholerae* can persist within the aquatic environment for months or years, often in a viable but dormant state, nonculturable by usual techniques. Environmental factors, including temperature, salinity, pH and sea-water nutrients, affect the persistence, abundance and viability of the organisms, and hence have a striking influence on human epidemics.

Under conditions of population crowding, poor sanitation and lack of clean water, cholera can have a devastating impact, as was shown by the massive outbreak of El Tor cholera in Rwandan refugees in Goma, Zaire, which caused 12 000 deaths in July 1994.[44]

The organism can be carried by humans, who sometimes have few or no symptoms, and introduced into new regions. Trade probably also plays a critical role. Ballast water, picked up by boats in multiple locations and discharged at another time and place, carries a wide range of species, including many that have no direct impact on human health.[45,46] In studies of the ballast and bilge of cargo ships in the USA Gulf of Mexico, researchers were able to identify *V. cholerae* identical to the strains causing epidemic disease in Latin America.[47]

Food-borne disease

The globalization of the food market means pathogens from one region can appear in another; some are common pathogens with a worldwide distribution but others are not. An outbreak of cholera in Maryland, USA, was traced to imported, contaminated commercial frozen coconut milk.[48] Alfalfa sprouts grown from contaminated seed sent to a Dutch shipper caused outbreaks of infections with *Salmonella* spp. on two continents, in at least Arizona and Michigan in the USA and in Finland.[49] Commercial movement of fruits and vegetables redistributes resistance factors along with the microbes. Tracing the source after an infection has been diagnosed can be convoluted and often is not carried out unless disease is severe, lethal or epidemic or involves a highly visible person or population.

Travel and trade are key features in the epidemiology of the infection *Cyclospora*, a cause of gastroenteritis. Recognized for many years in



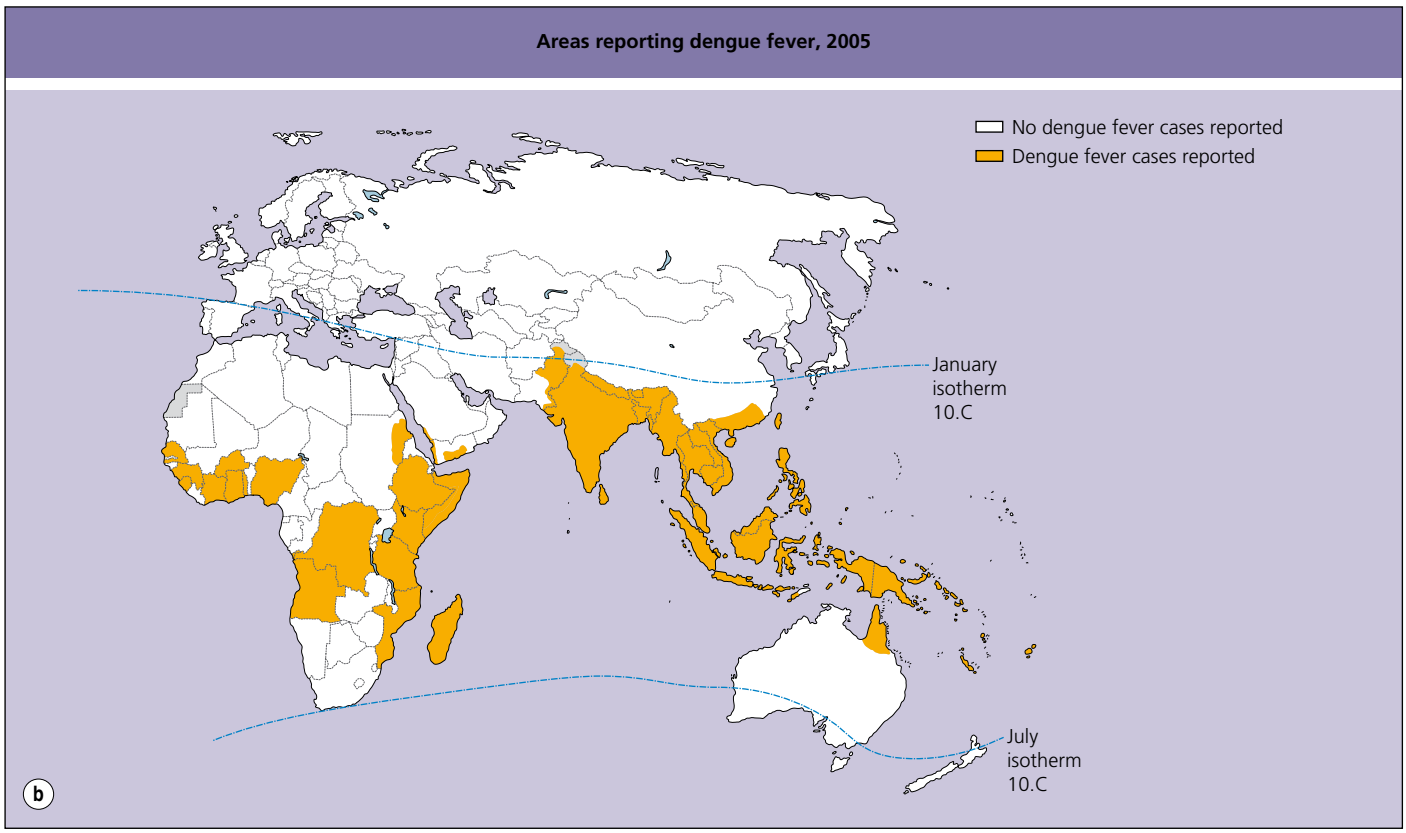


Fig. 101.6 (a, b) Areas reporting dengue fever, 2005. Many areas with a competent vector do not report dengue epidemic activity. Copyright World Health Organization 2008.



Fig. 101.7 (a, b) Worldwide distribution of malaria (updated CDC map). Data from Centers for Disease Control and Prevention.[24]

multiple regions of the world, cases were often associated with living in or travel to areas where sanitary facilities were poor. In the summer of 1996, a large US outbreak occurred in persons who had not traveled. Over a period of a few months, 1465 cases of cyclosporiasis were reported from 20 states. The outbreak was linked to eating raspberries imported from Guatemala.[50] During some seasons of the year up to 70% of selected fruits and vegetables sold in the USA come from developing countries.

Visceral leishmaniasis

In the past, visceral leishmaniasis in Brazil was primarily a rural disease. Recently, however, several cities have reported large outbreaks of visceral leishmaniasis.[51] Reasons for the change in epidemiology include geoclimatic and economic factors (drought, lack of farm land, famine), leading to migration of large numbers of persons, who settle in periurban areas where they live in densely crowded shanties, lacking basic sanitation. The presence of domestic animals, such as dogs, chickens and horses, in and adjacent to human dwellings provides ample sources of blood meals for the sand fly, the vector of leishmaniasis. Outbreaks have occurred in many cities in Brazil, including Teresina, São Luis and Natal. Children and young people have been most affected. Malnutrition can also contribute to the severity of the disease.

Disease–disease interactions can also alter the epidemiology of infections. Visceral leishmaniasis has become an important infection in HIV-infected people in Spain and other areas where the two infections coexist.[52] The presence of HIV leads to increased risk of progression of infection; late appearance of disease can occur years to decades after exposure in an endemic region, leading to the appearance of cases of leishmaniasis in regions distant from endemic areas. A common consequence is missed or delayed diagnosis.

Movement of vectors and other species

Movement today involves all forms of life and the movement of nonhuman species can affect infections in humans. Importation of wild animals from Ghana into the United States led to an outbreak of monkeypox, an infection previously known to exist in Africa. Humans became infected by handling prairie dogs (sold as pets) that had been housed with the imported wild animals from Africa.[53] *Aedes albopictus* introduced into the USA via used tires shipped from Asia[54] has since become established in at least 21 contiguous states of the USA and in Hawaii. *Aedes albopictus* can transmit dengue and chikungunya viruses, as described above, and is a competent laboratory vector of La Crosse, yellow fever and other viruses. It is also hardier than many other mosquito species and therefore may spread widely and be extremely difficult to eradicate. Multiple strains of eastern equine encephalitis virus have been isolated from *Aedes albopictus* in Florida.

An example from the past illustrates the potential consequences of the introduction of a mosquito vector into a new region. In March 1930, an entomologist in Natal, Brazil, came upon *Anopheles gambiae* larvae in a small, wet, grassy field between a railway and a river.[55] He was surprised, because the usual habitat for this mosquito was Africa. Investigation revealed that the probable route of entry into South America was via boats that made mail runs between Dakar in Senegal

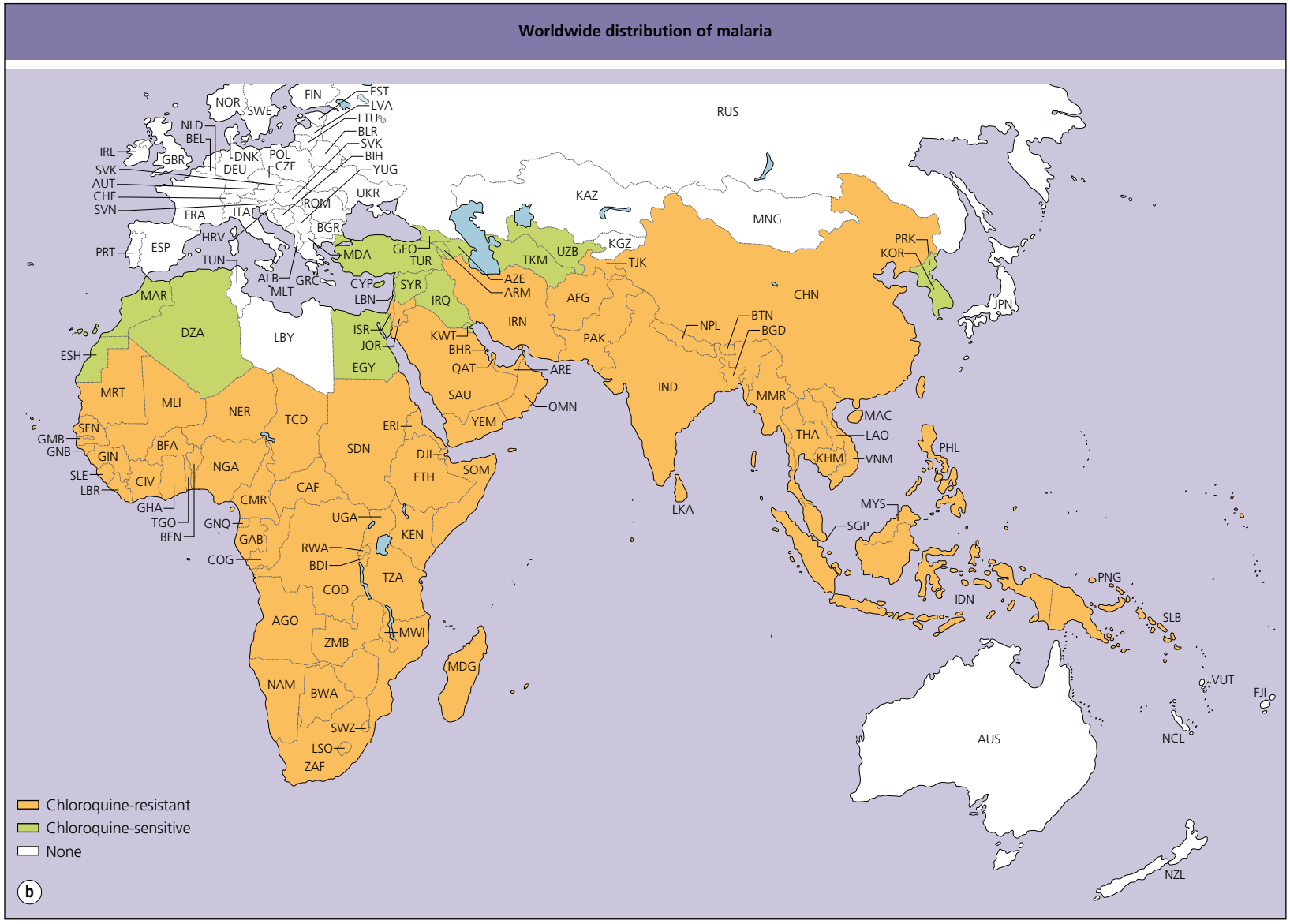


Fig. 101.7—cont'd.

and Natal in Brazil, covering the 3300 km in less than 100 hours. In Dakar the boats were anchored a distance from the shore within easy flight range of *A. gambiae*. In Brazil, over the ensuing years, the mosquito spread along the coastal region and inland. Natal, as an ocean port, terminus of two railway lines and the hub of truck, car and river transportation, was well suited for dissemination of *A. gambiae* into the region. Although malaria already existed in the region, the local mosquitoes were not efficient vectors. *Anopheles gambiae*, in contrast, lived in close proximity to humans, entered houses, sought human blood and was an efficient biter. In 1938 and 1939, devastating outbreaks of malaria killed more than 20 000 persons. In this instance, the simple introduction of a new vector into a region led to severe problems. Fortunately, an intensive (and expensive) eradication campaign was effective.

Current transportation systems regularly carry all forms of life, including potential vectors, along with people and cargo. In an experiment carried out several years ago, mosquitoes, house flies and beetles in special cages were placed in wheel bays of 747 aircraft and carried on flights lasting up to 7 hours. Temperatures were as low as –62°F (–52°C) outside and ranged from 46°F to 77°F (8–25°C) in the wheel bays. Survival rates were greater than 99% for the beetles, 84% for the mosquitoes and 93% for the flies.[56] Occasional cases of so-called airport malaria – cases of malaria near airports in temperate regions – attest to the occasional transport and survival of a commuter mosquito long enough to take at least one blood meal in the new environment.

In the USA, transportation of raccoons in the late 1970s from Florida to the area between Virginia and West Virginia (in order to stock hunting clubs) unintentionally introduced a rabies virus variant into the animals of the region. From there, the rabies enzootic spread for hundreds of miles, reaching raccoons in suburban and densely populated regions of the north-east USA. Spill-over of the rabies virus variant into cats, dogs and other animal populations and direct raccoon–human interactions have had extremely costly and unpleasant consequences.[57]

Today highly pathogenic avian influenza A (H5N1) is a global concern.[58] It is entrenched in poultry populations in Asia and Africa and has caused outbreaks in Europe and the Middle East. Although the virus causes high mortality in infected humans, thus far H5N1 has not been able to establish sustained transmission from person to person. Most humans appear to have been infected via close contact with poultry or their products. Although the virus can be carried by migratory birds,[59] most introductions appear to have been related to movement of poultry and poultry products. A model designed to map risk in South East Asia found risk was associated with duck abundance, human population and rice cropping intensity.[60]

GEOGRAPHIC INFLUENCES ON DIFFERENTIAL DIAGNOSIS

Geographic exposures influence how one thinks about probable diagnoses in a given patient. In Mexico, for example, more than 50% of patients with late-onset seizures have CT evidence of the parasitic infection, neurocysticercosis.[61] In Peru, 29% of persons born outside Lima who had onset of seizures after 20 years of age had serologic evidence of cysticercosis.[62] In northern Thailand, melioidosis is a common cause of sepsis, accounting for 40% of all deaths from community-acquired sepsis.[63]

In considering the consequences of exposures in other geographic regions, relevant data in assessing the probability of various infections include the duration of visit, activities and living conditions during the stay and the time lapsed since the visit. Among British travelers to West Africa, the relative risk of malaria was 80.3 times higher for persons staying for 6–12 months than among those staying 1 week.[64] In Malawi, the risk of schistosome infection increased directly with duration of stay. Seroprevalence was 11% for those present for 1 year or less, but this increased to 48% among those present for 4 years or longer.[65] In a study of persons with cysticercosis, the average time between acquisition of infection and onset of symptoms was about 7 years.[66]

For malaria, it is necessary to know not only whether infection can be acquired in a specific location but also the specie of parasite present and the patterns of resistance to antimalarial agents. As chloroquine resistance has spread, maps now typically highlight the few remaining areas of chloroquine sensitivity. Because the resistance to antimalarial agents is a dynamic process, with levels of resistance generally increasing over time (involving *Plasmodium vivax* in some areas as well as *P. falciparum*), it is essential to base decisions about chemoprophylaxis and treatment on up-to-date information. Figure 101.7 shows the distribution of malaria and resistance patterns globally as of 2005. Recent analysis of data from a network that uses travelers as a sentinel population found marked differences in the spectrum of disease in relation to the place of exposure.[20]

Expression of disease may vary depending on age of first exposure, immunologic status of the host, genetic factors and the number and timing of subsequent exposures. Temporary residents of endemic regions have different patterns of response to a number of helminths from those of long-term residents. In cases of loiasis, temporary residents have immunologic hyperresponsiveness, high-grade eosinophilia and severe symptoms that are not seen in long-term residents of the same area.[67] Genetic factors can affect susceptibility to infection or expression of disease. Some persons, for example, are genetically resistant to infection with parvovirus because they lack appropriate receptors on their erythrocytes.[68] Persons lacking Duffy factor cannot be infected with the malarial parasite, *P. vivax*.

CONCLUSION

Knowledge about the geographic distribution of diseases is essential for informed evaluation and care of patients, who increasingly have had exposures in multiple geographic regions. Recent travel and trade patterns have led to more frequent contact with populations (human and nonhuman species) from low latitude areas, regions with greater species richness.[69] Infectious diseases are dynamic and will continue to change in distribution. Changes in virulence and shifts in resistance patterns will also require ongoing surveillance and communication to health-care providers. Multiple factors favor even more rapid change, perhaps in unexpected ways, in the future: rapidity and volume of travel, increasing urbanization (especially in developing regions), the globalization of trade, multiple technologic changes that favor mass processing and broad dispersal, and the backdrop of ongoing microbial adaptation and change, which may be hastened by alterations in the physicochemical environment.

REFERENCES

References for this chapter can be found online at http://www.expertconsult.com

Chapter 102

David R Hill

Pretravel advice and immunization

Pretravel care of international travelers is entirely preventive medicine.[1] It should be the goal of all travel medicine practitioners to maintain the health of the more than 800 million persons who cross international borders each year (World Tourism Organization, www.unwto.org). The first step in providing this care is to make a risk assessment of the traveler's journey: determining the itinerary and duration of travel, the accommodation and planned activities, and the health status of the traveler. A research expedition to the shores of Kenya's Lake Victoria will expose the traveler to more health risks than a short business trip to Nairobi. During longer trips, and especially expatriate residence, there will be a cumulative risk of disease as well as the possibility that a traveler will become lax in preventive measures such as malaria chemoprophylaxis. In order to determine destination-specific risks, the global epidemiology of infectious disease health risks can be found in several authoritative publications such as those published by the US Centers for Disease Control and Prevention (CDC)[2] and the World Health Organization (WHO)[3] and from Internet sites (see Sources of Information) (Fig. 102.1).[4]

The next step in risk assessment is to review the traveler's health. Although at least 25% of individuals travel with chronic medical conditions, these generally do not interfere with travel plans. Nevertheless, consideration of specific activities, vaccines or prophylactic medications will need to be balanced against their health.

Once a risk assessment has been made, the health professional should provide the traveler with the tools to manage this risk. These usually include vaccines, self-treatment and prophylactic medications, and preventive advice. Education about illness avoidance may be the most cost-effective measure, but it is difficult to ensure compliance.[5] Each traveler should also be informed how to access medical care during travel and on return as many travelers will need to seek medical care during and after their trip.[6,7]

With constantly changing epidemiology of disease and patterns of microbial resistance, complexity of travel itineraries, and newly released vaccines and preventive medications, most travelers should be cared for in specialized travel clinics that have trained personnel, carry all vaccines and provide accurate preventive advice.[1,8]

IMMUNIZATIONS

It is helpful to divide immunizations into three categories:

- recommended as part of routine health maintenance irrespective of international travel;
- required for entry into a country under International Health Regulations (2005) or by an individual country; and
- recommended because of risk during travel.

Immunizations are often the reason that an individual comes for pretravel care, although the risk of most vaccine-preventable illness is less than one case per 1000 journeys. Adequate records should be kept: the type and dose of vaccine, date of administration, manufacturer and lot number, site of administration and administrator's signature. Adverse reactions to vaccines should be reported to the appropriate national monitoring agency (e.g. in the US to the Vaccine Adverse

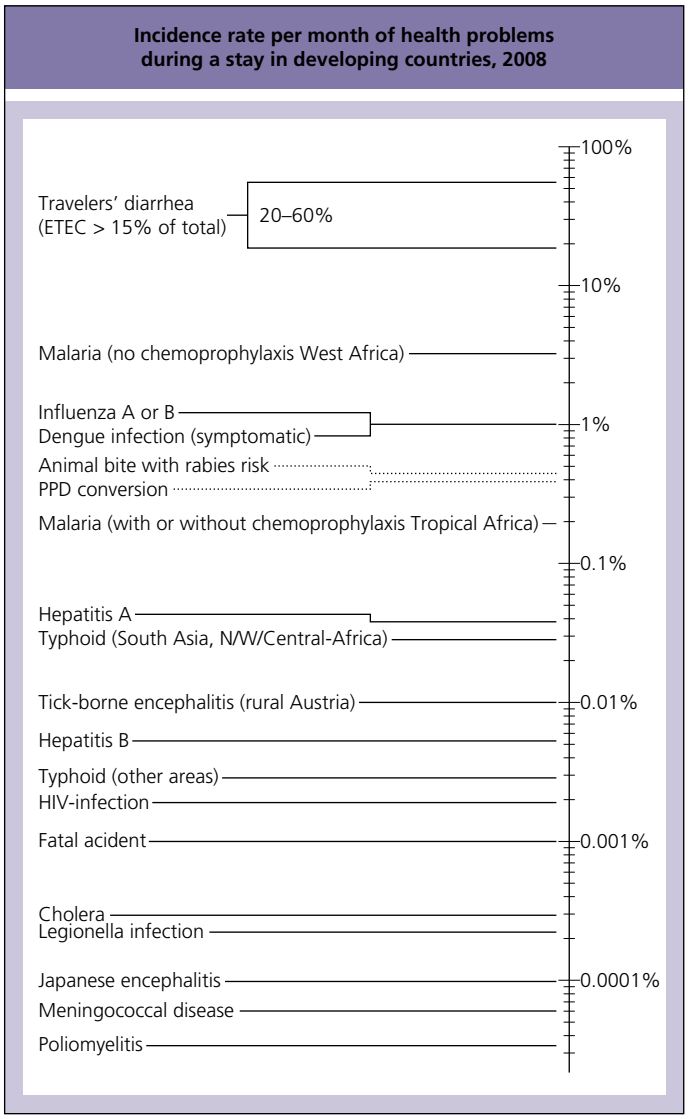


Fig. 102.1 Incidence rate per month of health problems during a stay in developing countries, 2008. From Steffen *et al*.[4]

Event Reporting System, http://vaers.hhs.gov/, and in the UK to the Medicine and Healthcare products Regulatory Agency, www.mhra.gov.uk/index.htm). Prior to administration of any vaccine patients should undergo informed consent. The use of vaccine information sheets will help to explain to travelers the benefits and risks of each vaccine. These are often available from vaccine manufacturers and in the US can be downloaded from the CDC: www.cdc.gov/vaccines/pubs/vis/default.htm.

Most vaccines may be administered simultaneously at different sites. Patient tolerance, therefore, usually dictates how many may be given at any one time. A few rules apply. Live viral vaccines should be either given simultaneously or separated by at least 1 month. Immune serum globulin (now only rarely used for prevention of hepatitis A infection) should not be given less than 3 months before or less than 2 weeks after measles, mumps and rubella or varicella vaccines.[9] Other specific considerations are discussed later. Vaccines used in travel medicine, their administration schedule and adverse events are listed in Table 102.1. Full manufacturer's prescribing information should always be consulted before administration of a vaccine, as schedules, doses and products will often differ between countries.

Immunizations for routine health maintenance

The pretravel visit is an ideal time to update routinely recommended immunizations according to age standards. In most areas of the world this includes tetanus, diphtheria, pertussis, measles, mumps and rubella, *Haemophilus influenzae* type b, polio and pneumococcal vaccines for infants, and influenza and pneumococcal vaccines for older adults. However, standards will differ between countries: the USA includes influenza, varicella, rotavirus and hepatitis A and B vaccines for infants and quadrivalent meningococcal conjugate and human papilloma virus (HPV) vaccines for adolescents, whereas the UK provides meningococcal C conjugate vaccine and bacille Calmette–Guérin (BCG) (for high risk) for infants and has recently introduced HPV for adolescents.

Issues of inadequate coverage and waning immunity also impact the choice of vaccines. Measles is no longer endemic to the USA and nearly all cases are imported or linked to imported infections. Although important gains have been made in global control of measles, particularly in Africa, ongoing measles outbreaks in both low- and high-income regions threaten efforts to control the disease.[10,11] Waning immunity against pertussis has led to an increase in cases in older individuals and the introduction of adult formulations of pertussis vaccine combined with tetanus and diphtheria (Tdap).[12] Where available, this vaccine should be used to boost travelers in need of any one of the three antigens.

Children should receive vaccines that are age recommended; however, the schedule may be advanced if a child is traveling before they would have received a scheduled vaccine and the risk of the vaccine-preventable illness during their trip is sufficient (Table 102.2).[9] In the case of measles, a single dose may be given from 6 to 11 months for travel to high-risk destinations, with the routine schedule still administered beginning at 12–15 months.

Older adults (those ≥65 years) make up an increasing proportion (as high as 15%) of international travelers. In addition, there are many travelers with chronic medical conditions such as HIV/AIDS, diabetes or chronic pulmonary, renal, hepatic or cardiac disease. These travelers should be vaccinated with influenza and pneumococcal vaccines. The recognition that influenza is one of the most common vaccine-preventable illnesses during travel has led to the consideration of administering vaccine to healthy travelers going to areas of seasonal influenza (year round in tropical regions, December to March in the northern hemisphere and April to September in the southern hemisphere).[13] Outbreaks can occur out of season when persons from diverse regions of the world congregate in close quarters, such as on cruise ships or during mass gatherings, e.g. the Hajj. The practicalities of obtaining vaccine out of season or vaccine that is matched to influenza strains at the destination may be difficult. Seasonal influenza vaccine is not expected to provide protection against highly pathogenic avian influenza A (H5N1). The role of antivirals used as self-treatment or prophylaxis has not been thoroughly evaluated.[14]

Required immunizations

Vaccination may be required under International Health Regulations (IHR 2005)[15] or by an individual country as a condition of entry. The only vaccine that currently may be required under IHR (2005) is yellow fever (YF) vaccine. There is a risk of YF transmission in countries throughout the Amazon basin of South America, and in sub-Saharan Africa between 15° north and 10° south of the equator. YF is re-emerging and expanding into new regions, as has been seen in South America in 2008 with expansion of disease into new regions of Brazil, Argentina and Paraguay (Pan American Health Organization, www.paho.org/English/AD/DPC/CD/yf-fa.htm). Although YF disease risk maps are published by both the CDC and the WHO, determining the actual risk of a traveler's journey can be difficult.[16] In general, travelers to rural regions in areas with a risk of YF transmission (either an endemic area where there are the appropriate mosquito vectors and nonhuman primate hosts for transmission, or infected areas where there are cases of YF reported) should receive vaccine. Other countries that are not at risk of YF transmission may require vaccination of travelers arriving from YF-risk countries. Country-specific requirements can be found in the CDC[2] and WHO[17] publications, and on their respective websites (see Sources of Information). Health professionals must therefore decide if the vaccine is required for entry and/or recommended because of risk. YF vaccination must be recorded in the revised (as of June 2007) International Certificate of Vaccination or Prophylaxis (see Sources of Information).[18] In addition to vaccine, travelers should protect themselves against the daytime-biting *Aedes* spp. mosquito (Table 102.3).

In 2001 newly recognized severe adverse events following administration of YF vaccine to first-time vaccine recipients were reported. There have been two patterns, viscerotropic and neurologic. Viscerotropic reactions occur within 2 weeks following vaccination (median of 4 days) and are characterized by dissemination of vaccine virus with multiorgan failure; about 50% of cases are fatal. In these cases there are high levels of vaccine virus, prolonged viremia and an unregulated inflammatory response.[19,20] The neurologic adverse events are characterized by a meningoencephalitis that begins up to 4 weeks following vaccination (median 14 days); nearly all cases recover.[21] Acute disseminated encephalomyelitis and Guillain–Barré syndrome also occur with neurologic events. The risk factors do not appear to be related to mutation of vaccine virus, but rather to an alteration in the host response. Older age, particularly 60 years and older, and the absence of a thymus (a contraindication to vaccination) carry an increased risk for both viscerotropic and neurologic reactions.

Cholera vaccine is no longer required under IHR (2005) for international travel, and smallpox vaccine has not been required since 1982 following the global eradication of smallpox in 1977. Meningococcal vaccine is required for religious pilgrims to Saudi Arabia (see following section).

Immunizations recommended because of risk

Vaccines discussed in this section are recommended because there may be a risk of exposure during travel. There are four vaccine-preventable diseases transmitted because of poor food and water hygiene: hepatitis A, typhoid, cholera and polio.

The risk of hepatitis A during travel has declined in many countries, particularly Latin America and East and South East Asia, with transition from high to lower endemicity.[22,23] Nevertheless, vaccination can be considered for most travelers as hepatitis A vaccines are well-tolerated, highly effective, give long-term (perhaps lifelong) protection and are being incorporated into routine immunization schedules.[24]

Hepatitis A vaccines are protective even if given shortly prior to departure. Immune serum globulin that provides immediate protection, with antibodies that circulate for 2–6 months, is not usually used for protection. If available, testing for previous exposure to hepatitis A can be performed in travelers with a high likelihood of previous hepatitis A infection, i.e. those born and raised in countries with high endemicity for hepatitis A and those with a history of jaundice.

Protection against both hepatitis A and B may be achieved with the use of a combined antigen vaccine, Twinrix® (GlaxoSmithKline), in a three-dose schedule. Two doses of vaccine should be given before departure to ensure protection against hepatitis A since a lower concentration of antigen is used in this preparation compared with the single antigen hepatitis A vaccines. The course may be accelerated over 3 weeks.

Hepatitis E is enterically transmitted, particularly during periods of high rainfall. In travelers, most cases have originated from India, although mild and subclinical infection may be more common than appreciated. Pregnant women have a high mortality. Immunoglobulin and the current hepatitis vaccines do not prevent hepatitis E so food and water hygiene is the best prevention (see Chapter 154).

Typhoid and paratyphoid are imported diseases in most high-income countries. The highest risk is in travelers to South Asia (India, Pakistan and Bangladesh); however, cases also originate from Latin America and Africa.[25,26] Multidrug-resistant *Salmonella typhi* is common. There are two vaccines with similar efficacy (60–70%): an oral live-attenuated (Ty21a) vaccine and a polysaccharide (Vi antigen) injectable vaccine.[27] The Ty21a vaccine is well-tolerated, effective in children older than 4 years and provides protection for 5 years; the Vi antigen vaccine is given in a single intramuscular dose and is effective for 2–3 years. Conjugate vaccines in development should provide higher levels of protection, particularly in children under the age of 2 years. Typhoid vaccines do not reliably protect against *S. paratyphi*, which may be more commonly imported than *S. typhi*.[26]

Cholera is endemic throughout Asia and Africa and parts of the Middle East; however, it is rarely a risk for travelers. *Vibrio cholerae* 01 is endemic in all regions, whereas *V. cholerae* 0139 has circulated in Asia. More than 90% of cholera cases are reported from Africa, although some countries in Asia, such as Bangladesh which has endemic cholera, do not report cases to the WHO.[28] There is one cholera vaccine now available: an oral vaccine that combines killed *V. cholerae* with the binding (B) subunit of cholera toxin (Dukoral®, Crucell). This vaccine is well tolerated and in efficacy trials provided 60–85% protection against cholera depending upon the age of the recipient and the time interval measured.[29] Because of the very low risk of cholera in travelers, vaccine should be reserved for those who will work in refugee settings or who will travel in cholera-endemic regions and will be remote from medical care. There has been interest in the use of this vaccine to protect against the syndrome of traveler's diarrhea. While there is cross protection against *Escherichia coli* expressing the heat labile enterotoxin (LT), the protection is modest and the vaccine should not generally be used for this indication.[29]

Although global efforts at polio eradication have been successful in several WHO regions of the world (the Americas, Western Pacific and European), the disease remains endemic in India, Pakistan, Afghanistan and Nigeria, with regional spread to other countries in Africa and Asia. In addition to the endemic countries, in mid-2008 the following countries reported cases or circulation of imported poliovirus: Angola, Benin, Central African Republic, Chad, Democratic Republic of the Congo, Ethiopia, Niger and Sudan in Africa, and Burma and Nepal in Asia (World Health Organization: http://www.polioeradication.org/). Travelers to risk areas should have completed a primary series against poliomyelitis and have a booster according to their national guidelines. Saudi Arabia requires polio vaccination in travelers age 15 years and younger coming from countries reporting wild-type polio.

Other immunizations recommended because of potential exposure during travel include those against hepatitis B, Japanese B encephalitis, *Neisseria meningitidis*, rabies, tick-borne encephalitis and tuberculosis.

Many countries now administer hepatitis B vaccine as part of routine childhood immunization. Unimmunized travelers who will be exposed to blood or body fluids (e.g. health-care workers, expatriate families and children, those receiving medical or dental care and those with unprotected sexual exposure) should receive vaccine. Protection against both hepatitis A and B is available in a combination vaccine (see section on hepatitis A). The vaccination schedule for hepatitis B (Engerix® only, GlaxoSmithKline) may be accelerated to 0, 1 and 2 months with a booster at 6–12 months, and it can be further accelerated by giving doses at 0, 7 and 21 days; 65% will seroconvert at 28 days.[30]

Japanese B encephalitis is a viral encephalitis in Asia transmitted by the *Culex* spp. mosquito. The complete listing of risk areas and seasons of transmission may be found in *Health Information for International Travel*[2] or on the CDC website (wwwn.cdc.gov/travel). Prolonged residence in endemic areas or engaging in high-risk activities such as camping, bicycling or field work are indications for vaccine. Rural Asia, particularly where rice and pigs are farmed, is the highest risk area; pigs act as a reservoir for the virus and the rice fields as a breeding ground for the *Culex* mosquito vector. The most widely used vaccine, JE-VAX®, manufactured by Biken, is a mouse brain-derived vaccine whose production has been discontinued. To replace this vaccine there are two products in development: a vero cell, inactivated vaccine (Ixiaro®, Intercell) that was released in 2009, and a live-attenuated chimeric vaccine (Acambis) that uses yellow fever virus as the backbone.[31,32] JE-VAX has been associated with rare (approximately 0.1–5 episodes per 1000 doses) hypersensitivity reactions, including anaphylaxis, urticaria, angioedema and respiratory distress, that can occur at intervals ranging from minutes to as long as 1 week or more after vaccination.[33] The traveler should avoid traveling within 10 days of completing the series in case a reaction occurs during flight or on arrival in the country of destination. Patients who have a history of allergies or urticaria may be at a slightly higher risk of severe reaction, so they should be vaccinated only after careful consideration.

Meningococcal vaccine is recommended for travelers to areas highly endemic for meningococcal disease such as the meningitis belt of sub-Saharan Africa (particularly during the months from December through June). Following the global spread of meningococcal W135 disease that was traced to Hajj pilgrims in 2000, Saudi Arabia requires vaccination with a quadrivalent vaccine containing serogroups A, C, Y and W135 for religious pilgrims arriving for the Hajj or Umrah.[34] In the USA there is a conjugated quadrivalent vaccine that has been incorporated into routine immunization of adolescents. Some European countries and Canada use a conjugated group C meningococcal vaccine for protection of infants.

All travelers should be counseled about avoiding exposure to rabies virus. In most low-income regions of the world, rabies is transmitted to humans by the bite of a dog (see Chapter 160), although other mammals (e.g. bats, cats, foxes) can also transmit the virus. In North America, bat-transmitted rabies is most common. Rabies-risk countries can be determined by consulting the CDC or WHO websites or reviewing other online resources such as the Country Information Pages of the National Travel Health Network and Centre (http://www.nathnac.org/ds/map_world.aspx). Pre-exposure protection against rabies should be considered for those whose risk is increased by type of activity (e.g. running, cycling), occupation (e.g. veterinarians) and longer duration of stay. Children may be at increased risk as they are less likely to avoid contact with animals and to report a bite or lick. The intramuscular route of administration is preferred to ensure adequate development of immunity.

In order to decrease risk of transmission, all bites should be thoroughly washed with soap and water; postexposure rabies treatment should then be obtained. A traveler who has received pre-exposure vaccine will not need rabies immune globulin (RIG, either human or equine) which is difficult to obtain or unavailable in many areas of the world. If vaccine has not been received before travel, both vaccine and RIG will need to be given. Regimens may differ, but if postexposure treatment is administered properly the traveler should be protected.[35] Travelers who had an overseas rabies exposure and treatment should be evaluated upon return. They can have serology checked and post-exposure treatment initiated while awaiting serologic evidence of protection (see Chapter 160).

Table 102.1 Immunizations for foreign travel*

Vaccine	Type	Route	Schedule	Indications	Precautions and contraindications†	Side-effects
Toxoids						
Tetanus–diphtheria	Adsorbed toxoid	im	Primary: 3 doses; first 2 doses, 4–8 weeks apart; 3rd dose 6–12 months later Booster: every 10 years. A single dose may be given at age 50 years to those who have completed full pediatric schedule	All adults	GBS <6 weeks after previous dose of tetanus-containing vaccine Severe allergic reaction to previous dose or vaccine component	Local reactions Occasional fever, systemic symptoms Arthus-like reactions if history of multiple boosters Rare: systemic allergy
Tdap‡	Combination toxoids of diphtheria and tetanus with acellular pertussis	im	Primary: not used Booster: single dose for adolescents and adults who have completed childhood DTP or DTaP course	For adolescents and adults aged 11–64 years who need boosting for any of the three antigens	Severe allergic reaction to previous dose or vaccine component Encephalopathy within 7 days of receipt of vaccine with pertussis component GBS <6 weeks after previous dose of tetanus-containing vaccine History of Arthus reaction after tetanus- or diphtheria toxoid-containing vaccine administered <10 years previously	Local reactions: erythema, swelling, and pain Occasional fever, headache, fatigue and gastrointestinal symptoms
Inactivated bacterial vaccines						
Cholera (*Vibrio cholerae*)	Killed, whole cell *V. cholerae* O1 of several serotypes and biotypes, combined with recombinant B subunit of cholera toxin	oral	Primary: 2 doses at 1 to 6 week interval Booster: 2 years	Humanitarian or relief workers; those who will be remote from medical care in areas of cholera outbreaks Modest protection against heat labile toxin-producing *E. coli* No longer required under International Health Regulations No protection against *V. cholerae* 0139	Hypersensitivity to previous dose	Mild gastrointestinal side-effects
Streptococcus pneumoniae	Polysaccharide containing 23 serotypes	sc or im	Primary: 1 dose Booster: 1 dose recommended for high-risk patients after 5 years	≥2 years old and at increased risk of pneumococcal disease and its complications Healthy adults 65 years or older	Severe allergic reaction to previous dose	Mild erythema and pain at injection site in about 50% Systemic reaction in <1% Arthus-like reaction with booster doses

Neisseria meningitis	Polysaccharide containing serotypes (A, C, Y, W135) (conjugate serotype C vaccine available in some countries)	sc	Primary: 1 dose Booster: 5 years in adults and children ≥5 years; 2–3 years in children vaccinated at 2–4 years.	Travel to areas with epidemic meningococcal disease Religious pilgrims to Saudi Arabia Asplenia or certain complement deficiency states	Severe allergic reaction to previous dose	Infrequent, mild local reactions
Neisseria meningitis	Conjugated polysaccharide containing serotypes (A, C, Y, W135)	sc	Primary: 1 dose Booster: not officially recommended	Travel to areas with epidemic meningococcal disease Religious pilgrims to Saudi Arabia Asplenia or certain complement deficiency states Children aged 11–12 years	Severe allergic reaction to previous dose	Local reactions in 10–60% Systemic reactions of fever, headache and malaise
Typhoid	Vi polysaccharide	im	Primary: 1 dose Booster: every 2 years	Risk of exposure to typhoid fever	Safety in pregnancy not known Severe allergic reaction to previous dose	Local pain and induration in 7% Headache in 16% Fever in <1%
Attenuated live bacterial vaccine						
Typhoid	Attenuated Ty21a strain of *Salmonella typhi*	oral	Primary: 1 capsule every other day for 4 doses Booster: every 5 years	Risk of exposure to typhoid fever	Safety in pregnancy not known Immunosuppression[§] Children <6 years Acute febrile or gastrointestinal illness Antibiotics or mefloquine (separate the doses by ≥24h) Capsules must be refrigerated	Infrequent gastrointestinal upset, rash
Attenuated live virus vaccines						
Influenza (Flumist)	Attenuated live virus: trivalent	intranasal	Annual vaccination with current vaccine Age 2–8 years (no previous influenza dose), 2 doses 4–6 weeks apart Age 9–49 years one dose per season	Routinely recommended in US for persons 2–49 years Can consider for travelers at risk	Severe allergic reaction to previous dose including eggs Immunosuppression[§] Persons <2 years or ≥50 years Pregnancy Children age 5–17 years taking aspirin History of GBS, asthma, chronic pulmonary, cardiac, metabolic or renal disease	Mild upper respiratory symptoms: rhinitis, nasal stuffiness, congestion

(Continued)

Table 102.1 Immunizations for foreign travel*—cont'd

Vaccine	Type	Route	Schedule	Indications	Precautions and contraindications†	Side-effects
Measles	Attenuated live virus: monovalent form or combined with rubella (MR) ± mumps (MMR)	sc	Primary: 2 doses, 1st at 12–15 months, 2nd at 4–12 years of age For adults, 2 doses separated by ≥1 month Booster: none	People born after 1956 (in US) who have not had documented measles or received 2 doses of live vaccine	Severe allergic reaction to previous dose or to gelatin or neomycin Pregnancy Immunosuppression§ (can be considered for asymptomatic HIV-infected persons) Recent (<3 months) administration of immune globulin for hepatitis A prevention	Temperature ≥39.4°C, 5–21 days after vaccination in 5–15% Transient rash in 5% Local reaction if previously immunized with killed vaccine (1963–67): 4–55%
Mumps	Attenuated live virus	sc	Primary: 2 doses (usually given as part of MMR vaccine) Booster: none	People born after 1956 (in US) who have not had documented mumps	Severe allergic reaction to previous dose or to gelatin or neomycin Pregnancy Immunosuppression§ Recent (<3 months) administration of immune globulin for hepatitis A prevention	Mild allergic reactions uncommon Rare: parotitis
Rotavirus	Attenuated live virus (2 products, RotaTeq®, Merck, and Rotarix®, GlaxoSmithKline)	oral	RotaTeq: 3 doses, 1st dose at 2 months, 2nd at 4 months and 3rd at 6 months; complete series by 32 weeks of age Rotarix: 2 doses, 1st dose at 6 weeks, 2nd at least 4 weeks later; complete series by 24 weeks of age	Must complete the series by 24–32 weeks of age depending upon vaccine product	Severe allergic reaction to previous dose Immunosuppression§ Previous history of intussusception Delay vaccination with moderate to severe gastroenteritis	Gastrointestinal upset
Rubella	Attenuated live virus	sc	Primary: 1 dose (usually given as part of MR or MMR) Booster: none	All, particularly women of child-bearing age, without documented illness or receipt of live vaccine at ≥12 months of age	Severe allergic reaction to previous dose or to gelatin or neomycin Pregnancy Immunosuppression§ Recent (<3 months) administration of immune globulin for hepatitis A prevention	Postpubertal women: up to 25% have joint pains, transient arthritis, beginning 3–25 days after vaccination, persisting 1–11 days Arthritis in <2%
Varicella	Attenuated live virus	sc	Primary: 2 doses; 1st at 12–15 months, 2nd at 4–6 years; unvaccinated older children and adults, 2 doses at 1–2 month interval	≥12 months old and no history of varicella	Severe allergic reaction to previous dose Pregnancy Immunosuppression§ Rare transmission of vaccine virus to susceptible hosts Recent (<3 months) administration of immune globulin for hepatitis A prevention	Local pain and induration in 20% Fever in 15% Localized or systemic mild varicella rash in 6%

Yellow fever	Attenuated live virus	sc	Primary: 1 dose, 10 days to 10 years before travel Booster: every 10 years	As required by individual countries or recommended because of risk	Severe allergic reaction to previous dose or to eggs Immunosuppression (can be considered in HIV infection with specialist advice)[§] Thymus disorders Avoid in pregnant women, unless high-risk travel Infants <9 months Caution in persons ≥60 years old	Mild headache, myalgia, fever, 5–10 days after vaccination in 25% Rare: immediate hypersensitivity, viscerotropic and neurologic reactions (see text)
Zoster	Attenuated live virus	sc	Primary: 1 dose ≥60 years Booster: none	Severe allergic reaction to gelatin or neomycin immunosuppression[§] Previous vaccination with varicella vaccine	Local reactions: erythema, pain, swelling Rare varicella-like rash	
Inactivated virus vaccines						
Hepatitis A	Inactivated virus; several products available (combination with hepatitis B, see below. Some countries have combination with Vi antigen typhoid vaccine)	im	Primary: 2 doses, 2nd dose after 6–24 months probably provides lifelong protection Booster: not recommended	Travel to low-income countries Routine childhood immunization in US Some travelers may benefit from prevaccine hepatitis A testing (see text)	Severe allergic reaction to previous dose Safety in pregnancy is not known	Local reaction of pain and tenderness in <20% Occasional fever in <5%
Hepatitis B	Recombinant HBsAg; several products available (also combination with hepatitis A, see below)	im	Primary: 3 doses at 0, 1 and 6 months. Can accelerate schedule (see text) Adolescents age 11–15 years can receive 2 doses at 6-month interval Booster: not routinely recommended	Health-care workers Residence or sexually active in areas of endemicity for HBsAg Contact with blood, body fluids or blood-contaminated medical or dental instruments	Severe allergic reaction to previous dose	Mild local reactions in 3–30% Occasional fever and headache
Hepatitis A and B antigens combined	Inactivated virus (A) plus recombinant HBsAg	im	Primary: 3 doses at 0, 1 and 6 months Can accelerate schedule (see text) Booster: not currently recommended	Travelers ≥18 years at risk for both hepatitis A and B Give at least 2 doses of vaccine before departure to protect against hepatitis A	Severe allergic reaction to previous dose Safety in pregnancy is not known	Local reactions in ~35% Systemic symptoms of headache and fatigue
Poliomyelitis	Inactivated virus, trivalent	sc	Primary: 3 doses, 1st two at a 4–8 week interval; 3rd 6–12 months after 2nd Booster: 1 lifetime dose	Travel to polio-endemic countries	Severe allergic reaction to previous dose Safety in pregnancy not known	Mild local reaction
Human papillomavirus	Inactivated virus (two products available)	im	Primary: 3 doses at 0, 2 and 6 months. Booster: none	Routine administration to females age 11–12 years. Also given to females age 13–18 years if not previously vaccinated	Severe allergic reaction to previous dose or yeast Safety in pregnancy not known	Local reactions Occasional systemic reactions or fever

(Continued)

Table 102.1 Immunizations for foreign travel*—cont'd

Vaccine	Type	Route	Schedule	Indications	Precautions and contraindications†	Side-effects
Influenza	Inactivated virus	im	Annual vaccination with current vaccine	Children age 6–59 months Healthy adults >50 years old Persons with chronic cardiovascular, pulmonary and metabolic conditions Health-care workers Can consider for travelers at risk	Severe allergic reaction to previous dose or eggs GBS following a previous dose of influenza vaccine	Mild local reactions in 10–60% Occasional systemic reaction of fever, malaise and myalgia
Japanese B encephalitis	Inactivated virus (tissue culture-derived virus; mouse brain purified vaccine no longer manufactured)	sc	Primary: 2 doses at days 0 and 28 Booster: not currently determined	Travelers ≥18 years to areas of risk with rural exposure or prolonged residence (see text)	Severe allergic reaction to previous dose or vaccine component Safety in pregnancy is not known	Local mild reactions Headache and myalgia common
Rabies	Inactivated virus	im or id	Pre-exposure: 3 doses at days 0, 7, and 21 or 28 Booster: not routinely recommended for travel. Other groups: depends upon risk category and is based upon serologic testing at specified intervals	Itinerary and activities that place travelers at risk of rabies	Severe allergic reaction to previous dose May be given in pregnancy if indicated id route should be completed ≥30 days before travel id route should not be used with concurrent chloroquine or mefloquine id dosing no longer available in USA	Local reactions in ~30% Mild systemic reactions: headache, nausea, aches, dizziness in ~20% Immune-complex reactions with booster doses of human diploid cell vaccine occurring 2–21 days after vaccination in 6%
Tick-borne encephalitis	Inactivated virus	im	Primary: 3 doses at 0, 1–3 months and 5–12 months (can be accelerated) Booster: 3 years	Hiking, camping in areas of risk; practice tick avoidance measures	Severe allergic reaction to previous dose or eggs Safety in pregnancy not known Hypersensitivity to previous doses	Occasional local reactions of swelling, redness or swollen regional lymph nodes Infrequent fever, headache Rare: neurologic adverse events
Passive prophylaxis						
Immune serum globulin	Fractionated Ig (primarily IgG)	im	Travel <3 months: 0.02 ml/kg Travel >3 months: 0.06 ml/kg every 4–6 months	For prevention of hepatitis A Some travelers may benefit from pretravel hepatitis antibody testing	Not to be given less than 2 weeks after, or 3 months before, measles, mumps and rubella, or varicella vaccines	Transient local discomfort Rare: systemic reaction

*Manufacturer's full prescribing information should be consulted. Dosing and vaccines are generally for adults and based on US guidelines; pediatric doses and vaccines may vary.
†Moderate or severe acute illness with or without fever is a vaccine precaution.
‡In the UK, Td is combined with IPV for adolescent and adult boosting: TdIPV.
§Persons immunocompromised because of congenital immunodeficiency diseases, leukemia, lymphoma, generalized malignancy, and untreated HIV infection or AIDS, or persons immunosuppressed as a result of therapy with corticosteroids, alkylating agents, antimetabolites, or radiation. Persons with a history of thymectomy, thymus diseases or myasthenia gravis should not receive yellow fever vaccine.
DTP, diphtheria, tetanus, pertussis; DTaP, diphtheria, tetanus, acellular pertussis; GBS, Guillain–Barré syndrome; HBsAg, hepatitis B surface antigen; id, intradermal, im, intramuscular; IPV, inactivated polio vaccine; sc, subcutaneous.

Table 102.2 Accelerated immunization of children under 2 years of age*

Vaccine	No. of doses after which protection may be achieved	Earliest age at which dose may be given	Interval (weeks)
Diphtheria-tetanus-pertussis	3	6 weeks	≥4
Measles	1	6 months	
Polio (inactivated)	3	6 weeks	≥4
Haemophilus influenzae type b	2	6 weeks	≥4
Hepatitis A	1	6 months	
Hepatitis B	3	Newborn	≥4 for doses 1 and 2; ≥8 for doses 2 and 3

*The data shown are for children who will travel to developing areas and require protection faster than by the routine schedule. Protection conferred by these schedules may not be complete. Children vaccinated with measles at under 12 months of age should be revaccinated at 12–15 months. In polio-endemic countries, the first oral polio dose may be given in the newborn period but three additional doses should be given, the first at 6 weeks of age and then at 4-week intervals.

Tick-borne encephalitis is a viral meningoencephalitis spread by *Ixodes* spp. ticks throughout forested areas of Eastern and Central Europe, and Siberia in the spring and summer months. Unpasteurized dairy products in endemic areas may also transmit the virus.[36] There are two inactivated vaccines (Encepur®, Chiron, and TicoVac®, Baxter AG), but they have limited availability outside of continental Europe and require three doses over a year to achieve full protection, which is not practical for most travelers. Accelerated vaccine courses exist. Travelers to risk areas should exercise precautions against ticks by the use of protective clothing, repellents and insecticides. These measures will help to prevent Lyme disease, which is also transmitted by the bite of *Ixodes* ticks.

The risk of acquiring tuberculosis during travel is greatest in travelers to countries of high endemicity (e.g. incidence of >40 cases/100 000 population), those who will stay for a long period (>1–3 months) and those who will have close contact with potentially infectious persons (e.g. health-care workers, and those visiting and staying with friends and relatives). The risk of infection has ranged from about 1 case/1000 persons/month in Peace Corps volunteers to 8 cases/1000 persons/month in health-care workers.[37,38] Many children reared outside the USA have received the BCG vaccine. The UK has set guidelines for travelers: children under the age of 16 and health-care workers to high endemic regions who have not previously received BCG.[39] Travelers who are not vaccinated should be offered pre- and post-travel tuberculin (purified protein derivative) skin testing or interferon gamma testing[40] to check for conversion and, therefore, infection. The post-travel skin test should be administered 1 month or more after return.

Table 102.3 Mosquito avoidance*

Repellents	DEET-containing products at 20–50% give 6–12 hours protection Picaridin (20%) also effective for approximately 6 hours Lemon eucalyptus oil is effective, but requires frequent application Apply to exposed areas of skin
Protective clothing	Wear loose-fitting, long sleeves and trousers Clothing may be treated with insecticide sprays or solutions
Screens and netting	Sleeping quarters can be screened Netting should be treated with insecticides
Pyrethroid insecticide sprays and coils	May be sprayed or burned in enclosed areas

*For avoidance of *Anopheles* (malaria) and *Culex* (Japanese encephalitis) mosquitoes, precautions should be exercised from dusk to dawn. *Aedes* spp. mosquitoes (dengue and yellow fever) are active in the daytime hours.

Immunization in special groups

Two important groups of travelers require special consideration before immunization – pregnant women and immunocompromised hosts, particularly those who have HIV or AIDS. For pregnant women, inactivated vaccines can usually be given if the risk is determined to be sufficient (Table 102.4).[2,9] Measles, mumps and rubella, and the varicella vaccine should not be given, although data have not clearly demonstrated adverse outcomes when women have received rubella vaccine. Yellow fever vaccine should be avoided unless there is high-risk travel; seroconversion to YF virus during pregnancy may be lower, necessitating revaccination after delivery.[41]

HIV-infected patients are another group to consider separately (Table 102.5).[2,42] All travelers should be asked about HIV risk factors before vaccination; the safety, immunogenicity and efficacy of the vaccine being considered then need to be balanced against the risk of the disease. Vaccine immunogenicity decreases with advanced disease from HIV; a CD4 lymphocyte count of <200–400 cells/ml or <15–25% by age-specific percentages correlates with decreased immunogenicity. Although it has not been clearly studied, this may also be a cut-off point for an increased risk of adverse consequences of live viral vaccines. If assurance of immunity is needed, then postvaccination serology should be obtained.

In addition to vaccination, HIV-infected travelers should consider the health risks associated with travel to developing regions. Many enteric infections, such as *Salmonella*, *Cyclospora* and *Cryptosporidium*, and systemic infections such as leishmaniasis and tuberculosis, are more prevalent and can be prolonged and difficult to treat in HIV-infected individuals. There may also be a decreased ability to access sophisticated medical care.

TRAVELERS' DIARRHEA

Travelers' diarrhea (TD) is the most common illness during visits to low-income regions of the world and affects 30–60% of travelers.[43] Illness usually begins in the first week after arrival and is typically

Table 102.4 Immunization of the pregnant traveler

Vaccine		Safely given to pregnant women at risk?	Notes
Bacterial	Tetanus–diphtheria	Yes	Tdap can also be given, but preference for vaccination in second and third trimesters
	Pneumococcal polysaccharide	Yes	
	Meningococcal		
	Polysaccharide	Yes	
	Conjugate	Yes	Data on safety are not available but may be given with high-risk
	Typhoid		
	Live-attenuated	Not known	Vaccination should generally be avoided
	Vi polysaccharide	Yes	Vaccine of choice in pregnancy
	Cholera, oral killed	Not known	May be given with high-risk exposure
	BCG	No	
Viral	Hepatitis A		
	Inactivated	Yes	Data on safety not available, but may be given with risk
	Immunoglobulin	Yes	
	Hepatitis B	Yes	
	Human papillomavirus	No	
	Influenza, inactivated	Yes	
	Japanese B encephalitis	Not known	Data on safety not available, but may be given with high-risk exposure
	Measles, mumps, rubella (MMR)	No	Should wait 4 weeks after MMR before becoming pregnant
	Poliomyelitis, inactivated	Yes	
	Rabies	Yes	
	Tick-borne encephalitis	Not known	May be given with high-risk exposure
	Varicella	No	Should wait 4 weeks after varicella before becoming pregnant
	Yellow fever	Yes	Should generally be avoided, but may be given with high-risk exposure

BCG, bacille Calmette–Guérin; DTaP, diphtheria, tetanus, acellular pertussis.
Adapted from CDC and WHO guidelines: Centers for Disease Control and Prevention, 2007 #6558; World Health Organization, 2008 #7181.

mild, characterized by three or more loose to watery stools accompanied by another symptom of nausea, abdominal cramping or malaise. Fever is usually less than 101°F (38°C) and vomiting is not common. In most cases, illness is self-limiting over 3–5 days. Dysentery with tenesmus and bloody stools occurs in less than 10% of patients. Although most individuals can continue with their activities, about 25% will need to alter plans. Enterotoxigenic *Escherichia coli* (ETEC) accounts for 20–30% of the known causes, and a newly described *E. coli*, enteroaggregative *E. coli*, causes another 15–20%.[44,45] *Shigella*, *Salmonella* and *Campylobacter* spp. are the most common other bacteria (see Chapter 34). Viruses (typically rotavirus and norovirus) cause 5–20% of cases and protozoa (*Giardia*, *Cryptosporidium* and, less commonly, *Cyclospora*) cause 5% or less and are usually associated with longer duration of travel. Although the overall incidence of TD does not decline with increasing time of residence in developing areas, partial immunity will develop to ETEC diarrhea.

Prevention

A full description of the prevention and treatment of TD can be found in Chapter 34 and Practice Point 17. Taking care in the selection of food and liquids will help prevent many cases of TD; however, this is often difficult to do consistently. In addition, the risk of TD seems increasingly related to the level of sanitation at the destination.[46] Foods and liquids that are likely to be contaminated are ground-grown greens, vegetables and fruits, incompletely cooked or poorly stored meats and seafood, untreated water and ice cubes, unpasteurized milk products and food from street vendors. Travelers should restrict themselves to commercially bottled or heated beverages, recently and thoroughly cooked meats and greens, and fruits that can be peeled by the traveler. Water may be purified by bringing it to a boil or by halogenating (iodine or chlorine preparations) and then filtering it with a filter of pore size ≤1 mm.[47] The cysts of *Cryptosporidium* and *Cyclospora* (and eggs of helminths) are likely to be halogen resistant, so water potentially contaminated with these parasites should be filtered or boiled.

Several nonantimicrobial agents have been used to prevent diarrhea. Bismuth subsalicylate in tablet (2 tablets (262 mg/tablet), four times daily) or liquid form (1 oz (262 mg/15 ml), four times daily) decreases the incidence of diarrhea by about 65%. Individuals who are allergic to or taking large doses of salicylates, or who are on anticoagulant therapy should not take the drug. It can decrease the absorption of doxycycline. Ingestion of probiotics does not confer reliable protection and antimotility agents such as loperamide should not be taken preventively.

Antibiotics are effective in prophylaxis against TD, but they may be associated with adverse events, contribute to bacterial resistance and have not been studied in travelers going for more than 2–3 weeks. These factors, combined with the effectiveness of prompt self-treatment have led to the consensus that antibiotic prophylaxis should not be recommended for most travelers. For highly selected persons in whom an episode of TD would have substantial adverse consequences, it may be considered. Fluoroquinolones have an evidence

Table 102.5 Immunization in HIV infection

Vaccine		Safely given to persons with HIV infection at risk?	Notes
Bacterial	Tetanus–diphtheria	Yes	
	Pneumococcal polysaccharide and conjugate	Yes	
	Meningococcal polysaccharide and conjugate	Yes	
	Typhoid		
	Live-attenuated	No	Data on safety are not available and vaccination should be avoided
	Vi polysaccharide	Yes	
	Cholera, oral killed	Yes (see notes)	Only limited data on safety
	BCG	No	
Viral	Hepatitis B	Yes	
	Hepatitis A		
	Immunoglobulin	Yes	
	Inactivated	Yes	
	Human papillomavirus	Yes	
	Influenza, inactivated	Yes	
	Japanese B encephalitis	Yes	Data on safety are not available but may be given with risk
	Measles	Yes (see notes)	Can be given to persons who are asymptomatic and with a CD4 count ≥200 cells/mm^3
	Poliomyelitis, inactivated	Yes	
	Rabies	Yes	
	Rotavirus	Yes	
	Tick-borne encephalitis	Yes	Only limited data on safety, but may be given with risk
	Varicella (adults)	Yes (see notes)	Can be given to persons who are asymptomatic and with a CD4 count ≥200 cells/mm^3
	Yellow fever	See note	Can be given to persons who are asymptomatic and with a CD4 count ≥200 cells/mm^3

BCG, Bacille Calmette–Guérin.
Adapted from CDC and WHO guidelines: Centers for Disease Control and Prevention[2]; World Health Organization[3].

base supporting their use for most areas of the world; however, *Campylobacter* resistance limits their effectiveness in South and South East Asia. Rifaximin, a poorly absorbed rifamycin derivative, can be considered in regions where *E. coli* predominates, such as Latin America and Africa.[48]

There is no vaccine to prevent all causes of TD. The oral cholera vaccine, Dukoral, provides modest protection against LT-producing ETEC.[29] An experimental transcutaneously administered vaccine consisting of LT toxin alone has demonstrated 75% protective efficacy against moderate to severe TD.[49]

Treatment

Prompt treatment of TD should be initiated with hydration. Commercial rehydration packets combining electrolytes, sugar and buffer are easy to use and are appropriate for young children, the elderly and those with chronic medical conditions. Infants should continue breast-feeding. As diarrhea improves, the diet can be increased by adding bland foods (bread, cereals, potatoes, soups, bananas, fish and chicken) in frequent small meals.

Mild disease can be treated with bismuth subsalicylate. This reduces the number of loose stools by about 50%, but can have a delay in effectiveness of about 4 hours. The antimotility agent loperamide will rapidly decrease cramping and the number of loose stools. Loperamide should be avoided if there is blood in the stool or a fever of >101.5°F (38.5°C). Because most episodes of diarrhea are self-limiting, symptomatic therapy alone may be sufficient.

A short course of an antibiotic will improve diarrhea within 20–36 hours. Antibiotics combined with loperamide have controlled symptoms within hours.[50] The wide prevalence of sulfonamide and tetracycline resistance has made fluoroquinolones such as ciprofloxacin, norfloxacin, ofloxacin and levofloxacin the most commonly recommended antibiotics in treatment. In South and South East Asia where *Campylobacter* is a common etiology and is often resistant to fluoroquinolones, azithromycin can be used.[51] Azithromycin can also be safely given to children. Antibiotics are prescribed for up to 3 days, but single-dose therapy is often sufficient. Persons with dysentery should seek medical care if self-treatment does not result in improvement within 24 hours, or in cases of severe dehydration. Diarrhea that persists after return should be evaluated (see Chapter 36 and Practice Point 19 for a full discussion of persistent diarrhea) for causes ranging from infection with *Giardia*, *Cryptosporidium* or *Clostridium difficile*, to tropical sprue, to irritable bowel disease.

MALARIA PREVENTION

Malaria is one of the most important diseases to prevent as it can be fatal. The type of malaria and the risk of acquisition vary by destination and reason for travel, but worldwide there are approximately

7500 cases each year in returned travelers. Of the five recognized species of malaria in humans (*Plasmodium falciparum*, *P. vivax*, *P. ovale*, *P. malariae* and, rarely, *P. knowlesi*), *P. falciparum* is the most severe form and is also the most frequently imported species. Over 80% of *P. falciparum* cases are acquired by travelers on trips to Africa, and especially West Africa where transmission can occur in both urban and rural areas. Travelers with the highest risk for acquiring malaria are those who return to their country of birth to visit friends and relatives, termed VFR travelers; they are usually traveling to West Africa where they are most likely to acquire *P. falciparum* or to South Asia where they usually get *P. vivax*.

Malaria in travelers can almost always be prevented by following an 'ABCD' approach: 'a'wareness of the risk of malaria, mosquito 'b'ite avoidance measures, 'c'ompliance with appropriate 'c'hemoprophylaxis, and prompt 'd'iagnosis of malaria and initiation of treatment.[52]

Bite avoidance measures

Malaria is transmitted by the female *Anopheles* mosquito, which is most active during the nighttime hours from dusk to dawn. During this period travelers should wear loose-fitting cotton clothing that covers their arms and legs, apply repellents to exposed areas of skin and sleep in enclosed areas behind screens or under netting (see Table 102.3). The most effective repellents are those that contain DEET (*N*,*N*-diethyl-3-methylbenzamide).[53] A concentration of 20–50% will provide protection lasting from 6 to 12 hours. DEET-containing repellents are safe to use in children and pregnant women, but should be applied carefully in these hosts to avoid systemic absorption: they should not be applied to mucous membranes or irritated skin and should be washed off when coming indoors. Picaridin-based repellents are also effective. Bed nets should be treated with a residual insecticide (e.g. permethrin-containing compounds). This can also be applied to clothing and will kill insects rather than only repel them. Mosquito coils and sprays containing pyrethroids can be used in enclosed sleeping areas.

Chemoprophylaxis

Chemoprophylaxis needs to be taken on a regular basis during travel and for a period of time after return that depends on which medications are taken (Table 102.6). Most cases of malaria in returned travelers occur because the traveler fails to take any chemoprophylaxis, takes incorrect prophylaxis or does not comply with their prophylaxis. The choice of a chemoprophylactic regimen should be based on risk of exposure, types of parasite prevalent in the travel destination and health conditions of the traveler. Up-to-date sources (including authoritative websites) should be consulted before prescribing any antimalarial.[2,17]

Chloroquine as a single agent is effective only in areas where *P. falciparum* remains sensitive or is not present: Mexico, Central America west and north of the Panama Canal, the Dominican Republic and Haiti, Egypt, most areas of the Middle East and parts of China. Travelers to all other risk areas in Africa, Asia and Latin America will need to take atovaquone–proguanil (Malarone™), doxycycline or mefloquine.

Atovaquone–proguanil and doxycycline are effective in treatment and prophylaxis of all malaria species, although experience using atovaquone–proguanil with non-falciparum species is limited.[54] Both drugs are started 1–2 days before exposure to malaria and then taken daily. Doxycycline is continued for 28 days after leaving the malarious area, but atovaquone–proguanil can be discontinued after 7 days because it has a causal prophylactic effect: it kills developing hepatic stage parasites, but not the dormant, hypnozoite forms of *P. vivax* or *P. ovale*. These may emerge from the liver many months after return from the place of exposure. Atovaquone–proguanil is ideally targeted

Table 102.6 Prophylaxis of malaria*

Drug	Adult dose	Pediatric dose
Chloroquine	300 mg base (500 mg salt) po, once per week, beginning 1 week before travel, weekly whilst traveling and for 4 weeks after travel	5 mg/kg base (8.3 mg/kg salt), once per week (not to exceed adult dose)
Mefloquine	250 mg salt (1 tablet) po, once per week, beginning 1–2 weeks before travel, weekly whilst traveling and for 4 weeks after travel	≤9 kg, 5 mg salt/kg/week 10–19 kg: ¼ tablet/week 20–30 kg: ½ tablet/week 31–45 kg: ¾ tablet/week ≥46 kg: 1 tablet/week
Atovaquone/proguanil (A/P) (Malarone™)	250 mg A/100 mg P (1 adult tablet) q24h, beginning 1–2 days before travel and for 7 days after travel	62.5 mg A/25 mg P (1 pediatric tablet) 5–8 kg: ½ tablet q24 h 9–10 kg: ¾ tablet q24h 11–20 kg: 1 tablet q24h 21–30 kg: 2 tablets q24h 31–40 kg: 3 tablets q24h ≥41 kg: adult dosing
Doxycycline	100 mg po q24h, beginning 1–2 days before travel and for 4 weeks after travel	≥8 years of age: 2 mg/kg po q24h, not to exceed adult dose
Proguanil	200 mg po q24h Used in combination with chloroquine for areas of low levels of chloroquine-resistant *P. falciparum* (see text)	100 mg (one tablet) 6–15 kg: ¼ tablet q24 h 16–24 kg: ½ tablet q24h 25–44 kg: ¾ tablet q24h ≥45 kg: 1 tablet q24h
Primaquine (alternative for chemoprophylaxis; usually used to eradicate hypnozoites of *P. vivax* and *P. ovale*)	30 mg base (52.6 mg salt) (2 tablets) po q24h, beginning 1–2 days before travel and for 7 days after travel Must check G6PD before prescribing	0.5 mg/kg base (0.8 mg/kg salt) po q24h, beginning 1–2 days before travel and for 7 days after travel Must check G6PD before prescribing

*Full manufacturer's prescribing instructions should be consulted on dosing guidelines as they may vary between countries.
G6PD, glucose-6-phosphate dehydrogenase.

for short-term travelers to areas of risk. Doxycycline cannot be given to children under the age of 8 or to pregnant women. It should be thoroughly swallowed in the upright position to prevent esophageal irritation. Doxycycline may predispose to vaginal yeast infection and can act as a photosensitizer.

Mefloquine is highly effective in preventing malaria, including chloroquine-resistant *P. falciparum*. When it is taken in prophylactic doses, minor GI and neuropsychiatric events occur in 5–30% of users.[55] The neuropsychiatric side-effects can include sleep disturbance, vivid dreams, mood changes, anxiety, headache and dizziness. Serious adverse events such as psychosis are rare with an occurrence of about one case per 6000–20 000 users.[56] Travelers should be screened for contraindications to mefloquine before prescribing it: a known hypersensitivity to the drug, a history of seizures or psychiatric disorder, or an underlying cardiac conduction abnormality. Some travel health consultants prescribe mefloquine 2–3 weeks before departure to assess patient tolerance and allow a switch to other agents if there is a problem. Seventy per cent of adverse reactions occur during the first three doses. Loading doses of the drug are not advocated.

The combination of chloroquine plus proguanil has been recommended by practitioners in the UK for areas in which there are low levels of chloroquine-resistant *P. falciparum* malaria and only a small risk of acquisition, such as most areas of risk in India. In low risk areas, some European experts do not recommend taking any chemoprophylaxis; rather they suggest being observant to febrile episodes and carrying stand-by self treatment.[56] US and UK authorities do not advocate this approach. Health practitioners should consult the appropriate expert recommendations for their country.

Primaquine is an alternative chemoprophylactic in persons intolerant of other options and can be considered following specialist advice.[57] It is a causal prophylactic and will prevent the establishment of liver hypnozoites of *P. vivax* and *P. ovale*. The drug is taken daily beginning 1–2 days before exposure and can be discontinued 7 days after leaving the risk area. It is a requirement that glucose-6-phosphate dehydrogenase (G6PD) deficiency is ruled out in all persons for whom primaquine is prescribed.

Rural, forested areas of Thailand that border Burma and Cambodia, western Cambodia (including Siem Reap) and southern areas of Vietnam that border Cambodia, have multidrug-resistant *P. falciparum* malaria. Travelers to these areas should take atovaquone–proguanil or doxycycline for prophylaxis.

All travelers should understand that no antimalarial is 100% protective and that they can develop malaria in spite of being compliant with prophylaxis. If they develop a fever or flu-like illness overseas that could be malaria, they should seek medical care. The evaluation needs to include a blood smear performed by a competent laboratory, because the sensitivity of symptoms or physical findings alone is low. The use of self-administered rapid diagnostic kits is not generally recommended as travelers have difficulty using and interpreting these tests; they are not currently licensed for this purpose. In circumstances where travelers cannot obtain prompt medical care, self-treatment can be considered. The self-treatment drug(s) should differ from what the traveler is taking for chemoprophylaxis. Options include atovaquone–proguanil, artemether–lumefantrine (Riamet™), and quinine plus doxycycline.[56]

Travelers who have had prolonged exposure to *P. vivax* and *P. ovale* malaria can consider primaquine to eradicate dormant hepatic-stage parasites. Chloroquine-resistant *P. vivax* is unusual, but has been described from Papua New Guinea and Papua (formerly Irian Jaya) with sporadic cases from Burma, India, Guyana and Brazil.

Pregnant women should not travel to malarious areas unless absolutely necessary, because of the added risk of complications of malaria during pregnancy. Chloroquine and proguanil (with added folate) are safe. Mefloquine can be taken after the first trimester, and may be acceptable in the first trimester if there are no alternatives. Doxycycline is contraindicated and there is insufficient safety data on atovaquone–proguanil during pregnancy. Primaquine should not be given because the G6PD status of the fetus cannot be determined.

ENVIRONMENTAL RISK

Travel to the tropics is associated with increased heat and humidity; the effects that these changes can have on health should be taken into account. These range from a feeling of malaise and tiredness to increased loss of salt and water with resultant dehydration. Travelers should maintain hydration, limit exercise and sleep in a cool environment, particularly if they are elderly or have chronic medical problems. Excessive sun exposure should be avoided by wearing loose-fitting cotton clothing to cover exposed skin, wearing hats and using sunscreens with a sun protection factor of at least 15. Water insolubility may extend the life of the sunscreen. If the patient is taking doxycycline for malaria prophylaxis, it is particularly important to limit sun exposure. Skin should be kept dry and clean to avoid cellulitis and dermatophyte infection.

Travelers to altitudes above 2500–3500 meters (8200–11 500 feet) can experience acute mountain sickness (AMS) or the more severe and potentially fatal conditions of high-altitude pulmonary edema (HAPE) and high-altitude cerebral edema (HACE).[58,59] AMS is characterized by headache, nausea, vomiting, insomnia and lassitude and may affect up to 50% of persons who rapidly ascend above 4000 meters. The risk of illness can be lessened by acclimatization: spending a few days at intermediate altitudes of 1500–3000 meters and gradually ascending, sleeping at elevations no more than 300–500 meters higher each night. Acetazolamide, a carbonic anhydrase inhibitor, can be taken to assist acclimatization, particularly when persons must ascend quickly. It is given at a dose of 125–250 mg orally twice daily, starting 2 days before being at altitude and for several days at altitude.[59,60] It has also been used to treat mild symptoms of AMS. Dexamethasone can be used to treat AMS but in severe illness the safest course is always to descend. Acetazolamide is contraindicated in persons with sulfonamide allergy.

Jet lag is a common problem, particularly when more than five time zones are crossed. It is easier to travel west and lengthen the day than to travel east and shorten the day. In order to help with jet lag, several methods have been proposed: light exposure, short-acting hypnotics and melatonin.[61] When traveling east, exposure to bright light in the late morning and early afternoon may aid in the adjustment. A short- to intermediate-acting benzodiazepine or benzodiazepine receptor agonist can facilitate and maintain sleep in the new time zone, decreasing the contribution of fatigue to the effects of time zone adjustment. Melatonin, which is secreted during the night hours, has been extensively studied. A dose of 3–8 mg taken 2–3 hours before bedtime for the first few nights in the new time zone may be helpful. However, the purity and effectiveness of over-the-counter preparations have not been documented.

Deep venous thrombosis (DVT) and pulmonary embolism are recognized complications of long-haul travel. DVTs can occur in as many as 3% of persons flying for 10–15 hours and who have cardiovascular risk factors; oral contraceptive pills, pregnancy, recent surgery, certain coagulation disorders and malignancy also contribute to risk.[62,63] Some long-haul travelers will develop a pulmonary embolism.[64] In order to decrease risk, travelers should maintain their hydration and exercise their lower extremities at regular intervals. Fitted, below-the-knee support stockings will decrease the incidence of DVT and for highest risk passengers low molecular weight heparin can be considered. Aspirin is not recommended.

BEHAVIORAL RISK

Although there is much focus on infectious illness, the most important contributor to severe morbidity and mortality, particularly in young adults, is accidents and injuries, with those related to road traffic accidents and drowning most common.[65,66] Road safety can be improved by using safety belts in vehicles, avoiding excessive

speed, not driving at night or after drinking alcohol, and not riding on motorcycles and mopeds. When swimming, travelers should be aware of undercurrents and never dive into unknown waters. To prevent assault and theft, travelers should not wear jewelery and clothing that draws attention, and they should travel in groups, avoiding high-risk urban areas particularly at night. The US State Department and the UK Foreign and Commonwealth Office post travel advisory and safety information (see Sources of Information). In all situations, alcohol contributes to increased risk behavior.

Sexually transmitted diseases, including HIV, gonorrhea, syphilis and chancroid, are prevalent in many destination countries in Africa, Asia and Latin America. Travelers should be counseled about safe sexual practices, and take condoms with them on their trip.[67,68] Postexposure prophylaxis against HIV may be necessary,[69] and women may need emergency contraception (information can be found on the Marie Stopes website: http://www.mariestopes.org.uk/).

OTHER DISEASES AND CONSIDERATIONS

Dengue fever, a viral disease (see Chapter 127) transmitted by *Aedes* mosquitoes, has seen a resurgence, particularly in Asia, the Caribbean and Latin America, and is a theoretical risk in the south-eastern USA. Dengue is characterized by the sudden onset of fever, headache, myalgias and arthralgias, abdominal discomfort, rash and mild liver abnormalities.[70] Severe disease (dengue hemorrhagic fever and dengue shock syndrome) is characterized by thrombocytopenia and vascular leak, and usually occurs in children following a second infection with a different dengue serotype. There is no vaccine available for prevention, so travelers need to exercise precaution against this daytime feeding mosquito. Complying with bite avoidance measures outlined in Table 102.3 will help to prevent not only dengue but a clinically similar viral infection, chikungunya virus, that in recent years has been a problem on the islands of the Indian Ocean and in countries of South and South East Asia.[71] Other less common insect-transmitted diseases such as leishmaniasis, trypanosomiasis, filariasis and rickettsial infection will also be prevented by insect bite avoidance. African trypanosomiasis has been documented in travelers to East African game parks, and African tick-bite fever caused by *Rickettsia africae* is a risk in southern Africa.[72]

Schistosoma spp. (see Chapter 112) can infect travelers who swim in fresh-water lakes and rivers, particularly in endemic areas of east and southern Africa.[73] Travelers to risk areas should avoid fresh-water swimming unless it is in a chlorinated pool. Letting water stand for 48 hours or warming it to 122°F (50°C) for 5 minutes will render it safe from the *Schistosoma* parasites. Fresh-water swimming, particularly after periods of flooding, can be a risk for acquisition of leptospirosis.

Although the viral hemorrhagic fevers, Ebola, Lassa and Marburg (see Chapter 126), garner a great deal of media attention, they are a rare risk for travelers. Current outbreaks of disease can be identified by reviewing information on ProMED or by checking the disease outbreak sites of the WHO, CDC and NaTHNaC web pages (see Sources of Information). Any returning traveler who is suspected of having a viral hemorrhagic fever should be managed according to WHO guidelines.[74]

Tattooing, injections and dental instruments should be avoided to decrease the risk of acquiring blood-borne pathogens such as hepatitis B and C and, less likely, HIV.

Travelers need to know how to access medical care during their trip. They should purchase a travel health insurance package that includes help in locating medical care, paying for the care upfront and, if necessary, providing for emergency evacuation. There are several air ambulance and insurance companies (http://travel.state.gov/travel/tips/brochures/brochures_1215.html). Embassies or consulates can assist in locating medical services, and mission hospitals can be a source of care. The International Association for Medical Assistance to Travellers (www.iamat.org) will provide a list of English-speaking physicians throughout the world. A small first-aid kit that contains analgesics, bandages, a thermometer and any frequently used over-the-counter medications is helpful as it can be difficult to find the simplest medicines overseas. All travelers should carry an extra supply of their prescription medications, and avoid putting them into checked luggage.

Post-travel illness

Travelers should report illness upon return and heath practitioners should be able to recognize common syndromes: fever, diarrhea, respiratory illness and rash.[1,7,75] Although routine post-travel follow-up for short-term travelers is usually not necessary, anyone who experienced major illness overseas or new-onset illness after return should be evaluated. A differential diagnosis can be developed by considering geography (the locations visited), activities undertaken, incubation periods of disease, frequency of occurrence, and preventive measures taken (e.g. immunizations, compliance with prophylaxis).[76] Practitioners who are not able to pursue complex investigations should refer their travelers for appropriate specialist care. A more detailed discussion of individual syndromes and diseases is provided in other chapters.

SOURCES OF INFORMATION

World Health Organization

- Home page: http://www.who.int/en
- International travel and health. Available online at: http://www.who.int/ith/en/index.html
- Disease outbreaks: www.who.int/csr/don/en/
- Weekly Epidemiologic Record: www.who.int/wer/en/
- Global Health Atlas (disease epidemiology): http://www.who.int/globalatlas/default.asp

United States

Centers for Disease Control and Prevention

- Home page: http://www.cdc.gov/
- Travel medicine home page: http://wwwn.cdc.gov/travel/default.aspx
- Health information for international travel. Available online at: http://wwwn.cdc.gov/travel/contentYellowBook.aspx
- Morbidity and Mortality Weekly Report: http://www.cdc.gov/mmwr/

US State Department

- Travel page: http://travel.state.gov/
- Medical Information for Americans Abroad (has information on travel medical insurance and air ambulance companies): http://travel.state.gov/travel/tips/brochures/brochures_1215.html

US Food and Drug Administration

- Vaccine Adverse Event Report System: http://www.fda.gov/cber/vaers/vaers.htm

Canada

- Health Canada – Travel Medicine: http://www.hc-sc.gc.ca/hl-vs/travel-voyage/index-eng.php
- Committee to Advise on Tropical Medicine and Travel: http://www.phac-aspc.gc.ca/tmp-pmv/catmat-ccmtmv/index.html
- Canada Communicable Disease Report: http://www.phac-aspc.gc.ca/publicat/ccdr-rmtc/index-eng.php
- Adverse Events Following Immunization Reporting: http://www.phac-aspc.gc.ca/im/aefi-form-eng.php

United Kingdom

- National Travel Health Network and Centre: www.nathnac.org/
- Fit for Travel (Health Protection Scotland): www.fitfortravel.scot.nhs.uk/
- Foreign and Commonwealth Office travel page: http://www.fco.gov.uk/en/travelling-and-living-overseas/
- Department of Health – Travel Advice: http://www.dh.gov.uk/en/Healthcare/Healthadvicefortravellers/index.htm
- Health Protection Agency: http://www.hpa.org.uk/
- Vaccine Adverse Event Reporting (Medicine and Healthcare products Regulatory Agency, Yellow Card Scheme): http://www.mhra.gov.uk/Safetyinformation/Reportingsafetyproblems/Medicines/Reportingsuspectedadversedrugreactions/index.htm

Surveillance

- European Centre for Disease Prevention and Control: http://ecdc.europa.eu/
- European Surveillance (Eurosurveillance): www.eurosurveillance.org
- European Travel and Tropical Medicine Network: http://www.istm.org/eurotravnet/main.html
- GeoSentinel: global surveillance network of the International Society of Travel Medicine and the Centers for Disease Control and Prevention: http://www.istm.org/geosentinel/main.html
- ProMED-mail: global electronic reporting system for outbreaks of emerging infectious diseases (International Society for Infectious Diseases): http://www.promedmail.org/pls/otn/f?p=2400:1000
- National Travel Health Network and Centre: http://www.nathnac.org/countrysearch.aspx
- TropNetEurop (European Network on Imported Infectious Disease Surveillance): http://www.tropnet.eu/
- WHO Disease outbreaks: www.who.int/csr/don/en/

International Association for Medical Assistance to Travellers

- IAMAT; advice for travelers about health risks: www.iamat.org

International Society of Travel Medicine

- Home page: www.istm.org/
- Directory of travel clinics: http://www.istm.org/clinicdir/clinicdir.aspx
- *Journal of Travel Medicine*: http://www.istm.org/publications/jtm.aspx

American Society of Tropical Medicine and Hygiene

- Homepage: www.astmh.org/
- Travel clinic directory: http://www.astmh.org/clinicians/clinics.cfm
- *American Journal of Tropical Medicine and Hygiene*: http://www.ajtmh.org/

Royal Society of Tropical Medicine and Hygiene

- Home page: www.rstmh.org/
- *Transactions of the Royal Society of Tropical Medicine and Hygiene*: http://www.rstmh.org/transactions.asp

Royal College of Physicians and Surgeons of Glasgow

- Faculty of Travel Medicine: http://www.rcpsg.ac.uk/Travel%20Medicine/Pages/mem_spweltravmed.aspx

Commercial travel medicine databases

- Gideon (Global infectious disease database): www.gideononline.com/
- Exodus Software Ltd (Ireland): www.exodus.ie
- International SOS: www.internationalsos.com/en/emergencies.htm
- MASTA (England): http://www.masta-travel-health.com/
- Travax and Travax EnCompass (Shoreland Inc, US): www.shoreland.com/gotrav_f.html
- Travax (Health Protection Scotland): www.travax.scot.nhs.uk/
- Tropimed® (Switzerland, Germany, USA): www.tropimed.com

Travel medicine texts

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- Jong E, McMullen R, eds. The travel and tropical medicine manual, 4th ed. Philadelphia: Saunders, 2008.
- Jong EC, Zuckerman JN. Travelers' vaccines. Hamilton, Ontario: BC Decker; 2004.
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Practice point | 49 |

Christopher JM Whitty

Fever in a returned traveler from Kenya or the Congo

Patients returning from East or Central Africa with fever potentially have a very wide differential diagnosis, but it is important to get the probabilities in perspective. By some measure the most common treatable and potentially serious infection causing a fever in people recently returned from most of sub-Saharan Africa is malaria and this must always be excluded. Clinicians not working in Africa often underestimate how common this is: in many parts of Central, West and East Africa the average person will get a clinical attack of malaria several times a year. Case series from the Hospital for Tropical Diseases in London suggest that around one in five travelers returning from Africa with fever will have malaria.[1]

Since it is impossible to exclude malaria on the basis of clinical symptoms or signs (see Chapter 111), a blood film for malaria should always be carried out. Clinicians should not be put off the diagnosis by other potentially localizing signs or symptoms since malaria can coexist with other infections. Clinicians should also be aware that roughly half of those with proven malaria do not have a fever at the time of presentation, so a normal temperature on presentation does not exclude the diagnosis of malaria. Around 95% of all malaria from Central and East Africa will be falciparum malaria and the great majority of cases will present within a month of return though later presentations are possible.[2]

Once malaria has been excluded, however, the two key points are to take a proper travel history and a very exact record of timings of different exposures. Many of the infections that can be acquired in East and Central Africa present simply with an undifferentiated fever and a general feeling of malaise. Initial treatment decisions therefore have to be made on the basis of probabilities, and these depend on epidemiologic exposure.

The simplest timing cut-off is between those who returned within the last 21 days and those presenting more than 21 days after return. With the exception of HIV, all the tropical viruses of importance and the great majority of tropical bacterial infections such as typhoid will present within 21 days of return.

In those presenting more than 21 days after return, three parasitic diseases dominate the common tropical presentation: malaria, amebic liver abscess and Katayama syndrome due to acute schistosomiasis exposure. Tuberculosis is the other major infection to exclude, and occasionally brucellosis, although this is relatively uncommon from this part of Africa. In those presenting more than 21 days after return from the tropics, a blood film to exclude malaria, a chest X-ray looking for the raised right diaphragm suggestive of amebic liver abscess as well as for tuberculosis, and a blood culture are the essential investigations. Katayama syndrome almost always presents with an urticarial rash and eosinophilia. A history of exposure in fresh water 3–8 weeks prior to presentation is strongly suggestive. There is currently no laboratory test that can exclude Katayama syndrome, which is generally self-limiting. Further investigations in those presenting more than 21 days after return from Kenya or the Congo are best guided by localizing symptoms or signs. The great majority of those who do not have malaria will have an infection unrelated to their travel.

In those presenting within 21 days of return from East or Central Africa, malaria remains the most likely diagnosis that requires treatment. There is, however, a much longer list. Some of the more common causes are outlined in Table PP49.1. In addition to those diagnoses mentioned above, there is a range of possible tropical arboviruses (transmitted by mosquitoes). Of these, the most important clinically (in the sense that it may require treatment) is dengue. This is much less common in Africa than in South East Asia and South America but does cause cases in travelers from this part of Africa.[3] Most arboviruses are self-limiting and missing the diagnosis makes no difference to patient outcome.

Table PP49.1 Approximate incubation periods for febrile illnesses from East and Central Africa

Incubation period	Infection
Short (<10 days)	Acute gastroenteritis (bacterial, viral) Respiratory tract infection (bacterial, viral including avian influenza) Meningitis (bacterial, viral) Arboviral infections, e.g. dengue, chikungunya Rickettsial infection, e.g. tick typhus, scrub typhus Relapsing fever (*Borrelia*)
Medium (10–21 days)	Protozoal • Malaria (*Plasmodium falciparum*) • *Trypanosoma rhodesiense* • Acute Chagas disease Viral • HIV, CMV, EBV, viral hemorrhagic fevers Bacterial • Enteric fever (typhoid and paratyphoid fever) • Brucellosis • Q fever • Leptospirosis
Long (>21 days)	Protozoal • Malaria (including *Plasmodium falciparum*) • Amebic liver abscess • Visceral leishmaniasis Viral • Viral hepatitis • HIV

CMV, cytomegalovirus; EBV, Epstein–Barr virus.

Several bacterial diseases are also possible, and these generally will need treatment. Typhoid and paratyphoid occur in this part of Africa, although they are rare compared to other bacterial diseases and are a much less common cause than in, for example, patients returning from South East Asia with a fever.[4] Typhoid is often overdiagnosed in travelers in Africa, and patients having had a fever investigated locally are often told that they have typhoid based on a positive Widal test. As this is almost invariably positive because people have typhoid vaccinations prior to travel, this should not be overinterpreted. Typhoid and paratyphoid do occur in East and Central Africa but they are very rare. Non-typhi *Salmonella* infections are much more common, and in immunocompetent people tend to present with a food-poisoning picture rather than as an undifferentiated fever.

Four things are worth asking about specifically:

- travel in game parks;
- swimming in fresh water;
- whether the returned traveler was caring for sick people; and
- high-risk sexual encounters.

Fresh-water exposure increases the risk of Katayama syndrome. Exposure in game parks increases the risk of one common and unpleasant (although generally self-limiting) infection, African tick typhus. It also increases the risk of a very rare but potentially very serious infection, trypanosomiasis. Typhus is suggested by a combination of a rash (occurring in about 90% of classic African tick-typhus cases) or having seen ticks, or an eschar. The only way of excluding tick typhus is a serologic test; however, as the results may take some time to get back to most centers, if tick typhus is suspected, treatment with doxycycline is worth trying presumptively. It is one of the most common causes of febrile illness in travelers from Africa.[3] If the patient is not better within 48 hours, tick typhus is essentially excluded. Human African trypanosomiasis should be considered but is extremely unlikely.[5] The acute rhodesiense trypanosomiasis which is found in Kenya and in game parks more generally will normally be diagnosed on a blood film. Serologic tests from trypanosomiasis have a very low positive predictive value, with the great majority of positives being false-positives in travelers.

Patients who are also health-care workers returning from East and Central Africa are clearly at higher risk of a wide range of infections than the general population. Generally, these are obvious and not specifically tropical – examples are fecal–oral infections, meningococcus and tuberculosis. In rural areas the exceptionally low probability of viral hemorrhagic fever (Marburg, Ebola) should be considered in health-care workers, especially those working in rural parts of the Democratic Republic of Congo (DRC). Lassa is not thought to occur in this part of Africa. If this seems a possibility, do not take blood tests, and telephone a specialist centre for advice. However, do keep the risk in perspective – it is exceptionally unlikely.

A surprisingly large number of travelers choose to have unprotected sexual contacts in Africa, despite knowing that HIV and other sexually transmitted infections are common in most East and Central African countries.[6] HIV seroconversion and secondary syphilis should be considered, especially where there is a rash. Those who are known to be HIV positive who travel to East and Central Africa are not at particularly increased risk of exotic tropical infections, but non-typhi *Salmonella* infections (especially *S. typhimurium*), pneumococcal infections and tuberculosis are all more common than in Europe in HIV-positive individuals.

Clinical signs can be helpful in diagnosing some of the tropical infections. Where patients present with a rash or jaundice with a fever, having recently returned from East or Central Africa, this may point to particular diagnoses and some of the possibilities are outlined in Table PP49.2. Jaundice and fever should be assumed to be complicated malaria until proved otherwise. Leptospirosis and ascending cholangitis are other common causes. In most cases of viral hepatitis, fever precedes the jaundice, and thinking on the basis of jaundice that a patient has hepatitis A when they have malaria is a potentially fatal mistake.

It is important to stress that, once malaria has been excluded, the great majority of those with an acute fever returning from East and Central Africa will have a self-limiting infection, most of which are nonspecific viral illnesses which they probably would have had as much chance of catching in the UK as when in Africa itself.

Table PP49.2 Acute fever and rash, ulcer or jaundice in those returning from East or Central Africa

Rash	Infection
Maculopapular	Drug reaction, e.g. antimalarials Arboviral infection, e.g. dengue, chikungunya 'Childhood viral illness', e.g. measles, rubella, parvovirus (all more common in Africa) Infectious mononucleosis group, e.g. EBV, CMV, HIV seroconversion Rickettsial spotted fevers, e.g. African tick typhus Secondary syphilis Viral hemorrhagic fever, e.g. Ebola
Vesicular	Rickettsial spotted fever Monkeypox (very rare)
Erythroderma	Sunburn Early dengue
Purpuric	Meningococcal infection Gonococcal infection Hemorrhagic herpes zoster Dengue hemorrhagic syndrome Viral hemorrhagic fever, e.g. Lassa, Ebola, Congo–Crimean hemorrhagic fever, Rift Valley fever Rickettsial spotted fevers (severe) Severe sepsis ± disseminated intravascular coagulation
Ulcer	Eschar: rickettsiosis, anthrax Chancre: *Trypanosoma rhodesiense*, bubonic plague Skin ulcer: superinfected bacterial ulcer, anthrax, cutaneous diphtheria Genital ulcer: syphilis
Jaundice	Severe *Plasmodium falciparum* malaria Hepatitis A–E, but generally the fever precedes the jaundice Leptospirosis (Weil's disease) Septicemia including pneumococcal sepsis EBV, CMV Enteric fever (typhoid and paratyphoid) Typhus Relapsing fevers (*Borrelia* spp.) Viral hemorrhagic fever Ascending cholangitis Malaria *Mycoplasma* *Bartonella* Hemolytic–uremic syndrome (*Shigella, Escherichia coli*) Sickle cell crisis with infective trigger

CMV, cytomegalovirus; EBV, Epstein–Barr virus.

REFERENCES

References for this chapter can be found online at http://www.expertconsult.com

Practice point **50**

Roderick J Hay

Skin rashes in a returned traveler from Ecuador

DEFINITION OF THE PROBLEM

X is a 38-year-old male who returned from a visit to Ecuador 2 weeks ago. He presents to your clinic with a 10-day history of a moderately itchy rash, worse on his right lower leg. He is otherwise fit and has not been taking medications. What are the possible diagnoses and implications for management?

HISTORY TAKING

In any patient with skin disease it is important to follow a logical history-taking format. This must include finding out about a past history of skin problems as, in many patients presenting with skin lesions after travel, the travel is incidental and they have a relevant past history (e.g. eczema or acne), possibly exacerbated by a hotter climate.

In addition, it is important to establish some key facts relevant to their recent travel which include:

- *Where has the patient been?* Try to establish the nature of the travel and determine, for instance, if they were trekking 'up country' or staying in a four-star city hotel. In addition, the type of terrain they have visited (e.g. camping by a fast-running river) may be important.
- *Has anyone else on the trip been affected?* Knowing that others have a similar problem is useful and also potentially indicative of common exposure
- *Have they been taking any medications?* This includes both recent and long-term treatments. Sun sensitivity conditioned by drugs, photodermatitis, can appear first after prolonged sun exposure.

CLINICAL EXAMINATION

Examine the patient, noting the morphology and distribution of the rash. Particularly look for evidence of rash affecting sites exposed to the sun or to insect bites

Key steps in the examination involve answering the following questions:

Is there a rash?

No evidence of rash

Pruritus may be the first sign of evidence of internal disease often not relevant to travel (e.g. chronic renal failure, liver disease). However, it may also be a sign of urticaria (symptomatic dermographism). Itching may also be the earliest sign of skin invasion by schistosomes, including nonhuman pathogens such as avian cercariae contracted while swimming, which do not cause further problems.

There is a rash but is it generalized or localized?

Localized itchy rash

Localized severe itching may be the earliest sign of acute papular onchodermatitis, the early phases of onchocerciasis. The rash is subtle with diffuse dermal edema and papule formation, often confined to one limb or body region (Fig. PP50.1); the shoulder or head may be involved in South American infections. Look for evidence of nodules (unlikely in a traveler) but a skin snip or skin biopsy from a papule is potentially diagnostic (Fig. PP50.2). A history of exposure by a river is important.

Plant- or animal-induced allergy may present with a localized itchy rash. Sometimes in the former case sun exposure may accentuate the rash which present with blisters or bullae. Jellyfish stings present with an itchy localized rash which is often arranged in linear streaks, reflecting the distribution of tentacles. The patient will have been sea bathing.

New dermatophyte infections are unusual in a patient after a short visit overseas. However, exposure to a warm climate may trigger tinea pedis or tinea cruris. Look for an inflammatory raised margin to the rash. Secondary Gram-negative infection of tinea pedis is also common in a warm humid environment. In such cases the itching may diminish and local tenderness or frank pain develops. The skin between the toe webs is eroded and raw but sometimes there is a diagnostic green



Fig. PP50.1 Acute papular onchodermatitis in a traveler.

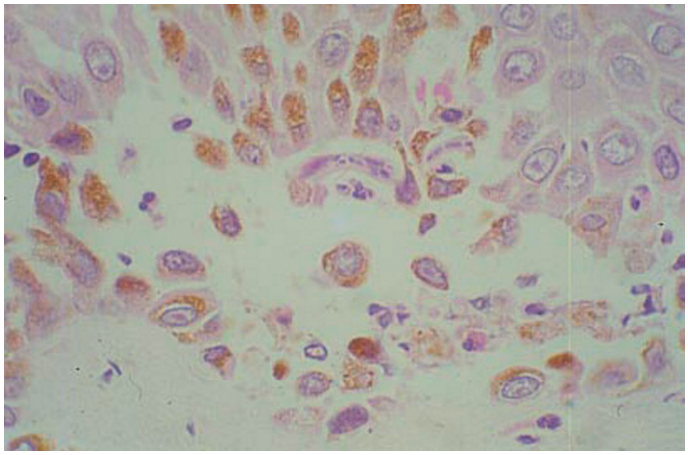


Fig. PP50.2 Skin biopsy (hematoxylin and eosin) showing edema and inflammatory infiltrate near the dermal–epidermal junction. A microfilaria can be seen.



Fig. PP50.3 *Pseudomonas* infection secondary to tinea pedis.

tinge, indicating the presence of *Pseudomonas* (Fig. PP50.3). This is important to recognize as antifungals alone are ineffective and it is necessary to use an antiseptic such as povidone–iodine or 1% acetic acid.

If the itch is localized or confined to the foot and there is a visible nodule, ask about other sensations such as a feeling of movement; in myiasis (*Dermatobia* spp.) infection often presents in this way. Tungiasis, due to *Tunga penetrans*, may present in travelers with a localized pustular lesion, usually on the foot, with a central dark area (Fig. PP50.4). If the rash is localized to the legs or buttocks but has a sinuous morphology, cutaneous larva migrans is a possibility. The patient will usually have been sitting or lying on a beach (Fig. PP50.5).

There are other diseases that may affect a visitor to Ecuador that present with a localized skin rash which is only moderately itchy. Contrary to classic teaching, itching may be an early presenting sign, although it is seldom severe, in certain tropical conditions.

Cutaneous leishmaniasis. This sometimes starts as an itchy localized nodule which develops slowly into an indurated lesion or a localized ulcer. Patterns vary but multiple lesions may develop (Fig. PP50.6) or a lymphangitic form can present. Patients often ascribe the lesion to unresolved insect bites. Taking a biopsy or a curetting from such lesions for histology or polymerase chain reaction (PCR) can be very helpful.

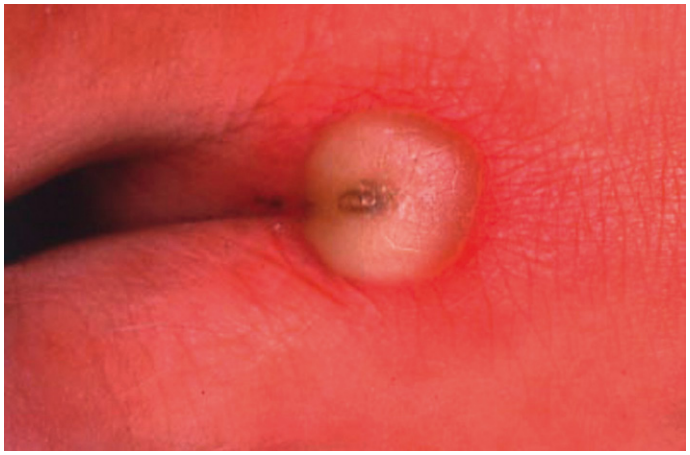


Fig. PP50.4 Local lesion of tungiasis.



Fig. PP50.5 Cutaneous larva migrans.



Fig. PP50.6 Cutaneous leishmaniasis presenting with clustered lesions (*Leishmania braziliensis* complex).



Fig. PP50.7 Lymphangitic sporotrichosis.

Sporotrichosis. This may start as a localized itchy nodule. While less common in travelers it is well recognized on those staying for a longer period and particularly those doing volunteer work in rural areas. Sporotrichosis classically presents with an ascending lymphangitic pattern of clinical lesions with nodules or ulcers along the course of a lymphatic and a primary distal lesion (Fig. PP50.7). On the face it also develops induration or ulceration and can closely resemble leishmaniasis. Sporotrichosis is often sparsely populated with yeast form fungi and culture is the best way of reaching a diagnosis.

Open wounds/sores. Open wounds or sores that itch seen in the more adventurous traveler who has been trekking may suggest a potential diagnosis of ecthyma (Fig. PP50.8). Again, these are often described as nonhealing bites but they may be covered by a dark crust which overlies a deep but regular crater. In a tropical environment streptococcal infection is the common cause.

Generalized itchy rash

It is important to note the morphology of the lesions. Possibilities in a traveler from Latin America include the following:

- Widespread insect bites. These are usually papular but may have necrotic centers. If some persist, consider cutaneous leishmaniasis (see above).
- Small target-like lesions symmetrically distributed on the face and peripheries and shallow oral ulcers might suggest erythema multiforme. This is most commonly triggered by reactivation of human herpesvirus 1 infection following sun exposure (Fig. PP50.9). However, some endemic mycoses may trigger this response – for example, histoplasmosis in the acute phase may present with erythema multiforme. A history of cave exploring might suggest this possibility.

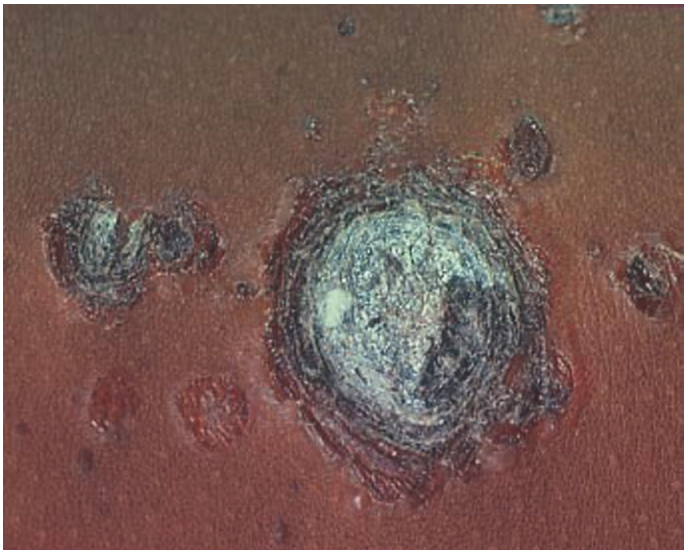


Fig. PP50.8 Streptococcal ecthyma.



Fig. PP50.9 Early erythema multiforme triggered by human herpesvirus 1.

While ultimately diagnosis will often depend on appropriate investigations, a careful history may well provide the clue to the diagnosis.

FURTHER READING

Further reading for this chapter can be found online at http://www.expertconsult.com

Practice point | 51 |

Jeremy Day

Jaundice in a returned traveler from Nepal

BACKGROUND

Nepal is an increasingly popular destination for travelers attracted by its spectacular scenery and culture. There are 500 000 tourist visitors each year, and the number is rising with the ending of political conflict. Although a major earner of foreign currency, the average spend per tourist is low at around 50 USD/tourist/day, and Nepal remains extremely poor. There has been improvement in access to drinking water, but problems remain, including contamination with arsenic and human waste. Moreover, ancient traditional water sources such as the spouts in Figure PP51.1 remain popular, often prized for their taste. Outside Kathmandu the mountainous terrain is a major obstacle to communication and the development of modern sanitation and safe water supplies. Thus water-borne disease is an important cause of illness. In the south of the country where the elevation is lower the climate is more tropical and malaria is endemic. Comparative country health indicators are illustrated in Table PP51.1.

POTENTIAL CAUSES OF JAUNDICE IN NEPAL

There is a multitude of causes of jaundice. In addition to contact with pathogens perhaps not found in the traveler's normal environment (e.g. hepatitis E virus, *Salmonella enterica* serovar Typhi), holiday travel is associated with increased recreational risk-taking behavior, including sexual activity and drug taking (alcohol included).



Fig. PP51.1 Collecting water from traditional water spouts in Patan, Nepal.

NON-INFECTIOUS CAUSES OF JAUNDICE

This section focuses on the differential diagnosis of infectious causes of jaundice, but of course there are many non-infectious causes. Additional non-infectious causes for the tropical traveler include drug-related hepatitis which has been described in patients using mefloquine, amodiaquine and atovaquone–proguanil for malaria chemoprophylaxis. It is also well recognized with fluoroquinolones, sometimes prescribed for self-treatment of travelers' diarrhea. Sale of pharmaceuticals is less regulated than in industrialized countries, with increased opportunity for self-medication.

INFECTIOUS CAUSES OF JAUNDICE

Potential infectious causes of jaundice are listed in Table PP51.2. This is not exhaustive! Early diagnosis and treatment are key in obtaining a good outcome from most infectious diseases. Treatable potentially fatal diseases such as malaria and enteric fever should always be considered and excluded in any patient returning from the tropics.

Table PP51.1 Comparative country indicators 2006 (UNICEF)

	Nepal	Vietnam	USA
Gross national income per capita, US dollars	290	690	44 970
Infant mortality rate/1000 live births	46	15	6
Life expectancy at birth, years	63	74	78
Proportion of population using improved drinking water, percent	89	92	100
Proportion of population with improved sanitation, percent	27	65	100
Proportion of government expenditure allocated to health, percent	5	4	24
Total adult literacy rate, percent	49	90	–

Table PP51.2 Infectious causes of jaundice

Pathogen		Comment
Viral	Hepatitis A virus Hepatitis B virus Hepatitis C virus Hepatitis D virus Hepatitis E virus Epstein Barr virus Cytomegalovirus Human immunodeficiency virus	Requires co-infection with HBV
Bacterial	*Listeria monocytogenes* *Neisseria* spp. Pneumococcus	As part of disseminated disease, i.e. severe sepsis
	Coxiella burnetti *Rickettsia conori*	Cause granulomatous hepatitis but jaundice rare. No reported *Coxiella* infections from Nepal
	Treponema pallidum	In secondary syphilis. Primary disease may be asymptomatic depending upon the site of the lesion Fitz-Hugh–Curtis syndrome
	Chlamydia spp. *Brucella* spp. *Leptospira* spp. *Salmonella enterica* *Mycobacterium tuberculae*	Typhi and Paratyphi A The commonest cause of granulomatous hepatitis worldwide
Parasitic	*Entamoeba histolytica* *Falciparum* spp. *Leishmania* spp. *Fasciola hepatica/gigantica*	If there is compression of bile ducts by abscess The presence of jaundice defines severe malaria Can cause granulomatous hepatitis, jaundice is rare Abnormal liver enzymes common in acute disease, jaundice can be a feature of established infection

Hepatitis E

Hepatitis E virus (HEV) is endemic in the developing world and is the commonest viral cause of acute hepatitis in Nepal. Probably only one-quarter of infections are symptomatic, but disease can be severe, particularly in pregnant women, with a death rate up to 25%. There have been large-scale outbreaks reported from several countries in recent years, including Nepal, but the vast majority of disease burden is accounted for by sporadic cases. The predominant route of infection is fecal–oral, but parenteral, vertical, person-to-person and zoonotic (domestic swine and rodents) routes may also be important.

In Nepal, some reports have attributed up to 90% of cases of acute infectious jaundice to HEV, but the incidence (and relative prevalence) seems to vary from year to year. The incidence is higher in the rainy season, perhaps related to fecal contamination of drinking water. Four genotypes of HEV have been described, but only one serotype, which offers the hope of development of a broadly specific vaccine.

The presentation and clinical course of hepatitis E disease, with the exception of the high mortality in pregnancy, is similar to that of hepatitis A. Large outbreaks are not such a feature of hepatitis A, and knowledge of any outbreak in the country visited by the traveler is helpful. The incubation period is in the range of 2–8 weeks. However, HEV cannot be distinguished clinically from other causes of acute hepatitis, and definitive diagnosis relies on serologic testing. Some reports have suggested the period of viremia can be prolonged; however, resolution of biochemical hepatitis seems to correlate strongly with clearance of virus from stool.

There is no specific treatment for HEV. Vaccines are in development, and a recent study in Nepal found good tolerability and efficacy from a course of three doses with a recombinant HEV vaccine.

Hepatitis A

Hepatitis A virus (HAV) is endemic in Nepal. One survey found between 3% and 8% of acute hepatitis to be due to HAV. It would be expected that the incidence of this disease would be falling in travelers thanks to the availability of effective vaccines. Spread is fecal–oral, and the incubation period is 2–6 weeks. Most infected adults (70%) become jaundiced, although children under 2 years are usually asymptomatic. Fulminant hepatic failure is rare (0.2%).

A study in 1995 found HAV to be the predominant cause of acute jaundice in foreigners in the Kathmandu valley, which contrasted with the indigenous population (predominantly HEV), but this may have changed with increased HAV vaccination.

Hepatitis B

Hepatitis B vaccination is increasingly offered to travelers. Risk factors for HBV infection are clearly defined – sex, contaminated medical equipment/blood products, intravenous drug use and vertical transmission – and a careful history may be revealing. The sex tourism industry is a growth area in South Asia. Diagnosis is through serologic testing. The incubation period is 2–6 months.

HBV accounts for between 2% and 15% of cases of acute infectious hepatitis in Nepalese patients, but the risk for local Nepalese of HBV infection is unlikely to be the same as that for visitors to the country.

Hepatitis C

Hepatitis C virus (HCV) accounts for 3–5% of acute infectious hepatitis cases in Nepal. There are few data, but most infections are due to genotypes 1 or 3. HCV is predominantly transmitted via blood, blood

products and intravenous drug use. There is a very low risk of sexual transmission. In addition, virus can be transmitted through tattooing, but this risk is often not recognized by travelers. The incubation period is between 2 weeks and 6 months. Most infections with HCV lead to chronic disease (80%) and are initially asymptomatic. Less than 25% of infections are acutely jaundiced.

Early diagnosis and treatment of HCV may result in increased cure rates, and the diagnosis should be excluded in any jaundiced patients or any asymptomatic patient with a significant exposure history.

Enteric fever

Enteric fever is the commonest identifiable cause of fever in patients presenting to Patan Hospital in Lalitpur, Kathmandu. A survey of febrile patients in 2001 identified a pathogen in 37%, and 13% of all patients had culture-confirmed enteric fever. Between 1% and 2% of patients with enteric fever are jaundiced. The incubation period is 1–4 weeks.

The past 10 years have seen the increasing importance of *Salmonella enterica* serovar Paratyphi A as a cause of enteric fever, and worryingly rates of drug resistance for this organism seem to be outstripping those of *Salmonella enterica* serovar Typhi. However, later generation fluoroquinolones, such as gatifloxacin, are effective. Marrow culture is the most sensitive diagnostic test for enteric fever. There may be only one colony-forming unit of *S. enterica* in 5 ml of blood, and diagnostic sensitivity is improved through taking a large volume of blood for culture. In Patan, about a third of patients with a clinical diagnosis of enteric fever have positive blood cultures.

Leptospirosis

Leptospirosis is widely distributed in both temperate and tropical countries and has been associated with recreational activities in travelers, including white water rafting, canoeing and triathlons. One survey of blood donors and army recruits in eastern Nepal found 12% of the population to be seropositive. Jaundice occurs in a subset of patients, although many infections are subclinical. Serologically confirmed leptospirosis accounted for 4% of all cases of fever in adults at Patan Hospital. The incubation period is between 5 and 14 days.

Headache and fever are the most frequent symptoms; other manifestations include cough, pharyngitis, myalgia, abdominal pain, hepatosplenomegaly and conjunctival suffusion. Clearly the clinical features are very similar to enteric fever, although jaundice is more common.

Diagnosis is made through paired sera; isolation of the organism is difficult but blood cultures may be positive if taken early in disease. Nucleic acid amplification techniques, including real-time polymerase chain reaction (PCR), have been developed as diagnostic tests, but are not widely available. Doxycycline or benzyl penicillin are commonly used for treatment.

Malaria

Hyperbilirubinemia is a manifestation of severe malaria. There are around 10 000 cases of malaria each year in Nepal. Most of these (90%) are *Plasmodium vivax*; *P. falciparum* accounts for the remainder. Transmission is limited to elevations of less than 1500 m, and there is seasonal variation in the transmission rate, the incidence being highest in June and August, and lowest from November to March. The Terai region has the highest transmission rates.

The risk to travelers is low; the Kathmandu valley and Himalayan districts, most frequently visited by tourists, are probably malaria free. However, travelers cannot always accurately recall their travel itinerary, exclusion of malaria is cheap and simple, and failure to diagnose malaria results in delay in treatment with potentially fatal consequences. Malaria should be excluded from all unwell travelers returning from endemic countries. If there is doubt about the infecting species, the patient should receive therapy active against *P. falciparum*, i.e. an artemisinin derivative or quinine. The SEAQUAMAT trial demonstrated the superiority of artesunate over quinine for the treatment of severe malaria.

Rickettsial disease

There is serologic evidence of possible acute infection with *Rickettsia typhi* (murine typhus) or *Orientia tsutsugamushi* (scrub typhus) in up to 27% and 23% of patients, respectively, presenting with febrile illness to hospitals in the Kathmandu area. Polymerase chain reaction for *R. typhi* is less frequently positive – 7% of febrile patients presenting to Patan Hospital. There are no data for *O. tsutsugamushi*.

In a series from Thailand, jaundice occurred in 35% of patients with scrub typhus, but this seems to be a rare feature in Nepal, where the presenting syndrome is similar to enteric fever. Treatment is with doxycycline.

Brucellosis

Granulomatous hepatitis can be a feature of brucellosis. The prevalence of brucellosis in Nepal is not known, but it has been reported. Presentation can be similar to enteric fever. The diagnosis should be considered in those with an appropriate exposure history. Risk factors for exposure are consumption of unpasteurized milk products and contact with animal carcasses or aborting animals. The incubation period is variable, and can be protracted, but is usually between 5 and 60 days.

Diagnosis is through culture of the organism – from marrow, blood or other affected tissue. Persistent negative serology excludes infection, but interpretation of low-level positive serologic results is difficult. A recent Cochrane meta-analysis found combined treatment with a tetracycline and streptomycin to be associated with better cure and lower relapse rates than treatment with rifampin (rifampicin)-based regimens.

SUMMARY

The most likely infectious cause of acute jaundice in Nepal is hepatitis E virus. While rare, potentially life-threatening infections that less commonly cause jaundice, such as malaria and enteric fever, must be excluded in all patients. Meticulous history taking with particular attention to exposures and risks is key in developing a coherent differential diagnosis and instituting timely treatment.

FURTHER READING

Further reading for this chapter can be found online at http://www.expertconsult.com

Practice point | 52 |

Willem Sturm

Sexually transmitted infection in a returned traveler from Durban

CASE STUDY

A 42-year-old man seeks medical assistance because of the development a single lesion on his penis. Three weeks ago he returned home from a 3-day business trip to Durban, South Africa. He noticed the lesion 3 days ago. He admits that he had unprotected sex with a South African woman whom he met in a bar. The lesion is painful, bleeds easily and its edges are undermined (Fig. PP52.1). Your clinical diagnosis is chancroid. You collect a specimen from the ulcer base and send this to the diagnostic laboratory for polymerase chain reaction (PCR) together with a blood specimen for syphilis and HIV serology. The results of the laboratory tests are shown in Table PP52.1.

Table PP52.1 Results of laboratory tests

PCR	*Haemophilus ducreyi* *Treponema pallidum* Herpes simplex virus 2 *Chlamydia trachomatis*, biovar LGV	Negative Negative Negative **Positive**
Serology	HIV Fluorescent treponemal antibody absorption (FTA-ABS) Rapid plasma reagin	Negative Negative 1:1

BACKGROUND INFORMATION

Durban is the second largest city in South Africa and has the largest harbor on the African continent. It is situated in the province of KwaZulu-Natal. The province has the highest prevalence of HIV in South Africa and, although no recent comparative surveys have been done, it is likely to have the highest prevalence of other sexually transmitted infections (STIs) as well.

The general concept is that STIs circulate within a limited number of individuals in a population. However, the existence of such a core group in KwaZulu-Natal is challenged by the presence of nonulcerative STIs in 35% of consecutive first-time antenatal clinic attendees who perceive themselves as asymptomatic. These figures show that STIs are widespread among members of the KwaZulu-Natal population and are not restricted to individuals that seek medical care for such a disease.

Studies on the prevalence of the different organisms that cause the different syndromes in patients attending the specialized STI clinic in the centre of Durban are shown in Table PP52.2. Although these relative prevalences change over time, all known causative agents of STIs are present in the KwaZulu-Natal population and Table PP52.2 shows the most recent available data. *Haemophilus ducreyi*, the most prevalent cause of genital ulcers a decade ago, has disappeared. Multiple infections are found in approximately 10% of patients.



Fig. PP52.1 Lymphogranuloma venereum with chancroid-like ulcers.

Table PP52.2 Etiology of the three main STI syndromes in Durban, South Africa

	PREVALENCE (%) IN PATIENTS WITH		
	Male urethritis	**Vaginal discharge**	**Genital ulcer syndrome**
Neisseria gonorrhoeae	52	5	
Chlamydia trachomatis, biovar OG	16	5	
Mycoplasma genitalium	5		
Trichomonas vaginalis		20	
Bacterial vaginosis		52	
Chlamydia trachomatis, biovar LGV			6
Treponema pallidum			4
Haemophilus ducreyi			0
Herpes simplex virus 2			34
Calymmatobacterium granulomatis			6

Susceptibility testing of *Neisseria gonorrhoeae* isolates reveals high levels of resistance to penicillins, tetracycline and fluoroquinolones, borderline susceptibility for spectinomycin but susceptibility to cephalosporins. Therefore, the only effective treatment available for a patient with gonorrhea contracted in Durban is a third-generation cephalosporin (cefepime or ceftriaxone).

GENERAL APPROACH

Since STIs are so prevalent in KwaZulu-Natal, each traveler to Durban who is sexually active during this visit with a South African partner can become infected with one or more STI pathogens. This is not restricted to sex with a commercial sex worker. The infections result in one or more of three clinical syndromes: male urethritis (MUS), vaginal discharge (VDS) and genital ulcer syndrome (GUS). MUS and VDS are caused by the same group of microbes while GUS has a different spectrum of pathogens. Although each of these infections has their 'textbook' clinical presentation, it has been well established that the clinical presentation is a poor predictor of the etiology for all three syndromes. Apart from atypical presentations, pathogens causing MUS and VDS also cause asymptomatic infections in a significant proportion of infected individuals.

An etiologic diagnosis is dependent on the laboratory diagnosis. Since microscopy and culture has low sensitivity or is not available, nucleic acid amplification tests need to be performed. This technology is only available in specialized laboratories except for *Chlamydia trachomatis*. In patients with GUS who have acquired their infection in Durban, asymptomatic MUS or VDS should also be considered.

PRINCIPLES OF MANAGEMENT OF SEXUALLY TRANSMITTED INFECTIONS

There are two approaches to management of STIs: a syndromic approach and an etiologic approach. Which one to apply is determined by the prevalence of STIs in the patient population and the availability of advanced laboratory tests.

The syndromic approach is practiced in the public health sector in South Africa since the prevalence is high and the availability of diagnostic laboratory capacity is limited. The government advocates this approach for use in the private sector also. In this approach, patients are diagnosed at syndrome level and treated for all causes of that particular syndrome in the part of the world where the acquisition of infection took place. The organisms to be covered for the different syndromes for disease acquired in Durban are summarized in Table PP52.2. Syndromic management protocols need regular revision based on local surveillance data.

Table PP52.3 Differential diagnosis for nonhealing genital ulcers

Sexually transmitted infection	Other causes
Noncompliance to treatment	Malignancy
Inadequate duration of treatment	Tuberculosis
Resistant STI microbes	Hydradenitis in the groin
Herpes simplex virus in an immunocompromised patient	Pyodermas
Secondary bacterial infection	Behçet's disease

For patients developing symptomatic STIs in countries like the UK where the prevalence of these infections is much lower and laboratory capacity higher, the etiologic approach is more appropriate.

MANAGEMENT OF THE PATIENT PRESENTED IN THE CASE STUDY

The results of the laboratory tests indicate that this patient has lymphogranuloma venereum (LGV). The PCR applied includes a restriction step that allows differentiation between the different biovars of *C. trachomatis*. A rapid plasma reagin (RPR) test of 1:1 with a negative fluorescent treponemal antibody absorption (FTA-ABS) test is not in keeping with syphilis as positivity in the FTA-ABS precedes a positive RPR, the RPR titer is very low and the *Treponema pallidum* PCR on ulcer secretions was negative. Repeat syphilis serology on the follow-up visit at 3 weeks should be considered.

The classic clinical presentation of LGV is a small, self-healing ulcer followed by lymphadenopathy. However, in Durban LGV presents with chancroid-like ulcers. Management of patients with LGV is 3 weeks of doxycycline or erythromycin. Patients should be seen weekly for follow-up till the ulcer has healed. If no healing tendency is observed at 2 weeks from the start of treatment, an alternative diagnosis should be entertained (Table PP52.3). The approach to possible asymptomatic infection of the urethra in this patient is to collect a urethral specimen for diagnosis.

FURTHER READING

Further reading for this chapter can be found online at http://www.expertconsult.com

Practice point | 53 |

James Seddon

Eosinophilia in a returned traveler from West Africa

INTRODUCTION

Eosinophils are bone marrow-derived leukocytes that have differentiated into first myeloid and then granulocyte (basophil/eosinophil) lineages. They are present in body tissues, especially those of the gut and lungs, at concentrations much higher than the peripheral circulation. They are produced and activated through stimulation from interleukin (IL)-4, IL-5 and IL-13 which are secreted by type 2 T-helper lymphocytes (Th2). These cells have differentiated from T-helper lymphocytes under direction from IL-4. In contrast, cytotoxic Th1 responses are driven by interferon gamma which also cross-inhibits Th2 responses.

Eosinophils seem to have evolved to defend against the tissue stages of parasitic invasion where the organism is too large to be phagocytosed. IgE is synthesized by activated plasma cells which initially become attached to the membranes of mast cells and basophils. Once the parasite has been opsonized by IgE the mast cell degranulates, releasing inflammatory mediators. One of the main cells drawn to the site of this mediatory release is the eosinophil. The parasite is also opsonized by IgG and complement which, together with IgE, activate the eosinophil, causing release of lytic proteins and superoxides. One by-product of this response is the development, in certain individuals, of hypersensitivity to non-pathologic antigens, leading to allergy, atopy and high circulating levels of IgE, mast cells and eosinophils.

Eosinophilia is generally defined as the presence of greater than 450–600 eosinophils/mm^3 of peripheral blood. Although this absolute number is probably of most value, a white blood cell count in which greater than 7% are eosinophils is also significant.

DEFINITION OF THE PROBLEM

A 26-year-old aid worker returned from Ghana where he had spent 9 months working on a rural development project. Prior to departure there were no outstanding health problems and he had taken appropriate malaria prophylaxis for the duration of his trip. During his time there he had stayed in fairly basic accommodation, eaten locally produced food, walked barefoot on numerous occasions and had swum in freshwater rivers and lakes.

Two weeks after return he started to develop headaches, evident in the morning but resolving during the day. He had episodes of sweating and fever as well as sore muscles and joints. After a further 2 days he woke with an urticarial rash over his torso, which came and went as the day progressed. The next morning he woke with mild swelling of his lips and tongue. At this point he decided to consult his general practitioner who sent him to hospital for investigation. A malaria slide was negative but a full blood count revealed an eosinophil count of 900 eosinophils/mm^3 with a 14% fraction.

CAUSES

Without any history of travel, a raised eosinophil count would probably hint towards an atopic condition such as hay fever, asthma or eczema. However, in a traveler returning from a developing country it should suggest helminth infection to the investigating physician. Nevertheless, it is important not to miss other causes, some of which may be as a consequence of the travel and some incidental to it. It is also important to bear in mind that the absence of a raised count in no way excludes a helminth infection. A full list of causes of a raised eosinophil count is provided in Table PP53.1, with the pathophysiology, epidemiology and treatment of the infectious causes being detailed more extensively in the main text.

HIV and human T-cell lymphotrophic virus 1 (HTLV-1) may be accompanied by a raised eosinophil count in certain situations. Resolving scarlet fever is said to be associated with eosinophilia, as are brucellosis and lepromatous leprosy. Unicellular protozoan parasites almost never cause eosinophilia, the occasional exceptions being *Isospora belli* and *Dientamoeba fragilis*. Aspergillosis, in the form of allergic bronchopulmonary aspergillosis, and primary or disseminated coccidioidomycosis can be associated with an elevated eosinophil count, as can scabies and myiasis infestations.

In terms of non-infectious causes, it is always important to consider drug reactions, especially as travelers have often been taking unfamiliar medications. Common culprits are sulfa-based drugs, which include Septrin and Fansidar, but quinine, β-lactams and tetracyclines have also been implicated. Adrenal insufficiency, as well as numerous inflammatory, neoplastic and hypersensitivity conditions, should be considered; however, in the majority of cases, in those returning from the tropics, the cause will be helminthic.

INVESTIGATION AND MANAGEMENT

Any patient found to have eosinophilia needs to be questioned carefully and examined thoroughly. Whilst the history should be comprehensive, there are certain useful questions that should be considered which may direct the physician to a particular diagnosis. These are outlined in Table PP53.2 with the indicated diagnosis. In a similar way the examination needs to be complete, covering all systems but certain findings may indicate a specific condition. These are outlined in Table PP53.3.

Table PP53.1 Causes of a raised eosinophil count

Non-infectious causes		
Drug reactions	Sulfa-based drugs • Fansidar (pyrimethamine–sulfadoxine) • Septrin (trimethoprim–sulfamethoxazole) β-Lactams Quinine and quinidine Non-steroidal anti-inflammatory drugs Tetracyclines Aspirin Beta-blockers	
Atopic/allergic conditions	Urticaria Pemphigoid Asthma/hayfever Eczema Loeffler's syndome Chronic eosinophilic pneumonia Tropical pulmonary eosinophilia Idiopathic hypereosinophilic syndrome	
Neoplasms	Lymphoma – especially Hodgkin's Chronic myeloid leukaemia Acute myeloid leukaemia Eosinophilic leukaemia Some solid-organ malignancies	
Connective tissue diseases	Rheumatoid arthritis Churg–Strauss syndrome Midline granuloma Sarcoid Polyarteritis nodosa Dermatomyositis Wegener's granulomatosis	
Endocrine	Addison's disease	
Non-helminth infectious causes		
Fungal	Aspergillosis – allergic bronchopulmonary aspergillosis Coccidioidomycosis – primary or disseminated	
Ectoparasites	Scabies Myiasis	
Bacterial and viral	Scarlet fever – resolving Brucellosis Leprosy – lepromatous HIV Human T-cell lymphotropic virus 1	
Protozoa	*Isospora belli* *Dientamoeba fragilis*	
Helminth causes		
Nematodes (roundworms)	Intestinal	*Ascaris lumbricoides* Hookworms • *Ancylostoma duodenale* • *Necator americanus* *Strongyloides stercoralis* *Oesophagostomum bifurcum* Visceral larva migrans • *Toxocara canis* • *Toxocara cati* Cutaneous larva migrans • *Ancylostoma braziliense*
	Tissue	*Wuchereria bancrofti* *Brugia malayi* *Mansonella perstans* *Loa loa* *Onchocera volvulus* *Trichinella spiralis* *Gnathostoma* spp.
Trematodes (flukes)	*Schistosoma mansoni, haematobium, japonicum, mekongi* • Chronic infections • Acute infections – Katayama fever Avian and mammalian schistosomiasis – swimmers' itch (schistosome dermatitis) *Clonorchis sinensis* *Opisthorchis viverrini* *Fasciola hepatica* *Paragonimus westermani*	
Cestodes (tapeworms)	*Taenia saginata* *Taenia solium* *Diphyllobothrium latum* *Hymenolepis nana* *Echinococcus granulosus*	

Table PP53.2 History to be considered

Geography traveled	Equatorial West Africa Sub-Saharan Africa, Arabian peninsula and some areas of elevated Central America Southwestern United States, northern Mexico South East Asia	*Loa loa* Onchocerciasis Coccidioidomycosis *Gnathostoma* spp. and *Clonorchis*
Activities	Swimming in fresh water Walking barefoot Sheep-rearing	Schistosomiasis Cutaeous larva migrans, hookworm, *Strongyloides* *Echinococcus granulosus*
Food	Pork Boar, bear, horse, walrus, wart hog Beef Crab Fish, seafood or animals fed on fish Watercress Horse chestnuts	Trichinosis, *Taenia solium* Trichinosis *Taenia saginata* *Paragonimus* Fish tapeworm, *Gnathostoma* *Fasciola* *Fasciolopsis*

Investigations

Various approaches to investigation have been proposed but it is probably best to start with a basic laboratory workup. Other tests can then be added if there are specific findings on history and examination, or if something is discovered on the initial tests (Fig. PP53.1).

It is obviously better to identify a specific cause and treat it with targeted anthelmintics as this leads to more effective treatment and less unnecessary toxicity. However, if no diagnosis is forthcoming

Table PP53.3 Examination findings and possible pathology	
Hydroceles, unilateral lower limb swelling	Lymphatic filariasis
Myositis, periorbital edema, splinter hemorrhages	*Trichinella*
Nodules	Onchocerciasis, *Loa loa*
Rash, pruritus	Onchocerciasis, *Loa loa*, cutaneous larva migrans, allergy (particularly to foods), vasculitis
Subcutaneous swellings	*Loa loa* or *Gnathostoma*
Larva currens	*Strongyloides*
Pulmonary findings – wheeze/cough/shortness of breath	*Ascaris*, *Strongyloides*, *Paragonimus*, tropical pulmonary eosinophilia, filariasis, asthma, aspergillosis
Hepatomegaly/tender right upper quadrant	Visceral larva migrans, schistosomiasis, liver flukes
Neurologic signs	Cysticercosis, trichinosis, schistosomiasis, strongyloidiasis

after extensive investigation or if resources are limited, then two options are available depending on the clinical status of the patient:

- first, the tests are repeated after an interval of 3 months; and
- second, presumptive treatment with albendazole and/or praziquantel can be given, targeted towards the perceived likely pathogens.

Complications

Normally eosinophilia does not lead to complications. However, if the levels have been severely raised or raised for a prolonged period of time, such as with the idiopathic hypereosinophilic syndrome, then organ damage can occur. The heart is the organ most commonly affected, resulting in endomyocardial fibrosis, pericarditis and valvular pathology. Other organs can also be involved, leading to neuropathy, skin lesions, encephalopathy, respiratory problems, enteritis, hepatitis and cholangitis.

FURTHER READING

Further reading for this chapter can be found online at http://www.expertconsult.com

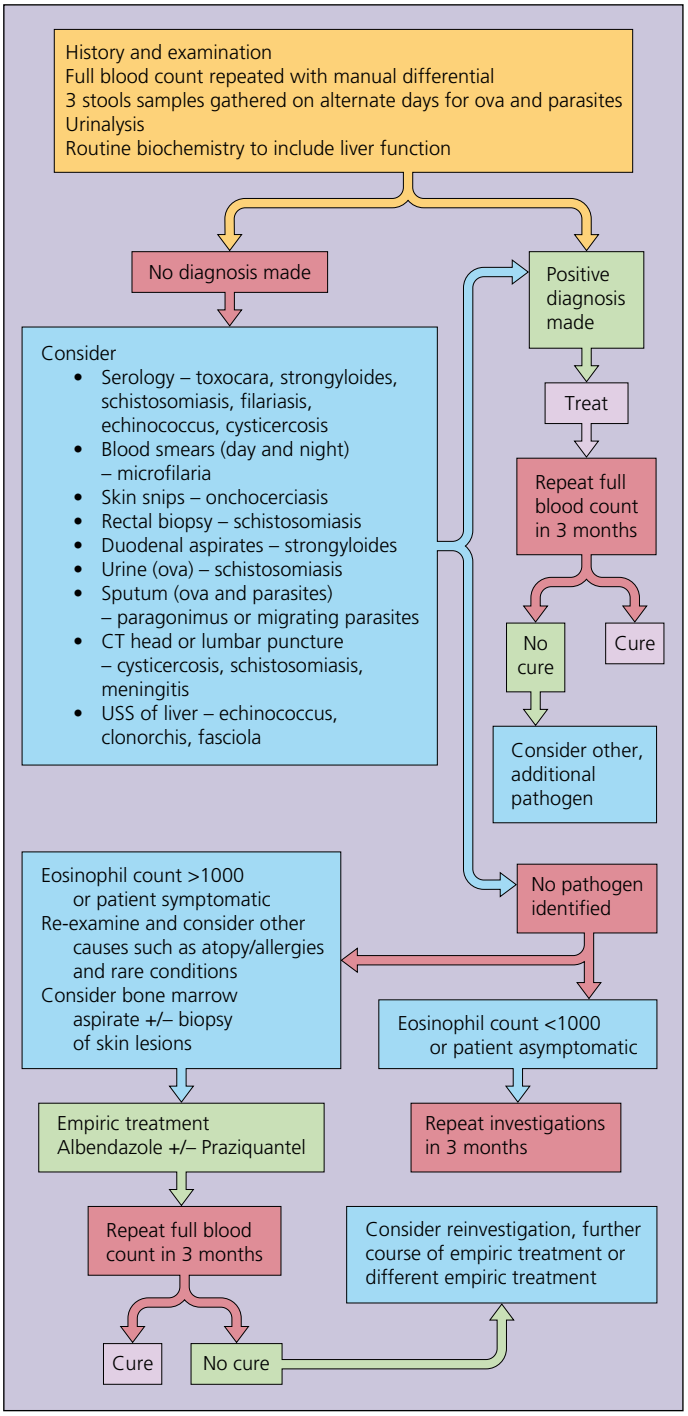


Fig. PP53.1 An algorithm for the investigation and management of eosinophilia.

Practice point | 54 |

Tom Doherty

Lymphadenopathy, splenomegaly and anemia in a returned traveler from Sudan

CASE HISTORY

A 32-year-old Caucasian man who had worked for an international aid agency in rural parts of Sudan for the last 3 years presented to hospital. He had grown up in the UK but had spent considerable periods of his working life overseas, mostly in East Africa. He complained of a fever with night sweats for the previous 6 weeks and, despite maintaining his appetite, had lost 8 kg in weight. He had no significant past medical history and, apart from doxycycline as prophylaxis against malaria, he was taking no regular medication. He smoked 10 cigarettes a day, drank alcohol in moderation and had no concerns regarding the possibility of having acquired HIV infection. He had no cough or other respiratory complaints and had not experienced any gastrointestinal symptoms.

On examination, he looked pale and unwell. He had an axillary temperature of 101.3°F (38.5°C) but his pulse rate and blood pressure were normal. He had generalized, nontender lymphadenopathy with palpable nodes in his neck, both axillae and groin, none of which was more than 1 cm in diameter. There were no peripheral stigmata to suggest HIV/AIDS. His throat looked normal, his chest was clear and examination of his cardiovascular system was unremarkable. He had marked splenomegaly with a spleen that was palpable 8 cm below the left costal margin, but no hepatomegaly. He was oriented in time, place and person and had no neurologic deficit.

Preliminary investigations revealed a pancytopenia, with a hemoglobin of 11 g/dl, total white cell count of 2.4 × 10^9/l and a platelet count of 131 × 10^9/l. A malaria parasite screen was negative on both thick and thin films. Both his erythrocyte sedimentation rate and his C-reactive protein were elevated above 100. His renal function was normal and, apart from a slight elevation in his alanine transaminase concentration, his liver function tests were unremarkable. However, his albumin concentration was low at 28 g/l. His international normalized ratio (INR) was 0.97. Chest X-ray was reported as normal.

DISCUSSION

In the context of any patient with suspected imported infection, it is often helpful to answer the four questions which comprise the maxim attributed to Professor Eldryd Parry: 'Why does this person, from this place, develop these symptoms at this time?' With regard to this case, the 'person' was a Caucasian professional who would be expected to have little or no immunity to malaria; in addition, he did not appear to be at particularly high risk of HIV infection. The 'place' was rural Sudan, an area where many of the classic tropical diseases such as malaria, tuberculosis, leishmaniasis, brucellosis, relapsing fever and schistosomiasis would all be highly prevalent. The 'symptoms' were a febrile illness, characterized by generalized systemic upset with night sweats and weight loss, with anemia and splenomegaly as prominent signs. The duration of the symptoms, 6 weeks, would imply that this was a significant illness rather than a simple viral infection, for example. Taken together, the history, physical findings and laboratory investigations would strongly suggest a diagnosis of visceral leishmaniasis in an HIV-negative individual.

Other diagnoses would need to be considered. Apart from visceral leishmaniasis, the three other recognized causes of splenomegaly of this magnitude would be hyperreactive malarious splenomegaly (previously known as tropical splenomegaly syndrome), chronic myeloid leukemia and myelofibrosis. Hyperreactive malarious splenomegaly is thought to be more common in women than men, would be uncommon among a Caucasian population and might have been prevented by the antimalarial prophylaxis that this man was taking. Chronic myeloid leukemia would be unusual in a man of 32 and, like myelofibrosis, would not be expected to present with such florid systemic symptoms, nor with pancytopenia. In this particular case, the diagnosis was confirmed both serologically and by performing a splenic aspirate. The serologic tests comprise a direct agglutination test (DAT) looking for IgG against *Leishmania donovani* and a commercial K39 strip test (DiaSys Europe Ltd, Wokingham, Berkshire, UK) which detects 39 consecutive amino acids from the kinetoplast of *L. donovani*; taken together, these two serologic tests are virtually 100% sensitive and specific in an HIV-negative individual. The splenic aspirate using a 21-gauge needle and 0.5 ml of normal saline which is then stained with Giemsa permits a quantification method of the number of amastigotes found in the spleen.

An HIV test would be necessary as, if this were positive, this would have implications for his treatment. Chronic schistosomiasis secondary to *Schistosoma mansoni* would also need to be excluded and brucellosis is another possibility. A lymphoproliferative disorder should also be included in the differential diagnosis of anyone presenting with systemic symptoms, lymphadenopathy and splenomegaly.

A list of conditions that are associated with lymphadenopathy is given in Table PP54.1. Table PP54.2 lists those conditions associated with splenomegaly with some reference to the degree of splenic enlargement, and Table PP54.3 outlines a schedule for investigations for splenomegaly and/or anemia in a patient recently returned from abroad.

Table PP54.1 Causes of lymphadenopathy

	Causes	Notes
Common	Local sepsis	Cause is usually obvious
	Tonsillitis	Particularly streptococcal
	Epstein–Barr virus	Palatal petechiae, splenomegaly, atypical lymphocytes
	Nonspecific viral infection	
Less common but important	HIV seroconversion illness	Transient lymphadenopathy
	HIV (established disease)	Permanent lymphadenopathy
	Kaposi's sarcoma	
	Tuberculosis	
	Trypanosomiasis	Localized lymphadenopathy in early infection with chancre; generalized lymphadenopathy in late infection
	Toxoplasmosis	
	Secondary syphilis	
	Dengue fever	Lymphadenopathy occurs in 50% of cases; usually mild
	Chancroid	Usually inguinal lymphadenopathy
	Lymphoproliferative disorder	
	Neoplasia	Usually metastatic lymphadenopathy
	Sarcoidosis	Particularly hilar lymphadenopathy
	Leishmaniasis	Cutaneous and visceral
	Typhus	Usually painful eschar
Uncommon	*Wuchereria bancrofti*	
	Brugia malayi	
	Parvovirus	
	Q fever	
	Lymphogranuloma venereum	
	Castleman's disease	
	Rheumatoid arthritis	
	Systemic lupus erythematosus	
	Leptospirosis	
	Still's disease	
	Pityriasis rosea	
	Cat-scratch fever	

Table PP54.1 Causes of lymphadenopathy—cont'd

	Causes	Notes
Rare	Lepromatous leprosy	
	Erythema nodosum leprosum	
	Bartonellosis	Often tender
	Tularemia	
	Anthrax	
	Diphtheria	
	Plague	Extremely tender 'buboes'
	Kikuchi's disease	
	Ehrlichiosis	
	Behçet's syndrome	
	Wegener's granulomatosis	
	Midline granuloma	
	Whipple's disease	
	Weber–Christian disease	
	Podoconiosis	
Infections usually not associated with lymphadenopathy	Brucellosis	
	Loiasis	
	Onchocerciasis	Secondary infection of scratched skin may result in lymphadenopathy
	Malaria	
	Hepatitis	
	Typhoid	Lymphadenopathy has been reported in chronic salmonellosis
	Tuberculoid leprosy	
	Schistosomiasis	May occur in acute disease
	Flaviviruses	
	South American trypanosomiasis (chronic form)	
	Intestinal helminths	
	Strongyloides spp.	
	Hydatid disease	
	Cholera	
	Tetanus	
	Melioidosis	
	Cysticercosis	
	Amebiasis	
	Fascioliasis	

Table PP54.2 Causes of splenomegaly*

Mild splenomegaly	*Malaria* *Epstein–Barr virus* *Hepatitis* *Typhoid and other salmonelloses* *Tuberculosis* *Dengue fever* *Katayama fever* *Toxoplasmosis* *Cytomegalovirus* *HIV* *Leptospirosis* *Brucellosis* *Sepsis* *Trypanosomiasis* *Histoplasmosis* Rheumatoid arthritis/Still's disease Systemic lupus erythematosus Hemoglobinopathies Sarcoidosis Lepromatous leprosy and erythema nodosum leprosum Amyloidosis
Moderate splenomegaly	Lymphoproliferative disorder Subacute bacterial endocarditis Splenic abscess Portal hypertension due to chronic schistosomiasis
Marked splenomegaly	*Visceral leishmaniasis* *Hyperreactive malarious splenomegaly* Myelofibrosis Chronic myeloid leukemia Glycogen storage diseases

*Each section reflects the importance and/or frequency of the various conditions. The italic type shows the conditions that are more likely to occur in travelers.

Table PP54.3 Suggested investigations for splenomegaly and/or anemia in a patient recently returned from abroad

Initial investigations	Thick and thin blood films for malaria (repeated several times) Blood cultures (repeated several times) Full blood count (red cell indices, white cell count, evidence of hypersplenism) Blood film examination (atypical lymphocytes; red cell morphology; evidence of hemolysis)
If anemia predominates	Serum iron Transferrin, total iron binding capacity Red cell folate Vitamin B12 Fecal occult blood (repeated several times) Hemoglobin electrophoresis Also consider: bone marrow aspirate endoscopy
Serologic investigations	Epstein–Barr virus Toxoplasmosis Cytomegalovirus Malaria immunofluorescent antibody test Schistosomal ELISA *Brucella* *Leishmania* HIV Histoplasmosis Acute and convalescent sera for dengue fever and other flaviviruses Beware false-positive autoimmune disease serology
Imaging	Abdominal ultrasound and CT Chest radiography Indium-labeled white blood cell scan
Tissue diagnosis	Liver biopsy Splenic aspiration

ELISA, enzyme-linked immunosorbent assay.

FURTHER READING

Further reading for this chapter can be found online at http://www.expertconsult.com

Practice point | 55 |

Jane Davies
David G Lalloo

History of an animal bite in a returned traveler from Burma

DEFINITION OF THE PROBLEM

A 26-year-old female presents to acute medical services 14 days after returning from a year long back-packing trip; for the past 7 weeks she has been in rural Burma. She reports being bitten by a stray but friendly dog on her ankle about 3 weeks ago. There is a visible bite wound and she complains of some pain around the bite area. She otherwise feels well. She is unsure of her pretravel immunization history.

HISTORY TAKING

When approaching this clinical scenario, the most crucial aspect of initial assessment is a detailed history of events. In this case we know the animal involved was a dog and therefore the most important aspect immediately becomes assessment of the risk of rabies. Successful management involves minimizing the patient's risk of developing symptomatic rabies. It is relatively common for an individual not to be clear about what has bitten them and then a much wider range of possibilities needs to be considered including snake, spider and scorpion bites as well as mammal bites. Terrestrial animals in the majority of South East Asia, Africa and South America are classed as high risk for rabies (see http://www.who-rabies-bulletin.org for up-to-date information by country). Bats can also carry classic and rabies-related lyssaviruses depending on species and location.

Rabies accounts for between 40 000 and 70 000 deaths per year and dog bites are responsible for 99% of these deaths.[1] Once symptomatic, apart from one or two documented exceptions, rabies is fatal; however, prompt postexposure prophylaxis is effective. The incubation period from bite to symptomatic diseases is usually between 3 and 12 weeks but has been documented up to 19 years.[2]

INITIAL ASSESSMENT (SEE ALSO FIG. PP55.1)

The location of the bite is important as the rabies virus reaches the brain by traveling centripetally along the peripheral nerves. This means bites on the neck or face have a shorter incubation time than those on hands or feet. Rabies cannot be transmitted through intact skin; however, infection has been documented after mucous membrane exposure as well as superficial scratches and licking episodes.[2]

The behavior of the biting animal is also important (Fig. PP55.2); did it appear sick, was it obviously limping or was it being uncharacteristically friendly? Did it appear to be coughing or have something stuck in its throat (hydrophobia)? In rabies-endemic countries, dogs are often reluctant to approach humans because of mistreatment; if a dog approaches humans it may be a sign that it is unwell.

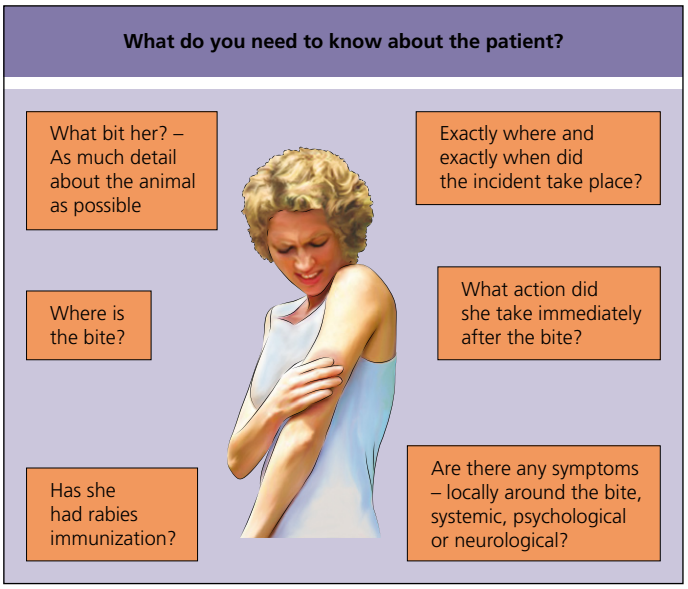


Fig. PP55.1 Initial assessment of a patient bitten by an animal.

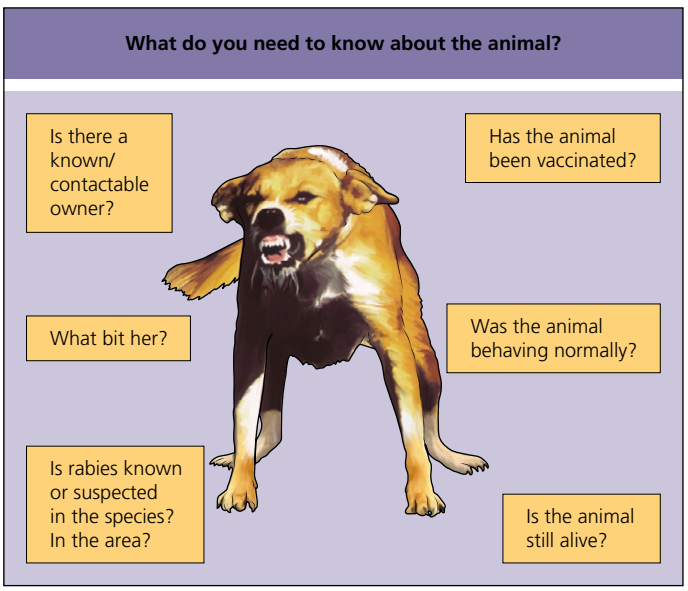


Fig. PP55.2 Description of the biting animal.

If the owner of the animal is known, is it possible to contact them and establish if it has been vaccinated? If it is still alive, is it being kept under observation? If a dog or cat remains healthy for 15 days after the incident it is unlikely that it was infected with rabies at the time of the bite.[1]

MANAGEMENT OF ANY BITE WOUND

Initial management

- Wash thoroughly, ideally for ~15 minutes.
- Clean with chlorhexidine.
- Do not suture.
- Assess tetanus need.
- Assess need for antibiotics.
- Assess need for rabies postexposure prophylaxis.

Rabies-specific management

Postexposure prophylaxis consists of vaccination and administration of human rabies immunoglobulin (HRIG) (Table PP55.1). As the patient described above has never been vaccinated, HRIG should be administered immediately based on her weight at a dose of 20 IU/kg.[3] As much as possible of the dose should be infiltrated around the site of the wound, the remainder given as an intramuscular injection. HRIG is not necessary if the patient has completed a full vaccination course or received a booster within the previous 5 years, however high the risk or if more than 7 days have elapsed since the first dose of postexposure vaccination.

Postexposure vaccination should be commenced as soon as possible. This consists of five doses of 1 ml of vaccine intramuscularly (not gluteal); however, only two doses are necessary if the patient has been previously vaccinated. Postexposure prophylaxis should be administered if indicated irrespective of the time delay to presentation as incubation periods can be prolonged.[4] The two products licensed in the UK are human diploid cell vaccine (HDCV) and purified chick embryo cell vaccine (PCEC) although other efficacious vaccines (e.g. Vero cell vaccines) are available.[3]

DIAGNOSIS

Virologic diagnosis of rabies during life can be difficult; in most cases it is the characteristic clinical picture and history which make the diagnosis (Table PP55.2). Saliva, cerebrospinal fluid (CSF) and skin biopsies taken from the nuchal area of the neck containing hair follicles and peripheral nerves and corneal impression smears can all be tested using various methods. Fluorescent antibody testing to detect viral antigen is most commonly used; however, sensitivity is limited. Antibody tests are available but can be difficult to interpret in the immunized patient. Reverse transcriptase polymerase chain reaction (RT-PCR) can be done on saliva, CSF and skin biopsies and is available in some countries via national reference laboratories.

Table PP55.2 Clinical presentation of rabies

Furious	Paralytic
Confusion, agitation, aggression	Ascending paralysis
Autonomic stimulation – sweating, excess saliva, terror	Fasciculation
Phases of arousal and lucid intervals	Loss of reflexes
Spasms, hydrophobia, aerophobia	Sphincter dysfunction
Cranial nerve lesions	Sweating, fever
Paralysis	Bulbar paralysis
Coma	Respiratory paralysis

TREATMENT

Symptomatic furious rabies can be very distressing with a combination of painful muscle spasms, hydrophobia and feelings of terror. There is no known curative treatment so symptom control with heavy sedation and analgesia is the mainstay of treatment. There have been recent reports of survival of a very limited number of patients by implementation of what has become known as the Wisconsin protocol.[5] The utility of this approach remains unproven.

OTHER CONSIDERATIONS

Macaque monkeys found in Burma and much of South East Asia, India and Japan commonly carry B virus. This is predominantly asymptomatic in monkeys but can rarely cause human disease through bites, scratches and mucous membrane splashes. Incubation is between

Table PP55.1 Postexposure prophylaxis

IMMUNIZATION STATUS		Full pretravel immunization*	No or inadequate/unclear pretravel immunization
Location	Type of exposure		
Any	Contact, licks to skin but no scratch or bite	No treatment	No treatment
High- or low-risk country	Licks, scratches or abrasions, minor bites, i.e. covered areas of arms, trunk or legs.	Commence postexposure vaccine only: two doses days 0 and 3	Commence postexposure vaccine: five doses days 0, 3, 7, 14 and 28 (plus rabies immunoglobulin if high-risk country†)
High- or low-risk country	Licks of mucosa or any major bite, or any exposure where animal known to have rabies	Commence postexposure vaccine only: two doses days 0 and 3	Commence postexposure vaccine: five doses days 0, 3, 7, 14 and 28 and rabies immunoglobulin

*Full pretravel immunization = three doses on days 0, 7 and 28.
†Use of rabies immunoglobulin may sometimes be restricted to high-risk cases when shortages occur.
Adapted from Health Protection Agency guidelines.[3]

2 days and 5 weeks and end-stage disease consists of diffuse encephalomyelitis with an untreated mortality rate of 80%.[5] Prevention is predominantly by prompt and through cleaning and irrigation of the wound. Postexposure prophylaxis with valaciclovir is recommended for high-risk exposures; however, it is unproven in humans. Treatment with intravenous aciclovir or ganciclovir if central nervous system signs are present improves survival.[6]

Controversy still exists about whether to give prophylactic antibiotics to bite patients. It is generally agreed that high-risk bites such as facial or hand injuries, extensive or crush injuries and those with deep penetration warrant antibiotic prophylaxis. The drug of choice is co-amoxiclav in view of its effectiveness against the bacterial flora of animal mouths commonly implicated in these infections, such as *Pasteurella multocida* and anaerobes, as well as streptococci and staphylococci.

REFERENCES



References for this chapter can be found online at http://www.expertconsult.com

Chapter 103

Warwick J Britton

Leprosy

EPIDEMIOLOGY

Leprosy is a chronic infection of the skin and nerves with *Mycobacterium leprae*, which, although rarely fatal, is a significant cause of disability. Over the past 15 years there have been dramatic changes in the prevalence of leprosy since the introduction of multidrug therapy (MDT).[1,2] As a result of the shorter duration of therapy and more intensive control programs, the number of registered leprosy patients receiving chemotherapy has fallen from 10–12 million to 225 000 in 2006.[3] The annual case detection rate of new cases remained high for many years, but this has recently fallen from 763 000 in 2001 to 259 000 in 2006. This rapid fall may reflect reduced active case finding activities following the integration of leprosy control into general health services, as well as a true decline in the incidence of leprosy worldwide. In addition, there is a pool of 2–3 million patients with permanent nerve impairment as a consequence of leprosy.

Leprosy is widely distributed in tropical and warm temperate countries and >1 billion people live in regions where there is active transmission of *M. leprae.* There has been a reduction in the number of endemic countries (prevalence rate >1/10 000) from 122 in 1985 to 4 in 2006,[3] mainly Brazil, Democratic Republic of Congo (DRC), Mozambique and Nepal. Currently 85% of registered cases occur in only six countries:

- India (accounting for 54% of all registered cases); and
- Brazil, Indonesia, DRC, Bangladesh and Nepal in order.[3]

Because of the long incubation period of leprosy an individual from an endemic country may develop leprosy years after migration elsewhere. Delay in diagnosis is usually longer in nonendemic than endemic regions, and therefore leprosy should be considered as a diagnostic possibility in any person who is from an endemic country and has chronic lesions of the skin or impaired function of peripheral nerves.

Subclinical infection with *M. leprae* is far more common than overt disease.[4] Analysis of *M. leprae*-specific immune responses[5,6] has demonstrated that *M. leprae* infection is common after exposure, but the majority of individuals control the infection. Currently there is no specific test to distinguish latent leprosy infection from latent tuberculosis infection, but *M. leprae*-specific T-cell assays are being developed.[7]

The major mechanism of transmission of *M. leprae* is thought to occur through nasal secretions, particularly from lepromatous patients.[4] Organisms probably enter through the mucosa of respiratory tracts and, if not controlled, disseminate to the skin and peripheral nerves. Other possible modes of transmission include breast milk from mothers with untreated lepromatous disease and uncommon cases of cutaneous inoculation. Although infection with *M. leprae* has been documented in wild armadillos in the USA and some primates, zoonotic transmission does not contribute to human disease.[6]

Proximity to leprosy patients is important in transmission, and the relative risk for disease for household contacts is 8- to 10-fold greater for lepromatous cases and 2- to 4-fold greater for tuberculoid cases.[4] Nevertheless, the majority of leprosy cases are sporadic.

Genetic factors influence both the development of leprosy and the pattern of disease. Genome-wide linkage analysis in multicase families has defined susceptibility alleles in the genes for lymphotoxin-α and the ubiquitin and proteosome related enzymes, PARK2 and PACRG.[8] Susceptibility loci have also been identified on chromosome 10p13, close to the gene for the mannose receptor C type 1,[9] and the HLA region with linkage to both the HLA Class II and tumour necrosis factor genes,[10] in Indian and Brazilian patients respectively. The HLA locus also affects the pattern of disease: HLA-DR2 and -DR3 are associated with tuberculoid disease and HLA-DQ1 with lepromatous leprosy. Racial and geographic factors also influence the type of leprosy, with lepromatous leprosy being less common in Africans than Indians, and most common in Chinese and Caucasians.

The incidence of leprosy peaks in two age groups (10–15 and 30–60 years of age) and there is a male predominance in most regions of about 2:1.[4] The incubation period varies widely from months to over 30 years, but is usually prolonged, averaging 4 years for tuberculoid and 10 years for lepromatous leprosy. In contrast to tuberculosis there is no definite evidence for increased HIV prevalence in leprosy patients or a change in the clinical spectrum of leprosy among co-infected patients, although some studies suggest there is an increase in leprosy reactions in HIV-infected patients.[11] Leprosy can occur as an immune reconstitution syndrome in co-infected patients after starting highly active antiretroviral therapy.

PATHOGENESIS AND PATHOLOGY

Although *M. leprae* was the second bacterium to be associated with a human disease, it still cannot be cultivated *in vitro*. The organism is capable of limited multiplication in mouse footpad, with a doubling time of 11–13 days, and this has permitted drug sensitivity studies.[6,12] *M. leprae* is an acid-fast Gram-positive bacillus and is an obligate intracellular parasite with tropism for macrophages and Schwann cells. The bacilli show preference for growth in cooler regions of the body. The unique characteristic of *M. leprae* is its predilection to infect Schwann cells. The receptor complex on the Schwann cell is the G-domain of the laminin α2 chain in the basal lamina of Schwann cells and the laminin receptor, α-dystroglycan.[13] A number of ligands on the surface of *M. leprae* bind to this complex including the specific trisaccharide of PGL-I and a 21 kDa surface protein.

Genetic and structural analyses have confirmed that *M. leprae* is a member of the family Mycobacteriacae. A major advance has been the sequencing of the genome of an Indian isolate of *M. leprae*.[14] The genome contains 1605 genes encoding proteins and 50 genes for stable RNA molecules. Remarkably, half the functional genes in the *M. tuberculosis* genome are absent, being replaced by many inactivated

or pseudogenes. This gene decay has removed entire metabolic pathways and regulatory genes, particularly those involved in catabolism. This may render the leprosy bacillus dependent on host metabolic products and may explain its long generation time and inability to grow in culture.[14] Comparison of single nucleotide polymorphisms in the *M. leprae* genome between multiple isolates from different continents suggests that leprosy infection of humans originated in East Africa or the Near East and then spread through successive human migrations.[15] Individual isolates of *M. leprae* can be distinguished by the variable number of tandem repeat sequences within their genome, but the extent of variability within individual patients for isolates collected from different sites or at different times limits the use of these repeat tandem sequences as tools for molecular epidemiologic studies.[16]

The availability of the *M. leprae* and other mycobacterial genomes has major implications for the development of new antimycobacterial drugs and *M. leprae*-specific diagnostic reagents.[6,7] The complex cell wall contains important targets of the immune response, including a species-specific phenolic glycolipid-I (PGL-I) and immunomodulatory lipoarabinomannan. The cell wall biosynthetic pathways are relatively intact in *M. leprae*, despite the loss of other genes, indicating that these represent the essential genes for the formation of a minimal mycobacterial cell wall.[17] *M. leprae* is relatively inert, and the host immune response is responsible for most of the tissue damage.

The manifestations of leprosy form a wide clinical spectrum determined by immunopathologic responses to the organism (Fig. 103.1).[18] Patients who have the polar forms of tuberculoid (TT) and lepromatous leprosy (LL) are immunologically stable, but those who have the intermediate types of borderline-tuberculoid (BT), mid-borderline and borderline-lepromatous (BL) leprosy are immunologically unstable and subject to either a gradual decline towards the lepromatous pole or upgrading reversal reactions (RRs; see Fig. 103.1). In TT a vigorous cellular response to *M. leprae* limits the disease to a few well-defined skin patches or nerve trunks.[5] The lesions are infiltrated by interferon gamma secreting $CD4^+$ T lymphocytes, which form well-demarcated granulomas containing epithelioid and multinucleate giant cells around dermal nerves. Few, if any, bacilli are demonstrable. Cellular immunity may be confirmed by *in-vitro* lymphocyte responses to *M. leprae* antigens or skin test reactivity. Intradermal injection of heat-killed *M. leprae* causes a transient swelling at 48 hours in a sensitized subject (Fernandez reaction), followed by the development of a granulomatous nodule at 3–4 weeks (Mitsuda reaction).[5] The latter confirms an individual's capacity to mount a T-lymphocyte response to *M. leprae.* Antibody responses to *M. leprae* are absent or low titer.

The hallmark of LL is the absence of *M. leprae*-specific cellular immunity, and this results in uncontrolled proliferation of the bacilli with extensive infiltration of the skin and nerves and numerous lesions.[5] Histologically, the dermis contains foamy macrophages filled with multiple bacilli and a scattering of $CD4^+$ and $CD8^+$ lymphocytes, but no organized granulomas.[18] There are high titers of antibodies to *M. leprae*-specific PGL and protein antigens. In borderline cases a progressive reduction in cellular responses is associated with a greater bacillary load, more frequent skin and nerve lesions, and increasing antibody levels.

The dynamic nature of the immune response to *M. leprae* is responsible for spontaneous fluctuations in the clinical pattern, termed leprosy reactions[5]:

- a type 1 reaction is usually an 'upgrading' RR caused by increased cellular reactivity to mycobacterial products, results in edema and acute inflammation of skin lesions and nerves, is most common in borderline patients and is a major cause of nerve damage[19]; and
- a type 2 reaction or erythema nodosum leprosum (ENL) is a systemic inflammatory response associated with the deposition of extravascular immune complexes leading to neutrophil infiltration and activation of complement in multiple organs.[20] This is accompanied by high circulating levels of tumor necrosis factor with systemic toxicity.

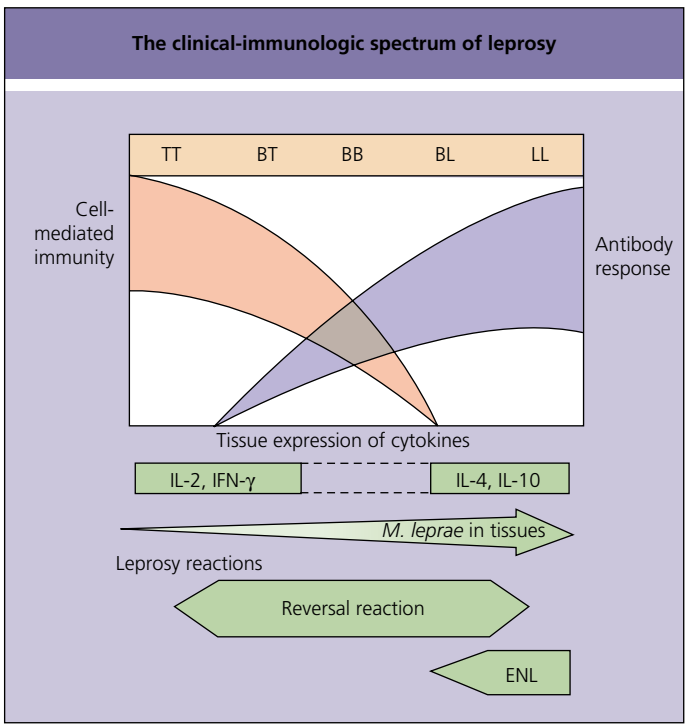


Fig. 103.1 The clinical–immunologic spectrum of leprosy. This reflects the underlying host immunity as measured by the T-cell and antibody responses to *M. leprae*. Spontaneous fluctuations in the immune response are responsible for reversal reactions and erythema nodosum leprosum (ENL). TT, tuberculoid leprosy; BT, borderline tuberculoid; BB, mid-borderline leprosy; BL, borderline lepromatous leprosy; LL, lepromatous leprosy; IFN, interferon; IL, interleukin.

PREVENTION

The chief means of preventing leprosy is the interruption of transmission by treating those with infectious leprosy early. Multidrug therapy was introduced because of the increasing spread of primary and secondary dapsone resistance worldwide.[1] Its advantages are its proven efficacy[2] and improved compliance, which is related to the limited duration of therapy and its monthly observed component (see Management, below). Furthermore, early treatment before the onset of nerve damage reduces the long-term disability associated with leprosy.[21] The effectiveness of MDT prompted a World Health Organization co-ordinated campaign to implement MDT in all endemic countries, with the aim of reducing the prevalence rate of leprosy to less than 1/10 000.[3] This has been successful, with only four countries left to attain this goal. Importantly, the case detection rate has been slower to fall, probably because of the prolonged incubation period of clinical leprosy, indicating that leprosy control must be sustained through case detection and treatment of leprosy within integrated health programs.

The response to immunization with *M. leprae* bacille Calmette–Guérin (BCG) has been variable, but in a major trial in Malawi, BCG induced 50% protective efficacy against clinical leprosy, both tuberculoid and lepromatous forms.[22] Re-immunization enhanced the protective effect by a further 50%. Recent meta-analysis has confirmed the significant protective effect of BCG against leprosy in both clinical trials and case-control studies.[23] Extensive BCG immunization of children in endemic countries has probably made a significant contribution to the decline of leprosy. The addition of heat-killed *M. leprae* to BCG did not increase the observed protective efficacy of BCG in two trials.[22] Other experimental vaccines are being tested against leprosy infection at present.

Chemoprophylaxis may also be useful in the control of leprosy, particularly in low endemic regions. A large, randomized control trial in Bangladesh showed that a single dose of rifampicin given to the close contacts of newly diagnosed leprosy patients resulted in a significant reduction of 57% (95% CI 33–72) in the incidence of leprosy in the contacts at 4 years.[24] Leprosy is commonly associated with poverty and overcrowding, and improved socioeconomic conditions have also contributed to the decline of leprosy in Europe and some Asian countries.

CLINICAL FEATURES

Types of leprosy

Indeterminate leprosy

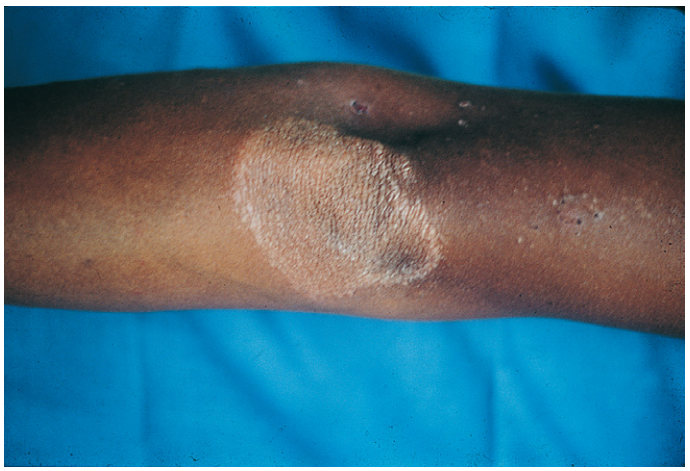
This is the earliest form and occurs as a single, slightly hypopigmented ill-defined macule in children, who are often contacts of leprosy patients.[25] The majority of these lesions are self-limiting and resolve without therapy. A minority (<25%) develop into defined lesions within the clinical spectrum.

Tuberculoid leprosy

These lesions occur as one to three large asymmetric macules or plaques with sharply defined borders and hypopigmented anesthetic centers (Fig. 103.2).[25,26] Although leprosy lesions are usually hypopigmented, in light skins the macules may appear erythematous or dyschromic. Involvement of sweat glands and hair follicles results in dryness and loss of hair. Enlarged cutaneous nerves may be palpable at the edge of the lesion, but nerve trunk involvement is minimal.

Borderline tuberculoid leprosy

This is the commonest form of leprosy. The skin lesions resemble those in TT leprosy, but are more frequent and variable in appearance and their borders are less well demarcated (Fig. 103.3). The outline may be irregular with adjacent 'satellite' lesions suggesting local spread. Occasionally, large patches of BT leprosy may involve a whole limb. Asymmetric enlargement of several peripheral nerves is usual and patients may present with muscle weakness or trauma secondary to sensory impairment. Progressive nerve damage is common.



f0015 **Fig. 103.2** Tuberculoid leprosy. Single hypopigmented anesthetic plaque with raised border and dry surface.



Fig. 103.3 Borderline tuberculoid leprosy. Three large well-defined erythematous patches with reduced sensation, spreading borders and satellite lesions.

Mid-borderline leprosy

This is the most immunologically unstable form with the propensity to shift rapidly towards BT leprosy during a reversal reaction or to downgrade towards BL leprosy. The skin lesions are numerous and vary in size, shape and distribution. They may be hypopigmented or erythematous. The characteristic 'target' lesion has a broad, erythematous border with a vague outer edge and 'punched-out' pale center with sensory impairment (Fig. 103.4).

Borderline lepromatous leprosy

In borderline lepromatous leprosy there are numerous small erythematous macules, which initially may be limited in distribution, but become progressively more symmetric.[25] Papules, nodules and succulent plaques may develop and, in contrast to tuberculoid leprosy, the lesions have normal sensation. The intervening skin is normal. Widespread nerve involvement is typical, especially if the patient has downgraded from BT leprosy.

Lepromatous leprosy

This is a systemic disease with a generalized bacteremia leading to widespread involvement of the skin and other organs.[25,26] The first manifestation may be a diffuse infiltration of the dermis causing a smooth shiny appearance of the skin. More typically, there are numerous symmetrically distributed macules, papules or nodules, and sensation may be retained in lepromatous lesions (Figs 103.5, 103.6). Progressive thickening of the skin results in coarsening of the facial features and nodular thickening of the ear lobes. With time the eyebrows and eyelashes become thinned.



Fig. 103.4 Mid-borderline leprosy. Characteristic target lesion with raised erythematous annular border and 'punched-out' central area with impaired sensation.



Fig. 103.5 Lepromatous leprosy. Multiple, small, slightly erythematous macules with intact sensation and symmetric distribution. The skin smears of both the lesions and intervening skin are positive for acid-fast bacilli.



Fig. 103.6 Nodular lepromatous leprosy. Diffuse infiltration of the skin by multiple nodules of varying size, each teeming with bacilli.

Bacillary infiltration is responsible for gradual tissue damage in the involved organs. The nasal mucosa is infiltrated at an early stage, resulting in discharge and obstruction. Erosion of the cartilage and nasomaxillary bones results in perforation of the nasal septum, collapse of the nose and saddle-nose deformity. Laryngeal involvement produces hoarseness and stridor. Direct bacillary involvement of the eye causes keratitis and iritis.

Infiltration of the dermal nerves results in a peripheral sensory loss similar to that of a 'glove and stocking' neuropathy,[25] which leaves the skin susceptible to ulceration and secondary infection. Reactional episodes cause edema of the feet, shins and hands. Dactylitis develops in the hands and feet, and, together with trauma and osteomyelitis, results in phalangeal erosion.

Both testicular infiltration and orchitis contribute to testicular atrophy and secondary gynecomastia. Glomerulonephritis may occur and is usually associated with ENL. Secondary amyloidosis is a consequence of recurrent ENL reactions.

Peripheral nerve involvement

The nerves of predilection occur at superficial sites where the nerve trunks are cooler, more readily traumatized and often anatomically constricted.[25] These include the:

- ulnar nerve at the medial epicondyle of the humerus;
- median nerve at the wrist;
- Lateral popliteal nerve at the neck of the fibula;
- posterior tibial nerve behind and inferior to the medial malleolus; and
- radial nerve in the humeral groove posterior to the deltoid insertion.

Easily palpated superficial cutaneous nerves include the:

- superficial radial nerve at the wrist;
- greater auricular nerves;
- supraorbital nerve; and
- sural nerves.

These nerves should be examined for enlargement and associated weakness and sensory loss. The resulting muscle imbalance results in the characteristic deformities of clawhand, footdrop, clawtoes and wristdrop. Autonomic nerve dysfunction contributes to impaired sweating and dry skin, which is subject to cracking, infection and poor healing. The combination of insensitive feet and clawtoes leads to recurrent plantar ulceration, a major cause of disability.

In pure neural (PN) leprosy the nerve trunks are affected without any skin lesions. On biopsy the neural lesions tend to be 'lepromatous' in appearance and PN leprosy involving more than one nerve should be treated as multibacillary (MB).

Before and during therapy the function of the commonly involved nerves should be assessed at regular intervals by voluntary muscle and sensory testing (preferably with nylon monofilaments) to determine whether there is ongoing nerve function impairment. This may presage the onset of a reversal reaction before nerve pain or typical skin lesions develop. Nerve conduction studies commonly detect subclinical neuropathy in BT–BL leprosy patients and these tests may become abnormal weeks before the development of clinical neuropathy.[27] Nerve function impairment may develop or worsen despite effective chemotherapy, and early recognition and therapy prevent permanent nerve damage.[19,21] Patients with pre-existing nerve damage at diagnosis and MB patients are at greatest risk for new nerve function impairment and should be carefully monitored.[28]

Leprosy reactions

Reversal reactions

These develop in about one-third of patients who have BT–BL leprosy, usually within the first year of treatment, but monitoring for new nerve function impairment should occur for 2 years.[27,29] They present with:

- increased inflammation in established BT–BL skin lesions or new swollen lesions in BL and subpolar LL patients (Fig. 103.7);
- acute neuritis with pain or tenderness in the involved nerve and loss of function; and
- recent (<6 months) or progressive nerve function impairment in the absence of painful nerves.



Fig. 103.7 Reversal reaction. Erythema and edema in the facial lesions of a patient who has borderline-tuberculoid leprosy undergoing an upgrading reversal reaction.

Silent neuritis responds to therapy for the RR.[30] Patients at particular risk for developing RR are those who have MB leprosy involving more than two body areas, established nerve function impairment at diagnosis, facial patches or *M. leprae*-specific IgM anti-PGL antibodies.

Erythema nodosum leprosum

This once affected 30–50% of BL and LL patients, but the frequency and severity of ENL have reduced since the regular use of clofazimine in MDT.[2] ENL may develop at any stage of therapy, but usually within the first year and is often recurrent.[31] An episode begins with fever and malaise and the rapid emergence of painful erythematous nodules, typically over the extensor surfaces of the limbs.[5,25] In severe cases widespread nodules may form pustules and ulcerate (Fig. 103.8). Painful neuritis is the most common complication. Erythema nodosum leprosum has features of widespread immune complex deposition and these may include small vessel vasculitis, iridocyclitis, polyarthritis, orchitis, lymphadenitis and glomerulonephritis.

Recurrent or uncontrolled ENL reactions can result in the development of secondary amyloidosis (amyloid A protein) within 3 months.

Eye involvement

Involvement of branches of the facial and trigeminal nerves results in lagophthalmos and corneal anesthesia, respectively, and if combined there is a considerable risk of ulceration and infection of the exposed insensitive cornea.[32]

In 25–30% of patients who have LL, infiltration of the anterior segment of the eye causes a superficial punctate keratitis and iridocyclitis, which may be painless and only recognized by slit-lamp examination. Iridocyclitis is exacerbated during episodes of ENL, but can occur independently of overt reactions. The iritis may be complicated by glaucoma or cataract, both of which contribute to leprosy-associated blindness (see Chapter 17).



Fig. 103.8 Erythema nodosum leprosum. Tender papules associated with fever, arthralgia and acute neuritis in a patient who has lepromatous leprosy.

DIAGNOSIS

A diagnosis of leprosy is usually straightforward if it is suspected as a cause of any skin or peripheral nerve lesion in a person from an endemic country. The cardinal signs of leprosy[5,33] are:

- skin patch with sensory loss;
- nerve enlargement; and
- acid-fast bacilli (AFB) in the skin.

The presence of one or more of these features establishes the diagnosis, which should be confirmed with a full-thickness skin biopsy. Approximately 70% of all leprosy patients can be diagnosed by the single sign of a skin patch with sensory loss, but 30% of patients, including many MB patients, may not present with this sign, indicating the importance of nerve enlargement as an additional sign and the importance of clinical suspicion for the diagnosis.[33]

Acid-fast bacilli are best demonstrated in slit-skin smears, which should be taken from the edges of at least two lesions and both ear lobes. If these are not available a skin biopsy should be stained for AFB with a modified Wade–Fite stain. The extent of the bacillary load can be quantitated as a bacterial index[12] on a logarithmic scale of 1+ to 6+. The percentage of solid staining AFB in smears, the morphologic index,[12] is an indirect measure of the viability of leprosy bacilli. In PN leprosy a biopsy from a sensory nerve such as the superficial radial nerve may be diagnostic. Polymerase chain reaction (PCR) can be used to identify *M. leprae* DNA and, together with PCR-based detection of rifampin (rifampicin)-resistant strains, it is a valuable tool for epidemiologic studies.[6,34]

Lepromin testing and serology may be used for accurate classification of patients in research studies. Antibodies to PGL and other *M. leprae*-specific protein antigens are present in MB patients and their titer falls with effective therapy.[5] In patients who have BL and LL, evidence of chronic inflammation includes anemia, hypergammaglobulinemia, elevated serum amyloid A protein and positive antinuclear and anticardiolipin autoantibodies.

Other skin diseases can be differentiated from tuberculoid leprosy by the absence of anesthesia in the lesions and the presence of nerve involvement elsewhere.[25,26] Lepromatous skin lesions are not anesthetic and biopsy may be necessary to distinguish these from those due to other systemic infections, such as leishmaniasis and secondary syphilis and other nodular or infiltrative skin conditions. Other causes of nerve enlargement, such as primary amyloidosis and familial polyneuropathy, are excluded by biopsy and family history.

MANAGEMENT

Successful management of leprosy requires prolonged drug treatment and careful monitoring for complications, and it is essential to enlist the patient as an ally in this process. The patient should be educated about:

- the importance of compliance;
- the first symptoms of a reaction; and
- the elements of self-care needed to prevent secondary tissue damage if there is sensory nerve impairment.

The most important step in disability prevention is the early initiation of bactericidal drug therapy.

Antileprosy drugs

Dapsone

This is an important antileprosy drug because of its bactericidal effect at full dosage, low cost and low toxicity.[2] When used alone, stepwise dapsone resistance emerges as a major problem, owing to mutations in *folP1*, encoding dihydropteroate synthase,[6,35] but this is prevented by combination therapy. Mild hemolytic anemia is common, but is only severe in the presence of glucose-6-phosphate dehydrogenase

deficiency, which should be excluded where possible. Occasionally dapsone allergy, and rarely agranulocytosis, may develop after 2–6 weeks of therapy.

Rifampin

Rifampin (rifampicin) is a key component of MDT because it is the most effective bactericidal drug against *M. leprae* when given either daily or monthly.[2,12] Toxicity is low with monthly dosage, although thrombocytopenia, hepatitis and a flu-like syndrome occasionally occur. It must be used with at least one other effective drug to prevent rifampin resistance owing to mutations in RNA polymerase.[34] Tuberculosis should always be excluded before monthly rifampin is started (see Chapters 30 and 143). Rifapentine is a long acting rifamycin-derivative, which is more bactericidal than rifampin in mice, and is currently being tested in humans.

Clofazimine

This is a fat-soluble dye that is deposited within the skin, fat stores and macrophages. It has similar bactericidal activity to that of dapsone[2,12] and also a significant anti-inflammatory effect. It is relatively nontoxic and its only disadvantage is the associated development of a reddish skin pigmentation, which resolves after the drug has been discontinued. When used in high doses for prolonged periods clofazimine is deposited in the small intestinal wall and can cause diarrhea and pain.

Other drugs

Three additional drugs have proved effective against *M. leprae* in human and mouse studies:[5,6]

- the fluoroquinolones, ofloxacin and moxifloxacin, have moderate bactericidal activity and infrequent side-effects involving the gastrointestinal tract and central nervous system;
- minocycline, the only fat-soluble tetracycline, has moderate anti-*M. leprae* activity. It has proved effective as an alternative drug in patients with LL and has low toxicity in adults when used as long-term therapy for acne, and so is a useful alternative drug for leprosy; and
- clarithromycin has modest bactericidal activity.

Multidrug therapy

The principle underlying MDT is the use of three drugs when the bacterial load is high in MB leprosy to treat and prevent the emergence of dapsone-resistant strains. Two drugs are sufficient for paucibacillary disease. Since its introduction in 1982,[1] MDT has proved highly effective and over 10 million patients have been treated with few treatment failures and remarkably low relapse rates of about 0.1/100 patient years.[3,36]

Multibacillary multidrug therapy

This is recommended for adult patients with BB, BL, LL, smear-positive BT and PN leprosy. In leprosy control programs in endemic countries, a simplified form of classification is employed, based on the number of patches so that MB leprosy >5 patches and paucibacillary (PB) ≤5 patches. Multibacillary MDT comprises:

- rifampin, 600 mg once a month, supervised administration;
- dapsone, 100 mg/day, self-administered; and
- clofazimine, 300 mg once a month, supervised administration; 50 mg/day, self-administered.

Originally MB-MDT was continued for at least 2 years and then until the skin smears became negative.[1] In subsequent field trials a fixed duration of MB-MDT for 2 years was as effective[2] and was utilized in control programs with very low rates of relapse.[36] Patients who have MB leprosy and a skin smear bacterial index of 4+ or over may require a longer duration of therapy.[2] In 1998, WHO recommended MB-MDT of 12 months duration for use in control programs,[36] and 12-month MB-MDT is currently used in leprosy control programs in endemic countries.

Some authorities in developed countries prefer to use more frequent doses of rifampin and continue to treat until the smears are negative, which can take many years. One approach is to double the dose of rifampin to 600 mg daily for two consecutive days each month, while others use daily rifampin 450–600 mg for 2 years with dapsone and clofazimine. There is no evidence, however, that daily rifampin is more effective than when given once monthly.

If clofazimine is unacceptable because of pigmentation or if dapsone hypersensitivity occurs, minocycline (100 mg daily) or ofloxacin (400 mg daily) may be substituted.[36] Patients who have rifampin intolerance require two new drugs, minocycline and ofloxacin, along with clofazimine (50 mg/day) for 6 months and then either drug with clofazimine for another 18 months.

Paucibacillary multidrug therapy

This is recommended for indeterminate, TT and smear-negative BT leprosy[2] and in the control programs for patients with 5 patches. PB-MDT comprises of:

- rifampin, 600 mg once a month, supervised administration; and
- dapsone, 100 mg/day, self-administered.

This is continued for 6 months. If the skin smear is positive at any site, the patient is given MB-MDT. If a RR develops after completion of chemotherapy, MDT should be recommenced while on prednisone.

In some countries patients with solitary leprosy skin lesions are common. A large field study in India established that a single dose of the combination of rifampin (600 mg), ofloxacin (400 mg) and minocycline (100 mg) (ROM) was almost as effective as PB-MDT in the treatment of patients with single smear-negative skin lesions and no nerve involvement, although the follow-up period was only for 18 months.[37] This regimen should be reserved only for carefully selected patients in endemic countries with a high proportion of single lesion cases.

Treatment for reactions

Patients who have RRs, including silent nerve function impairment, require high-dose corticosteroids for a prolonged duration to permit nerve function recovery.[19] Prednisone is started at 40 mg/day and increased to 60 mg/day if there is no response, and then to 120 mg/day if necessary. Once there is evidence of improvement on serial voluntary muscle and sensory testing, the dose is reduced over 6 weeks to 20 mg/day and this is continued for some months before gradual removal. There are few clinical trials on the therapy for RRs, but one randomized study demonstrated that outcome is improved if corticosteroids are continued for at least 5 months.[38] Longer durations are often required for RRs complicating MB leprosy. It is important to maintain treatment with antimycobacterial drugs to reduce the bacillary load. Adequate analgesia is essential along with physical support during the period of active neuritis. This therapy can be successfully administered without admission if other infections and medical problems are excluded. The overall recovery rate for nerve function is 60–70%,[39] but may be up to 88% in patients with no nerve damage at diagnosis who develop acute neuropathy during MDT.[21] Recovery is less in those with pre-existing nerve function impairment or with chronic or recurrent reactions.

Mild ENL may respond to aspirin or nonsteroidal anti-inflammatory drugs, increased clofazimine dosage and rest. Moderate or severe episodes and those with neuritis require prednisone, usually starting at 40–60 mg/day. The response is rapid, but as ENL is liable to become corticosteroid dependent, the prednisone should be withdrawn over 2–3 months. Clofazimine at a higher daily dose of 300 mg suppresses ENL after 4–6 weeks, and can be used to prevent further episodes.

If the ENL is poorly controlled or recurs, it usually responds to thalidomide, 400 mg/day for 2–3 weeks, and then 100–200 mg/day

as maintenance.[40] Thalidomide inhibits the release of tumor necrosis factor from macrophages and modulates T-cell function, and is of proven and prompt efficacy for ENL in clinical trials. Its use, however, is severely limited by its teratogenicity, and thalidomide should be restricted to male and postmenopausal patients under strict supervision. Thalidomide may cause a peripheral neuropathy, but this neurotoxic effect has not been evaluated in leprosy patients.

Eye involvement is common and iritis requires local treatment with corticosteroid and atropine drops.[32]

Other therapies

Prevention of disability is an important component of care.[41] Regular monitoring of nerve function will reveal early reversible RRs. Patients with irreversible nerve function impairment must learn to care for insensitive hands and feet and be provided with appropriate footwear. Plantar ulceration requires prolonged rest for healing. Physiotherapy and reconstructive surgery for clawhands and clawfeet, footdrop and lagophthalmos may prevent further tissue damage and restore appearance, and facial deformity can be corrected by plastic surgery.[41] Community-based rehabilitation is proving effective in assisting patients with persistent nerve impairment to return to full participation in their own communities.[42]

Websites

- http://www.who.int/lep/: Provides access to WHO documents on the current epidemiology, clinical features and treatment of leprosy with detailed information on MDT and leprosy control.
- http://www.ilep.org.uk/fileadmin/uploads/Documents/techforum.pdf: International Leprosy Association Technical Forum: critical evaluation of clinical diagnostic criteria and therapy for leprosy.
- http://www.leprosy-review.org.uk/: Website of *Leprosy Review* – a journal contributing to the better understanding of leprosy and its control.

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Chapter 104

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Endemic treponematoses

The endemic treponematoses include yaws (also known as buba, framboesia, parangi and pian), endemic syphilis (also known as bejel, dichuchwa and sklerjevo) and pinta (also known as azul, carate and mal de pinto), all of which are chronic bacterial infections. The causative organisms (*Treponema pallidum* subsp. *pertenue*, *T. pallidum* subsp. *endemicum* and *T. carateum*, respectively) are morphologically and serologically indistinguishable from *T. pallidum* subsp. *pallidum*, which is the causative organism of venereal syphilis.[1]

In the 1990s, small genetic differences (single base pair changes) were identified between the organisms of the venereal and nonvenereal treponematoses but these genetic variations did not allow differentiation of one *T. pallidum* subsp. from another.[2] Recent molecular genetic studies indicate that *T. pallidum* subsp. *pallidum*, subsp. *pertenue*, subsp. *endemicum* and a simian *T. pallidum* (Fribourg–Blanc) strain can be differentiated through subspecies-specific genetic signatures in multiple genes.[3,4]

A phylogenetic analysis indicates that *T. pallidum* subsp. *pertenue* arose first in history causing yaws in our anthropoid ancestors in the tropical belt of the Old World; it spread as yaws to the New World and as endemic syphilis to North Africa, Eastern Europe and the Middle East (through divergence of subsp. *pertenue* to subsp. *endemicum*). It finally evolved in the Americas as the modern subsp. *pallidum* strain (or a progenitor) which was introduced into the Old World as a result of the European exploration of the Americas, and disseminated as venereal syphilis all over the world.[4]

Nevertheless, in clinical practice the treponematoses (venereal and nonvenereal) continue to be diagnosed based on their distinctive clinical and epidemiologic characteristics.[1]

EPIDEMIOLOGY

Historic perspective

The endemic treponematoses, because of the disfigurement and disability they cause, were a major public health problem in the pre-antibiotic era. In 1948, the World Health Organization (WHO) and the United Nations International Children's Emergency Fund (UNICEF) sponsored a global control program. This involved 46 countries and brought these diseases under control with the help of long-acting penicillin. Unfortunately, the diseases were not eradicated and the lack of continuing vigilance allowed persistence of endemic foci in some countries, resulting in localized resurgence of these diseases since the 1980s. In 2007, WHO launched a renewed attempt to eliminate yaws.[5]

Geographic distribution

The endemic treponematoses are now largely confined to communities in remote rural areas living in poor, overcrowded and unhygienic conditions. Yaws occurs mainly in the warm, humid areas of Africa, South East Asia and the Pacific, the Caribbean and Central and South America. In the South East Asia region, India successfully eliminated yaws in 2006, and yaws now remains present only in two countries, Indonesia and Timor-Leste.[6,7] Endemic syphilis occurs mainly in the arid areas of sub-Saharan Africa and among the nomadic people of the Arabian peninsula. Pinta occurs mainly in Central and South America (among Indian tribes in the Amazon region and adjacent areas). Cases of imported yaws and endemic syphilis are sometimes encountered in the countries of the northern hemisphere, where clinicians may need to include them in the differential diagnosis.

INCIDENCE AND PREVALENCE

Accurate incidence and prevalence data are unavailable. The last estimate by WHO in 1995 yields a global prevalence of 2.5 million cases, including 460 000 infectious cases. The situation today remains unknown.

Age and sex

Yaws and endemic syphilis usually occur in children aged 2–14 years. For pinta the range is 10–30 years. Yaws affects boys more than girls; for the other diseases there is probably no sex difference.

Mode of transmission

Yaws and pinta are transmitted by direct skin-to-skin contact with infectious lesions, transmission being facilitated by a breach in the skin of the recipient. The role of nonbiting flies in the transmission is uncertain. In the case of endemic syphilis, because the initial lesions are often in or around the mouth, the infection spreads by direct contact (e.g. older children kissing their younger siblings) and by indirect contact through infected communal eating or drinking utensils.

PATHOLOGY

Yaws and endemic syphilis affect skin and bones, whereas pinta is confined to the skin.

The basic pathology in endemic treponematoses is the same as in venereal syphilis. However, the vascular changes in the endemic treponematoses are less marked. Skin biopsies from yaws patients show numerous plasma cells but few T and B cells; the treponemes are found mostly in the epidermis, whereas in venereal syphilis they are demonstrated mainly in the dermis and dermal–epidermal junction.[8] Because these differences are relative, they cannot be used to differentiate yaws from venereal syphilis.

In pinta, there is loss of melanin in basal cells, the presence of many melanophages in the dermis and the absence of inflammatory cells and treponemes in the achromic lesions.[9]

Endemic treponematoses are not believed to be associated with congenital transmission or with involvement of the cardiovascular and nervous systems.

PREVENTION

Essential in prevention of the endemic treponematoses is not only to identify and treat clinical cases but also to recognize that the presence of clinical cases in a community necessitates an immediate search for further clinical and latent cases, which also must be treated.

The supportive measures for control include:

- strengthening and improving accessibility of the primary health-care facilities;
- training of clinicians to detect and treat patients and to examine and treat household and other obvious contacts;
- health education of the population;
- improvement in the standard of living and in personal and environmental hygiene; and
- provision of soap and water and clothing to children.

CLINICAL FEATURES

The incubation period is 9–90 days (mean 21 days).

Yaws

Early stage

The initial or primary lesion ('mother yaw') appears at the site of infection on an exposed part of the body. It may be a localized maculopapular eruption or a papule, which may develop into a large papilloma 2–5 cm in diameter. It is painless but itchy and may ulcerate as a result of scratching. It may heal in 3–6 months with or without scarring. Secondary lesions, which are the result of lymphatic and hematogenous spread of organisms, appear a few weeks to 2 years after the primary lesion. They may consist of multiple excrescences, often resembling the initial papilloma. The papillomas may ulcerate and the exudate may dry to form a yellow crust which, when removed, gives the lesion an appearance of a raspberry (hence the name 'framboesia'). The lesions may be irregular, crescentic or discoid in shape and on moist areas may mimic condylomata lata of venereal syphilis. They are rather florid and become more numerous in the rainy season (Fig. 104.1). They may last up to 6 months and heal with or without scarring. Infectious relapses may occur for 5 years and, rarely, for 10 years.

Other manifestations include:

- regional lymphadenopathy;
- palmar and plantar lesions, which may be painful, resulting in a crab-like gait; and
- osteoperiostitis of the proximal phalanges of the fingers (dactylitis) or of long bones, causing nocturnal bone pains.

The patient may at any time enter latency, with only serologic evidence of the infection.

Late stage

About 10% of patients develop late lesions after 5 years or more of untreated infection. The late stage is characterized by gummatous lesions of skin, bones and overlying tissues. The manifestations, some of which also occur in early stage but are now more destructive, include:

- hyperkeratosis of palms and soles with deep fissuring;
- juxta-articular subcutaneous nodules;



Fig. 104.1 Mixed early yaws lesions: papillomata, ulceropapillomatous lesions and squamous macules. Courtesy of WHO, Geneva.

- more extensive osteoperiostitis of long bones (e.g. sabre tibiae);
- hyperostosis of the nasal processes of the maxillae ('goundou'); and
- ulceration of the palate and nasopharynx (rhinopharyngitis mutilans; Fig. 104.2) with secondary infection resulting in foul-smelling discharge ('gangosa').

Endemic syphilis

The primary lesion is seldom seen. The early manifestations include mucous patches (i.e. shallow painless ulcers on the lips and in the



Fig. 104.2 Gangosa in late stage of yaws (rhinopharyngitis mutilans; occurs also in endemic syphilis). Courtesy of WHO, Geneva.



Fig. 104.3 Angular stomatitis (also called split papules) of endemic syphilis; these lesions are also found in early yaws. Courtesy of Dr GM Antal.

oropharynx) and other mucocutaneous and bone lesions resembling those of venereal syphilis and yaws. Papillomata favor warm and moist areas and occur as split papules or angular stomatitis at the labial commissures (Fig. 104.3). Later, the patient may enter the latent phase, which may be prolonged; after this some patients develop late lesions, which are similar to those seen in yaws.

Pinta

Pinta is confined to the skin and is the mildest of all treponematoses. The primary lesion, appearing at the site of entry of *T. carateum* on an exposed part of the body, is an itchy, red, scaly papule, sometimes associated with satellite lesions and regional lymphadenopathy. The secondary stage develops several months later, in other areas, with the appearance of pintids, which are similar to the initial lesions. These are also itchy. In due course, they undergo a variety of color changes from red to copper-colored, gray and bluish-black. The lesions remain infectious for many years.

The late lesions are characterized by varying degrees of hypochromia, discoloration, atrophy and achromia. Sometimes these features are seen in the same area.

Attenuated endemic treponematoses

In areas of reduced transmission, the clinical expression of endemic treponematoses can be much milder (a few or even a single papilloma) or many of the infected subjects can be asymptomatic.[1,10] In the Gambia, 9.3% of pregnant women were seropositive for a treponemal infection; children of seropositive mothers showed no signs of congenital syphilis and there was no increase in perinatal, neonatal or child deaths. No clinical signs of endemic treponematoses were found, indicating the asymptomatic nature of the infection.[11,12]

In a number of countries of West and Central Africa, high prevalence rates for syphilis have recently been found in pregnant women based on rapid treponemal tests; clinical cases of endemic treponematoses (yaws or bejel) are not reported or not known to exist in those areas. A serosurvey in children 2–15 years of age could help to indicate that this seroreaction is probably due to past (asymptomatic) endemic treponematoses or might be venereal (latent) syphilis.

HIV infection and endemic treponematoses

As yet, no information is available on any interaction between HIV infection and endemic treponematoses.

DIFFERENTIAL DIAGNOSIS

In endemic areas, an accurate clinical diagnosis can be made in the presence of classic lesions. This will, however, necessitate appropriate training of clinicians, especially in view of the rather milder forms being encountered. The difficulties arise when there are no clinical lesions (i.e. latent cases), when venereal syphilis is also locally prevalent and when the patient is an immigrant from an endemic area presenting at a clinic in a nonendemic country. Differentiation from venereal syphilis is important because of social stigma implications. A careful and detailed history (including that of mother, father and siblings when appropriate) and thorough physical examination are always essential.

Apart from venereal syphilis, the conditions to be considered for differential diagnosis include:

- skin sepsis, scabies, fungal infection, lichen planus, plantar warts, psoriasis and tungiasis in a patient who has early skin lesions;
- tropical ulcer, cutaneous leishmaniasis, mycotic lesions and leprosy in a patient who has gummatous ulceration; and
- tuberculosis and sickle cell disease in a patient who has dactylitis.

Pinta may need to be differentiated from pityriasis versicolor, tinea corporis, vitiligo, leprosy and chloasma.

DIAGNOSIS

There is no test that can differentiate the treponematoses (including venereal syphilis) from one another. The diagnosis of treponemal infection is confirmed by the demonstration of treponemes (but beware of nonpathogenic commensals) in a wet preparation of the material from early lesions by darkfield microscopy or in the biopsy material stained by the silver impregnation technique.

Serologic tests – rapid plasma reagin (RPR) or VDRL nontreponemal tests, *T. pallidum* hemagglutination assay (TPHA) or fluorescent treponemal antibody absorption (FTA-ABS) treponemal (i.e. specific) tests – should be carried out in all cases, but their interpretation requires expertise. The treponemal tests are particularly useful to confirm a reactive nontreponemal test (exclusion of false-positives). A reactive treponemal test may indicate a current infection or a past infection ('serologic scar'). Simple and rapid treponemal tests became recently available in the form of immunochromatographic strips; as they can use whole blood and do not require refrigeration they are extremely useful in the field.[13] Radiologic evidence of osteoperiostitis may assist in the diagnosis.

If the differentiation from venereal syphilis is difficult, the patient should be managed as for venereal syphilis.

MANAGEMENT

Penicillin remains the drug of choice. Long-acting benzathine penicillin G, given intramuscularly in a single session, is preferred. The dose is 600 000 units for children under the age of 6 years, 1.2 million units for those aged 6–15 years and 2.4 million units for those over 15 years. The dose may be divided, half to be given into each buttock. While it is recognized that treponematoses have remained exquisitely sensitive to penicillin, there is a report of penicillin treatment failures of yaws in Papua New Guinea.[14] A few penicillin treatment failures have also been observed in Ecuador.[15]

The distinction between relapse, re-infection or true resistance is difficult to make but these clinical failures are worrisome and should be further researched. Oral penicillin V (50 mg/kg in four divided doses) for 10 days was used in rural Guyana and 16 out of 17 children with yaws were cured.[16] However, such a regimen with multiple doses

has a potential compliance problem and is not suitable as epidemiologic treatment (e.g. in elimination campaigns).

There is little information on the use of drugs other than penicillin to treat these conditions in case of penicillin allergy, but regimens recommended for treatment of venereal syphilis should be efficacious. Azithromycin in a single dose of 2 g orally was shown to be highly efficacious in adults but emergence of azithromycin-resistant *T. pallidum* should be monitored.[17] Oral erythromycin or tetracycline 500 mg every 6 hours for 15 days or oral doxycycline 100 mg every 12 hours for 15 days is also likely to be effective; children between 8 and 15 years of age should receive half that dose. Tetracyclines (including doxycycline) are not recommended for pregnant and breast-feeding women and for children under the age of 8 years. For children younger than 8 years, azithromycin and erythromycin should be given in doses adjusted for their body weight.

Contacts

Arrangements should be made to examine and, if appropriate and after proper explanation, to treat the household contacts and other close contacts.

Prognosis and follow-up

The lesions become noninfectious within 24 hours after the injection of penicillin. Whereas treatment in early stages should result in cure in almost 100% of patients, it will not reverse any destructive change in late stages. Rapid plasma reagin (or VDRL) titers should decline within 6–12 months, becoming negative in about 2 years. However, in a small proportion of cases, especially if treated in late stages, the RPR (or VDRL) may remain positive, albeit in low titer (below 1:8). The specific tests (i.e. TPHA, FTA-ABS, rapid treponemal tests) will remain positive throughout life.

REFERENCES


References for this chapter can be found online at http://www.expertconsult.com

Chapter 105

David G Lalloo

African trypanosomiasis

EPIDEMIOLOGY

African trypanosomiasis (sleeping sickness) causes considerable mortality and morbidity across much of the African continent. Approximately 18 000 cases are reported annually to the World Health Organization (WHO) and the estimated burden of disease is 50 000–70 000 cases annually.[1] The number of new cases has fallen over the past 10 years due to intensive control activities.

African trypanosomiasis occurs in two distinct clinical forms: an acute form, caused by *Trypanosoma brucei rhodesiense*, transmitted in endemic situations by savanna *Glossina morsitans* group flies and in epidemic situations by a 'riverine' species, *Glossina fuscipes*; and a more chronic form caused by *Trypanosoma brucei gambiense*, transmitted by the riverine species *Glossina palpalis*, *G. tachinoides* and *G. fuscipes* (Fig. 105.1).[2] *T. b. rhodesiense* occurs in east and central Africa between Ethiopia and Botswana. It is a zoonosis with several game animal reservoirs; cattle are important reservoirs in epidemics. *T. b. gambiense* occurs from Uganda west to Senegal and south through Zaire to Angola; humans are the prime reservoir of infection (Fig. 105.2, Table 105.1). Over 95% of reported African trypanosomiasis cases are caused by *T. b gambiense*.

PATHOGENESIS

Trypanosomes are blood and tissue parasites of vertebrates and are transmitted by blood-sucking insects. A developmental cycle occurs in the gut and sometimes in the salivary glands of the insect vectors, producing infective metacyclic trypanosomes; mammals acquire the infection by the bite of the tsetse fly vector. Trypanosomes are microscopic, varying in length from 15 µm to 35 µm, and are highly active when observed under the microscope.

Metacyclic trypanosomes inoculated during tsetse feeding multiply locally in extracellular spaces and induce the typical 'chancre' (Fig. 105.3), with a marked local tissue response characterized by vasculitis, perivascular mononuclear cell infiltration, edema and local tissue damage. Trypanosomes enter the lymphatics and multiply within lymph nodes, leading to parasitemia 5–12 days after infection. Antigenic variation of the surface glycoproteins causes successive waves of parasitemia as the parasite evades the host immune response.[3]



Fig. 105.1 The causative organisms of sleeping sickness in humans. Reproduction of Dutton's original drawing of *Trypanosoma brucei gambiense* from the blood of a man. The organisms possess nucleus kinetoplast and flagella, and their relative size can be assessed from the red blood cell diameter of 7 µm.

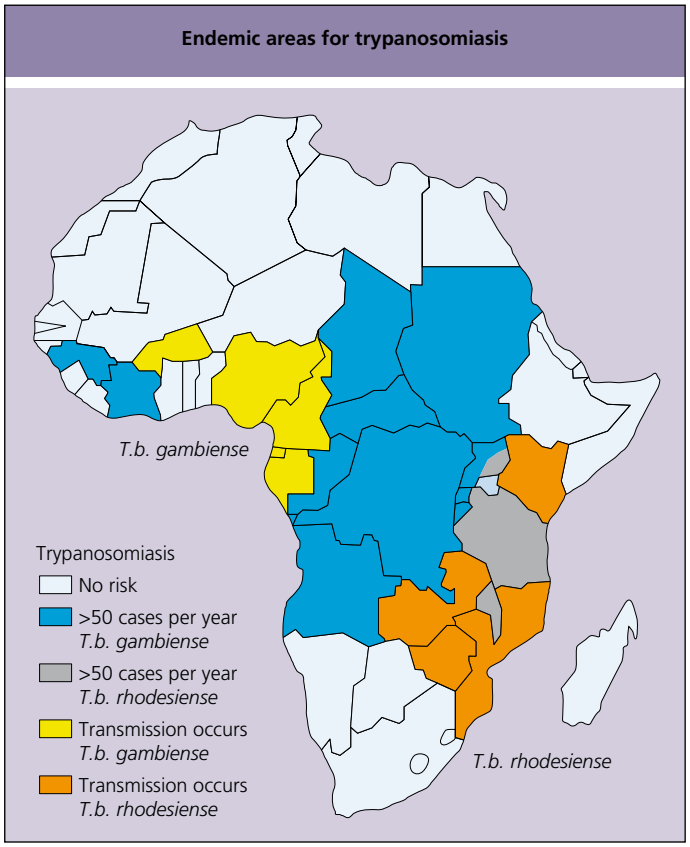


Fig. 105.2 Endemic areas for trypanosomiasis. Adapted from World Health Organization.[1]

Table 105.1 Epidemiology and distribution of the human trypanosomiases

	Trypanosoma brucei gambiense	*Trypanosoma brucei rhodesiense*
Distribution	Uganda to Senegal and Angola	Ethiopia south to Botswana and east of Rift Valley
Vector	*Glossina palpalis*, *tachinoides*, *fuscipes*; feed on any available host	*Glossina morsitans*, *pallidipes*, *swynnertoni*, (*fuscipes* in epidemics); host is game or cattle
Acquisition	Sites of high human–vector contact: river crossings, sacred groves, streams; end of dry season in Guinea savannas; peridomestic transmission in derived humid savanna; plantations of coffee and cocoa	Human penetration into savanna woodland; associated occupations (e.g. hunting, fishing, gathering of firewood and honey)
Epidemic characteristics	Lack of control (surveillance, diagnosis, treatment and vector control) as a result of expansion of human reservoir	Changes of habitat; intense human–fly–cattle contact for *G. fuscipes* transmission; encroachment on human habitation for *G. morsitans* transmission
Animal reservoirs	Pigs; to a lesser extent other domestic animals, rarely game animals	Game animals in endemic situation (bushbuck and hartebeest); cattle in epidemic situation



Fig. 105.3 Typical chancre of a patient infected with *Trypanosoma brucei rhodesiense*. The chancre develops at the site of the infecting fly bite.

Parasites enter the central nervous system (CNS) via the choroid plexus or by transcytosis across endothelial cells to cause a lymphocytic meningoencephalitis, which particularly affects the brain stem, although cortical areas and the cerebellum are also involved.[4] Perivascular infiltration with lymphocytes, plasma cells, macrophages and characteristic morular cells occurs; microglia and astrocytes proliferate and there is neuronal destruction and demyelination. Similar lesions also occur in the heart, serous membranes and endocrine organs.

Polyclonal activation of B lymphocytes leads to elevation of IgM concentrations. Heterophile antibodies, rheumatoid factor, immune complexes and autoantibody production may occur. Some neuropsychiatric manifestations may be biochemically induced; elevated prostaglandin D_2 concentrations have been found in advanced *T. b. gambiense* infection, which may be responsible for the circadian sleep disorders.[4,5]

PREVENTION

There are two major components of sleeping sickness control: detection and treatment of cases, and vector control. In *T. b. rhodesiense* areas, patients who present with symptoms of early parasitemia (passive surveillance) can be treated at local rural centers. In epidemics, rapid deployment of active surveillance using blood film screening and the establishment of local treatment centers is important (Fig. 105.4). In *T. b. gambiense* areas, limited clinical symptoms in the early stages require active surveillance. Individuals can be screened using gland aspiration or rapid antigen tests (e.g. the card agglutination test for trypanosomes, CATT). Although prophylactic measures such as 6-monthly intramuscular injections of pentamidine have been suggested for populations most at risk, concerns about development of drug resistance and the masking of second-stage infections means that this is no longer advocated.[6]

Vector control using insecticide-impregnated traps and targets has been demonstrated to be effective in epidemics of *T. b. gambiense* in the Congo[7] and in epidemics of *T. b. rhodesiense* transmitted by *G. fuscipes* in Uganda.[8] Sterile insect release methods may also be useful in reducing vector populations.[9] Residual insecticide application to *Glossina* resting sites, insecticide spraying and the clearing of riverine habitats have been used in the past but resource and environmental considerations mean that these can no longer be considered. In *T. b. rhodesiense* epidemics, treatment of the cattle reservoir with trypanocides may prevent human sleeping sickness.[10]



Fig. 105.4 Emergency treatment center Uganda. This center was established to provide facilities for the care and treatment of patients with sleeping sickness during the epidemic in Busoga.

CLINICAL FEATURES

A local skin lesion, the chancre, may develop at the site of inoculation (see Fig. 105.2). This is a raised, tender, edematous papule that rapidly increases in size with surrounding local edema, erythema and local lymphadenopathy. Chancres resolve after 2–3 weeks; they are common in *T. b. rhodesiense* infections, but rare in *T. b. gambiense* infections. Trypanosomes subsequently invade lymphatics and blood, leading to the hemolymphatic stage (stage I) and may invade the central nervous system and cerebrospinal fluid (CSF), leading to meningoencephalitis (stage II). *Trypanosoma b. gambiense* causes a mild but protracted illness over months or years, which is followed by the late development of meningoencephalitis. In contrast, *T. b. rhodesiense* causes a severe, acute, febrile disease, with rapid progression to meningoencephalitis and death within months.[6,11]

Hemolymphatic trypanosomiasis (stage I)

The characteristic clinical features of hemolymphatic trypanosomiasis comprise:

- episodes of fever, often accompanied by chills and rigors, malaise and prostration;
- headache;
- joint pains; and
- loss of weight.

In *T. b. gambiense* infections the disease slowly increases in severity as it progresses. Lymphadenopathy occurs in *T. b. gambiense* infections and enlargement of the posterior cervical glands is characteristic (Winterbottom's sign). Lymph nodes are moderately enlarged, firm and discrete. Splenomegaly occurs in around one-third of cases, and may be associated with hepatomegaly. A fleeting erythematous rash ('circinate erythema') on the trunk or upper extremities is evident in light skinned people. There are focal areas of edema, facial puffiness, dependent edema and serous effusions including ascites, pleural effusions and pericardial effusions. Myocarditis occurs especially in *T. b. rhodesiense* infections.[11]

Meningoencephalitic trypanosomiasis (stage II)

In *T. b. rhodesiense* infections, cerebral involvement occurs within weeks of the onset of infection, whereas in *T. b. gambiense* infections meningoencephalitis may be delayed several years after initial infection. Headache becomes more severe and protracted. Personality changes may occur early, especially in *T. b. gambiense* infections, with apathy, lack of attention, loss of appetite, antisocial behavior and paranoid or delusional states. Abnormalities of sleep are characteristic and result from disturbance of normal circadian rhythms; daytime inappropriate somnolence is often associated with nocturnal insomnia.

Neurologic features include tremors, muscle fasciculation, increased muscle tone and rigidity, choreic and athetotic movements. The gait is affected early with progressive ataxia. Speech is slow, slurred or incoherent. Convulsions and hemiplegia can occur. Pyramidal tract and cranial nerve lesions are less common. Kerandel's sign refers to deep hyperesthesia, often with a delayed response to painful stimuli. Intractable pruritus is a feature of *T. b. gambiense*, especially in the later stages. Ocular manifestations can include iritis, chorioretinitis and papilledema.

The course of the disease is a relentless deterioration to a stuporose state, with cachexia, wasting and progressive malnutrition (Fig. 105.5). Patients become increasingly difficult to rouse and pass into deepening coma and death. This is relatively rapid in *T. b. rhodesiense* but may be protracted over months or years in *T. b. gambiense*. Intercurrent infections, especially bronchopneumonia, are common in the late stages of the disease. In *T. b. gambiense*, amenorrhea and impotence are common.[11,12]



Fig. 105.5 Comatose terminal stage patient with sleeping sickness. Note the degree of cachexia.

Sleeping sickness in special groups

Although sleeping sickness is less common in children than adults, the clinical course of infection in children may be more severe and, with *T. b. rhodesiense* infection, progression to meningoencephalitis may be even more rapid than in adults.[13] Congenital infection has been described in *T. b. gambiense*.

Trypanosomiasis caused by *T. b. rhodesiense* or *T. b. gambiense* may occur in tourists and visitors to endemic areas. Severe early infections with high peripheral parasitemia are most common, characterized by a severe systemic illness with fever, anemia, biochemical abnormalities and circulating immune complexes. Thrombocytopenia is common and coagulopathy and disseminated intravascular coagulation may occur. Most travelers do not develop CNS involvement; however, delayed diagnosis may be a problem outside endemic areas, especially if no chancre is present.[14]

DIAGNOSIS

Routine laboratory tests demonstrate normal white cell counts, raised erythrocyte sedimentation rate, anemia, thrombocytopenia, low serum albumin and elevated serum IgM.

Parasitologic diagnosis[15]

Diagnosis of sleeping sickness is dependent on finding trypanosomes in the chancre, blood, lymph gland juice or CSF – either in unfixed or unstained preparations, when moving parasites are observed, or in stained preparations. Blood films are usually positive in *T. b. rhodesiense* infection but parasitologic diagnosis may be difficult in chronic *T. b. gambiense* disease; repeated examination and concentration techniques such as microhematocrit centrifugation or the quantitative buffy coat (QBC) improve diagnostic sensitivity. *Trypanosoma b. gambiense* may also be diagnosed by finding parasites in aspirates from enlarged cervical lymph glands.

Diagnosis of second stage (CNS) disease requires examination of CSF. Before lumbar puncture, a single dose of suramin eliminates trypanosomes from the blood, avoiding the risk of contamination of CSF with trypanosoma.[6] Parasites may be found in the centrifuged deposit, which should be examined within 15 minutes of the lumbar puncture. A raised CSF cell count (>5/mm^3) or raised CSF protein also indicates CNS infection.

Immunologic diagnosis

A number of serologic tests have been developed; CATT is particularly useful for rapid preliminary screening of large populations for *T. b. gambiense* infection, but positive results must be confirmed parasitologically.[16] An antigen detection test (CIATT) can detect *T. b. rhodesiense* in serum and CSF. This may be useful for following the response to therapy.[17]

Differential diagnosis

In endemic areas, *T. b. rhodesiense* is most common in occupational groups such as hunters, game wardens and fishermen, although all members of the community may be affected. Transmission of *T. b. gambiense* occurs where there is contact between humans and water, and both sexes and all age groups are affected. Outside endemic areas, trypanosomiasis caused by *T. b. rhodesiense* should be considered in travelers from eastern and southern Africa (especially those who have visited game parks) who present with an acute severe febrile illness. *Trypanosoma b. gambiense* infections may present months or years after exposure, with a febrile illness or neuropsychiatric features.

Chancres may be confused with insect bites, skin infections, an eschar or cutaneous anthrax. Early hemolymphatic disease mimics a wide range of febrile illnesses, especially malaria, relapsing fever, typhoid, brucellosis and arboviral infections. Occasionally, myocarditis may dominate the clinical picture. The differential diagnosis of meningoencephalitis includes a wide range of inflammatory cerebral infections; in particular, cryptococcal or tuberculous meningitis complicating HIV infection needs to be excluded. The neuropsychiatric presentations of trypanosomiasis may be confused with a variety of psychiatric syndromes, especially when personality change or psychotic behavior predominates. Late-stage disease may also mimic focal neurologic disorders, parkinsonism and space-occupying cerebral lesions.

MANAGEMENT

Treatment of sleeping sickness depends upon the stage of disease; a lumbar puncture is therefore crucial to determine whether there is evidence of CNS involvement. At least one dose of suramin or pentamidine should be given to clear blood parasites before a lumbar puncture is performed to prevent inoculation of parasites into the CSF at the time of lumbar puncture. There have been few recent advances in the chemotherapy of trypanosomiasis and the number of available drugs is limited (Table 105.2).[18]

Most treatment regimens are relatively toxic and it is therefore important to diagnose and treat nutritional deficiencies or intercurrent infections; routine anthelmintic and antimalarial treatment is often given. Hemolymphatic trypanosomiasis is treated with suramin or pentamidine; the latter is only effective in *T. b. gambiense* infection. Meningoencephalitis requires treatment with drugs that cross the blood–brain barrier in trypanocidal concentrations. The mainstays of therapy are arsenical drugs, particularly melarsoprol, used since the beginning of the 20th century. Eflornithine and nifurtimox are also used for the treatment of late-stage *T. b. gambiense* infections. In most individuals, drugs rapidly clear parasitemia or CNS parasites, but relapses occur and 2-year cure rates are the best estimate of drug efficacy. Treatment responses vary according to the drug, severity of illness and geographic location.

Hemolymphatic trypanosomiasis

Suramin is given intravenously as a 10% solution. Fever, nausea, vomiting and urticaria are common but the most severe side-effect is an idiosyncratic anaphylactic reaction, which is infrequent (1 in 2000–4000) but may be more common in the presence of onchocerciasis. Proteinuria and renal failure may also occur. Intramuscular or intravenous pentamidine are alternatives to suramin in *T. b. gambiense* infection and are usually well tolerated. Side-effects include a histamine-like reaction with hypotension and circulatory collapse, hypoglycemia and, after prolonged administration, hyperglycemia and diabetes; adrenaline (epinephrine) and glucose should be available when pentamidine is administered.

Meningoencephalitic trypanosomiasis

Melarsoprol is a trivalent arsenical compound that crosses the blood–brain barrier. A number of treatment schedules have been used, most lasting for 4–5 weeks. However, shorter 10-day treatment regimens have recently been shown to be effective in *T. b. gambiense* infection.[19,20] Thrombophlebitis and cellulitis following extravascular leakage are common (Fig. 105.6). The most important side-effect is reactive arsenic encephalopathy, which occurs in 2–10% of patients, usually after the third or fourth dose. Presentation is usually as an acute deterioration in conscious level, often heralded by convulsions. There is an associated case fatality rate of 10–50%. Prophylactic prednisone (prednisolone) reduces the incidence of reactive arsenic encephalopathy in *T. b. gambiense* (but not *T. b. rhodesiense*) infection.[21] Other toxicity is also common – a peripheral neuropathy occurs in up to 10% of patients and skin reactions, hepatic and renal toxicity are also seen; hemolysis may occur in glucose-6-phosphate dehydrogenase deficiency. Treatment with melarsoprol normally leads to a striking improvement in the mental and physical condition of patients with sleeping sickness. However, recent data suggest that rates of relapse following melarsoprol therapy are increasing.[18]

Eflornithine (difluoromethyl-ornithine, DFMO) is an ornithine decarboxylase inhibitor that is effective in the treatment of stage I and stage II *T. b. gambiense* infection but is poorly effective against *T. b. rhodesiense*. Most current regimens use intravenous administration,

Table 105.2 Drugs currently used for the treatment of sleeping sickness

Drug	Species	Indication	Route	Typical dosage regimen	Side-effects
Suramin	*Trypanosoma brucei rhodesiense (Trypanosoma brucei gambiense)*	Stage I	Slow iv infusion	Day 1: 5 mg/kg test dose Days 3, 10, 17, 24, 31: 20 mg/kg Alternative: 5 mg/kg day 1 10 mg/kg day 3 20 mg/kg days 5, 11, 17, 23, 30	Anaphylaxis Cutaneous reactions
Pentamidine	*T. b. gambiense*	Stage I	im/iv	7–10 doses of 4 mg/kg q24h (or alternate days)	Local reactions (im) Hypotension Hypoglycemia
Melarsoprol	*T. b. rhodesiense*	Stage II	iv infusion	Three series of four daily doses of 1.2–3.6 mg/kg, each separated by 7–10 days	Arsenical encephalopathy
	T. b. gambiense			As for rhodesiense or short course 2.2 mg/kg q24h for 10 days	Peripheral neuropathy Dermatitis
Eflornithine	*T. b. gambiense*	Stage II	iv infusion (po)	400 mg/kg q24h in four divided doses for 7–14 days	Diarrhea Pancytopenia Convulsions
Nifurtimox	*T. b. gambiense*	Stage II	po	15–20 mg/kg q24h in three divided doses for 14 days	Anemia Neurologic side-effects



Fig. 105.6 Treatment of a patient with an intravenous injection of melarsoprol. It is vital to adhere to the schedules of treatment and to have a scrupulous technique of injection in order to avoid destruction of local tissue as a result of leakages of the melarsoprol suspended in propylene glycol.

although oral preparations are being evaluated. The drug is less toxic than melarsoprol. Standard courses last 14 days; 7-day courses have been advocated but appear to be less effective in new cases of sleeping sickness.[22] Eflornithine may be safer and more effective than melarsoprol in some settings, although it is not as effective in those who are HIV positive.[23,24] It may also be useful for the treatment of late-stage *T. b. gambiense* infection when the organism has become resistant to melarsoprol.

Nifurtimox is a drug that has occasionally been used in the treatment of arsenic-refractory *T. b. gambiense*. On its own, it has high relapse rates, but a low-dose melarsoprol regimen combined with nifurtimox was superior to standard dose regimens.[25] Combination with nifurtimox allows a reduction in the dose and duration of eflornithine therapy with no loss of efficacy and lower adverse event rates.[26]

Post-treatment follow-up

Following treatment, patients should be reviewed every 3 months for 6 months and then 6-monthly for 2 years to identify episodes of relapse, which may occur after both early- and late-stage disease. In late-stage infection, the CSF abnormalities improve slowly after treatment and values usually return to normal within 1–2 years. CSF examination should be repeated before discharge and routinely at follow-up to detect parasites or a rising cell count. Relapse in *T. b. gambiense* following treatment with suramin or pentamidine is often treated with melarsoprol; eflornithine can also be used. Relapse in *T. b. rhodesiense* is usually treated with a second course of melarsoprol; nifurtimox may be effective if further relapse occurs but data are limited. Combination therapy has also been used in the treatment of relapse but there are inadequate data to determine its role at present.

REFERENCES

References for this chapter can be found online at http://www.expertconsult.com

Chapter 106

Euan M Scrimgeour

Other parasitic infections of the central nervous system

This chapter reviews parasitic infections of the central nervous system (CNS), with brief reference to trypanosomiasis and cerebral malaria (see Chapters 105, 111 and 118).

EPIDEMIOLOGY

Numerous species of protozoa and helminths parasitize man, especially in tropical and developing countries where environmental conditions promote mass transmission. Many sources of infection exist (Table 106.1). Table 106.2 lists parasites that can invade the CNS as adults, larvae or ova, depending upon the species, and gives their principal geographic distribution. It excludes parasitic Diptera, whose maggots occasionally invade the brain from the eye, nose or ear, and ticks that cause tick bite paralysis.

Many parasitic diseases that may affect the CNS are zoonoses. Examples, identifying the mammalian hosts, include:

- toxoplasmosis (cats and rodents);
- South American trypanosomiasis (domestic and wild animals);
- Rhodesian trypanosomiasis (antelopes, cattle);
- angiostrongyliasis (rodents);
- gnathostomiasis (fish-eating mammals);
- trichinosis (pigs and rats);
- toxocariasis (dogs and cats);
- schistosomiasis japonica (domestic animals and rodents); and
- echinococcosis (dogs and herbivores).

Gambian trypanosomiasis, schistosomiasis mansoni and schistosomiasis haematobium are essentially anthroponotic. In taeniasis solium (*Taenia solium*) humans are the definitive host but the larval cysticerci are found in pigs.

Relatively few parasites commonly involve the CNS. In some (e.g. *Plasmodium falciparum*, *Trypanosoma* spp., *Toxoplasma gondii*, *Angiostrongylus cantonensis* and *Taenia solium* (cysticercosis)), it is part of the life cycle. In others, it is accidental (e.g. eosinophilic meningoencephalitis caused by the raccoon ascarid *Bayliscaris procyonis*).

Because CNS involvement is often underdiagnosed and silent, it is difficult to estimate prevalence and incidence rates. However, autopsy studies of patients with cysticercosis in Peru, Mexico, India and Zimbabwe have shown CNS infection rates of 0.5–3%, usually without clinical sequelae; in Mexico, 80% were asymptomatic.[2] Schistosomiasis japonica affects 70 million people and 2–5% of cases develop CNS complications.[3] Clinical evidence of CNS involvement is uncommon in infections with *S. mansoni* and *S. haematobium*, but autopsy studies in Zimbabwe, Nigeria, Egypt and Brazil have revealed ova in the brain in 3–28% and in the spinal cord in 0.3–2% of cases.[4] Worldwide, cerebral malaria is the most commonly diagnosed manifestation of CNS parasitism; in African children with severe falciparum malaria, 30–70% have this complication.[5]

PATHOGENESIS AND PATHOLOGY

Parasites invade the CNS as adults, larvae or ova, through the systemic circulation or retrogradely via the vertebral venous system (Fig. 106.1). Clinical sequelae depend upon the nature and number of parasites and the immune response. Immune evasion is exhibited by many parasites (e.g. *Toxoplasma gondii*, *Trypanosoma* spp.), with minimal tissue reaction.

In falciparum malaria, maturation of the trophozoite to the schizont requires sequestration of the parasitized erythrocyte by cytoadherence in capillaries, including those of the CNS. This is usually without clinical sequelae in immune subjects, but in cerebral malaria massive sequestration obstructs capillaries, the most plausible explanation for coma.[5] In acute African trypanosomiasis, trypomastigotes rapidly invade the brain. They may become dormant but later, in stage II of the disease, especially in Gambian trypanosomiasis, they cause nonsuppurative encephalomyelitis (see Chapter 105).

Migrating larvae of the nematodes *Angiostrongylus cantonensis*, *Gnathostoma spinigerum*, *Toxocara canis* and *Toxocara cati*, *Trichinella spiralis*, occasionally *Loa loa*, *Mansonella perstans*, *Ascaris lumbricoides* and *Dirofilaria immitis* (the dog heartworm) produce transient focal cerebral lesions or eosinophilic meningoencephalitis.[6,7] *Bayliscaris procyonis* and the saprophytic soil nematode *Micronema deletrix* have also been incriminated. *Angiostrongylus cantonensis*, and others that cannot complete their life cycle in humans, die after a few weeks.

Certain trematode ova and larvae can involve the CNS. In early schistosomiasis, anomalous migration of worms to the CNS is followed by a cell-mediated response to ova deposition to form a periovular granuloma.[8] When the subsequent humoral response to adult worms and egg antigens is excessive, Katayama fever occurs, especially in *Schistosoma japonicum* infection, with fever, eosinophilia and self-limiting encephalopathy or myelopathy. In chronic schistosomiasis retrograde passage of ova and occasionally worms through Batson's vertebral venous plexus may result in myelopathy or cerebral lesions. This also occurs in *S. haematobium* infection with obstructive uropathy. When hepatosplenic schistosomiasis develops, ova pass through portopulmonary anastomoses to the lungs and reach the systemic circulation through arteriovenous shunts or by pulmonary veins (see Fig. 106.1). Schistosomal cor pulmonale also promotes cerebral embolization of ova. Ova in the CNS may incite little histologic reaction, but the development of a granuloma, focal vasculitis, localized infarction or rarely subarachnoid or cerebral hemorrhage can result.[4] In paragonimiasis, flukes migrate from lung to brain through the soft tissues of the neck.

In cysticercosis, the larval oncospheres reach cerebral and meningeal capillaries and mature to cysticerci in the gray matter and meninges. The spinal cord is largely spared. Cysticerci survive silently for 2–10 years. Intense inflammation follows their death and antibodies appear in the cerebrospinal fluid (CSF). Healing, with fibrosis

Table 106.1 Sources of parasite infection and resulting disease

Source of Infection		Disease
Food	Salads	Amebiasis, angiostrongyliasis, ascariasis, cysticercosis
	Raw aquatic plants	Fascioliasis
	Uncooked vegetables	Angiostrongyliasis, echinococcosis, cysticercosis
	Uncooked pork	Trichinosis, taeniasis solium, sparganosis
	Uncooked beef	Toxoplasmosis
	Uncooked freshwater fish	Gnathostomiasis, diphyllobothriasis, heterophyiasis, meganonimiasis
	Uncooked freshwater crayfish or crabs	Paragonimiasis, angiostrongyliasis
	Uncooked snakes, frogs*	Sparganosis
	Uncooked land molluscs (e.g. *Achatina fulica* snails)	Angiostrongyliasis
Fresh water	Skin contact	Schistosomiasis
	Nasal contact	*Naegleria fowleri* meningoencephalitis
	Consumption	Sparganosis, dracontiasis, amebiasis
Other environmental sources	Geophagy	Toxoplasmosis, toxocariasis, ascariasis, hydatidosis
	Soil	Strongyloidiasis
	Airborne	*Acanthamoeba castellani* meningoencephalitis, ascariasis
Arthropods	Mosquito (*Anopheles* spp.)	Falciparum malaria
	Mosquito (*Culex* spp.)	Bancroftian filariasis, dirofilariasis
	Midge (*Culicoides* spp.)	Mansonellosis
	Tsetse fly (*Glossina* spp.)	Rhodesian and Gambian trypanosomiasis
	Blackfly (*Simulium* spp.)	Onchocerciasis
	Chrysops flies	Loiasis
	Reduviid bugs (e.g. *Triatoma* spp.)	American trypanosomiasis (Chagas' disease)
Other sources	Blood transfusion/contaminated syringes and needles	Falciparum malaria, American trypanosomiasis, toxoplasmosis
	Cardiac, renal transplant	Toxoplasmosis
	Lung, liver, kidney	Microsporidiosis
	Transplacental	American trypanosomiasis (Chagas' disease), toxoplasmosis
	Autoinfection†	Cysticercosis, strongyloidiasis

*In South East Asia, frogs applied as a poultice may transmit sparganosis.
†Patient with taeniasis solium inadvertently consumes eggs he or she has passed in feces.

and calcification, follows. If the oncosphere enters the subarachnoid space or ventricles, then chronic meningitis with hydrocephalus may result.[2] Rarely, parasites produce large cystic lesions (e.g. in paragonimiasis, hydatidosis and coenurosis). In dracontiasis an extradural abscess containing an adult worm may compress the spinal cord, and in diphyllobothriasis an adult tapeworm competes with the host for vitamin B12 and can produce myelopathy (see Chapter 113).

Immunodeficiency and central nervous system parasitism

Immunodeficient patients are susceptible to the same parasites as the immunocompetent. Falciparum malaria is not more common in patients with HIV/AIDS but the parasite count may be increased and vertical transmission of the virus from mother to baby enhanced. In toxoplasmosis, following acute infection, cysts containing the resting phase bradyzoites remain dormant in muscle, heart, brain or choroid for decades. (The risk of a nonimmune subject developing toxoplasmosis following organ transplant from a seropositive donor is 50% for cardiac and 20% for renal transplant.) In immunosuppressed states (e.g. AIDS, leukemia and lymphomas, inherited immunodeficiency syndromes, systemic lupus erythematosus, immunosuppressive drug therapy, radiotherapy, etc.), reactivation of bradyzoites produces multiplying tachyzoites, with cerebral abscess formation. Reactivation results from impaired interferon gamma (IFN-γ) dependent cell-mediated and humoral immunity.[9] In AIDS, this occurs when the CD4 lymphocyte count falls below 350 cells/mm^3. Other parasite infections have followed transplantation, including microsporidiosis (lung, liver, kidney) and malaria (kidney and bone marrow).[10] *Trypanosoma cruzi* spares the brain in the immunocompetent, except children, but in immunodeficiency (in AIDS, CD4 cell count <200/mm^3), often after symptom-free decades, reactivation produces cardiomyopathy and/or acute, multifocal, necrotizing meningoencephalitis or a cerebral granuloma.[11] Although *Leishmania* spp. have been incriminated in various neurologic manifestations including peripheral neuropathy, optic nerve involvement, and even meningitis, reported from India, Egypt and the Sudan,[10] in most cases supportive evidence is not strong since other unidentified, opportunistic infections might have been responsible. Other opportunistic parasites that invade the CNS include free-living amoebae (e.g. *Acanthamoeba culbertsoni* and *Balamuthia mandrillaris*), which produce chronic granulomatous meningoencephalitis,[12] and the intestinal microsporidium *Encephalitozoon cuniculi*, which disseminates to produce encephalopathy.[13] *Strongyloides stercoralis* larvae normally hatch in the small intestine, penetrate the mucosa, migrate through the lungs and return to the jejunum as adults. In immunodeficiency, especially in human T-cell lymphoma virus 1 (HTLV-1) infection, *Strongyloides* hyperinfection may develop with massive tissue invasion, encephalitis and complicating *Escherichia coli* meningitis.

Table 106.2 Parasites that cause generalized or focal or space-occupying lesions and their principal geographic distribution

Parasite		CNS disease	Geographic distribution
Protozoa	*Acanthamoeba castellani**	Meningoencephalitis	Worldwide
	Balamuthia mandrillaris	Meningoencephalitis	Worldwide
	Encephalitozoon cuniculi	Encephalitis	Worldwide
	*Entamoeba histolytica**	Meningoencephalitis	Tropics, subtropics
	*Naegleria fowleri**	Meningoencephalitis	Worldwide
	Plasmodium falciparum	Cerebral malaria	Tropics, subtropics
	Toxoplasma gondii	Encephalitis, SOL brain	Worldwide
	Trypanosoma brucei gambiense	Encephalitis	West Africa eastward to Rift Valley
	Trypanosoma brucei rhodesiense	Encephalitis	Central and East Africa
	Trypanosoma cruzi	Meningoencephalitis	Central and South America
Helminths			
Nematodes	*Angiostrongylus cantonensis*	Meningoencephalitis	South East Asia, Oceania
	*Ascaris lumbricoides**	SOL brain	Worldwide
	*Bayliscaris procyonis**	Meningoencephalitis	North America
	*Mansonella perstans**	Meningoencephalitis	Tropical Africa
	*Dirofilaria immitis**	Meningitis	Worldwide
	*Dracunculus medinensis**	SOL spinal cord	Tropical Africa, Asia and Brazil
	*Loa loa**	Meningoencephalitis	Central and West Africa
	Gnathostoma spinigerum	Meningoencephalitis	Far East
	*Micronema deletrix**	Meningoencephalitis	North America
	*Onchocerca volvulus**	SOL brain	West Africa, South America, Yemen
	*Strongyloides stercoralis**	Meningoencephalitis	Tropics, subtropics
	Trichinella spiralis	Meningoencephalitis	Worldwide
	Toxocara canis, Toxocara cati	SOL brain	Worldwide
	*Wuchereria bancrofti**	SOL brain	Tropics, subtropics
Trematodes	*Fasciola hepatica**	SOL brain	Worldwide, sheep-farming countries
	*Heterophyes heterophyes**	SOL brain and cord	Far East, Middle East
	*Metagonimus yokogawi**	SOL brain and cord	Far East, Europe
	Paragonimus westermani	SOL brain and cord	Far East, tropical Africa, South America
	Schistosoma japonicum	SOL brain and cord	Far East, Philippines, Indonesia (Sulawesi)
	Schistosoma mansoni	SOL brain and cord	Africa, Middle East, South America
	Schistosoma haematobium	SOL brain and cord	Africa, Middle East
	Schistosoma intercalatum	Myelopathy	Sâo Tomé e Príncipe
Cestodes	*Diphyllobothrium latum**	Myelopathy	Russia, Canada and subarctic Europe
	Echinococcus granulosus (hydatidosis)	SOL brain and cord	Worldwide
	*Echinococcus multilocularis**	SOL brain and cord	Northern Europe, Canada, Japan
	Spirometra spp. (sparganosis)*	SOL brain and cord	South East Asia, North America
	Taenia multiceps (coenurosis)*	SOL brain and cord	Worldwide
	Taenia solium (cysticercosis)	SOL brain and cord	Worldwide, South America

Parasites of the order Diptera, whose maggots may invade the CNS, and ticks that cause tick bite paralysis are excluded. Note that related species of some parasites listed produce similar symptomology (e.g. *Angiostrongylus mackerrasae* in Queensland, Australia, *Angiostrongylus malayensis* in Malayasia and Indonesia, and numerous *Paragonimus* spp. in different parts of the world).
*CNS disease is infrequent or rare. SOL, space-occupying lesion.

PREVENTION

Important personal precautions (see also Chapter 102) for the control and prevention of parasite infestations are outlined in Table 106.3.

CLINICAL FEATURES

Most parasitic CNS infections lack specific diagnostic features. Diagnosis depends on suspecting a parasitic etiology and obtaining a history of residence in an endemic area (at any time from recent to remote, depending upon the parasite suspected), when exposure to infection may have occurred. The latter often requires a searching inquiry because patients are usually ignorant of the ways in which infection is contracted. Clinicians unfamiliar with this complex field must refer to a differential diagnosis checklist, remembering that multiple parasite infections are common in the tropics.

Major neurologic syndromes

Cerebral malaria

Fever, coma, absence of meningitis or focal neurologic signs (in the early stages) and presence of falciparum trophozoites in the blood suggest cerebral malaria. Alternative causes in a person who coincidentally has falciparum parasitemia include encephalitides, various systemic and toxic infections, and heat stroke.[5]

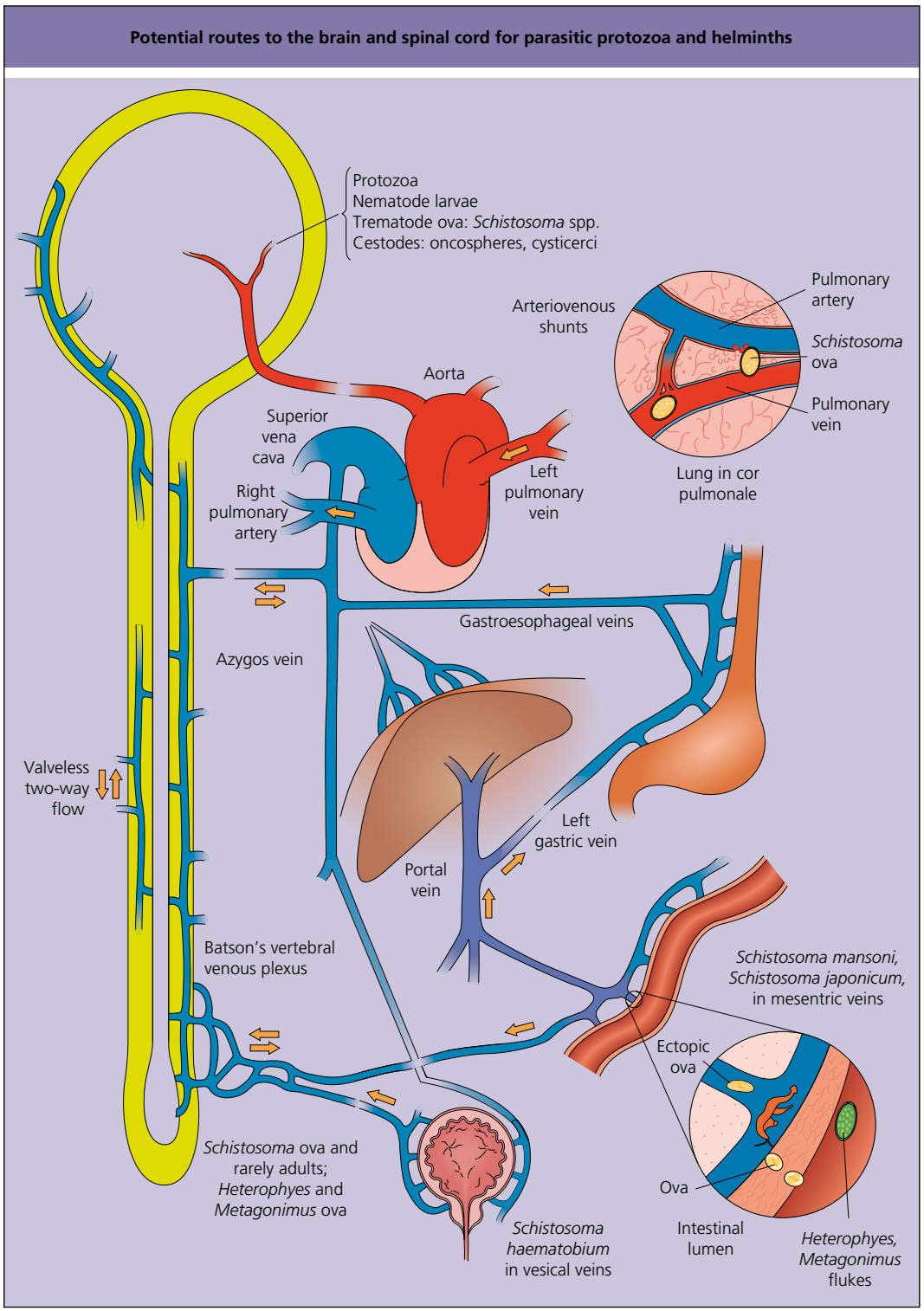


Fig. 106.1 Potential routes to the brain and spinal cord for parasitic protozoa and helminths. Collateral circulation (e.g. in hepatosplenic schistosomiasis with portal hypertension) allows ova to embolize via portopulmonary anastomoses to the lung and thence to the systemic circulation. Batson's vertebral venous plexus allows retrograde access to the spinal cord and brain by parasites and/or ova.

Trypanosomiasis

In stage II of African trypanosomiasis (sleeping sickness), chronic encephalomyelitis develops. This presents with change of personality, apathy, extrapyramidal signs including tremor, chorea, expressionless facies and reversal of sleep rhythm (see Chapter 105). Acute American trypanosomiasis causes meningoencephalitis in children.

Meningitis or meningoencephalitis

A specific helminthic cause is suggested by periorbital edema and generalized myositis (trichinosis) or subcutaneous migratory swellings followed by CNS complications (gnathostomiasis).[6] In angiostrongyliasis – history of consumption of uncooked *Achatina* or (in Thailand) *Pila* snails, or paratenic hosts of the nematode (e.g. freshwater prawns)

Table 106.3 Important personal precautions for the control and prevention of parasite infestations

- Avoidance of drinking water or eating salads or uncooked food (vegetables, meat, freshwater fish, crustaceans or terrestrial molluscs) in regions where contamination by various protozoa and helminths is probable
- Efforts to discourage children from geophagy (risk of toxoplasmosis and toxocariasis from cat and dog excreta, respectively)
- Regular anthelmintic treatment of pet dogs and cats
- Prevention of skin exposure to fresh water in regions endemic for schistosomiasis
- Prevention of arthropod bites (protective clothing, use of insect repellent creams and mosquito nets)
- Effective prophylaxis for falciparum malaria
- Screening patients who have been exposed to strongyloidiasis prior to immunosuppressive therapy
- Trimethoprim–sulfamethoxazole prophylaxis for toxoplasmosis in AIDS when the CD4 count falls below 350 cells/mm^3.

– signs may fluctuate markedly; the patient may have headache, confusion, severe generalized dysesthesia, neck stiffness and various focal neurologic signs but later the same day the patient may be almost symptom free. Fever is often absent.[1,7] Other migrating larval nematodes can produce similar variable features.

Focal or space-occupying lesions

Parasitic space-occupying lesions in the CNS are usually without diagnostic features (see Table 106.2). However, cysts in the third ventricle (e.g. cysticercus, hydatid or coenurus cysts) cause intermittent internal hydrocephalus with periodic headache and loss of consciousness.

Toxoplasmosis

Immunodeficient patients developing reactivation toxoplasmosis, with abscess formation usually in the basal ganglia, present with low-grade fever, headache, seizures, raised intracranial pressure and hemiparesis. Diffuse encephalitis is less frequent.

Cysticercosis

Acute infection presents with headache, diffuse hyperesthesia and myalgia. The first manifestation may be seizures, developing months or years after exposure to infection. Careful examination of the whole skin surface may reveal firm, painless, pea-sized, subcutaneous nodules in 50% or more of patients.[2] Differentiation from other causes of seizures is required.

Schistosomiasis

In *S. japonica* infection, and less frequently in *S. mansoni* and *S. haematobium* infections, ova in the CNS may cause seizures or present as a space-occupying lesion. There may be no other evidence of schistosomiasis.

Myelopathy

Myelopathy is uncommon in parasitic infection, except in schistosomiasis.[14] Asymptomatic deposition of ova in the spinal cord is frequent in *S. haematobium* infection but *S. mansoni* is the usual cause of myelopathy; it is uncommon in *S. japonicum* infection. *S. intercalatum* was incriminated in two cases from Sâo Tomé e Príncipe. The usual presentation is acute, flaccid, areflexic paraparesis caused by a granuloma in the conus medullaris but spasticity is present in higher lesions. Acute massive necrosis of the lower cord, presumably immunologically mediated, has been described in Brazil. The differential diagnosis includes tuberculosis, neoplasia and HTLV-1 infection. In diphyllobothriasis, megaloblastic anemia is present in association with posterior column degeneration.

Table 106.4 Ophthalmic involvement by parasites

Disease	Parasite
Iritis	*Toxoplasma gondii, Trypanosoma gambiense* and *rhodesiense, Leishmania donovani, Onchocerca volvulus, Wuchereria bancrofti, Dirofilaria immitis, Toxocara* spp., *Gnathostoma spinigerum* and cysticerci
Choroidoretinitis	*T. gondii* (bilateral, congenital; unilateral, acquired), *O. volvulus, Angiostrongylus cantonensis, Toxocara* spp., *Loa loa, Schistosoma mansoni*, cysticerci and *Armillifer armillatus* (pentastomid larvae)
Optic neuritis	*T. gondii* , *Trypanosoma gambiense* and *rhodesiense, O. volvulus, A. cantonensis* and *Paragonimus westermani*
Orbital myositis	*Trypanosoma cruzi*
Orbital or retro-orbital mass or lesion	*Leishmania* spp., *Trichinella spiralis, G. spinigerum, S. mansoni, Fasciola hepatica, P. westermani, Echinococcus granularis* cyst, cysticerci and *Taenia brauni* coenurus (in tropical Africa)

Ophthalmic involvement by parasites

Reports of direct or immunologic involvement of the eye by parasites are extensive; specific diseases are outlined in Table 106.4. In addition, the maggots of various Diptera can invade the eye (e.g. *Oestrus ovis*), ear or nose (e.g. *Cochliomyia hominivorax*), rarely extending to the brain.

Tick bite paralysis

In Australia, Africa, America and south east Europe, various hard (ixodid) and soft (argasid) ticks possess salivary neurotoxins. Between 1 and 6 days after it starts feeding, symmetric, ascending, flaccid paralysis appears, reaching the facial and bulbar muscles. Pain, fever and sensory abnormalities are absent. Death may result from respiratory failure. Recovery follows early removal of the tick.

DIAGNOSIS

Cerebral malaria

The demonstration of ring forms of *Plasmodium falciparum* in the blood and exclusion of other causes of coma (including normal CSF) are the basic diagnostic criteria.

African trypanosomiasis: sleeping sickness

Trypanosomes may be present in blood films. The enzyme-linked immunosorbent assay (ELISA) IgM is positive in 90%. Suramin is given prior to lumbar puncture (LP) to destroy blood trypomastigotes that might otherwise enter the CSF. The CSF contains lymphocytic pleocytosis, raised protein and normal glucose levels and occasionally motile trypomastigotes. Polymerase chain reaction (PCR) may detect *Trypanosoma* DNA in CSF. CT and MRI scans show brain edema (see also Chapter 105).

Meningitis or meningoencephalitis

Examination of the CSF is essential. This is often deferred if papilledema or CT scan evidence of raised intracranial pressure is present, but if an early diagnosis is critical (e.g. to exclude tuberculous meningitis) cisternal or LP to aspirate a small sample of CSF is justified. In protozoal infections, lymphocytosis, normal glucose and mildly

raised protein levels are typical. Similar findings are present in helminthic meningoencephalitis, but eosinophilia is usually present. Nonparasitic causes of eosinophilic meningitis include Hodgkin's disease, polyarteritis nodosa and occasionally bacterial or viral meningitis. Micro-organisms observed include amebae in primary amebic meningoencephalitis, trypomastigotes in African trypanosomiasis and in *T. cruzi* infections in children (and immunodeficient adults), and occasionally larval worms in angiostrongyliasis and in disseminated strongyloidiasis.

Other investigations may point to the diagnosis. Stool may contain larvae in strongyloidiasis and sputum ova or larval worms in many helminthic infections (see Chapters 108 and 184). In filarial diseases, microfilariae may appear in the peripheral blood at night (Bancroftian filariasis), at noon (loiasis) or at any time (mansonellosis) or in a skin snip (onchocerciasis). In trichinosis, biopsy of a tender muscle may reveal larvae.

Focal or space-occupying lesions

Basic parasitologic investigations may explain focal or space-occupying lesions. The stool may contain ova in *S. mansoni* and *S. japonicum* infections, paragonimiasis, fascioliasis, heterophyiasis, metagonimiasis and diphyllobothriasis. The terminal drops of urine may contain ova of *S. haematobium*. Multiple rectal snips may reveal schistosome ova of all species. In paragonimiasis, and occasionally schistosomiasis, the sputum contains ova.

The CSF in toxoplasmosis and cysticercosis reveals a lymphocytic pleocytosis and raised protein and occasionally reduced glucose levels. In schistosomiasis, a modest lymphocytosis is typical and protein and glucose levels are usually normal, whereas in paragonimiasis, eosinophilia is accompanied by raised protein levels. Eosinophilia is present in only 25% of cases of cerebral hydatidosis.

Immunodiagnostic tests for *protozoa* and helminths (see Chapters 183 and 184)

When organisms cannot be detected, antibody or antigen detection tests in serum and/or CSF confirm exposure or support diagnosis.[14] Immunodiagnosis is especially useful in the following:

- toxoplasmosis (ELISA, PCR for antigens);
- trypanosomiasis;
- neurocysticercosis (ELISA is 90% specific in blood and almost 100% in CSF; immunoblot may be negative if few cysticerci are present);
- echinococcosis (indirect hemagglutination test positive in 60% of sera); and
- schistosomal myelopathy (ELISA in the CSF is positive in >75% of cases).

To confirm active infection with *T. cruzi*, xenodiagnosis is performed utilizing laboratory-raised vector reduviid bugs to feed on the patient (see Chapter 118).

Ultrasonography of the liver or CT or MRI scans of the abdomen detect amebic and hydatid hepatic cysts (the latter calcify), *Fasciola hepatica* flukes in bile ducts or periportal fibrosis (pathognomonic of hepatosplenic schistosomiasis). Chest radiography and CT scans may reveal pulmonary paragonimiasis, hydatid cyst (non-calcifying) or schistosomal cor pulmonale. In schistosomal myelopathy, myelography typically reveals an intramedullary lesion of the conus with a complete block between T12 and L1. CT or MRI may identify a granuloma; occasionally diffuse cord edema is observed, as in the Katayama syndrome.

CT and MRI scans assist diagnosis of cerebral parasitic space-occupying lesions. Toxoplasmosis presents as one or more low-density lesions, usually ring enhancing (Fig. 106.2). In early cysticercosis, small nonenhancing hypodense lesions are present. Later, a hyperdense center (the scolex) develops (Fig. 106.3), with ring enhancement when it dies (Fig. 106.4). Finally, calcification supervenes.[2] Plain radiographs may show multiple calcified cysticerci in muscle. In hydatid cysts,

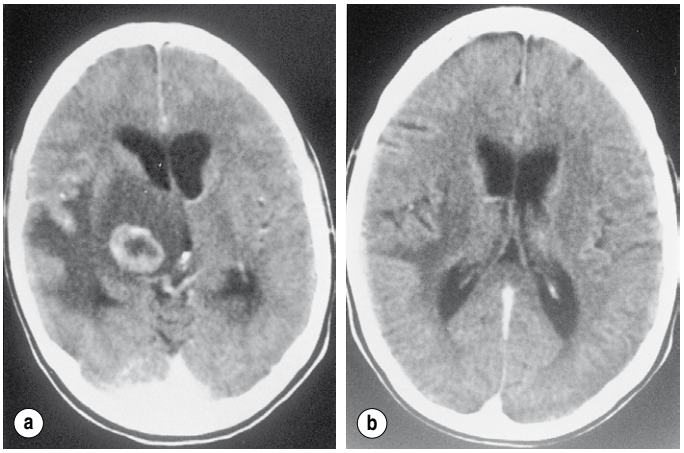


Fig. 106.2 *Toxoplasma* abscess. (a) This CT brain scan shows a *Toxoplasma* abscess in the left internal capsule, compressing the lateral ventricles. Contrast demonstrates a typical ring-enhancing effect. (b) Same patient after 17 days of treatment with pyrimethamine and sulfonamide showing resolving abscess.

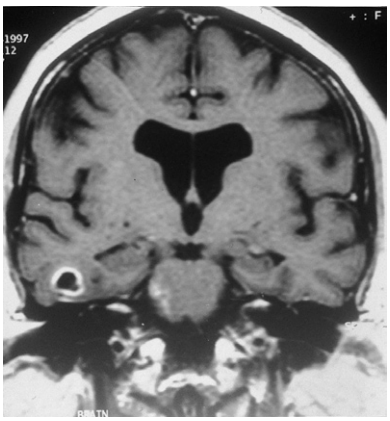


Fig. 106.3 Coronal MRI of brain showing living cysticercus, with the scolex appearing as a hyperintense center ('pea in the pod' appearance). There is no visible inflammatory reaction.

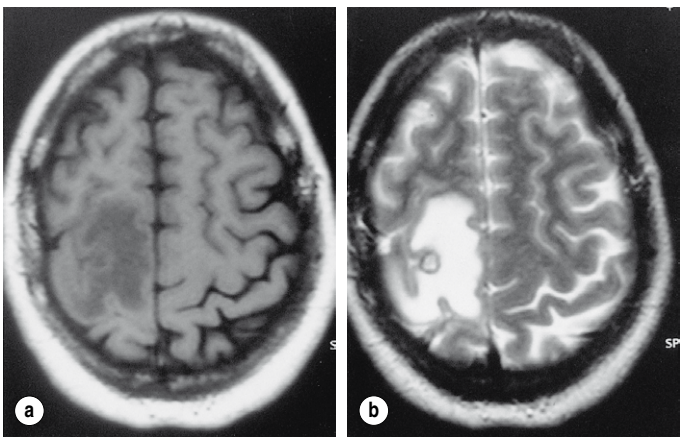


Fig. 106.4 Cerebral cysticercus. (a) This MRI of brain shows a dying cysticercus surrounded by intense inflammation. (b) Postcontrast MRI T2-weighted image demonstrating the isointense wall of the cyst and surrounding hyperintense edema.



Fig. 106.5 Extensive necro-hemorrhagic lesions of the right cerebral hemisphere in an AIDS patient with reactivated acute Chagas' disease. Courtesy of Professor L Chimelli.

CT scan is the most definitive investigation; dead cysts calcify. The CT/MRI scan in paragonimiasis reveals cystic calcified lesions containing flukes, in schistosomiasis a contrast-enhancing granuloma, and in sparganosis a granuloma containing a worm. Other investigations (e.g. gallium-67 scans and positron emission tomography) are useful but nonspecific for identifying inflammatory foci.

Finally, whenever the diagnosis is in doubt, brain or spinal cord biopsy under CT guidance is required.

Immunodeficient patients

Meningitis in immunodeficiency (e.g. AIDS) is more likely to be caused by cryptococcosis or tuberculosis than parasites. In meningoencephalitis in South or Central American patients, Chagas' disease should be considered. Rarer causes include chronic granulomatous meningoencephalitis (caused by *Acanthamoeba culbertsoni* or *Balamuthia mandrillaris*) diagnosed by brain biopsy. Encephalopathy in disseminated microsporidiosis is diagnosed by identifying *Encephalitozoon cuniculi* cysts in the urine, and neuroimaging may show multiple ring-enhancing lesions in gray and white matter.

Cerebral space-occupying lesions are most likely to be caused by toxoplasmosis (10% of cases in North America, up to 50% in Africa) or tuberculoma. In North America, 2% are due to primary CNS lymphomas; the specific investigation is thallium-201 single photon emission CT. In South America, a 'chagoma' caused by *T. cruzi* should be considered (Fig. 106.5). Many other opportunistic infections can produce space-occupying lesions (e.g. nocardiosis, cryptococcoma, aspergilloma and syphilitic gumma). Myelopathy is most likely to be caused by tuberculosis but many other causes, including toxoplasmosis and HTLV-1 infection, must be considered.

MANAGEMENT

Specific treatment of parasite infections listed in Table 106.1 is discussed in the relevant chapters. Here, the management of the major CNS parasitic disease syndromes is summarized.

Cerebral malaria

Any comatose patient who has suspected or diagnosed falciparum malaria should be treated at once with intravenous quinine (or in North America, quinidine), while monitoring blood glucose, pending further evaluation (see Chapter 111). Corticosteroid drugs are withheld as they worsen the prognosis.

African trypanosomiasis: sleeping sickness

Suramin is given to clear trypanosomes from the blood. After 1 week, melarsoprol is administered. Eflornithine is effective in Gambian trypanosomiasis only (see Chapters 105 and 150).

Meningitis and meningoencephalitis

When a parasitic cause (excluding cerebral malaria) is diagnosed, corticosteroid treatment (prednisolone 1–2 mg/kg/day) should be considered if there is raised intracranial pressure or if the patient is seriously ill. If specific treatment is lacking (e.g. in angiostrongyliasis, trichinosis, gnathostomiasis and toxocariasis), it may be the only option other than repeated LP to reduce pressure. Diethylcarbamazine and thiabendazole have been tried in toxocariasis and albendazole in gnathostomiasis (together with corticosteroids) with some evidence of benefit.

Focal or space-occupying lesions

In cysticercosis, when living cysticerci are present, praziquantel (without corticosteroids, which reduce its efficacy) 10–20 mg/kg q8h for 21 days, sometimes increased to 25 mg/kg q8h for up to 30 days,[2] is advocated. Albendazole (with preceding corticosteroid therapy) 15 mg/kg/day for 1 week has replaced praziquantel in some centers. Seizures are controlled by anticonvulsants. Despite the apparent benefit of these drugs, if untreated, the majority of cysts disappear without sequelae within 1 year. Surgery should be avoided unless the cysticercus is blocking the third ventricle.

In schistosomiasis, praziquantel 40 mg/kg is routinely given as a single dose; 60 mg daily for 3 days may eradicate worms more completely. Suspected schistosomal myelopathy should be treated conservatively with praziquantel. When this develops acutely in the Katayama syndrome, with edema of the spinal cord (Fig. 106.6), adjunctive, high-dose corticosteroid treatment is essential. Schistosomal myelopathy may improve even after several months of paraplegia. Surgery is required if there is deterioration despite treatment. If schistosomal myelopathy is encountered unexpectedly at operation, only a biopsy should be obtained.[4] It is difficult to know when schistosomiasis has been cured; antibody titers decline slowly and antigen detection may be negative although some worms persist (see Chapter 112).

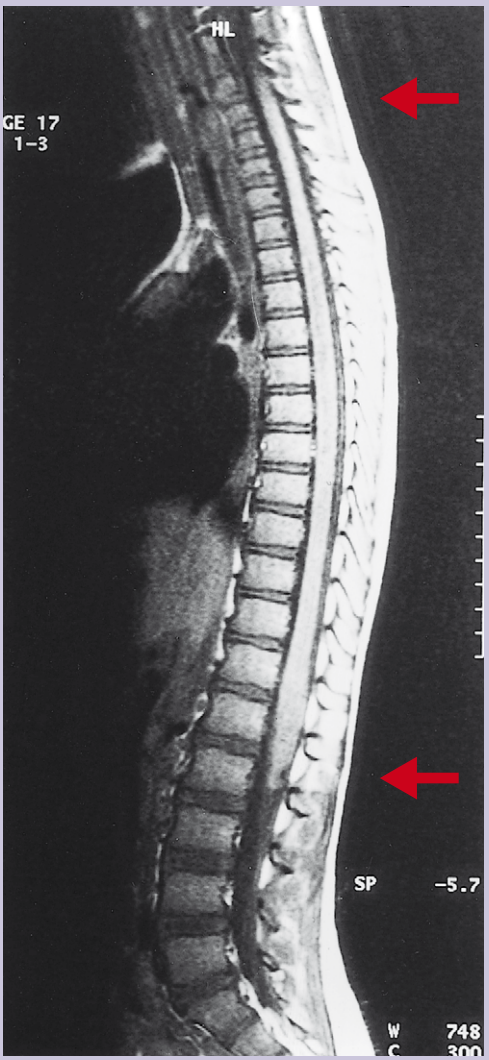
Cerebral paragonimiasis is treated conservatively with praziquantel or bithionol. Hydatid cysts are completely excised, first administering praziquantel or albendazole. Surgical resection is required for a sparganum and for a coenurus (with adjunctive praziquantel).

Treatment of central nervous system parasitism in immunodeficiency

Standard treatment applies for most parasitic infections. The immune status should be improved if possible (e.g. administer highly active antiretroviral treatment in AIDS). If brain biopsy is not possible, empiric treatment for suspected toxoplasmosis in an IgG-seropositive patient (IgM remains negative) is pyrimethamine and sulfadiazine or high-dose trimethoprim–sulfamethoxazole. A convincing response within 7–10 days supports the diagnosis. Lifelong trimethoprim–sulfamethoxazole prophylaxis follows recovery. When *Toxoplasma* IgG is negative, an intracerebral space-occupying lesion is treated as either a tuberculoma or a primary CNS lymphoma. In South America, early treatment of Chagas'

Fig. 106.6 MRI of the spinal cord of a 9-year-old Omani boy with acute schistosomiasis mansoni, who presented with transverse myelitis (vertical scale in cm). Extensive cord edema from the first thoracic vertebral level to the conus medullaris is present (arrows). He improved and was ambulant after 2 weeks treatment with prednisone and praziquantel but had persistent incontinence of urine.

MRI of the spinal cord of a 9-year-old boy with acute schistosomiasis mansoni



meningoencephalitis (which may coexist with toxoplasmosis) with benznidazole is imperative. No effective treatment for chronic granulomatous meningitis exists (see Chapters 118 and 182). Cerebral microsporidiosis (*Encephalitozoon cuniculi*) is almost invariably fatal, but has responded temporarily to albendazole 400 mg q12h (Chapters 150 and 181). Hyperinfection with strongyloidiasis responds to ivermectin, and broad-spectrum antibiotics for the usual coexistent *E. coli* meningitis.

REFERENCES

References for this chapter can be found online at http://www.expertconsult.com

Chapter 107

Robin Bailey

Eye infections in the tropics

This chapter discusses the contribution of infection to the major blinding diseases of the tropics and the ocular features associated with common tropical infections. In tropical practice, visual prognosis in both major blinding infections and simple trauma is often worsened by late presentation, secondary infection and inappropriate use of traditional eye medicines.

Major Blinding Infections of the Tropics

Cataract, vitamin A deficiency, trachoma and onchocerciasis are classically considered to be causes of blindness in developing countries. An increasing number of countries, including recently Pakistan, Nigeria and Ethiopia,[1–3] have now undertaken community-based surveys to identify the causes of blindness, showing that cataract, universally, is by far the greatest cause of blindness. Trachoma and onchocerciasis are considered to account for 3.6% and 0.8%, respectively, of the global burden of blindness.[4] In these two diseases, and in two other important tropical infectious diseases, leprosy and measles, infection and the host response to it play prominent and contrasting roles in the pathogenesis of blindness and other ocular complications. These are discussed below.

TRACHOMA

Trachoma is a chronic follicular keratoconjunctivitis caused by infection with *Chlamydia trachomatis*, almost exclusively of serotypes A, B, Ba and C (the 'genital' *C. trachomatis* serotypes D–K may cause disease that is indistinguishable from trachoma, but this is rare). Host tissue tropism in *C. trachomatis* is strongly related to the capacity to synthesize tryptophan, with all ocular strains having inactivating mutations in the *trpBA* tryptophan synthetase genes.[5] Trachoma is characterized by scarring sequelae in the conjunctiva after repeated infections.

Epidemiology

Trachoma is one of the most common infectious diseases: the WHO estimates (2008) that there are 84 million people infected worldwide and that 8 million people are irreversibly visually impaired due to trachoma. Several million more individuals are projected to become visually impaired due to trachoma in the next 20 years as a result of demographic trends.[6] The global picture of trachoma has changed considerably in recent decades but information from a number of countries remains scanty. India and China in particular have some evidence of a trachoma problem, and because of their very large populations, could be very influential in the estimates if accurate data on their trachoma burden were forthcoming. It is a disease of poverty, associated with poor personal and environmental hygiene, and it was common in much of Europe and North America during the 19th century. The map (Fig. 107.1) represents the best available information on the current distribution of active trachoma. Trachoma is found principally in sub-Saharan Africa, with additional foci in Asia, in South America and among aboriginal Australians.

In trachoma-endemic communities, the main reservoir of infection is the eyes of affected children: active trachoma is unusual among adults, and there is evidence that most transmission of trachoma occurs within the family[7,8] as a result of close contact between young children and their mothers and other caregivers. Transmission is favored by poor environmental and personal hygiene, lack of water for washing, inadequate sleeping space, inadequate disposal of rubbish or sewage, and the proximity of domestic animals. It is considered to take place via fingers, fomites and flies: the relative importance of these means of transmission probably varies from one community to another.

Pathogenesis

There is evidence that the pathologic features of trachoma are not the result of direct tissue damage but are immunologically mediated. A single infection with *C. trachomatis* usually leads to a self-limiting follicular conjunctivitis, and repeated episodes may be necessary for the development of intense inflammation and scarring sequelae. The most characteristic histologic finding in active trachoma is the presence of follicles, which resemble germinal centers in the superior tarsal conjunctiva. Subsequently subconjunctival scarring occurs and contraction of scars causes distortion of the tarsal plate, entropion and trichiasis.

Evidence from animal models suggests that cellular immune mechanisms are of primary importance in limiting and clearing chlamydial infection. Why some individuals clear infection without sequelae, while others develop scars is not known. There is some evidence that scarred subjects have a reduced capacity for clearance of chlamydial infection, which occurs by way of specific T helper-1 lymphocyte and interferon gamma responses.[9] Genetic variants at the interleukin (IL)-10 locus which potentiate IL-10 transcription increase the risk of scarring.[10,11] A series of other observations implicate tumor necrosis factor (TNF), which has proinflammatory and antichlamydial activity, and matrix metalloprotease 9 (MMP-9; a mediator in the extracellular matrix breakdown pathway linking inflammation and tissue remodeling) in the process by which repeated episodes of intense disease and infection lead to scarring.[12–16] Antibodies to a chlamydial heat shock protein, Hsp 60, are also associated with scarring sequelae of infection in both the eyelid and the genital tract, but it is uncertain whether these reflect a causal role for this antigen or an epiphenomenon.

Prevention

The goal of prevention is to reduce transmission to such a level that exposure to reinfection does not occur often enough to cause blinding trachoma. Measures focused on improving personal and community

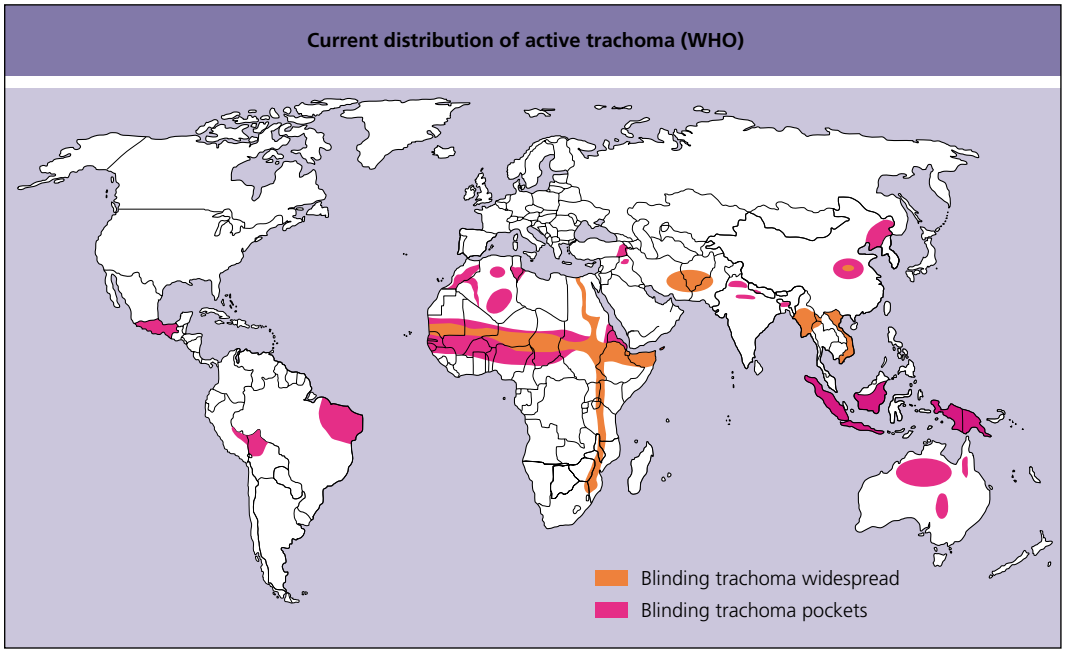


Fig. 107.1 Current distribution of active trachoma (WHO).

hygiene such as the provision of adequate water supplies, fly control and latrines are likely to reduce the incidence of trachoma. A study of community education targeted at face washing showed that, with intense effort, reductions in trachoma prevalence are possible but not well sustained.[17] Treatment of active cases with antibiotics may be effective temporarily but even high coverage mass treatment may fail due to re-exposure to untreated individuals.[18] The WHO-endorsed strategy is known by the acronym SAFE: Surgery, Antibiotic treatment, Facial cleanliness and Environmental improvement. There is no vaccine at present.

Clinical features

In endemic communities *C. trachomatis* infection is usually acquired early in childhood and the progressive scarring and distortion of the eyelid may lead to corneal scarring and blindness, usually in late middle age. In severely affected communities signs of trachoma can be found in over 90% of children aged between 1 and 2 years and blindness rates may approach 25% in those over 60 years. In most endemic areas, there is no sex difference in prevalence or incidence until after adolescence, but both active trachoma and its scarring sequelae are commoner in women, probably reflecting their closer contact with children.

Subjects who have trachoma typically have few symptoms until the final stages when inturned eyelashes (trichiasis) develop. Secondary bacterial infection may play a role in this progression, and in the mucopurulent conjunctivitis, nasal discharge and chronic otitis media seen in some patients.

The most prominent sign of active trachoma is the presence of lymphoid follicles, which are usually found on the superior tarsal conjunctiva. These can be easily visualized by everting the upper eyelid, although they may occasionally be found at the corneoscleral junction (limbal follicles). The presence of five or more of these pale off-white spots with a diameter <0.5 mm is needed in the central area of the superior tarsal conjunctiva to meet the accepted definition of trachomatous inflammation of follicular grade (TF) (Fig. 107.2). Active trachoma is also associated with capillary congestion of the conjunctiva, visible either as small red dots (papillae) or as obscuration of the normally visible tarsal blood vessels by inflammatory thickening. If these blood vessels are obscured over more than half of the central area over the tarsal plate, trachomatous inflammation of intense grade



Fig. 107.2 Everted eyelid showing follicular trachoma (TF). Courtesy of the WHO Program for the Prevention of Blindness.

(TI) is said to be present (Fig. 107.3). Neovascularization of the cornea or 'pannus' is associated with active *C. trachomatis* infection and in trachoma typically involves the superior corneal margin.

Conjunctival scars – which, if clearly visible, would be graded trachomatous scarring (TS) – are initially small and stellate, but eventually become broad and confluent (Fig. 107.4). The scars contract, causing distortion of the tarsal plate and loss of its normal protective functions, resulting in inturning of the lashes (trichiasis, which is graded TT if any lash is deviated towards the eyeball), which rub on the cornea (Fig. 107.5). This can lead to corneal opacity and ultimately to blindness from abrasion of the cornea. Limbal follicles resolve to leave small depressions at the limbus known as 'Herbert's pits' (see Fig. 107.4).

Trachoma may be confused with other conditions producing a follicular conjunctivitis (Table 107.1). With the exception of viral conjunctivitis, which is acute and self-limiting, the other conditions in Table 107.1 are never endemic in a community. However, limbal follicles or Herbert's pits (see Fig. 107.4) are the only clinical sign unique to trachoma[19] and these do not occur even in a majority of cases.

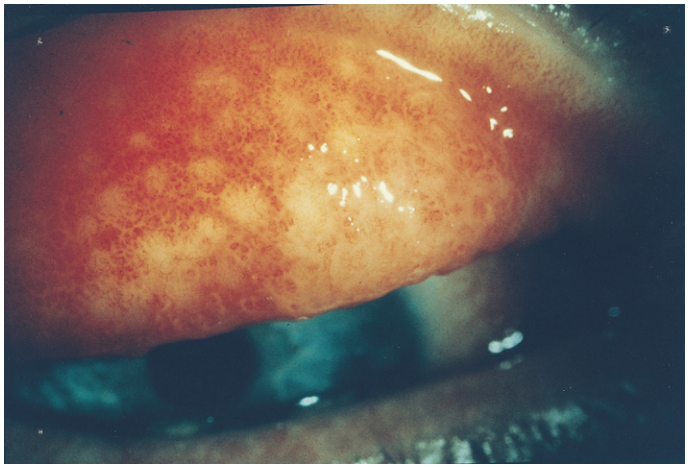


Fig. 107.3 Everted eyelid showing intense inflammatory trachoma. Follicles are also present.



Fig. 107.4 Everted eyelid showing trachomatous scarring (TS). There are also Herbert's pits visible at the corneoscleral junction. Courtesy of the WHO Program for the Prevention of Blindness.

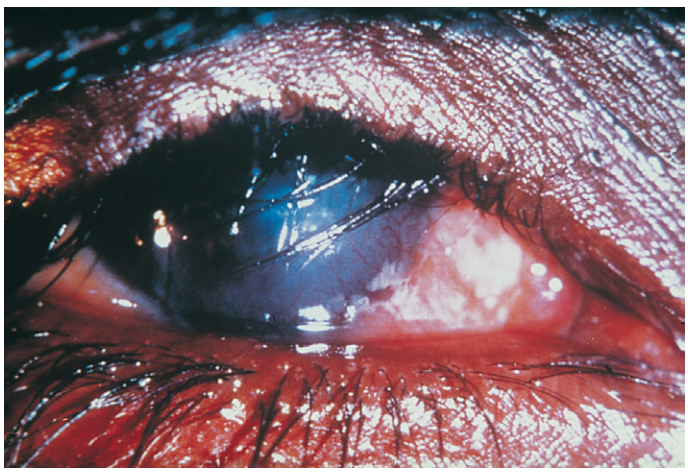


Fig. 107.5 Trachomatous trichiasis (TT) and secondary corneal opacity. Courtesy of the WHO Program for the Prevention of Blindness.

Diagnosis

Trachoma is usually diagnosed on clinical grounds. The simplified grading scheme including TF, TI, TS and TT has been developed by the World Health Organization for public health purposes.[20] Several laboratory tests can be used to confirm a diagnosis of trachoma, but these are rarely available in endemic areas. Chlamydial infection is characterized by blue intracytoplasmic inclusions in Giemsa-stained epithelial cell scrapings. The organism can be cultured in cell monolayers, visualized in smears using direct fluorescent antibody methods or detected by enzyme immunoassay. Nucleic acid amplification tests (NAATs) targeting DNA sequences in either the common plasmid pCT1 of *C. trachomatis* or, more recently, ribosomal RNA sequences[21] are the most sensitive methods for demonstrating ocular chlamydial infection.

Table 107.1 Causes of follicular conjunctivitis

Cause	Comments
Folliculosis	Follicles are few and occur in the inferior fornix without inflammation or hyperemia; a common finding
Viral infections	Acute, self-limiting with signs of resolution in 2 weeks
Trachoma	
'Inclusion conjunctivitis' and other ocular chlamydial infections	Also caused by ocular chlamydial infection, often with genital serotypes; frequently unilateral
'Toxic' follicular conjunctivitis:	
Molluscum contagiosum	Caused by spillage of contents of molluscum lesions on eyelids
Drug induced	Follows use of eye medications for months or years
Eye cosmetics	Granules of cosmetics seen in follicles
Bacterial infections: *Moraxella* spp. and others	Angular blepharitis with *Moraxella lacunata*; seen in adolescent girls who share eye make-up
Axenfeld's chronic follicular conjunctivitis	Reported in institutionalized children and native Americans, probably a mild form of trachoma[13]
Chronic follicular conjunctivitis of Thygeson	Outbreak in a Californian high school that contained trachoma cases; features compatible with mild active trachoma[13]
Parinaud's oculoglandular syndrome	Associated with pathogens invading through the conjunctivae; associated with systemic malaise and gross pre-auricular lymphadenopathy; some cases associated with exposure to cats and may be due to feline strains of *Chlamydia psittaci*; many other causes (e.g. syphilis, lymphogranuloma vereneum, tuberculosis, tularemia, cat-scratch disease)
Vernal catarrh	Occurs in atopic subjects; characteristic appearance with giant 'cobblestone' papillae

Management

Individual sporadic cases may be treated with 1% tetracycline eye ointment topically q12h for 6 weeks. A single oral dose of azithromycin (20 mg/kg) is just as effective.[22] Mass treatment with azithromycin is recommended by WHO for all inhabitants of any district, neighborhood or community in which the prevalence of TF exceeds 10% in children aged 1–9 years. As of 2008, over 77 million doses of azithromycin, donated by the manufacturer, have been distributed through the International Trachoma Initiative for this purpose.

Trichiasis can be treated by epilation, but this normally provides only temporary relief and lid surgery is usually needed to prevent blindness. Tarsal rotation[23] is the operation of choice.

ONCHOCERCIASIS

Pathogenesis

In the eye, as in the rest of the body, pathology is due to an inflammatory reaction to dead microfilariae of *Onchocerca volvulus*. As these can be found in the cornea, the anterior chamber, the iris, the lens, the retina and the choroids, onchocercal lesions may involve all these sites. In the conjunctiva and iris, an immunohistochemical study has found that chronic ocular onchocerciasis is associated with predominant infiltration by $CD8^+$ lymphocytes and is associated with major histocompatibility complex class II expression in the resident cell population, suggesting activation.[24] Regulatory T cells may have a role in suppressing the immune response.[25]

Clinical features

Eye involvement in onchocerciasis is usually bilateral and affects men more commonly than women.

Punctate or 'snowflake' keratitis occurs as inflammatory cells accumulate around dead microfilariae and it may respond to topical corticosteroids. Sclerosing keratitis, in which neovascularization and scarring develop nasally and temporally in the cornea and then extend inwards from the inferior limbal margin to involve the whole surface in a total corneal scar, has been a common cause of blindness, particularly in savannah regions. Microfilarial movement may be visible in the anterior chamber with a slit-lamp, often closely associated with the posterior corneal surface. Inflammatory reactions may produce iritis, and cataract may also contribute to reduced vision. In the posterior segment of the eye, choroidoretinal atrophy, with clumping and breaking up of the retinal pigment epithelium, and associated optic atrophy may follow. These changes contribute further to visual loss and have no specific treatment.

MEASLES

Pathogenesis

Measles virus infection and its consequences are a major risk to sight in tropical practice. In Africa they cause about 50% of childhood blindness, usually from corneal scarring.[26] Although measles virus infects the corneal epithelium and the conjunctiva, its devastating effects on the cornea are the results of secondary processes which include infection, acute vitamin A deficiency, exposure and the effects of traditional eye medicines. Measles virus-associated immunosuppression appears to be responsible for reactivation of herpes simplex virus, which has been found in corneal ulcers after measles,[27] and gut involvement appears to precipitate acute vitamin A deficiency in those with marginal reserves.

Clinical features

The direct effects of measles on the eye are a punctuate keratitis and sometimes conjunctival lesions that are analogous to Koplik's spots, which normally resolve without sequelae. Corneal ulceration and keratomalacia with liquefaction of the cornea and the whole eye may supervene in acute vitamin A deficiency. Secondary ulceration due to herpes simplex may be typically dendritic or modified by other factors as discussed below. If subjects are too sick or too dehydrated to close their eyes, corneal dryness and exposure ulceration may result, and in tropical practice, traditional medicines are frequently applied and may contribute to secondary infection and a worse prognosis. Subjects who have ocular complications of measles should be treated with vitamin A, topical antibiotics and measures to avoid corneal exposure.

LEPROSY

Pathogenesis

In leprosy three main mechanisms operate in the eye to cause pathology. Overwhelming bacterial infection in lepromatous leprosy may lead to atrophy of the involved tissues. The eye may be involved in type 1 reactions (reversal reactions), in which motor and sensory nerve loss is prominent, and in type 2 reactions, in which inflammation within the eye is prominent. As elsewhere in the body, it is the reactions that cause most damage, manifesting as visual loss and blindness in the case of the eye.

Clinical features (see also Chapter 103)

Lepromatous leprosy may be characterized by limbal lepromata, painless yellow or pink nodules at the corneoscleral junction. Chalky deposits may be associated with corneal invasion by *Mycobacterium leprae*. The iris may become thin and atrophic, and pathognomonic 'iris pearls' (which are calcified foci of dead leprosy bacilli) appear as white nodules on the surface of the iris.[28] Type 1 reactions involving the fifth cranial nerve can result in corneal anesthesia, and lagophthalmos (inability to close the eyes) may occur if the seventh cranial nerve is involved. Together, they produce a cornea that is both anesthetic and exposed, and therefore requires protection. Eye health education, blinking exercises, protective spectacles and surgical procedures such as tarsorrhaphy may all be needed.[28] Type 2 erythema nodosum leprosum (ENL) reactions may also cause acute or chronic iritis, which requires treatment with topical corticosteroids, or scleritis which may require systemic treatment with corticosteroids and clofazimine.

Clinical Aspects of Eye Involvement in Common Tropical Infections

BACTERIAL INFECTIONS

Tuberculosis

Primary tuberculosis may affect the conjunctiva with nodular lesions and associated chronic conjunctivitis that is not responsive to standard topical treatment. In miliary disease, tubercles may be found in the conjunctiva, the iris or the choroid. Phlyctenular conjunctivitis and granulomatous uveitis may be associated with tuberculosis but are not specific for it. Optic neuritis may complicate tuberculous basal meningitis.

Sexually transmitted diseases

Ocular gonococcal infection, usually as a result of autoinoculation from the genital tract, causes an acute purulent conjunctivitis that may progress rapidly to corneal ulceration and perforation in the absence of appropriate treatment.

Gonococcal ophthalmia neonatorum, acquired by an infant passing through an infected birth canal, is similarly threatening to sight and usually presents in the first week of life as a bilateral purulent conjunctivitis.[29]

Ocular autoinoculation from genital *C. trachomatis* infection causes a clinical picture identical to trachoma, except that it is commonly unilateral. Among infants born to infected mothers, 30% develop

chlamydial ophthalmia neonatorum, which is usually a self-limiting bilateral mucopurulent conjunctivitis presenting within 2 weeks of delivery. In a small proportion of cases, pneumonia and permanent lung sequelae follow. Thus chlamydial and gonococcal ophthalmia neonatorum require both systemic and topical therapy.

Iritis, retinal vasculitis, optical neuritis and disseminated chorioretinitis may be features of secondary acquired syphilis.

Other bacterial infections

Petechiae may be seen at the conjunctiva in meningococcal meningitis: conjunctivitis, anterior uveitis and even panophthalmitis may complicate meningococcaemia. Diphtheria may present with a membranous conjunctivitis, lid edema and the local effects of exotoxin. Cholera with rapid dehydration has been associated with the acute development of cataracts. Rose spots in typhoid fever may involve the conjunctiva. The clinical picture known as Parinaud's syndrome (follicular or granulomatous conjunctivitis and preauricular lymphadenopathy, often with systemic malaise) is associated with pathogen invasion via the conjunctiva and may be seen with a number of infections, including tuberculosis, syphilis, tularemia, cat-scratch disease and lymphogranuloma venereum. Brucellosis has been associated with chronic granulomatous uveitis. Dilatation of the conjunctival vessels and subconjunctival hemorrhages may be presenting features of leptospirosis or typhus.

PARASITIC INFECTIONS

Retinal hemorrhages and retinal whitening are components of a specific malarial retinopathy[30] and may be the earliest sign of cerebral involvement. Unilateral edema of the eyelid (Romana's sign) may be seen in American trypanosomiasis if the inoculation site is in the region of the eye. Chorioretinitis is the most common ocular manifestation of toxoplasmosis. An ocular larva migrans syndrome (larvae migrating within the eye) may be seen with *Toxocara* and *Gnathostoma* spp., and *Loa loa* typically migrates under the conjunctiva. Egg granulomas may occur in the conjunctiva or choroids in schistosomiasis. Cysticerci of *Taenia solium* may occur within the eye, often subretinally.

VIRAL INFECTIONS

Herpes simplex virus, which has a worldwide distribution, can have devastating effects on the eye. In many tropical environments this is the commonest cause of corneal ulceration, often occurring as a complication of measles or causes of high fever such as malaria. A narrow, branching dendritic ulcer that is best seen with fluorescein staining is typical (Fig. 107.6); however, in tropical practice the time to presentation and inappropriate use of traditional eye medicine often modify this, leading to larger ameboid ulcers. If available, idoxuridine or aciclovir drops should be given very frequently until epithelial healing takes place.

Infection with HIV may itself cause a retinopathy with cotton wool spots, indicating damage to the retina. Cytomegalovirus retinopathy does not seem to be common in tropical practice, but syphilis, tuberculosis and herpes simplex virus and their associated ocular manifestations are commoner among HIV patients. Kaposi's sarcoma may involve the eyelid or conjunctiva.

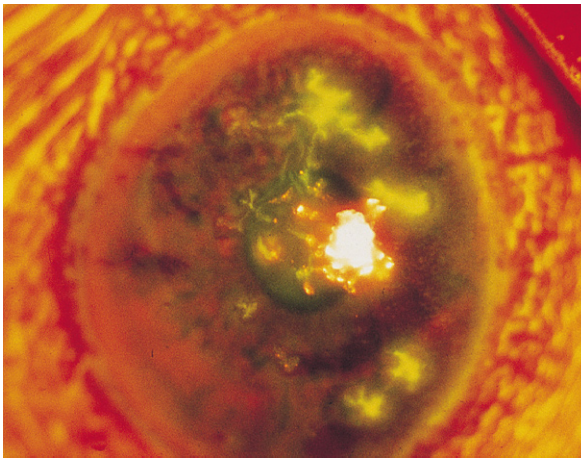


Fig. 107.6 Typical dendritic ulcer caused by herpes simplex virus, visualized with fluorescein staining.

MICROBIAL KERATITIS

Although contact lens use is the most important predisposing factor for microbial keratitis in developed country settings, in tropical practice microbial keratitis is usually the result of corneal trauma, and may have been mismanaged in the traditional sector before presentation. Patients present with a history of preceding trauma, together with photophobia, pain, loss of vision and purulent discharge in the affected eye, with cells and pus in the anterior chamber (hypopyon). The corneal defect may contain bacteria (*Staphylococcus aureus, Streptococcus, Proteus* or *Pseudomonas* spp. are commonly reported) or fungi.[22] Fungal keratitis, often with *Aspergillus* and *Fusarium* spp., has been reported associated with trauma involving plant or organic matter. If available, microscopy or culture of a corneal scrape, or local knowledge,[31] may help to diagnose the cause. Medical treatment with frequent application of topical antibiotic solutions and/or antifungals may prevent corneal scarring or perforation.

REFERENCES

References for this chapter can be found online at http://www.expertconsult.com

Practice point | 56 | Brian Greenwood

Managing an outbreak of meningococcal disease in an African village

For over 100 years, major epidemics of meningococcal disease have occurred every few years in countries of the African Sahel and sub-Sahel, the African 'meningitis belt' (Fig. PP56.1). Epidemics, which may be very large, start during the hot, dry season and subside with the coming of the rains. Epidemics often have a focal start, beginning in a cluster of villages, before spreading rapidly to involve a district, region or country. This review addresses the management of a local outbreak involving a cluster of villages within a country of the African meningitis belt.

In countries of the African meningitis belt, since the clinical features of meningitis are well recognized by the community, the first indication of an imminent outbreak may be a community-based report of cases of meningitis within a group of villages. In several countries of the meningitis belt, a more formal, facility-based reporting system has been established through which cases of meningitis are reported to a national center on a weekly basis. Thus, the first indication that a district medical officer receives of a possible outbreak may come either directly from the community or from an increase in the number of cases of meningitis reported from an individual health center during the preceding week. Deciding when to raise the alarm is a difficult decision but some simple guidelines have been devised by the World Health Organization to help. Based on past experience, these set out the attack rates which, in different epidemiologic circumstances, are likely to predict an epidemic (Fig. PP56.2).

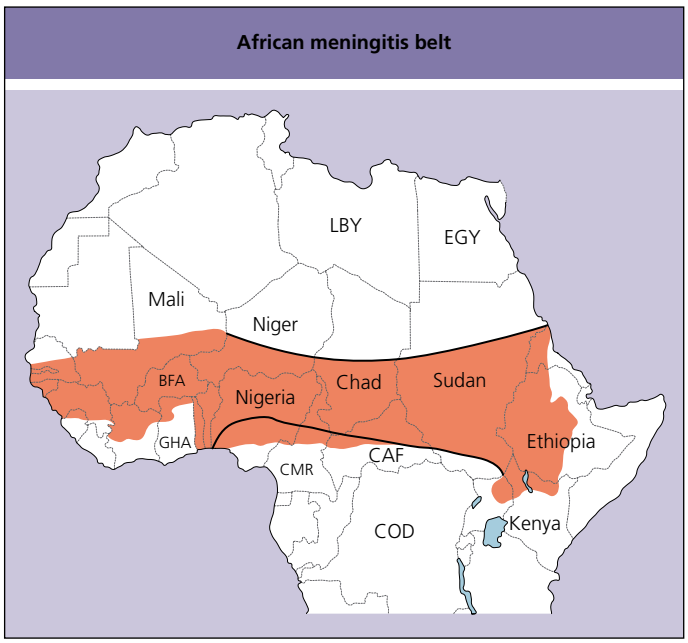


Fig. PP56.1 The African meningitis belt, modified from Lapeysssonnie.

Epidemic threshold of meningococcal disease

<30 000 Population	>30 000
• 5 cases in one week • Doubling of cases each week over a three week period	• 15 cases 100 000 population per week or • 10 cases per 100 000 population per week if – no epidemic for 3 years – threshold reached early in the dry season

WHO Guidelines

Fig. PP56.2 Numbers of cases of meningitis predicting an outbreak of meningococcal disease in a country in the African meningitis belt.

Table PP56.1 Steps to be followed on the report of a suspected outbreak of meningococcal disease

- Confirm that the cases being reported have meningitis

If this is the case:

- Determine the cause of the meningitis

If this is a meningococcus:

- Notify local, national and international authorities using established reporting procedures
- Establish or activate an epidemic management team
- Establish additional treatment facilities and obtain the necessary supplies of drugs
- Make urgent arrangements for vaccination of the affected communities if the outbreak is caused by serogroup A, C or W135 meningococcus

Once it has been established that an outbreak is likely, the district health team must undertake a number of actions (Table PP56.1). Urgency is essential as outbreaks can progress with frightening speed.

Step 1

Step 1 involves establishing that the cases being reported are cases of meningitis and, if so, determining their cause. This requires lumbar puncture in a selected number of patients at the health facility where cases are being seen and examination of their cerebrospinal fluid

(CSF). Detection of turbid CSF confirms the diagnosis of meningitis, and rapid diagnostic tests can be used for determination of the cause if conventional microbiologic support is not available. It cannot be assumed that clusters of cases are caused by the meningococcus as, within the meningitis belt, pneumococci (especially those of serotype 1) can cause similar outbreaks. Rapid diagnostic tests that are currently commercially available are based on latex technology and are relatively expensive; work is in progress to develop simpler dipstick assays.

Collection of CSF into transport medium allows bacteria to survive transport to a central laboratory where they can be cultured and further characterized using serologic and molecular biologic methods. Determination of the capsular serogroup of the meningococcus responsible for the outbreak is essential in order to guide vaccination policy (see below). Isolates may be sent to an international reference laboratory for more detailed molecular typing. Collection of laboratory samples should be accompanied by acquisition of simple epidemiologic data on the cases such as their age, sex, residence and recent travel history.

Step 2

If an outbreak of meningococcal disease is confirmed, regional, national and international authorities should be informed using established reporting procedures.

Step 3

Confirmation of an outbreak that has passed the epidemic threshold should initiate epidemic control procedures. In many epidemic-prone regions, a standing committee will be in place which needs only to be activated. In other areas, an emergency committee may need to be established. This committee must be broad based and include representatives of all groups likely to be affected by an epidemic (Table PP56.2).

Step 4

An epidemic of meningococcal disease can rapidly gain momentum and overwhelm the ability of dispensaries or a small district hospital to cope with the large number of sick patients that it generates. Thus, emergency treatment facilities may be needed, utilizing school buildings, tents or other convenient places. For many years, the standard treatment for cases of epidemic meningococcal disease has been oily chloramphenicol given as a single injection but this is being superseded by a single injection of ceftriaxone, which is easier to give and which is becoming cheaper. It may be necessary to recruit relatively untrained staff (e.g. teachers) to help with simple patient care such as ensuring that patients are well hydrated.

Step 5 – Chemoprophylaxis

Because outbreaks often cover an area with rapidly expanding and ill-defined boundaries, chemoprophylaxis is not usually recommended for outbreak management unless this occurs in a defined institution, such as a residential school or military barracks, when chemoprophylaxis with rifampin (rifampicin) might be used.

Table PP56.2 Potential members of an epidemic management team

- District medical officer
- Officer in charge of the local hospital or dispensaries
- Senior nursing officer
- Pharmacist
- Officer responsible for the local expanded program on immunization
- Representative of the civil administration
- Representative of the traditional leaders
- Representative of the local educational authority
- Religious leaders
- Representative of the World Health Organization
- Representative of any local nongovernmental organizations involved in health-care delivery
- Representative of the local media – newspaper, radio



Fig. PP56.3 Meningococcal vaccination in an African village.

Step 6 – Vaccination

Bivalent (serogroup A + C), trivalent (serogroup A, C, W135) and quadrivalent (serogroup A, C, W135, Y) meningococcal polysaccharide vaccines are available but the last is too expensive for epidemic control and is used primarily for vaccination of pilgrims. Both the bivalent and trivalent vaccines have been used in epidemic control and shown to be partially effective. Thus, if initial investigation shows that the outbreak is caused by a serogroup A, C or W135 meningococcus, vaccination of the affected communities should be undertaken as soon as possible (Fig PP56.3).

Vaccination is usually given to those aged 1–29 years old, the main group at risk. Defining the extent of the population to be vaccinated can be difficult. Speed is essential if vaccination is to have its maximum impact. Obtaining the vaccine and syringes needed for epidemic control rapidly has been facilitated by the creation of the International Coordinating Group (ICG), a group of donors and implementing agencies.

When implemented rapidly, vaccination with polysaccharide vaccines can halt an epidemic and save many lives; however, there is no evidence that the widespread use of polysaccharide vaccines in the meningitis belt over many years has reduced the frequency of epidemics. A more effective preventive strategy is needed. The dramatic success of a serogroup C meningococcal polysaccharide/protein conjugate vaccine in eliminating serogroup C meningococcal disease in the UK and elsewhere in Europe has raised expectations that the serogroup A vaccine, currently being developed by the Meningitis Vaccine Project, will be equally successful when deployed in mass vaccination campaigns in countries of the African meningitis belt. Immunization campaigns using this vaccine will commence in 2010.

FURTHER READING

Further reading for this chapter can be found online at http://www.expertconsult.com

Chapter 108

Carmel M Curtis
Peter L Chiodini

Parasitic infections of the gastrointestinal tract

INTRODUCTION

This chapter examines the parasitic causes of gastrointestinal infection in the nonimmunocompromised host. The organisms involved are a very heterogeneous group of protozoa and helminths. They vary in size from microsporidia, which are barely visible using light microscopy, to long multicellular organisms such as *Taenia saginata*, which can reach 25 m in length (Fig. 108.1). The clinical approach is emphasized here because the parasites themselves are discussed elsewhere (see Chapters 180, 181 and 184), and diagnosis in amebic infections is covered in Chapter 110. Parasitic gastrointestinal infections in HIV infection are discussed in Chapter 91. The parasites discussed in this chapter are listed in Table 108.1. Bacterial causes of chronic diarrhea and tropical sprue are discussed in Chapter 36.

EPIDEMIOLOGY

In affluent settings, parasitic infections appear to be uncommon causes of gastrointestinal illness. Certain populations are particularly likely to be affected:

- returned travelers;
- day-care workers and patients;
- migrant workers; and
- men who have sex with men.

The most common causes of parasitic gastrointestinal infection are *Giardia intestinalis* and *Cryptosporidium* spp.

In resource-poor settings the impact of parasitic infections is of much greater significance in relation to morbidity, mortality and economic impact. Their epidemiology is varied and their control presents a complex sociopolitical problem. The most important infections are *Entamoeba histolytica*, *G. intestinalis*, hookworms, *Ascaris* spp. and tapeworms.

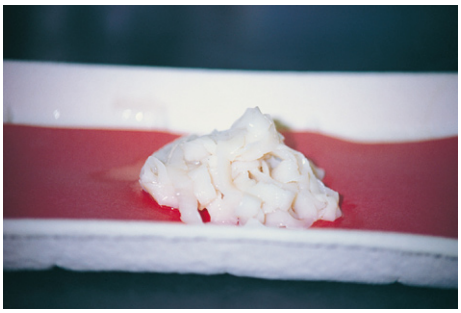


Fig. 108.1 Adult beef tapeworm (*Taenia saginata*) passed in a patient's feces.

Geographic distribution of parasites

The distribution of a parasite depends upon:

- human behavior;
- the physical environment; and
- the biologic environment.

Human behavior

Behavioral factors are often dominant but the distribution of vectors, intermediate hosts and reservoir hosts is also to some extent under human control. Some parasites are worldwide in their distribution, such as *E. histolytica* and *G. intestinalis*, whereas others are very localized.

Table 108.1 Gastrointestinal parasites

Intestinal protozoa	Amebae	*Entamoeba histolytica*; *Entamoeba dispar*
		Commensals: *Entamoeba coli*, *Entamoeba hartmanni*, *Endolimax nana*, *Iodamoeba butschlii*
	Bigyra	*Blastocystis hominis*
	Flagellates	*Giardia intestinalis*
		Dientamoeba fragilis
	Ciliate	*Balantidium coli*
	Coccidia	*Cryptosporidium parvum/C. hominis*
		Cyclospora cayetanensis
		Isospora belli
	Microsporidia	*Enterocytozoon bieneusi*
		Encephalitozoon intestinalis (formerly *Septata intestinalis*)
Intestinal helminths	Nematodes (round worms)	*Ascaris lumbricoides*
		Enterobius vermicularis
		Hookworms: *Ancylostoma duodenale*, *Necator americanus*
		Trichuris trichiura
		Strongyloides stercoralis
	Trematodes (flukes)	*Fasciolopsis buski*
		Heterophyes heterophyes
	Cestodes (tapeworms)	*Taenia solium*
		Taenia saginata
		Hymenolepis nana
		Diphyllobothrium latum

Physical environment

The physical environment also affects the distribution of parasites. Temperature and humidity affect the viability of parasites in the external environment; viability is lost in cold or over-hot temperatures and most parasites require moist, aerobic conditions. Examples of parasites for which these factors are critical include:

- the cysts of protozoa with direct life cycles, such as *E. histolytica, G. intestinalis, Balantidium coli* and the various gut commensal species (*Entamoeba coli, Entamoeba hartmanni, Endolimax nana, Iodamoeba butschlii*);
- the oocysts of *Isospora belli*;
- the eggs and larvae of soil-transmitted nematodes (*Ascaris* spp., hookworm[1] and *Strongyloides stercoralis* (see Fig. 108.3));
- the eggs of trematodes (*Fasciolopsis buski* and *Heterophyes heterophyes*) and cestodes before their entry into the aquatic environment; and
- the eggs of cestodes before their ingestion by the intermediate hosts (*Taenia solium* and *Taenia saginata*).

Temperature and humidity also affect sporulation of oocysts, the rates of embryonation of nematode, cestode and trematode eggs, and the development times for hookworm and *Strongyloides* larvae. Parasites that have a soil stage are affected by the particular physical properties of the soil, including its particle size and water-holding capacity, and by factors such as rainfall. Susceptibility to anaerobic conditions is important for some parasites when 'night soil' (human feces) is used raw or after composting as a fertilizer.

The aquatic environment is important in the life cycle of many parasites. In the trematodes, the cercariae of *Heterophyes* spp. must survive long enough to infect the fish or shrimp and the metacercariae of *F. buski* must survive long enough to infect the human or pig hosts. *Strongyloides* larvae live in the capillary water films in soil and on low vegetation.

Biologic environment

The biologic environment also affects the epidemiology of these gastrointestinal parasites. The distribution in nature of appropriate vectors, intermediate hosts and reservoir hosts can obviously affect the distribution of parasites. Examples of animal reservoirs are *F. buski* in dogs, pigs or rabbits; *B. coli* in pigs; *C. parvum* in domestic animals, particularly cattle; and *H. heterophyes* in fish-eating mammals. Secondary hosts include snails for *F. buski* and freshwater fish for *H. heterophyes*. An important biologic factor is the presence of dung beetles that take the parasitic ova or larvae underground to a more favorable physical environment.

Human factors

Population density and urbanization

The agricultural revolution in developing countries has produced large resident human populations with the potential for direct person-to-person spread of infection and greater environmental contamination by feces. In addition, animal husbandry has created other cycles for parasite transmission, for example *Cryptosporidium* spp. in calves. Rapid urbanization, especially in the tropics, is often associated with increased poverty, poorer housing and unsanitary conditions. The result is that people may be living in a more fecally polluted environment than in rural areas, encouraging such diseases as amebiasis and giardiasis. Epidemics, such as outbreaks of cryptosporidiosis, may occur when public water supplies become fecally contaminated. *Cyclospora cayetanensis* is transmitted via contaminated produce and contaminated drinking water.[2] The soil-transmitted nematodes *Ascaris lumbricoides* and *Trichuris trichiura*[3] are often more common in towns and cities. Overcrowding favors direct transmission of *Hymenolepis nana* and *Enterobius vermicularis*, especially in children when levels of hygiene and sanitation are poor.[4]

Population movements

Population changes associated with mining, political unrest or industrialization may cause people to move into at-risk areas; travelers may also visit such areas.

Dams and irrigation

Development programs in the tropics frequently involve irrigation projects, where contaminated water supplies reach greater numbers of people and larger water-borne outbreaks may occur. Irrigation and poor drainage facilitate the breeding of flies that may have a role in spreading fecal material.

Agriculture

Cattle raising may be complicated by bovine cysticercosis (*T. saginata*), which renders carcasses unsaleable, and calves may be a source of human infection. Pigs can allow *T. solium*, the pork tapeworm, to be spread and *F. buski*, the intestinal fluke, to prosper. *Balantidium coli* is also acquired from close contact with pigs. Fish farms in which water plants such as water calthrop are grown transmit *F. buski*, especially if human or pig feces are used as fertilizer. Foods implicated in outbreaks of cyclosporiasis in the USA include fresh raspberries, mesclun lettuce and Thai basil.[5] In endemic areas cases of cyclosporiasis have also been associated with drinking contaminated water or to recreational water contaminated with human sewage. To date there is no known association with animal husbandry or animal feces.[6] The use of untreated human night soil enables soil-transmitted helminths to enter the human food chain.

Domestic environment

Sanitation, water supplies and domestic customs in hygiene and food preparation are all very important. Children are at risk of parasitic infections because of poor hand washing after defecation, finger sucking and playing with soil. Local dietary behavior is critical for parasite transmission. Ingestion of certain fish, crustacea, mollusks and aquatic vegetation can lead to fluke infection. Tapeworms are contracted by ingestion of undercooked pork or beef (*Taenia* spp.) or certain freshwater fish (*Diphyllobothrium* spp.). People who have occupations involving sewage, water or soil contact are at increased risk of parasitic infection.

Host susceptibility

Host susceptibility is affected by many factors such as nutritional status, intercurrent disease, pregnancy, immunosuppressive drugs and malignancy. Previously mild or clinically inapparent infections can produce dangerous disease when host immunity falls, such as occurs in strongyloidiasis, in which the parasite is capable of multiplying by autoinfection within its host, and fatal amebiasis that may occur if corticosteroids are administered in error.

Some protective immunity is usually acquired by the host but its effectiveness is variable. The absence of symptomatic giardiasis in adults in places where the infection is common is good evidence for acquired immunity. Re-infection and superinfection, possibly by different gastrointestinal parasite strains, is certainly common in areas of endemic infection. Immunodeficiency associated with HIV infection is of paramount importance in some of the more recently recognized gastrointestinal parasitic diseases such as cryptosporidiosis and microsporidiosis (see Chapter 91).

PATHOGENESIS AND PATHOLOGY

Gastrointestinal parasites cause disease in a variety of ways. Most are present in the lumen of the gut or attached to the mucosa of the gut wall and are not capable of invasion. The coccidian parasites such

Table 108.2 Anatomic location of gastrointestinal parasites

Lumen only	Small bowel (normally) Large bowel	*Ascaris lumbricoides* *Entamoeba histolytica/dispar* *Balantidium coli* *Enterobius vermicularis*
Mucosal attachment	Small bowel Large bowel	*Giardia intestinalis* Tapeworm Hookworm *Fasciolopsis buski* *Heterophyes heterophyes* *Trichuris trichiura*
Epithelial cell invasion	Small bowel	*Isospora belli* *Cyclospora cayetanensis* *Cryptosporidium parvum/ C. hominis* *Microsporidia*
Mucosal invasion	Small bowel Large bowel	*Strongyloides stercoralis* *Entamoeba histolytica* *Balantidium coli*

as cryptosporidia can invade the epithelial cells of the small bowel. Others, such as *E. histolytica*, *S. stercoralis* and occasionally *B. coli*, do invade the mucosa (Table 108.2).

Gastrointestinal protozoa

Amebiasis

Amebic ulcers mostly develop in the cecum, appendix or adjacent ascending colon, although the sigmoidorectal region can be involved.[7] Amebic ulcers are formed on the mucosa. They are usually flask shaped with a small, raised opening and a larger area of mucosal destruction below. The mucosa between abscesses is normal but lesions can be confluent. Pathogenic amebae are able to resist complement-mediated lysis and they possess other virulence factors such as attachment lectins, cysteine proteases and other enzymes[8] which, it is believed, assist the trophozoites with apoptosis of mammalian intestinal cells. Amebae have tissue-lysing enzymes on their surfaces that can be released from lysosomes or after amebic rupture. Recent studies *in vitro* and in mice have suggested that alterations in intestinal permeability may be mediated by pore-forming proteins called amebapores as well as alterations in tight junction proteins; this has yet to be confirmed in humans.[9]

In a study in Bangladeshi children, acquired immunity to amebiasis was associated with the appearance of an intestinal IgA response to the parasite Gal/GalNAc lectin, the virulence factor required for attachment of *E. histolytica* trophozoites to human cells. An intestinal IgA response to this lectin was associated with a 70% reduction in infections in children over the 2-year study period. On the other hand, those children who developed anti-amebic IgG antibodies in the serum were more susceptible to amebiasis. Interestingly, serum anti-lectin IgG antibodies cluster in families, supporting the presence of an inherited component to susceptibility to amebic disease.[10] Based on work with *Entamoeba invadens*, a parasite of reptiles, Eichinger has suggested that interactions of amebae with intestinal mucin glycoproteins promote encystment of the parasite and thus prevent invasion.[11] This is in keeping with the finding that *E. histolytica* cyst passers are asymptomatic.[12]

Giardiasis

The histopathology of the upper small bowel varies from normal to subtotal villous atrophy in giardiasis. *Giardia* spp. seem unable to penetrate the mucosal wall in humans but are able to attach to the mucosa of the small bowel. In symptomatic cases there is increased mucus secretion and dehydration.[13] *Giardia intestinalis* may undergo antigenic variation, thereby evading the human immune response.[14] Giardiasis is more common in the immunodeficiency syndromes, particularly in common variable hypogammaglobulinemia, although there is no particular increase in incidence among the HIV-infected population.

Balantidium coli

The trophozoite of *Balantidium coli* causes mucosal inflammation and ulceration, invading the distal ileal and colonic mucosa. Ulceration may be superficial or involve the full thickness of the bowel, leading to perforation.[15] Invasion may be enhanced by hyaluronidase produced by the parasite. Other products liberated by the parasite as well as host factors, such as the recruitment of mucosal inflammatory cells, may also be important.[16]

Cryptosporidiosis

Cryptosporidial infection in humans is most commonly due to *Cryptosporidium parvum* and *Cryptosporidium hominis* (formerly *Cryptosporidium parvum* genotype 1,[17]) but *C. felis, C. muris, C. canis* and *C. meleagridis* have been identified in immunocompromised individuals.[17] *C. hominis* has been linked to more severe symptoms in sporadic outbreaks, in HIV-positive individuals and in a study of Brazilian children. In a study of more than 500 Peruvian children, *C. hominis* was the predominant cryptosporidial species and subtype Ib was associated with more symptoms of nausea, vomiting and general malaise in association with diarrhea than other subtypes (Ia, Id and Ie).[18] Different subtypes of *C. hominis* have varying effects in those patients with HIV-associated cryptosporidiosis (see Chapter 91).

Cryptosporidium infects the intracellular, extracytoplasmic area of host epithelial cells of the small bowel. The intracellular stage of *C. parvum* resides within a parasitophorous vacuole in the microvillus region of the host cell. Oocysts undergo sporogony while in the host cells. Approximately 20% of the oocysts do not form the usual environmentally resistant oocysts but are released as sporozoites that are capable of penetrating the microvillus regions of other cells within the intestine. This explains the ability of *C. parvum* to cause severe diarrhea in some patients, particularly in the immunocompromised.[19] Small bowel histology shows villous atrophy and crypt hyperplasia, usually with a mixed inflammatory cell infiltrate in the lamina propria. There is impaired absorption and enhanced intestinal secretion. There appears to be *Cryptosporidium*-associated apoptotic epithelial cell death.

The precise molecular mechanism for cryptosporidial diarrhoea is not yet elucidated. There appears to be enterotoxin-like activity and it appears that attachment of *Cryptosporidium* to epithelial cells induces survival signals in those cells so that the organism is able to multiply, while at the same time causing alterations such as apoptosis in adjacent uninfected cells which impair absorption and secretion by those cells and result in clinical disease; however, no enterotoxin has yet been isolated.[20]

Cyclosporiasis

The mechanism of diarrhea production has not been clearly established for *Cyclospora cayetanensis*. The organism is found within enterocytes. There is reduction in villus height with associated mucosal inflammation and increased numbers of intraepithelial lymphocytes, which suggests a direct effect on the intestinal mucosa.[21]

Isosporiasis

Mild to subtotal villous atrophy and crypt hyperplasia occurs in *I. belli* infection. Histologically, there may be infiltration of the lamina propria with large numbers of eosinophils, plasma cells, lymphocytes and polymorphs. Dilated lymphatics may be seen.[22]

Microsporidiosis

Intestinal microsporidia infect enterocytes in the small bowel and undergo sporogony, which leads to enterocyte degeneration, vacuolation and loss of the brush border. These cells are sloughed off and the spores are capable of infecting further enterocytes.[23,24]

Gastrointestinal helminths

Nematode infections

Ascariasis (roundworm infection)

The embryonated eggs of *A. lumbricoides* are ingested and hatch in the stomach and duodenum, from where the larvae penetrate the intestinal wall (Fig. 108.2). They are carried to the lungs in the circulation and usually cause no symptoms unless there are a large number of larvae, in which case pneumonitis can ensue. The larvae then break out of the lung tissue and may cause some bronchial epithelial damage. Intense tissue reaction with infiltration of eosinophils, macrophages and epithelioid cells occurs.[25] Jejunal histology shows shortened villi, a decrease in the villus:crypt ratio and cellular infiltration of the lamina propria.[25]

Hookworm infection

Hookworm disease is caused by *Ancylostoma duodenale* and *Necator americanus*. Vesiculation and pustules can occur on the skin at the site of entry of the filariform larvae. Asthma and bronchitis occur during migration through the lungs, with small hemorrhages into the alveoli and infiltration of eosinophils and leukocytes. Adult hookworms attach firmly to the small bowel mucosa; *A. duodenale* does this by means of well-developed mouth parts and *N. americanus* by means of cutting plates. There tends to be chronic blood loss at the site where the worm attaches.

Trichuriasis (whip worm)

The egg of the nematode *T. trichiura* hatches in the small intestine and the larva penetrates the villi causing no pathologic reaction. It re-emerges after 1 week and migrates to the cecum and colorectum. When few worms are present there is little damage, but with heavy infections there is hemorrhage, mucopurulent stools and symptoms of dysentery, sometimes with rectal prolapse (the *Trichuris* dysentery syndrome).[26]

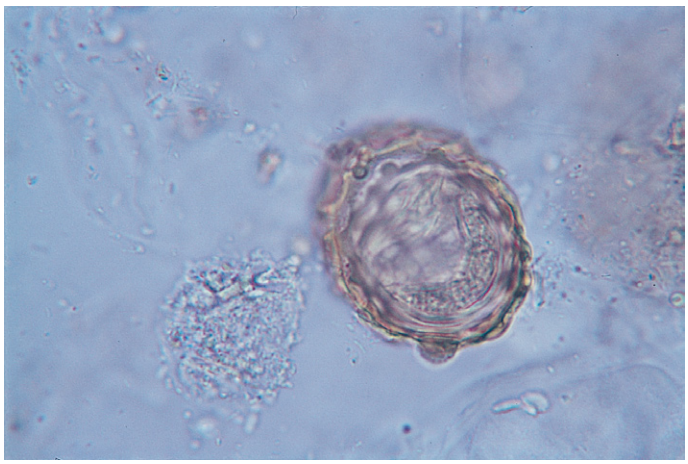


Fig. 108.2 *Ascaris lumbricoides* ovum in feces. The ovum measures 50–70 × 40–50 μm and is elliptical. The rough albuminous coat gives it a mammillated appearance.

Strongyloidiasis

The life cycle of *S. stercoralis* is complex (Fig. 108.3). Human infection is acquired when filariform larvae in the soil penetrate the skin. This can cause petechial hemorrhages, congestion and edema at the site of entry. The larvae migrate into cutaneous blood vessels and are carried to the lungs, where they break out of the pulmonary capillaries and sequentially enter the alveoli, trachea, pharynx and then the mucosa of the duodenum and upper jejunum. There can be pathologic findings similar to those of bronchopneumonia with lobar consolidation. The females mature in the intestine, invade the tissues of the bowel wall and lay their eggs, which hatch and release first-stage (rhabditiform) larvae in the feces (Fig. 108.4). In certain situations, the rhabditiform larvae mature in the intestine to the filariform stage, and these parasites bore into the wall of the duodenum and jejunum and initiate another cycle of infection, which eventually results in there being more adult worms in the small bowel. Filariform larvae that penetrate the bowel wall can spread throughout the lymphatic system to the mesenteric lymph glands and can enter the general circulation and hence the liver, lungs, kidneys and gallbladder. They can cause granulomas in the gastrointestinal tract and mesenteric glands. There are abscesses in the lungs and there may be granuloma in the liver. Migrating larvae may cause the patient to die from sepsis arising from the normal intestinal bacterial flora.

Trematode infections (flukes)

Fasciolopsis buski *infection*

The giant intestinal fluke *F. buski* is contracted by humans and pigs through eating metacercariae attached to water plants. The parasite excysts and attaches to the mucosa of the jejunal and duodenal wall causing mechanical injury and inflammation, which can lead to ulcers, bleeding or abscesses. There may be a mild anemia and low levels of serum vitamin B12, owing to the parasite's competition for the vitamin or impairing its absorption.

Heterophyes heterophyes *infection*

Heterophyes heterophyes is a trematode that infects humans and is found mainly in Asia. The pathology depends on the degree of infection acquired through ingestion of pickled or raw fish. The metacercariae excyst and attach to the walls of the small intestine. The adults may cause only a mild inflammatory reaction. Eggs may enter the circulation because the adults are attached deeply into the intestinal wall. Ectopic eggs can provoke granuloma formation, especially if they lodge in the heart or brain.[27]

Cestode infections (tapeworms)

Taenia solium *and* Taenia saginata

The pork tapeworm (*T. solium*) and the beef tapeworm (*T. saginata*)[28] are acquired by the human host by ingestion of poorly cooked meat that contains the encysted larvae (cysticerci). The larva is digested in the stomach and the head of the tapeworm evaginates in the upper small intestine, attaches via the scolex to the intestinal mucosa and feeds by absorbing nutrients from the bowel. The scolex of *T. solium* has four suckers and a rostellum that contains a double row of hooks; *T. saginata* has the suckers only. Very little pathology is caused by these well-adapted adult worms, which may reach 7 m and 25 m in length, respectively. The pathologically significant stage for humans is the cysticercus of *T. solium*. The eggs passed in feces are ingested and hatch when they are exposed to gastric juice. The oncospheres that are released penetrate the intestinal wall and are carried via the bloodstream and can potentially form a cysticercus in any organ. The cysticercus is an ovoid, milky-white bladder with the parasite head invaginated inside. The pathology is described in detail in Chapter 113.

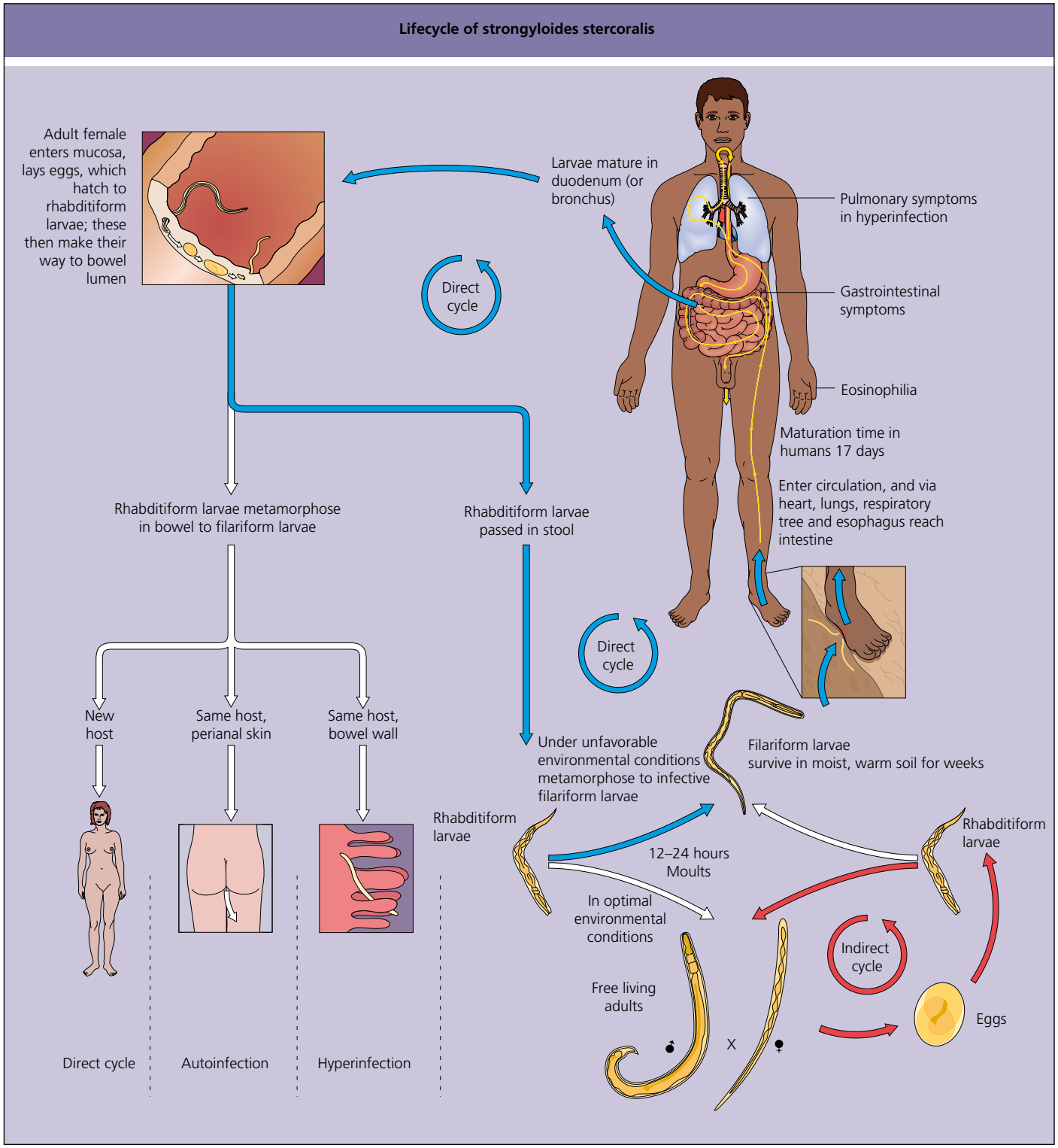


Fig. 108.3 Life cycle of *Strongyloides stercoralis*.

Dwarf tapeworm (Hymenolepis nana)

Once the ova of the dwarf tapeworm *H. nana* are ingested they encyst in the small intestinal villus mucosa. The adult worm reaches a length of 3–4 cm and begins egg production. Heavy infections of more than 100 worms can cause some symptoms, and the competition for nutrients has been linked with growth retardation in children, although this may be more the result of unsanitary conditions, poverty and malnutrition.

Fish tapeworm (Diphyllobothrium latum)

The fish tapeworm *D. latum* has a life cycle involving a first intermediate host of tiny aquatic invertebrates, a second intermediate host of predominantly freshwater fish and a definitive host that includes humans, other terrestrial mammals and less commonly marine mammals (believed to become infected by eating anadromus fish). The tapeworm attaches to the small intestinal mucosa by means of two longitudinal slit-like suckers (bothria). Infections are commonly

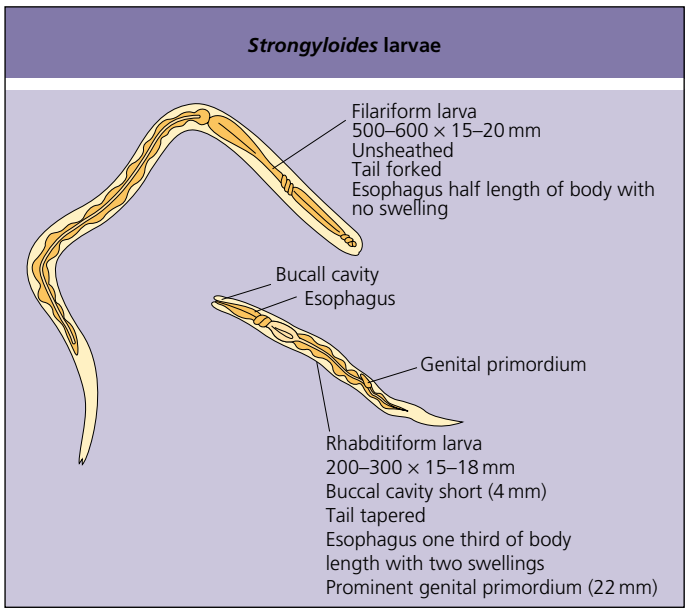


Fig. 108.4 *Strongyloides* larvae.

multiple and may reach more than 100 individual worms, each measuring up to 10 m in length. The pathology is minimal from the local effects. Tapeworm anemia has been described exclusively in Finland and has now all but died out as a result of control measures. The anemia is caused by vitamin B12 deficiency, with worms competing for the limited dietary vitamin B12, and it is strongly associated with gastritis and achlorhydria; there is probably a genetic predisposition to this condition.

PREVENTION

The prevention and control of parasitic gastrointestinal disease can be achieved through improvement of living standards, personal hygiene and better sanitary conditions.[29] In immunocompromised patients, different recommendations need to be considered (see Chapter 90).

Public health hygiene measures

Disposal of human sewage and waste water is fundamental to the control of parasitic gastrointestinal infections. Fecal material contaminates agricultural food crops or water supplies if it is passed promiscuously in the fields or close to habitation or if it is deliberately used unprocessed on fields as fertilizer ('night soil'). Any of the gastrointestinal parasites can be transferred in this way. Following rain, fecal material is washed into rivers and pools. *Giardia* cysts are found in surface water in many parts of the world.

The provision of latrines can reduce these sources of environmental contamination but, unfortunately, unless they are well constructed and maintained, latrines can themselves become important foci of infection. The prevalence of *Ascaris* infection is often higher among urban latrine users than among rural nonusers. The eggs of *A. lumbricoides* and also those of *T. trichiura* are very often resistant and can remain viable in the latrine environment for long periods. In addition, the moist soil around a latrine favors the survival of hookworm larvae[30] and the free-living cycle of *S. stercoralis*.

Domestic waste water (and the excreta that it accompanies) when used for irrigation puts crops and workers at risk. Sewage may enter lakes, rivers and ponds and the water then be used for drinking or irrigation. *Cryptosporidium* oocysts are particularly difficult to eliminate from water and require an efficient filtration system. Boiling water is the most reliable method of killing oocysts.

Composting is another reliable way of killing the infective forms of parasites. This takes 3–4 months. Cysts, eggs and larvae are quite rapidly killed at temperatures of 131 °F (55 °C) and die within a few days at 113 °F (45 °C). The compost heap needs turning and good maintenance or the periphery may become an intense transmission focus. Chemical treatment of excreta has been used, for example 12–24 hours in ammonium sulfate, but proper control is important to maintain efficacy and avoid toxicity to fish and plant life. Solid waste from sewage treatment plants is used as agricultural fertilizer and can contain viable parasites if inadequately processed. Sewage treatment removes or destroys parasites by sedimentation and the creation of completely anaerobic conditions. The eggs of *Taenia* spp. and *A. lumbricoides* are notoriously resistant and sometimes survive in the solid wastes taken from sedimentation tanks or ponds.

Personal hygiene measures

Most gastrointestinal diseases could be prevented if it were easy to modify human behavior. In practice this is often very difficult, but health education can be very effective and it can take many forms. Schools can be targeted or the local press used for a health awareness campaign. The fecal–oral route of transmission is very important, especially for intestinal protozoa. For example, infected food handlers are disseminators of *G. intestinalis* and *E. histolytica* cysts. There is high prevalence of infection in institutions for children or the mentally subnormal, where personal hygiene is poor. Those intestinal helminths whose life cycles can lead to fully embryonated eggs being released or formed very soon after entry into the environment (*H. nana* and *E. vermicularis*) are also spread in this way. Microsporidia are probably spread by the fecal–oral or urinary–oral route. Simple hand washing before preparing and eating food prevents transmission of these infections. The soil-to-skin route of infection can be inhibited by wearing shoes.[31] Infective forms of hookworm and *Strongyloides* larvae will be stopped from entering the skin. Persons dealing with composted human feces must use boots and gloves.

Good food hygiene is essential. Prevention of most of the parasites transferred by the fecal–oral route can be achieved by washing all salad vegetables and fruit before consumption. Kitchen utensils and hands should be washed frequently. Avoidance of wild-grown watercress and other water plants prevents *F. buski* infections. Proper cooking of meat and fish removes the risk of flesh-derived parasites such as *H. heterophyes* and tapeworms. Problems arise when cultural preferences dictate consuming these products raw, undercooked, salted, dried or pickled. The inspection of meat can detect the presence of cysticerci in the carcasses and allow infected meat to be condemned. Stopping the consumption of fish that is raw, pickled or salted is often impossible if it is part of a deep cultural tradition. Deep freezing of meat and fish at temperatures colder than −4 °F (−20 °C) for 24–48 hours kills all these parasites.

Prevention by chemotherapy

The treatment of these gastrointestinal parasites with appropriate chemotherapy is helpful in the prevention of further cases. This is particularly so in developed parts of the world. In areas where parasitic disease is endemic, generally only symptomatic patients are treated. Patients that are asymptomatic, those in whom the diagnosis is incidental and those who do not have heavy infections are usually not treated, as re-infection is probably inevitable. Mass chemotherapy has been used in amebiasis, soil-transmitted

nematodes (*A. lumbricoides*,[32] hookworm, *T. trichiura*) and tapeworms (*T. saginata*, *T. solium*, *D. latum*).[33] Success depends upon several factors:

- the chemotherapeutic agent used should have a broad spectrum of activity and be given annually as a single oral dose;
- the drugs should be cheap to purchase and administer; and
- the drugs should have few side-effects.

In the past, mass chemotherapy was used in an attempt to eradicate amebiasis but, because treatment required a prolonged course of luminal amebicides, compliance was poor and reinfection in endemic areas was high. *Fasciolopsis buski* infection in Indonesia has been treated by community-based praziquantel treatment but rapid re-infection followed. Selective or targeted chemotherapy may be of more use. *Enterobius vermicularis* infection in one child requires treatment not only of that child but also all family contacts together with education in personal hygiene. In 2003 a national program of soil-transmitted hookworm control (*A. lumbricoides* and *T. trichiura*) was implemented in Uganda, which has shown a sharp reduction in intensity of infection following annual mass drug administration of albendazole to children and high-risk adults. By 2006–2007 approximately 43 million treatments had been administered/[34] Ivermectin (in two separated doses) has been used successfully as mass treatment in north-east Brazil to control both intestinal helminths (including strongyloidosis) and ectoparasites including scabies.[35] A more recent study in Ecuador of school-age children demonstrated that treatment annually and biannually with ivermectin over a period of up to 17 years may have had a significant impact on *T. trichiura* infection.[36]

Vaccines

As yet no vaccines have been used in humans for the control of gastrointestinal parasitic diseases.[37]

Role of prophylaxis

There is no evidence for prophylactic use of chemotherapy being helpful in prevention of these gastrointestinal parasitic diseases.

Advice for travelers on preventive measures is discussed in Chapter 102.

CLINICAL FEATURES

The symptoms produced by gastrointestinal parasites are diverse. The most common presentation is of poorly localized abdominal pain; less commonly there is nonspecific diarrhea (Table 108.3). The severity varies from asymptomatic carriage to life-threatening gastrointestinal disease, as occurs in amebiasis and cryptosporidiosis. It is important to emphasize that the identification of an infection by stool microscopy does not necessarily imply that it is the cause of a patient's symptoms. This is particularly so in the case of light helminth infections, which are often completely symptomless. Other pathologies should be excluded before attributing the patient's symptoms to the gut parasite. Some of these infections present with extraintestinal manifestations, such as iron-deficiency anemia, wasting or (in the case of hookworm infection) growth retardation.

Gastrointestinal disease caused by protozoa

Amebiasis (*Entamoeba histolytica*)

It is now recognized that there are two species of amebae that were formerly termed pathogenic and nonpathogenic *E. histolytica*.[38] Methods used to separate these include biochemical, immunologic and genetic data. These are now called *Entamoeba histolytica* and *Entamoeba dispar*. Only *E. histolytica* is capable of causing disease. Asymptomatic cyst carriers may excrete cysts for a variable length of time, usually only a few weeks. Confirmation that the parasite previously described as *Entamoeba histolytica* is in reality two species, *Entamoeba dispar* which is nonpathogenic and *Entamoeba histolytica* which is a pathogen, meant that the natural history of amebiasis needed reassessment.[12] Haque *et al.* studied 300 preschool children in Dhaka, Bangladesh.[10] Over a 2-year period, new *Entamoeba histolytica* infections were found in half the children. Of those who were asymptomatic cyst passers, one in 10 subsequently developed diarrhea within 2 months of the start of infection; 10% of the children had diarrhea in association with *E. histolytica* and 4% were found to have amebic colitis.

Table 108.3 Gastrointestinal parasites associated with diarrhea

Diarrhea and fever	*Entamoeba histolytica* *Cryptosporidium parvum/C. hominis* *Isospora belli* *Cyclospora cayetanensis* (occasionally)
Diarrhea with blood in stool	*Entamoeba histolytica* *Trichuris trichiura* *Strongyloides stercoralis* (rarely)
Chronic diarrhea	*Entamoeba histolytica* *Giardia intestinalis* *Cryptosporidium parvum* *Isospora belli* *Cyclospora cayetanensis* *Microsporidia* *Trichuris trichiura* Hookworm (*Necator americanus* and *Ancylostoma duodenale*) *Strongyloides stercoralis*

Amebic intestinal disease

The incubation period of invasive amebiasis varies from a few days to 1–4 months. There is a good correlation with the presence of *E. histolytica* trophozoites containing ingested red blood cells. Patients may be asymptomatic or present with colicky abdominal pain, frequent bowel movements and tenesmus. Amebic dysentery is characterized by blood-stained stools with mucus occurring up to 10 times a day. The duration of the dysentery can be very variable and may last for only a few days or for several months with concomitant weight loss and debility. The symptoms may be confused with inflammatory bowel disease. In acute cases the clinical picture may mimic appendicitis, cholecystitis, intestinal obstruction or diverticulitis.

Amebic extraintestinal disease

Amebic dysentery progresses to invasive amebic disease in between 2% and 8% of patients. Symptoms can be gradual in onset, with right upper quadrant pain and fever. Weakness, weight loss, dry cough and sweating are less common. Tender hepatomegaly is often seen with liver function tests that are normal or only slightly abnormal. Jaundice is very unusual. The site most commonly involved is the upper right lobe of the liver, and abscesses are mostly solitary. A raised right diaphragm may be found on chest radiograph. The abscess is visualized by ultrasound, CT or MRI. Hematogenous spread to the brain (see Chapter 106), lung, pericardium and other sites is possible.

Giardiasis (*Giardia intestinalis*)

The clinical spectrum of giardiasis ranges from asymptomatic infection, through acute gastrointestinal infection to severe chronic diarrhea[39] with intestinal malabsorption.[40] The average incubation period is 9 days and the acute infection is self-limiting.[41] Common symptoms include nausea, diarrhea, flatulence and upper abdominal cramps with

distention and nausea. Weight loss is common and there can be signs of malabsorption (steatorrhea, disaccharidase deficiency and vitamin B12 deficiency).

Blastocystis infection (*Blastocystis hominis*)

There is controversy surrounding *B. hominis*; it is unclear whether it causes gastrointestinal disease or not. When large numbers are found in stool in the absence of other parasites, bacteria or viruses it may be the cause of diarrhea, cramps, nausea, fever, vomiting and abdominal pain. Data from Canada indicate that, although it is commonly seen in stools, it is not pathogenic.[42] This finding is further supported by a large Australian study which found no correlation between clinical symptoms and the presence of *B. hominis* in immunocompetent adults.[43] It is possible that a small subset of *B. hominis* organisms have virulence factors that are missing in most.[44]

Dientamoeba fragilis infection

Like *Blastocystis*, the pathogenicity of *Dientamoeba fragilis* is uncertain. It has been associated with a wide range of symptoms. In children, symptoms may include intermittent diarrhea, abdominal pain, nausea, anorexia, malaise, fatigue, poor weight gain and unexplained eosinophilia.[45]

Cryptosporidiosis (*Cryptosporidium parvum* and *C. hominis*)

The incubation period of *Cryptosporidium* infection averages 3–6 days. Symptoms include a flu-like illness, diarrhea, malaise, abdominal pain, anorexia, nausea, flatulence, malabsorption, vomiting, mild fever and weight loss. In the 1993 Milwaukee water-borne outbreak of cryptosporidiosis, which affected around 403 000 people, the mean duration of illness was 12 days and the median maximum number of stools per day was 12.[20] Oocyst excretion generally occurs for 3–30 days (average 12 days) and occurs at the same time as the symptoms. Generally the symptoms are self-limiting, and prolonged disease is uncommon.[46] In immunocompromised patients the situation is different and there can be intractable, profuse, life-threatening diarrhea.[47,48]

Cyclosporiasis (*Cyclospora cayetanensis*)

There is epidemiologic evidence that *Cyclospora cayetanensis* is the cause of persistent diarrhea in immunocompetent patients as well as in immunocompromised patients. Initially, the clinical findings do not distinguish cyclosporal diarrhea from other causes of diarrhea. Other common symptoms are abdominal pain, nausea, vomiting and anorexia.[49] *Cyclospora* infection can last for 1–8 weeks.[21]

Isosporiasis (*Isospora belli*)

The predominant clinical symptom is diarrhea, which may be intermittent and last for months or even years. Stools are watery, soft, foamy and offensive, which may suggest malabsorption. There is associated weight loss, abdominal pain and fever.[50,51] In HIV-infected patients, chronic infection occurs.

Microsporidiosis

Microsporidia may cause acute self-limiting diarrhea in immunocompetent persons,[23] in patients who have immunodeficiency other than AIDS and in the elderly.[24] In HIV infection, chronic diarrhea and wasting are common.[52] *Enterocytozoon bieneusi* is one of the most important intestinal pathogens in severely immunodeficient HIV-infected patients; it is present in 7–50% of those who have otherwise unexplained chronic diarrhea. In one large Australian study there has been a reported decline in incidence of microsporidiosis from 11% in 1995 to 0% in 2004.[53] There are also increasing reports of intestinal microsporidial infections in immunocompetent people.[54] *Encephalitozoon intestinalis* (formerly *Septata intestinalis*) is a less commonly recognized cause of chronic diarrhea. *Encephalitozoon cuniculi* intestinal infection has been reported. These are covered in Chapter 181.

Balantidiasis (*Balantidium coli*)

Balantidium coli, the largest and least common of the human protozoan pathogens, is capable of causing an infection resembling amebic colitis. It is particularly prevalent among people living in close association with pigs in South America, Iran, Papua New Guinea and the Philippines. Up to 80% of persons carrying the organism are asymptomatic carriers.[16] Acute diarrhea with blood and mucus begins abruptly and is associated with nausea, abdominal discomfort and marked weight loss. There can be inflammatory changes and ulceration in the proctosigmoid region. Peritonitis and colonic perforation can progress rapidly to death. A chronic infection occurs with intermittent diarrhea and infrequent bloody stools.

Helminthic gastrointestinal disease

The World Bank regards intestinal helminth infections as the main cause of disease burden in children from 5 to 14 years old in developing countries.

Ascariasis (*Ascaris lumbricoides*)

Most cases of ascariasis are asymptomic; symptomatic infections are more common in children than adults. When large numbers of larvae migrate to the lungs in a short time period, *Ascaris* pneumonitis can result. This is the clinical picture of Löffler's syndrome, which is characterized by dyspnea, dry cough, wheezing or coarse rales, fever up to 104°F (40°C), transient eosinophilia and a chest radiograph that is suggestive of viral pneumonia and that resolves within a couple of weeks. In addition, eosinophils, Charcot–Leyden crystals and larvae may rarely be found in the sputum. Symptoms of asthma and urticaria may continue during the intestinal phase of ascariasis. The adult worms occasionally migrate from the small bowel and cause biliary or hepatic ascariasis. Rarely, adult worms migrate into the biliary tree with secondary sepsis and abscess formation. Pancreatitis may result from migrating ascarids that obstruct the pancreatic duct. In people who have large numbers of adult worms intestinal obstruction can occur owing to the sheer bulk of worm bodies. Adult worms migrate more in the presence of a stimulus such as a fever of over 102°F (38.9°C) or the use of a general anesthetic, and they may block the bile duct or pancreatic duct or enter the liver or peritoneal cavity. In children, nutritional deficiencies such as reduced appetite, kwashiorkor (protein-energy malnutrition), vitamin A deficiency and growth deficit are related to the burden of the adult worms.[55]

Enterobiasis (*Enterobius vermicularis*)

Infection with *Enterobius vermicularis* (threadworm or pinworm) causes few or no symptoms in the vast majority of people. The predominant symptom is nocturnal pruritus ani, caused by migration of the female worms from the anus to the perianal skin in the process of laying their eggs. Scratching may be intense and secondary infection may ensue. Pruritus vulvae caused by pinworms entering the vulva is occasionally seen. In children, insomnia, loss of appetite, loss of weight, emotional instability, enuresis and irritability may also be found.[55]

Hookworm infection (*Ancylostoma duodenale*, *Necator americanus*)

Hookworm causes ground itch, a moderate-to-severe pruritus of the skin, usually of the feet, as the hookworm larvae penetrate. Secondary infection can occur if the vesicular lesions are excoriated by scratching. A pneumonitis, caused by alveolar migration of larvae, is less common, less severe and causes less sensitization than that seen with *Ascaris* or *Strongyloides* infection. Symptoms of the intestinal phase are fatigue, nausea, vomiting, abdominal pain, diarrhea with occult bleeding, and weakness. Heavy worm burdens may have serious sequelae in young children.[56] This is particularly problematic with *A. duodenale* infection. Eosinophilia is usually present. According to the degree of worm burden, chronic infection leads to an iron deficiency anemia[30] and hypoproteinemia with pallor, edema of the face and feet, listlessness, koilonychia, cardiomegaly, heart failure and rarely mental retardation. Iron deficiency in children is associated with impaired academic performance at school and in adults with weakness and fatigue, leading to reduced productivity.

Trichuriasis (*Trichuris trichiura*)

Light infections with this common, ubiquitous infection rarely cause symptoms. In heavy infections (more than 10 000 eggs per gram of feces), epigastric pain, vomiting, distention, flatulence, anorexia and weight loss may occur. Rarely the *Trichuris* dysentery syndrome may occur, with blood and mucus in the stools and, in heavy infections, prolapse of the rectum. Reduced childhood growth rates, reduced food intake, iron deficiency and gastrointestinal protein loss are seen in heavy infections. The diagnosis is made when numerous worms are seen on the rectal mucosa or eggs are found in the stool. A 'honeycomb' effect of the small intestine has appearances similar to Crohn's disease. There may be deformity of the proximal colon and also the ileum or appendix.

Strongyloidiasis (*Strongyloides stercoralis*)

Strongyloides stercoralis largely causes asymptomatic infection of the small intestine, which can last for 30 years or longer.[57] The prepatent period from infection through the skin to the appearance of rhabditiform larvae in the stools is 1 month or more. Symptoms only develop with high intestinal worm loads, which can be the result of several factors. In people debilitated by concurrent disease or malnutrition, there may be massive invasion of the tissues by *S. stercoralis*. Treatment with immunosuppressive drugs in a patient harboring *S. stercoralis* can also lead to the same effect.[58] Infection with human T-cell leukemia virus 1 is important in predisposing to massive infection by *S. stercoralis*. Interestingly, infection with HIV is not a common cause of the *Strongyloides* hyperinfection syndrome; this may be due to the decreased likelihood of intestinal larval migration in the presence of CD4 lymphopenia seen in HIV infection.[59] Symptoms include watery mucous diarrhea, with the severity depending on the intensity of the infection. Sometimes diarrhea alternates with constipation. Malabsorption of fat and vitamin B12 with a chronic diarrhea and protein-losing enteropathy has also been described and is rapidly reversed by treatment.

There are two types of skin rash. The first is larva currens, which occurs on the trunk or near the anus, and is a linear eruption in which the larvae migrate under the skin causing an itchy, nonindurated wheal with a red flare that moves rapidly and disappears in a few hours. This contrasts to the indurated and persistent track of nonhuman hookworm larvae (cutaneous larva migrans). The second type of rash is urticaria.

Features of the strongyloidiasis hyperinfection syndrome (see Fig. 108.3) include severe diarrhea, malabsorption, edema, hepatomegaly and paralytic ileus. Gram-negative sepsis is a recognized complication. In very severe cases encephalopathy and even secondary pyogenic meningitis have been described.

Fasciolopsis buski infection

Fasciolopsis buski is confined to Asian countries, particularly Thailand. Symptoms are more frequent in children than adults owing to their greater exposure to water plants while at play. Like many other intestinal parasites, the majority of infected people have very minor symptoms or none at all. In heavier infections, clinical features can include diarrhea, abdominal pain, vomiting, flatulence, poor appetite, fever and eosinophilia. In very severe cases there may be ascites, edema of the face, abdomen and legs, anemia, anorexia, weakness and even intestinal obstruction. Intestinal ulceration may cause malabsorption and lead to malnutrition and wasting.[27]

Heterophyes heterophyes infection

Heterophyes heterophyes causes few symptoms unless the infection is heavy, which is dependent on the quantities of pickled or uncooked fish eaten in endemic areas (mostly the Middle and Far East). The adult worms produce abdominal pain, diarrhea with mucus and ulceration of the intestinal wall.

Beef and pork tapeworm infection (*Taenia saginata* and *Taenia solium*)

The clinical features of *T. saginata*, the beef tapeworm, and *T. solium*, the pork tapeworm, are similar. The adult worms in the gastrointestinal phase of both organisms usually cause no symptoms, but carriers can sometimes feel a proglottid emerging from the anus; the motile proglottid may be upsettingly obvious in the feces. Other associated symptoms, such as abdominal pain and distention, nausea and anorexia have been attributed to the tapeworm. There is occasionally a mild eosinophilia but there is no anemia, even in long-term infection. Cysticercosis complicates infection with *T. solium* only. Ingestion of *T. solium* eggs leads to the dissemination of oncospheres in the bloodstream; these can become lodged anywhere in the subcutaneous and intramuscular tissues,[60] where they become cysticerci; symptoms depend on the particular body site involved. The clinical findings of neurocysticercosis are discussed in detail in Chapter 113.

Dwarf tapeworm infection (*Hymenolepis nana*)

As with other tapeworms, there are usually few symptoms in *H. nana* infection. Symptoms that may occur include abdominal pain, anorexia, irritability and headache. Eosinophilia is common. Symptoms are more common in heavy infections and may cause growth retardation in children.

Fish tapeworm infection (*Diphyllobothrium latum*)

There are few if any symptoms from infection with *D. latum*. Symptoms including diarrhea, headache and nonspecific malaise all appear to be somewhat more common than in uninfected people. Tapeworm-associated anemia is probably related to vitamin B12 deficiency caused by competition for the vitamin between the tapeworm and a genetically predisposed host; however, this is now exceedingly rare.

DIAGNOSIS (TABLE 108.4)

Microscopic examination of the stool is fundamental to the diagnosis of all the gastrointestinal infections. A minimum of three stool specimens, examined by trained personnel using a concentration and a permanent stain technique, should be used.

An ELISA for *Strongyloides* is useful in screening patients who have suggestive symptoms or eosinophilia. In microscopically proven

Table 108.4 Methods of diagnosis of gastrointestinal parasites

Intestinal protozoa	
Entamoeba histolytica and nonpathogenic amebae (*E. dispar*, *E. hartmanni*, *Entamoeba coli*, *Iodamoeba butschlii* and *Endolimax nana*) Methods to differentiate *Entamoeba histolytica* and *E. dispar*	Fecal microscopy for cysts Also possible to scrape or aspirate material from mucosal surfaces at sigmoidoscopy for microscopic examination Microscopy of fresh feces (within 20 min) which must contain blood and mucus to detect trophozoites containing ingested red cells enables *E. histolytica* to be distinguished from *E. dispar* ELISA of fecal samples (to detect galactose adhesin) PCR (often only available in reference centers) Culture and zymodeme pattern analysis (reference standard test)[61] Serologic tests are valuable in cases of suspected amebic abscess (and ameboma); the indirect fluorescent antibody test is positive in over 95% of cases after 14 days but should be confirmed by the cellulose acetate precipitin test
Giardia intestinalis	Fecal microscopy for cysts (and occasionally trophozoites); if negative, a string test, duodenal aspirate or biopsy may be positive ELISA to detect *Giardia* antigens in fecal specimens (sensitivity and specificity of 87–100%) Indirect immunofluorescence test on fecal specimens using a cyst-specific monoclonal antibody[62] Direct fluorescent antibody test (MERIFLUOR DFA) against cell wall antigens to *Giardia* in fecal specimens demonstrated increased sensitivity and specificity of over 95%[63] Immunochromatographic assays are also available for qualitative detection in fecal specimens[63] Serology tests are also available
Blastocystis hominis *Dientamoeba fragilis* *Balantidium coli* *Cryptosporidium parvum/C. hominis*	Fecal microscopy with Romanowsky stain Fecal microscopy with Romanowsky stain Fecal microscopy Fecal microscopy or intestinal biopsy. Staining of oocysts (~5 μm) with auramine, modified ZN or immunofluorescence using monoclonal antibodies[64] Immunochromatographic assays are also available for qualitative detection in stool specimens[63] PCR is still principally reference laboratory technique
Cyclospora cayetanensis	Fecal microscopy or intestinal biopsy. Cysts are visible using auramine, modified ZN (~8 μm) and are bright blue autofluorescent under UV light (330–380 nm)
Isospora belli	Fecal microscopy or intestinal biopsy. Stain with modified ZN or auramine under UV light
Enterocytozoon bieneusi and *Encephalitozoon intestinalis* (formerly *Septata intestinalis*)	Fecal microscopy with a modified trichrome stain or a fluorescent chromotrope stain Electron microscopy or PCR is needed for species identification
Nematodes (roundworms)	
Ascaris lumbricoides *Ancylostoma duodenale* *Necator americanus* (hookworms) *Trichuris trichiura* *Enterobius vermicularis* *Strongyloides stercoralis*	Depends on identification of ova, larvae or worms in feces on light microscopy Samples should be mixed well before examination and a concentration method is usually used Parasites are identified by appearance and size Visualized by Sellotape slide or perianal swab Fecal microscopy for larvae and fecal culture can also be performed A string test can also be undertaken as can serologic tests
Trematodes (flukes)	
Fasciolopsis buski *Heterophyes heterophyes*	Fecal microscopy looking for ova and identification of worms Serology is also available in some centers Fecal microscopy for ova and identification of worms
Cestodes (tapeworms)	
Taenia solium *Taenia saginata* *Hymenolepis nana* *Diphyllobothrium latum*	Fecal microscopy for ova and proglottids to differentiate between the pork and beef tapeworm Serology available for cysticercosis but not for intestinal tapeworm infection Fecal microscopy for ova and segments Fecal microscopy for ova Fecal microscopy for ova and segments

ZN, Ziehl–Neelsen stain.

disease, the sensitivity of *Strongyloides* serology has been shown to be best in immigrants with chronic infection when compared to travellers to endemic countries.[65] Cross-reactions occur occasionally with ascariasis and filarial infection. The filarial ELISA cross-reacts in some cases of strongyloidiasis. Serum antigiardial antibody detection is not clinically useful in highly endemic areas because people may be seropositive from past infection. The giardial immunofluorescent antibody test gives good titers if the disease has caused mucosal damage, and it is helpful in the investigation of parasite-associated malabsorption.

MANAGEMENT

Gastrointestinal protozoa

The management of gastrointestinal protozoa is summarized in Table 108.5. Treatment regimens are discussed in detail in Chapter 150.

Entamoeba histolytica (see Chapter 110)

Treatment of *E. histolytica* infection is divided into two types. Luminal amebicides, such as diloxanide furoate, act on organisms in the intestinal lumen and are not effective against organisms in tissue. Tissue amebicides, such as metronidazole and tinidazole, are effective in treating invasive amebiasis but less effective in the treatment of organisms in the bowel lumen. There is some controversy about treating asymptomatic patients. Ideally, any amebic cysts should be tested to identify whether they are *E. histolytica* (in which case the infection should be treated to avoid the risk of developing invasive disease and to prevent secondary spread) or *E. dispar* (which does not require any treatment). However, until ELISA or PCR tests become widely deployed it will continue to be usual to treat asymptomatic patients in nonendemic areas with diloxanide furoate (see Table 108.5). When asymptomatic cyst carriage persists after treatment for amebic dysentery or liver abscess, further treatment with a luminal amebicide is mandatory, otherwise relapse is frequent. The treatment of asymptomatic cyst carriers in endemic areas is of questionable value because of the high rate of re-infection. A second-choice intraluminal amebicide is paromomycin.[66] New broad-spectrum antiparasitic agents are being introduced as infection with multiple parasites is a common occurrence in the tropics. Nitazoxanide, a compound structurally similar to nitrobenzamide, is reported to act on a wide range of intestinal parasites, including some cestodes, nematodes and protozoa. In a study in Mexican children, Davila-Gutierrez reported a higher eradication rate for treatment of *E. histolytica/ dispar* with nitazoxanide 100 mg twice daily for 3 days than with quinfamide 100 mg as a single dose.[67] Further work is needed to determine whether or not nitazoxanide will become a first-line treatment for amebic cyst passage.

Proven amebic dysentery should always be treated. Drugs of choice are metronidazole or tinidazole followed by diloxanide furoate (see Table 108.5). Amebic liver abscess is treated with metronidazole, 400–500 mg orally q8h, followed by diloxanide furoate as above. Tinidazole is an alternative; chloroquine (150 mg base q6h for 2 days, then 150 mg base q12h for 19 days) can also be used.

Giardia intestinalis (see Chapter 180)

Treatment is often unnecessary because most healthy, immunocompetent patients have a self-limiting disease and recover by their own natural host defense mechanisms. Treatment of symptomatic patients reduces the duration and severity of symptoms. The treatment of asymptomatic cyst carriers is controversial in endemic areas. Generally, in a nonendemic area, asymptomatic *Giardia* cyst carriers are treated. The 5-nitroimidazole derivatives metronidazole or tinidazole are the treatment of choice and can be used in short courses.[41] Albendazole, 400 mg daily for 5 days, has been shown to have useful antigiardial activity. Mepacrine, an acridine dye, has a similar efficacy but is generally less well tolerated with an incidence of 1.5% of acute psychosis. Furazolidone, a nitrofuran, has lower efficacy but is well tolerated. Newer thiazolide antiparasitic agents such as nitazoxanide, which is licensed by the Food and Drug Administration (FDA) for the treatment of *G. intestinalis* in children, appear well tolerated with good oral bioavailability.[68] *In-vitro* and *in-vivo* resistance of *G. intestinalis* has been demonstrated, although rarely, to conventional therapy, particularly to the 5-nitroimidazoles such as metronidazole and tinidazole.[69–71]

Table 108.5 Treatment of gastrointestinal protozoal infection*

Condition	Drug	Dosage
Amebiasis		
Asymptomatic carrier of intestinal cysts		
1st choice	Diloxanide furoate	500 mg q8h for 10 days
2nd choice	Paromomycin (aminosidine)	500 mg q8h for 10 days
Intestinal infection (amebic dysentery or ameboma)	Metronidazole or Tinidazole followed by Diloxanide furoate or Paromomycin	750–800 mg q8h for 5 days 2 g daily for 2–3 days 500 mg q8h for 10 days 500 mg q8h for 10 days
Amebic liver abscess	Metronidazole or Tinidazole followed by Diloxanide furoate	400–500 mg q8h for 5–10 days 2 g daily for 3–5 days 500 mg q8h for 10 days
Giardiasis		
1st choice	Tinidazole or Metronidazole	2 g single dose 400–500 mg q8h for 3 days
2nd choice	Nitazoxanide	500 mg q12h for 3 days
3rd choice	Albendazole	400 mg q24h for 5 days
4th choice	Mepacrine	100 mg q8h for 5–7 days
***Balantidium coli* infection**		
1st choice	Tetracycline	500 mg q6h for 10 days
Alternatives	Ampicillin, metronidazole or paromomycin	
***Cyclospora cayetanensis* infection**		
1st choice	Trimethoprim–sulfamethoxazole (co-trimoxazole)	960 mg q12h for 7 days
Alternatives	Ciprofloxacin	500 mg q12h for 7 days
***Isospora belli* infection**		
1st choice	Trimethoprim–sulfamethoxazole (co-trimoxazole)	960 mg [160 mg (TMP)/800 mg (SMX)] q12h for 7–10 days (q6h in immunosuppressed patients)
2nd choice (if intolerant to sulfonamides)	Furazolidone	100 mg q6h for 10 days

*All treatments are adult dosage and given orally unless stated otherwise.

Blastocystis hominis

If *B. hominis* is present in the stool, the physician must not stop looking for another cause of diarrhea. Whether any treatment is required is controversial. Metronidazole seems to be the most appropriate drug.

Dientamoeba fragilis

In adults infected with *D. fragilis*, improvement can be seen with tetracycline; in children, metronidazole is appropriate.

Balantidium coli

Tetracycline is effective against *B. coli*. Doxycycline 100 mg daily for 10–14 days is an alternative. Other drugs to which *B. coli* is sensitive are ampicillin, metronidazole and paromomycin. Surgery may be required for fulminant disease with perforation or abscess formation.

Cryptosporidium spp. (see Chapter 91)

Cryptosporidium spp. infection is self-limiting in those who have normal immunity. It presents a severe problem when it occurs in patients who have AIDS.[47] Where available, treatment is with HAART (highly active antiretroviral therapy) with or without antiparasitic agents. No currently available drug reliably eradicates cryptosporidial infection, but suppression of infection is possible. Agents currently in use are paromomycin, azithromycin[72] and nitazoxanide.[73] In one randomized control trial of immunocompetent adults and children with *C. parvum* infection, the parasitologic and clinical cure rate with nitazoxanide (500 mg q12h for adults, 100–200 mg q12h for children) was significantly greater than placebo.[74]

Cyclospora cayetanensis

Many cases of *C. cayetanensis* infection are self-limiting. When treatment is felt to be necessary trimethoprim–sulfamethoxazole (co-trimoxazole) has been found to be effective, eradicating the oocysts from 94% of 16 patients in 7 days compared with 12% of 17 patients who received placebo.[75] Relapse is common in the immunocompromised but responds to a second course of treatment. Given the propensity of sulfonamide-containing drugs to cause side-effects in HIV-infected individuals, an alternative agent to co-trimoxazole is required. Verdier *et al*. compared the activity of trimethoprim–sulfamethoxazole (TMP–SMX) 160/800 mg orally twice daily for 1 week with ciprofloxacin 500 mg twice daily for 1 week in HIV-positive patients infected with *Cyclospora cayetanensis*.[76] Nine received TMP–SMX and 11 received ciprofloxacin. Diarrhea ceased in all patients receiving TMP–SMX and in 10 of 11 patients receiving ciprofloxacin. At 7 days all those who received TMP–SMX had negative stool examinations, four of 11 treated with ciprofloxacin had persisting oocysts, one of whom still had diarrhea. All four responded to open treatment with TMP–SMX. Patients who had shown a complete response at 7 days were given maintenance therapy with TMP–SMX 160/800 mg three times weekly or ciprofloxacin 500 mg three times weekly for 10 weeks. No recurrences were observed in the TMP–SMX group but one of seven patients who received ciprofloxacin experienced a recurrence after 4 weeks. Whilst ciprofloxacin is not as effective as TMP–SMX against *Cyclospora*, it provides an acceptable alternative for individuals who cannot tolerate a sulfonamide.

Isospora belli

Treatment of *I. belli* infection may be necessary in the immunocompromised, and oral trimethoprim–sulfamethoxazole eliminates the parasite in most cases; relapse is common but retreatment is usually effective. Prophylactic trimethoprim–sulfamethoxazole may then be necessary. Pyrimethamine–sulfonamide combinations have also proven to be effective.[77] If the patient is intolerant to sulfonamides, furazolidone 100 mg four times daily for 10 days is an alternative. The macrolide antibiotic roxithromycin (2.5 mg/kg q12h) was reported to be successful in a single patient with AIDS and *I. belli* infection who had not responded to co-trimoxazole or pyrimethamine therapy. The 5-nitrothiazole derivative nitazoxanide is also reported to be effective in isosporiasis in a dose of 7.5 mg/kg (500 mg for adults; 200mg for children less than 12 years of age) twice daily for 3 days. Biliary isosporiasis may require intravenous co-trimoxazole as both oral co-trimoxazole and oral nitazoxanide have been reported to fail in the presence of malabsorption and cholangitis due to *I. belli*.[78,79]

Microsporidia

The treatment of microsporidiosis in the immunocompetent patient is not required. Evidence and experience in treating these infections comes from HIV-infected patients where albendazole has been shown to be useful with some species of microsporidia, notably *Encephalitozoon intestinalis*.[80] Fumagillin has shown activity against *Enterocytozoon* bieneusi,[81] as has nitazoxanide, although the evidence has been limited to case reports.[82]

Gastrointestinal helminths

The management of gastrointestinal helminths is summarized in Table 108.6 (see Chapter 150).

Table 108.6 Treatment of gastrointestinal helminthic infection*

Condition	Drug	Dosage
Nematodes		
Round worms		
Ascaris lumbricoides	Albendazole	400 mg, single dose
	Mebendazole	100 mg q12h for 3 days
	Levamisole	150 mg, single dose
	Piperazine hydrate	4.5 g, single dose
Enterobius vermicularis	Mebendazole	100 mg, single dose
	Piperazine phosphate	4 g, single dose
	Pyrantel pamoate	10 mg/kg, single dose
Hookworms		
Ancylostoma duodenale	Mebendazole	100 mg q12h for 3 days
Necator americanus	Albendazole	200 mg q24h for 3 days
	Levamisole	150 mg, single dose (less effective against *N. americanus*)
	Pyrantel pamoate	10 mg/kg, single dose
Trichuris trichiura	Mebendazole	100 mg q12h for 3 days or 600 mg, single dose
	Albendazole	400 mg, single dose
Strongyloides stercoralis	Ivermectin	200 μg/kg, daily for 2 days
	Albendazole	400 mg q12–24h for 3 days
	Thiabendazole	25 mg/kg (max 1.5 g) q12h for 3 days
Trematodes (flukes)		
Fasciolopsis buski	Praziquantel	15 mg/kg, single dose
Heterophyes heterophyes	Praziquantel	10–20 mg/kg, single dose
Cestodes (tapeworms)		
Taenia solium	Praziquantel	10 mg/kg, single dose
	Niclosamide	2 g, single dose
Taenia saginata	Praziquantel	10 mg/kg, single dose
	Niclosamide	2 g, single dose
Hymenolepis nana	Praziquantel	20 mg/kg, single dose
	Niclosamide	2 g on day 1 then 1 g/q24h for 6 days
Diphyllobothrium latum	Praziquantel	10 mg/kg, single dose
	Niclosamide	2 g, single dose

*All treatments are adult dosage and given orally unless stated otherwise.

Ascaris lumbricoides

Treatment is effective only against the adult worm. It is usual to treat any established infection. The drugs used are albendazole, mebendazole, levamisole or piperazine in a single adult dose of 4 g of piperazine phosphate or 4.5 g of piperazine hydrate, or pyrantel pamoate. *Ascaris* pneumonitis responds dramatically to prednisolone therapy and anthelmintics should be given 2 weeks after lung involvement. Surgery is sometimes required for bowel perforation or obstruction.

Enterobius vermicularis

Enterobius vermicularis infection is treated with mebendazole (100 mg, single dose, which is repeated if necessary after 2–3 weeks), piperazine phosphate (4 g, single adult dose repeated after 14 days) or pyrantel pamoate (10 mg/kg, single dose). The whole family should be treated simultaneously, fresh bed linen and night clothes should be provided and the nails kept short and scrubbed. Repeat treatment may be required because recurrence is common.

Hookworm

Hookworm infection is treated by eliminating the adult worms and treating anemia if present; these treatments can be carried out concurrently. In endemic countries where re-infection is inevitable, light infections are treated only in children, not in adults. Mebendazole and albendazole are effective against both *A. duodenale* and *N. americanus*. In a single-dose comparison of albendazole and mebendazole, albendazole gave better cure and egg reduction rates.[83] Levamisole is less effective against *N. americanus*, and pyrantel pamoate is preferred. In a study from Ivory Coast, Utzinger *et al*. reported a reduction in the prevalence and intensity of hookworm infections in schoolchildren receiving praziquantel for *Schistosoma mansoni* infection. If their results are replicated they will have a significant impact upon helminth control programs.[84]

Trichuris trichiura

In symptomatic patients and in asymptomatic carriers who have high numbers of eggs, trichuriasis is treated with mebendazole or albendazole. In undernourished children who have moderate infection intensities in Jamaica, albendazole treatment also resulted in improvement in some tests of cognitive ability and in school attendance and school performance, even after controlling for socioeconomic status.[85]

Strongyloides stercoralis

Strongyloides stercoralis infection should be treated in both symptomatic and asymptomatic people because of its ability to cause hyperinfection if immunosuppression occurs. Ivermectin is the drug of choice. This regimen has proven very effective in a prospective randomized trial comparing the efficacy of ivermectin and thiabendazole in Cambodian refugees who had symptomatic chronic strongyloidiasis[86] and in a prospective randomized trial comparing a 7-day course of oral albendazole with a single dose of ivermectin in 42 Thai adults with chronic *Strongyloides*.[87] Ivermectin also looks very promising in HIV-positive patients infected with *S. stercoralis*. Albendazole is also effective. Thiabendazole, orally twice daily for 3 days, is effective but often poorly tolerated. Subcutaneous ivermectin (unlicensed in humans) has proven effective in a case of life-threatening *Strongyloides* hyperinfestation.[88,89] Although there are no currently known minimally effective concentrations of ivermectin for *Strongyloides* treatment in humans, repeated doses subcutaneously[90] and orally[91] appear to have a cumulatively increasing antiparasitic effect.

Fasciolopsis buski

Fasciolopsis buski infection is treated with praziquantel which is highly effective. Niclosamide has also been used.

Heterophyes heterophyes

Heterophyes heterophyes infection is treated with a single dose of praziquantel. Niclosamide is an alternative.

Taenia solium and *Taenia saginata*

Patients who have *T. solium* infection should be evaluated for the presence of cerebral cysticercosis before commencing therapy against the intestinal tapeworm. Praziquantel is effective therapy for the adult worm. Niclosamide has also been widely used.

Hymenolepis nana

Hymenolepis nana infection can be treated with a single oral dose of praziquantel. Niclosamide is also successful.

Diphyllobothrium latum

Diphyllobothrium latum infections are treated with praziquantel. Niclosamide was extensively used in the past.

REFERENCES

References for this chapter can be found online at http://www.expertconsult.com

Chapter **109**

Christiane Dolecek

We wish to acknowledge the contribution of John Richens, the author of the previous chapter.

Typhoid fever and other enteric fevers

EPIDEMIOLOGY

Typhoid fever and paratyphoid fever are Gram-negative septicemias caused by *Salmonella enterica* serovar Typhi (*S. typhi*) and *Salmonella enterica* serovar Paratyphi (*S. paratyphi*) A, B and C. Typhoid and paratyphoid fever are summarized as enteric fevers. Whilst *S. typhi* and *S. paratyphi* A and B infections are restricted to humans, *S. paratyphi* C can affect a variety of animals.

Typhoid fever is endemic in Africa, Asia, Central and South America and is found in parts of the Middle East, southern and eastern Europe.[1] Infections seen in Europe, Australia and North America are usually acquired abroad, mostly from the Indian subcontinent, South East Asia and South America (Fig. 109.1).

Estimates from the World Health Organization (WHO) suggest that the worldwide incidence of typhoid fever is approximately 21 million cases annually with 210 000 deaths and paratyphoid fever causes an additional 5 million diseases.[2] It is likely that the proportion of disease caused by *S. paratyphi* A has been underestimated; recent data show that enteric fevers were caused in 64% in China, in 24% in India and in 33% in Nepal by *S. paratyphi* A.[3,4]

In population surveillance studies from Asia the typhoid incidence (per 100 000 persons per year) among 5–15 year olds in Vietnam and China was low (24 and 29, respectively) and high in Indonesia, Pakistan and India (180, 413 and 493, respectively).[5] In endemic areas typhoid fever has been considered to be a disease of schoolchildren and young adults; however, recent data emphasize that it is a common cause of morbidity in children between 1 and 5 years. Transmission of typhoid fever occurs via the fecal–oral route by ingesting contaminated water or food or through direct person-to-person contact. Risk factors for acquiring enteric fever are contaminated water (Fig. 109.2), eating food prepared outside the home, shellfish from polluted water, vegetables fertilized with human waste and having a relative with a recent history of typhoid fever living in the same house. Chronic typhoid carriers involved in food handling are an important reservoir of infection.

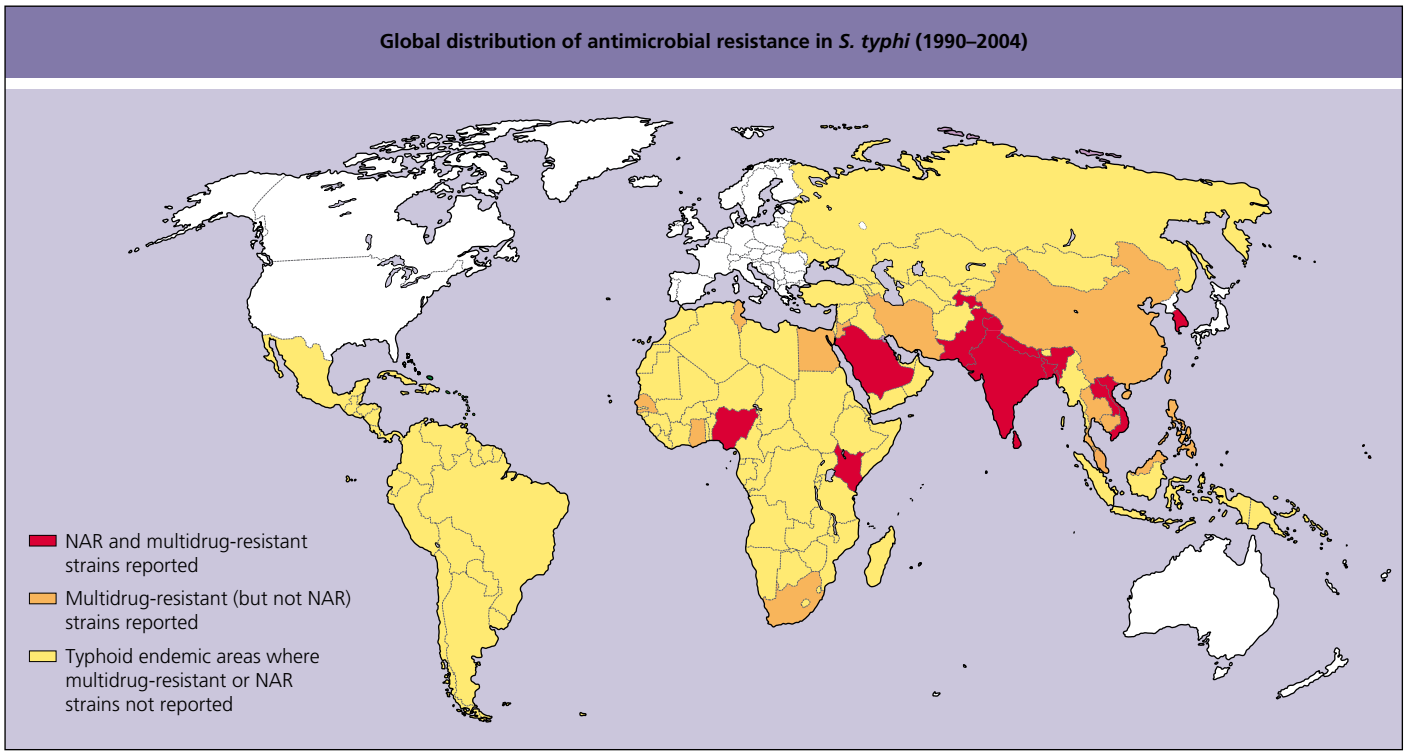


Fig. 109.1 Global distribution of antimicrobial resistance in *Salmonella typhi* (1990–2004). Reproduced with permission from Bhan *et al.*[9]



Fig. 109.2 Community water spouts in Kathmandu, Nepal. Villagers, mostly women and children, are seen collecting their daily water provision from the community's stone spouts. This water is usually not boiled.

PATHOGENESIS AND PATHOLOGY

The species *Salmonella enterica* (see Chapter 169) is divided into six subspecies (*enterica, salamae, arizonae, diarizonae, houtenae* and *indica*) and contains more than 2400 serotypes.[6] Most of the Salmonellae that cause disease in humans (including *S. typhi*, *S. paratyphi* and *S. typhimurium*) are in the subspecies *enterica*.

S. typhi and S. paratyphi are flagellated, non-spore-bearing, facultative anaerobic Gram-negative bacilli, identified by a characteristic biochemical pattern and confirmed by serologic identification of their somatic lipopolysaccharide (O) and flagellar protein (H) antigens. *S. typhi* and *S. paratyphi* C sometimes possess a polysaccharide capsular Vi (virulence) antigen that coats the O antigen and potentially protects it from antibody attack.

The genome of *S. typhi* is 4.8 million base pairs in length and encodes approximately 4000 genes.[7] There are several large insertions, believed to originate from bacteriophages or plasmids, termed the *Salmonella* pathogenicity islands which encode genes that are important for survival in the host. *Salmonella* has two type III secretion systems (TTSS) encoded by *Salmonella* pathogenicity islands SPI-1 and SPI-2. The invasion of epithelial cells is mediated by the SPI-1 TTSS which injects virulence proteins into the host cell, leading to macropinocytosis of the bacteria and cytoskeletal rearrangement of the host cell to allow translocation. SPI-2 TTSS secretes effectors required for survival and replication in *Salmonella*-containing vesicles inside phagocytes and epithelial cells.[8]

S. typhi also has more than 200 pseudogenes. The inactivation of these genes may explain the host restriction of *S. typhi*.

Volunteer studies have shown that the infective dose of *S. typhi* is 10^3 to 10^9 bacteria. Gastric acidity destroys the organisms but gastric hypoacidity (following gastrectomy, intake of histamine-2 receptor antagonists or proton pump inhibitors) allows a greater number of organisms to enter the small intestine. In a study from India, *Helicobacter pylori* infection has been associated with an increased risk of typhoid fever.[9]

After ingestion in water or food, *S. typhi* bacteria reach the small intestine where they adhere to the mucosal epithelial cells. They penetrate the mucosal epithelium via the M (membranous) cells (specialized cells overlying the Peyer's patches), enterocytes or via a paracellular route. They are taken up by macrophages and multiply in the mononuclear phagocytic cells of the small intestine, are drained into mesenteric lymph nodes and reach the general circulation (causing an asymptomatic primary bacteremia) by lymph drainage from the thoracic duct. During the incubation period, which varies between 7 and 14 days, the bacteria reside and multiply within the organs of the reticuloendothelial system (spleen, liver, bone marrow and lymph nodes, especially the Peyer's patches of the terminal ileum). Bacteria are then shed into the bloodstream, marking the onset of fever and symptomatic disease. During the symptomatic stage, *S. typhi* can be cultured from blood, although this is made difficult by the low bacterial load that characterizes this infection (less than one colony-forming unit per ml of blood).

If left untreated, the *S. typhi* bacteremia persists for several weeks. In this phase the organism disseminates widely to the organs of the reticuloendothelial system. *S. typhi* infection produces hyperplasia of the Peyer's patches in the first week, which can resolve or progress to necrosis. Ulcers can lead to perforation and hemorrhage, usually in the third week, although these may occur earlier or later during the disease.[1]

The majority of patients mount local and systemic humoral and cellular immune responses but these do not confer complete protection against relapse or re-infection. The mortality of untreated typhoid used to be about 10–20%.

PREVENTION

Typhoid and paratyphoid fever can be prevented by the provision of safe water, good food hygiene and safe sewage disposal. As chronic carriers pose a special risk to the community, programs to detect and treat chronic carriers are needed.[10]

Vaccines are another tool to prevent enteric fever. There are two licensed vaccines for typhoid fever, the parenteral Vi polysaccharide and the oral Ty21 vaccines.

The live oral Ty21 vaccine is available in enteric-coated capsules or liquid formulation. It is administered in three doses (four doses in the USA and Canada) 2 days apart and is licensed for adults and children above 6 years. Field studies with the oral Ty21 vaccine in the 1980s showed a protective efficacy of 96% after 3 years in Egypt, 77% in Chile after 3 years when using the liquid formulation and 53% in Indonesia after 2.5 years.[1] Herd protection was demonstrated during the field trials in Chile. Ty21a cannot be used in immunocompromised patients and pregnant women, and antibiotics should be avoided 7 days before and after immunization. Booster doses are recommended every 5 years.

The injectable capsular polysaccharide Vi vaccine is given in a single dose (25 µg) and is immunogenic and licensed above 2 years of age. A single intramuscular injection conferred a protective efficacy of 55% after 3 years in South Africa and 72% after 17 months in Nepal.[10] Booster doses are recommended every 3 years.

The Vi conjugate vaccine is immunogenic in children under 2 years and had a protective efficacy of 91.5% after 27 months in field trials in Vietnam,[10] but is not yet commercially available.

None of the currently licensed typhoid vaccines protects against *S. paratyphi* A, an emerging infection in Asia, and there is no licensed paratyphoid vaccine available. A polyvalent panenteric vaccine is urgently needed.

CLINICAL FEATURES

Enteric fever presents with fever, headache, anorexia and abdominal discomfort with either diarrhea or constipation. This can be accompanied by nausea, vomiting and a dry cough. Profuse diarrhea has been described in typhoid patients with HIV infection.[1] Abdominal tenderness, hepatomegaly (40–70% of patients) and splenomegaly are common. It is rare for patients with typhoid fever not to have any abdominal symptoms, but even in the absence of these symptoms typhoid fever cannot be excluded.

Less than 5% of patients show rose spots, small blanching erythematous maculopapular lesions of about 2–4 mm diameter on the trunk (Fig. 109.3). Hemoglobin levels, white cell counts and platelet counts are normal or reduced. Liver enzymes (aspartate aminotransferase, alanine aminotransferase) are often elevated two to three times the upper limit of normal and bilirubin is normal or slightly raised. Abdominal sonography may demonstrate enlargement of liver and spleen and prominent mesenteric lymph nodes.

Although it has been frequently cited in the literature that *S. paratyphi* causes a milder disease, recent prospective clinical trials have

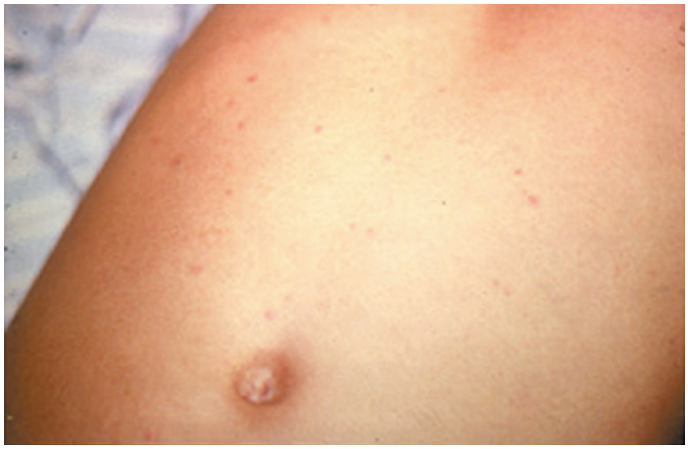


Fig. 109.3 Typhoid rose spots. These are small, blanching maculopapular lesions, ~1–4 mm in size, usually seen on the trunk. They may also take on a more purpuric, nonblanching character. Courtesy of CM Parry, Liverpool, UK.

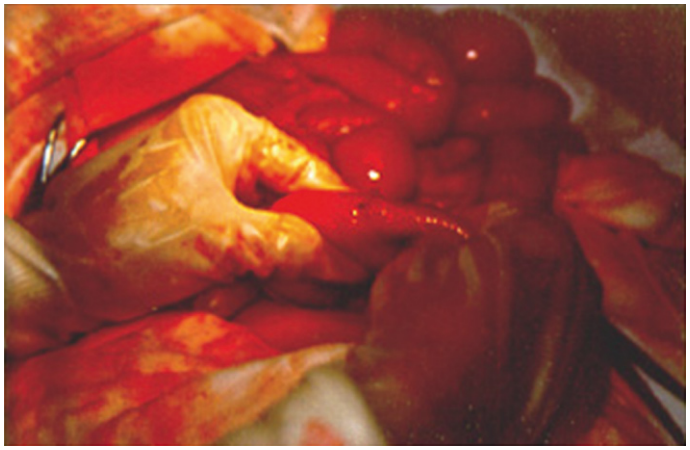


Fig. 109.4 Gastrointestinal perforation, usually of the terminal ileum, is one of the most serious complications of typhoid fever. The picture shows the surgical inspection of a perforating necrotic ulcer. Courtesy of John Wain, Cambridge, UK.

reported that enteric fever caused by *S. typhi* and *S. paratyphi* A are clinically indistinguishable.[4,11] *S. paratyphi* A has also been described as a cause of severe enteric fever in Indonesia.[12]

Complications and severe typhoid fever

Complications of typhoid fever are more likely to occur in patients who have been sick for longer periods without receiving treatment and in patients with nonsusceptible *S. typhi* isolates who do not receive appropriate treatment (Table 109.1), and may develop in up to 10% of hospitalized patients.[10]

Septic shock and acute respiratory distress syndrome are serious complications when treatment is delayed.

Table 109.1 Important complications of typhoid fever

Abdominal complications
Gastrointestinal bleeding
Intestinal perforation and shock
Hepatitis with/without jaundice
Cholecystitis
Genitourinary complications
Retention of urine
Glomerulonephritis
Cardiovascular complications
Asymptomatic ECG changes
Myocarditis
Sudden death
Respiratory complications
Pneumonia
Bronchitis
Hematologic complications
Anemia
Disseminated intravascular coagulation (DIC)
Focal infections
Abscesses of brain, liver, spleen etc.
Neuropsychiatric complications
Encephalopathy – reduced consciousness levels
Meningitis
Seizures

Gastrointestinal bleeding (occurs in about 10% of patients), intestinal perforation and typhoid encephalopathy are the most common complications. Gastrointestinal bleeding results from a necrotic Peyer's patch eroding the wall of an enteric blood vessel. Often the bleeding resolves without intervention, but in about 2% of cases the bleeding requires blood transfusion. Gastrointestinal perforation, usually at the terminal ileum (Fig. 109.4), is the most serious complication; it occurs in about 1–3% of hospitalized cases.[1] It manifests as acute abdomen or as worsening of abdominal pain accompanied by shock. Perforation is associated with a high mortality and needs urgent surgical intervention.

A reduced level of consciousness or encephalopathy, often accompanied by shock, is a serious complication, associated with a mortality of up to 50%.[12] Typhoid encephalopathy encompasses a wide range of symptoms ranging from agitation to delirium and coma. These symptoms might be possibly caused by liver failure, hematogenic dissemination to the brain or other unknown mechanisms.

The incidence of this presentation varies between different countries, ranging from 10% to 40% of hospitalized cases in Indonesia and Papua New Guinea but less than 2% in Pakistan and Vietnam. This variation is unexplained.[1] In patients with encephalopathy, cerebrospinal fluid should be obtained and meningitis (including tuberculous meningitis) and encephalitis excluded.

The prognosis of typhoid fever during pregnancy has been improved through antimicrobial treatment. Typhoid fever acquired through intrauterine infection can lead to neonatal typhoid fever, a severe sepsis with a mortality of up to 10% or to asymptomatic persistent secretion.[1]

Relapse

The recurrence of symptoms within 1 month after treatment has been completed and symptoms have resolved is considered a relapse. Relapse rates vary considerably (between 0% and 10%) and are related to the efficacy of the antibiotic treatment.[1,13,14]

Chronic carriage

Between 1% and 5% of patients with a history of enteric fever become chronic carriers, harboring *S. typhi* or *S. paratyphi* in their gallbladder and shedding bacteria intermittently in their stools after the illness.[10] These carriers are an important reservoir of infection and are usually asymptomatic. Up to 25% of chronic carriers do not have a history of typhoid infection. The rate of carriers is higher among females and patients with cholelithiasis. An association between urinary carriage

of *S. typhi* and *S. paratyphi* A and schistosomiasis has been described, caused by obstructive lesions of the urinary tract.

Case fatality

A recent WHO report has estimated the case fatality rate in typhoid fever at 1%.[2] There seems to be considerable geographic variation; in Vietnam and Pakistan the case fatality rate among hospitalized patients is less than 2%, but in patients with severe typhoid fever in Papua New Guinea and Indonesia it can be as high as 30%.[1] The most important contributor to a poor outcome is probably a delay in instituting effective antibiotic treatment.

Differential diagnosis

Typhoid fever presents with unspecific symptoms and this makes the diagnosis difficult. Other endemic illnesses, most importantly malaria, have to be ruled out. Typhoid can occasionally present as a gastroenteritis with vomiting and diarrhea at its initial presentation. Differential diagnosis includes leptospirosis, typhus (rickettsial disease), tuberculosis, brucellosis, other bacterial sepsis, encephalitis, amebic liver abscesses, visceral leishmaniasis, viral diseases (dengue fever, infectious mononucleosis, hepatitis, influenza) and lymphoproliferative disease.

ANTIMICROBIAL DRUG RESISTANCE

In 1948 the introduction of chloramphenicol revolutionized the management of typhoid fever.[15] Chloramphenicol was effective for more than 20 years, but then in the 1970s outbreaks of chloramphenicol-resistant typhoid fever occurred in Mexico, India and Vietnam. In the late 1980s there were outbreaks of typhoid fever that were resistant against all 'first-line' antimicrobials (multidrug resistance is defined as resistance to chloramphenicol, ampicillin and trimethoprim–sulfamethoxazole).[1] These multidrug-resistant (MDR) *S. typhi* isolates have been responsible for numerous outbreaks in countries in the Indian subcontinent, South East Asia and Africa.[9] All MDR strains so far examined have been harboring plasmids of the incHI1 incompatibility group.

The fluoroquinolone class of drugs has become the treatment of choice for typhoid fever. The fluoroquinolones show excellent tissue penetration, accumulation in monocytes and macrophages and high drug levels in the gallbladder. However, there have been reports from Vietnam, India and Tajikistan of the emergence of *S. typhi* isolates that respond less well to the fluoroquinolones.[1,9] In 1997, an epidemic in Tajikistan caused by resistant strains caused illness in more than 10 000 people and 108 deaths. Technically these isolates are still within the breakpoints set for fluoroquinolone susceptibility by the Clinical Laboratory Standard Institute (CLSI),[16] but they are resistant to nalidixic acid (the prototype quinolone) and show higher minimum inhibitory concentrations (MICs) to the fluoroquinolones. Patients infected with these isolates show a poor clinical response when treated with ciprofloxacin or ofloxacin.

Nalidixic acid resistance is usually caused by single point mutations in the bacterial target enzyme *gyrA*, either at codon 83 or 87.[17] High-level ciprofloxacin-resistant *S. typhi* with double mutations at position 83 and 87 from the Indian subcontinent has been described.

Recently there have been reports of *S. typhi* isolates with reduced susceptibility to the fluoroquinolones that test nalidixic acid sensitive,[9] suggesting another mechanism of resistance. There have been sporadic reports of ceftriaxone-resistant *S. typhi* fever, but these isolates remain rare.[18] A decreasing trend of chloramphenicol-resistant *S. typhi* has been reported in recent years from the Indian subcontinent.[9]

MDR as well as nalidixic acid-resistant *S. paratyphi* A is an emerging problem in the Indian subcontinent.[3,4]

DIAGNOSIS

The diagnosis of enteric fever requires the isolation of *S. typhi* or *S. paratyphi* from blood, bone marrow or an anatomic lesion. Blood culture is the gold standard in the diagnosis of enteric fever. As a low bacterial load is a characteristic feature of typhoid fever, obtaining a large volume of blood – ideally 10–15 ml for schoolchildren and adults and 2–4 ml for preschool children – is recommended. The sensitivity of blood culture is estimated to be 50–60%; bone marrow culture is more sensitive (up to 80%), because of the higher concentration of bacteria in the bone marrow.[10]

Serology

The Widal tube agglutination test, described more than 100 years ago, is based on the presence of agglutinating antibodies to the O and H antigens of *Salmonella*. It is usually performed in settings where culture facilities are not available. In the original format, paired sera (acute and reconvalescent) were required and a fourfold increase of antibodies (to O and H antigens) supported the diagnosis of typhoid fever. However, the test is usually performed on acute serum and lacks sensitivity and specificity. This is because not everybody mounts a detectable antibody response to *S. typhi*, healthy populations in endemic areas and people who received typhoid vaccine show a high level of antibodies and there is cross-reactivity to other *Salmonella* serotypes. Rapid tests (Tubex, Typhidot and Typhidot M) have been developed with varying sensitivity and specificity.[19]

MANAGEMENT

Enteric fevers are systemic infections and antimicrobial treatment should be initiated early. It is important to provide supportive measures, such as oral and intravenous fluids, appropriate nutrition and antipyretics. More than 90% of patients are managed at home with oral antibiotics, and reliable and close medical follow-up for complications or failure to respond to therapy. Patients with persistent vomiting, severe diarrhea and abdominal distention need admission to hospital.[10]

The management of patients with enteric fever should include blood and stool cultures after completion of treatment to check for convalescent stool carriage, as well as follow-up to identify chronic fecal carriage.

The choice of treatment depends on the antimicrobial susceptibility of the isolates, but is also influenced by cost and availability, which are important factors, especially in endemic regions.

Table 109.2 shows the recommendations of the World Health Organization for the treatment of typhoid fever, updated with evidence from recent randomized controlled trials.[13,14,20]

The fluoroquinolones are the most effective treatment for typhoid fever. In patients infected with nalidixic acid-susceptible isolates, fever usually resolves within 4 days, cure is achieved in 96% of patients and rates for fecal carriage and relapse are below 2%.[1]

The fluoroquinolones are also recommended for the treatment of children with typhoid fever. Concerns that the fluoroquinolones might cause cartilage damage in children have led to their cautious use in many countries. However, extensive experience with the fluoroquinolones in children with cystic fibrosis, typhoid fever and bacillary dysentery has provided a body of evidence suggesting that these antibiotics are safe in children.[1,10]

For infections with nalidixic acid (quinolone)-resistant *S. typhi* and *S. paratyphi*, azithromycin, newer generation fluoroquinolones (gatifloxacin) and third-generation cephalosporins (ceftriaxone) are effective.

Two trials[13,20] and one recent trial from Nepal (Basnyat B, unpublished results; this trial included monitoring for dysglycemia) show that the 8-methoxy-fluoroquinolone gatifloxacin is safe and efficacious in patients with enteric fever (91% and 96% overall treatment success, respectively). A retrospective study from Canada reported a high risk

Table 109.2 Treatment of uncomplicated typhoid fever*

	OPTIMAL THERAPY			ALTERNATIVE EFFECTIVE DRUGS		
Susceptibility	**Antibiotic**	**Daily dose (mg/kg)**	**Days**	**Antibiotic**	**Daily dose (mg/kg)**	**Days**
Fully sensitive	Fluoroquinolone, e.g. ofloxacin or ciprofloxacin†	15	5–7‡	Chloramphenicol Amoxicillin Trimethoprim–sulfamethoxazole	50–75 75–100 8/40	14–21 14 14
Multidrug resistance	Fluoroquinolone	15	5–7	Azithromycin Ceftriaxone	10–20 75	7 10–14
Quinolone (nalidixic acid) resistance§	Azithromycin or newer fluoroquinolone, e.g. gatifloxacin#	10–20 10	7 7	Ceftriaxone	75	10–14

*Based on the World Health Organization (WHO)[10] recommendations for the treatment of typhoid fever, updated with evidence from recent randomized controlled trials in typhoid fever.[13,14,20] Cefixime, an oral third-generation cephalosporin that was included in the WHO recommendations of 2003, has been removed due to recent evidence.[20]
†The available fluoroquinolones (ofloxacin, ciprofloxacin, fleroxacin, pefloxacin) are all highly active and equivalent in efficacy, with the exception of norfloxacin, which has inadequate oral bioavailablity and should not be used in typhoid fever.[10]
‡Three-day courses have also been shown to be effective, but should be reserved for epidemic containment.[10]
§Prolonged courses of high-dose fluoroquinolones (e.g. ofloxacin 20 mg/kg q24h) have been recommended[10] and are standard treatment in many contries for nalidixic acid-resistant *S. typhi* and *S. paratyphi* A; however, a recent trial using ofloxacin at 20 mg/kg q24h for 7 days has shown treatment success in only 64% of patients and high rates of convalescent fecal (19%) carriage (at day 8).[14]
¶Azithromycin used at 20 mg/kg q24h for 7 days achieved better cure rates (93% and 91%, respectively)[13,22] than azithromycin 10 mg/kg q24h in multidrug-resistant and nalidixic acid-resistant typhoid fever.
#Two published trials[13,20] and one recent trial from Nepal (Basnyat B, unpublished results; this trial included monitoring for dysglycemia) show that the newer 8-methoxy-fluoroquinolone gatifloxacin is safe and efficacious in patients with typhoid fever (91% and 96% overall treatment success, respectively). A retrospective study from Canada reported that gatifloxacin used in elderly patients (mean age 77 years) with type 2 diabetes has a high risk of inducing dysglycemia;[21] this was not seen in the typhoid treatment trials in young and otherwise healthy patients.

of gatifloxacin-induced dysglycemia in elderly patients (mean age 77 years) with type 2 diabetes.[21] These side-effects were not seen in the young and otherwise healthy patients in these trials.

Prolonged courses of fluoroquinolones (e.g. ofloxacin 20 mg/kg/day) have been recommended[10] and are standard treatment in many countries for nalidixic acid-resistant *S. typhi* and *S. paratyphi* A. However, a recent trial using ofloxacin at 20 mg/kg/day for 7 days has shown treatment failure in 36% of patients and high rates (19%) of immediate post-treatment fecal carriage (at day 8).[14]

Cefixime (oral third-generation cephalosporin) should not be used. A recent trial observed high treatment failure rates of 38% and the trial was stopped early by the trial's Independent Data Safety and Monitoring Board.[20]

Ceftriaxone should be used for 10–14 days. Ceftriaxone therapy for 7 days was associated with a relapse rate of 14%.[9]

There are few data on the treatment of typhoid fever in pregnancy. Ampicillin for fully susceptible isolates and ceftriaxone are considered safe for this indication.[10]

Management of severe typhoid fever

Both inpatients and outpatients should be closely monitored for the development of complications. The parenteral fluoroquinolones are probably the first choice for the treatment of severe typhoid fever[10] and should be given for a minimum of 10 days, but there are no randomized trials to date. For patients infected with nalidixic acid-resistant isolates, ceftriaxone is effective.[10]

A trial conducted in the 1980s showed a dramatic beneficial effect of high-dose dexamethasone treatment (3 mg/kg for the first dose given over 30 minutes and 1 mg/kg every 6 hours for 48 hours) in severe typhoid fever patients with encephalopathy and shock, given in addition to chloramphenicol. Dexamethasone treatment reduced the mortality from 56% to 10% when compared to placebo.[12] Hydrocortisone at a lower dose was not effective.

Intestinal perforation is a surgical emergency. Early intervention is crucial, and mortality rates increase as the delay between perforation and surgery lengthens, varying between 10% and 32%.[10] Metronidazole should be added to the antibiotic regimen.

Patients with intestinal hemorrhage need intensive care, monitoring and blood transfusion. Intervention is not needed unless there is significant blood loss, but cross-matched blood should be ready and the surgical team alerted.

In patients presenting with a relapse, which is defined as the re-occurrence of acute illness after the patient has been treated successfully, cultures should be obtained and patients should be treated according to the susceptibility pattern of the infecting isolate.

Typhoid carriers

Chronic carriers play an important role in the transmission of typhoid and paratyphoid fever. As *S. typhi* and *S. paratyphi* are only shed intermittently in the feces, a series of stool cultures should be obtained to detect typhoid carriers. In order to eradicate carriage, long antimicrobial treatment courses of 6 weeks should be given according to the susceptibility of the isolates. In susceptible isolates, clearance was achieved in up to 80% with the administration of 750 mg ciprofloxacin or 400 mg norfloxacin twice daily for 28 days.[10] If cholelithiasis is present the patient may require cholecystectomy as well as antibiotic therapy.

REFERENCES

References for this chapter can be found online at http://www.expertconsult.com

Chapter 110

David C Warhurst

In memory of Peter Sargeaunt and Mehreen Zaki, who died in 2008.

Amebic infections

INTRODUCTION

Amebiasis ranks as the fourth most common protozoan cause of death, behind malaria, African trypanosomiasis and leishmaniasis.[1] The motile trophozoites (amebae) live in the lumen of the large intestine, where multiplication by binary fission takes place. Trophozoites of *Entamoeba histolytica* can invade the colonic mucosa producing ulceration and dysentery (Fig. 110.1). Through blood-borne spread they can give rise to extraintestinal lesions, usually abscesses in the liver (see Fig. 110.5). The course of dysentery is usually self-limiting, but amebic liver abscess is potentially fatal unless diagnosed promptly and treated appropriately. During their life in the large intestine, the trophozoites of *Entamoeba* spp. develop into cysts, a dormant stage, resistant to environmental stress, which is passed out in the feces and serves to infect other humans after ingestion in contaminated water or food.

This organism was first observed by Lösch[2] in 1875, who reported from St Petersburg on a fatal dysentery in a patient with persistent diarrhea, fever, general weakness and tenesmus. However, not until the last decade of the 20th century was it generally accepted that amebae termed *E. histolytica* living in the human colon and producing 4-nucleate cysts between 10 and 20 µm in diameter comprised three distinct species, *E. histolytica*, *E. dispar* and *E. moshkovskii*, of which only *E. histolytica* was capable of causing dysentery and other pathologic manifestations. *E. dispar* had been proposed as a nonpathogen, separate from but morphologically identical to *E. histolytica*, by Brumpt in 1925:[3] the separation of potentially pathogenic *E. histolytica* from morphologically similar amebae was supported by Sargeaunt and colleagues[4] in 1978 on the basis of isoenzyme patterns, then on nucleic acid sequence by Diamond & Clark[5] in 1993. The World Health Organization (WHO)[6] redefined amebiasis in 1997 as infection with *E. histolytica*, with or without clinical manifestations, and noted the importance of distinguishing it from the morphologically identical but nonpathogenic *E. dispar*.



Fig. 110.1 Pathology specimen from a fatal case of human amebic colitis. Deep ulcerations into the submucosa have produced abundant hemorrhages. Courtesy of Dr Jesús Aguirre García, Hospital General de México, Secretaria de Salud.

It has since become clear that *E. moshkovskii* represents a third morphologically similar nonpathogenic species, isolated from humans, but able to grow in culture at ambient temperatures (10–25°C) as well as 37°C, unlike *E. histolytica* or *E. dispar*.[7] Although initially it was thought that human infections with *E. moshkovskii* might be rare and restricted to temperate zones, it now appears that the organism may infect humans over a much wider geographical range.[8,9] Worldwide *E. histolytica* prevalence data gathered from stool examinations for cysts during the last century are therefore misleading. However, mortality data from confirmed cases of invasive disease are likely to be reliable. Similarly, prevalence data based on serology remain valuable indicators of distribution because natural infections with noninvasive species of *Entamoeba* do not elicit an antibody response.[10]

Clinically it is clearly desirable, when symptoms such as bloody diarrhea or colitis are seen and amebae are present, that:

- we should be able to confirm the presence of the pathogen, *E. histolytica*, ideally by reliable specific tests; and
- in the absence of a confirmed *E. histolytica* infection, the true cause of the symptoms (e.g. shigellosis) should be found and treated, incidentally avoiding unnecessary chemotherapy for amebiasis.

Because the three species have only recently been differentiated, it is an epidemiologic priority to understand their worldwide distribution and fully evaluate their individual significance.[11]

DIAGNOSIS

Amebic colitis

Although diarrhea alone may be the presenting feature, amebic colitis, like shigellosis, classically presents with bloody diarrhea or dysentery; this should be differentiated from carcinoma, necrotizing colitis, cytomegalovirus (CMV) colitis (in AIDS), antibiotic-associated colitis and inflammatory bowel disease (misdiagnosis and corticosteroid treatment can be fatal here). Laboratory tests are essential for definitive diagnosis. Detection of specific antibody (IgG) in serologic tests can be extremely valuable for confirming invasive amebic disease of the colon or extraintestinal amebiasis.

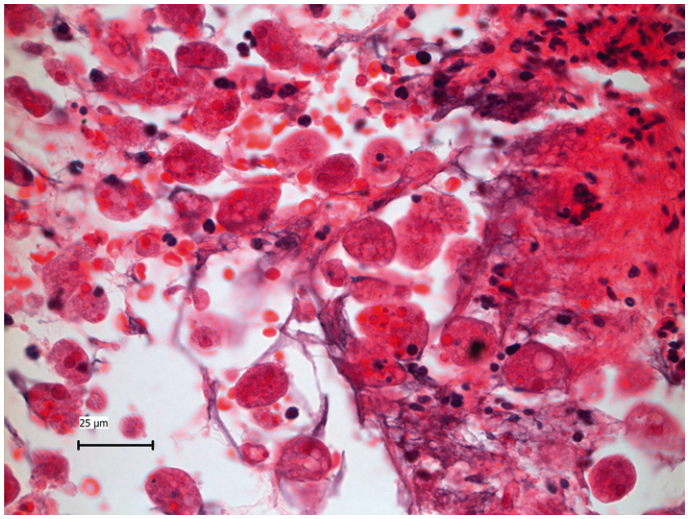


Fig. 110.2 Section through bleeding colonic ulcer showing *E. histolytica* trophozoites with ingested red blood cells in human colonic submucosa and undermined mucosa. Leukocytes from the cellular reaction are visible, and they show typically strongly stained nuclei and sparse cytoplasm. The technique is Ehrlich's alum hematoxylin with eosin, which stains erythrocytes well but does not clearly show the amebic nuclei. Courtesy of John Williams, LSHTM.

In classic fecal microscopy, nonpathogenic *Entamoeba* spp. such as *E. coli, E. hartmanni, E. polecki*-like amebae (including *E. chattoni*)[12] and also *Iodamoeba butschlii* and *Endolimax nana* can be distinguished from *E. histolytica, E. dispar* and *E. moshkovskii* by the size of cysts, nuclear number and/or nature of inclusions. Cysts of the last three species are all quadrinucleate and average 12 µm in diameter (10–20 µm). Morphology of the trophozoite is usable, since to find amebae which have ingested erythrocytes in fresh samples of mucoid or bloody mucoid stools, or in a rectal scrape/rectosigmoidoscopy sample from suspected amebic colitis (Fig. 110.2) is diagnostically highly predictive of *E. histolytica* infection.[13] However, the laboratory worker must be sufficiently skilled not to mistake erythrophagocytosis in human leukocytes with densely staining polymorphic or monocytic nuclei for the same process in the generally larger, vacuolated and motile amebic trophozoites, each with a poorly staining nucleus which is difficult to detect. When recognizable amebic trophozoites without ingested red blood cells are seen well away from bleeding areas, in advance of the main inflammatory response, it can also be strongly suggestive of invasive disease (Figs 110.3, 110.4). The nonmicroscopic molecular biologic technique of nested polymerase chain reaction (PCR) and the simpler, but still expensive, technique of specific *E. histolytica* antigen detection, which can both be carried out on stool samples, are recommended[14] in preference to microscopy. Buss *et al.*[15] found that the TechLab *E. histolytica* II antigen-detection test based on Eh-adherence lectin detected *E. histolytica* infections more accurately than another similarly based test. Antigen detection test kits are unfortunately not as sensitive as PCR.

Amebic liver abscess diagnostics

Amebic liver abscess is usually accompanied by right hypochondrial pain and tender liver. The diaphragm is often seen to be raised on chest X-ray. A neutrophil leucocytosis (>10 000) and a raised alkaline phosphatase may be detected (see Table 110.2). There is usually a space-occupying lesion in the liver on ultrasound and CT scan. If an aspirate is done, amebic trophozoites may be detected microscopically, by antigen detection or by PCR, in the atypical 'pus' (mainly necrotic liver parenchyma with few neutrophils or macrophages). Serology for antiamebic antibody, which is elevated in more than 90% of cases, is very helpful.[16]

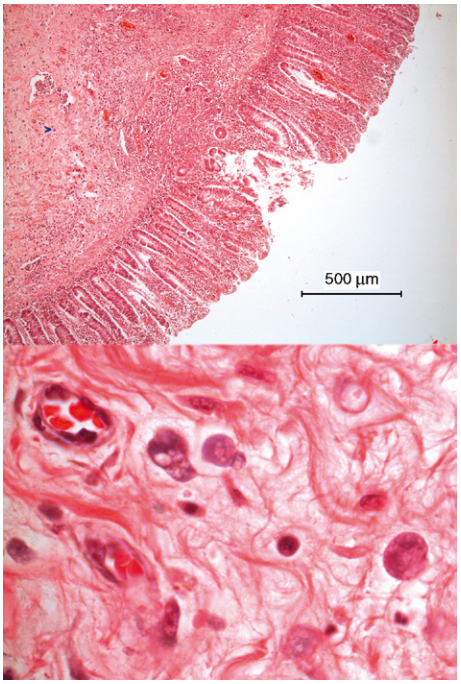


Fig. 110.3 Small mucosal ulcer, with early submucosal spread. Deep in the submucosa, all we see suggestive of a cellular response is in lymphatic vessels, near the blue arrow which shows the position of the advancing front of invading amebae. In the detail of this area (below) no leakage of blood or obvious damage to tissues has taken place, and the amebae have ingested no red blood cells. The technique is Ehrlich's alum hematoxylin with eosin, which stains erythrocytes well but does not clearly show the amebic nuclei. Courtesy of John Williams, LSHTM.



Fig. 110.4 *E. histolytica* trophozoites in human colonic submucosa. A few leukocytes from the cellular reaction, with heavily stained nuclei and sparse cytoplasm, are visible. Weigert's iron hematoxylin and eosin highlights the dark staining of a chromatin ring just inside the nuclear membrane in each ameba. The abundant cytoplasm contains vacuoles with host-cell material in the process of digestion. Courtesy of John Williams, LSHTM.

Differential diagnosis

Pyogenic abscess and neoplasm of the liver are commonly the alternative diagnoses. Pyogenic abscess is more common in older patients with a previous history of hepatobiliary or abdominal disease. The presenting features may be jaundice, pruritus and septic shock, with

no hepatomegaly or elevated diaphragm in the chest X-ray, while amebic serology is negative. Needle aspiration allows microscopy and culture in such circumstances.

Where the patient is febrile and wasted and has vague abdominal discomfort, liver neoplasm is suspected. On CT scanning, the distinct image may be indicative, and blood samples may test positive for alpha-fetoprotein, carcinoembryonic antigen or other tumor markers.[16] Stool microscopy, antigen testing or PCR should be carried out, to search for the concomitant asymptomatic intestinal ameba infection commonly found in amebic liver abscess cases.

EPIDEMIOLOGY

Entamoeba histolytica (*sensu lato*) is one of the 10 most common intestinal infections in the world. Recent WHO[1] estimates give 48 million clinical cases per year, resulting in at least 70 000 deaths (mortality in clinical disease = 0.15%). Using recent advances in molecular genetics, the potentially pathogenic *E. histolytica* can be distinguished from the nonpathogenic similar species. This allows scrutiny of important and previously obscure pathogen–host interactions, and a number of studies from around the world are now available to provide a better understanding of the epidemiology of the disease (see website for further information).

Although invasive amebiasis is much less common in industrialized countries than in the developing world, it is still important to identify an *E. histolytica* infection there, as misdiagnosis can lead to death. Missed intestinal amebiasis, mistaken for chronic ulcerative colitis, may be treated disastrously with corticosteroids. Infection rates in immigrant groups can be significant, and outbreaks can occur in institutions such as schools or psychiatric hospitals where hygiene is compromised. A major increase in intestinal amebic infections reported in male homosexual populations in USA, Canada and England has mainly been asymptomatic, caused by the nonpathogenic *E. dispar*. In 136 suspected or confirmed HIV-positive inpatients in Tanzania, *E. histolytica*, *E. moshkovskii* and *E. dispar* were all detected without evidence of invasive amebic disease.[9] In Japan and Taiwan, however, invasive amebiasis due to *E. histolytica* is certainly found in groups of sexually active male homosexuals, though there is no evidence that symptoms are more serious in HIV.

PATHOGENESIS

Stanley,[17] Ackers & Mirelman[18] and Guo *et al*.[19] have recently reviewed amebiasis in general, and in particular the expanding scenario of pathogenesis and invasion.

The first stage in pathogenesis is the attachment of the trophozoite to the human target cell, erythrocyte, enterocyte or leukocyte which is made by a surface-located Eh-adherence lectin and is necessary for all subsequent changes. The adherence of the binding domain to target cells can be inhibited experimentally by D-galactose or *N*-acetyl D-galactosamine, and naturally by colonic mucin. This lectin, often termed 'Gal/GalNAc lectin' or 'Eh-adherence lectin' is also used by the amebae for binding bacteria prior to phagocytosis. The heavy subunit of the Eh-adherence lectin has a carbohydrate recognition domain (CRD) of 104 residues which is only 89% conserved in the homologous sequence expressed by nonpathogenic *E. dispar*.[20,21]

It is probable that Eh-adherence lectin responds to attachment by stimulating cytoskeletal changes in the ameba, associated with phagocytosis of the target cell and also the transfer of cysteine proteinases (EhCP-) and amebapores (EhAP-) from granules in the amebic cytoplasm to the membrane or external medium.

Subsequently, amebae produce a range of neutral cysteine proteinases[18] which are expressed on the cell membrane or are secreted to the exterior. They have the ability to break down matrix collagen, allowing the spread of amebae in the submucosa. Attracted (and activated) by interleukin 8 (IL-8) or other cytokines, neutrophils (and to a lesser extent macrophages) invade the mucosa and ease the entry of the amebae to the submucosa and deeper layers of the colonic wall.

The transcriptional framework of virulence[22]

Recent studies have shed considerable light on the molecular basis of virulence in amebiasis (see website for additional information), and now that the draft genome sequence of *E. histolytica* has been obtained[23] and is undergoing secondary editing, comparison with nonvirulent *E. histolytica* clones, *E. dispar* and *E. moshkovskii* is progressing. New regulatory genes,[24] retrotransposons and new attachment proteins are being discovered.

TRANSMISSION AND PREVENTION

Although the organism can infect other animals, there is effectively no reservoir of *E. histolytica* other than humans. All age groups are susceptible to infection with *E. histolytica*; persons with asymptomatic intestinal infection are most likely to be excreting cysts. Young children in particular are likely to infect their carers (mainly mothers and female relatives) and healthy infected adults (male or female) capable of work may be involved under relatively insanitary conditions in preparing food sold on the streets or in small retail outlets. In endemic areas poor education, poverty, overcrowding, inadequate washing facilities and shortage of uncontaminated water supplies for washing and drinking, together with poor sanitation, favor fecal–oral transmission.

When the fully developed and infective cysts are passed in the stools, they can be killed by desiccation. Cysts in fecal material die within 10 minutes on the surface of the hands, but can survive under (long) fingernails for 45 minutes. Conditions of high relative humidity will prolong these survival times. Although the cysts are killed by freezing, they may survive for more than 100 hours at 25°C in moist conditions or in water. Survival in water at low temperatures above freezing can be for at least 3 months, but above 50°C it is a matter of minutes. Pasteurization or brief boiling is sufficient to eliminate the possibility of transmission from milk or water. Residual concentrations of chlorine sufficient to eliminate bacterial contamination from drinking water supplies will not kill the cysts: 3 mg/l is needed for 30 minutes. Cysts may be removed from water by slow filtration through biologic sand filters or rapid sand filtration after flocculation.

Feachem *et al*.[25] classify *Entamoeba* spp. on the basis that the cysts are infective when passed, and they are resistant to stomach acid. Thus the direct fecal–oral route is probably more important than for geohelminths (where development to the infective stage generally needs to take place outside the body) and compared to bacteria the inoculum size can be small (<<100). Since the cysts are susceptible to desiccation their persistence in the environment is limited. When, eventually, uncontaminated water supplies for drinking and hand washing become available, and health education increases personal hygiene, the importance of the direct fecal–oral route would be expected to diminish together with the prevalence of infection. Risk factors for infection in endemic areas have been reported to be crowding, illiteracy, lack of safe drinking water and inadequate disposal of human waste. It appears that focusing on availability of suitable water supplies for drinking and hand washing, as well as health education, should be the priority, together with early diagnosis and treatment.[26]

Apart from the desirability of preventive treatment of asymptomatic persons in an endemic area,[27] there will be reservations over its practicality in view of the high re-infection rate. Recommendations for treatment of asymptomatic carriers in nonendemic areas do not usually include a tissue amebicide, which avoids major ethical objections on the grounds of side-effects. Two alternatives are available:[29] paromomycin 25–35 mg/kg q8h for 7–10 days, or diloxanide furoate (if available) 500 mg q8h for 10 days.

Some success has been achieved in the laboratory, using recombinant preparations of the Eh-adherence lectin to immunize experimental animals. However, the method will need much more development before it is ready for preventive trials in humans.

CLINICAL FEATURES

Amebic invasion more commonly remains localized in the colon but can metastasize via the portal vein to the liver and more rarely, by extension from a liver abscess into pleura, lungs and/or pericardium. The amebae invade the colonic mucosa through small punctures in the mucosal surface and spread laterally into the submucosa, producing typical flask-shaped ulcers (see Fig. 110.3). Extension may occur rarely from the colon to large abdominal vessels and other viscera including the urogenital system, or extremely rarely to the brain by hematogenous spread. Extension to the skin can take place through rupture of an untreated liver abscess or from a perforated colonic segment. Primary infection of the skin can also be acquired by direct contact with an infected subject during vaginal or anal intercourse.[16]

Intestinal amebiasis (Table 110.1)

The spectrum of invasive disease ranges in severity from mild diarrhea to life-threatening fulminating necrotic colitis (see Fig. 110.1) and perforation of the colon, which are the main causes of death in invasive intestinal amebiasis. Other complications include direct extension to the skin and dissemination, mainly to the liver (Figs 110.5, 110.6). Severe intestinal amebiasis is twice as frequent in adults as in children,[16,28] where it may be associated with undernourishment.

Usually an acute rectocolitis characterizes invasive intestinal disease. Most patients present with a nontoxic dysenteric syndrome (a 'walking dysentery') and general symptoms are not as prominent as in *Shigella* dysentery. The onset of acute rectocolitis is gradual and 85% of patients develop intense abdominal pain (Table 110.1).[16,28]

Initially there are loose watery stools, but these rapidly become blood-stained and contain mucus. Tenesmus associated with rectosigmoidal involvement occurs in 50% of patients. Watery diarrhea or loose stools without blood may be present for a few days, particularly if the distal colon is involved. Ameboma is an abnormal response, probably to prolonged infection and manifesting as a marked granuloma, mimicking colon carcinoma, except in its rapid response to metronidazole. Usually there is a marked serologic antiamebic titer. With some symptoms resembling the toxic colon of nonspecific ulcerative colitis, invasive amebic colitis is an emergency which may progress to life-threatening fulminant colitis with perforation and development of amebic peritonitis, the most serious condition associated with invasive amebiasis of the intestine. This is apparently influenced by nutritional factors, age and susceptibility, and, probably, strain differences in virulence. Ulceration and necrosis can affect all layers of the colon wall; perforations can be multiple and can give rise to purulent peritonitis. In a series of 62 patients with fulminant amebic colitis seen by Guarner[31] from 1963 to 1970, 57.5% showed a single perforation, 28% multiple perforations and none were found in 14.5%.

When the luminal surface of the mucosa has been damaged, the amebae may undermine the mucosa to give more ulceration, producing abundant microhemorrhages. Normal mucosa can still be seen macroscopically between the sites of invasion. Ulcers are initially superficial, with hyperemic borders and a necrotic base (see Fig. 110.3). Progression of the lesions may result in a loss of mucosa and submucosa covering the muscle layers and lead to rupture of the serosa.

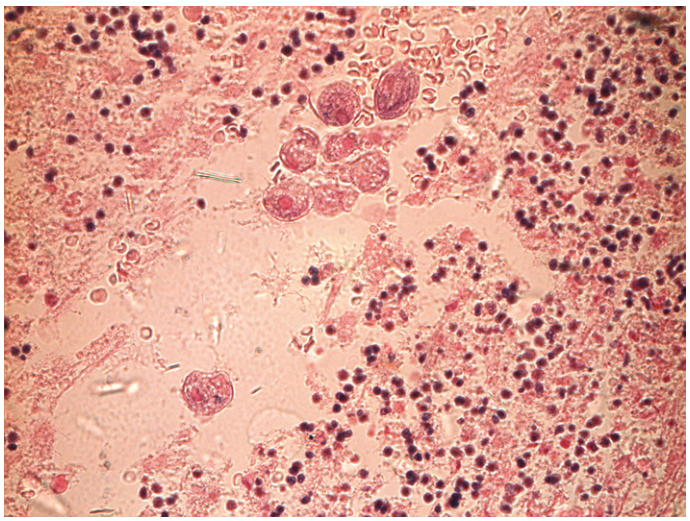


Fig. 110.6 Liver abscess section stained with Ehrlich's hematoxylin and eosin. Many dark-stained nuclei of necrotic liver cells are seen around the liquid content of the abscess. A group of amebic trophozoites is seen adjacent to an area of bleeding.

Table 110.1 Clinical features of acute amebic colitis

Clinical feature		Percentage of cases
Symptoms for	0–1 week	48
	2–4 weeks	37
	>4 weeks	15
Diarrhea		100
Dysentery		99
Abdominal pain		85
Lower back pain		66
Fever		38
Abdominal tenderness		83

From Adams & McLeod.[30]

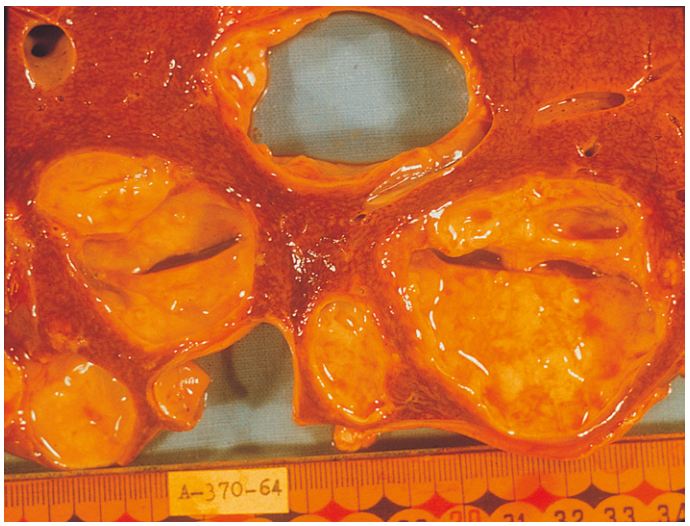


Fig. 110.5 Human amebic liver abscess. Multiple abscesses, one cavitated, can be observed occupying virtually all lobes of the liver parenchyma, which is replaced by a semisolid material. Courtesy of Dr Jesús Aguirre García, Hospital General de México, Secretaría de Salud.

Amebic liver abscess

Liver abscess is the most common extraintestinal form of invasive amebiasis, which may occur in all age groups, but is 10 times more frequent in adults, with three to five times as many cases seen in men.[16] Lesions are usually single and localized to the right lobe of the liver in the posterior, external and superior portions.[28]

Amebae probably spread from the intestine to the liver through the portal circulation. Although liver abscess develops after intestinal infection, patients rarely have associated amebic rectocolitis, but the large intestine is colonized with *E. histolytica* in more than 70% of cases.

In most patients, mainly those under 30 years of age and children,[16] the clinical presentation and course of the disease are typical (Table 110.2). The onset is abrupt, with pain in the upper abdomen and high fever. The pain is intense and constant, radiating to the scapular region and right shoulder; it increases with coughing, deep breathing or when the patient rests on the right side. When the abscess is located in the left lobe, the pain tends to be felt in the epigastrium and may radiate to the left shoulder. Fever is present in most cases; it varies between 100°F (38°C) and 104°F (40°C), frequently in spikes, but is sometimes constant over several days, with rigors and profuse sweating. There is anorexia and rapid weight loss and approximately one-third of patients have a nonproductive cough. Nausea and vomiting may occur and in some cases there may be diarrhea or dysentery. Physical examination reveals a pale wasted patient who has an enlarged tender liver. Digital pressure in the right lower intercostal spaces produces intense pain and there is often marked tenderness on percussion over the right lower ribs in the posterior region. Movement of the right side of the chest and diaphragm is greatly restricted, as is the intensity of respiratory sounds. Older patients may present with a chronic and milder nonspecific febrile illness, hepatomegaly, anemia and abnormal liver function tests.[16]

Table 110.2 Clinical features of amebic liver abscess

Clinical feature		Percentage of cases
Symptoms for	<2 weeks	37–66
	2–4 weeks	20–40
	4–12 weeks	16–42
	>12 weeks	5–11
Fever		71–98
Abdominal pain		62–98
Diarrhea/dysentery		14–66
Cough		10–32
Weight loss		33–53
Tender liver		80–95
Hepatomegaly		43–93
Epigastric tenderness		22
Rales, rhonchi		8–47
Jaundice		10–25
White cell count >10 000/mm^3 (10 × 10^9/1)		63–94
Elevated transaminases		26–50
Elevated alkaline phosphatase		38–84
Elevated bilirubin		10–25
Increased erythrocyte sedimentation rate		81

Based on Martínez-Palomo & Ruíz-Palacios G.[28]

Complications

Amebic liver abscesses commonly produce thoracic complications, particularly pleurisy with a nonpurulent pleural effusion, rupture into the bronchial tree and, less commonly, rupture into the pleural cavity or amebic pericarditis. Rupture into the abdomen occurs in approximately 8% of patients who have amebic liver abscess; only rarely do abscesses rupture into the gallbladder, stomach, duodenum, colon or inferior vena cava. Occasionally, an abscess may erode through the abdominal wall and reach the skin. Secondary infection of amebic liver abscesses is an uncommon complication, which can be suspected when the patient presents with a severe toxic state and there is lack of response to antiamebic chemotherapy.[28] Although acute appendicitis is a common cause for emergency surgery, amebic appendicitis, even in Mexico City where amebic colitis is endemic, is a rare ocurrence (see website for further information).

MANAGEMENT

Because they are efficiently absorbed from the small intestine and reach high concentrations in the tissues, metronidazole and related 5-nitroimidazole compounds are the drugs of choice for treatment of invasive amebiasis. They are termed 'tissue amebicides'. Concentrations in the large intestinal content do not reach amebicidal levels, and only those trophozoites invading the mucosa and deeper tissues are affected. Since they do not prevent the production of infective cysts in the lumen, the tissue amebicides are often reported to be 'inactive against the cysts'. Actually, none of the available amebicides is active against the cysts themselves. The 5-nitroimidazoles have few negative features: adverse interactions are seen with alcohol (also warfarin and phenytoin) and they are known to be mutagenic against bacteria in the Ames test. Although cancer has been reported in mouse testing, no carcinogenic effects have been reported in humans. Although their effect on fetal development is unknown, it is recommended that, because of their ability to cross the placental barrier and rapidly enter the fetal circulation, they should be avoided in the first trimester and prescribed under strict supervision during the second and third trimesters. Similarly, because of secretion in breast milk, breast-feeding should be suspended if 5-nitroimidazoles are prescribed.

The tissue amebicides emetine hydrochloride and dehydroemetine (cardiovascular and gastrointestinal adverse effects), combined with chloroquine, are seldom used now except in resistance. Despite some isolated reports of failure in the treatment of amebic liver abscess with metronidazole, and even though *in-vitro* studies confirm that some established and long-cultivated isolates of the organism are indeed metronidazole resistant,[32] clinical metronidazole resistance is vanishingly rare.

Use of luminal amebicides after treatment with tissue amebicides for elimination of intestinal infection with *E. histolytica*

Since 5-nitroimidazole tissue amebicides are ineffective against the trophozoites in the colonic lumen, complete treatment for both intestinal and extraintestinal infection should be followed by a course of a luminal amebicide. Two alternatives are available[29] (see above): diloxanide furoate (if available) in adults, 500 mg orally q8h for 10 days, or paromomycin 25–35 mg/kg orally q8h for 7–10 days. Both these agents are poorly absorbed from the intestine and so reach optimal amebicidal concentrations within the intestinal lumen, without troublesome side-effects. Paromomycin is the usual choice, as the availability of diloxanide is patchy. These have also been recommended for the treatment of asymptomatic infections in food handlers and when infection control measures are being

applied within families and close associates of infected persons. The use of these agents in asymptomatic persons is controversial in endemic areas, considering the high risk of re-infection. However, the approach, when operationally possible, appears to be ethical in view of the safety of these drugs.

Fulminant amebic colitis

Guarner[31] reports on a series of 47 patients with known or suspected perforation who had surgical treatment. Of 20 who received conservative surgery (i.e. without removal of part of the colon), 16 (80%) died (7 had colostomy, 6 ileostomy, 1 exteriorization of the affected segments, 5 suture of a single perforation and 1 surgical drainage). Where the treatment included resection (i.e. removal of a damaged part or whole of the colon), mortality was 56%. He stresses the importance of making an adequate evaluation of the magnitude of the colonic lesions, as well as the possibility of liver lesions: 'Surgery should be immediate and radical.' When the right colon is involved, right colectomy is indicated; when both sides are involved, total colectomy is indicated. In a total colectomy, ileostomy and suprapubic exteriorization of the rectal segment ought to be performed. Primary anastomosis is not advised; it is better to exteriorize. The patients should always be nursed in intensive care to allow maintenance of vascular tone and osmotic pressure, and to avoid respiratory problems. Antibacterial and antiamebic therapy should be instituted at an early preoperative stage, using a broad-spectrum antibiotic and 500 mg metronidazole intravenously q8h with intravenous fluid replacement. Guarner supports the use of high-dose corticosteroids if the patient enters a state of shock.[31]

Further advice on amebic peritonitis supporting the above surgical approach is given by Cook[33] who refers to case reviews by Shukla *et al*.[34] Gastric suction is recommended. Overall mortality may be over 50%, but resection of the necrotic area with exteriorization of both cut ends of the bowel allowed survival in six of nine cases.

Ameboma

This amebic granuloma often localizes in the ascending colon. Guarner[31] recommends the physician should make a precise clinical diagnosis and take a cautious attitude to surgical intervention in ameboma. He reports a series of 71 cases, 42 treated medically and 29 surgically. In the surgical group, 16 had exploratory laparotomy, 8 required hemicolectomy, and in 5 drainage of a concurrent amebic liver abscess was carried out. All of those medically treated were cured by chemotherapy, whereas there was 17% mortality in the surgical group. (Among these may, of course, have been the worst cases.)

Amebic liver abscess

Amebic liver abscess should be treated with chemotherapy; surgery is rarely indicated. It is not recommended to remove the 'pus' from an amebic liver abscess (as is often seen in pyogenic abscesses). Only 2% of amebic liver abscesses are reported to be contaminated with bacteria, and surgical intervention itself can be responsible. A marked reduction in amebic liver abscess mortality followed introduction of metronidazole and reduction in surgical intervention.[31] Aspiration of the abscess might be necessary in some cases where the abscess is more than 10 cm in diameter and also when in the left liver lobe. Percutaneous drainage is usually sufficient and safer than the open surgical approach, which may be needed if the abscess is ruptured.[35] Surgery should be reserved for patients with a ruptured abscess, with bacterial superinfection or with abscesses that cannot be reached safely by the percutaneous route.

Since those who develop amebic liver abscess are usually carrying an asymptomatic intestinal infection of *E. histolytica*, the importance of elimination of intestinal carriage using luminal amebicides in addition to tissue amebicides has been emphasized, to prevent recurrence of the abscess, which occasionally does happen, and to protect the close associates of the patient from infection.

A prompt diagnosis and adequate chemotherapy will control most cases of liver abscess produced by *E. histolytica*. In general, a full clinical recovery and disappearance of the liver lesions (as confirmed by CT scanning) can be expected for uncomplicated cases. In 85% of cases liver imaging reveals resolution of amebic abscesses within 6 months of treatment; the remaining 15% of cases still show imaging defects 3 years after treatment.[28]

Percutaneous drainage

Indications for percutaneous drainage[31] of an amebic liver abscess are:

- imminent rupture of a large abscess;
- as a complementary therapy to shorten the course of the disease when the response to chemotherapy has been slow; and
- when pyogenic or mixed infection is suspected (e.g. persisting fever on treatment).

Drainage should be carried out under ultrasound or CT guidance. Catheters should *not* be left in for drainage and should be rapidly removed to avoid contaminating the track and skin.

Surgical drainage

Indications for surgical drainage[31] include:

- imminent rupture of an inaccessible liver abscess, especially of the left lobe;
- a risk of peritoneal leakage of necrotic fluid after aspiration;
- rupture of a liver abscess.

When surgical drainage is indicated in abscess of the left lobe, with the patient lying supine, a 5–10 cm incision on the midline will isolate the drainage point.

The study of Guarner[31] should be consulted in case of pleuropulmonary complications or amebic pericarditis. Similarly, for advice on extremely rare cases of hematogenously spread cerebral amebiasis, see also Guarner.[31]

REFERENCES

References for this chapter can be found online at http://www.expertconsult.com

Practice point | 57 |

Sherwood L Gorbach

Diarrhea in a returned traveler from Mexico

DEFINITION OF THE PROBLEM

A 21-year-old woman returned from Mexico with diarrhea which had started 10 weeks previously while she was attending a summer course in Cuernavaca. The initial episode started abruptly with 5–10 bowel movements (BM) per day, accompanied by intestinal cramps, fever of 39°C and mild headache. The feces were watery at first, and then turned mushy. No blood was observed. She consulted a local physician who prescribed ciprofloxacin 500 mg twice daily for 3 days, which she took faithfully. The diarrhea improved, but remained at 3–5 BMs per day with mild cramps until she returned to the USA 2 weeks later, and then persisted for an additional 8 weeks, at which time she consulted another physician.

Her history revealed that she had been in good health without any bowel disorders. While in Mexico she ate at local restaurants for most lunches and dinners but carefully avoided fresh fruits and vegetables and drank only bottled water. Her only medication was a birth-control pill. Physical examination by her physician was unremarkable except for some mild periumbilical tenderness. Rectal examination and later colonoscopy were normal. Her laboratory values, including complete blood count and differential, liver function tests and hepatitis screens, were all negative. Stool examination on three occasions for bacterial pathogens (including *Clostridium difficile*) and for parasites were all negative.

She was treated with azithromycin, followed by metronidazole, without any change in her symptoms. She did gain some relief with loperamide. She was seen by a gastroenterologist at 3 months and then 6 months later. Many gastrointestinal studies, including small bowel radiographs and CT scan of the abdomen as well as repeats of the earlier studies were all negative. She continues to have 3–5 BMs per day without blood, mild abdominal pain, occasional constipation, but mostly mushy stools. Her weight has remained stable at 60 kg.

EPIDEMIOLOGY

Persistent diarrhea is a common occurrence in travelers returning from a journey to a high-risk area, such as Mexico, where up to 40–50% of visitors from North American are stricken with acute travelers' diarrhea.[1] When strict Rome criteria[2] are applied to make a diagnosis of what is now recognized as irritable bowel syndrome (IBS), a recent meta-analysis of eight studies found an incidence of 9.8% in persons with a history of acute infectious gastroenteritis compared to 1.2% in controls.[3] In two prospective studies specifically done in returning travelers,[4] the incidence of IBS was 10%[5] and 14%.[6] The diarrhea symptoms tend to predominate in the returning traveler with only occasional intervals of constipation (alternating cycles of diarrhea/constipation are more frequent in noninfectious IBS). Care in eating and drinking should be followed by travelers to high-risk areas, as practiced by our patient, since it may prevent serious illness; however, such virtue is not rewarded by avoidance of diarrhea, as has been shown in several studies.[1] She had a particularly severe and prolonged course of travelers' diarrhea in Mexico and this is known to increase the risk of IBS.[4]

IBS should be a diagnosis of exclusion in a returning traveler since it is based on clinical criteria without the support of any specific laboratory or imaging tests and many other causes must be considered (Table PP57.1). An alternative diagnosis in the returning traveler with diarrhea is a persistent intestinal pathogen, either bacterial or parasitic. (Viral pathogens do not tend to persist, except cytomegalovirus, which is rare in a normal host; fungal pathogens are also unlikely in a previously healthy person.) Among the bacteria, *Campylobacter*, *Salmonella* and *Shigella* can cause symptoms and continuing pathology for weeks and months until they are treated with an appropriate antibiotic. *Giardia* is arguably the most common cause of persistent diarrhea in a traveler.[7]

INVESTIGATIONS AND MANAGEMENT

Our patient underwent several stool examinations in expert laboratories and was also treated expectantly with metronidazole. *Entamoeba histolytica* is frequently diagnosed clinically but rarely present by

Table PP57.1 Causes of persistent diarrhea in a returning traveler

- Bacterial infection, which may be untreated or associated with an antibiotic-resistant strain, most commonly *Campylobacter*, *Shigella* or *Salmonella*
- Parasitic infection, especially *Giardia lamblia*, followed by *Cryptosporidium parvum, Cyclospora cayetanensis or Entamoeba histolytica*
- Acquired disaccharidase deficiency, especially lactase deficiency
- Postinfectious irritable bowel syndrome
- Inflammatory bowel disease, new onset of Crohn's disease or ulcerative colitis
- *Clostridium difficile* diarrhea or colitis
- Medication-associated diarrhea (e.g. magnesium, sorbitol, various antibiotics)
- Tropical sprue (also known as tropical malabsorption)
- Bowel carcinoma

laboratory testing – amebiasis has been called by Elsdon Dew, 'the refuge of the diagnostically destitute'. *Cryptosporidium* and *Cyclospora* were also excluded by stool examination. Acquired lactase deficiency is surprisingly common in returning travelers and it can be a permanent condition. It can be suspected in the first instance by relief upon avoidance of dairy products, and proven by a hydrogen breath test. Many patients with Crohn's disease or ulcerative colitis date the onset of their symptoms to an episode of acute gastroenteritis and these conditions must be ruled out by endoscopy and imaging studies.

Diarrhea and colitis caused by *C. difficile* have been reported in returning travelers who have been treated with antibiotics such as a fluoroquinolone. In one report, the symptoms persisted in five of six patients for 25–62 days before gaining relief.[8] Other drugs, herbs and micronutrients (e.g. magnesium) have been implicated in persistent diarrhea. Tropical sprue (also known as tropical malabsorption) has been diagnosed in returning travelers, usually those on long journeys or expatriates. Weight loss is a common finding and the condition is associated with laboratory studies showing malabsorption of fat, carbohydrates and/or vitamin B12. A final consideration is an occult bowel cancer.

In our patient the other diagnoses were excluded and the clinical picture fitted the persistent diarrhea form of IBS. Continued symptoms for more than 6 months and even for years has been reported in other forms of postinfectious IBS,[9] including IBS following *Salmonella* gastroenteritis.[10] While some relief is gained with loperamide and similar drugs, the long-term prognosis for persistence of grinding diarrhea remains grim.

REFERENCES

References for this chapter can be found online at http://www.expertconsult.com

Practice point | 58 |

Carmel M Curtis
Peter L Chiodini

Amebic cysts in the stool

INTRODUCTION

Amebiasis is considered by the World Health Organization as one of the top three causes of mortality from parasitic disease with estimates of 40 000–100 000 deaths per year. Approximately 10% of the world's population is thought to be infected and although occasional cases in animals have been recorded, humans are thought to be the definitive host. The causative agent is the protozoan parasite *Entamoeba histolytica* and it presents as either amebic colitis or amebic abscess. It is most commonly spread via fecal–oral means through the contamination of food and water sources with infectious cysts from human feces. Less developed regions with poor sanitation and hygiene have a high prevalence of amebic illness. Person-to-person spread within families and amongst other household contacts is also well documented. Transmission between men who have sex with men (MSM) is increasingly recognized, but is still a minor route of infection worldwide. This condition should also not be forgotten in the returning traveler who has visited an endemic area.

Other amebae present in the stool that are considered nonpathogenic include *Entamoeba dispar*, *Entamoeba coli*, *Entamoeba hartmanni*, *Iodamoeba butschlii* and *Endolimax nana*. It is now commonly recognized that the majority of people with detectable *Entamoeba* are colonized with *E. dispar* which is nonpathogenic and nondisease causing, even in the most immunocompromised patients with AIDS.[1] These organisms in the stool are, however, a marker that the patient has come into contact with water or food contaminated with human feces.

PATHOGENESIS

Entamoeba histolytica exists in two stages within the human host, the ameboid trophozoite and the infectious cyst. The conditions that cause the transition from one form to the other are poorly understood. Once the cysts are ingested by the host they are protected from gastric acid degradation by the cyst wall. They then pass through the small intestine and excyst in the colon into the trophozoite stage where they multiply by binary fission. Trophozoites are motile, engulfing bacteria and food particles, and can potentially invade the host gut mucosal wall using a combination of proteolytic, phagocytic and cytolytic properties, disrupting tight junctions causing changes in permeability, and ultimately resulting in host cell apoptosis.[2] The trophozoites can exit via the stool and although they can be seen occasionally, they do not survive outside the body. More typically they encyst within the colon and are excreted in large numbers in host feces.

CLINICAL FEATURES

Many *E. histolytica* infections are asymptomatic or self-limiting. The incubation period is thought to be a minimum of 7–10 days after cyst ingestion, although it can extend longer than that. The acute intestinal form of infection results in a diarrheal illness (often but not always with blood and mucus) and an acute proctocolitis, with inflamed, edematous mucosa and numerous discrete flask-shaped ulcers which develop as the disease progresses. These are often separated by normal tissue and the findings are not grossly dissimilar to the appearance of inflammatory bowel disease. Typically there is also associated abdominal tenderness and colicky pain. If the infection is prolonged and severe, peritonitis, intussusception and hemorrhage may occur. Fever and an associated leukocytosis are more commonly seen in a more fulminant course of the infection, which, although rare, is sometimes associated with severe malnutrition and in those who are immunocompromised or on steroid treatment. It manifests as areas of confluent ulceration and colonic necrosis and has a significant associated mortality. Chronic intestinal sequelae include colonic stricture and post-amebic colitis.

Amebic infection can also become invasive either through direct invasion through the gut wall or via hematogenous spread. Breaches in the gut mucosal surface and spread through the portal circulation can result in the formation of a liver abscess. They commonly present with fever, right upper quadrant pain, rarely with jaundice and if left untreated can discharge through the skin, rupture into the peritoneum or extend through the diaphragm. Approximately 20% of cases have a history of dysentery and 10% have a history of diarrhea at presentation. Abscesses have also been identified in both the lung and brain, although these are much rarer.

DIAGNOSIS OF CYST TYPE

The diagnosis of amebic dysentery and amebic liver abscess is discussed in Chapter 110. This practice point deals only with the detection of amebic cysts (Fig. PP58.1). Light microscopy of fecal specimens remains the traditional mainstay of amebic cyst diagnosis, but has the limitation of being unable to differentiate between *E. histolytica* and the nonpathogenic *E. dispar*, the cysts of which are morphologically identical. The importance of identifying pathogenic species is clear as there is both a requirement to minimize unnecessary treatment and a need to delineate the epidemiology. The cysts of nonpathogenic amebae other than *E. dispar* can be distinguished by their size and morphology.

E. histolytica/*E. dispar* cysts are typically round, approximately 10–15 μm in diameter and have four nuclei, each with a central karyosome, when mature (Fig. PP58.2). A trichrome stain is required to

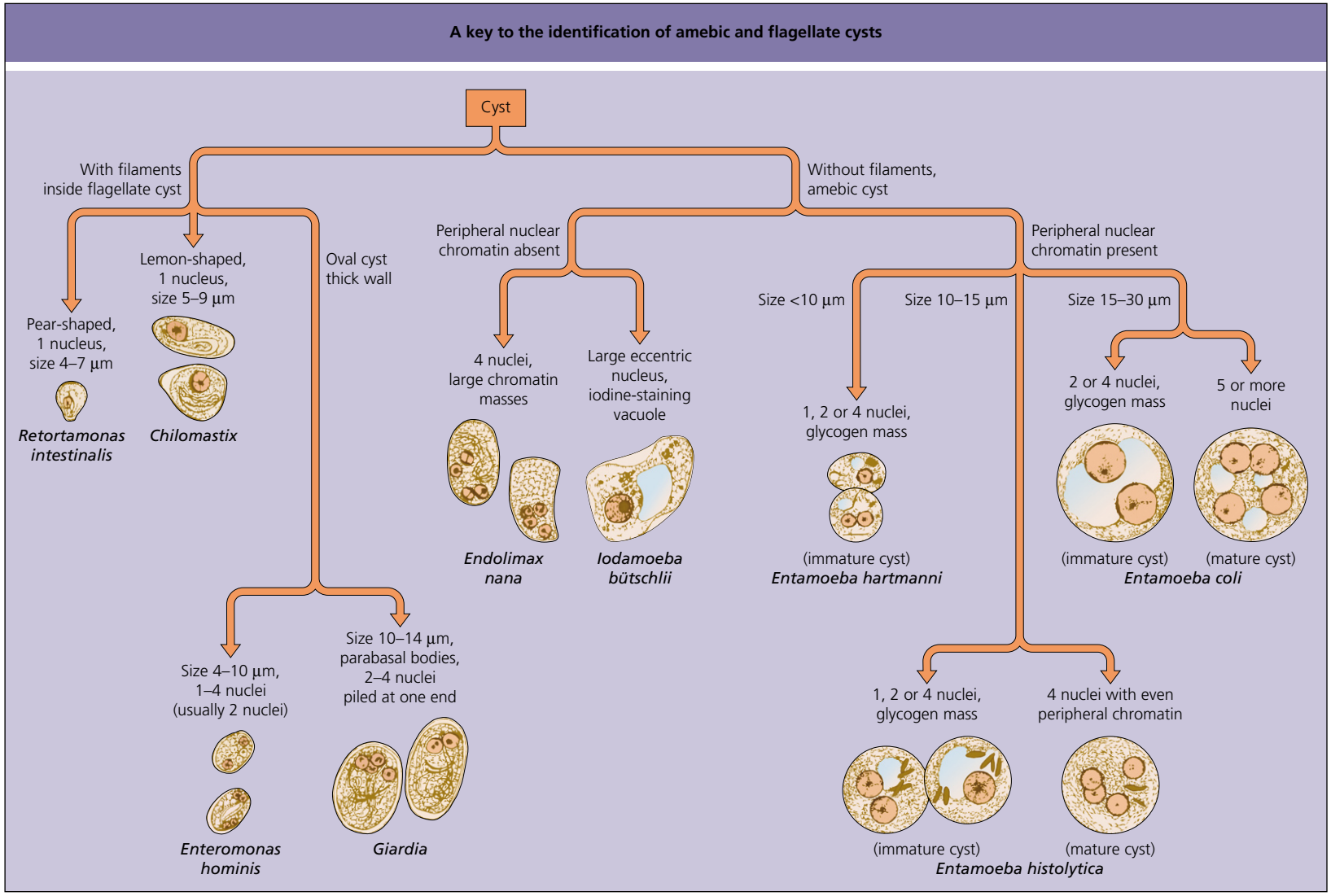


Fig. PP58.1 A key to the identification of amebic and flagellate cysts. (Courtesy of WHO – http://whqlibdoc.who.int/publications/9241544104_(part 2).pdf.)

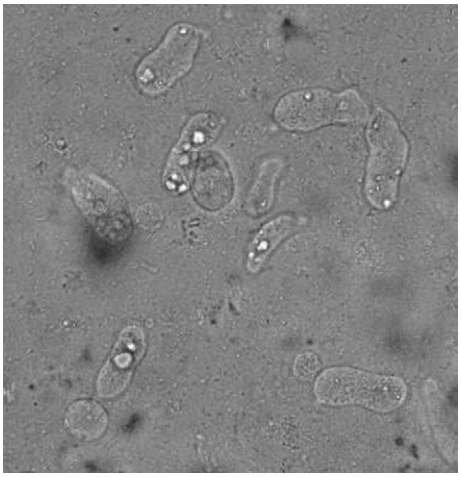


Fig. PP58.2 *Entamoeba histolytica* trophozoites in culture.

demonstrate detailed nuclear morphology which shows fine peripheral chromatin and a central karyosome.

Many diagnostic laboratories now use a commercially available enzyme-linked immunosorbent assay (ELISA) stool antigen detection method (to detect galactose adhesin) to differentiate *E. histolytica* from *E. dispar*.

More recently, polymerase chain reaction (PCR) assays have reportedly shown improved sensitivity and specificity over other methods of cyst detection,[3] although the data are somewhat conflicting between different centers.[4] Currently many of these molecular assays are only performed in reference laboratories.

MANAGEMENT

The presence of *E. histolytica* cysts in the stool does not necessarily indicate the presence of amebic dysentery, for which the presence of trophozoites is required (see Chapter 110). Furthermore, the absence of intestinal cysts does not exclude a diagnosis of amebic dysentery or amebic liver abscess. *Entamoeba histolytica* is detected in the stools of patients with amebic liver abscess less than 50% of the time using light microscopy, although in studies this rises to 75% if the stool is cultured.[5]

Essential points

- *Entamoeba histolytica* is the most significant amebic cyst in the stool
- Microscopy does not differentiate between *E. histolytica* and *E. dispar*
- ELISA stool antigen detection tests or PCR are needed to separate *E. histolytica* from *E. dispar*
- *E. dispar* cysts do not need treatment
- Metronidazole or tinidazole (used to treat amebic dysentery and abscess) do not kill luminal forms of *E. histolytica* reliably
- The agent of choice for *E. histolytica* cyst passage is diloxanide furoate. Alternatives are paromomycin or nitazoxanide

Asymptomatic cyst passers of *E. histolytica* can be treated with diloxanide furoate 500 mg q8h for 10 days for adults and 20 mg/kg for children in three divided doses. An alternative is paromomycin 25–30 mg/kg/day in three divided doses for 7 days. A new agent available for luminal use is nitazoxanide 500 mg q12h for 3 days.[6] The decision whether or not to treat asymptomatic carriage with *E. histolytica*/*E. dispar* partly depends on whether or not the person lives in an endemic area and is likely to become re-infected fairly quickly. Consideration also needs to be given to the cost which may outweigh any benefit to the individual, given that many cases of *E. histolytica*/*E. dispar* infection are in fact *E. dispar* which would lead to unnecessary drug administration. However, elimination of cyst passage is clearly indicated following treatment for amebic dysentery or amebic liver abscess where failure to eradicate the intestinal phase of the infection can lead to relapse.

Treatment of amebic dysentery and amebic abscess are discussed in Chapter 110.

REFERENCES

References for this chapter can be found online at http://www.expertconsult.com

Chapter 111

Arjen Dondorp
Lorenz von Seidlein

Malaria

INTRODUCTION

Malaria is a parasitic disease caused by the coccidian protozoa of the genus *Plasmodium*, and transmitted by *Anopheles* spp. Human malaria can be caused by *Plasmodium falciparum*, *P. ovale*, *P. vivax* and *P. malariae* (Fig. 111.1), but clusters of malaria caused by *P. knowlesi* jumping species from long-tailed macaque monkeys to men have been described in South East Asia.[1] The life cycle of the parasite is summarized in Figure 111.2. Although it is increasingly recognized that *P. vivax* is able to cause severe disease in humans, including severe anemia, pulmonary edema, hemoglobinuria and rarely coma,[2] the vast majority of severe disease is caused by *P. falciparum*, resulting in 1–2 million deaths per annum. The majority (>90%) of these casualties are children in sub-Saharan Africa, where transmission intensity is high. The total burden of malaria disease, however, is similar in Asia, where transmission is low, but the population size much larger.

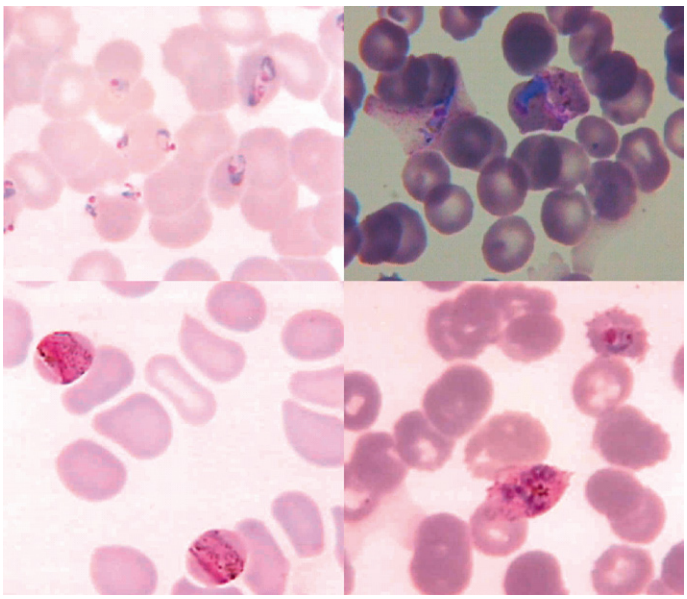


Fig. 111.1 Asexual stages of (clockwise, starting left upper corner) *Plasmodium falciparum*, *P. vivax*, *P. ovale* and *P. malariae*. Courtesy of Dr Kesinee Chotivanich.

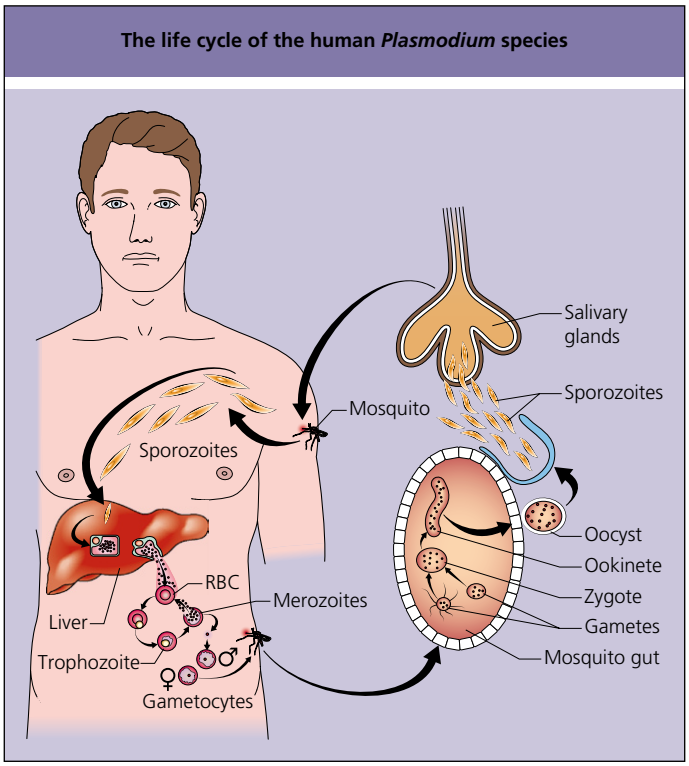


Fig. 111.2 The life cycle of the human *Plasmodium* species. Adapted from White NJ. Antimalarial drug resistance. J Clin Invest 2004;113:1084–92.

EPIDEMIOLOGY

Geographic distribution

Malaria control efforts during the last century eliminated malaria from North America, Europe and Russia, but it has remained a major problem throughout the tropics. At the end of the 20th century, with increasing problems of antimalarial drug and insecticide resistance, childhood mortality caused by malaria was on the increase. However, at the beginning of this millennium a surge in efforts towards malaria control, including the wide distribution of impregnated bed nets and

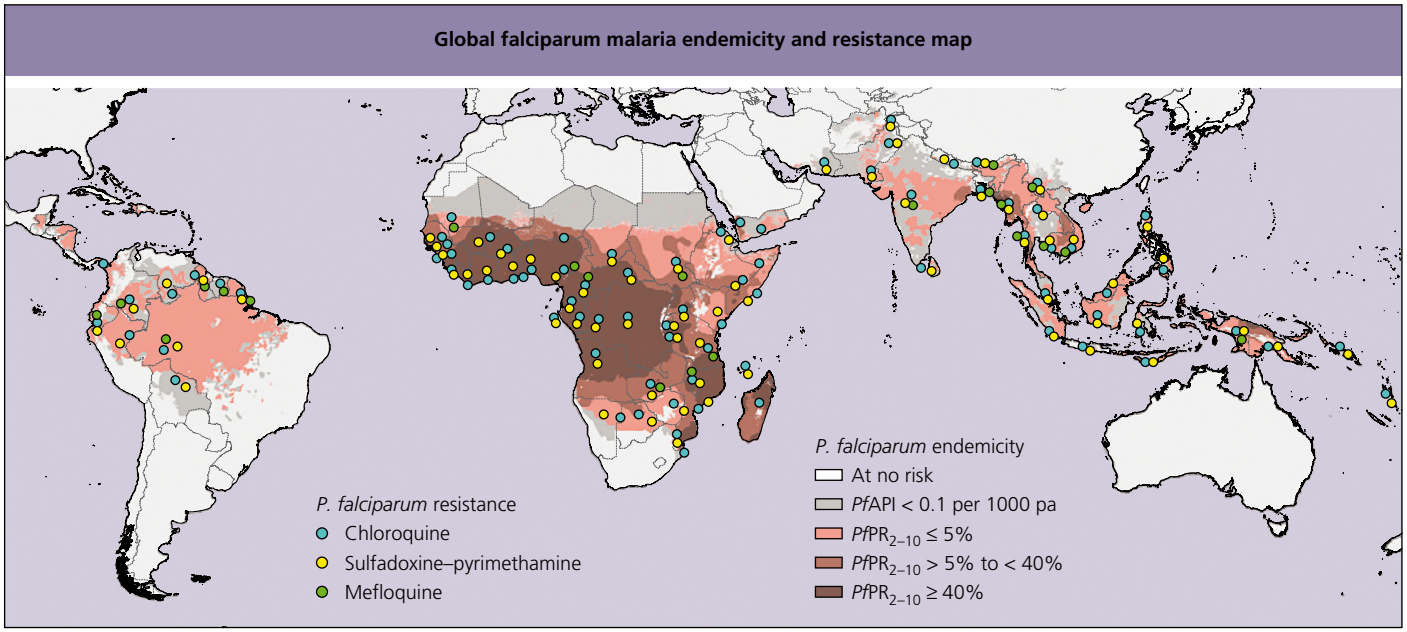


Fig. 111.3 Global falciparum malaria endemicity and resistance map. Endemicity data were compiled using both prevalence data and climatologic information. The spatial distribution of *P. falciparum* malaria prevalence rates, age standardized to 2–10 years ($PfPR_{2-10}$) are categorized as low risk $PfPR_{2-10}$ ≤5%, light red; intermediate risk $PfPR_{2-10}$ >5% to <40%, medium red; and high risk $PfPR_{2-10}$ ≥40%, dark red. The rest of the land area was defined as unstable risk (medium gray areas, where *Pf*API <0.1 per 1000 pa) or no risk (light gray). From Hay *et al*.[4] Data on resistance are from the WHO.[5] Mefloquine resistance data outside South East Asia are currently under review.

the deployment of effective treatment with artemisinin-based combination therapy, has started to reduce malaria-attributable mortality in several African and Asian countries.[3]

Measuring the malaria burden of disease is difficult. Modern epidemiologic methods estimate malaria incidence and its distribution from a combination of case-incidence data and parasite prevalence, medical intelligence and the use of plausible biologic constraints for malaria transmission by the *Anopheles* vector based on temperature data and information on vegetation and population[4] (Fig. 111.3). In 2007, 2.37 billion people lived in areas at risk for *P. falciparum* transmission. In large parts of sub-Saharan Africa transmission is moderate to high, with *P. falciparum* malaria prevalence rates above 50%, and the number of infectious mosquito bites (expressed as the entomologic inoculation rate or EIR) ranging between 10 and 1000 per year. Outside Africa, *P. falciparum* malaria prevalence is largely hypoendemic, with less than 10% parasite rates, corresponding to an EIR of <1 per year.

In Asia *P. vivax* malaria is responsible for approximately 50% of the total malaria prevalence, and is also common in Central and South America, North Africa and the Middle East. Mixed infection with *P. falciparum* is common in South East Asia. *P. vivax* is rare in West Africa, where the majority of people lack the erythrocyte surface Duffy-antigen/chemokine receptor (Duffy blood group negative), which is required for red cell invasion. The prevalence of malaria caused by *P. ovale* (mainly West Africa and Asia) and *P. malariae* is always much lower than the other species. Mixed infection with *P. falciparum* occurs frequently.

With modern air travel, individuals with malaria can be rapidly transported within hours to any part of the world, and malaria is a common imported infection occurring in travelers (import malaria). Occasionally airplanes carry infected mosquitoes, which can give unexpected infections in the neighborhood of airports in nonendemic countries (airport malaria). Malaria can also be transmitted by blood and blood products (transfusion malaria).

Epidemiologic factors

Malaria epidemiology depends upon a complex interplay between the vector (female *Anopheles* mosquitoes), the host (humans) and the malaria parasite. Malaria transmission does not occur at temperatures below 61°F (16°C) or above 91°F (33°C) ambient temperature, and at altitudes above 2000 m of sea level, because sporogony (the development of the sporozoite in the mosquito) cannot take place. Longevity of the vector, which is climate dependent, has to be at least a week, since after the sexual forms of the parasite (gametocytes) are engulfed during a blood meal, sporogony takes 6–12 days (shorter at higher temperatures). Of the over 400 species of *Anopheles*, only 80 of these can transmit malaria, and approximately 45 are considered important vectors. Each vector has its own behavior and blood-feeding patterns, although most bites occur in the evening, at night or in the early morning. For example *Anopheles gambiae*, an important malaria vector in Africa, feeds mainly on humans (anthropophilic), bites mainly after 23:00 hours and mostly indoors (endophagic) where it also tends to rest (endophilic).

Seasonal rainfall dramatically increases the breeding of mosquitoes. Depending on the *Anopheles* species, larvae spend most of their time at the surface of fresh water and salt water marshes (mal-aria means 'bad air'), mangrove swamps, grassy ditches, rice fields, puddles or other water collections, like discarded car tires. In the human host a subpopulation of gametocytes appears after a series of asexual cycles in *P. falciparum*, but earlier in *P. vivax*, another important factor in malaria transmission. Gametocyte density is higher in patients with high parasitemia, in nonimmune individuals (children) and with the use of an antimalarial drug in the presence of drug resistance.

Certain genetic hemoglobinopathies protect against the development of severe falciparum malaria. This has led to a state of 'balanced polymorphism' in populations living in malaria endemic areas, where the disadvantage of the presence of the hemoglobinopathy, especially in the homozygotes, is balanced by the malaria protective effect, which is mainly evident in the heterozygotes. This has been most convincingly shown for sickle cell disease, thalassemia and Melanesian ovalocytosis.

Where malaria prospers, human societies prosper the least, and there is a striking correlation between malaria and poverty. The effects of malaria are felt on diverse areas including fertility, population growth, savings and investments, worker productivity, medical costs, absenteeism, subtle developmental retardation in children and premature mortality.

PATHOGENESIS AND PATHOLOGY

The parasite

The sporozoite form of the parasite is injected into the host when the female *Anopheles* mosquito is probing for a blood meal. After inoculation the parasite hides and replicates in the liver for 5–7 days in *P. falciparum*, after which between 10^5 and 10^6 merozoites are released into the bloodstream. In malaria caused by *P. vivax* and *P. ovale*, but not *P. falciparum*, some parasites stay behind in the liver; these hypnozoites can cause a relapse of the disease long after treatment of the blood stage infection. The merozoites quickly invade circulating erythrocytes, where the erythrocytic cycle of the parasite begins. The parasite matures from a small ring form to the pigment-containing trophozoite, and develops into the mature schizont after division of the nucleus. In *P. falciparum* after 48 hours the erythrocyte ruptures and 6–36 merozoites are released, which will invade passing erythrocytes (Fig. 111.4). In *P. vivax* and *P. ovale* infections this cycle also takes 48 hours, whereas it takes 72 hours in *P. malariae*. There is then a massive expansion of the infection in the human host, with a multiplication factor of around 8 to 10, but sometimes up to 20, per new generation, as observed in early studies with *P. falciparum* as a treatment for syphilis. About 12–13 days after inoculation the parasite number has increased from about 10 parasites to between 10^8 and 10^{10} parasites, and the patient starts to have fever. In the nonimmune patient the disease can quickly progress into severe disease if the infection is not treated, with an increase in the total number of parasites in the body up to 10^{12} to 10^{13}.[6]

Cytoadherence

Plasmodium falciparum is the human *Plasmodium* species responsible for the vast majority of severe disease and is also the only species that induces cytoadherence to vascular endothelium of erythrocytes containing the mature forms of the parasite. As the parasite matures, parasite proteins are transported and inserted into the erythrocyte membrane. The high molecular transmembrane protein *P. falciparum* erythrocyte membrane protein 1 or *Pf*EMP1 is the most important ligand for cytoadherence.[7] Cytoadhesion begins at approximately 12 hours of parasite development, 50% of the maximum effect is obtained at 14–16 hours, and adherence is highly effective in the second half of the parasite life cycle.[8] As a result, late stages of the parasite are only sparsely detected in a peripheral blood slide, and when they do appear in significant numbers (>20% of the total parasites) this is a poor prognostic sign representing a large sequestered parasite load.[9]

*Pf*EMP1 is encoded by the highly variable *VAR* gene family, comprising around 60 genes. The high switch rate between these genes gives rise to a new variant *Pf*EMP1 in 2% of the parasites every new cycle, and this clonal antigenic variation helps the parasite escaping the immune system.[10] *Pf*EMP1 is expressed on the surface of 'knobs', which can be identified by electron microscopy as protrusions from the erythrocyte membrane acting as points of attachment to the vascular endothelium (Fig. 111.5). Other surface proteins that might play a role in cytoadherence are sequestrin, rifin and surfin. On the vascular endothelium numerous receptors that can bind *Pf*EMP1 have been identified, with different distributions in various organs and different contributions to rolling, tethering and finally stable binding of the parasitized erythrocyte. Of these, only CD36, which is constitutionally expressed on most vascular beds but remarkably absent in brain vessels, and chondroitin sulfate A (CSA), the main receptor in the placenta, are able to support firm adhesion under flow conditions.[11] The intercellular adhesion molecule 1 (ICAM-1) is the most important receptor on brain endothelium, and its expression is upregulated by the proinflammatory cytokine tumor necrosis factor (TNF).

Recently it has been suggested that platelets, which express CD36, might serve as a sticky bridge between infected erythrocytes and the endothelium, which could be particularly important in the brain microvasculature lacking CD36.[12] (11). Sequestration of parasitized erythrocytes in capillaries and post-capillary venules causes heterogenic blockage of the microcirculation, which can be demonstrated in living patients by video microscopy[13] and is the cause of retinal bleaching on ophthalmoscopy.[14] Sequestration is not distributed equally throughout the body and is greatest in the brain; however, it is also prominent in the heart, eyes, liver, kidneys, intestines and adipose tissue.[15] Autopsy studies have found that patients dying from cerebral malaria have more prominent sequestration in the brain microvasculature compared to severe but noncomatose fatal cases.[16,17] Autopsy studies

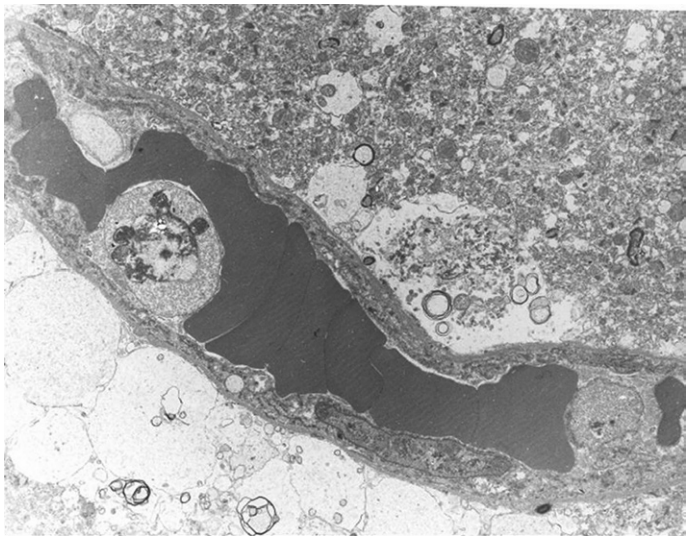


Fig. 111.5 Microcirculatory obstruction in falciparum shown in a post-mortem needle specimen from a fatal case of adult severe malaria. The capillary lumen is reduced by the rigid sequestered erythrocyte containing the mature parasite. Rigid uninfected cells will be hampered in squeezing through the narrowed lumen that remains. Adhesive forces between infected and uninfected red blood cells (rosetting) and between adjacent infected red blood cells (autoagglutination) could further reduce microcirculatory flow. Courtesy of Dr Emsri Pongponrat. Reproduced with permission from Dondorp AM, Pongponrat E, White NJ. Reduced microcirculatory flow in severe falciparum malaria: pathophysiology and electron-microscopic pathology. Acta Trop 2004;89(3):309–17.

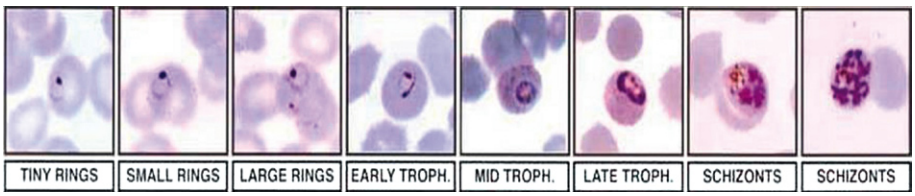


Fig. 111.4 Developmental stages of *Plasmodium falciparum* during the 48-hour asexual life cycle. Mature stages, trophozoites (troph.) and schizonts efficiently adhere to the endothelial lining in the microcirculation, so that these stages are rarely seen in the peripheral blood. If present in the peripheral blood, this denotes a large parasite biomass, associated with severe disease. Courtesy of Dr Kamolrat Silamut.

in children dying from cerebral malaria in Malawi describe, in addition to erythrocyte sequestration, intravascular accumulation of platelets, as well as mononuclear leukocytes and fibrin deposition.[18]

Red cell deformability, rosetting and autoagglutination

Sequestration of parasitized erythrocytes will compromise the microcirculation in vital organs. In addition, the deformability of both parasitized and uninfected erythrocytes is markedly reduced in severe malaria.[19] Acting synergistically with the reduction in lumen caused by sequestration, rigid erythrocytes presumably further reduce blood flow in the microcirculation of vital organs, causing dysoxia with lactic acidosis, organ dysfunction and death. Lactic acidosis is a strong and reproducible predictor for death in falciparum malaria, both in children and adults.[20] In addition, formation of erythrocyte clumps through rosetting and autoagglutination could further compromise flow. Rosette formation is the *in-vitro* phenomenon in which uninfected red blood cells adhere to erythrocytes containing the mature forms of the parasite. However, whereas all erythrocytes containing the mature parasite cytoadhere, not all rosette. Rosette-forming strains are associated with severe disease,[21] although other studies have not found this association[22] and their pathophysiologic role is not clearly established.

Autoagglutination is the aggregation of parasitized red blood cells, which is mediated via platelet CD36. Presence of this phenotype has been associated with disease severity in both Kenya and Thailand.[23] In general, hemodynamic shock is not a contributor to microcirculatory failure until very late in the disease (<10%). Cerebral blood flow in larger vessels is not decreased in cerebral malaria.[24] Axonal accumulation of β-amyloid precursor protein as a measure of impaired axonal transport observed in autopsy studies may represent a final common pathway leading to, in essence, reversible neurologic dysfunction in cerebral malaria. Whether focal dysoxia is the cause of this axonal dysfunction has not been established.

Permeability and intracranial pressure

There is a mild generalized increase in systemic vascular permeability in severe malaria, but the blood brain barrier (BBB) in adults with cerebral malaria is functionally grossly intact.[25] Studies in African children with cerebral malaria do show a subtle increase in BBB permeability with a disruption of endothelial intercellular tight junctions. Imaging studies reveal that most adults with cerebral malaria have no cerebral edema. In African children cerebral edema is a more frequent, although not a consistent finding. Similarly, opening pressures on lumbar puncture are usually normal in adult patients, but are elevated in over 80% of children with cerebral malaria.[26]

There is only one published controlled trial evaluating the use of mannitol in pediatric cerebral malaria, which did not show a beneficial effect.[27] Additionally, raised intracranial pressure in children is more likely a feature developing in the later stages of cerebral malaria, rather than a primary cause for coma.

Immunologic factors and cytokines

Despite the enormous intravascular antigenic load in malaria, with the formation and deposition of immune complexes and variable complement depletion, there is little evidence of a specific immunopathologic process in severe malaria. As in other severe infections, blood concentrations of proinflammatory cytokines like TNF, interleukin (IL)-1, interferon gamma (IFN-γ), IL-6 and IL-18 are raised, as well as anti-inflammatory Th2 cytokines (IL-4, IL-10), but there is an imbalance in patients with a fatal course of the disease.[28]

A potent stimulator inducing proinflammatory cytokine production by leukocytes is the glycosylphosphatidylinositol (GPI) anchor of *P. falciparum*. GPI stimulates the production of TNF and possibly also the lymphokine 'lymphotoxin'. Both cytokines can upregulate the expression of ICAM-1 and vascular cell adhesion molecule 1 (VCAM-1) on endothelium cells, and could thus promote sequestration of parasitized erythrocytes in the brain, contributing to coma. High plasma concentrations of TNF in patients with falciparum malaria correlate with disease severity, including coma, hypoglycemia, hyperparasitemia and death. However, a trial using monoclonal antibodies against TNF did not show a beneficial effect on either mortality or coma duration, but was associated with a significant increase in neurologic sequelae.[29] Moreover, concentrations of TNF are also high in paroxysms of uncomplicated vivax malaria.

Further downstream in the cytokine cascade, nitric oxide (NO) production is increased via inducible NO synthase (iNOS), and iNOS expression is increased in the brain in fatal cerebral malaria. NO has been proposed as a cause for coma through interference with neurotransmission, but more recent studies have shown reduced levels of NO and its precursor, L-arginine, in severe malaria, related to endothelial dysfunction in these patients.[30] With our present knowledge proinflammatory cytokines seem to be related to overall disease severity, and are clearly involved in the pathogenesis of fever and possibly other complications like dyserythropoiesis, adult respiratory distress syndrome (ARDS), renal failure and hypoglycemia, but not in the pathogenesis of coma per se. Other researchers in the field contest this view.[31]

PREVENTION AND MALARIA CONTROL

Malaria prevention and control have three principal components:

- reduction of contact between vector and human host;
- prevention of disease through prophylactic use of antimalarial drugs; and
- adequate treatment (described below) of malaria episodes to minimize the risk for transmission.

Currently no vaccines to protect against malaria are licensed but promising candidates are in development (see below).

Minimizing contact between vector and human host

The affluent traveler has a range of effective options to prevent mosquito bites, which include screened windows, air conditioning, protective clothing and insect repellents (e.g. DEET). Few of these are permanently available to the majority of residents in malaria endemic regions who depend on indoor residual insecticide spraying (IRS) and bed nets impregnated with insecticides (ITNs) to prevent mosquito bites. IRS is highly effective as long as vectors remain susceptible and insecticides are funded and delivered, neither of which has been the case in sub-Saharan Africa. Sophisticated IRS programs rotate insecticides which usually include pyrethroids and dichlorodiphenyltrichloroethane (DDT) to minimize the emergence of resistance. IRS can be highly effective[32] but has not been sustainable and has therefore mixed popularity among malaria control experts. Despite these concerns the current wave to support for malaria control programs has led to a renaissance of IRS programs in sub-Saharan Africa.

A second approach to prevent mosquito bites is the use of insecticide-impregnated bed nets. A series of randomized trials have clearly demonstrated the high effectiveness of ITNs, and wide scale introduction has importantly contributed to a decrease in malaria transmission in many African countries. Bed nets requiring regular dipping in insecticide solutions are now being replaced by 'long-lasting insecticidal nets', which remain effective for extended periods (years). Delivery of these nets through social marketing has been advocated, but increasingly experts think that free distribution of nets is needed to show impact.[33] Efficacy of bed nets will depend on the timing of biting of the predominant Anopheles vector, since ITNs can only protect as long as the individual is covered by the bed net.

Prevention of disease through prophylactic use of antimalarial drugs

Travelers visiting malaria endemic countries are recommended to take prophylactic antimalarial drugs. The choice of drug will depend on pharmacokinetic/dynamic properties, safety profiles and the prevailing drug resistance patterns in the area. Since these patterns change over time, the recommendations in Table 111.1 can only serve as a guideline. For people living in malaria endemic countries, targeted prophylactic use of antimalarial drugs, rather than widespread drug administration, has recently gained popularity as a measure for malaria control in the form of intermittent presumptive treatment (IPT). IPT targets two populations at high risk for malaria attacks: infants (IPTi) and pregnant women (IPTp). The experience of IPT is mostly based on use of sulfadoxine–pyrimethamine (S/P) in sub-Saharan Africa, which is increasingly losing its efficacy on the continent.[34] As widespread use of S/P is likely to accelerate the emergence of S/P resistance, the balance between benefit and risk of adverse effects becomes more important. Trials using drugs other than S/P are currently in progress.[35]

Vaccine development

Despite the accepted need and great promises of a vaccine that can protect against malaria there is still no malaria vaccine licensed. *Plasmodium falciparum* is genetically complex and antigenic surface proteins are highly variable. Yet protective immunity can be obtained with age in populations residing in malaria endemic areas. Immunoglobulin purified from the blood of immune adults from endemic regions can passively transfer protection against *P. falciparum*,[36] but cell-mediated immunity is thought to also play an important role.

The current most promising vaccine candidate is known as RTS,S, a recombinant protein pre-erythrocytic stage vaccine, based on *P. falciparum* circumsporozoite antigen. RTS,S, combined with the ASO1E adjuvant, has an adjusted vaccine efficacy of 53% (95% CI 28–69) against developing clinical malaria in children aged 5–17 months during a mean duration of 8 months follow-up. In addition, early phase trials with vectored vaccines aiming at liver stage parasites are currently underway.[37] Vaccinations with irradiated sporozoites have been shown to be highly protective. Producing irradiated sporozoites in sufficient quantities, which have to be isolated from infected mosquitoes, is a daunting challenge. However, the persistence of the developers and the increased availability of funds for malaria vaccines have resulted in plans for clinical trials with this candidate vaccine in the near future.[38]

A malaria vaccine cannot replace other malaria control efforts since no malaria vaccine candidate will be 100% protective or provide lifelong protection. No malaria vaccine on its own can reduce transmission sufficiently to eliminate malaria. However, the addition of a vaccine to other tools for malaria control has the potential to drive malaria transmission to very low levels, even in high transmission areas.

Table 111.1 Currently available drugs with antimalarial activity

Arylaminoalcohols	
4-aminoquinolines	Chloroquine Amodiaquine *Amopyraquine* *Pyronaridine* *Piperaquine* Quinine Quinidine Mefloquine Halofantrine*
8-aminoquinolines	Lumefantrine (Benflumetol) Primaquine *Tafenoquine* (*Etaquine*)
Dihydrofolate reductase inhibitors	Pyrimethamine Proguanil *Chlorproguanil* Pyrimethamine–sulfadoxine *Chlorproguanil–dapsone*
Artemisinin and derivatives	Artemisinin Artemether Artesunate *Artemether* *Dihydroartemisinin*
Hydroxynaphthaquinones	Atovaquone Atovaquone–proguanil
Antibiotics with antimalarial activity	Sulfonamides Tetracycline–doxycycline† Chloramphenicol Fluoroquinolones (weak) Rifamycins (weak) Macrolides Clindamycin Lincomycin

Drugs in italics are either still investigational or are not widely available.
*Halofantrine is not recommended as first-line treatment for uncomplicated malaria because of cardiotoxicity.
†Doxycycline should not be used in children under 8 years of age.

CLINICAL FEATURES

The clinical manifestations of malaria are critically dependent on the immune status of the host. In areas of stable high transmission (sub-Saharan Africa) severe falciparum malaria occurs predominantly between 6 months and 3 years of age; mild symptoms are seen in older children, and a state of partial immunity (or 'premunition') means that adults are usually asymptomatic. With transmission intensity decreasing (moderate transmission) the period with symptomatic disease shifts to a slightly older age. In low transmission areas, severe and symptomatic disease occurs at all ages, and will affect especially young adults. Pregnant women are at increased risk of developing symptomatic and severe malaria in all transmission settings.

Uncomplicated malaria

In most cases, the incubation period for falciparum and vivax malaria is around 2 weeks, but this can vary widely. The majority (>90%) of *P. falciparum* infections in travelers occur within 8 weeks of leaving an epidemic area. Some *P. vivax* strains (var. *hibernans*) in a few areas of China and North and South Korea have extremely long incubation times (up to 9–12 months).

The clinical features of all four human malarias start nonspecifically and resemble influenza. Headache, muscular ache, vague abdominal discomfort, lethargy and dysphoria often precede the fever. Rising temperatures initially cause shivering, mild chills, worsening headaches, malaise and loss of appetite. If the infection is untreated, the fever in *P. vivax* and *P. ovale* regularizes to a 2-day cycle (tertian malaria; fever on the third day if the starting day is counted as number one) and *P. malaria* fever spikes occur every 3 days (quartan malaria; fever on the fourth day). The fever pattern in *P. falciparum* is generally much more variable, since the infection tends to be less synchronized. Classic 'paroxysms' are therefore more common in tertian and quartan malaria, and consist of an abrupt steeply rising temperature to >102°F (39°C), with intense headache and highly uncomfortable 'cold chills' with peripheral vasoconstriction, and dramatic rigors with shaking limbs and teeth chatter. This is followed by a 'hot stage' during which the patient may have a temperature well over 104°F (40°C), with peripheral vasodilatation, often with restlessness and vomiting. During defervescence, the patient has profuse transpiration and feels exhausted. As the infection continues,

the spleen and liver enlarge and anemia develops. Mild abdominal discomfort is common in malaria. In routine clinical practice in malarious areas malaria is rarely the cause of lymphadenopathy, pharyngitis or a rash.

Severe malaria

The vast majority of severe malaria is caused by falciparum malaria in nonimmune individuals, although severe vivax malaria is relatively common in areas with chloroquine resistance.[2] Severe malaria is a multisystem disease. In a minority of patients cerebral malaria is an isolated presentation and generally combined with other severity signs. In areas of high transmission in sub-Saharan Africa severe malaria is mainly a pediatric disease. Important symptoms in children are severe anemia, hypoglycemia and coma with convulsions. Main prognostic indicators are coma, respiratory distress (which includes a Kussmaul pattern breathing related to metabolic acidosis) and to a lesser extent severe anemia.[39]

In low transmission areas such as South East Asia, young adults are the most affected group. Cerebral malaria, metabolic acidosis, renal failure, severe jaundice and ARDS are the most prominent complications in this group. Coma and acidosis have the strongest prognostic significance, whereas patients developing ARDS or renal failure have a high risk of dying. Definitions of the clinical manifestations of severe falciparum malaria are summarized in Table 111.2.

Cerebral malaria

The clinical picture is that of a diffuse encephalopathy with unrousable coma; focal signs are relatively uncommon. In young children coma can develop rapidly, with a mean onset after only 2 days of fever. One or more generalized seizures, which cannot be distinguished clinically from febrile convulsions, often precede the coma. In adults the onset in usually more gradual, with high fever (mean duration of 5 days) and increasing drowsiness, but sometimes agitation. Convulsions are present in about 15% of the cases, whereas more than 50% of pediatric cases have convulsions. Convulsions are most frequently generalized, but in small children approximately 25% have subtle or subclinical convulsions, with seizure activity on electroencephalography, but only minor convulsive movements of limbs or facial muscles. These patients often have deviated eyes, excessive salivation and irregular breathing patterns. Signs of meningism are absent, although passive resistance to neck flexion is not uncommon. The eyes often show a divergent gaze. Bruxism with grinding of the teeth and a positive pout reflex are common in cases with deep coma. Various forms of abnormal posturing can be present, with either a decorticate pattern with flexor rigidity of the arms and extension of the legs or a decerebrate pattern with abnormal extensor responses in arms and legs with or without opisthotonos.[40]

In areas of high transmission a high background prevalence of peripheral parasitemia can hamper the diagnosis of 'cerebral malaria'. A positive blood slide in a febrile comatose child in this setting does

Table 111.2 Manifestations and complications of severe falciparum malaria, including current opinions about pathophysiology and treatment

Manifestations and complications	Pathophysiology	Treatment
Coma (Glasgow Coma Score <11; Blantyre Coma Score <3) and convulsions	Sequestration of parasitized red blood cells in the cerebral microcirculation and other factors	Hypoglycemia and other causes of meningo/encephalitis should be excluded Good general intensive nursing care, including close observation of breathing, eye care Give nasogastric tube, Foley catheter If feasible: intubation to protect airway Frequent monitoring of blood glucose Treat convulsions (e.g. diazepam); prophylactic anticonvulsive treatment is not recommended
Anemia (Hct <20%, in presence of parasitemia >100 000/µl)	Loss of parasitized red blood cells, increased splenic clearance of uninfected red blood cells (decreased red cell deformability, immunologic factors?), dyserythropoiesis	General recommendation: transfusion if in distress, or Hct <20% (adults), or Hb <5 g/dl in children
Hyperparasitemia (>10% infected red blood cells)	Host immunologic factors and parasite virulence factors (multiplication rate, red cell selectivity)	Start parenteral antimalarial drugs promptly in effective doses (artemisinins; if quinine: give loading dose) Exchange transfusion (?)
Hypoglycemia (blood glucose <40 mg/dl)	Increased use, decreased production (?) Quinine-related hyperinsulinism	Glucose 10%, 4 mg/kg bodyweight
Acute renal failure (plasma creatinine >3 mg/dl)	Acute tubular necrosis Prerenal component (dehydration)	Record input/output (Foley catheter) Check biochemistry (BUN, electrolytes), start or transfer for renal replacement therapy (hemofiltration or hemodialysis preferred over peritoneal dialysis)
Severe jaundice (bilirubin >3.0 mg/dl, with parasitemia >100 000/µl)	Mainly in adults; multifactorial	No specific treatment Monitor blood glucose
Fluid/electrolyte imbalances, metabolic acidosis (venous plasma bicarbonate <15 mmol/l or lactate >4 mmol/l)	Dehydration, SIADH (?) Only minor increase in capillary permeability, compromised microcirculation by sequestration and other factors causing anaerobic glycolysis	Careful fluid resuscitation Bicarbonate administration only if pH ≤7.15 Dialysis as treatment for severe acidosis has been advocated

Table 111.2 Manifestations and complications of severe falciparum malaria, including current opinions about pathophysiology and treatment—cont'd

Manifestations and complications	Pathophysiology	Treatment
Respiratory distress and pulmonary edema	Acidosis-related deep breathing Pulmonary edema (ARDS) mainly in adults and pregnant women Etiology unknown; cytokine mediated (?)	See also acidosis ARDS: do not overfill, positive pressure mechanical ventilation with PEEP etc. Do not allow 'permissive hypercapnia' (brain swelling) Distinguish from pneumonia
Black water fever	Related to severe malaria, quinine use and G6PD deficiency	Transfusion if needed Bicarbonate administration (?)
Circulatory shock (systolic BP <90 mmHg [80 mmHg in children] with cold extremities)	Rare in malaria (nitric oxide binding by free hemoglobin?) Consider concurrent septicemia	Fluids, inotropic drugs (do not use adrenaline [epinephrine] because of lactic acidosis), antibiotics
Abnormal bleeding	Diffuse intravascular coagulation: consider concomitant sepsis Isolated thrombocytopenia (very common) not enough explanation	No specific treatment Packed red cell transfusion if indicated

Aspects of pathophysiologic mechanisms or treatment that are still controversial are marked with a '?'.
ARDS, adult respiratory distress syndrome; G6PD, glucose-6-phosphate dehydrogenase; Hct, hematocrit; PEEP, positive end-expiratory pressure; SIADH, syndrome of inappropriate antidiuretic hormone secretion.
Adapted from White *et al.*[52]

not always adequately exclude other possible diagnoses, and bacteremia can be present in up to 20% of these patients.[41] The presence of retinal hemorrhages on ophthalmoscopy can sometimes be useful here because of its specificity for malaria, although a majority of patients will lack this finding[18] (Fig. 111.6).

In surviving patients the median time to full recovery of consciousness is approximately 24 hours in children, compared to 48 hours in adults. Neurologic sequelae are rare in adults recovering from cerebral malaria (<1%) (Fig. 111.7), but more frequent in children, with approximately 12% still having symptoms at the time of discharge, including hemiplegia, cortical blindness, aphasia and cerebellar ataxia.[42] These symptoms will completely resolve over a period from 1 to 6 months in over half of the children, but a quarter will be left with major residual neurologic deficits. More subtle cognitive impairments as a late consequence of cerebral malaria are common in children, especially in those cases presenting with a combination of coma, hypoglycemia and seizures.[43]



Fig. 111.6 Retinal hemorrhages are a common finding in patients with severe and cerebral malaria (around 15%). Because of its specificity, it can sometimes be a useful tool to distinguish malaria from other causes of febrile illnesses with coma. Courtesy of Dr Abdullah Abu Sayeed.



Fig. 111.7 Adult severe malaria has a high case fatality rate of around 20% in developing countries. However, surviving patients, even those presenting with a deep coma (left), usually recover without sequelae (right). Neurologic sequelae are more common in pediatric cases.

Acidosis

Metabolic acidosis is a major cause of death in severe malaria.[20] Acidosis results from accumulation of lactic acid and other unidentified organic acids as a result of tissue ischemia, compounded by reduced hepatic clearance. Renal impairment can further aggravate the metabolic acidosis. Lactic acidosis is often associated with hypoglycemia.

Anemia

Anemia is mainly a complication in young children and its pathogenesis is multifactorial. Red cells will be destroyed at the moment of schizont rupture, but the rapid decline in hematocrit is more importantly determined by an accelerated destruction of nonparasitized red

cells. Moreover, reticulocyte counts are low in the acute phase of the disease because of bone marrow dyserythropoiesis. Increased clearance of uninfected erythrocytes is associated with their increased rigidity and possibly with neomembrane antigens resulting from aborted merozoite invasion, which makes them more prone to removal in the sinusoids of the spleen.[44]

Anemia in African children is only partly explained by malaria. In addition, bacteremia, hookworm, HIV infection and deficiency of vitamin A and vitamin B12 are all associated with severe anemia.[45]

Hypoglycemia

Hypoglycemia is most prominent in small children and pregnant women with severe malaria. Demand is increased because of increased anaerobic glycolysis (also causing lactic acidosis), increased metabolic demands of the febrile illness, and the malaria parasites which use glucose as their major fuel. Supply is reduced as a result of reduced oral intake, vomiting, and failure of hepatic gluconeogenesis, although this has been contested. Hepatic glycogen is exhausted rapidly. The net result is hypoglycemia in 20–30% of children with severe malaria. In patients treated with quinine, this is compounded by quinine-stimulated pancreatic β-cell insulin secretion.[46] Untreated hypoglycemia can cause neurologic damage and is associated with residual neurologic deficit in survivors.

Renal impairment

Acute renal failure is a feared complication of adult severe malaria and most frequently results from acute tubular necrosis. Usually the patient is oliguric for a median of 4 days, but nonoliguric renal failure may also occur. Renal microvascular obstruction and cellular injury consequent upon sequestration in the kidney and the filtration of free hemoglobin, myoglobin and other cellular material are contributing factors.[47] Elevated plasma TNF has also been associated with renal impairment. Significant glomerulonephritis is very rare, except in chronic *P. malariae* malaria which can cause an immune complex glomerulonephritis with a nephrotic syndrome.

Respiratory distress

Respiratory distress manifesting as deep breathing or tachypnea is associated with poor outcome in children with severe malaria.[39] There may be intercostal recession, use of the accessory muscles of respiration and flaring of the alae nasi.

Respiratory distress is often present as respiratory compensation for a profound metabolic acidosis, but can also be caused by the presence of severe anemia or a concomitant lung infection. Pulmonary edema is rare in children. Respiratory depression can be caused by an overuse of anticonvulsants, particularly phenobarbital in combination with benzodiazepines. Identification of these different causes of respiratory distress is important as each requires a different treatment modality.

In adults with severe falciparum malaria, but occasionally in severe vivax infections, ARDS with increased pulmonary capillary permeability can develop, which has a high mortality (Fig. 111.8). Pregnant women are especially prone to this complication. Overhydration aggravates the condition, but is not the cause; the pulmonary capillary wedge pressure is usually normal.

Blackwater fever

A minority of patients (6% in adult severe malaria) develop massive intravascular hemolysis and the passage of 'Coca-Cola'-colored urine (Fig. 111.9). The pathogenesis of Blackwater fever could be related to a build-up of oxidative stress in the erythrocyte membrane. Risk factors are glucose-6-phosphate dehydrogenase (G6PD) deficiency and the use of oxidative drugs such as primaquine.[48] Blackwater fever may contribute to the development of acute renal failure, although in the majority of cases renal function remains normal.

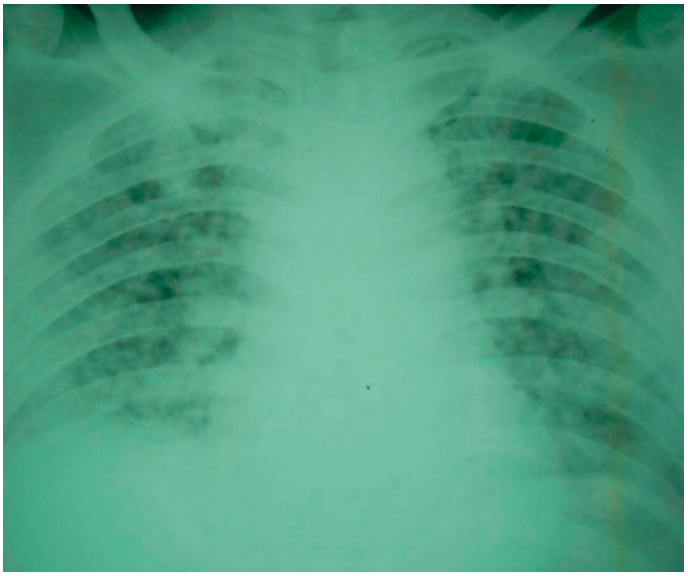


Fig. 111.8 Chest X-ray of an adult patient with adult respiratory distress syndrome (ARDS), a feared complication of severe falciparum malaria in adults.



Fig. 111.9 Blackwater fever in an adult patient with severe malaria. Massive intravascular hemolysis of both infected and uninfected erythrocytes causes 'Coca-Cola'-like discoloring of the urine.

Shock

Shock or 'algid malaria' is relatively rare in severe malaria (<10%). In most cases the blood pressure of patients with malaria is at the lower end of the normal range, probably due to vasodilatation. Marked hypotension in a few cases may be the result of dehydration, but is more commonly due to concomitant sepsis, and possible sources (e.g. respiratory and urinary tract infections) should be investigated.

Bleeding

Bleeding due to the commonly occurring thrombocytopenia in malaria is rare. Bleeding is more likely to occur in the setting of disseminated intravascular coagulation, which should prompt the clinician to consider concomitant sepsis as a cause. More often there is only

subtle activation of the coagulation cascade with a reduction in antithrombin III concentration, an increase in thrombin–antithrombin III complexes and a reduction in factor XII and prekallikrein activities, which do not appear to be clinically significant.[49]

Differential diagnosis

Malaria is a great mimic and must enter the differential diagnosis of a number of clinical presentations.

- In the acute presentation, fever due to malaria needs to be differentiated from typhoid, viral illnesses such as dengue fever and influenza, leptospirosis, brucellosis and respiratory and urinary tract infections. Less common causes of tropical fevers include leishmaniasis, trypanosomiasis, rickettsial infections and relapsing fevers.
- In the case of cerebral malaria, it is of utmost importance to exclude the presence of hypoglycemia as a contributing factor. The principal differential diagnosis in tropical areas is of a bacterial or viral meningoencephalitis. If the patient presents with any sign of meningeal involvement a lumbar puncture should be performed. Especially in small children this implies that in most cases a lumbar puncture will be necessary. Some African centers treating pediatric cerebral malaria postpone lumbar puncture fearing herniation related to raised intracranial pressure which is present in a majority of their cases. These centers start empiric antibiotic coverage in all children until results of lumbar puncture become available. Other differential diagnoses include enteric fevers, trypanosomiasis, brain abscess and other causes of coma.
- The anemia of malaria can be confused with other common causes of hemolytic anemia in the tropics such as that due to the hemoglobinopathies (e.g. sickle cell disease, thalassemia), G6PD deficiency and the South East Asian form of ovalocytosis. The anemia of malaria must be differentiated from that of iron, folate or vitamin B12 deficiency. Hookworm and HIV infection are also associated with severe anemia.
- The renal failure of malaria must be distinguished from renal impairment due to sickle cell disease, leptospirosis, use of traditional herbal medicines and chronic renal disease resulting from glomerulonephritis and hypertension.
- The jaundice and hepatomegaly of malaria must be distinguished from that of viral hepatitis (A, B and E, cytomegalovirus and Epstein–Barr virus infections), leptospirosis, yellow fever, biliary disease and drug-induced disease, including alcohol.

DIAGNOSIS

Because of the high prevalence of the disease in the tropics and the danger of developing severe disease, a traveler with fever who has been in a malarious area within the previous 2 months must be considered to have malaria unless proved otherwise. Similarly, in patients living in endemic countries who present with a fever the suspicion of malaria should be high. On the other hand there tends to be overdiagnosing of malaria in high transmission malarious countries, partly because of the lack of diagnostic facilities. A clinical diagnosis of both uncomplicated and severe malaria is not reliable because of the nonspecific presentation of the disease.

As such, the diagnosis malaria should be confirmed by either a rapid diagnostic test or microscopy of stained thin and thick blood films, at a magnification of 1000. The intraerythrocytic parasites must be identified and counted. In severe malaria, the developmental stage of the parasites and the percentage of neutrophils containing malarial pigment have prognostic significance. A negative blood smear makes the diagnosis very unlikely, unless the patient has received antimalarial treatment before presentation. If there is still uncertainty the slide should be repeated every 12 hours for 48 hours. Microscopy with fluorescent staining of the buffy coat (quantitative buffy coat analysis or QBC) has a higher sensitivity to detect low parasitemias, but this is seldom needed. Dipstick detection of the *P. falciparum* antigens *Pf*HRP2 and *p*LDH (Paracheck, Binax NOW, ParaSight-F, ICT Malaria Pf, OptiMAL) has a diagnostic sensitivity similar to that of microscopy, but does not require an experienced microscopist. Parasitemia and parasite stages cannot be assessed in this way. *Pf*HRP2 remains circulating weeks after cure, which can lead to false-positive results in high transmission settings in patients with a recent malaria attack.

MANAGEMENT

Uncomplicated malaria

Malaria caused by *Plasmodium falciparum*

Today, resistance of *P. falciparum* against chloroquine has reached most parts of the tropics and resistance against the usual next alternative, sulfadoxine–pyrimethamine, is rapidly spreading. To ensure efficacy and to limit the chances of de-novo appearance and spread of antimalarial drug resistance, falciparum malaria should be treated with combinations of two or more blood schizontocidal drugs with independent modes of action. Artemisinin combination therapy (ACT) has become the standard recommendation for treatment of uncomplicated malaria, endorsed by the WHO.[5] The potent antimalarial capacity of the artemisinins quickly reduces the total body parasite number, which will not only relieve symptoms rapidly, but will also reduce the chance of emergence of clones resistant against the partner drug. The partner drug mutually protects the artemisinin component against resistance development.[50]

To ensure treatment courses that do not exceed 3 days, the partner drug to the artemisinin derivative should have a longer half-life in order to kill the remaining parasites (about 10^3 to 10^5 of the initial 10^8 to 10^{13}). Possible partner drugs in ACT regimens include amodiaquine, atovaquone–proguanil, clindamycin, doxycycline, lumefantrine, mefloquine, piperaquine, pyronaridine, chlorproguanil–dapsone, proguanil–dapsone, sulfadoxine–pyrimethamine, and tetracyclines. Of these partner drugs, lumefantrine, mefloquine and piperaquine have proven efficacy even in areas of multidrug-resistant *P. falciparum*, although piperaquine is presently only licensed in Asia. The combination of an artemisinin derivative with amodiaquine or sulfadoxine–pyrimethamine has been shown to be effective only in areas where amodiaquine and sulfadoxine–pyrimethamine monotherapy failure rates do not exceed 20%. Chloroquine cannot be recommended because of widespread high-level resistance. Of these combinations, artemether–lumefantrine, dihydroartemisinin–piperaquine, artesunate–mefloquine and artesunate–amodiaquine are now available as fixed combinations. Artesunate–pyronaridine will be registered in the near future. Wide deployment of fixed combinations will preclude the use of artemisinin monotherapy, which is an important strategy to reduce the spread of drug resistance. In 2008 the first well-documented cases of reduced *in-vivo* sensitivity of *P. falciparum* for artesunate were reported.

The following ACTs are currently recommended for treatment of uncomplicated falciparum malaria:[5]

- artemether + lumefantrine
- artesunate + amodiaquine
- artesunate + mefloquine
- artesunate + sulfadoxine–pyrimethamine.

An effective combination therapy not containing an artemisinin derivative is atovaquone–proguanil (Malarone), which can be used in the treatment of imported uncomplicated falciparum malaria in returning travelers in nonendemic countries.

Tables 111.1 and Tables 111.3–111.6 summarize the different drug combinations and dosing schemes for the treatment of uncomplicated falciparum malaria. Table 111.7 summarizes treatment of uncomplicated falciparum malaria in returning nonimmune adult travelers and Table 111.8 in pregnant women. If the patient is vomiting and not

Table 111.3 Dosing schedule for artemether–lumefantrine (fixed combination)

Body weight in kg (age in years)	NO. OF TABLETS RECOMMENDED AT APPROXIMATE TIMING OF DOSING*					
	0 h	8 h	24 h	36 h	48 h	60 h
5–14 (<3)	1	1	1	1	1	1
15–24 (3–9)	2	2	2	2	2	2
25–34 (9–14)	3	3	3	3	3	3
>34 (>14)	4	4	4	4	4	4

*The regimen can be expressed more simply for ease of use at the program level as follows: the second dose on the first day should be given any time between 8 h and 12 h after the first dose. Dosage on the second and third days is twice a day (morning and evening). The drug should be taken with food or milk.
Adapted from White *et al.*[52]

Table 111.4 Dosing schedule for artesunate plus amodiaquine

Age	DOSE IN NO. OF TABLETS ARTESUNATE (AS)/AMODIAQUINE (AQ) FIXED COMBINATION			
		Day 1	Day 2	Day 3
5–11 months	AS: 25 mg with AQ: 67.5 mg	1	1	1
1–6 years	AS: 25 mg with AQ: 67.5 mg	2	2	2
7–13 years	AS: 100 mg with AQ: 270 mg	1	1	1
>13 years	AS: 100 mg with AQ: 270 mg	2	2	2

Table 111.5 Dosing schedule for artesunate plus mefloquine*

Age	DOSE IN MG (NO. OF TABLETS)					
	ARTESUNATE			MEFLOQUINE (BASE)		
	Day 1	Day 2	Day 3	Day 1	Day 2	Day 3
5–11 months	25 (½)	25	25	–	125 (½)	–
1–6 years	50 (1)	50	50	–	250 (1)	–
7–13 years	100 (2)	100	100	–	500 (2)	250 (1)
>13 years	200 (4)	200	200	–	1000 (4)	500 (2)

*A fixed combination is now also available.
Adapted from White *et al.*[52]

Table 111.6 Dosing schedule for artesunate plus sulfadoxine-pyrimethamine

Age	DOSE IN MG (NO. OF TABLETS)					
	ARTESUNATE			SULFADOXINE–PYRIMETHAMINE		
	Day 1	Day 2	Day 3	Day 1	Day 2	Day 3
5–11 months	25 (½)	25	25	250/12.5 (½)	–	–
1–6 years	50 (1)	50	50	500/25 (1)	–	–
7–13 years	100 (2)	100	100	1000/50 (2)	–	–
>13 years	200 (4)	200	200	1500/75 (3)	–	–

Adapted from White *et al.*[52]

Table 111.7 Recommendations on the treatment of falciparum malaria in nonimmune adult travelers

For travelers returning to nonendemic countries	• Atovaquone–proguanil (1 g/400 mg q24h for 3 days) • Artemether–lumefantrine (1.5 mg/12 mg/kg q12h for 3 days) • Quinine (10 mg/kg q8h) plus doxycycline* (3.5 mg/kg q24h) or clindamycin (10 mg/kg q12h) • All drugs to be given for 7 days
For severe malaria	• The antimalarial treatment of severe malaria in travelers is the same as the general recommendation for severe malaria • Travelers with severe malaria should be managed in an intensive care unit • Hemofiltration or hemodialysis should be started early in acute renal failure • Positive pressure ventilation should be started early if there is any breathing pattern abnormality, intractable seizure or acute respiratory distress syndrome

*Doxycycline should not be used in children under 8 years of age.

Table 111.8 Treatment of uncomplicated falciparum malaria in pregnancy

First trimester	Quinine (10 mg/kg q8h), preferably plus clindamycin (5 mg/kg q8h) for 7 days An artemisinin-based combination therapy (ACT) should be used if it is considered that the benefits of treating outweigh the risks of not treating when the only drug option available is an ACT
Second and third trimesters	ACT known to be effective in the country/region or artesunate (2 mg/kg q24h) plus clindamycin for 7 days, or quinine plus clindamycin for 7 days
Severe malaria	As in non-pregnant individuals (see Table 111.7) Increased risk of hypoglycemia in quinine-treated patients
Vivax malaria	
Chloroquine sensitive	Chloroquine 25 mg base/kg divided over 3 days Primaquine is contraindicated during pregnancy Chloroquine prophylaxis (300 mg base once per week) should be continued until delivery
Chloroquine resistance	No clear guidelines Quinine, artesunate and amodiaquine can be used

tolerating oral treatment, parenteral treatment with artesunate, artemether or quinine is warranted. Hyperparasitemia above 4% is, for some authors, also an indication for parenteral treatment, even in the absence of any other severity sign. Others recommend an oral ACT under close supervision.[5] Hyperparasitemia above 10% should be treated with parenteral antimalarials.

Malaria caused by *P. vivax*, *P. ovale* and *P. malariae*

P. vivax and *P. ovale* form hypnozoites, parasite stages in the liver that can result in multiple relapses of infection, weeks to months after the primary infection. Radical cure of the infection targets both the blood stage and the liver stage, preventing relapse as well as recrudescence. In general, *P. vivax, ovale and malariae* are sensitive to chloroquine, which remains the treatment of choice; however, radical cure can only be achieved by the addition of primaquine. Important chloroquine-resistant vivax malaria has been reported, especially from Papua New Guinea and parts of Indonesia. In these settings, dihydroartemisinin–piperaquine and artemether–lumefantrine are an effective treatment for vivax malaria.[51]

The following are currently recommended for treatment of uncomplicated vivax malaria (Table 111.9):

- chloroquine + primaquine
- amodiaquine + primaquine (for chloroquine-resistant), *or*
- artemisinin-based combination therapy (dihydroartemisinin + piperaquine or artemether + lumefantrine; not recommended is artesunate + sulfadoxine–pyrimethamine).

The recommended treatment for malaria caused by *P. ovale* is the same as that given to achieve radical cure in vivax malaria, i.e. with chloroquine and primaquine. *P. malariae* should be treated with the standard regimen of chloroquine as for vivax malaria, but does not require radical cure with primaquine.

Severe malaria

Antimalarial treatment

Prompt start of parenteral antimalarial treatment in full doses is essential in this life-threatening condition. Available recommended parenteral drugs include artesunate, artemether and quinine. Chloroquine and sulfadoxine–pyrimethamine are no longer recommended for the treatment of severe malaria because of widespread resistance. Treatment of the acute phase of severe vivax malaria is the same as for severe falciparum malaria. If the patient is recovering and able to take oral medication, parenteral treatment can be changed to oral treatment. A full course of an ACT can be chosen as follow-on oral treatment.

Quinine

Parenteral quinine or artesunate are the drugs of choice for treating severe malaria in African children, and the results of a large trial comparing these two treatments can be expected in 2010. Because of its cardiotoxicity, intravenous infusion should be carried out over 4 hours. Where intravenous infusion is not practical, quinine can be given by deep intramuscular injection in the upper thigh. Quinine is a relatively toxic drug with a narrow therapeutic ratio.

Table 111.9 Treatment of uncomplicated vivax and ovale malaria

- Chloroquine 25 mg base/kg divided over 3 days, combined with primaquine 0.25 mg base/kg, taken with food once daily for 14 days is the treatment of choice for chloroquine-sensitive infections. In Oceania and South East Asia the dose of primaquine should be 0.5 mg/kg
- Amodiaquine (30 mg base/kg divided over 3 days as 10 mg/kg single daily doses) combined with primaquine should be given for chloroquine-resistant vivax malaria
- In moderate glucose-6-phosphate dehydrogenase (G6PD) deficiency, primaquine 0.75 mg base/kg should be given once a week for 6 weeks. In severe G6PD deficiency, primaquine should not be given
- Where artemisinin combination therapy has been adopted as the first-line treatment for *Plasmodium falciparum* malaria, it may also be used for *P. vivax* malaria in combination with primaquine for radical cure. Artesunate plus sulfadoxine–pyrimethamine (S/P) is the exception, since S/P is generally not effective against *P. vivax*

Table 111.10 Antimalarial treatment of severe malaria

Artesunate*	2.4 mg/kg iv or im stat, at 12 and 24 h, then daily (artesunic acid (60 mg) is dissolved in 0.6 ml 5% sodium bicarbonate and injected iv as a bolus, or diluted to 5 ml with 5% dextrose for im injection; 1 ampoule = 60 mg)
Artemether	3.2 mg/kg im stat, followed by 1.6 mg/kg daily; **not** for iv administration (1 ampoule = 80 mg)
Quinine	20 mg/kg dihydrochloride salt by iv infusion over 4 h, followed by 10 mg/kg infused over 2–8 h q8h
Quinidine	10 mg base/kg infused at constant rate over 1–2 h, followed by 0.02 mg/kg/min as constant infusion, with electrocardiographic monitoring

*Parenteral artesunate is the drug of choice for the treatment of severe malaria in low transmission areas or outside malaria endemic areas. For children in high transmission areas there is as yet insufficient evidence to recommend any of the above antimalarial medicines over another.

Quinine-induced hyperinsulinemic hypoglycemia is a particular problem in patients with severe malaria, especially during pregnancy, and is impossible to diagnose clinically in the already unconscious patient. Frequent monitoring of blood glucose concentrations is therefore essential.[46] It is also important that parasitocidal drug levels are obtained as quickly as possible, so in patients who have not been given a dose of quinine <12 hours before admission an initial loading dose of 20 mk/kg should be administered.[52] In severe malaria the dose should be reduced by one-third after 48 hours if there is no clinical improvement or if there is renal failure. Dosing schemes are summarized in Table 111.10.

Artesunate and artemether

The artemisinin derivatives are rapidly parasitocidal, and crucially unlike quinine they kill young circulating parasites before they sequester in the deep microvasculature. In adult patients with severe malaria, treatment with intravenous artesunate is importantly superior over quinine in preventing death[53] and is the drug of choice for the treatment of severe malaria in low transmission areas and in pregnancy in the second and third trimesters.[5] For pediatric severe malaria the results of a large African trial comparing artesunate with quinine are awaited. A good manufacturing practice (GMP) formulation of artesunate has recently become available for compassionate use in the USA.

Intramuscular (fat-soluble) artemether, unlike intramuscular (water-soluble) artesunate, is erratically absorbed in patients with severe disease, and might explain why trials in both adults and children have shown similar mortality in patients treated with intramuscular artemether compared to quinine.[54] There are insufficient trials with the other parenteral fat-soluble artemisinin derivative artemotil (artemether) to allow firm recommendations. Dosing schemes of artesunate and artemether are summarized in Table 111.10.

Convulsions

Seizures in cerebral malaria should be treated with rectal diazepam, intravenous lorazepam, paraldehyde or other standard anticonvulsants, after high flow oxygen and appropriate airway management have been initiated. Prophylactic phenobarbital was shown to reduce seizure incidence in adult cerebral malaria, but a study in children using a single intramuscular dose of 20 mg/kg increased mortality, possibly through respiratory depression caused by an interaction with diazepam.[55] Prophylactic anticonvulsive therapy is therefore currently not recommended.

Anemia

Benefits of blood transfusion should outweigh the risks (especially HIV and other pathogens). There is no clear evidence supporting specific hemoglobin cut-off levels, and a number of figures are quoted in reviews and guidelines. In adults the threshold for blood transfusion is commonly set at a hematocrit below 20%, in African children at a hemoglobin level below 4 mg/dl (absolute threshold) or 5 mg/dl if there is coexisting respiratory distress, impaired conscious or hyperparasitemia.

Acute renal failure

Renal replacement therapy can save lives in those cases with acute renal failure, a common complication in adult patients, with an untreated mortality of over 70%. Hemofiltration, when available, is superior to peritoneal dialysis in terms of mortality and cost-effectiveness.[56]

Hemodynamic shock

Shock in severe malaria ('algid malaria') should be treated initially with oxygen and fluids (with monitoring of central venous pressure if available). A septic screen including blood cultures should be performed, and appropriate broad-spectrum antibiotics commenced to cover the possibility of bacterial sepsis. Massive hemorrhage from the gastrointestinal tract or rarely a ruptured spleen should be excluded. Dopamine, dobutamine and noradrenaline (norepinephrine) can be used as inotropic medication, but adrenaline (epinephrine) should be avoided as it induces serious lactic acidosis.[57]

Bacterial superinfection

Bacterial superinfection is common in malaria and must be suspected, particularly if the fever remains high despite antimalarial treatment or if there is evidence of septicemia or focal sepsis (e.g. pneumonia or urinary tract infection).

Supportive treatment

Severe malaria is a multiorgan disease and adequate supportive treatment is crucial for survival. Good nursing care is essential, with particular attention to fluid balance, management of the unconscious patient, and detection of potentially lethal complications such as hypoglycemia. Mechanical ventilation in the unconscious patient helps to protect the airway. The role of aggressive fluid resuscitation in the management of severe malaria, particularly in children, is unclear. The debate centers around whether children with severe malaria are intravascular hypovolemic and to what extent this contributes to impairment of the microcirculation. Many 'resource poor' countries are at a stage where basic intensive care support in regional hospitals is becoming feasible, a development which has great potential to further reduce mortality.

Other adjunctive treatments

Many adjunctive treatments have been tried in often underpowered clinical trials, but none has been shown convincingly to improve survival in severe malaria. Exchange blood transfusion is a popular adjunctive therapy, particularly in well-resourced settings. There are a number of rationales for its use in severe malaria, including removal of parasitized erythrocytes, removal of cytokines and other soluble toxins and mediators, and improving the rheology of the blood unparasitized erythrocytes by replacing unparasitized erythrocytes with reduced deformability. However, a meta-analysis of small studies and case series showed no clear benefit.

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Chapter 112

Alan Fenwick
Yaobi Zhang

Schistosomiasis

INTRODUCTION

Schistosomiasis or bilharzia, caused by infection with trematode *Schistosoma* spp., is one of the most debilitating helminthic diseases among rural populations, particularly in sub-Saharan Africa. Schistosomiasis can cause a wide range of symptoms and consequences depending on the species, the worm burden and the length of time infected. There are three major species which affect humans, of which two are predominant in Africa (*Schistosoma haematobium* and *Schistosoma mansoni*) and one is only found in the Far East, e.g. China and the Philippines (*Schistosoma japonicum*). Another two less widespread species – *Schistosoma mekongi* in South East Asia and *Schistosoma intercalatum* in Africa – are considered to be less of a public health problem.

EPIDEMIOLOGY

Schistosomiasis is prevalent in 76 countries and territories in tropical and subtropical regions (Fig. 112.1), with an estimated 207 million people infected and 779 million people at risk.[1] Approximately 85% of infections are found in sub-Saharan Africa.[2] The geographic distribution of *S. haematobium* and *S. mansoni* within endemic areas is dependent on the presence of suitable aquatic intermediate host snails (*Bulinus* and *Biomphalaria* spp., respectively). Human water contamination activities, such as urination and defecation, lead to infection in these snails while exposure during activities such as swimming, fishing or irrigation lead to cercariae from snails infecting humans and completing the cycle. In the case of *S. japonicum*, a reservoir of infection exists in domestic and wild animals and the snail intermediate hosts are amphibious *Oncomelania* species.

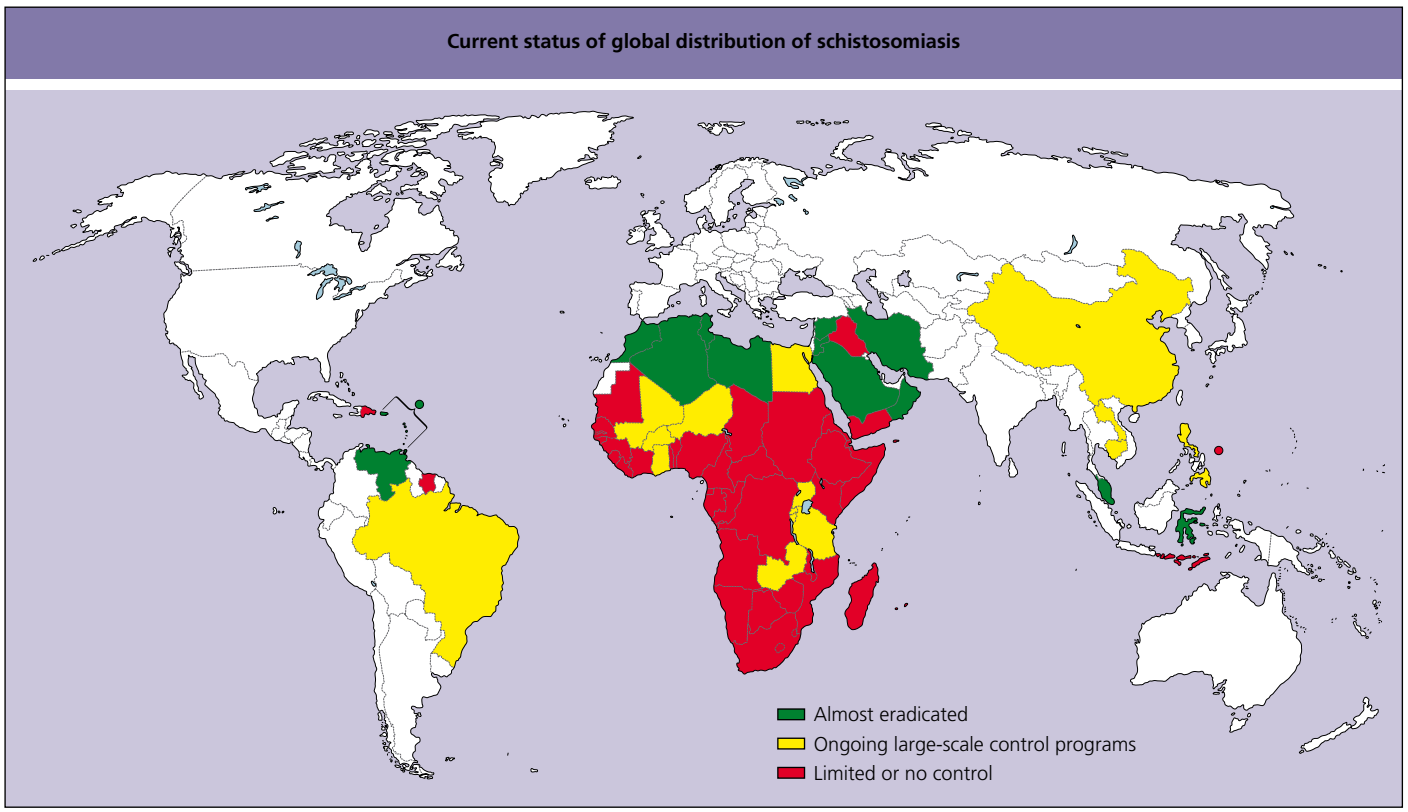


Fig. 112.1 Current status of global distribution of schistosomiasis. From the World Health Organization, with modification.



Fig. 112.2 Water contact activities. (a) Girls collecting water and washing in Lake Victoria in Uganda. (b) Mass fishing activities in Burkina Faso.

Transmission levels are determined by cultural, social and behavioral activities (Fig. 112.2). Recent infections in new areas have resulted from new irrigation projects[3] and may probably be accelerated by climate change.[4] Detailed information on geographic distribution in each endemic country/area has been obtained through rapid epidemiologic mapping[5] or using geographical information system (GIS) technology.[6] *S. mekongi* is present only in the Mekong River basin in South East Asia, though latest research suggests that the distribution may be a little wider, with intermediate host *Neotricula aperta* snails. Finally, *S. intercalatum* is present in limited areas in Central and West Africa with *Bulinus* spp. as snail host.

In endemic areas, children may acquire infection in infancy,[7] much earlier than previously believed, probably through being bathed in the infested water by their mothers (Fig. 112.3). Prevalence of infection usually increases with age, peaks in age 10–20 years and then decreases in adults. Intensity of infection, which is measured as the number of eggs excreted (a surrogate estimate of worm load) in urine (*S. haematobium*) or feces (*S. mansoni*, *S. japonicum*, *S. mekongi* and *S. intercalatum*), follows a similar pattern to prevalence: increasing with age through childhood and then decreasing in adults. Schistosomiasis does, however, result in exceptional consequences when epidemiologic conditions are extreme – for example, car washers in Kenya present a unique situation of repeated heavy infections,[8] despite frequent treatment.



Fig. 112.3 Water contact – baby bathing. A baby is being washed by the mother near Lake Victoria in Uganda. Courtesy of Dr J Russell Stothard.

There are two schools of thought about this 'normal' age-specific pattern of prevalence and intensity whereby peak infection in children and decline in adults may be explained by either acquired immunity due to existing infections, or a reduction in water contact patterns in older age groups. Those who favor the second argument of water contact as a major factor point to the gender-related patterns in prevalence and intensity of infection which vary in different areas according to behavioral, professional, cultural and religious factors.

Another epidemiologic feature of schistosome infections is that the frequency distribution of the number of worms in individuals in endemic areas is aggregated or overdispersed.[9] This means that in most populations the majority of infected individuals harbor a relatively low worm burden and only a minority (5–15%) are heavily infected (as measured by eggs in stool or urine). Consequently, this minority group of individuals is most likely to show severe morbidity. What determines the predisposition to heavy or light infection is not known, but may be attributed to age, innate and acquired immunity and/or genetic background of individuals.[10]

PATHOGENESIS AND PATHOLOGY

Disease manifestations caused by schistosome infections are multiple, depending on the parasite species, but can be divided into acute and chronic phases.

Acute schistosomiasis is seen most commonly soon after exposure to an infested water body by previously unexposed individuals, and is more common in *S. japonicum* and *S. mansoni*. The typical acute symptoms are observed after the percutaneous penetration of cercariae which can cause cercarial dermatitis, a local hypersensitivity reaction to the penetrating parasites at the site of entry. Subsequently, Katayama fever, a systemic hypersensitivity reaction, can then occur during the next month as larvae migrate through the lungs. The exact pathogenesis is still unknown, but is probably a serum sickness-like illness caused by circulating immune complexes and proinflammatory cytokines in response to the migrating schistosomula and the egg antigens.[11] Classically, egg laying begins approximately 6 weeks after infection, and the onset of oviposition can cause severe bloody diarrhea.

In chronic schistosomiasis, caused by continued infection with mating worm pairs, pathologic lesions are mainly localized in specific sites within infected individuals. While the adult worms are living in the blood vessels and are feeding on blood, it is the eggs and the subsequent host granulomatous reactions to the eggs trapped in tissues which cause the damage. Mature worms reside in the venous tributaries of the vesical (*S. haematobium*) or mesenteric (*S. mansoni*, *S. japonicum*, *S. mekongi* and *S. intercalatum*) plexuses and continue oviposition for life. Through the secretion of proteolytic enzymes, and perhaps aided by urinary tract or intestinal movement, the eggs rupture capillary vessels and then pass

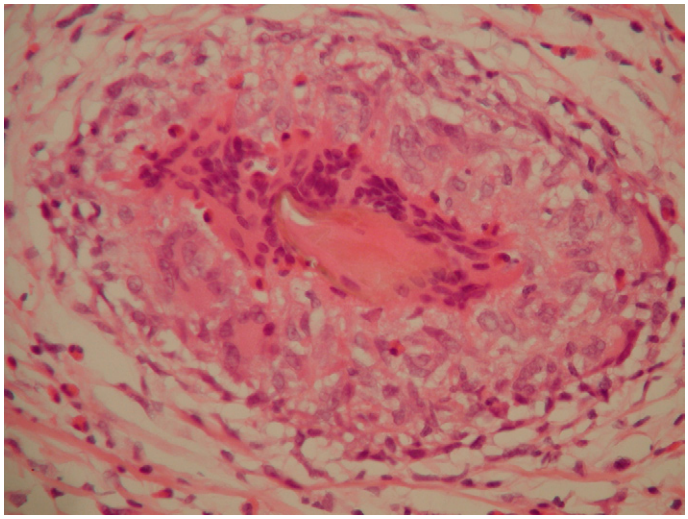


Fig. 112.4 Multiple granulomata surrounding eggs. Courtesy of Dr Robert Goldin.

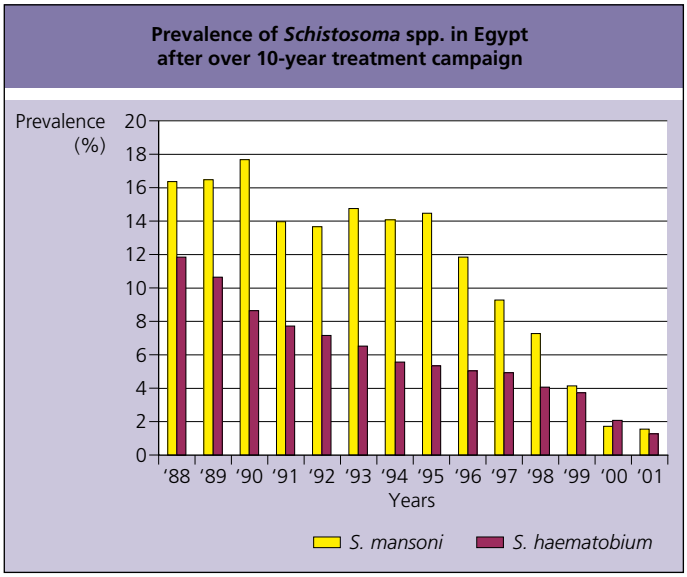


Fig. 112.5 Decreasing prevalence of *Schistosoma* spp. in Egypt (1988–2001) in the course of an ongoing treatment campaign. Redrawn from Fenwick *et al*.[14] Copyright Elsevier 2003.

through the tissues to the lumen of these viscera where they are carried to the outside via urine or stools. This process causes bleeding, which in the short term causes overt blood in the urine and blood in the stool (less easily visible) and in the longer term contributes to anemia. A significant proportion of parasite eggs, however, are either retained in local tissues (bladder or gut walls) or carried via venous blood to distant organs such as the liver and lung, and are trapped there due to their relatively large size (60–170 µm). As long as the worms are alive, the numbers of trapped eggs continue to accumulate in the tissues. At these sites, miracidia in the trapped eggs secrete soluble antigens which diffuse out of the egg shell, triggering a marked inflammatory and granulomatous response from the host immune system. As a result, the individual eggs (or cluster of eggs in the case of *S. japonicum*) are enclosed by the aggregated immune cells, including eosinophils, forming granulomas (Fig. 112.4).

The granulomatous response is a form of delayed-type hypersensitivity reaction and is dependent predominantly on $CD4^+$ T cell-mediated mechanisms.[12] Granulomas are tumor-like inflammatory nodules which can cause intrahepatic portal vein obstruction, leading to portal hypertension, or obstruct urine flow through the ureters, leading to hydroureters and hydronephrosis. The miracidia within the eggs, however, survive for only 6–8 weeks and then die, thus eliminating the continuous antigenic stimulation. The granulomas may then disperse and fibrous tissues form. The tissue damage caused by scattered eggs does not cause a significant problem, but when numerous eggs are trapped the damage becomes significant. Therefore, the severity of morbidity is closely related to the intensity of infection and the passage of time.

PREVENTION

Prevention of infection by improved water supplies and sanitation is fundamental to the elimination of schistosomiasis, as was achieved several decades ago in Japan and more recently in Puerto Rico.[13] In the meantime, current control programs in less developed countries target prevention of morbidity using chemotherapy, with elimination some way in the future. After 20 years of regular chemotherapy, Egypt has significantly reduced the prevalence of infection and reduced the public health importance of schistosomiasis (Fig. 112.5).[14]

Without human contact with fresh water there would be no infection. For nonindigenous travelers into endemic areas, avoidance of all freshwater sources is most prudent, because a single exposure to many cercariae can have severe consequences. However, for indigenous residents in endemic areas the risks are more complex and multifaceted, often reflecting the degree of education, socioeconomic status and cultural and recreational practices. In many areas in sub-Saharan Africa, the snail-infested water bodies may be the only water sources in their daily life, and for fishermen, farmers and domestic washing, contact is unavoidable. How can people with regular water contact escape infection? Strategies considered for prevention may include molluscicide to kill intermediate host snails, chemotherapy of infected individuals to reduce transmission sources, health education to improve hygiene, provision of latrines and sewage disposal, installation of a clean water supply, and economic and social development in general. Each of these strategies has its limitations and should be used in conjunction with others in an integrated manner according to local conditions. In recent years, despite investments to develop antischistosome vaccines, and promise in laboratory studies using a mouse model, a successful vaccine and a practical vaccination strategy are still some way away.

To reduce morbidity, a preventive chemotherapy strategy has been recommended by the World Health Organization[15] to reduce the intensity of infection in the population by targeted mass chemotherapy. This strategy to prevent morbidity should be based first on targeting school-aged children and populations at high risk. Since children can acquire infection at a very young age and severe morbidity usually takes years to develop, repeated annual treatment during school-aged years should prevent the devastating consequences of organ damage at a later stage of life. Since 2002, the Schistosomiasis Control Initiative (SCI) based at Imperial College London, has implemented a sustainable integrated chemotherapy strategy using praziquantel against schistosomiasis and adding in albendazole to control the concurrent intestinal helminth infections,[16] and approximately 40 million treatments were dispensed in six countries by mid 2008. Since 2006, control of schistosomiasis by chemotherapy has been 'absorbed' into a rapid-impact package covering seven major neglected tropical diseases including schistosomiasis, and this is now being implemented in several sub-Saharan African countries.[17]

CLINICAL FEATURES

The clinical manifestations of schistosomiasis are related not only to the parasite species but also to the intensity of infection and genetic make-up of the infected individuals. Disease manifestations have

multiple stages and also interact with other co-infections such as HIV and malaria, and nutritional conditions.

The first clinical manifestations occur as individuals are acquiring infection, as cercarial penetration of the host skin can provoke a temporary urticarial rash that can manifest within hours of exposure (contacting cercariae-infested water) and sometimes persist for days as maculopapular lesions. Such cercarial dermatitis, also called 'swimmers' itch', usually occurs in those who have not previously been exposed, e.g. tourists and migrants. The lesions are usually self-limited and often go unrecognized in endemic areas.

Interestingly, in temperate climate zones (e.g. in the lakes in North America) a similar 'swimmers' itch' is seen and is due to penetration of skin by schistosomal cercariae that normally infect birds or other small animals and cannot fully develop in humans. The severity of the lesions is dependent on the degree of exposure. After heavy exposure to the infested water, a delayed-onset dermatitis as urticaria or angioedema may occur within 1–12 weeks.

A few weeks to months after a primary schistosome infection, systemic manifestations of acute schistosomiasis, Katayama fever, can develop. Early infections are mostly asymptomatic, but in some cases of heavy naive infections the disease starts suddenly with nonspecific symptoms, such as fever, fatigue, myalgia, malaise, nonproductive cough, eosinophilia and patchy pulmonary infiltrates on chest radiography. Abdominal symptoms may develop later when egg deposition begins. Most patients recover spontaneously after 2–10 weeks, but some may develop persistent and more serious disease with weight loss, dyspnea, diarrhea, diffuse abdominal pain, bloody diarrhea, hepatosplenomegaly and widespread rash.

Chronic schistosomiasis and its liver and bladder sequelae may develop within months but usually it takes years after infection depending on the accumulated intensity of infection or the length of exposure to infection. Symptoms, signs and pathologic lesions are species specific.

Urinary schistosomiasis

Urinary schistosomiasis is caused by infection with *S. haematobium*. Most infected individuals show some symptoms, the most common of which is blood in the urine – haematuria (Fig. 112.6a). Other early signs include frequency of urination, dysuria and proteinuria. Haematuria in school-aged children in sub-Saharan Africa, although usually present, is not considered important, because it is often unrecognized as a disease symptom. In girls, it may be confused with menstruation; in boys, it may be considered as a sign of puberty in local perception. The prevalence of these symptoms varies according to the degree of endemicity in each local setting but rates of almost 100% in infected children are not unusual.

Ultrasound examination of the urinary tract in longstanding infections usually indicates thickening of the urinary bladder wall (Fig. 112.6b) and granulomas, and sometimes hydronephrosis and evidence of bladder and ureteric calcification can be seen. Kidney function is usually well preserved, but may be complicated by frequent urinary tract bacterial infection and bladder or ureteric stone formation. There is strong epidemiologic association with squamous bladder cancer, which occurs characteristically in older age groups, especially historically in Egypt, where there is now evidence that with the recent decline of *S. haematobium*, the cancer has decreased.[18]

Intestinal/hepatic schistosomiasis

Intestinal/hepatic schistosomiasis is caused by infection with *S. mansoni*, *S. japonicum*, *S. mekongi* or *S. intercalatum*, with most of the reports of the former two species. There is no definitive symptom such as blood in the urine, and so in many cases with light infections, symptoms and signs are nonspecific and unrecognized. In a relatively small proportion of individuals with heavier infections and/or with a long history of exposure, chronic or intermittent abdominal pain and discomfort, loss of appetite and diarrhea with or without blood may be common.

The serious long-term consequences of infection are liver disease which manifests as early inflammatory and late fibrotic stages. The early stage is typified as hepatomegaly due to the granulomatous response – sharp-edged enlargement of the left liver lobe and perhaps splenomegaly. At this stage liver function can be normal. Ultrasound examination may reveal mild forms of diffuse fibrosis. With the progression of the disease, massive deposition of the diffuse collagen deposits in the periportal spaces leads to pathognomonic periportal or Symmer's pipe-stem fibrosis (which is classic *S. mansoni* pathology). This in turn leads to portal hypertension, splenomegaly, collateral venous circulation and esophageal varices. The liver is not necessarily enlarged and may even be shrunk, and liver function tests remain largely unaffected, in contrast to other causes of liver disease. Ultrasonography will reveal typical fibrotic streaks and portal vein dilatation. The late stage of liver disease may be associated with extensive splenomegaly, ascites and repeated episodes of hematemesis which is often fatal (Fig. 112.7).

Unusual consequences of schistosomiasis

Schistosomiasis may also appear in other forms, e.g. pulmonary, genital or neurologic. These are caused by the deposition of schistosome eggs or worms in these organs/systems and subsequent host granulomatous responses. A common and yet only recently recognized complication is female genital schistosomiasis, which is caused by *S. haematobium* infections in women, with up to 75% of those infected having schistosome eggs in the uterine cervix, vagina or vulva. It usually manifests as mucosal grainy sandy patches, mucosal bleeding and inflammation, and later as ulcerative lesions in these genital areas.

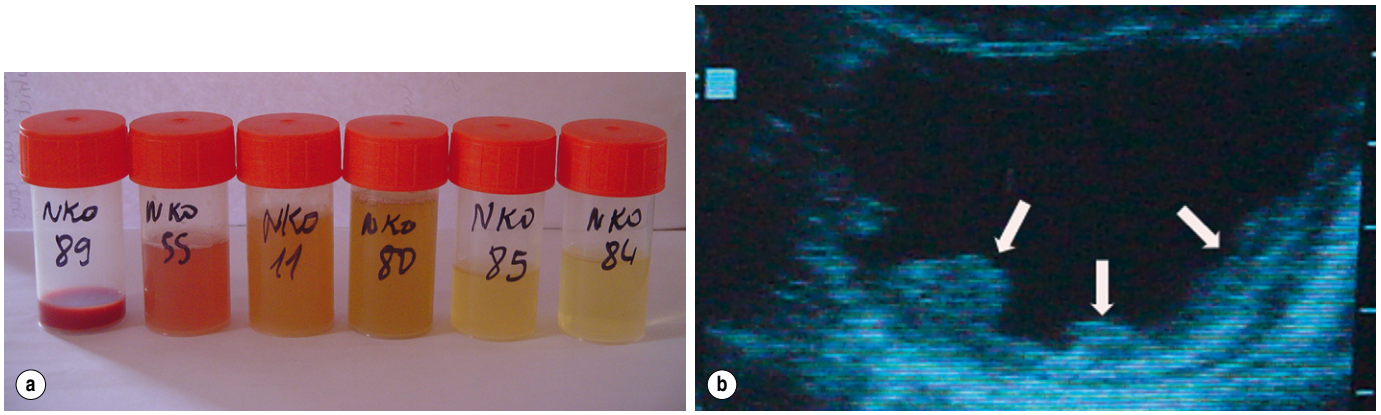


Fig. 112.6 Urinary schistosomiasis. (a) Blood in urine samples collected during a school survey in Burkina Faso. (b) Ultrasound image of the bladder wall from a child infected with *S. haematobium*.

Investigations have shown that genital schistosomiasis can significantly increase the chances of HIV infection.[19]

Another serious possible complication is neurologic schistosomiasis, which can be present in infections with all three main species.[20] Cerebral complications associated with *S. japonicum* can occur during all phases of infection from acute to chronic. These include delirium, loss of consciousness, seizures, dysphasia, visual field impairment, focal motor deficits and ataxia. Spinal complications such as myelopathy are primarily caused by *S. mansoni* but can complicate later stages of *S. haematobium* infection and occur during the acute phase of egg production (see Chapter 106). Severe myelopathy can provoke a complete flaccid paraplegia with areflexia, sphincter dysfunction and sensory disturbances.

DIAGNOSIS

Travelers from nonendemic areas reporting with nonspecific malaise after visiting Africa need to be questioned about water contact. Apart from blood in the urine, infection with different schistosome species manifests only a variety of nonspecific clinical features, and therefore a personal history of possible contact with infested water in endemic areas in tropical or subtropical regions is an important indicator for a suspicion of schistosomiasis.

Parasitologic examination of urine (for distinctive eggs of *S. haematobium*) or stool (for *S. mansoni, S. japonicum, S. mekongi* and *S. intercalatum* eggs) by microscopy is still the gold standard for the diagnosis of schistosomiasis (see also Chapter 184). However, it must be noted that in some cases with advanced stage of infection the eggs may not be present in urine or stools. In addition, microscopic examination of stool has a relatively low sensitivity and repeated samples or multiple tests may be required to detect eggs in those with low intensity of infection. For *S. haematobium*, urine should preferably be collected around noon, when egg passage is maximal, and generally 10 ml should be concentrated through filtration onto a nitrocellulose filter for examination under a microscope and eggs counted. The intensity of infection can be expressed as number of eggs per 10 ml urine. A less sensitive but useful method for *S. haematobium* is detecting hematuria with a reagent strip which reacts to blood. This practice, however, is more useful for survey work in highly endemic areas than for returning occasional travelers in the developed world because of the multiple etiologies of hematuria.

For intestinal schistosomes, eggs in the feces is the definitive diagnosis; unfortunately, failure to find these eggs does not necessarily mean that the individual is negative. The most commonly used stool examination method is the Kato–Katz thick smear, which examines 41.7 mg feces in each smear, so when the number of eggs are counted, the egg load can be quantified. The intensity of infection is then expressed as number of eggs per gram of feces, and this is assumed to be proportional to the number of worms.

Serologic tests using schistosome antigens to detect antibodies are sensitive but cannot distinguish current from past infection as antibodies can remain in circulation for a long period, even after chemotherapeutic cure, and can also cross-react with other helminth infections. Antigen detection techniques which use labeled monoclonal antibodies to detect circulating anodic antigens or circulating cathodic antigens in serum or urine have been developed. The methods are quantitative and can be used to estimate the worm burden, but they have low sensitivity for light infections.

Clinical diagnosis by ultrasonography of the liver or urinary tract pathology is a valuable tool. Standard protocols (Niamey protocols)[21] for ultrasound examination have been developed to classify schistosome hepatic fibrosis and urinary tract lesions. It is very useful for evaluating the treatment effect and the reversal of schistosome pathology, but requires specific expertise. In hospital settings, cystoscopy and endoscopy may be used to visualize tissue lesions. Tissue samples may be obtained from rectal mucosa, bladder and liver biopsies, and examined for schistosome eggs.



Fig. 112.7 Intestinal/hepatic schistosomiasis. (a) Ultrasound image of the liver of a child infected with *S. mansoni*. (b) Advanced case of *S. mansoni* infection of a child with blood in the stool and ascites. (c) Advanced case of *S. mansoni* infection of an adult.

MANAGEMENT

Treatment of schistosomiasis is relatively inexpensive and safe using the drug praziquantel, which is active against all adult schistosome species at a single oral dose of 40 mg/kg given after food. Praziquantel rarely causes significant side-effects; however, in those with heavy worm burdens, abdominal pain, nausea and vomiting may occur. Recently the World Health Organization has declared that praziquantel is safe for pregnant women and young children (≤4 years of age or ≤94 cm in height). The drug is not effective against eggs and immature worms. After a single treatment, 70–100% of individuals cease to excrete eggs in urine or feces; in those not cured, the intensity of infection (egg counts) should be reduced by over 90%. Artemisinin (or one of its derivatives) has been reported as effective against immature schistosome worms, but not against adult worms. This is a drug currently in use against malaria and so a minor effect on prevalence in endemic areas may be observed.

For acute early cases, treatment should be given to suppress the hypersensitivity reaction, and a combination of praziquantel and artemisinin might be given to kill adult and immature worms. Praziquantel therapy in early chronic infections will result in reversal of pathologies such as hepatomegaly, bladder wall thickening or hydroureters. However, in late stage infections with severe pathologic conditions, such as portal hypertension or extensive hydronephrosis, general medical care will be required. In extreme cases, corrective surgical procedures for portal hypertension or urinary tract anatomic alterations may be necessary.

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Chapter 113

David J Diemert

Cestode and trematode infections

INTRODUCTION

Cestodes (tapeworms) and trematodes (flukes) are ubiquitous flatworms that are able to infect both vertebrates and invertebrates. The life cycles of both types of helminth are complex and are characterized by morphologically distinct developmental stages of the parasite being harbored by different host species. Most of these worms are restricted in the range of host species that they can infect, particularly in the definitive host of the adult reproductive worms. From among the large number of parasitic flatworms, humans are the preferred host of just a few, although more are capable of causing incidental (paratenic) human infection.

Despite this variety, the life cycles of the flatworms that infect humans have several features in common:

- all trematodes undergo asexual reproduction in aquatic snails, the intermediate host, and sexual reproduction in a definitive mammalian host;
- the geographic distribution of infections with the various host flukes corresponds to the range of their intermediate snail; and
- when eggs passed in the waste products (usually feces) from a definitive host infected with an adult fluke come into contact with fresh water, they hatch to release larvae that then infect snails.

Flukes are categorized according to their habitat in the human host: blood flukes (*Schistosoma* species), intestinal flukes (*Fasciolopsis*), liver flukes (*Clonorchis, Opisthorchis* and *Fasciola*) and lung flukes (*Paragonimus*). In the case of schistosomiasis, larvae (known as cercariae) released by snails penetrate skin or mucous membranes that come into contact with infected water. Other human trematode parasites cause infection through consumption of food – usually a form of fish or seafood – contaminated with encysted intermediate stages (metacercariae). They are mainly found throughout the tropics and subtropics, although a few species are also found in temperate climates.

Adult cestodes are tapeworms that are parasitic in the intestinal tracts of various vertebrate hosts. Intermediate hosts are infected by ingesting eggs, which develop into a cystic larval form in host tissues. The life cycle is completed when these infected tissues are eaten by a suitable definitive host. Tapeworms are flat, segmented worms, composed of a head (scolex) and a series of symmetric segments, known as proglottids. The scolex may be equipped with hooks, suckers or elongated grooves to facilitate attachment to the host's intestine. Each segment possesses a complete set of both male and female reproductive organs; sperm are typically transferred between adjacent segments, giving rise to gravid proglottids that contain thousands of embryonated eggs. Gravid segments detach from the parent worm and either exit intact via the feces, or disintegrate before leaving the host, releasing embryonated eggs. Cestodes lack a functional digestive tract; the tegument, or body covering, serves as a metabolically active layer through which nutrients are absorbed. Although adult tapeworms do not cause much pathology, the encysted larval forms of cestode parasites (termed cysticerci) often have serious adverse consequences for the intermediate human host, especially neurocysticercosis caused by the larval stages of the pork tapeworm, *Taenia solium*.

Schistosomiasis is discussed in Chapter 112 and echinococcosis in Chapter 114.

TAPEWORM INFECTIONS

Taeniasis

For both the beef tapeworm, *Taenia saginata* (Fig. 113.1), and the pork tapeworm, *T. solium*, humans are the only definitive hosts. Infection is acquired by eating raw or undercooked beef or pork, respectively, that contain living cysticerci. The clinical consequences of taeniasis are usually mild and are limited to minor abdominal symptoms, such as epigastric fullness, nausea and vomiting with *T. saginata*, and abdominal pain, distention and diarrhea with *T. solium*. *T. saginata* proglottids tend to migrate spontaneously from the anus, causing considerable alarm in the host. *Taenia* spp. do not appear to have any significant nutritional effect on their host. The principal complication of infection with *T. solium* is that eggs may be passed in the feces and subsequently be ingested and cause cysticercosis (see below) either in the same host or in others living in close proximity.

DIPHYLLOBOTHRIASIS

Diphyllobothrium latum is the longest parasite that infects humans: adult worms can achieve a length of more than 33 feet (10 m) in the small intestine. Maintenance of the life cycle of *D. latum* requires that feces of infected hosts be discarded into fresh water that contains the appropriate crustacean and fish intermediate hosts and that the infected fish be eaten raw by definitive hosts. Although *D. latum* was previously

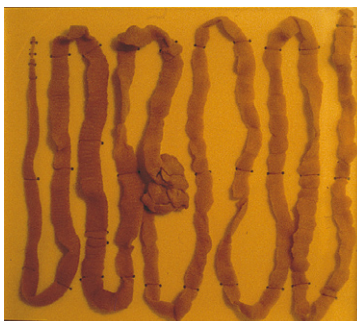


Fig. 113.1 *Taenia saginata*. A mature worm may be up to 33 feet (10 m) long. Courtesy of Guy Baily.

a common infection in Scandinavia, the incidence there has been reduced markedly because of improved sanitation, so that most cases now occur in Russia, Brazil and Japan.[1] Humans are the most important definitive host for this tapeworm, although bears, dogs and other carnivores may serve as reservoir hosts. Adult worms cause minimal clinical disease, although they may compete with the host for absorption of vitamin B12 from the intestinal lumen. Megaloblastic anemia due to depletion of vitamin B12 has been reported in the past as a consequence of infection with *D. latum*, but this is now rarely seen. Various other *Diphyllobothrium* spp. that usually occur in wild animals may infect humans incidentally, principally in the sub-Arctic and the northern Pacific areas. Clinical consequences of these infections appear to be relatively minor.

Hymenolepis

Infection with the dwarf tapeworm (so-called because of its small size) *Hymenolepis nana* occurs worldwide, mostly in children living in conditions with poor sanitation. Rodents are the main reservoir. Infection is acquired either by ingestion of embryonated eggs (from rodents or humans) or an infected insect containing cysticercoid metacestodes. Ingested eggs hatch in the small intestine, releasing larvae that penetrate the lamina propria of the small bowel epithelium where they differentiate into cysticercoids that re-enter the intestinal lumen and attach to the surface of the villous tissue. These develop into new tapeworms approximately 4 cm in length. If an intact cysticercus is ingested, it attaches directly to the small intestinal wall and matures into an adult worm. Although most infections are asymptomatic, heavy infections may result in diarrhea.

Zoonotic tapeworms

Several tapeworms that are not primarily adapted to humans may nevertheless cause incidental human infection after accidental exposure. *Hymenolepis diminuta* is a tapeworm of rodents that uses their fleas as an intermediate host. Similarly, *Dipylidium caninum* passes between dogs and their fleas. Both are occasional sources of human infection worldwide, principally in children, through the accidental ingestion of fleas. There are no known serious clinical consequences with either tapeworm infection.

Diagnosis and management of tapeworm infections

Diagnosis of tapeworm infection is made by microscopic identification of characteristic eggs in the feces, although the species cannot be determined based on morphology since members of the genera *Taenia* and *Diphyllobothrium* produce identically shaped eggs (Fig. 113.2). Speciation can be made by examination of entire proglottids which may sometimes be found in the feces. Treatment with a single dose of praziquantel at 10 mg/kg is effective for all human tapeworms except *H. nana*, for which at least 25 mg/kg should be administered.

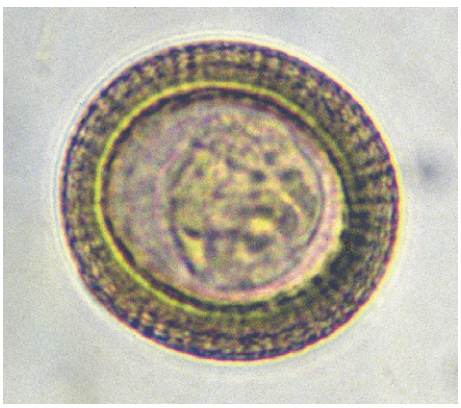


Fig. 113.2 *Taenia* egg. Speciation cannot be done based on morphology. Eggs measure between 30 and 40 μm in diameter.

CYSTICERCOSIS

Epidemiology and prevention

Cysticercosis is caused by ingestion of eggs of the pork tapeworm *T. solium*. Although food and water contaminated with human feces were previously thought to be the primary source of tapeworm eggs, recent evidence indicates that the most common source is an asymptomatic tapeworm carrier in the household.[2] Infected pigs serve to perpetuate the infection: pigs have access to contaminated human feces by their coexistence with humans in the domestic setting and the lack of adequate sanitation and sewage facilities. Cysticercosis remains endemic in most low-income countries, especially in Latin America and Asia (particularly China and South East Asia). The World Health Assembly in 2003 recommended several measures to control *T. solium* infection with the aim of reducing the incidence of neurocysticercosis, including mass or selected treatment with praziquantel to reduce the prevalence of adult worm infection in endemic areas, improved pig husbandry, enforced meat inspection and control, and treatment of infected animals.[3]

Pathogenesis

After ingestion, *T. solium* ova hatch upon exposure to gastric and duodenal contents, releasing oncospheres (embryos) that invade the intestinal wall, enter the vasculature and migrate to host tissues where they develop into larval cysts. In most tissues, cysts are eliminated by the host's immune system, except for sites such as the central nervous system and the eye, which are immunologically privileged, and both striated and cardiac muscle where development to the cysticercus stage is rapid. Cysts are normally approximately 1 cm in diameter and consist of a scolex (head) of the tapeworm larva that is surrounded by a vesicle formed by extension of the parasite's tegument, all of which is enclosed by a host-derived capsule.

Cysts can be found in any part of the central nervous system, although the majority are located in the brain parenchyma, where they initially form viable vesicles which may remain alive for years. However, the host's immune system eventually prevails and the cysticercus dies, a process called involution. First, the vesicular fluid becomes turbid and a thick collagen capsule forms around the dying cyst together with diffuse edema (colloidal stage). The scolex then shows signs of degenerating into coarse granules (granular stage), corresponding to resolution of the surrounding edema and eventual formation of a calcified nodule that represents a dead cysticercus. Significant clinical consequences of cysticercosis are related to this neurologic involvement (Fig. 113.3).

Clinical features

Most of the morbidity and mortality due to this infection is caused by neurocysticercosis, with seizures and sequelae of raised intracranial pressure being the most common clinical manifestations. Symptomatic

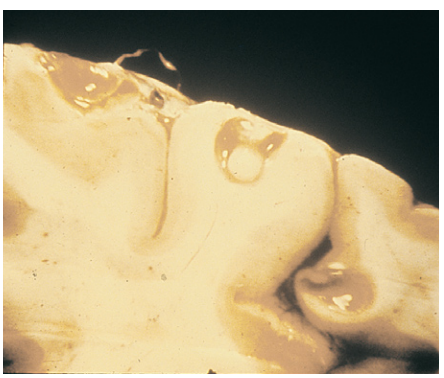


Fig. 113.3 Cysticerci in the brain. Courtesy of Guy Baily.

neurocysticercosis results from a combination of factors, including the number, stage and location of the cysts, together with the severity of the host's immune response against the parasites. As many as 70% of individuals with neurocysticercosis will present with seizures.[4] Other manifestations include those of intracranial hypertension (focal signs, dementia and seizures), caused primarily by hydrocephalus but also rarely by cysticercotic encephalitis. Hydrocephalus occurs most commonly with cysts that are located in the ventricles or subarachnoid space. If untreated, hydrocephalus may lead to permanent loss of cerebral function with dementia and cortical blindness. Various focal neurologic findings may also occur and usually follow a subacute or chronic course, although acute signs may arise due to a stroke.

Ophthalmic cysticercosis is not uncommon and typically manifests as intraocular cysts floating freely in the vitreous or the subretinal space, where they give rise to visual field defects or scotomata (Fig. 113.4). Development of uveitis or retinitis may result in permanent loss of vision.

Extraneural cysticercosis is usually asymptomatic and consists initially of nontender subcutaneous nodules or discrete swellings of particular muscles in which cysticerci have embedded. Several months after initially being noticed, the nodules may swell and become tender as the cysts begin to involute and die. Ultimately, the cysts will calcify; subcutaneous and intramuscular calcifications may persist for years after cysticercal death.

Diagnosis

Diagnosis of neurocysticercosis is usually made on the basis of imaging studies – CT or MRI – and confirmatory serology. Although MRI has better accuracy in making a diagnosis – especially for cysts in the ventricles or basal cisterns – CT is more commonly used in most endemic areas. On CT, viable cysts appear as hypodense lesions whereas degenerating cysts appear as contrast-enhancing lesions with a surrounding ring of edema (Fig. 113.5). The scolex may sometimes be visualized in the interior of viable cysts, but usually not in degenerating ones. The most commonly used serologic test is the enzyme-linked immunoelectrotransfer blot, which has a specificity of 100% and reported overall sensitivity of 98%, although the sensitivity is considerably less for individuals with a solitary lesion.[5]

Occasionally, evidence of extracranial cysticercosis may aid in the diagnosis, particularly direct visualization of ophthalmic cysticerci, palpation of subcutaneous cysticerci or evidence of cigar-shaped intramuscular calcifications seen on plain radiography (Fig. 113.6). A set of diagnostic criteria has been formulated to aid in the diagnosis of neurocysticercosis, which permits classification as either definitive or probable disease.[6]

Management

Treatment of neurocysticercosis consists of a combination of antiparasitic and symptomatic modalities and should involve specialists in infectious diseases, neurology, neuroimaging and neurosurgery.

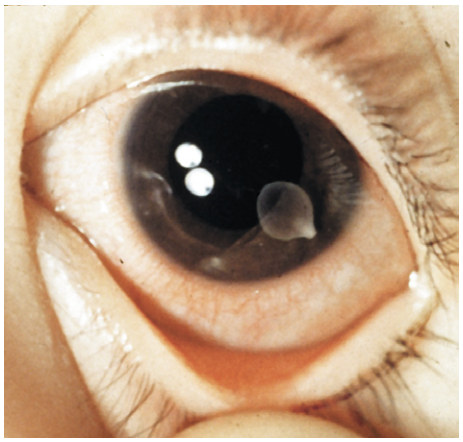


Fig. 113.4 Cysticercus of *Taenia solium* floating in the anterior chamber of the eye.

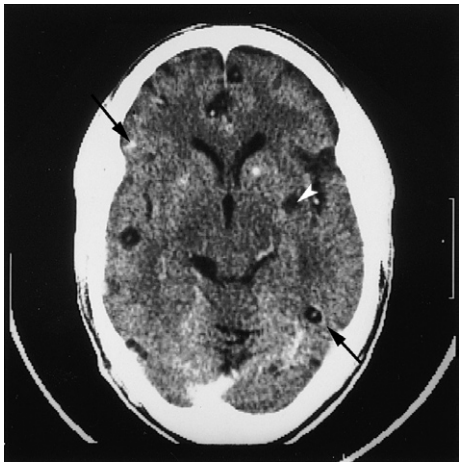


Fig. 113.5 CT image of neurocysticercosis. Viable cysts appear as radiolucent defects (arrowhead). The central protoscolex may be visualized as a radiodense spot (small arrow). Cysts that show ring enhancement are probably degenerating. Calcified cysts (large arrow) are dead and will not benefit from specific therapy. Courtesy of Guy Baily.



Fig. 113.6 Plain radiograph of lower leg with numerous calcified cysticerci of *Taenia solium.*

Antiparasitic measures include cysticidal medications (albendazole or praziquantel) and surgical resection of cysts, whereas symptomatic interventions can include antiepileptics, analgesics and placement of ventricular shunts.[7]

There has been considerable controversy over the use of cysticidal medications, mostly because treatment-associated parasite death can lead to an acute inflammatory reaction in the surrounding brain tissue which can result in seizures, raised intracranial pressure and even death. However, studies have now shown that treatment of viable intraparenchymal cysts results in reduced numbers of seizures and faster resolution of cysts.[8] Although in most cases single enhancing lesions resolve spontaneously,[9] many experts maintain the value of cysticidal treatment. Albendazole (15 mg/kg daily for 7 days) is preferred over praziquantel (100 mg/kg daily in three doses for 15 days) because of its higher cysticidal effect and the fact that serum concentrations of praziquantel are decreased with concomitant use of corticosteroids. Simultaneous administration of corticosteroids reduces the headache, vomiting and other complications that may result from the inflammation induced by dying parasites; their use is indicated in treatment of multiple intraparenchymal cysts.

Cysticercotic encephalitis should not be treated with cysticidal drugs because this may result in increased intracranial pressure. Treatment for this condition is with high-dose steroids and diuretics. Cysticidal treatment is also not indicated for individuals with calcified lesions alone since these represent degenerated parasites.

Treatment of extraparenchymal cysts is considerably more complex. Subarachnoid and Sylvian fissure cysts should be treated with cysticidal drugs and high-dose steroids, although placement of a ventricular shunt should be performed first in cases with hydrocephalus. Ventricular cysts should be excised surgically or by neuroendoscopy; adjunct antiparasitic treatment is controversial.

OTHER LARVAL CESTODE INFECTIONS

Coenurosis

Humans are occasionally accidentally infected with the metacestode (juvenile stage) of *Taenia multiceps, T. brauni, T. serialis* or other *Taenia* spp., the adult form of which normally infects dogs. Intermediate hosts include cattle and sheep or goats. The metacestode, known as a coenurus, often invades the central nervous system where it forms a large cyst, often more than 2 cm in diameter, with multiple invaginated protoscolices.

Human cases are rare and largely occur in Africa, although infections have been reported worldwide. Diagnostic criteria include epidemiologic, clinical, radiologic and, most often, biopsy findings; serology is not available. Treatment usually involves surgical excision if the cyst is accessible; specific anthelmintic therapy is unavailable.

Sparganosis

Infection by the migratory plerocercoid larvae of the cestodes *Spirometra mansonoides* and other *Spirometra* spp. is termed sparganosis. Although the definitive hosts remain unknown, intermediate hosts include reptiles, amphibians, birds and some mammals. Humans become infected from ingestion of the raw or undercooked flesh of an intermediate host such as inadequately cooked frog or snake meat, or from using the skin or flesh of such animals as a poultice for wounds or sore eyes, as is customary practice in parts of eastern Asia.

Clinically, a localized subcutaneous inflammatory swelling forms from entry of the larva into host tissue, which can slowly migrate and contains a single worm-like sparganum (Fig. 113.7). If the worm enters via a dressing placed over the eye, migration into the brain may occur along the optic nerve, with occasional devastating results. Treatment consists of surgical excision.

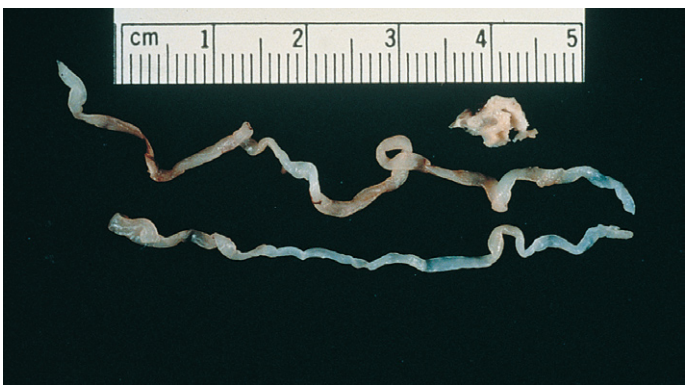


Fig. 113.7 Sparganum worm dissected from an inguinal mass. Courtesy of Guy Baily.

Trematode Infections

LIVER FLUKE INFECTIONS

Epidemiology

Clonorchis sinensis, Opisthorchis viverrini and *Opisthorchis felineus* are three closely related species which, for clinical purposes, can be considered as one. *C. sinensis* (Fig. 113.8) is the most common of the three, with over 25 million people infected in Japan, Korea, China, Cambodia, Laos and Vietnam. *O. viverrini* is found in northern Thailand and Laos, whereas *O. felineus* occurs throughout the Philippines, Japan, Vietnam, India and eastern Europe. All three species are acquired by ingesting raw or undercooked fresh water fish or crab harboring metacercariae, with dogs and cats serving as the most common reservoir hosts. Adult worms living in definite hosts produce eggs that are excreted in feces. Eggs that reach fresh water are eaten by the intermediate aquatic snail host, stimulating the miracidia to hatch and develop; motile cercariae subsequently released from the snails encyst under the scales of appropriate fresh water fish or crustaceae, completing the life cycle.

Pathogenesis and pathology

Ingested larvae excyst in the small intestine and transform into immature flukes that then migrate into the biliary tract, where they develop into hermaphroditic adult flukes that begin producing eggs after about 4 weeks. Adult worms may survive for decades within small bile ducts in the liver, where they feed on epithelium. Pathologic consequences arise from the mechanical injury and eosinophilic inflammatory reaction caused by adult worms present in the bile ducts. Over time, heavy infections lead to desquamation of the biliary epithelium, leading to hyperplasia, and eventual fibrosis or metaplastic changes that can lead to cholangiocarcinoma. This is especially true for heavy *O. viverrini* infections that may be associated with a 15-fold increase in the risk of developing cholangiocarcinoma.[10]

Clinical features

Most liver fluke infections are light and asymptomatic. Heavier infections may be associated with nonspecific epigastric or right upper quadrant pain, nausea, anorexia and diarrhea.[11] Heavy infections are also associated with recurrent ascending cholangitis and pancreatitis due to secondary bacterial infection and stone formation in chronically infected, fibrosed bile ducts.

Diagnosis and management

Eggs of liver flukes can be detected by microscopic examination of a concentrated sample of feces; differentiation between the eggs of the various species is often difficult. Eosinophilia and elevated levels of serum IgE are often present in heavy infections. On ultrasound, aggregates of flukes may be visualized as echogenic foci within bile ducts. Treatment with praziquantel 75 mg/kg/day in three doses for 2 days is highly effective, as is albendazole for 7 days.



Fig. 113.8 An adult liver fluke (*Clonorchis sinensis*). The worm is typically about 2 cm in length. Courtesy of Guy Baily.

FASCIOLIASIS

Humans are occasionally infected with the sheep liver fluke, *Fasciola hepatica* (Fig. 113.9), or the closely related cattle fluke, *F. gigantica*. Both are acquired by eating contaminated leafy wild plants (especially watercress) that grow in standing bodies of fresh water. *F. hepatica* is found worldwide, whereas *F. gigantica* is endemic in Africa and Asia. After excysting in the small intestine, metacercariae penetrate the intestinal wall and migrate to the surface of the liver which they penetrate to enter the parenchymal tissue. Migration of the immature worms to the liver can be associated with fever, eosinophilia and painful hepatomegaly that usually subsides within a month.[12] Ingested metacercariae occasionally migrate to tissues other than the liver such as the brain or kidney, where they may present as a small mass lesion.

Microscopic identification of eggs in the feces is the definitive method of diagnosis, although the sensitivity can be low because eggs are produced only intermittently. Ultrasonography or CT scan may reveal hepatic or biliary abnormalities due to fascioliasis. If no eggs are found in the stool, diagnosis can be made using serology (an ELISA), even in the acute stage of infection, although there is some cross-reactivity with antigens of other trematodes.[13] *Fasciola* is the only human flatworm that is not effectively treated with praziquantel. Bithionol is the recommended treatment, although side-effects are frequent and it is not readily available. Nitazoxanide and the veterinary drug triclabendazole have also been successfully used to treat fascioliasis.

LUNG FLUKE INFECTIONS

Epidemiology

The lung fluke, *Paragonimus westermani*, is found throughout eastern Asia and infects a wide variety of mammalian reservoir hosts. Numerous fresh water crustaceans such as crabs and crayfish serve as intermediate hosts and infection occurs when these are eaten raw or lightly cooked. Several other members of the *Paragonimus* genus routinely cause human infection, mostly in areas of eastern Asia, central and western Africa, and Latin America.

Pathogenesis and pathology

Ingested metacercariae excyst in the small intestine and penetrate into the abdominal cavity where they develop into immature flukes before crossing the diaphragm to enter the lungs and mature into reproductive adults within 2–3 months. *P. westermani* usually lives as pairs of worms within a host-derived fibrous capsule at the periphery of the lungs. Pathology results from the local effects of established adult worms and the surrounding inflammatory reaction characterized by eosinophilic infiltration. Occasionally, worms develop in ectopic sites such as the brain, muscle, liver or skin. Other species of *Paragonimus* can cause significant tissue damage as the larvae migrate through the viscera.

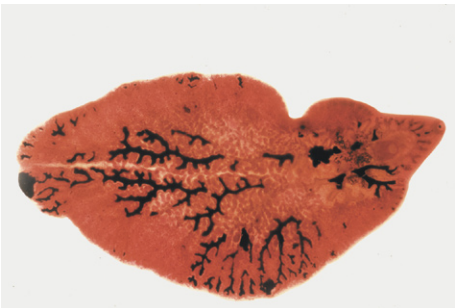


Fig. 113.9 A typical adult of *Fasciola hepatica*, measuring 30 × 14 µm.

Clinical features

Adult *P. westermani* worms may cause progressive disease characterized by chronic cough that is productive of blood-tinged sputum. Complications include recurrent bacterial pneumonia and lung abscess formation. Larval migration due to non-*P. westermani* species, especially in Asia, may give rise to migratory subcutaneous nodules and more seriously to an eosinophilic meningitis.[14]

Diagnosis and management

Diagnosis is by microscopic identification of eggs in the sputum or feces (due to swallowed sputum). Serologic tests are also available but do not differentiate between current and past infection. Chest radiography may reveal cavitary disease that must be distinguished from lung abscess due to other causes, especially tuberculosis. Eosinophilia is common. Praziquantel at a dose of 75 mg/kg in three divided doses for 2 days is the drug of choice and is effective against extrapulmonary disease.

INTESTINAL FLUKE INFECTIONS

Over 50 species of intestinal flukes have been reported in humans, but most cases are due to *Fasciolopsis buski*, *Echinostoma* and *Heterophyes* species. All are found throughout eastern Asia, whereas *Heterophyes* is also found in the Middle East and North Africa. All are zoonoses and infect humans only incidentally; for *F. buski*, the reservoir hosts include pigs and dogs. Humans acquire infection by consuming contaminated aquatic plants (especially bamboo shoots and water chestnuts for *F. buski*) or fish (*Echinostoma* and *Heterophyes*). Infections are usually asymptomatic, although heavy *Fasciolopsis* infections, often with more than 500 worms, can result in diarrhea alternating with constipation, abdominal pain, nausea and vomiting.[15] Intestinal obstruction or malabsorption may develop in the most severe cases.

Diagnosis is based on identification of characteristic operculated eggs on microscopic examination of feces. The treatment of choice for all intestinal flukes is praziquantel 75 mg/kg in three divided doses given in one day.

REFERENCES

References for this chapter can be found online at http://www.expertconsult.com

Chapter 114

Bruno Gottstein

Hydatid disease

Echinococcus spp. are cestode parasites commonly known as small tapeworms of carnivorous animals. Their medical importance lies in the infection of humans by the larval stage of the parasites, predominantly including two species:

- *Echinococcus granulosus*, which is the causative agent of cystic hydatid disease (or cystic echinococcosis, CE);[1] and
- *Echinococcus multilocularis*, which causes alveolar echinococcosis (AE).[2]

A few other species or genotypes, namely *E. equinus*, *E. ortleppi*, *E. vogel* (*vogeli*), *E. oligarthrus* and *E. shiquicus*, are only very rarely or not found in humans at all, and will thus not be covered in the present chapter.

EPIDEMIOLOGY

Echinococcus granulosus

Echinococcus granulosus parasitizes as a small tapeworm the small intestine of dogs and occasionally other carnivores. The shedding of gravid proglottids or eggs in the feces occurs within 4–6 weeks after infection of the definitive host (Fig. 114.1). Ingestion of eggs by intermediate host animals or humans results in the release of oncospheres into the gastrointestinal tract, which then migrate to primary target organs such as liver and lungs, and less frequently to other organs (Fig. 114.2). Usually, the fully mature metacestode (i.e. hydatid cyst) develops within several months or years.

Infections with *E. granulosus* occur worldwide, predominantly in countries of South and Central America, the European and African part of the Mediterranean area, the Middle East and some sub-Saharan countries, Russia and China. The annual incidence rates of diagnosed human cases/100 000 inhabitants vary widely, for example 13 in Greece, 143 in some provinces of Argentina, 197 in the Hinjang province of China and 220 in the Turkana district of Kenya. Most cases observed in Central Europe and the USA are associated with immigrants from highly endemic areas. Various strains of *E. granulosus* have been described, and differ especially in their infectivity for intermediate hosts such as humans. The most important strains for human infection include sheep (G1) and cattle (G5) as intermediate hosts.

Echinococcus multilocularis

The natural life cycle of *E. multilocularis* involves predominantly red and arctic foxes as definitive hosts (Fig. 114.3), but domestic dogs can also become infected and represent an important infection source for humans in highly endemic areas.[3] In the definitive host, egg production starts as early as 28 days after infection. After egg ingestion by a rodent or a human, larval maturation will occur practically exclusively within the liver tissue (see Fig. 114.2); subsequent metastases may affect adjacent or distant tissues, such as lungs or brain. Proliferation occurs by exogenous budding of metacestode tissue with a progressive tumor-like growth; central necrotic cavities may develop and cause differential diagnostic problems.

The geographic distribution of *E. multilocularis* is restricted to the northern hemisphere. In North America, the cestode is mainly present in the subarctic regions of Alaska and Canada, but an apparent expansion of distribution within the North–Central American continent was documented. In Europe, relatively frequent reports of AE in humans occur in central and eastern France, Switzerland, Austria and Germany. Within the past 10 years, the endemic area of Europe now includes many more countries such as Belgium, The Netherlands and Italy, and most former Eastern bloc countries as far up as Estonia. The Asian areas where *E. multilocularis* occurs include the whole zone of tundra, from the White Sea eastward to the Bering Strait, covering large parts of Russia, China and northern Japan.

Worldwide there are scant data on the overall prevalence of human AE. Some well-documented studies demonstrate a generally low prevalence among affected human populations. The annual mean incidence of new cases in different areas including Switzerland, France, Germany and Japan has therefore been reported to vary between 0.1 and 1.2/100 000 inhabitants.[1,2] The incidence of human cases correlates with the prevalence in foxes and the fox population density. Recently, a study documented that a fourfold increase of the fox population in Switzerland resulted in a statistically significant increase of the annual incidence of AE cases.[4]

PATHOGENESIS AND PATHOLOGY

Echinococcus granulosus

Cystic echinococcosis (cystic hydatid disease) is clinically related to the presence of one or more well-delineated spherical primary cysts, most frequently formed in the liver, but other organs such as the lungs, kidney, spleen, brain, heart and bone may also be affected (see Fig. 114.2). Tissue damage and organ dysfunction result mainly from this gradual process of space-occupying displacement of vital host tissue, vessels or parts of organs. Consequently, clinical manifestations are primarily determined by the site, size and number of the cysts, and are therefore highly variable. Accidental rupture of the cysts can be followed by a massive release of cyst fluid and hematogenous or other dissemination of protoscolices. This can result in anaphylactic reactions and multiple secondary cystic echinococcosis (as protoscolices can develop into secondary cysts within the same intermediate host).

The histology of a typical hydatid cyst exhibits the germinal layer as the primary site of parasite development (see Fig. 114.1). It is surrounded by a parasite-derived thick laminated layer, which is rich in aminocarbohydrates, as shown by periodic acid–Schiff (PAS) positivity. The germinal layer forms protoscolices and brood capsules within the

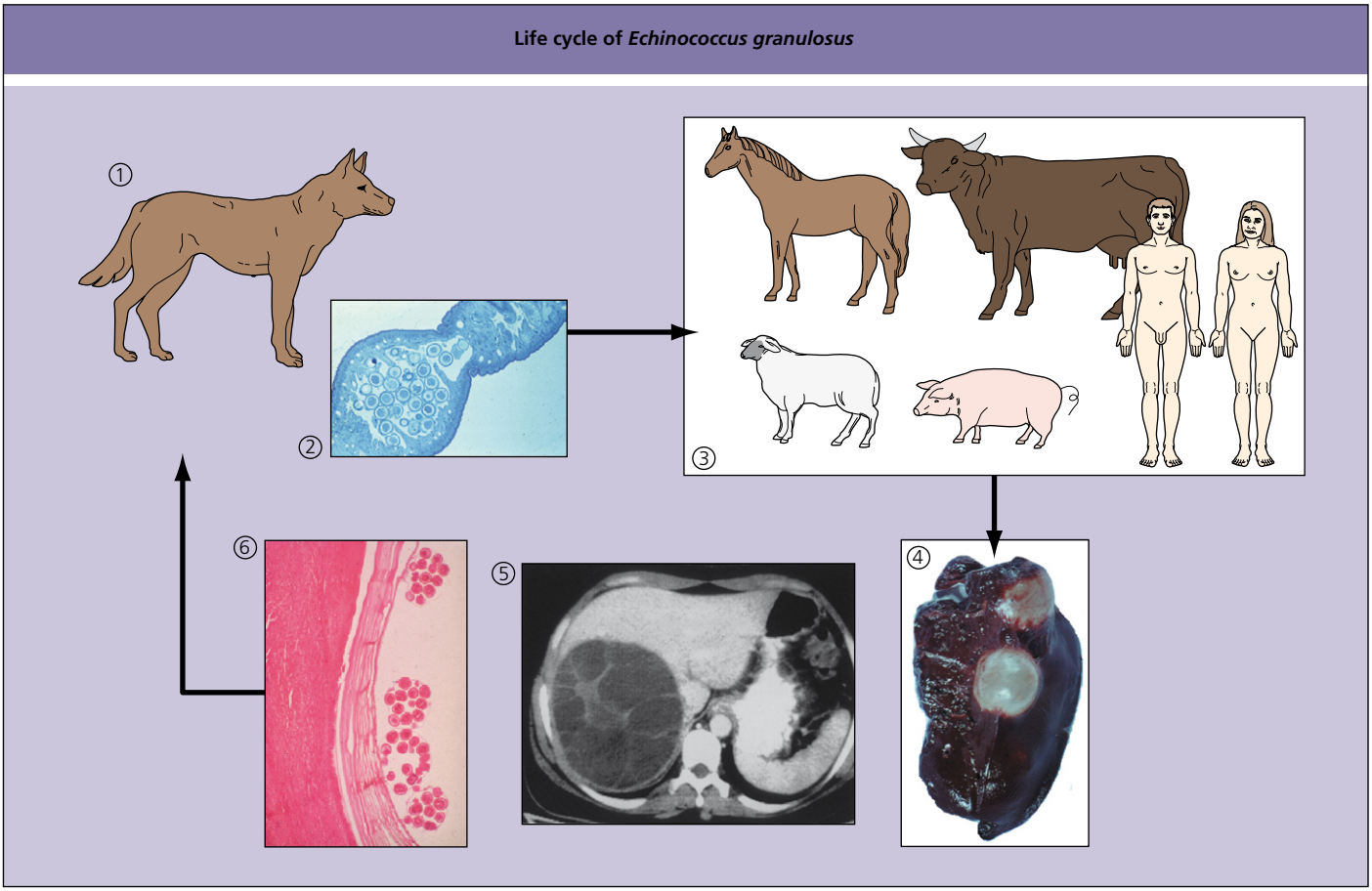


Fig. 114.1 Life cycle of *Echinococcus granulosus*. Adult tapeworms parasitize the small intestine of definitive hosts, mainly dogs (1). Parasite proglottids and eggs are shed with the feces (2), such eggs being infectious for intermediate hosts including humans (3). Hydatid cyst formation occurs predominantly in the liver (4), but also in lungs and other organs. Imaging techniques such as CT (5) demonstrate well-delineated, fluid-filled, usually unilocular bladder-like lesions. Internal daughter cysts may be visible in larger cysts as septated segments within the primary cyst. Histologically, the cyst by itself consists of a very thin inner germinal and nucleated layer with a predominantly syncytial structure (6). The germinal layer is externally protected by an acellular laminated layer of variable thickness. The endogenous formation of brood capsules and protoscolices is a prerequisite for completion of the life cycle (6), which occurs when definitive hosts ingest protoscolex-containing hydatid cysts.

cyst lumen. Granulae, calcareous corpuscles and occasionally free daughter cysts are often observed. The parasite evokes an immune response, which is involved in the formation of a host-derived adventitious capsule. This often calcifies uniquely in the periphery of the cyst, one of the typical features found in imaging procedures. In the liver there may be cholestasis. Commonly, there is pressure atrophy of the surrounding parenchyma. Immunologically, the coexistence of elevated quantities of interferon (IFN)-γ, interleukin (IL)-4, IL-5, IL-6 and IL-10 observed in most of hydatid patients supports Th1, Th17 and Th2 cell activation in CE. In particular, Th1 cell activation seemed to be more related to protective immunity, whereas Th2 cell activation was related to susceptibility to disease.[5]

Echinococcus multilocularis

In infected humans the *E. multilocularis* metacestode (larva) develops primarily in the liver (see Fig. 114.2). Occasionally, secondary lesions form metastases in the lungs, brain and other organs. The typical lesion appears macroscopically as a dispersed mass of fibrous tissue with a conglomerate of scattered vesiculated cavities with diameters ranging from a few millimeters to centimeters in size. In advanced chronic cases, a central necrotic cavity containing a viscous fluid may form, and rarely there is a bacterial superinfection. The lesion often contains focal zones of calcification, typically within the metacestode tissue and not in the periphery as in CE.

Histologically, the hepatic lesion is characterized by a conglomerate of small vesicles and cysts demarcated by a thin PAS-positive laminated layer with or without an inner germinative layer (see Fig. 114.3). Parasite proliferation is usually accompanied by a granulomatous host reaction, including vigorous synthesis of fibrous and germinative tissue in the periphery of the metacestode, as well as necrotic changes centrally. In contrast to lesions in susceptible rodent hosts, lesions from infected human patients rarely show protoscolex formation within vesicles and cysts.

Genetic and immunologic host factors are responsible for the resistance shown by some patients in whom there is an early 'dying out' or 'abortion' of the metacestode.[6] Therefore, not everyone infected with *E. multilocularis* is susceptible to unlimited metacestode proliferation, developing symptoms on average within 5–15 years after infection.[2,3] The host mechanisms modulating the course of infection are most likely of an immunologic nature, including primarily suppressor T-cell interactions. Thus, the periparasitic granuloma, mainly composed of macrophages, myofibroblasts and T cells, contains a large number of $CD4^+$ T cells in patients with abortive or died-out lesions, whereas in patients with active metacestodes the number of $CD8^+$ T cells is increased. An immunosuppressive process is assumed to downregulate the lymphoid macrophage system. Conversely, the status of cured AE is generally reflected by a high *in-vitro* lymphoproliferative response. The cytokine mRNA levels following *E. multilocularis* antigen stimulation of lymphocytes show an enhanced production of

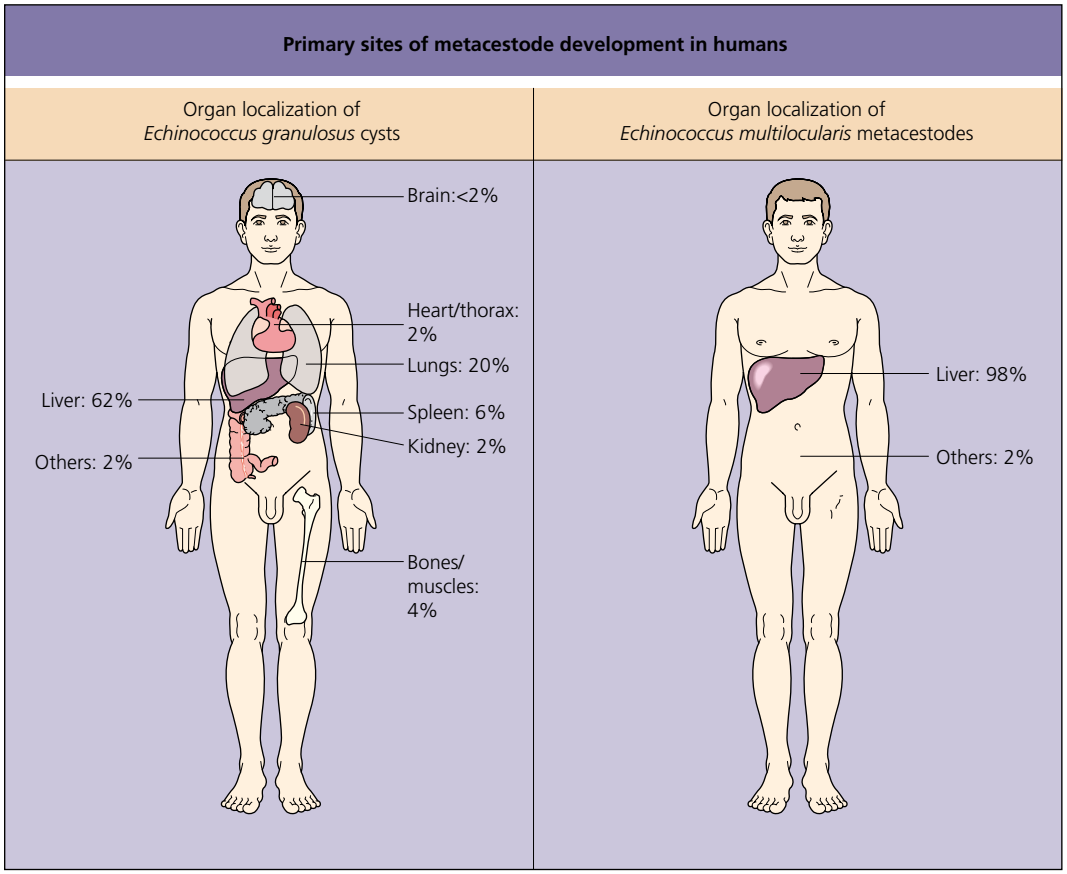


Fig. 114.2 Primary sites of metacestode development in humans. Organ distribution of the primary sites of metacestode development for *Echinococcus granulosus* (cystic echinococcosis) and *Echinococcus multilocularis* (alveolar echinococcosis) in human disease.

Th2-cell cytokine transcripts of IL-3, IL-4 and IL-10, including significant IL-5 mRNA expression in patients and not in healthy control donors.[7] A lack or deficiency of T helper (Th) cell activity such as in advanced AIDS is associated with a rapid and unlimited growth and dissemination of the parasite in AE,[8] and recovery of the T cell status in AIDS is prognostically favorable.[9]

PREVENTION

Prevention of both CE and AE focuses primarily on veterinary interventions to control the extent and intensity of infection in definitive host populations, which may be approached indirectly by controlling the prevalence in animal intermediate hosts. This includes regular pharmacologic treatment and taking sanitary precautions for handling domestic dogs to prevent infection and egg excretion, respectively.[10] Regular praziquantel treatment of wild-life definitive hosts may contribute to lowering of the prevalence in affected areas.[11] Finally, a vaccine to protect ruminant intermediate hosts from *E. granulosus* infection is available.[12]

CLINICAL FEATURES

The initial phase of primary infection is always asymptomatic. The infection may then remain asymptomatic for years or even decades depending upon the size and site of the developing cyst or metacestode mass. After a highly variable incubation period, the infection may become symptomatic due to a range of different events.

Cystic echinococcosis

The growing cyst can exert pressure on or induce dysformation of adjacent tissues, thus inducing dysfunction of the affected organ or vascular compromise. In the case of hepatic CE, signs and symptoms may include hepatomegaly with or without a palpable mass in the right upper quadrant, right epigastric pain, nausea, vomiting and occasionally cholestatic jaundice. In inoperable cases, hepatic compromise may lead to biliary cirrhosis and the Budd–Chiari syndrome.

Infestation of the lungs may present with chronic cough, hemoptysis, bilioptysis, pneumothorax, pleuritis, lung abscess and parasitic lung embolism.

Rare but often catastrophic infestations can affect the heart or the brain. In the heart this can present as a tumor, pericardial effusion including tamponade, complete heart block and sudden death. In the spine and brain presentation is as a tumor with neurologic symptoms. Hydatid disease should be considered as a cause of stroke in young patients.

A cyst may rupture and spill its content into the adjacent site. Rupture into the biliary tree will mimic biliary colic or result in cholestatic jaundice and cholangitis or pancreatitis. This is the presenting symptom in 5–25% of patients. Rupture in the liver but also in the lungs and other organs may result in acute anaphylactic shock reactions, which usually represent the initial and life-threatening manifestation. Each spilled protoscolex can develop into a secondary cyst. This is the most frequent reason for relapses that occur after surgical interventions.

A cyst can become superinfected; in hepatic CE this occurs in about 9% of patients and is an indication for rapid surgical intervention.[13]

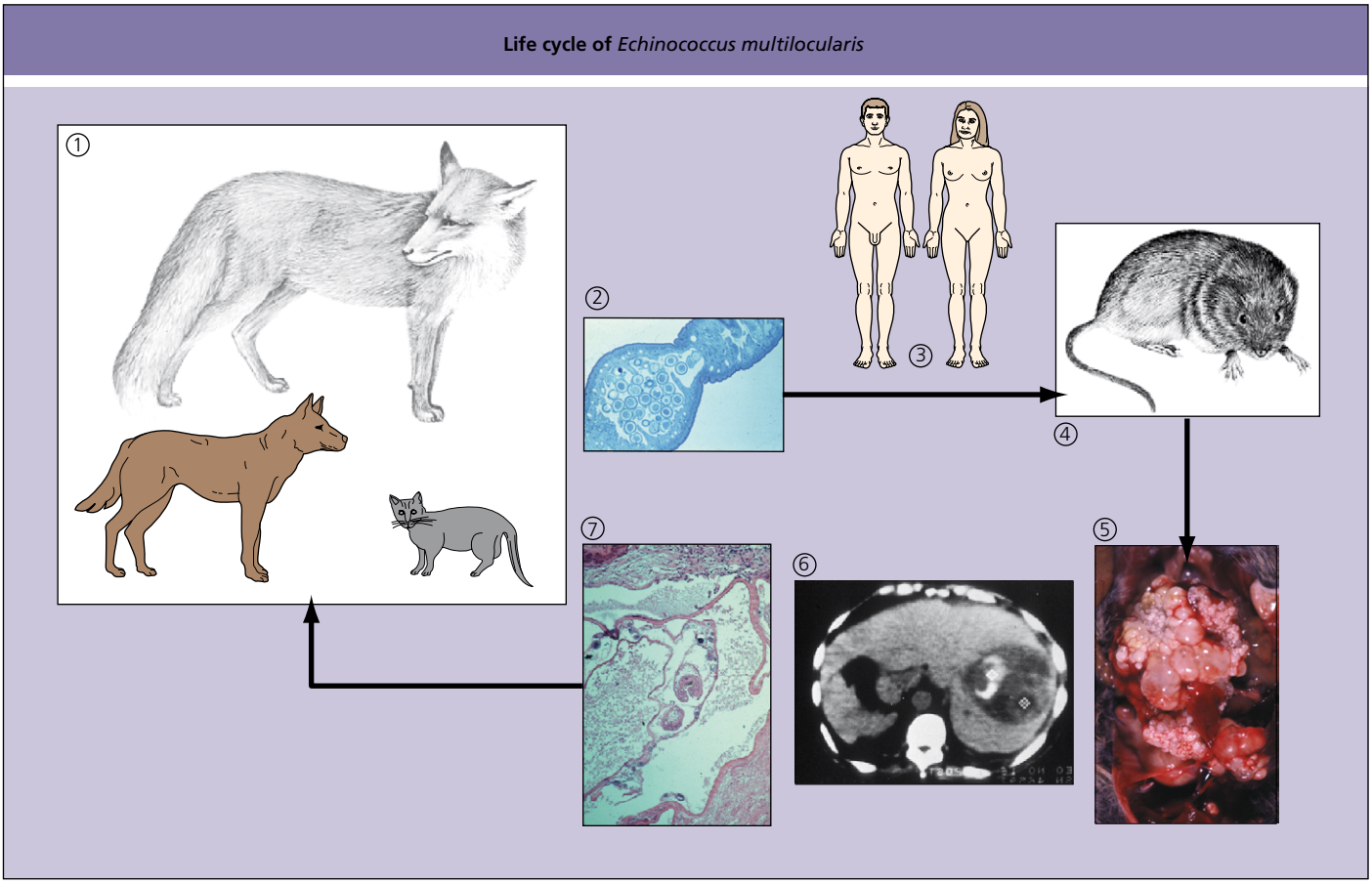


Fig. 114.3 Life cycle of *Echinococcus multilocularis*. This involves predominantly foxes as definitive hosts (1) and occasionally other carnivores such as domestic dogs or house cats. Egg production by the tapeworm starts as early as 28 days after infection (2). Eggs must be ingested by a suitable intermediate host, including humans (3) and various rodent species (4). As a result, the parasite metacestode primarily becomes established in the liver. Macroscopically, the typical lesion is characterized by a dispersed mass of fibrous tissue with a multitude of interconnected vesicles ranging from a few millimeters to centimeters in size (5). The lesion often contains focal necrotic zones with scattered calcifications, as demonstrated by CT (6). Histologically, the hepatic lesion consists of a conglomerate of small vesicles and cysts demarcated by a thin laminated layer with or without an inner germinal layer and, predominantly in the rodent intermediate host, protoscolex formation (7). Oral ingestion of protoscolex-containing metacestodes by definitive hosts completes the life cycle.

The majority of patients with CE have single organ involvement with solitary cysts. Simultaneous involvement of two or more organs is observed in 10–15% of patients, depending on the geographic origin of the patient and the strain of the parasite. In hepatic CE, the right lobe is more frequently affected than the left. Cyst size usually varies between 1 and 15 cm in diameter. Cyst growth ranges between a size increase of a few millimeters (1/3 of the patients) to approximately 10 mm (most of the patients); 1/10 of the patients exhibit a rapid increase with an annual average of 30 mm. In Europe, the average age of patients at diagnosis is 36 years. Approximately 10% of the CE cases occur in children, and the rate of lung involvement is significantly increased among this group. Pulmonary cysts occasionally become superinfected and this is best detected by CT scanning. The ratio of males to females may vary depending on the geographic area but is statistically not significant overall.

Hepatic alveolar echinococcosis

In patients with hepatic AE, the size of the liver lesion will range between a few millimeters up to 50 cm or more when patients present. A classification of the different types and stages of AE has recently been released.[14] Typical calcifications occur in 70% of cases and central or peripheral necrotic cavities are also found in approximately 70% of cases. Clinical signs at diagnosis include hepatomegaly–cholestasis–jaundice, secondary biliary cirrhosis, liver abscess, portal hypertension and Budd–Chiari syndrome. The disease starts frequently with nonspecific symptoms such as epigastric pain or cholestatic jaundice. In complicated cases, evidence of secondary biliary cirrhosis and/or cholangitis will be found. Evidence of cholestasis is frequently present, while transaminases are only rarely and moderately elevated, in particular when there is central necrosis. One of the most feared complications is infection of a necrotic cavity and/or obstructed bile ducts, which are associated with very high mortality due to development of septic shock. Distant metastases can occur late in the disease; these have been described in brain, spine, lung and bone. Metastatic disease occurs in approximately 10–20% of patients.

The growth rate of the metacestode tissue is usually slow in immunocompetent patients. Analysis by CT scans indicated an average volume increase of 15 ml/year for progressive forms of AE. In Europe, the average age of AE patients at diagnosis is 55 years.[2] Young children rarely develop AE, unless the cellular immune system is compromised.[8] The ratio of males to females varies geographically, but any variation is not statistically significant.

DIAGNOSIS

Echinococcus granulosus

In many cases, imaging procedures together with serology will yield the diagnosis. Sonography is the primary diagnostic procedure of choice for hepatic cases, although false-positives occur in up to 10% of cases due to the presence of nonechinococcal serous cysts, abscesses or tumors. An international classification of ultrasound images in CE has been released.[15] The main diagnostic features of hydatid disease include:

- separation of the membrane from the wall;
- daughter cysts; and
- ruptured cysts.

Computed tomography is the best investigation for detecting extrahepatic disease and volumetric follow-up assessment; MRI assists in the diagnosis by identifying changes in the intra- and extrahepatic venous systems. Ultrasonography is also helpful in following up treated patients as successfully treated cysts become hyperechogenic. Calcification of variable degree occurs in about 10% of the cysts.

Aspiration cytology appears to be particularly helpful in the detection of pulmonary, renal and other nonhepatic lesions for which imaging techniques and serology do not provide appropriate diagnostic support. The viability of aspirated protoscolices can be determined by microscopic demonstration of flame cell activity and trypan blue dye exclusion. Anti-Ag5 monoclonal antibody has been used for the detection of the respective antigen in diagnostic fine-needle aspiration biopsies (FNABs) from patients with suspected CE.[16] Immunodiagnostic tests to detect serum antibodies are used to support the clinical diagnosis of CE.[17] The indirect hemagglutination tests and the enzyme-linked immunosorbent assay using *E. granulosus* hydatid fluid antigen are diagnostically relatively sensitive for hepatic cases (85–98%). For pulmonary cysts and for cases in children the diagnostic sensitivity is markedly lower (50–60%); multiple organ involvement yields a very high sensitivity (90–100%). These tests are usually used for primary serologic screening. Specificity is low for other cestode infections. To increase specificity, primary seropositive sera are retested using a confirmation test such as immunoblotting for 8 kDa/12 kDa subunits of *E. granulosus* antigen B.[17]

Serologic studies to follow-up patients with CE postoperatively have shown that only a few antigens correlate with the activity status of a treated cyst, including antigen B[18] and antigen P29.[19]

Besides skin tests and basophil degranulation tests, diagnostic cellular immunodiagnosis has focused on *in-vitro* lymphoproliferative responses to *E. granulosus* antigens. The diagnostic sensitivity of cell-mediated immunodiagnosis is 75%, including the finding of seronegative patients with a positive proliferation test.[17]

Echinococcus multilocularis

Among the imaging procedures, ultrasonography, CT and MRI are of greatest diagnostic value, none of those being uniquely superior.[20] Irregularly dispersed clusters of calcifications on plain abdominal radiographs may give the first clue as to the etiology of the disease; the percentage of calcifications within the lesions increases from 30% to nearly 100% as the disease progresses. Hyperechogenic and hypoechogenic zones characterize the lesions. The cystic appearance may reflect central necrotic cavities. Similar findings can be found on CT and the lesions are typically not enhanced with contrast medium. The lesions are heterogeneous hypodense masses with irregular contours and lacking well-delineated walls. Hilar involvement can lead to liver atrophy, which is easily visualized by CT. Ultrasonography is the preferred imaging procedure for mass screening programs. Magnetic resonance imaging adds to diagnosis, in particular in cases with appropriate organ localization such as brain and bone, and to visualize pathologically altered microstructures in certain affected organs. Thus, MRI can give a precise analysis of the different components of the parasitic lesions such as necrosis and fibrosis.[20] However, in contrast to CT, microcalcifications are not visualized by MRI.

Assessing the parasite viability *in vitro* following therapeutic interventions may be of tremendous advantage when compared with the invasive analysis of resected or biopsied samples. Such alternatives may be offered by magnetic resonance spectrometry or positron emission tomography (PET). The latter technique has recently been used for assessing the efficacy of chemotherapy in AE.[21] PET positivity actually demonstrates periparasitic inflammatory processes due to remaining activity of the metacestode tissue.

Immunodiagnosis represents a valuable secondary diagnostic tool complementary to imaging procedures and is useful for confirming the nature of the etiologic agent.[17] Serologic tests are more reliable in the diagnosis of AE than CE. The use of purified *E. multilocularis* antigens such as the Em2 antigen and recombinant antigens from the family of EMR proteins (EmII/3-10, EM10, EM4 and Em18, all four of them harboring an identical immunodominant oligopeptide sequence) exhibits diagnostic sensitivities ranging between 91% and 100%, with overall specificities of 98–100%.[17] These antigens allow discrimination between the alveolar and the cystic forms of disease with a reliability of 95%. Seroepidemiologic studies reveal asymptomatic preclinical cases of human AE as well as cases in which the metacestode has died at an apparently early stage of infection (see above).[3] Serologic tests are of value for assessing the efficacy of treatment and chemotherapy only when linked to appropriate imaging investigations. Prognostically, disappearance of anti-II/3-10 or anti-Em18 antibody levels coupled to PET negativity indicates inactivation of AE.[22] Cellular immune tests show that the *in-vitro* lymphoproliferative response to *E. multilocularis* antigen stimulation is high in cured patients who have had radical surgery and in patients with dead lesions, and is significantly lower in patients who have had partial or no surgical resection.

Histopathologic and immunohistochemical procedures to analyze surgically resected samples or biopsies obtained by FNAB include the use of species-specific MAbs such as MAbG11 or polymerase chain reaction.[16]

MANAGEMENT

The management of CE and AE follows the strategy recommended in the manual on echinococcosis published in 2001 by the Office International des Epizooties and the World Health Organization.[10]

Echinococcus granulosus

Surgery remains the mainstay in the treatment of hepatic hydatid disease. Cystectomy and pericystectomy offer a good chance for cure and should be undertaken wherever possible. Occasionally, formal hepatic resection will be required. Radical surgery – either pericystectomy or resection – is possible in 50–85% of cases. In the absence of complications this can be achieved with little mortality and an acceptable morbidity. Laparoscopic pericystectomy has been demonstrated to be as safe and effective as open laparotomy in selected cases with hepatic and/or splenic involvement.[23]

If surgical removal of the cyst is contraindicated, treatment of CE has several alternatives such as PAIR (Puncture, Aspiration, Injection of a helminthicide and Re-aspiration), chemotherapy or 'wait and observe' approach.

Indications for hepatic surgery include:

- large liver cysts with putatively multiple daughter cysts;
- single liver cysts, situated superficially, which may rupture spontaneously or as a result of trauma;
- bacterially superinfected cysts;
- cysts communicating with the biliary tree and/or exerting pressure on adjacent vital organs;
- brain, heart and kidney cysts, and spinal and bone cysts.

Relative contraindications are inoperable cases as defined for surgical procedures in general, patients with cysts difficult to access and abortive cysts that are either partly or totally calcified.

A direct communication between the hydatid cyst and the biliary tree may contraindicate the use of protoscolicidal solutions, which can cause chemical cholangitis leading to sclerosing cholangitis. Formalin should not be used for this reason. Protoscolicides with a relatively low risk of toxicity are 70–95% ethanol or 15–20% hypertonic saline solution; effective killing of protoscolices is only achieved after a long-term contact with the agent. Thus, double percutaneous aspiration and injection of alcohol into the cyst without re-aspiration of the ethanol, which remains *in situ*, may prove more efficacious than PAIR (see below).[24]

Preoperative chemotherapy with albendazole for up to 3 months (with a minimum of 4 days) is indicated for reducing the risk of secondary echinococcosis after operation.[25] Therapy may additionally include praziquantel that exhibits a good antiprotoscolicidal activity. Diagnostic puncture of hydatid cysts increases the risk of cyst rupture and dissemination of protoscolices and is therefore not recommended.

PAIR has become well justified in selected cases (Table 114.1).[26]

Treatment with benzimidazoles (preferably albendazole) is highly recommended for 4 days prior to intervention. After successful instillation of protoscolicides and re-aspiration, benzimidazoles should be given for 3 months.

Treatment of nonresected cysts with benzimidazoles (albendazole or mebendazole) results in cyst disappearance in 30% of cases; in 30–50% of patients there is cyst degeneration or a significant reduction in cyst size and in 20–40% of patients the cysts show no morphologic change.[10] Indications for chemotherapy include inoperable patients as listed above.

The formerly conventional dosage of albendazole (10–15 mg/kg/day in several 1-monthly courses with 14-day intervals) included three courses at minimum, and more than six courses were usually not necessary. This strategy is more commonly replaced by continuous treatment, which demonstrated equal or improved efficacy without increased adverse effects when compared with cyclic treatment.[27] For mebendazole, the usual dosage is 40–50 mg/kg/day for at least 3–6 months. Praziquantel has been proposed as an additional antiprotoscolicidal drug to be given once a week in a dose of 40 mg/kg along with benzimidazoles. It is also recommended before and after surgery/PAIR when there is a risk of cyst rupture and release of protoscolices.

Table 114.1 Indications and relative contraindications for PAIR

Indications
• Patients refusing surgery
• Infected cysts not communicating with the biliary vessel system
• Inoperable patients (see contraindications for surgery, above)
• Pregnant patients
• Anechoic lesion ≥5 cm in diameter
• Cysts with a regular double laminated layer
• Cysts with more than five septal divisions
• Multiple cysts (≥5 cm in diameter) in different liver segments
• Relapse after surgery
• Failure to respond to chemotherapy
Relative contraindications
• Inaccessible or risky location of the cyst in the liver
• Multiple septal divisions
• Cysts with echogenic lesions
• Inactive cysts or calcified lesions
• Communicating cysts; cysts located in the lung and bones; and some others
• Presence of exophytic cysts or dilated bile ducts on preoperative imaging

Echinococcus multilocularis

The following strategies are commonly accepted for treatment of AE:

- the first choice of treatment is radical surgical resection of the entire parasitic lesion from the liver and other affected organs in all operable cases, with excision of the parasitic lesion following the rules of radical tumor surgery;
- concomitant chemotherapy is mandatory for all cases after radical surgery or after nonsurgical interventional procedures;[27] and
- long-term chemotherapy for inoperable or only partially resectable cases and all patients after liver transplantation.[10]

Presurgical chemotherapy is not indicated for AE. The daily dosage for albendazole and mebendazole treatment is the same as for CE. For albendazole, continuous treatment is well tolerated for a duration up to 6 years, and is replaced by the former discontinuous scheme (see above) only in cases with side-effects related to medication. For mebendazole, plasma drug levels should be over 74 ng/ml (250 nmol/l). Generally, the duration of treatment is at least 2 years after radical surgery or continuously for many years for inoperable cases or if resection is incomplete. In experimental trials, alternative compounds have been used to treat AE. Experimental studies included amphotericin B[28] and nitazoxanide.[29]

As an ultimate goal, liver transplantation has been proposed for a selected group of patients who have inoperable AE and chronic liver failure. However, the indications are limited and focus on cases with extensive lesions restricted to the liver and secondary liver disease leading to chronic liver failure;[30] relapse is frequent and caused by extrahepatic metacestodes, which rapidly proliferate under immunosuppressed conditions.

REFERENCES

References for this chapter can be found online at http://www.expertconsult.com

Chapter 115

Eric A Ottesen

Filariasis

INTRODUCTION

Lymphatic filariasis (LF), onchocerciasis and loiasis are the three most important filarial infections of humans (Table 115.1) and for two of these (lymphatic filariasis and onchocerciasis) there are now major public health initiatives to control or eliminate them completely.[1–3] All of these infections are caused by parasites transmitted by biting arthropods (mosquitoes or flies). Each goes through a complex life cycle that includes a slow maturation (often 3–12 months) from the infective larval stage carried by the insects, to the adult worm that resides either in the lymph nodes and adjacent lymphatics or in the subcutaneous tissue. The offspring of the adults, the microfilariae, are 200–350 µm in length and either circulate in the blood (lymphatic filariasis and loiasis) or migrate through the skin (onchocerciasis), awaiting ingestion by the insect vectors in which they develop infectivity for humans to continue this complex life cycle.

EPIDEMIOLOGY

Lymphatic filariasis

There are 120 million people in at least 80 countries of the world who are infected with lymphatic filarial parasites, and it is estimated that 1.2 billion people (20% of the world's population) are at risk of acquiring infection.[1] Of these infections, 90% are caused by *Wuchereria bancrofti*, whose only host is humans, and most of the remainder are caused by *Brugia malayi*. The major vectors for *W. bancrofti* are culicine mosquitoes in most urban and semiurban areas, anopheline mosquitoes in the more rural areas of Africa and elsewhere, and *Aedes* spp. in many of the endemic Pacific islands. For the *Brugia* parasites, *Mansonia* spp. serve as the major vector, but in some areas anopheline mosquitoes are responsible for transmitting the infection. *Brugia* parasites are confined to areas of eastern and southern Asia, especially India, Malaysia, Indonesia and the Philippines.

Onchocerciasis

There are almost 18 million people in 37 endemic countries who are infected with *Onchocerca volvulus*; 99% of these people live in sub-Saharan Africa.[2] Humans are the exclusive host for *O. volvulus*; there is no nonhuman reservoir of infection. Blackflies of several *Simulium* species are the vectors of infection, and because the larvae of these flies require fast-flowing water for their development, transmission is limited to those areas within the flight range of the flies that breed in such rivers. Thus, the distribution of onchocerciasis ('river blindness') is very much determined by river systems and tributaries in Africa, Yemen and the Americas, where the infection is endemic.

Loiasis

Loa loa infects approximately 12 million people in the rainforest belt of western and central Africa and equatorial Sudan, where the *Chrysops* vector flies can easily find breeding spots.[3] Although *L. loa*

Table 115.1 Principal filarial diseases

Disease	Number of people infected worldwide	Parasite	Vector	Principal clinical manifestation	Distribution	Location of micro-filariae within the body	Periodicity
Lymphatic filariasis	120 million	*Wuchereria bancrofti*	Mosquitoes	Lymphedema, elephantiasis, genital pathology (hydrocele)	Tropics worldwide	Blood	Nocturnal (95%), 'subperiodic' (5%)
		Brugia malayi	Mosquitoes	Lymphedema, elephantiasis	Asia, India, Philippines	Blood	Nocturnal (75%), 'subperiodic' (25%)
Onchocerciasis	18 million	*Onchocerca volvulus*	Blackflies	Dermatitis, blindness	Africa (99%), Americas, Yemen	Skin	Minimal
Loiasis	12 million	*Loa loa*	Deer flies	Angioedema, 'eyeworm'	Africa	Blood	Diumal

can be found in a number of nonhuman African primates, there is little or no cross-infection between the cycles of *L. loa* in humans and in nonhuman primates.

PATHOGENESIS AND PATHOLOGY

Pathology associated with the filariases results from a complex interplay of the pathogenic potential of the parasite (and its endosymbionts), the immune response of the host and external ('complicating') bacterial and fungal infections.[1–6]

Lymphatic filariasis

Although genital abnormalities (especially hydrocele) and lymphedema or elephantiasis are the most recognizable clinical entities associated with lymphatic filarial infections (Fig. 115.1), earlier changes can be detected when lymphoscintigraphy or ultrasound techniques are used,[4] including lymphatic dilatation with abnormal lymphatic function. Such subclinical changes can be seen even in young children[7] and can occur with or without a localized inflammatory response. Secondary host inflammation, including responses to bacterial and fungal superinfections of tissues with compromised lymphatic function, causes most of the progression and physical destruction associated with elephantiasis. Endotoxins released when the *Wolbachia* endosymbionts of the parasite are killed during natural or drug-induced parasite death may also play a role in inducing this local inflammation.

The pathology in lymphatic filariasis that is immune mediated most commonly derives from the lymphatic dysfunction consequences of the responses to dead or dying worms in the lymphatics; however, for the syndrome of tropical pulmonary eosinophilia, the pathogenesis is distinctly different,[8] the inflammation resulting from immunologic (allergic) hyperresponsiveness to the microfilaria-stage parasites.

Onchocerciasis

Most of the significant pathology in onchocerciasis affects the skin (Fig. 115.2) or the eyes.[2] There are both noninflammatory and inflammatory routes to tissue damage.[5] In 'steady-state' chronic *O. volvulus* infections, between 10 000 and 500 000 microfilariae are produced each day. These microfilariae migrate through the skin, inducing little inflammatory response; however, the elaboration of collagenases, elastases and other enzymatic molecules leads to hyperpigmentation, atrophy and thinning of the skin (see Fig. 115.2). Superimposed inflammatory reactions to dying microfilariae result in episodic papular dermatitis. In the eyes, these inflammatory responses result either in a characteristic punctate keratitis and, ultimately, anterior segment blindness or in uveitis and retinal lesions that can lead to posterior segment blindness. Again, the endosymbiont bacterium (*Wolbachia* spp.) within *O. volvulus* adult worms and microfilariae might also play a role in the pathogenesis of onchocerciasis skin and eye disease.[9]



Fig. 115.2 Onchocerciasis. Evidence of excoriation caused by the patient's trying to relieve the maddening pruritus caused by onchocerciasis. Note also the marked dermal atrophy associated with chronic infection.

Loiasis

'Calabar' swellings, the angioedematous lesions characteristic of loiasis, have been less well studied. Presumably, these are immune-mediated inflammatory responses to migrating subcutaneous adult worms (Fig. 115.3). Not common in untreated patients, but increasingly problematic in populations receiving ivermectin for co-endemic onchocerciasis, is a central nervous system (CNS) depression syndrome leading to coma or even death.[3] Its pathogenesis is suspected to involve inflammatory responses to dying microfilariae in cerebral vessels, but the details remain uncertain.

PREVENTION

Filarial infections can be acquired only from vector-borne infective larvae. Therefore, prevention of infection can be achieved either by decreasing contact between humans and vectors, generally through vector control efforts, or by decreasing the amount of infection the vector can acquire to pass on to uninfected individuals, through treating the human host.



Fig. 115.1 Elephantiasis. (a) Already advanced elephantiasis in a 14-year-old Indian girl who has bancroftian filariasis. Although such clinical expression of filarial disease is more commonly seen in adults, infection in endemic areas is usually established in early childhood. (b) Scrotal elephantiasis in an adult man who has bancroftian filariasis.

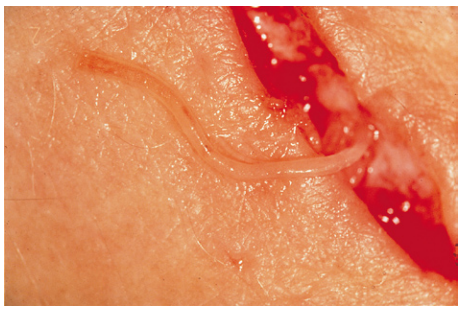


Fig. 115.3 *Loa loa* adult worm. The worm has been teased from the subcutaneous tissue after incision was made through a small pruritic papule (0.5 cm in diameter) in an expatriate patient who had loiasis. Such papules can occur spontaneously or after treatment with DEC.

Lymphatic filariasis

Population-based prevention

Efforts to decrease filariasis in populations through mosquito-vector control have usually proved ineffective owing to high cost and the long lifespan of the parasite (4–8 years). With the advent of extremely effective single-dose, once-yearly, two-drug regimens (albendazole 400 mg plus either ivermectin 200–400 µg/kg or diethylcarbamazine (DEC) 6 mg/kg), the alternative approach of treating whole populations (and thereby decreasing microfilariae available to the vectors) has become the norm.[10] Indeed, it is this strategy that forms the basis of the new Global Program to Eliminate Lymphatic Filariasis undertaken by the World Health Organization (WHO) and a Global Alliance of public and private sector partners.[1,10]

Individual-based prevention

Contact with infected mosquitoes can be decreased through the use of personal insect repellents, bed nets or insecticide-impregnated materials. Alternatively, suggestive evidence from animal models and some limited experience in human populations indicate that a prophylactic regimen of DEC (6 mg/kg/day for 2 days each month) could provide effective protection against infection.

Onchocerciasis

Population-based prevention

Despite its considerable expense, a highly successful program to prevent onchocerciasis based on large-scale insecticiding to control the vector blackflies was undertaken in 11 West African countries by the WHO, the United Nations Development Program and the World Bank. In its 27 years of existence this 'Onchocerciasis Control Program' was extraordinarily successful in 'reclaiming' both land and lives of people otherwise severely compromised by onchocercal disease.[2] However, this expensive and difficult approach to population-based prevention of onchocerciasis has been superseded by a new strategy that both treats and prevents infection through the use of once-yearly ivermectin in affected populations of all countries where onchocerciasis is endemic.[2]

Individual-based prevention

Decreased contact with infected blackflies through protective clothing and repellents is helpful in preventing infection. Although no prophylactic treatment regimen has yet been defined, studies employing monthly doses of ivermectin in cattle exposed to the related parasite *O. ochengi* show a dramatic prophylactic effect of ivermectin,[11] but studies have not yet been undertaken to see whether similar prophylactic efficacy can be shown for ivermectin in humans as well.

Loiasis

Population-based prevention

No specific prevention efforts in populations have been undertaken.

Individual-based prevention

There are good data that repeated use of DEC (300 mg weekly or 6 mg/kg/day for 2 days each month in adults) is effective prophylaxis against acquisition of *L. loa* infection.[12]

CLINICAL FEATURES

Lymphatic filariasis

Chronic manifestations

Hydrocele, even though found only with *W. bancrofti* (and not *Brugia*) infections, is the most common clinical manifestation of lymphatic filariasis. It is seen principally after puberty and there is a progressive increase in prevalence with age.[13] In some endemic communities, as many as 40–60% of all adult males have hydrocele. It often develops in the absence of overt inflammatory reactions and, indeed, many patients who have hydrocele also have microfilariae circulating in the blood. The localization of adult worms in the lymphatics of the spermatic cord leads to a thickening of the cord so that the cord is palpable on physical examination of most patients. Hydroceles can become massive but still occur in the absence of any lymphedema or elephantiasis in the penis and scrotum (see Fig. 115.1).[14]

Although lymphedema can also develop in the absence of overt inflammatory reactions and in the early stages be associated with microfilaremia, the development of elephantiasis (either of the limbs or the genitals) is most frequently associated with a history of recurrent inflammatory episodes. Patients who have chronic lymphedema or elephantiasis are rarely microfilaremic. Very important in the progression of these lesions is the fact that the redundant skin folds, cracks and fissures of the skin provide havens for bacteria and fungi to thrive and intermittently penetrate the epidermis, leading to either local or systemic infections.

Chyluria, another of the chronic filarial syndromes, is caused by the intermittent flow of intestinal lymph (chyle) through ruptured lymphatics into the renal pelvis and subsequently into the urine. The mechanisms underlying this have not been well defined and the clinical course is known to be intermittent. Nutritional compromise can, however, be severe in patients who have chronic chyluria; special diets (low-fat and high-protein, supplemented with fluids) can often be helpful.[1]

Acute manifestations

There are four distinct acute manifestations of lymphatic filariasis, each with a different set of causative mechanisms and pathogenic implications.

The first and most important is acute inflammation of the limbs or scrotum (sometimes with systemic symptoms) that is related to bacterial or fungal superinfection of tissues with already compromised lymphatic function.[14]

Often confused with this picture in the past, a second type of acute syndrome is characterized by initiation of inflammation in the lymph nodes (commonly the inguinal or axillary node) with 'retrograde' extension down the lymphatic tract and an accompanying 'cold' edema. Here the inflammation appears to be immune mediated around dying parasites in the nodes; it is much less frequent than the episodes of inflammation initiated by dermal infection.[14]

A third acute filarial syndrome is tropical pulmonary eosinophilia (Weingarton's syndrome), a distinctly different syndrome caused by an immunologic hyperresponsiveness to filarial infection.[8] It is characterized by:

- extremely high levels of peripheral blood eosinophilia;
- asthma-like symptoms;
- restrictive (and often obstructive) lung disease;
- very high levels of specific antifilarial antibodies; and
- an excellent therapeutic response to appropriate antifilarial treatment with DEC.

While occurring with a frequency of less than 1% of all cases of clinical filariasis, it is nevertheless a severe condition that can lead to chronic interstitial fibrosis and pulmonary failure.

The fourth (and least commonly recognized) form of acute inflammatory reaction is that seen early after infection, particularly in expatriates who are exposed to and acquire filarial infection for the first time. Lymphangitis occurs around developing larval and early adult stages in these patients, associated with acute eosinophilic inflammation.[8]

Asymptomatic presentations

Of all the patients who have lymphatic filariasis, at least half appear clinically asymptomatic. Some of these have microfilariae circulating in their blood, while others have infections identifiable only by filarial antigen in the circulation. Essentially all have hidden damage to their lymphatic or renal systems.[7,15] A precise understanding of the natural history of these 'asymptomatic' infections is still not available, despite their obvious importance in the development of clinical disease.

A variety of other syndromes coexisting with filariasis are found in filarial-endemic regions; because they sometimes show evidence of therapeutic response to DEC they have been regarded as possible manifestations of lymphatic filariasis. These include arthritis (typically monoarticular), endomyocardial fibrosis, tenosynovitis, thrombophlebitis, lateral popliteal nerve palsy and others. Although future studies may strengthen an etiologic relationship with filariasis, such presentations cannot now be confidently attributed to lymphatic filarial infection.

Onchocerciasis

Chronic presentations

Most damage from onchocerciasis occurs in the skin and eye. Subcutaneous nodules (generally 1–6 cm in diameter) can be palpated superficially, but most skin involvement is a waxing and waning of maculopapular rashes, essentially always accompanied by itching (see Fig. 115.2),[2] likely because of allergic responses to dying microfilariae. During the long course of infection, the skin becomes extensively damaged, losing much of its elasticity and even pigmentation. Indeed, when the skin over the inguinal nodes (which are often enlarged by their continued stimulation from dying microfilariae) becomes so atrophic that it cannot support the underlying lymph nodes, the clinical presentation of 'hanging groin' occurs.

In the eye, acute changes are those associated with dying microfilariae and the local inflammatory reactions that they induce.[2] In the cornea, 'fluffy opacities' (inflammatory cells associated with the dying microfilariae) can lead to punctate keratitis, and in prolonged and heavy infection inflammation in the cornea results in sclerosing keratitis; inflammatory responses localized elsewhere in the eye lead to iridocyclitis, choroidoretinitis or optic atrophy. Longer-term complications of these inflammatory eye processes also include glaucoma and cataract.

Loiasis

The two most characteristic clinical features of loiasis are the passage of an adult filarial worm across the eye ('eye worm'), often in an otherwise asymptomatic person, and Calabar swellings.[3] The latter are localized areas of erythema and angioedema that may be 5–10 cm or more in size. Often they occur in the extremities and may last for several days before regressing spontaneously. If the inflammatory reaction extends to nearby joints or peripheral nerves, corresponding symptoms may develop. Routine radiographs of people in endemic areas may reveal calcified dead worms lying between the metacarpals.

Expatriate syndrome

'Expatriate syndrome' is a term to describe the clinical and immunologic hyperresponsiveness found in expatriate visitors to regions endemic for loiasis and other filariases. These people manifest prominent signs and symptoms of inflammatory reactions (including allergic reactions) to the mature or maturing parasites. In loiasis, these manifestations have included primarily Calabar swellings, hives, rashes and occasionally asthma.[12] In bancroftian filariasis (when military personnel or other migrants to endemic areas have acquired these infections), the manifestations have usually been lymphangitis, lymphadenitis and genital pain (from inflammation of the associated lymphatics), with hives, rashes and other 'allergic-like' manifestations, including blood eosinophilia.[8]

DIAGNOSIS

Except for *W. bancrofti* infections, diagnosis of filarial infections depends principally on the direct demonstration of the parasite (almost always microfilariae) in blood or skin specimens using relatively cumbersome techniques and having to take into account the periodicity (nocturnal or diurnal) of microfilariae (see Table 115.1). Most alternative methods based on detection of antibodies by immunodiagnostic tests have not proved to be satisfactory because of their failure to distinguish between active and past infections and their problems with specificity. There is good evidence, however, that recombinant antigens will greatly improve the value of such antibody-based immunodiagnostics in the future.

Lymphatic filariasis

Antigen detection

Circulating filarial antigen detection, with its very high specificity and sensitivity, should now be regarded as the 'gold standard' for diagnosing *W. bancrofti* infections.[16] Two commercial forms of this assay are available: one is based on the methodology of an enzyme-linked immunosorbent assay and yields semiquantitative results; the other is based on a simple card (immunochromatographic) test and yields only qualitative (positive or negative) answers. No such test is currently available for brugian filariasis.

Microfilaria detection

Before the development of the circulating filarial antigen assay, detection of microfilariae in blood was the standard approach to diagnosing lymphatic filarial infection, and it is the one still required today for both brugian filariasis and those situations where the antigen detection test is not available for bancroftian filariasis. Such assessments must take into account the possible nocturnal periodicity of the parasites when choosing the optimal time for drawing blood; namely, between 10.00 pm and 2.00 am for most brugian filariasis and bancroftian infections.[8] The simplest technique for examining blood or other fluids (e.g. hydrocele fluid or articular effusions) is to spread 20 µl evenly over a clean slide that is dried and then stained with Giemsa or a similar stain. A wet smear may also be made by diluting 20–40 µl of anticoagulated blood with water or 2% saponin, which will lyse the red blood cells but allow the microfilariae to remain motile and thus more readily identifiable. The larger the blood volumes examined, the greater will be the likelihood of detecting low levels of parasitemia. Other concentration techniques are also available.[8]

Clinical diagnosis

Many lymphatic filariasis patients are amicrofilaremic and therefore the diagnosis of these infections must be made 'clinically'. For amicrofilaremic syndromes other than tropical eosinophilia syndrome, serologic findings based on detecting IgG_4 antibodies have proved helpful, because

this subclass of IgG has greater diagnostic specificity and is stimulated by the presence of active infection. Such antibody analyses also are particularly helpful in diagnosing the 'expatriate syndrome', in which background (i.e. pre-exposure) levels of IgG and especially of IgG_4 antibodies to filarial antigens are very low, so that elevated levels have significant diagnostic implications in association with the clinical presentation.[16]

Eosinophilia is a frequent concomitant of all filarial syndromes but is diagnostically helpful only when the eosinophil levels are extremely high (as in tropical eosinophilia or the expatriate syndrome).

Onchocerciasis

Parasitologic techniques are most commonly used to diagnose onchocerciasis.[2] Microfilariae can be visualized directly in the anterior chamber fluid of the eye by slit-lamp examination of patients who have been 'prepared' by remaining for 2 minutes in a head-down position to allow microfilariae to drift forward into the anterior chamber. Skin microfilariae can be visualized after a skin snip has removed the most superficial layers of skin with either a corneoscleral punch or a small needle and disposable razor blade to obtain approximately 1 mg of a bloodless piece of skin. This sample is then placed in saline or water for examination for the emergence of microfilariae after 30 minutes to 24 hours. Alternative tests include a polymerase chain reaction on skin-snip specimens or a patch test, in which DEC is incorporated into a cream that is placed on the skin, covered by gauze and the area later observed for development of papular dermatitis resulting from the death of the microfilarial parasites. Also, subcutaneous or deep nodules (generally 1–6 cm in size) can be detected by palpation or ultrasound, and the adult worms they contain can be identified histologically in specimens that have been removed surgically.

Loiasis

Diagnosis of loiasis remains dependent on direct parasitologic identification (most frequently microfilariae in the blood) or indirect serologic approaches in association with a compatible clinical presentation and exposure history.[3,17] *Loa loa* microfilarial periodicity in the blood means that blood sampling must be done near midday (usually between 12.00 pm and 2.00 pm). Treatment with DEC of some amicrofilaremic patients who have suspected loiasis can induce an inflammatory nodule which, on biopsy, frequently discloses an adult worm surrounded by acute inflammatory cells. Eosinophilia and antifilarial antibodies are important diagnostic tools in expatriates with extensive exposure to infection.[18] Radiographic assessment is of little diagnostic value in these patients, but because hypereosinophilia resulting from loiasis has been associated with endomyocardial disease, echocardiography is of value in establishing whether cardiac damage has occurred.

MANAGEMENT

Lymphatic filariasis

Treatment of the infection

Remarkable advances in treating lymphatic filarial infection have recently been achieved,[19] but most of these have focused not on individual patients but on the community reduction of microfilaremia through once-yearly treatment, as described above. As few clinical trials have focused on optimizing treatment for the individual patient, there are still insufficient data to permit formal recommendation for a change from the older treatment regimens (DEC 6 mg/kg/day for 12 days in bancroftian filariasis and for 6 days in brugian filariasis); importantly, however, essentially the entire effectiveness of such a 'course' of DEC results from the first dose.[20] Ivermectin, although very effective in decreasing levels of microfilaremia, appears not to kill adult worms and thus it cannot be expected to cure infection completely. Albendazole, on the other hand, appears to kill the adult worms after prolonged courses (2–3 weeks) and to inhibit production of microfilariae after single doses (400 mg).[19]

Therefore, for treating infection in individual patients, in spite of the older textbook advice to use 6- or 12-day courses of DEC, more recent expert opinion[20] recommends use of either of the two single-dose, two-drug regimens currently employed in the Global Program to Eliminate Lymphatic Filariasis. These are DEC (6 mg/kg) plus albendazole (400 mg), and ivermectin (200–400 μg/kg) plus albendazole (400 mg). Because the use of DEC in patients who have either onchocerciasis or loiasis can be unsafe (see below), it is important that individual patients who have bancroftian filariasis and who live in areas endemic for these other infections be examined for co-infection with these parasites before being treated with DEC. While there may, in addition, be a role for multi-week doxycycline treatment of individual patients to cure infections,[21] additional studies are required before such a recommendation can be advanced.

Both diethylcarbamazine and ivermectin, given at the doses necessary to treat microfilaremic patients, cause minimal or no drug-induced side-effects. However, their rapid killing of microfilariae releases enough antigen to overwhelm the modulating effects of the host's immune system and to induce a variety of side reactions.[22] These occur in proportion to the pre-treatment microfilarial levels and include headaches, fever, myalgia, lymphadenopathy and occasionally rash, itching and other symptoms. Although the most severely affected patients can also experience postural hypotension, generally these reactions are well managed through the use of antipyretics, antihistamines or, in the most severe instances, corticosteroids. In the tropical eosinophilia syndrome, as there are no microfilariae in the blood, there is no exacerbation of symptoms, but rather a steady improvement over the 2–4 weeks during which DEC is administered.

Treating the disease

Although it is important to cure the infection, management of the consequences of that infection (particularly the lymphedema, elephantiasis and genital pathology) is what is often of greatest concern to the patient. For early disease manifestations, it has been shown repeatedly that treatment of communities with either intermittent (monthly, 6-monthly or yearly) drug administration or the steady use of DEC-fortified table or cooking salt leads to clinical improvement, with decreases in hydrocele size and prevalence and in regression of early lymphedema.[14]

In more chronic states, patients with hydroceles or related urogenital pathology must be subjected to surgical procedures in order to obtain relief.[14]

The most dramatic change in managing patients with lymphatic filariasis has come from the recent recognition that bacterial and fungal superinfections of tissues with compromised lymphatic function play a prominent and progressively exacerbating role in disease development, so that careful attention to these infections can dramatically improve the outcome.[8,14] Rigorous hygiene in the affected limbs removes much of the excess stress on the lymphatic system and allows it (although still functionally compromised) to handle much more of the extracellular fluid effectively. Management regimens should include the following:

- twice-daily washing of the affected parts with soap and water;
- raising the affected limb at night;
- regular exercising of the affected limb to promote lymph flow;
- keeping the nails clean;
- wearing shoes; and
- using antiseptic or antibiotic creams to treat small wounds or abrasions.

The addition of elastic bandages and other adjunctive measures can further improve results.

These same intensive local hygiene efforts and antibiotic ointments can also decrease the frequency of recurrent infection episodes in patients

who have elephantiasis of the penis or scrotum. Unfortunately, however, specific guidelines for management have not yet been developed for successfully reversing such anatomic distortions caused by the infection.

Noninvasive management of chyluria relies on nutritional support, especially replacement of fat-rich diets with high-protein, high-fluid diets supplemented where possible with medium-chain triglycerides.[8] Surgery, the sclerosing effects of lymphangiography or, often, time alone can also lead to the cessation of the leakage of lymph into the renal pelvis, collecting system and urine.

Onchocerciasis

Complete cure of *O. volvulus* infections, though not yet the goal of public health programs or most physicians, is now very feasible for individual patients, since long-term treatment with doxycycline (100 mg/day for 6 weeks) kills the adult parasite by depleting it of its essential endosymbiont (*Wolbachia*) bacterium.[21]

However, for most patients, because the pathology is generally associated with the microfilarial stage of the parasite, treating to kill the microfilariae rids the patients of existing symptoms and protects them from development of further eye lesions. The safest, most effective microfilaricide is ivermectin at the recommended dosage of 150–200 µg/kg; in various settings it has been repeated at 12-, 6- or even 3-month intervals. For individual patients, the frequency of treatment can best be determined by the rate at which symptoms (primarily itching and rash) recur. For individual patients, optimal management requires a thorough eye examination before initial treatment to ensure that no microfilariae are present, since it is the inflammatory complications of treatment with microfilaricides that must be avoided, especially in the eye. If there are microfilariae in the eye, the most conservative approach to treatment would include administration of prednisone 1 day before the dose of ivermectin is given and for 2 days after it. Short courses of corticosteroids have little negative effect on the microfilaricidal activity of ivermectin, and they are clearly effective in diminishing the side reactions caused by killing the microfilariae.

The side reactions that follow treatment of onchocerciasis with ivermectin (or, earlier, with DEC) have been termed the Mazzotti reaction. They result from the rapid killing of microfilariae and consist primarily of headache, fever, pruritus, adenopathy, rash and, occasionally, postural hypotension.[23] Although pronounced after DEC, they are much milder after ivermectin and are self-limiting (beginning within hours of treatment and persisting as long as 4–5 days); they can be managed satisfactorily with antipyretics, analgesics, antihistamines and, if necessary, systemic corticosteroids.

Because adult worms, which are generally not killed by either ivermectin or DEC, continue to shed microfilariae for up to 12–15 years, symptoms may recur and require additional 'microfilaricidal' treatment over an extended period of time.

Loiasis

The approach to treatment of loiasis depends on the clinical presentation. In patients who do not have microfilaremia, DEC 6–8 mg/kg/day for 3 weeks is the optimal treatment and results in cure of approximately half of the patients. Repeated courses of the drug are indicated when patients become symptomatic again and each repeated treatment results in additional patient cures.[24]

For microfilaremic patients, the approach to treatment is more difficult because the side reactions induced by the dying microfilariae can include CNS complications and even death. Such severe reactions rarely, if ever, occur in patients who have blood microfilaria counts of less than 2000/ml of blood (drawn at the time of day for peak parasitemia). However, even in a very controlled hospital setting, when highly microfilaremic loiasis patients were treated with DEC (initially at very low dosages – 0.25 mg/kg – and then increased progressively), there were still some patients in whom there was development of a post-treatment encephalopathy and death. This was not prevented even when corticosteroids were co-administered.[25]

When ivermectin is used instead of DEC, the clearance of microfilaremia from the blood is very much slower and not so complete. While it is much safer than DEC, both in terms of the systemic side reactions that it elicits (similar to those of the Mazzotti reaction) and in terms of avoiding the catastrophic neurologic complications in patients with extremely high levels of microfilaremia (especially 15 000–100 000 mf/ml), ivermectin has still led to instances of CNS deterioration, coma and death. With optimal clinical care, the transient CNS compromise of such ivermectin-treated patients can be managed successfully and catastrophic results minimized.[3] However, the treatment of loa-endemic populations with ivermectin (usually as part of national programs linked to the African Program for Onchocerciasis Control[2] or the Global Program to Eliminate Lymphatic Filariasis[1]) often must be rendered in remote areas without access to optimal medical management. Therefore, this potential complication of treatment provides a major challenge that must be overcome before these massive public health initiatives can be successfully implemented in the loa-endemic regions of Africa.

If patients experience an adult *L. loa* crossing the eye below the conjunctiva, such worms can be removed through simple surgical incision of the conjunctiva, but because usually there are multiple parasites within the patient, a single procedure may not be curative.[26]

REFERENCES

References for this chapter can be found online at http://www.expertconsult.com

Chapter **116**

Graham R Serjeant

Infections in sickle cell disease

DEFINITION

Sickle cell disease is a 'generic' term that includes a group of genotypes characterized by pathology associated with the presence of sickle hemoglobin (HbS). This abnormal hemoglobin results from a single amino acid substitution of valine for glutamic acid at position 6 in the β-chain. Inheritance of this abnormal gene from one parent and a normal gene for HbA from the other results in the harmless carrier state, the sickle cell trait. Sickle cell trait (AS genotype) is excluded from the definition of sickle cell disease because it causes no clinical problems in the great majority of subjects unless they are exposed to hypoxic environments such as high altitude or respiratory depression. The principal genotypes of sickle cell disease include:

- homozygous sickle cell (SS) disease, in which the abnormal HbS gene is inherited from both parents;
- sickle cell-hemoglobin C (SC) disease, in which the gene for HbS is inherited from one parent and the gene for HbC from the other. HbC is the second most common abnormal hemoglobin among people of West African origin; and
- inheritance of the sickle cell gene with one of the genes for β-thalassemia.

The β-thalassemia genes reduce the synthesis of β-chains, the degree of reduction determining the presence and amount of HbA. In sickle cell β^0-thalassemia there is total suppression of β-chain synthesis, no HbA and a generally severe course similar to that of SS disease. The spectrum of sickle cell β^+-thalassemia results from a variety of molecular mutations differing in different populations and resulting in variable amounts of β-chain synthesis and hence HbA. Most common among peoples of African origin are mutations resulting in 20–30% of HbA and a very mild clinical course. In India, a more severe mutation results in only 3–5% HbA and in Greece, the commonest mutation results in 10–15% HbA. In both these phenotypes, there does not appear to be enough HbA to significantly reduce sickling and sickle cell β^+-thalassemia in both regions has a relatively severe clinical course, often similar to SS disease.

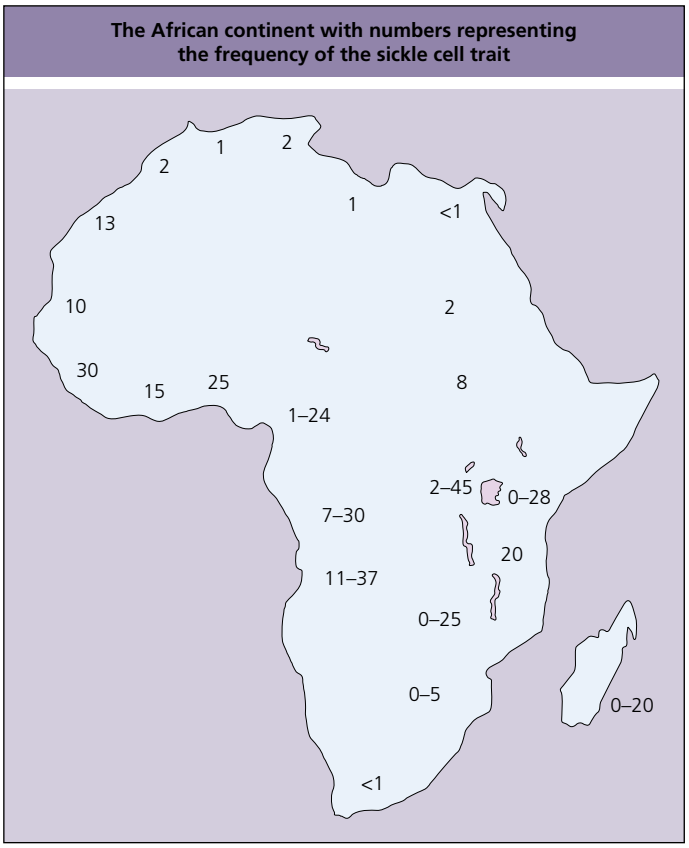


Fig. 116.1 The African continent with numbers representing the frequency of the sickle cell trait. Note the low levels in northern and southern Africa and high but variable levels in equatorial Africa.

DISTRIBUTION AND PREVALENCE

In equatorial Africa, the prevalence of the sickle cell trait commonly reaches 20–30% (Fig. 116.1). Contrary to the common belief that the sickle cell gene is confined to peoples of African ancestry, the gene is widespread in populations around the Mediterranean (Sicily, northern Greece, southern Turkey, the Levant and northern Africa), Saudi Arabia (especially eastern Saudi Arabia and the Arabian Gulf) and central India. HbC is a marker of west African ancestry, reaching a prevalence of 20% in parts of Ghana and Burkina Faso, falling to 3–5% in Nigeria, and not occurring in central or east Africa, Saudi Arabia, Greece or central India.

In Nigeria, where approximately 25% have the sickle cell trait, it is estimated that SS disease occurs once in every 50 births, or 100 000 births annually. The prevalence of SS disease at later ages is determined by the mortality of this genotype, which in Africa is often very high with infrequent survival to adult life. In Jamaica, the relative frequencies of the four major genotypes at birth (Table 116.1) are also influenced at later ages by their relative mortality which is greater in SS disease and $S\beta^0$-thalassemia. The prevalence of these four major genotypes among African-Americans and among the UK population

Table 116.1 Relative frequency of principal genotypes of sickle cell disease in Jamaica*

Genotype	Frequency at birth
Homozygous sickle cell (SS) disease	1 in 300
Sickle cell-hemoglobin C (SC) disease	1 in 500
Sickle cell β^+-thalassemia	1 in 3000
Sickle cell β^0-thalassemia	1 in 7000

*In these data, sickle cell β^+-thalassemia refers to the common Jamaican form with 20–25% HbA.

of Afro-Caribbean origin are similar to those in Jamaica (because of similar gene frequencies) although the increasing population of direct African origin has markedly increased trait and disease frequencies in the UK. In all populations, the prevalence of sickle cell disease at birth is determined by the gene frequency but at later ages is influenced by the relative survival of affected patients and by the relative severity which determines presentation in hospital-based populations.

PATHOGENESIS AND PATHOLOGY

On deoxygenation, HbS molecules form rigid linear structures (polymers), which increase the intracellular viscosity and deform red cells into an abnormal 'sickled' shape. This can be reversed on oxygenation but after several sickle–unsickle cycles, these cells may become permanently deformed. These less deformable red cells have difficulty negotiating the capillary beds where normal red cells with an average diameter of 7 µm have to bend and fold to traverse a capillary measuring 3 µm. As a result, these abnormal red cells become prematurely destroyed (hemolysis) and may also block blood flow (vaso-occlusion).

Accelerated hemolysis results in anemia, jaundice, an increased prevalence of pigment gallstones and marked bone marrow expansion with high metabolic demands. The consequences of vaso-occlusion are determined by the site of the occlusion, but may include strokes, retinal ischemia, acute chest syndrome, a variety of splenic pathologies and chronic leg ulcers. Some manifestations, such as bone marrow necrosis, are influenced by both the hemolytic and vaso-occlusive components which give rise to dactylitis, the painful crisis, avascular necrosis of the femoral head and of the sternum and ribs which may precede the acute chest syndrome. In these conditions, the pathology is initially confined to areas of active bone marrow and is presumed to be precipitated by the high metabolic demands outstripping the supply. The mechanism is still controversial but may result from vaso-occlusion or, more likely, by shunting of blood away from the active marrow.

The pathologies of particular relevance to infections in sickle cell disease are the accelerated bone marrow activity, the prevalence of leg ulcers, acute chest syndrome and – most important of all – the early loss of splenic function. These processes are most marked in SS disease, sickle cell β^0-thalassemia and severe forms of sickle cell β^+-thalassemia, and less in SC disease and mild forms of sickle cell β^+-thalassemia.

The spleen in sickle cell disease

The spleen acts like a filter in the circulation, removing damaged red cells and bacteria from the bloodstream. It achieves this function by requiring red cells to squeeze between endothelial cells (Fig. 116.2) as the blood traverses from the cordal tissue to the splenic sinuses before returning to the circulation.[1] In addition to the filtering mechanism,

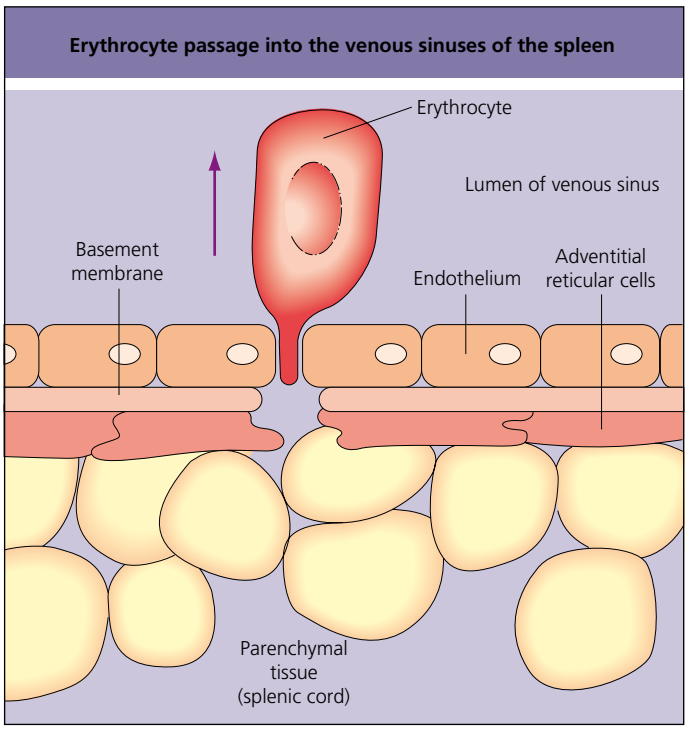


Fig. 116.2 Erythrocyte passage into the venous sinuses of the spleen. A red cell passing between endothelial cells (arrow shows direction of movement) from the cordal tissue to the vascular sinus. Sickle cells do not have this deformability, so they accumulate in the spleen. Modified from Weiss.[1]

the spleen also represents a large mass of reticuloendothelial tissue in intimate contact with the circulation and is important in the production of specific antibodies. These are especially important if the liver is to participate actively in the removal of blood-borne antigens. Splenectomy removes these protective mechanisms and in patients without sickle cell disease has been calculated to increase the risk of sepsis 50- to 60-fold.

In sickle cell disease, the abnormal red cells damage splenic function early in life. Even when clinically enlarged, splenic uptake of ^{99m}technetium sulfur colloid is abnormal;[2] elevated pitted red cell counts, which suggest abnormal splenic function, may occur as early as 6 months of age and are seen in 20% of SS children by 1 year of age and in 40% by 2 years of age.[3] This loss of splenic function appears to be directly related to the susceptibility to infection[4] and may be reversed by transfusion in young children (Fig. 116.3).[5] It is also delayed in SS patients with high levels of fetal hemoglobin (HbF), which inhibit sickling and allow persistence of splenic function in SS patients in eastern Saudi Arabia.[6]

CLINICAL FEATURES

Bacterial infection

Streptococcus pneumoniae

The susceptibility to invasive pneumococcal infection in SS disease is well recognized and was first reported in 1928. The relative risk has been calculated to be at least 20 times that in the general population, and age-specific incidence rates are highest before the age of 2 years and fall sharply after 5 years.[7] Infection is closely linked to the appearance of clinical splenomegaly before 6 months of age.[8]

Prophylactic penicillin markedly reduces this risk, whether given orally[9] or by depot monthly intramuscular injection,[10] and has now

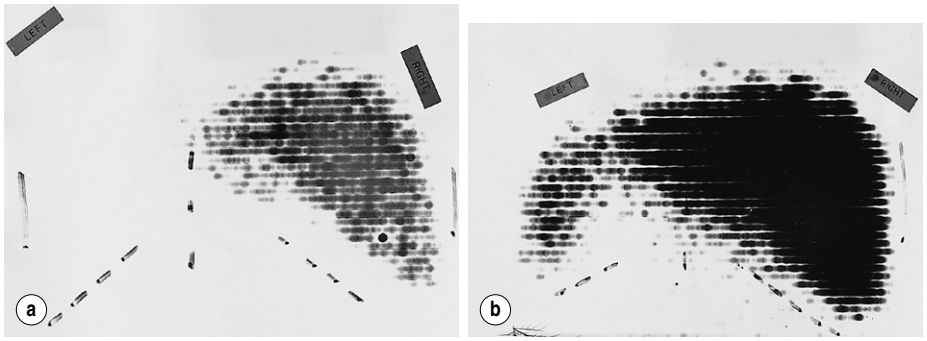


Fig. 116.3 ^{99m}Technetium sulfur colloid scans (posterior view) in a 2-year-old child with SS disease and splenomegaly. (a) The scan shows hepatic uptake but no splenic uptake. (b) A repeat scan 6 days after a blood transfusion shows restoration of splenic uptake of colloid. From Pearson *et al*.[5]

become routine for children with severe genotypes (SS disease, $S\beta^0$-thalassemia). Although there is some evidence of an increased risk in SC disease, it is not generally considered of sufficient magnitude to justify routine prophylaxis in the milder genotypes (SC disease, $S\beta^+$-thalassemia). The maximum risk from pneumococcal sepsis in Jamaica is in the first 3 years of life (Fig. 116.4) and depot injections of penicillin during that period prevent infection. It is unclear when to stop but the current Jamaican protocol provides intramuscular penicillin monthly from 4 months to 4 years with a single dose of 23-valent pneumococcal vaccine at the time of the last penicillin injection. Intramuscular injections are preferred in Jamaica to avoid the problems of compliance which may compromise the twice daily oral penicillin generally favored in the USA. Twice daily erythromycin may be given in children who are allergic to penicillin. The nonconjugated capsular polysaccharide pneumococcal vaccine does not confer adequate protection in young children, but its immunogenicity improves with the age of the patient, and preliminary data suggest that protective levels against many of the serotypes are achieved by the vaccine given at 4 years of age.

Two recent factors may cause these policies to be reassessed: the increasing prevalence of penicillin-resistant pneumococci, which may account for 20–50% of isolations in children with SS disease in the USA; and the development of a conjugate pneumococcal vaccine, which may be effective given at 2, 4 and 6 months. The availability of this vaccine has been associated with a significant decline in invasive disease[11] but potential disadvantages are the high cost and the less comprehensive coverage (7- or 9-valent) compared with the standard 23-valent nonconjugate vaccine.

This susceptibility to the pneumococcus has not been shown in all sickle cell populations and studies on the bacteriology of sepsis in SS disease in Nigeria[12–15] and Uganda[16] revealed a paucity of *Streptococcus pneumoniae*, the most common agents being *Klebsiella*, staphylococci, *Salmonella* and *Escherichia coli*. Possible interpretations of this infrequency of pneumococci include the early use of over-the-counter penicillin, the rapid demise of septicemic patients before they reach hospital, the environmental dominance of Gram-negative organisms or the intriguing postulate that malaria-induced splenomegaly may allow persistence of splenic function.[14] Regardless of the potential

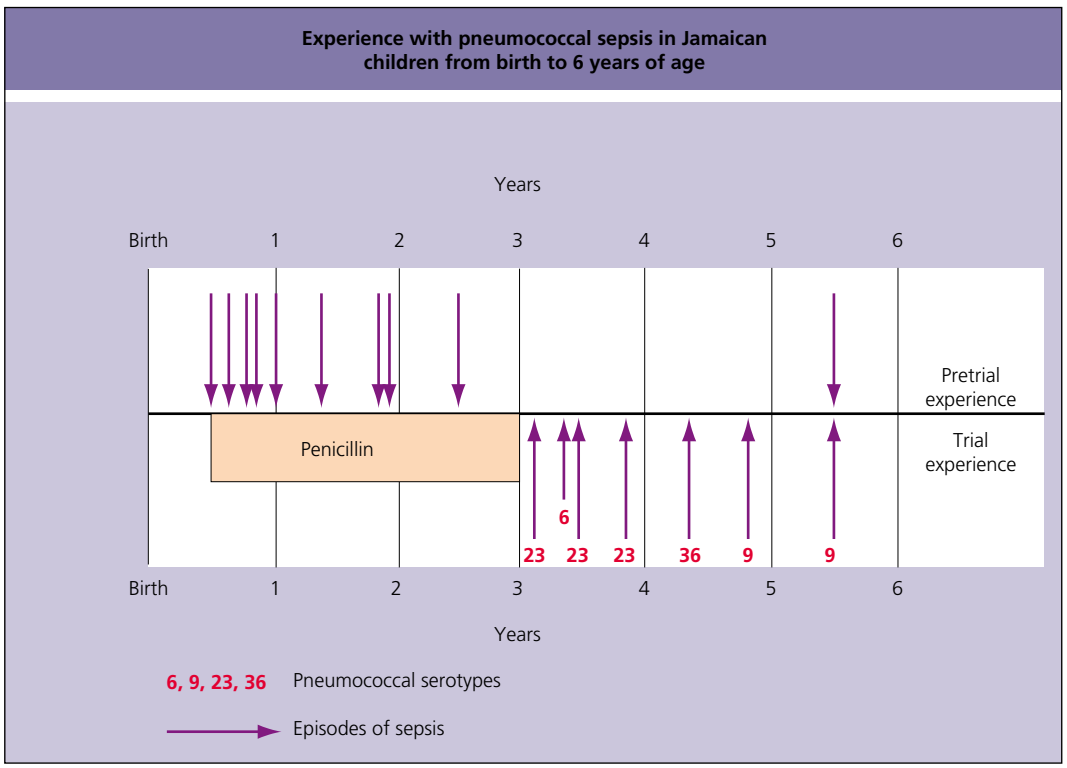


Fig. 116.4 Experience with pneumococcal sepsis in Jamaican children from birth to 6 years of age. Pretrial experience shows that sepsis episodes commenced at 6 months and 9 or 10 episodes occurred before 3 years. During penicillin prophylaxis from 6 months to 3 years, no episodes occurred but seven episodes occurred after cessation of penicillin. The numbers by the arrows refer to pneumococcal serotypes.

mechanism, there seems to be enough doubt to justify a trial of pneumococcal prophylaxis in SS disease in equatorial Africa if governments are to be persuaded to spend limited resources on pneumococcal prophylaxis in sickle cell disease.

Haemophilus influenzae type b

It seems likely that children with SS disease are also more prone to *Haemophilus influenzae* type b (Hib) infection, which has been increasing in importance with the advent of effective pneumococcal prophylaxis.[17] Extensive data from the US Cooperative Study of Sickle Cell Disease showed an incidence rate of 0.45 per 100 patient-years under 6 years of age, not significantly different from the incidence in the normal black population;[6] however, a susceptibility of 20–60 times that in the normal population has been proposed from other studies.[18] Although the risks of Hib infection in SS disease are unclear, prophylaxis appears justified and may be effected by conjugated Hib vaccines given between 2 and 6 months of age.[19]

Salmonella

Susceptibility to *Salmonella* osteomyelitis in SS disease has long been documented but an association with *Salmonella* sepsis in the absence of clinical bone involvement is less familiar. In a Jamaican study, half of the *Salmonella* isolations from blood occurred without obvious bone involvement and were associated with a 22% mortality because the potential significance of *Salmonella* spp. in a septic patient often went unrecognized.[20] On the other hand, none of the patients with *Salmonella* isolations associated with bone involvement died because the increased awareness of the association led to the early use of specific therapy against *Salmonella* spp.

Salmonella osteomyelitis is believed to be a secondary infection of avascular bone marrow and its distribution reflects that of the underlying bone marrow necrosis. The complication may become superimposed upon dactylitis in young children and should be suspected if the swelling is marked or there is high fever, in which case a surgical opinion should be sought regarding drainage; infection may be followed by a premature epiphysial fusion and a permanent shortening of affected small bones (Fig. 116.5). At later ages, osteomyelitis may follow bone marrow necrosis in the shafts of the long bones, ribs or sternum, pelvis and vertebrae. Infection of the avascular femoral head may be particularly difficult to diagnose, but this can lead to very rapid dissolution and extensive bone damage. Diagnosis depends on positive blood culture, and the diagnostic yield may be increased by invasive procedures such as bone marrow aspirate or trephine biopsies. The differential diagnosis of sterile avascular necrosis and osteomyelitis is a difficult clinical challenge and even bone scanning techniques have generally been unhelpful, the diagnosis resting on clinical judgment. Treatment is by specific antibiotics against *Salmonella* spp., such as chloramphenicol, ampicillin, trimethoprim–sulfamethoxazole (co-trimoxazole) or third-generation cephalosporins. Surgical drainage and the removal of sequestra may be necessary for complete healing and, even after apparent recovery, patients are prone to recurrence, sometimes years later, suggesting that the organisms remain dormant or loculated.

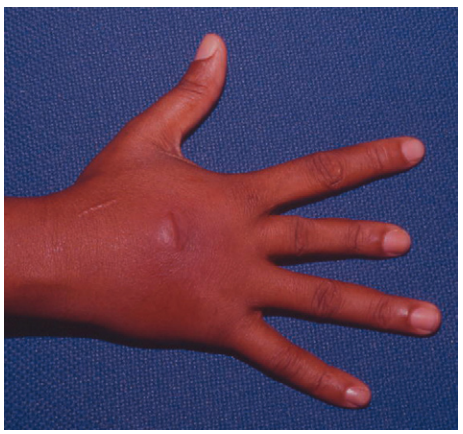


Fig. 116.5 Shortened third finger following infection complicating dactylitis. The scar represents surgical drainage of an apparent abscess.

The source of *Salmonella* organisms in SS disease remains unknown, but common speculations include microinfarction of the gut wall in patients carrying *Salmonella*, or gallbladder colonization associated with gallstones and an abnormal gallbladder wall. However, a recent study showed no association with gallstones or with indices of vasoocclusion, and *Salmonella* isolation from stools is only occasionally reported in patients with *Salmonella* osteomyelitis. The high prevalence of *Salmonella enteritidis*, which accounted for one-third of all isolations in the Jamaican series, suggests a dietary source and the possibility that *Salmonella* spp. may be carried by white cells raises the intriguing possibility that the organism could be introduced to the site of initially sterile avascular necrosis by the inflammatory process. More work is needed on the method of acquisition of *Salmonella* spp. in SS disease.

Escherichia coli

The extent of the increased risk of *Escherichia coli* infections in SS disease is unknown, but a significant excess of the SS genotype has been noted among black children admitted to hospital with serious infection and diagnosed as having *E. coli* sepsis.[21] *Escherichia coli* sepsis has also been associated with osteomyelitis and stroke. Urinary tract infections appear more common in SS disease and are a likely origin of *E. coli* sepsis.

Splenectomy and infection

Splenectomy, which may avoid morbidity and potential deaths in acute or chronic red cell sequestration, may be deferred because of concerns over loss of the splenic contribution to immune competence. However, the immune functions of the spleen in such patients are already compromised and no increase in severe infections or deaths occurred in 130 splenectomized SS patients when compared with 130 age and sex-matched SS controls followed over the same period.[22] If splenectomy is indicated in SS disease, either prophylactically in acute splenic sequestration or therapeutically in chronic hypersplenism, splenectomy should not be deferred for fear of losing any persisting splenic immune function. This practice has been satisfactory in nonmalarial areas but insufficient data on which to base recommendations are available in malarial environments, and both the role of splenectomy and of malarial prophylaxis should be studied separately in these areas.

Malaria

There is a special relationship between malaria and the HbS gene initially noted because malaria endemicity tended to coincide with high frequencies of the sickle cell trait in Africa. This has been the basis of many studies which have reached general agreement that during a critical period in early childhood (between the loss of passively acquired maternal immunity and the development of active immunity), the presence of the sickle cell trait confers some protection. The mechanism remains controversial and may be multifactorial, but increased sickling of parasitized red cells has been demonstrated and may serve to identify the host cell to the spleen and bring about its more effective removal. The maintenance of high frequencies of the sickle cell gene in areas of falciparum malaria led to the hypothesis of balanced polymorphism, proposing that a survival advantage in the sickle cell trait was balanced by the disadvantage and presumed loss of two genes in the early deaths occurring in SS disease. This protection is usually considered confined to the sickle cell trait but there is recent evidence that some resistance to infection may also occur in

SS disease. Once established in SS disease, malaria is believed to be a major cause of morbidity and mortality, exacerbating the already rapid hemolysis. It is unclear whether such patients die of malaria or succumb to symptoms of sickle cell disease precipitated by malaria.

Viral infection

Patients with SS disease are not believed to be intrinsically more prone to viral infections. Antibody responses to viral vaccines appear normal and infection rates by human parvovirus are similar in SS disease and AA controls.[23] More frequent transfusion-acquired viral infections such as hepatitis C virus and HIV may simply reflect the greater exposure to blood transfusion.

Human parvovirus infection appears to be the cause of aplastic crisis in SS disease, but this reflects a difference in response rather than an increased susceptibility. Human parvovirus infection colonizes and destroys red cell precursors in the bone marrow, but as the virus becomes neutralized by specific antibody, bone marrow function returns usually after 7–10 days. In SS disease, the red cell survival may be as short as 7–10 days and, unless oxygen delivery is maintained by transfusion, the aplastic crisis may result in death. Human parvovirus displays the general characteristics of a viral infection – most affected patients are under 15 years of age, in Jamaica epidemics occur at 3- to 4-year intervals and infectivity reaches 70–80% among nonimmune siblings. Close monitoring of at-risk siblings of affected cases is therefore recommended. A human parvovirus vaccine has been developed but it is still awaiting clinical trial. Human parvovirus infection in SS disease may be followed by cerebrovascular or renal complications in some cases.

Infection with atypical organisms

Mycoplasma pneumoniae and *Chlamydia pneumoniae* have been associated with the acute chest syndrome in patients with SS disease, especially in the autumn. The clinical course of these infections may be severe but it is unknown whether patients are more prone to develop these infections. The increased rate of hospital admission among SS patients also exposes them to an increased chance of hospital-acquired infections.

Other mechanisms of infection

Gallstones

The rapid hemolysis and consequent high excretion of bilirubin increases gallstones which occurred in 50% of SS patients in the Jamaican Cohort Study by the age of 25 years.[24] Jamaican experience suggests that most are asymptomatic, but complications include acute or chronic cholecystitis. The organism involved is usually *E. coli*, although anaerobes may also occur.

Leg ulceration

Chronic leg ulceration, a feature of SS disease, affects approximately 70% of Jamaican SS patients; they occur most commonly for the first time between 15 and 20 years of age and run a healing–relapsing course. The ulcer surface is commonly colonized by *Staphylococcus aureus*, *Pseudomonas aeruginosa* and β-hemolytic streptococci, but these ulcers are rarely associated with evidence of systemic infection. A possible association between ulcer-borne β-hemolytic streptococci and glomerular disease with proteinuria has been suggested, similar to the association between skin carriage of this organism and acute glomerulonephritis described from Trinidad; however, since both leg ulceration and proteinuria increase with age, correction for age in analyses did not confirm this relationship. Leg ulcers occasionally act as a portal of entry for tetanus.

The lack of evidence for systemic infection in leg ulceration suggests a limited role for antibiotic therapy, although infection with *P. aeruginosa* may occasionally be associated with ulcer deterioration and poor healing.

Acute chest syndrome

The acute chest syndrome is a pneumonia-like pathology with elements of infection, infarction, pulmonary sequestration and fat embolism. It is a major cause of morbidity and the commonest single cause of mortality at all ages after 2 years. The contribution of primary infection is controversial, and, although early studies reported pathogens in approximately half the cases in children aged under 3 years, recent studies have found evidence of bacterial infection in only 4–14% of episodes. Furthermore, the poor response to antibiotics and the striking improvement in many cases following transfusion has favored a vascular pathology rather than an infective one. Infections with the atypical agents *Mycoplasma pneumoniae* and *Chlamydia pneumoniae* have been mentioned above.

CONCLUSION

Patients with sickle cell disease are susceptible to some but not all infections. However, any infective illness coinciding with sickle cell disease may precipitate sickle-related complications, such as painful crisis, by inducing fever and possibly dehydration from vomiting and diarrhea.

REFERENCES

References for this chapter can be found online at http://www.expertconsult.com

Chapter 117

Robert N Davidson

Leishmaniasis

EPIDEMIOLOGY

Leishmania can cause cutaneous (CL), mucocutaneous (MCL) and visceral (VL, kala-azar) disease. The distribution of leishmaniases is shown in Figure 117.1; ~1.5 million cases of CL and 500 000 cases of VL occur annually.[1] Phlebotomine sandflies transmit leishmaniasis from a range of infected animals (= zoonotic) or from human to human (= anthroponotic). Transmission varies according to climate, habitat, season and occupation. All forms of leishmaniasis are increasing in many areas. In Brazil, the increase in VL and CL is due to deforestation, which brings humans into close contact with animal reservoirs and forest vectors of *Leishmania braziliensis* and other species. In North Africa and the Middle East, irrigation projects resulted in increased numbers of gerbils and construction of townships in these areas has led to increases in endemic CL caused by *Leishmania major*. Breakdown of the infrastructure in Afghanistan caused outbreaks of urban CL due to *Leishmania tropica*.

Visceral leishmaniasis

This is caused by *Leishmania donovani*, *Leishmania infantum* and *Leishmania chagasi* (see Fig. 117.1); the latter two species are indistinguishable. A reduction of dichlorodiphenyltrichloroethane (DDT) spraying against malaria vectors in India and Bangladesh heralded the start of the present epidemic of *L. donovani* VL, which affects hundreds of thousands annually. Population movement, famine, civil war and ecologic change caused epidemics of VL (*L. donovani*) in Sudan and Somalia.[2,3] In Europe prior to effective antiretrovirals, 20–70% of cases of VL (due to *L. infantum*) were co-infected with HIV, and 1.5–9% of AIDS patients had VL.[4] Co-infections of HIV and *Leishmania* are increasing in Africa (especially Ethiopia), India and South America.

Subclinical or self-healing infection occurs more frequently than clinical VL, particularly where *L. infantum* or *L. chagasi* is involved.[5] In epidemics involving *L. donovani*, most infections are symptomatic, and the mortality rate is high.[6]

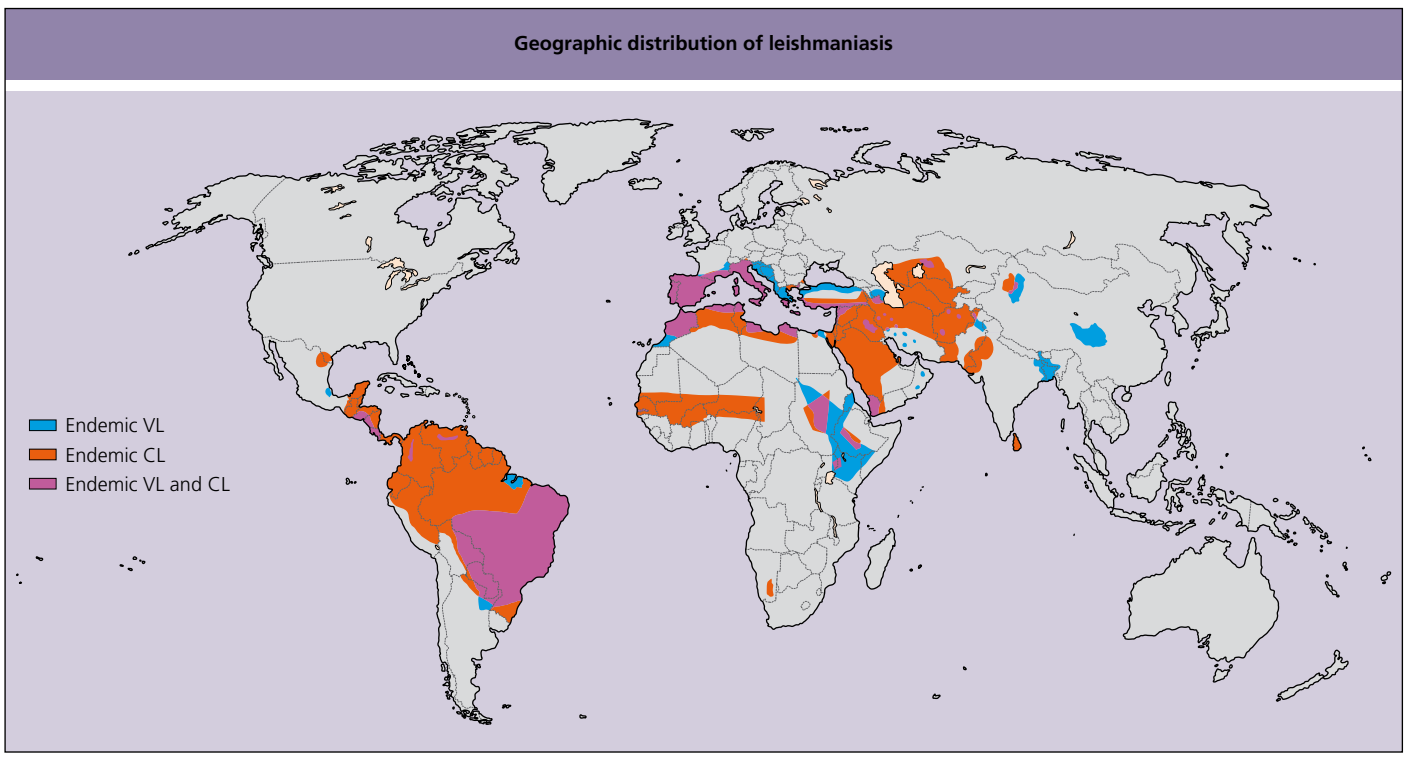


Fig. 117.1 Geographic distribution of leishmaniasis. More than 90% of VL cases occur in India/Nepal/Bangladesh, Sudan/Ethiopia and Brazil, and more than 90% of CL cases occur in Brazil/Peru, Algeria, Saudi Arabia and Syria/Iraq/Iran/Afghanistan. Courtesy of Pablo Martín-Rabadán and Emilio Bouza.

Cutaneous leishmaniasis

In the Old World, *L. tropica* causes anthroponotic CL in villages, towns and cities; *L. major* causes zoonotic CL in those living or working near gerbil burrows. Smaller numbers of CL cases are caused by *L. infantum* in Europe and *L. aethiopica* in Ethiopia and parts of Kenya. In the New World, CL is mainly caused by members of the *Leishmania mexicana* complex (*L. mexicana mexicana*, *L. m. amazonensis*, *L. m. venezuelensis*) and the *Leishmania braziliensis* complex (*L. braziliensis braziliensis*, *L. b. panamensis*, *L. b. guyanensis*, *L. b. peruviana*).[7]

PATHOGENESIS AND PATHOLOGY

Infected macrophages produce nitric oxide as an innate mechanism for killing *Leishmania* amastigotes; this is inhibited by the parasite, which multiplies in the parasitophorous vacuole. Eventually, infected macrophages rupture and amastigotes are taken up by new phagocytes. Macrophages and dendritic cells present *Leishmania* antigens to T cells and this results in either:

- an effective cellular immune response – a T-helper (Th)1 pattern; or
- an ineffective humoral response – a Th2 pattern.

In the Th1 response, T cells activate macrophages by releasing the cytokines interferon (IFN)-γ and interleukin (IL)-2. In the Th2 response, T cells release cytokines IL-4, IL-5, IL-10 and transforming growth factor (TGF)-β, which inhibit macrophages from killing amastigotes (see Chapter 2). Each *Leishmania* spp. produces a typical pattern of disease, and host cellular immunity (genetic factors, nutrition and co-morbidities all contribute) at the time of infection will determine whether:

- a clinical or subclinical infection results;
- the disease is visceral, cutaneous or mucocutaneous;
- lesions are few or diffuse; and
- response to treatment is complete or partial.[8]

PREVENTION

Individual protection against nocturnal sandfly bites is provided by insecticide-impregnated bed nets. Long sleeves and trousers, and permethrin-impregnated clothing may also help.[9]

Animal reservoirs of *Leishmania* can be controlled, for example by bulldozing gerbil burrows or killing infected dogs. Domestic dogs can be given deltamethrin-impregnated collars or 65% spot-on solution of permethrin.[10] Active case finding and treatment of patients with VL and post-kala-azar dermal leishmaniasis (PKDL) caused by *L. donovani* and CL caused by *L. tropica* reduce human-to-human transmission. Early case finding is possible using serologic tests, for example the direct agglutination test (DAT) and rapid test strips using a recombinant antigen, rK39.[11] Sandflies are susceptible to residual insecticides; spraying homes or fogging streets reduces the density of peridomestic sandflies.

Two doses of a vaccine, combining killed *Leishmania* promastigotes and live bacille Calmette–Guérin (BCG) were >70% protective against CL in Ecuador;[12] in Iran one dose of a similar vaccine was ineffective.[13] In Sudan a similar vaccine did not prove effective against VL,[14] but boosted cure rates in PKDL.[15]

CLINICAL FEATURES

Visceral leishmaniasis

After an incubation period of 2–8 months (range 10 days to >2 years), the patient develops pyrexia, wasting and hepatosplenomegaly, which may become massive (Fig. 117.2). Males outnumber females in Africa and India, partly because outdoor activities expose males to sandfly bites, but mainly because females are denied access to diagnosis and treatment. When treatment is provided free, or active case-finding done, the ratio of male:female cases approaches 1:1.[16] In Europe or Brazil, VL affects mainly infants (hence the name *L. infantum*) or adults co-infected with HIV.

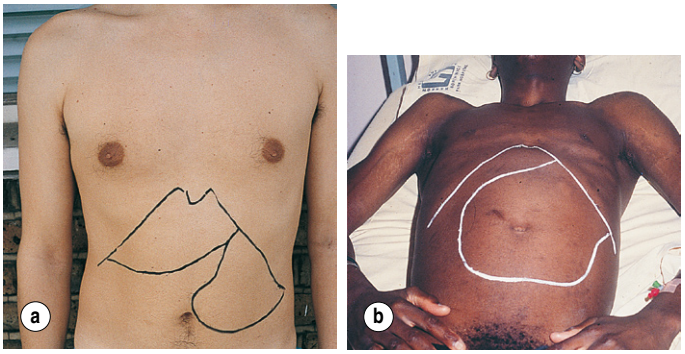


Fig. 117.2 Visceral leishmaniasis. (a) Hepatosplenomegaly and pallor in a 29-year-old Italian man. (b) Splenomegaly and pallor in a 23-year-old Angolan. Both complained of weight loss, fatigue and fever of several weeks' duration.

Visceral leishmaniasis in India, Kenya and Somalia often has an ill-defined onset, and months elapse before the patient presents with fever, discomfort from an enlarged spleen, abdominal swelling, weight loss, epistaxis, cough or diarrhea. Conversely, patients in Sudan or those affected during an epidemic characteristically experience high fever, progressing over a few weeks to prostration, weakness, dyspnea and acute anemia.

The physical signs (Table 117.1) depend upon the duration of the disease, the nutritional state of the patient and the presence of

Table 117.1 Features of visceral leishmaniasis (*Leishmania donovani*)*

Clinical feature	Proportion affected (%)
Age <9 years	22 (*L. infantum* and *L. chagasi* more commonly affect children and infants)
Age <15 years	44
Fever	83–100
Wasting	70–100
Uncomfortable spleen	81–88
Cough	72–83
Epistaxis	44–55
Diarrhea	25–55
Vomiting	2–37
Splenomegaly	93 (adults), 98 (children)
Lymphadenopathy	55–86 (uncommon outside Africa)
Jaundice	2–7
Edema	2–7
Laboratory findings	**Proportion (%)**
Globulin >30 g/l	98
Anemia	61–92
Albumin <30 g/l	88
Leukopenia	84
Thrombocytopenia	73
Elevated bilirubin	17
Elevated alkaline phosphatase	40
Positive *Leishmania* serology	95
Parasitologically proven	96

*The duration of symptoms is 2–4 months but is shorter in children

complications. Late cases are thin, wasted and cannot walk unaided. Hair changes and pedal edema accompany hypoalbuminemia, but ascites is rare. Nonspecific hyperpigmentation of the face, hands, feet and abdomen occurs ('kala-azar' means 'black sickness'). The spleen is enlarged in >90% of cases. Though massive spleens are often photographed, reaching the left or right iliac fossa, the average spleen in VL only reaches 5–10 cm below the left costal margin. It is smooth, firm and nontender unless there has been a recent infarct. The liver is moderately enlarged in one-third of cases. Lymphadenopathy is common only in African patients, in whom unimpressive, peanut-sized lymph nodes are often palpable in the groins. Jaundice, mucosal and retinal hemorrhage, and episcleritis are occasional features. Neurologic complications, such as confusion, tremor and ataxia, convulsions, footdrop and nerve deafness, are occasionally seen. After weeks to months of illness, most VL patients die, often as a result of uncontrolled bleeding (nasal, intestinal, intracranial), secondary bacterial pneumonia, anemic heart failure, tuberculosis or dysentery, or other infections (e.g. cancrum oris).

Leishmaniasis in patients who are immunosuppressed or have HIV infection

Visceral leishmaniasis is an AIDS-defining opportunistic infection. Currently, HIV/VL is commonest in north-eastern Africa (especially Ethiopia), India and Brazil.[4] In southern Europe numbers of co-infected cases fell dramatically after the adoption of highly active antiretroviral treatment (HAART). VL in HIV co-infected patients in Europe characteristically occurs at very low CD4 counts, and affects injecting drug users disproportionately because shared syringes spread the infection. The clinical features of HIV/VL in Europe are often atypical: the symptoms vague, the laboratory abnormalities nonspecific, and hepatosplenomegaly absent or unimpressive. *Leishmania* amastigotes may be found unexpectedly in circulating neutrophils or bone marrow aspirates of febrile HIV-positive patients, or in rectal, gastric or skin biopsies. *Leishmania* serology is negative in one-third of such patients. During treatment, patients may experience drug toxicity (especially pancreatitis on antimonials) and even if apparently responding well they are prone to relapse. With HAART, many HIV-infected patients with *L. infantum* achieve long-term cure; the danger of relapse recedes once their CD4 count is >200 cells/mm^3 for >3 months. A minority of HIV/VL patients never achieve a good CD4 response, despite effective HIV suppression, and relapse repeatedly. Eventually such patients become nonresponsive to all antileishmanial drugs.

L. donovani in Africa and India is more virulent than *L. infantum* in Europe, and the clinical picture of HIV/VL is indistinguishable from VL in HIV-negative patients. Those with HIV co-infection have high mortality during and after treatment, and higher rates of relapse and PKDL.[16,17] In Ethiopia, even good CD4 responses may not protect against repeated relapses.[18] Cutaneous leishmaniasis has been reported in patients who have HIV infection in Africa and South America. The lesions often resemble those of diffuse cutaneous leishmaniasis (DCL, see below).

VL may occasionally occur in patients immunosuppressed by drugs or who have undergone organ transplantation or thymectomy. There is usually no prior history of leishmaniasis, and travel to endemic areas may have been years previously.

Post-kala-azar dermal leishmaniasis

After successful treatment for VL due to *L. donovani* (but not *L. chagasi*/*L. infantum*), patients may develop a prolonged inflammatory reaction in skin and/or mucosae called post-kala-azar dermal leishmaniasis (PKDL).

In Sudan, PKDL occurs toward the end of apparently successful treatment or weeks to months later. Mild PKDL affects ~55% of VL patients, including those with subclinical VL.[19] In India, PKDL is less common, and occurs months to years after VL. Occasionally, PKDL is acute and severe, resulting in desquamation of skin and mucosae. More commonly, it is characterized by the development of hypopigmented patches, nodules and plaques. Parasites are infrequent or absent from the biopsies. Patients with PKDL are infectious to sandflies and may be the reservoir of *L. donovani* between outbreaks.

Cutaneous leishmaniasis

In CL (Fig. 117.3) amastigotes multiply in dermal macrophages near the site of inoculation, typically on the arms, legs, face or ears. The lesions may be:

- nodular or ulcerative; and
- single or with multiple satellite nodules or lymphangitic spread.

The most typical lesion of CL is a nodule or chronic ulcer with a diameter of 2–5 cm and indurated margins. The ulcer may be covered by a fibrinous crust or exudate. It is painful if large or secondarily infected.

The histologic picture is of an intense lymphoid and monocytic infiltrate with granulomas. A 'tissue-paper' scar remains after healing. The median spontaneous healing rate differs for each species and is typically:

- <5 months for *L. major*;
- <8 months for *L mexicana*; and
- ~1 year for *L. tropica* and *L. braziliensis*.

There are two chronic forms of CL. Diffuse cutaneous leishmaniasis is rare but disfiguring. Widespread plaques containing huge numbers of amastigotes persist for decades. People with DCL are anergic to leishmanin, but do not have visceral dissemination or systemic symptoms. DCL is caused mainly by *L. aethiopica* in Africa and *L. amazonensis* in South and Central America. Leishmaniasis recidivans is a chronic, nonhealing or relapsing cutaneous infection, seen mainly with *L. tropica* infection in the Middle East. These patients are hypersensitive to leishmanin and organisms are rarely identified (see Fig. 117.3).

Mucocutaneous leishmaniasis

Mucocutaneous leishmaniasis (MCL; Fig. 117.4) occurs in ~3–10% of cases of CL due to *L. b. braziliensis*, especially in Peru and Bolivia. MCL usually occurs months to years after CL has healed, but simultaneous CL and MCL can occur, as can MCL without previous CL. Usually the tip of the nose, nasal cartilage or upper lip are



Fig. 117.3 Cutaneous leishmaniasis. *Leishmania tropica* recidivans leishmaniasis lesions on the face and forearm of a Syrian girl. These had been present for 4 years with slow healing in the center and multiple recurrences despite courses of intralesional meglumine antimonate.



Fig. 117.4 Mucocutaneous leishmaniasis. A young man from Peru who had a 2-year history of slow enlargement of the lips and ulceration of the nostrils. Courtesy of Professor Luis Valda Rodriguez.

involved first with a painless induration or ulceration. The condition may remain static or may extend over months to years into the nasopharynx, palate, uvula, larynx and upper airways. The nose may be destroyed.

Biopsies show a chronic inflammatory and granulomatous infiltrate with very few amastigotes. Cultures of biopsies are usually positive for *L. braziliensis* but this may require repeated attempts. Less severe oral (e.g. tongue), nasal or laryngeal involvement occasionally occurs with other species (e.g. *L. infantum*) and this often indicates an underlying immune defect.

DIAGNOSIS

Parasitologic diagnosis

Clinical leishmaniasis is supported by serologic or skin tests but is ideally confirmed by finding or culturing the parasite. *Leishmania* spp. may be isolated from material taken from reticuloendothelial tissue or from biopsies of skin or mucosal lesions. Some of the sample is smeared onto glass slides stained with Giemsa and examined for amastigotes (Fig. 117.5). The rest of the sample is inoculated into suitable media and cultured at 78.8–82.4°F (26–28°C); a positive culture produces motile promastigotes within 2 weeks.

In VL, positive yields from smears of aspirates are of the following order: spleen, >95%; bone marrow or liver, 70–85%; lymph node (Africa), 58–65%; and buffy coat of blood (HIV/VL), ~70%.[11] Cultures yield about another 10% in good hands. The technique of splenic aspirate is shown in Figure 117.6.

In CL, DCL, PKDL and MCL, slit skin smears are taken from the raised edge of the CL ulcer or center of the CL nodule (Fig. 117.7; see Chapter 103). Amastigotes are most abundant in fresh CL lesions and are very numerous in DCL. Conversely, they are infrequent in old CL lesions, in MCL and in PKDL.

Immunologic diagnosis

In VL, 95% of cases have high titers of anti-*Leishmania* antibodies by the direct agglutination test (DAT), immunofluorescent antibody test (IFAT) or enzyme-linked immunosorbent assay. A major advance is the dipstick test using the rK39 antigen, which gives sensitivity and specificity for VL of >90% in India.[11] In Europe, ~30% of HIV/VL patients have negative serology for *Leishmania*; in Africa, *Leishmania* serology is positive in most HIV/VL patients.[18] In VL, the leishmanin skin test is invariably negative, indicating antigen-specific anergy. In CL, *Leishmania* serology may be weakly positive and the leishmanin skin test is usually positive. In MCL and PKDL, both serology and the leishmanin skin test are usually positive. In DCL, there is anergy and both serology and the leishmanin skin test are negative.

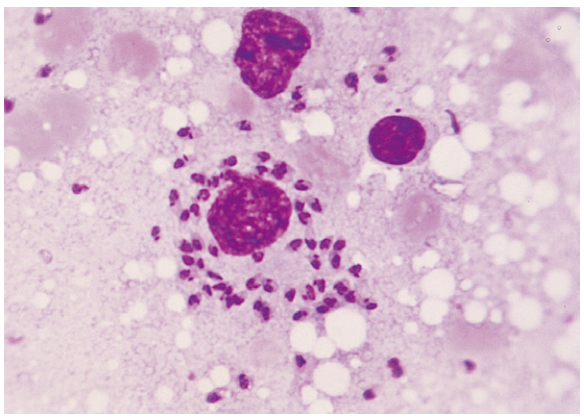


Fig. 117.5 Amastigotes (Leishman–Donovan bodies) in bone marrow aspirate from a patient who had *Leishmania infantum* visceral leishmaniasis and AIDS. The nucleus and kinetoplast stain deeply with Giemsa and give the organism its characteristic appearance. *Histoplasma* spp. are the main source of mistaken identification in bone marrow smears, but lack these structures. Amastigotes measure 2–3 mm in length and are found within macrophages in tissue sections, but usually lie free in smears because infected macrophages burst as they are smeared.



Fig. 117.6 Splenic aspiration. The picture shows a splenic aspirate being performed under field conditions on a child suffering from *Leishmania donovani* kala-azar in south Sudan. The procedure is simple, painless and safe if the prothrombin time is normal and the platelet count is above 40×10^9/l. Palpate the spleen and mark its outline. Using a 30 mm long 21-gauge needle attached to a 5 ml syringe, penetrate the skin over the spleen. Withdraw the plunger 1 ml and plunge the needle into the spleen upwards at an angle of 45° and withdraw immediately, maintaining suction. The tiny amount of material obtained is sufficient for culture and smear. Courtesy of Drs Robert Wilkinson and Jill Seaman.



Fig. 117.7 Slit skin smear. The picture shows a slit skin smear being taken from the edge of a chronic *Leishmania infantum* ulcer obtained in Malta. Smears are taken from the raised edge of the ulcer or center of the nodule, where amastigotes are most abundant. The skin is cleaned and then firmly pinched throughout the procedure to squeeze away blood. A 5 mm long and 3 mm deep incision is made and then the scalpel is turned through 90° and the blade is used to scrape the edge of the slit. A line of tissue scrapings is gently streaked on to a slide and the process is repeated until two or three lines of scrapings are present on at least two slides. Further scrapings and fluid oozing from the pinched slit are put into culture medium.

MANAGEMENT

Ideally, VL, like other life-threatening infections, should be treated with combination chemotherapy to improve cure rates, reduce duration and toxicity of treatment, reduce relapses and (in areas

of zoonotic transmission) delay the development of drug-resistant *L. donovani*. However, despite agreement among all experts on this principle, combination treatment has only been adopted in a few situations. [16]

Antileishmanial drugs

Pentavalent antimonials

Pentavalent antimonials (Sb^v) have been used since the 1940s. Sodium stibogluconate contains Sb^v 100 mg/ml; meglumine antimonate contains 85 mg/ml. In the systemic treatment of VL, CL and MCL, a single daily dose of 20 mg Sb^v/kg is used for 28 days. Intravenous injections are less painful than intramuscular injections. Courses of up to 3 months are used for PKDL. Primary resistance to Sb^v is seen in ~1% of cases in Africa and up to 60% in parts of India. Relapse rates should be <5%, but secondary Sb^v resistance is likely to develop in patients who relapse unless they are retreated very thoroughly.

Toxicity

Sb^v is reduced by parasite reductases to SbIII, and human reductases might perform a similar function. This may account for the unpredictable toxicity of Sb^v, as well as explaining why toxicity is rare in 'well' patients treated for PKDL, but common in 'ill' patients with VL, particularly those with HIV/VL. Sb^v-induced hyperamylasemia is common, and pancreatitis may be symptomatic and even fatal, especially in those co-infected with HIV.[20] Elevated liver enzymes, arthralgia and myalgia, thrombocytopenia, leukopenia, anorexia and thrombophlebitis all occur. Patients may experience symptoms indistinguishable from VL itself: lethargy, headache, nausea, vomiting. Electrocardiograph (ST-segment and T-wave) changes occur commonly; prolongation of the corrected QT interval to >0.5 s is an indication to temporarily discontinue therapy.[21] Acute renal failure, thrombocytopenia, arthritis, tremors and exfoliative dermatitis occur occasionally. If toxicity occurs, treatment should be changed to another agent. If only Sb^v is available, treatment should be stopped for 1–2 days; if toxicity recurs, the daily dose should be reduced.

Before starting treatment, ideally a full blood count, biochemistry profile and electrocardiograph should be obtained. Patients should be hospitalized during systemic Sb^v therapy where possible, and biochemical and hematologic parameters monitored twice weekly. This is usually impossible in endemic countries, where Sb^v is administered by a nurse to outpatients without the facilities for monitoring toxicity. Sudden deaths, thought to be due to arrhythmias, occur occasionally in VL patients. It is unclear what proportion of deaths in VL is due to Sb^v. However, in a randomized controlled study involving 580 VL patients, many of whom were HIV positive, the mortality was 10% among patients treated with Sb^v versus 2% among miltefosine recipients.[22]

Intralesional administration

When used intralesionally, ~1 ml of undiluted Sb^v is infiltrated into the base and edges of a CL lesion. The injections are repeated every 2–3 days for up to 2–3 weeks. There are no systemic side-effects, but the injections are painful.

Amphotericin B

Amphotericin B deoxycholate is a very powerful antileishmanial and a first-line drug in India, with good efficacy in Uganda, Nepal and elsewhere. Amphotericin B is remarkably nontoxic in the regimen of 15 doses of 1 mg/kg on alternate days.[23] The main limitation is the need for hospitalization and slow intravenous infusions. Where possible, renal function and electrolytes should be monitored weekly. Amphotericin B is the drug of choice for advanced MCL, for which Sb^v treatment is often ineffective, and total doses of 30 mg/kg are used. Amphotericin B has not been systematically assessed for CL or PKDL.

Lipid-associated amphotericin B

These particulate preparations of amphotericin B are taken up by macrophages and therefore target amphotericin B to the site of infection, achieving very high levels in liver and spleen. They have lower renal and infusion toxicity than amphotericin B but are more expensive. Liposomal amphotericin B (AmBisome®) has been extensively used, and is licensed for VL in many countries. It is rapidly effective and nontoxic for VL in Europe,[24] Sudan[25] and India.[26] The licensed regimen is a total dose of 20–30 mg/kg, given as at least five daily doses of 3–4 mg/kg over a period of 10–21 days. Liposomal amphotericin B can be dosed flexibly, since the drug has very low toxicity and a prolonged tissue half-life in spleen and liver (and, presumably, bone marrow).[27]

Very short courses of liposomal amphotericin B (two doses of 10 mg/kg) have been used in Europe.[27] While total doses as low as 5 mg/kg as a single dose have a high cure rate in India,[26] liposomal amphotericin B is not as effective in Sudan.[25,28] A few complicated cases of CL have been successfully treated with long courses of liposomal amphotericin B.

Amphotericin B cholesterol dispersion (Amphocil®) and amphotericin B lipid complex (Abelcet®) have also been used successfully in VL.

Combination therapy

Liposomal amphotericin B would be ideally suited as part of a combination therapy. Regimens currently under consideration are a single dose of AmBisome 5 mg/kg plus a short course of either intramuscular paromomycin sulfate or oral miltefosine.

Miltefosine

Miltefosine is an oral drug with good efficacy against VL;[29] it is licensed in several countries. The common side-effects are mild to moderate vomiting and diarrhea and modest elevation of liver enzymes. More rare are anorexia, nausea, abdominal pain and increase of urea and creatinine. Miltefosine should be avoided in pregnancy, and women of child-bearing potential must use effective contraception during and for 2 months after treatment.

The duration of miltefosine treatment is 28 days. The daily dose for adults and children >25 kg is 100 mg/day; for children 2–12 years (8–25 kg) it is 2.5 mg/kg/day (max 50 mg/day). Miltefosine should be taken in divided doses with meals.

Miltefosine (same regimen as above) is highly effective against *L. braziliensis* CL and MCL in South America[30,31] and against *L. major* CL in Iran.[32]

Paromomycin (aminosidine)

Paromomycin sulfate (previously called aminosidine sulfate) is an aminoglycoside antibiotic used since the 1950s, which is a highly effective and cheap antileishmanial for VL, though it has little efficacy in CL or MCL. It is licensed in India in the dose of paromomycin sulfate 15 mg/kg/day for 21 days.[33] This dose is equivalent to paromomycin (base) 11 mg/kg/day.

Paromomycin is well-tolerated. In clinical trials, no cases of renal impairment occurred, and subclinical ototoxicity (which was reversible) occurred in only 2%, with 6% developing mild, reversible elevation of liver enzymes.[33]

Combination treatment

A combination of Sb^v and paromomycin has been used extensively in Sudan with good effect.[16,34] The regimen is sodium stibogluconate 20 mg/kg/day plus paromomycin sulfate 15 mg/kg/day, both given intramuscularly daily for 17 days.

Pentamidine

Pentamidine is not routinely used for VL, although short courses – seven doses of 2 mg/kg on alternate days, or four doses of 3 mg/kg on alternate days – are effective for New World CL.[35]

Second-line oral agents

Ketoconazole is effective for CL caused by *L. major* and *L. mexicana*, but is less effective against *L. tropica*, *L. aethiopica* and *L. braziliensis*. Fluconazole[36] and itraconazole have similar efficacy and are better tolerated. Imidazoles cannot reliably cure VL or PKDL.

Topical treatment

Topical paromomycin in the treatment of CL has been evaluated in many studies. At best, it shows a modest benefit over placebo and is usually less effective than Sb^v.[37]

Monitoring response to treatment

Visceral leishmaniasis

Intercurrent infections such as malaria, tuberculosis and dysentery must be treated, and good hydration and nutritional supplements provided. Severely ill patients should receive empiric broad-spectrum antibiotics (e.g. ceftriaxone) to cover sepsis.

With effective antileishmanial treatment, the patient will be afebrile within 1 week and clinical and laboratory abnormalities will improve within 2 weeks. Liposomal amphotericin B acts faster than Sb^v.

After successful treatment, amastigotes will be absent from aspirates and cultures will be negative; a test-of-cure aspirate is not needed unless the patient has previously relapsed or has not shown a full clinical recovery. The patient should be reviewed during 6–12 months after treatment. Slight splenomegaly may persist for several months. A relapse rate of less than 5% is expected for immunocompetent patients but more than 80% for patients who also have HIV infection; most relapses occur within 6 months. Body weight, spleen size, full blood count, serum albumin concentration and erythrocyte sedimentation rate are all sensitive markers of recurrent VL.

Maintenance with intravenous pentamidine every 2–4 weeks or liposomal amphotericin B weekly may be useful to prevent or delay relapse for patients who have HIV infection, but efficacy is unproven. HIV co-infected patients should be started on HAART, irrespective of the CD4 count, since relapsing VL carries a high morbidity and mortality.

When treating a relapse of VL, a drug combination is advised, and parasite clearance should be confirmed by a test-of-cure aspirate.

Cutaneous leishmaniasis

Treatment is necessary if the lesions are large, multiple, disfiguring or overlie a joint. Intralesional Sb^v is cheap and usually effective but CL due to *L. braziliensis* should be treated systemically to reduce the risk of subsequent MCL. Most relapses of CL will occur within 12 months.

Mucocutaneous leishmaniasis

Untreated MCL will slowly progress to produce extensive mutilating lesions. Early lesions respond better to treatment[37] but the response is slow and relapses are common. Corticosteroids should be added if the larynx or airways are involved, to prevent edema complicating the start of treatment. Relapse may occur up to several years after treatment, so prolonged clinical follow-up is necessary.

REFERENCES

References for this chapter can be found online at http://www.expertconsult.com

Chapter 118

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Chagas disease (American trypanosomiasis)

DEFINITION

Chagas disease, first described by the Brazilian scientist Carlos Chagas in 1909,[1] is a vector-borne anthropozoonosis caused by the protozoan parasite *Trypanosoma cruzi*. Infection with *T. cruzi*, which is life-long, is characterized by three clinical phases:

1. acute phase which may be present with edema at the cutaneous or conjunctival portal of entry of the parasite;
2. indeterminate or latent phase which is asymptomatic and of a median duration of 10 years; and
3. chronic phase with the appearance of cardiac (chagasic cardiopathy) and digestive (megaesophagus and megacolon) pathology.

EPIDEMIOLOGY

Although control of Chagas disease has dramatically improved in the past two decades, this parasitic disease is widely disseminated in Latin America and is considered as a public health problem in many of its countries.[2] By the end of the 1980s it was estimated that 16–18 million people were infected, that 90–100 million more were at risk of infection, and that 450 000 new cases occurred each year. However, after the launch of the Southern Cone Initiative by six endemic countries in 1991 (Argentina, Bolivia, Brazil, Chile, Paraguay and Uruguay) and similar initiatives in Central America and the Andean Pact countries in 1997, Chagas disease cases have been declining.[3] Currently it is thought that less than 9 million people are infected, and Brazil, Uruguay and Chile have been declared free of transmission due to domestic *Triatoma infestans* (Fig. 118.1**)**. Nevertheless, around 40 million people remain at risk of infection, transmission is still present and national programs for the control of this disease are not present in countries such as Mexico, Peru, Colombia and Costa Rica.[2] In the USA it is estimated that around 100 000 persons are infected.[4] Although most of them are migrants who acquired the diseases during their childhood, vectors and animals infected with *T. cruzi* can be found in many parts of the country and at least six documented cases of autochthonous transmission have been reported.[5]

ETIOLOGIC AGENT

The causative agent of Chagas disease is the protozoan parasite *Trypanosoma cruzi*. Similar to other parasites of the order Kinetoplastida, *T. cruzi* is characterized by the presence of the kinetoplast, a DNA-containing organelle located within the mitochondrion at the posterior end of the parasite close to the flagellar base.

Biochemical and genetic studies have demonstrated that *T. cruzi* is actually a group of genetically diverse parasite populations. Isoenzyme and other analyses have led to classification of *T. cruzi* parasites in two major lineages, *T. cruzi* I and *T. cruzi* II, with the latter containing five subgroups (a–e).[6] In the Southern Core region, *T. cruzi* IIb, IId and IIe are the predominant agents although sporadic cases of *T. cruzi* I occur.[7] However, *T. cruzi* I is the predominant cause of Chagas disease in the Amazon region and in countries north of the Amazon region, where megasyndromes seem to be less commonly associated with chronic disease.[8]

Chagas disease is primarily a zoonosis in which wild and domestic animals serve as reservoirs. All mammals, but not birds or reptiles, are considered to be susceptible to *T. cruzi* infection. Mammals typically involved in sylvatic transmission include opossums, armadillos, raccoons, monkeys, wood rats and others. Domestic dogs, guinea pigs, cats, rats and mice may in some situations be important reservoirs. Chickens, although not infected, are a significant factor because they can sustain large infestations with the reduviid vectors.[9]

The life cycle of the parasite is complex. It circulates in the blood of infected mammals in an elongated trypomastigote form, about 20 µm long, and characterized by the presence of a flagellum that goes over an undulating membrane from the kinetoplast to the anterior end of the parasite (Fig. 118.2). Triatomine insects may ingest the parasite during a blood meal and in the midgut of the insect trypomastigotes transform into epimastigotes and multiply by binary fission. Epimastigotes then migrate and attach to the walls of the rectal sac where they produce nondividing metacyclic trypomastigotes that are eliminated with the insect feces. Infective metacyclic trypomastigotes gain entry into the mammalian host by penetrating mucous membranes or abraded skin. Trypomastigotes can then enter nonphagocytic or phagocytic cells, in which they transform into round amastigotes, without flagellum and of 0.5–4 µm in diameter, and divide by binary fission to produce a pseudocyst. Before rupture of the pseudocyst, amastigotes transform into trypomastigotes, which upon release re-enter cells or circulate in the blood, thus completing the life cycle (Fig. 118.3).

TRANSMISSION

The transmitting vectors of *T. cruzi* parasites are blood-sucking triatomine bugs of the order Hemiptera, family Reduviidae, subfamily Triatominae, and primarily genera *Rhodnius*, *Triatoma* and *Panstrongylus*. Around half of the 130 known triatomine species have been found to be naturally infected, although due to their physiologic similarities all of them are considered as potential vectors of Chagas disease.[10] The most common species are *Triatoma infestans* (Argentina, Bolivia, Brazil, Chile, Paraguay, Peru and Uruguay), *Rhodnius prolixus* and *Triatoma dimidiata* (northern South America and Central America), *Panstrongylus megistus* (central and eastern Brazil) and *Triatoma brasiliensis* (northeastern Brazil) (Fig. 118.4).

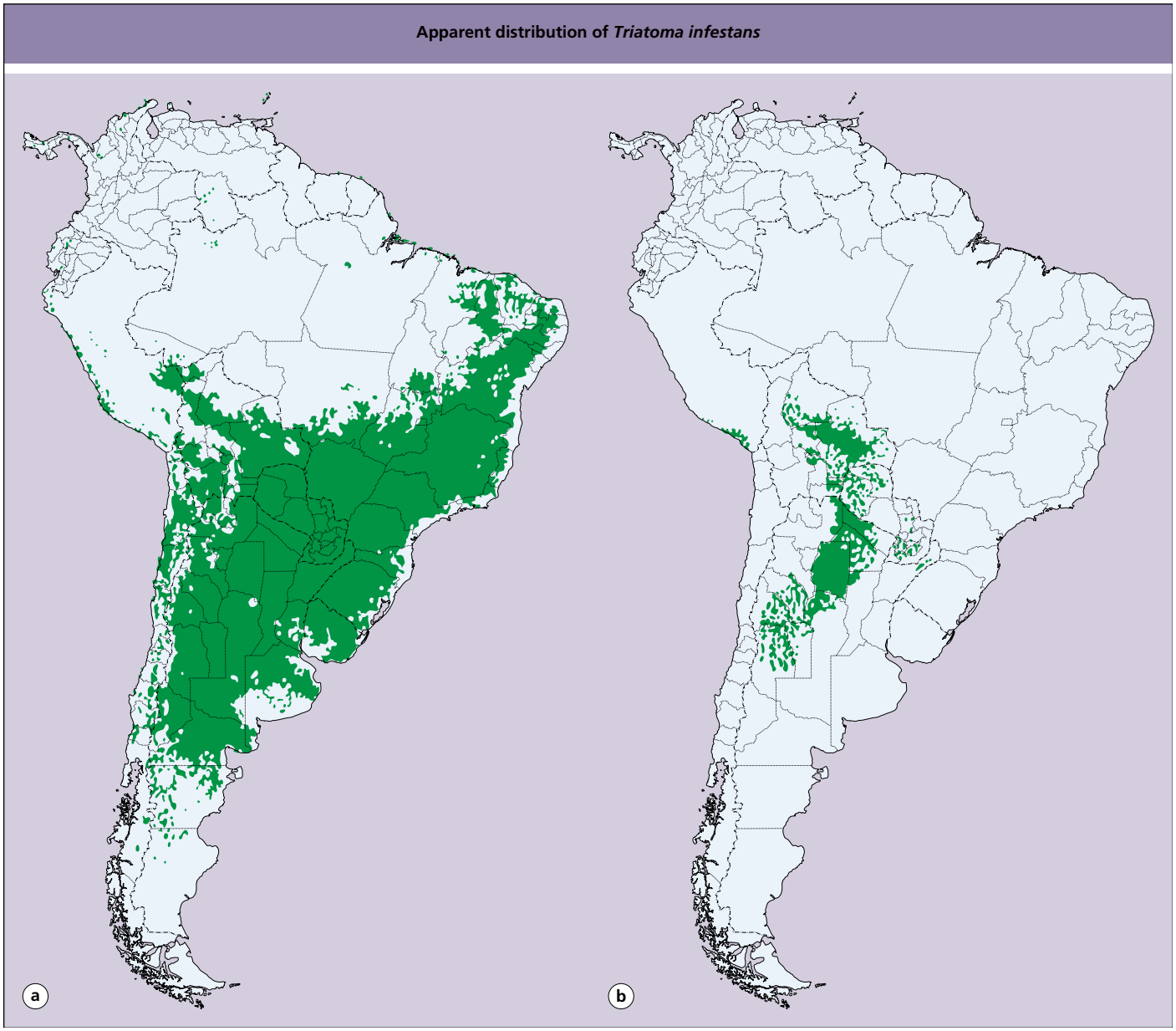


Fig. 118.1 Apparent distribution of *Triatoma infestans*. (a) Maximum predicted distribution in the absence of control interventions. (b) An estimate of current distribution based on report from the Intergovernment Commission for the Southern Cone Initiative. Reproduced with permission from Schofield *et al*.[2] Copyright Elsevier 2006.

Eggs from these insects reach complete development in around 134 days and adult forms can live for around 1 year. Adult triatomines are 1.5–3 cm long, with an elongated head, chitinous thorax and double wings. The natural habitats of triatomines are trees, burrows and rocks, where they feed on mammals, birds and reptiles. However, the presence of triatomines inside houses or huts constructed with vegetal materials favors the domestic transmission (Fig. 118.5).

Infection of triatomines with *T. cruzi* parasites occurs during a blood meal from a mammalian reservoir. Generally the vector is infective 20 days after ingesting parasites and continues to be infective for the rest of its life. Transmission of parasites occurs when infected triatomine insects defecate during a blood meal, depositing parasites close to the conjunctiva, mucosal tissues or the bite wound in the skin. Secondary routes of transmission to humans include blood transfusion, organ transplant and transplacental transmission. *Trypanosoma cruzi* can also be transmitted when mammalian hosts ingest infected insects or insect feces. This mechanism may be important in maintaining the sylvatic cycle[11,12] and occasionally causes significant outbreaks in humans.

PATHOGENESIS AND CLINICAL SYMPTOMS

Acute phase

The initial acute phase of *T. cruzi* infection may be asymptomatic and is not commonly reported, being diagnosed mainly in children younger than 10 years old. The incubation period between exposure to infection and the appearance of symptoms may be as short as 2 weeks. Metacyclic trypomastigotes that enter the organism are phagocytosed and multiply in local macrophages, inducing a localized inflammatory response causing unilateral conjunctivitis and periophthalmic edema (Romaña's sign) if parasites enter the conjunctiva (Fig. 118.6) or a cutaneous lesion (chagoma) if the portal of entry is the skin. Occasionally multiple chagomas may be seen in infants. Regional lymphadenopathy often accompanies acute infection, and systemic migration of parasites and invasion of other organs may result in fever, hepatosplenomegaly, generalized

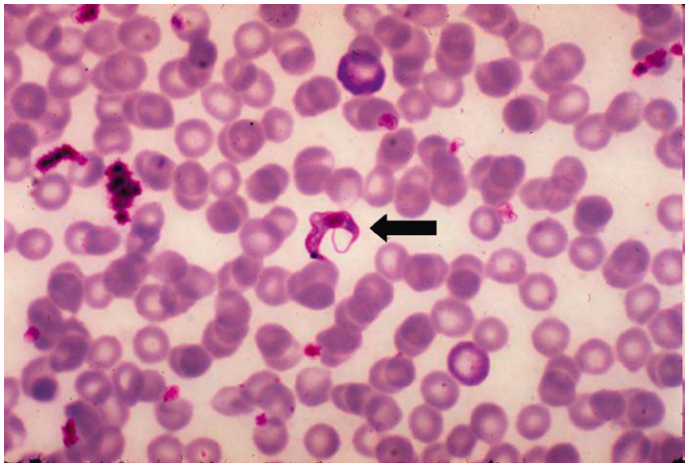


Fig. 118.2 *Trypanosoma cruzi* trypomastigote. Micrograph of *T. cruzi* (arrow) in a blood smear using Giemsa staining technique. Source: US Centers for Disease Control and Prevention (CDC), Atlanta, GA, USA. Public Health Image Library ID#3013.

lymphadenopathy, facial or generalized edema, rash, vomiting, diarrhea and anorexia. Acute infection in the heart may cause an acute myocarditis, with cellular infiltrate and patchy areas of necrosis and infected cells. There may be early ECG abnormalities, including sinus tachycardia, increased PR interval, T-wave changes and low QRS voltage. Acute phase infections are fatal in around 10% of cases in children. Once *T. cruzi* infection has been acquired, in the absence of treatment it is usually retained for life.[13]

Indeterminate or latent phase

In those surviving acute phase infection, intracellular multiplication and parasitemia in the blood subside, although trypomastigotes may still be detectable by sensitive methods. The majority of individuals who recover from the acute phase lead entirely normal lives without any further sequelae. However, after a symptom-free indeterminate phase of unpredictable duration which may last many years, ECG abnormalities typical of chronic Chagas disease arise in up to 30% of patients. There is no way to predict which individuals will progress to develop chronic Chagas.

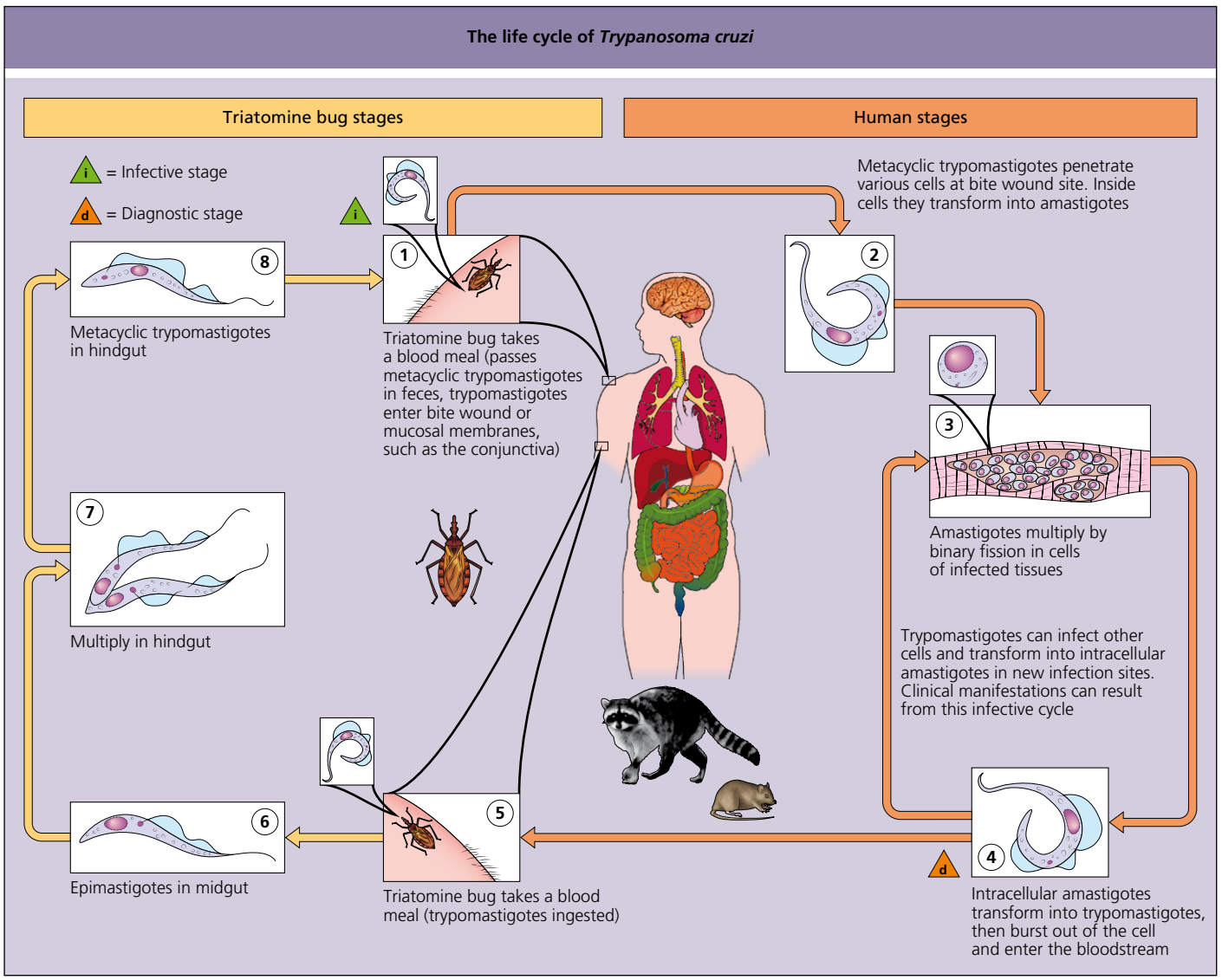


Fig. 118.3 The life cycle of *Trypanosoma cruzi*. Modified from US Centers for Disease Control and Prevention (CDC), Atlanta, GA, USA. Public Health Image Library ID#3384.

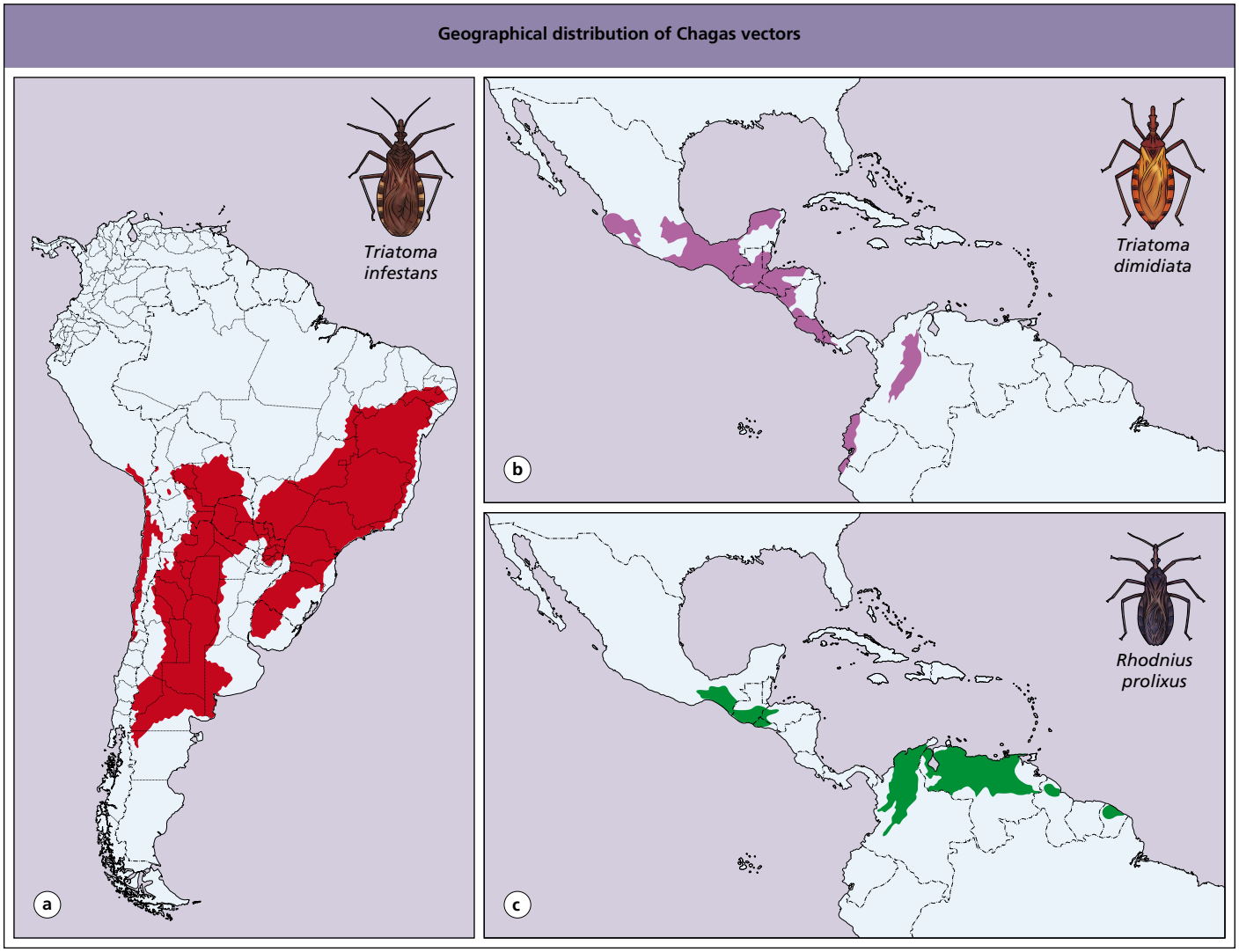


Fig. 118.4 Distribution of Chagas vectors. Maps indicating the historical distribution of (a) *Triatoma infestans*, (b) *Triatoma dimidiata* and (c) *Rhodnius prolixus*. Source: Pan American Health Organization/World Health Organization. 1989. Unpublished document WHO/VBC/89.967. Geneva, Switzerland.



Fig. 118.5 Example of a typical house construction which favors the domestic transmission of *Trypanosoma cruzi* parasites.



Fig. 118.6 Romaña's sign. Acute Chagas disease in a young child. Source: Special Programme for Research and Training in Tropical Diseases (TDR). World Health Organization Image ID#9305157.

Chronic phase

The pathogenesis of chronic Chagas disease is still somewhat enigmatic. Pathogenesis has been described as involving an inflammatory response, focal neurologic damage and, sometimes, autoimmunity.[14] *Trypanosoma cruzi* invades and replicates in macrophages, fibroblasts, Schwann cells, and smooth and striated myocytes, and parasite DNA can be found in heart muscle and smooth muscle of the alimentary tract in the chronic phase. It is known that antigens released from ruptured pseudocysts may spread from the immediate site of infection and be adsorbed to uninfected cells. This may lead to an extension of focal damage and the release of normally sequestered host antigens, which could precipitate autoimmunity. Candidate cross-reactive epitopes between *T. cruzi* and mammalian tissues have also been described, such as B13 and cruzipain in *T. cruzi* and host antigens in cardiac cells.[15] It is not clear whether autoantibodies are markers of pathology or have a causal role.

Focal lesions in the conducting system of the heart are associated, both clinically and experimentally, with corresponding ECG abnormalities. The pathogenesis of this 'neurogenic' form of chronic Chagas disease is thought to depend upon irreversible neuron loss in the acute phase, exacerbated by further loss with age, such that a threshold is reached beyond which organ function is perturbed. Gross pathology of the heart consists of megacardia and focal thinning of the myocardium, especially at the apex of the left ventricle, which may lead to apical aneurysm formation, which is considered to be pathognomonic of chronic chagasic cardiomyopathy (Fig. 118.7). Cardiac signs include dysrhythmias, palpitations, chest pain, edema, dizziness, syncope and dyspnea. The most typical ECG changes are right bundle branch block and left anterior hemiblock, but there may also be atrioventricular (AV) conduction abnormalities, including complete AV block. Many different types of dysrhythmia may occur, including sinus bradycardia, sinoatrial block, ventricular tachycardia, primary T-wave changes and abnormal Q waves.[16]

Associated chronic phase gastrointestinal symptoms result from denervation of hollow viscera and consequent dysfunction. Chagasic megaesophagus (Fig. 118.8) is more common than chagasic megacolon (Fig. 118.9) and either or both may be associated with chagasic cardiomyopathy.[17] Signs of megaesophagus include loss of peristalsis, regurgitation and dysphagia. Constipation and abdominal pain are typical symptoms of megacolon. Advanced megacolon may be associated with obstruction, perforation and sepsis.

The differential diagnosis of Chagas disease includes distinction from all other types of heart disease and ECG abnormalities, but changes such as right bundle branch block and left anterior hemiblock associated with a history of exposure to *T. cruzi* infection are indicative. Megacolon due to Hirschsprung's disease is usually recognizable, in part because of its rarity in adults.

Congenital infection

Perinatal transmission of *T. cruzi* parasites may occur in 2–10% of women of child-bearing age with indeterminate or chronic Chagas disease.[18] Characteristic signs of congenital infection are: hepatosplenomegaly, jaundice, anemia, interstitial pneumonia, myocarditis and neurologic signs. The ECG in congenital cases is usually normal but there may be low-voltage complexes, decreased T-wave height and increased AV conduction time.

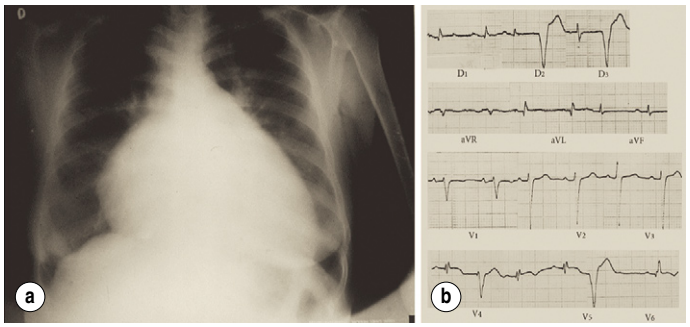


Fig. 118.7 Patient with advanced Chagas cardiomyopathy. (a) Radiography showing enlargement of heart. (b) ECG showing ventricular extrasystoles, atrioventricular block, ischemia and myocardial fibrosis in chronic Chagas' heart disease. Reproduced with permission from Coura.[31]

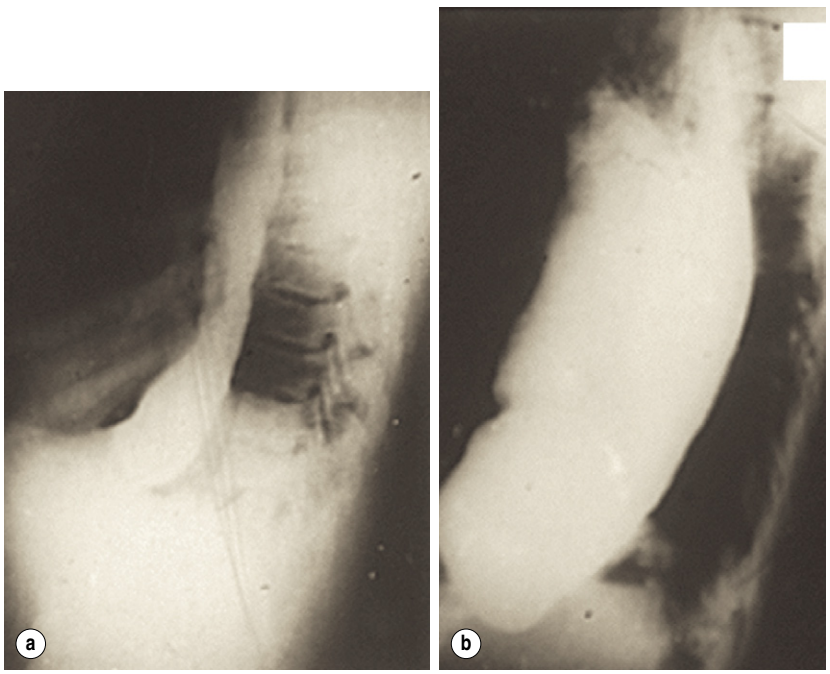


Fig. 118.8 Chagasic megaesophagus. Barium contrasted radiographs showing (a) megaesophagus grade III and (b) megaesophagus grade IV in a patient with chronic Chagas disease. Modified with permission from Coura.[31]



Fig. 118.9 Chagasic megacolon. Barium contrast radiography from the large intestine presenting a very large rectum–sigmoid segment filled with fecal mass. Reproduced with permission from Da-Costa-Pinto *et al*.[32]

Post-transfusion

Transfusion can be considered the second most common form of transmission for *T. cruzi*. The risk of parasite transmission by one unit of blood fluctuates between 12% and 20%.[18] The appearance of symptoms such as general lymphadenopathy and splenomegaly are common and occur several months after the blood transfusion. This is possibly due to the fact that trypomastigotes in contaminated transfused blood may less efficiently invade the heart or other organs.

Immunodeficient patients

An immunocompromised state may lead to reactivation of infection, producing symptoms typical of the acute phase. Meningoencephalitis is infrequent in immunocompetent adults but more common in infants and in immunocompromised adults. Chagas disease in HIV-infected patients is associated with advanced disease with CD4 counts <200 cells/mm^3. The central nervous system (CNS) and the heart are the most commonly affected sites and may account for 75% and up to 44% of chagasic disease in HIV patients, respectively.[19] CNS manifestations can include acute fatal meningoencephalitis, tumoral lesions or a granulomatous encephalitis called 'brain chagoma'. Cardiac manifestations are heart failure and arrhythmias.

Reactivation of Chagas disease is always associated with high parasitemias in contrast to the low parasitemias found in chronic immunocompetent patients. Treatment of *T. cruzi* infection in HIV-positive individuals should be started early in the reactivation process, although meningoencephalitis carries a very poor prognosis.[20]

DIAGNOSIS

The World Health Organization (WHO) recommends that individual diagnosis must be based on two conventional tests based on different principles and different antigens.[21] This is due to the suboptimal sensitivity and/or specificity reported for the existing tests. The diagnosis of acute, congenital and reactivated forms of Chagas disease is based on direct detection of parasites, while the diagnosis of chronic infection is generally based on serologic testing.[13]

Parasitologic diagnosis

Direct microscopy

Circulating trypomastigotes may be detectable by direct microscopy of unstained wet-blood preparations in around 90% of acute cases and virtually never in chronic cases. Several methods can be attempted to improve the sensitivity of parasitologic diagnosis. These include microscopic examination of Giemsa-stained thick blood films, buffy coat layer after centrifugation of hematocrit capillaries (with care to avoid exposure to infection), centrifugation sediment from recently separated serum (Strout's method) and centrifugation sediment after lysis of red blood cells with 0.87% ammonium chloride. All these methods may fail, even in the acute phase of infection, if the parasitemia is low. Microscopic examination of biopsies from lymph nodes is an alternative in cases of undetectable parasitemia.

Xenodiagnosis

A more sensitive method of parasitologic diagnosis is the process known as xenodiagnosis, in which triatomine insects from laboratory colonies maintained on birds are fed on the suspect patient. The sensitivity of this method is close to 90% during the acute phase and between 20% and 50% during the chronic phase of the disease. The insects are dissected about 20–25 days after feeding and the hind gut contents are examined for the presence of *T. cruzi* epimastigotes. Care must be taken to avoid infection during this procedure and insects should be dissected in a microbiologic safety cabinet. It is also necessary to ensure that insects in the colony are not infected with the monoxenous flagellate *Blastocrithidia triatomae*, which may be found in *T. infestans*.

In the chronic phase of Chagas disease, xenodiagnosis is still the parasitologic method of choice throughout most of Latin America. In areas where *R. prolixus* is a vector of *T. cruzi*, xenodiagnosis may yield the nonpathogenic human trypanosome *Trypanosoma rangeli*. Frequently, *T. rangeli* infections in *Rhodnius* spp. can be identified by the presence of long, slender (up to 80 mm) epimastigotes invading the insect salivary glands (*T. rangeli* is transmitted by inoculation, not by contamination).

Culture

Culture of venous blood on to a blood agar base medium may be used as an alternative to xenodiagnosis but demands better laboratory facilities, is difficult to perform under field conditions and seldom achieves the sensitivity of xenodiagnosis.

Serologic tests

Specific antibodies to *T. cruzi* are usually detectable within a few days of infection and usually persist for life unless the infection is eliminated by chemotherapy. Rarely, serologic reversion may occur without treatment. There is an initial IgM response and a sustained IgG response, which is detectable by a variety of assays. Commonly used tests include the immunofluorescent antibody test (IFAT), enzyme-linked immunosorbent assay (ELISA) and the indirect hemagglutination test (IHAT). Most of these tests use lysates of epimastigotes as target antigens but several are based on recombinant proteins. Their sensitivity ranges from 96.5% to 100% and their specificity from 87% to 98.9%. Cross-reactions may occur, especially with visceral and cutaneous leishmaniasis, which may coexist in the same geographic area with Chagas disease.[22] Infants born of seropositive mothers may be seropositive until up to 9 months of age due to transplacental transfer of IgG. Seropositivity in such infants using an IgM-specific conjugate suggests congenital infection. Short-term visitors to endemic areas are extremely unlikely to acquire Chagas disease and, if infection is suspected through exposure to triatomine bites or blood transfusion, serologic status may be used to exclude more likely causes of heart disease.[23]

Molecular methods

Detection of *T. cruzi* DNA by polymerase chain reaction (PCR) may be an adjunct to parasitologic diagnosis. Different assays targeting DNA sequences such as the 195 base pair satellite repeat or the kDNA

minicircle conserved region have been evaluated.[24] Although PCR-based assays have better sensitivity rates than xenodiagnosis, PCR methods have not been standardized and no kits are available commercially. For these reasons and because of the technical complexity of the testing, PCR is limited primarily to research settings and clinical utilization is minimal.[25]

Although several methods are available, current diagnostic approaches are insufficient to respond to the global Chagas disease challenge. There is an urgent need to develop and market new diagnostic tests that may be used in the remote areas where the disease is endemic and which perform well enough to direct clinical care.[26]

TREATMENT

Currently there are only two drugs available to treat Chagas disease: benznidazole and nifurtimox.[27] It has been estimated that both drugs produce parasitologic cure in 60–85% of acute cases. The use of either of these two drugs is recommended during the acute phase of infection and for chronic cases in patients under 15 years of age. Chemotherapy for chronic Chagas disease in adults is more controversial as the pathogenesis might be largely attributable to acute phase damage. Adult chronic cases are thus not always treated because the contribution of continued low-level infection to pathogenesis is uncertain, side-effects may cause interruption of treatment, treatment often fails to eliminate the organism and cure is difficult to prove (negative parasitology is not sufficiently sensitive to prove absence of infection and reversion of serology may take decades). Immunocompromised patients must be treated, and double or even higher dose rates, if tolerated, may be recommended to treat meningoencephalitis. Similarly, congenital cases also demand treatment. New therapeutic alternatives for *T. cruzi* elimination are being evaluated and work in this area has recently been extensively reviewed.[28]

Benznidazole

This orally delivered nitroimidazole should be used at doses from 5 to 10 mg/kg/day in two divided doses for 60 days. Children tolerate higher doses than adults. Common adverse events are rashes, fever, nausea, anorexia, weight loss, headache and insomnia. After 4 weeks of treatment dermatologic adverse events and peripheral neuropathy could occur and this should lead to treatment discontinuation. Laboratory testing for blood cell count should be performed before the start of treatment and every week during treatment to detect possible bone marrow suppression. This drug is contraindicated during pregnancy, in cases of hepatic or renal failure, and in cases of hematopathology or neuropathology.

Nifurtimox

Similar to benznidazole, nifurtimox is better tolerated by children. Recommended doses differ with age and are 15–20 mg/kg for children, 12.5–15 mg/kg for adolescents below 16 years old and 8–10 mg/kg for adults. Nifurtimox should be used at 25 mg/kg for the treatment of meningoencephalitis and at 10–20 mg/kg for congenital cases. The drug should be administered in three divided doses for 90 days in acute cases and 120 days in chronic cases. In adults it is recommended to start the treatment with low doses and increase by 2 mg/kg per week until reaching the maximal dose. Adverse events produced by nifurtimox are anorexia, weight loss, nausea, vomiting and abdominal discomfort. In some patients, symptoms of central nervous system toxicity could be present. These could be irritability, insomnia, disorientation, tremors, polyneuropathy, dizziness, vertigo and mood changes. Reversible psychiatric symptoms could also be present in elderly patients. This drug is not recommended during pregnancy.

Supportive treatment

Chemotherapy is often required for the treatment of adverse events such as fever, vomiting, diarrhea and convulsions. Management of acute meningoencephalitis may require anticonvulsants, sedatives and intravenous mannitol. Management of chronic chagasic heart disease may be a balancing act between patient management, drug administration and use of a pacemaker.[16] Sodium intake is restricted if there is acute-phase heart failure and diuretics and digitalis may be indicated.

Vasodilatation (angiotensin-converting enzyme inhibitors) and maintenance of normal serum potassium may be required initially; digitalis is advisable only as a last resort because it may aggravate dysrhythmias. Bradycardia that does not respond to atropine, atrial fibrillation with a slow ventricular response or complete AV block may necessitate a pacemaker. Surgical resection of dysrhythmic endocardial regions and of ventricular aneurysms has been suggested. Heart transplantation for end-stage chagasic cardiomyopathy has been shown to be a valuable treatment option.

Megaesophagus may improve with dietary control or respond to dilatation of the cardiac sphincter using probes, air or hydrostatic pressure. The surgical procedure for alleviating advanced megaesophagus involves selective removal of a portion of muscle at the junction between the esophagus and stomach. Severe megaesophagus may demand replacement of the distal esophagus with another part of the alimentary tract such as the jejunum.[17] The recommended surgical treatments for megacolon are the anterior rectosigmoidectomy and the modified Duhamel–Haddad operation.[29]

Table 118.1 Screening of blood donors for *Trypanosoma cruzi* in Latin America

	Coverage (%)	Seropositive (%)
Southern Cone countries		
Argentina	100	4.50
Bolivia	86	9.90
Brazil	100	0.61
Chile	75*	0.47
Paraguay	99	2.80
Uruguay	100	0.47
Andean Pact countries		
Colombia	99	0.98
Ecuador	100	0.15
Peru	99	0.26
Venezuela	100	0.67
Central America		
Belize	100	0.40
Costa Rica	100	0.34
El Salvador	100	2.46
Guatemala	100	0.79
Honduras	100	1.40
Nicaragua	100	0.49
Panama	98	0.90

* 98% in endemic regions.
From Schofield *et al.*[2] Copyright Elsevier 2006.

PREVENTION

There is no vaccine for Chagas disease. Crude or fractionated antigens can protect experimental animals against a fatal challenge infection. However, prospects for vaccine development are remote because of the alleged autoimmune pathogenesis of Chagas disease and the impracticality of vaccine trials.[30] Current preventive measures are focused on four main strategies:[3]

- the elimination of domestic vectors;
- the screening of blood donors;
- maternal screening and treatment of infected newborns; and
- active case detection and treatment among school-age children.

Chagas disease is maintained by poverty and poor housing (see Fig. 118.5), which prevent families and communities from controlling domestic triatomine populations. Prevention of new cases of vector-borne *T. cruzi* infection relies on insecticide spraying, health education and improved housing. Pyrethroids, which have low toxicity and high residual activity, are the insecticides of choice for killing triatomines.

Blood for transfusion and organ donors should be screened by one of the available serologic methods. In Latin America, ELISA is the principal method for the current extensive screening of blood donors for *T. cruzi* (Table 118.1). Chemical methods to sterilize donated blood were previously recommended; however, universal blood donor screening for *T. cruzi* infection is now the preferred strategy for the prevention of transfusion- and transplant-related Chagas disease.[23] In 2006, the US Food and Drug Administration (FDA) approved an ELISA from Ortho-Clinical Diagnostics for blood screening prior to transfusion.

REFERENCES

References for this chapter can be found online at http://www.expertconsult.com

Chapter 119

Sharon J Peacock

Melioidosis

Melioidosis is a serious bacterial infection caused by the Gram-negative bacillus *Burkholderia pseudomallei*. This organism has been designated a select agent by the US Centers for Disease Control and Prevention.

EPIDEMIOLOGY

The global distribution of melioidosis is shown in Figure 119.1. Most cases are diagnosed in South East Asia and Northern Australia, but the disease is underrecognized in many parts of the world (reviewed in[1]). Imported infection is seen mainly in immigrants or soldiers serving in endemic areas, but occasionally occurs in tourists.[1]

Melioidosis occurs as a result of inoculation, inhalation or ingestion of *B. pseudomallei* present in the environment. In Asia, melioidosis is most commonly seen in agricultural workers who probably acquire disease following contamination of existing wounds or new penetrating injuries. Evidence for infection via the respiratory route includes the development of melioidosis in US helicopter crews and servicemen during the war with Vietnam, and the shift towards more pneumonic melioidosis observed in Northern Australia during periods of extreme weather including heavy rainfall and winds.[2] Ingestion was implicated as the route of infection during an outbreak associated with contaminated drinking water,[3] although this appears to be rare. Near-drowning led to a cluster of melioidosis cases in survivors of the 2004 tsunami.[4] Mother-to-child and person-to-person transmission are very rare, while patient-to-patient transmission in the hospital setting has not been reported in the published literature. Rare laboratory-acquired infections have been described (reviewed in[5]).

Melioidosis is seasonal in the tropics where most cases present during the rainy season.[1] Males are more often affected than females, and infection can occur at any age with a peak incidence in people aged 40–60 years. Risk factors include diabetes mellitus, chronic lung or kidney disease, thalassemia, alcohol excess, malignancy and immunosuppressive treatment (including corticosteroids) (reviewed in[1]). An associated predisposition is present in around three-quarters of adults presenting with melioidosis.

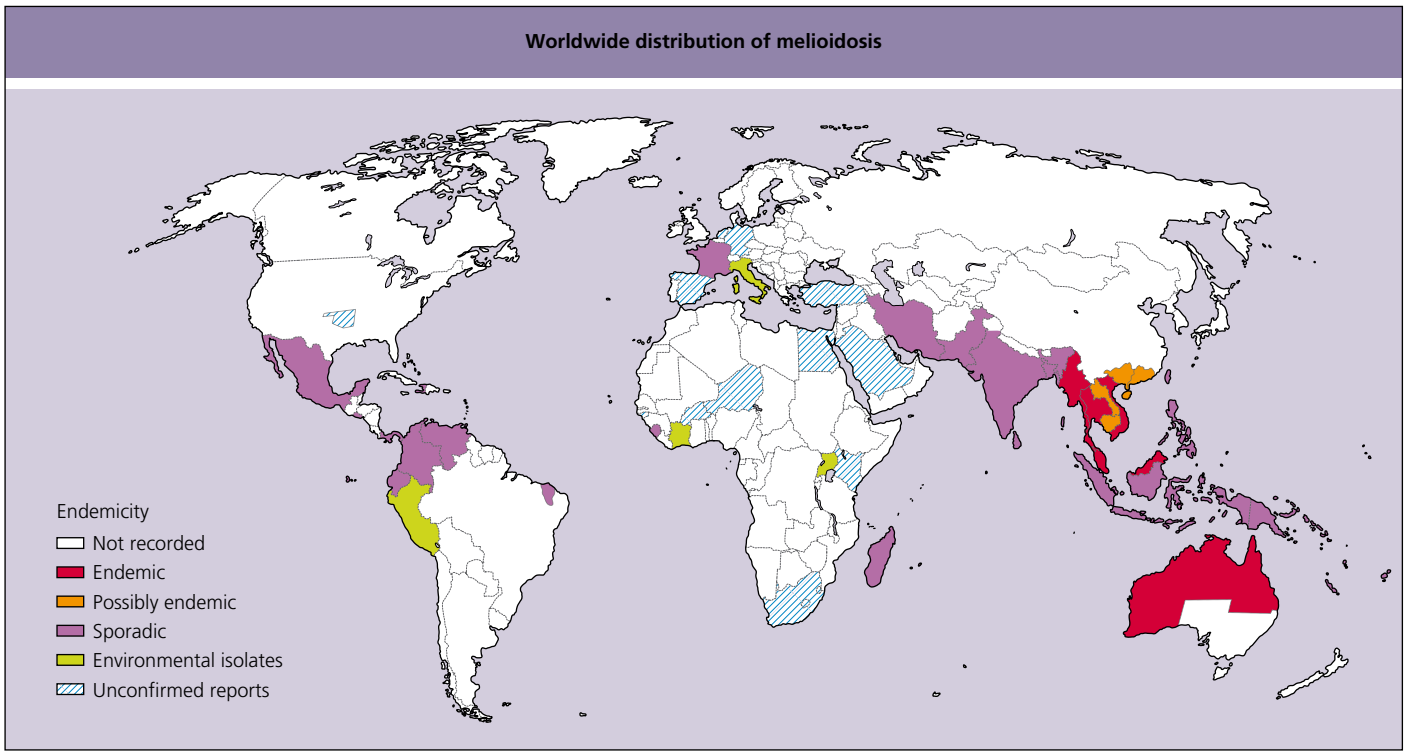


Fig. 119.1 Worldwide distribution of melioidosis. Reproduced with permission from Cheng & Currie.[1]

PATHOGENESIS AND PATHOLOGY

Study of the pathogenesis of melioidosis is in its infancy compared with many other infectious diseases. Key questions are:

- How does *B. pseudomallei* interact with the host immune response, and how does this relate to specific conditions that predispose to melioidosis?
- What is the basis for bacterial latency and persistence *in vivo*?

Putative virulence factors include quorum sensing, a type III secretion system (TTSS3) that shares homology to the *inv/spa/prg* TTSS of *Salmonella enterica* serovar Typhimurium and the *ipa/mxi/spa* TTSS of *Shigella flexneri*, capsular polysaccharide, lipopolysaccharide and flagella (reviewed in[6]).

Burkholderia pseudomallei is a facultative intracellular pathogen that can invade nonphagocytic cells and persist in phagocytes. Following uptake, the organism can escape endosomes and propel itself within and between cells by polar nucleation of actin and induce cell fusion. TTSS3 is central to this process (reviewed in[6]), and BimA is required for actin-based bacterial motility.[7] Reversible colony morphology switching associated with complex alterations in protein expression has been described, and this may be associated with adaptive changes that facilitate bacterial survival *in vivo*.[8] The cytokines interferon gamma (IFN-γ), tumor necrosis factor (TNF) and interleukins (IL)-12 and IL-18 play an important role in early resistance against experimental *B. pseudomallei* infection. These cytokines are expressed during human melioidosis, and may contribute to pathogenesis since high plasma levels of several cytokines are correlated with mortality.

The macroscopic pathology of melioidosis is primarily one of abscess formation. These may be single or multiple, and present in one or more organs. The histopathologic appearance of infected human tissue forms a spectrum from acute to chronic granulomatous inflammation. Samples taken at post-mortem were reported to show focal or diffuse acute necrotizing inflammation with varying numbers of neutrophils, macrophages, lymphocytes and 'giant cells' of unknown cell type.[9] Intracellular bacteria have been observed within macrophages and giant cells.[9]

PREVENTION

Burkholderia pseudomallei is ubiquitous in the environment in areas where disease is endemic and avoidance of the organism is virtually impossible. Protective gear (waterproof shoes or boots and protective gloves) are recommended for agricultural workers in affected geographical regions, but are rarely used because they cause discomfort when worn for prolonged periods in tropical climates. People with risk factors for melioidosis should be advised to stay indoors during periods of heavy wind and rain due to the potential for aerosolization of *B. pseudomallei*. These recommendations are based on known routes of transmission but their efficacy has not been evaluated to date. No *B. pseudomallei* vaccine is available for human use.

CLINICAL FEATURES

The period between an exposure event that results in melioidosis and clinical features of infection is highly variable and often difficult to define. A history of a specific inoculation event is often absent. In one study, 25% of cases reported a specific event from which an incubation period of 1–21 days (mean 9 days) was derived.[10] The incubation period following a high inoculum event such as near-drowning may be short, with a period of less than 24 hours from event to bacteremia in some cases. By contrast, exposure may be followed by a prolonged period of latency, the maximum recorded being 62 years.[11]

Time from onset of clinical manifestations to presentation to a medical facility is also highly variable. In northeast Thailand, around a third of patients have symptoms for less than 7 days, one half report being unwell for 7–28 days, and the remainder have symptoms for more than 28 days.[12] In Northern Australia, 13% of patients presenting for the first time had symptoms for more than 2 months.[10]

Manifestations of disease are extremely broad and range from an acute fulminant bacteremia to a chronic localized infection. Melioidosis is a great mimicker, and it is often impossible on clinical grounds to differentiate between this and other acute and chronic bacterial infections, including tuberculosis. The majority of adult patients develop an acute septicemia associated with bacterial dissemination that most commonly manifests as pneumonia and/or hepatosplenic abscesses. Multiple organ involvement, and the development of additional organ involvement following presentation, are common.

Bacteremia is detected in around half of patients with melioidosis. Septic shock is common in this group and is frequently complicated by the development of organ dysfunction. The lung is involved in approximately half of all cases, manifesting as pneumonia and/or lung abscess(es) (Fig. 119.2). Large or peripheral lung abscesses may rupture into the pleural space to cause thoracic empyema. Solitary or multiple abscesses are detected in the liver and/or spleen in around one-quarter of cases in Thailand when abdominal imaging is performed as routine. More than half of patients with hepatosplenic abscess(es) lack abdominal signs and symptoms. Renal abscesses are often associated with calculi and urinary tract infection. Infection involving the urinary tract is present in at least one-quarter of Thai patients based on a urine culture positive for *B. pseudomallei*, although only a quarter of positive cases have urinary symptoms such as urinary frequency, dysuria, haematuria, or flank or back pain.[13] Genitourinary infection is common in Australia, with prostatic abscesses occurring in 18% of male patients.[14]

A neurologic syndrome of meningoencephalitis with varying involvement of the brain stem, cerebellum and spinal cord has been reported in 5% of melioidosis cases in Darwin, Australia.[15] Prominent features on presentation were unilateral limb weakness, predominant cerebellar signs, mixed cerebellar and brain stem features with peripheral weakness, or flaccid paraparesis.[15] Peripheral motor weakness may mimic Guillain–Barré syndrome and unilateral limb weakness may mimic a stroke. Focal suppurative infections involving the central nervous system with or without meningitis have been reported. CNS infections occur in around 1.5% of melioidosis patients in Thailand,[16] but the syndrome of meningoencephalitis has not been defined in this setting.

Figure 119.3 shows serial CT brain scans with contrast performed on a patient with neurologic melioidosis. The patient initially presented with fever and a hemiparesis. Blood, throat swab and urine cultures were negative for *B. pseudomallei*. A CT scan was performed (a), and the patient was treated for a urinary tract infection and a cerebrovascular accident. The patient re-presented 2 months later with worsening headache, confusion and hemiparesis. Repeat CT scan (b) showed a ring-enhancing lesion with surrounding edema in the right frontoparietal lobe, pus from which grew *B. pseudomallei*. The patient went on to make a full recovery after prolonged parenteral antimicrobial treatment.

Osteoarticular involvement is diagnosed in around one-sixth of patients in Thailand, but only 4% of those affected in Australia.[14] The knee is the most common site for septic arthritis, and more than one joint is involved in a quarter of affected cases. Osteomyelitis is often secondary to infection of another organ, although localized osteomyelitis does occur.

Melioidosis in Thai children presents as acute suppurative parotitis in around one-third of cases, but this is uncommon in Thai adults and very rare in Australia. Parotitis is bilateral in 10% of cases and may be complicated by rupture into the auditory canal, facial nerve palsy and necrotizing fasciitis.[17]

Skin and soft tissue infections are found in a quarter of melioidosis patients in Thailand and one-sixth in Australia. This includes superficial pustules, subcutaneous abscesses and pyomyositis. Such foci may represent the source of systemic infection, or may result from hematogenous or local spread (Fig. 119.4). Soft tissue infection may run an aggressive course similar to that seen for necrotizing

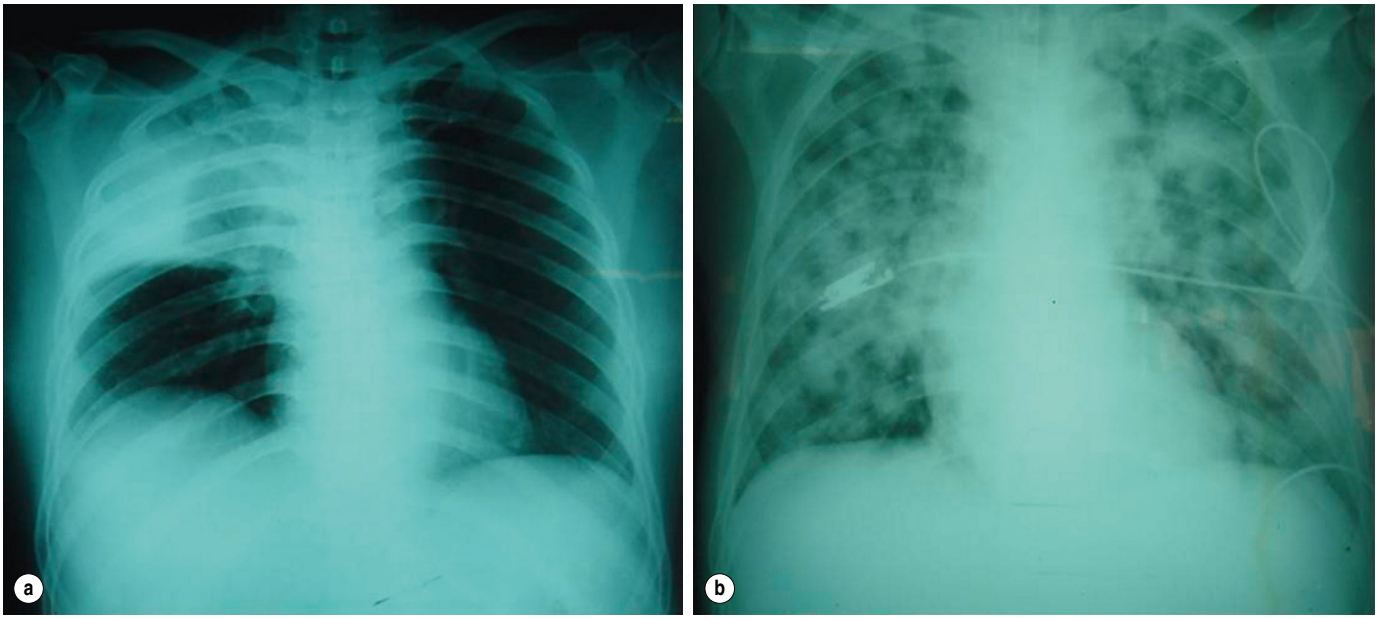


Fig. 119.2 Chest radiographs of two patients with melioidosis. (a) Right upper lobe consolidation in a patient with bacteremia, pneumonia, pyelonephritis and subcutaneous abscesses. (b) Widespread bilateral shadowing in a patient with bacteremia, pneumonia and multiple abscesses in liver and spleen. Courtesy of Dr Gavin Koh.

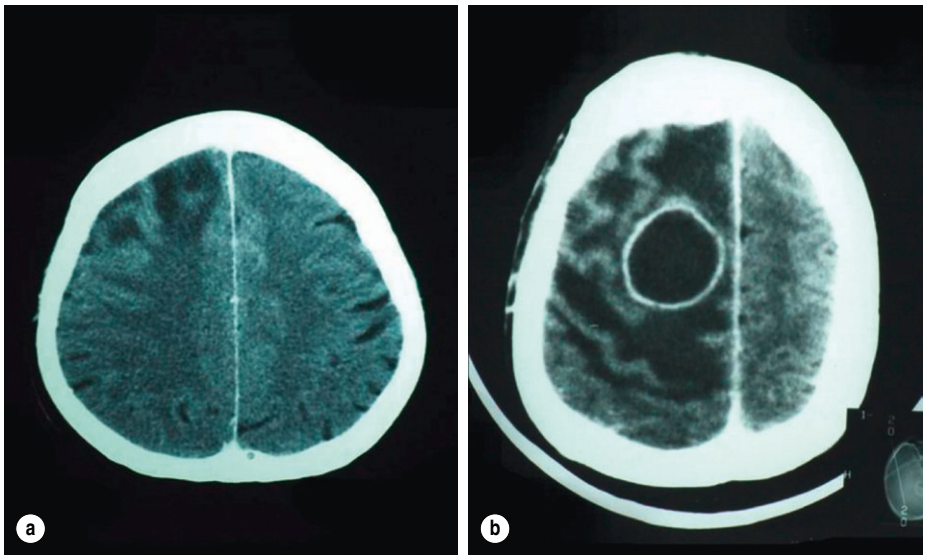


Fig. 119.3 CT brain scans. (a) Hypodensity in the right frontal lobe. (b) Ring-enhancing lesion with surrounding edema in the right frontoparietal lobe. Reproduced with permission from Limmathurotsakul *et al*.[16]

fasciitis caused by other organisms. Infections involving many other sites have been described, including lymphadenitis, mycotic aneurysm, mediastinal infection, pyopericarditis, orbital cellulitis, corneal ulcers, acute otitis media, sinusitis, and abscess formation in the breast, scrotum and deep tissues of the neck.

DIAGNOSIS

Given the myriad of possible presentations, a high index of suspicion is essential for clinicians working in areas that are endemic for melioidosis. This infection should also be considered when investigating the cause of fever in returning travelers or army personnel who could have been exposed to a contaminated environment. Microbiologic culture and identification of *B. pseudomallei* represents the diagnostic gold standard (Fig. 119.5).

A direct immunofluorescent microscopy assay has been described that provides a rapid presumptive diagnosis of melioidosis,[18] but the antibody used is not commercially available and the assay is restricted to a small number of laboratories in Thailand. Unlike the investigation of many other bacterial infections, culture should be performed on all available specimens including blood, urine, throat swab, respiratory secretions, pus and swabs from surface wounds. Growth of even a single *B. pseudomallei* colony from any sample is

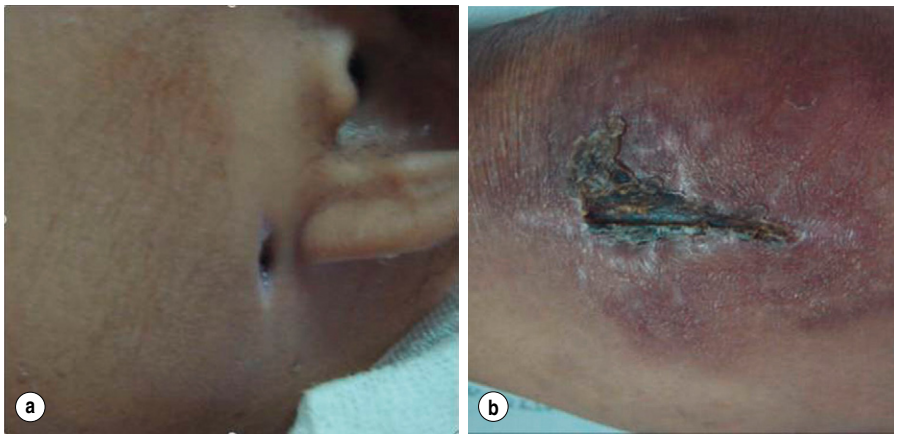


Fig. 119.4 (a) Abscess affecting the pre-auricular area. (b) Infected soft tissue of the heel. Courtesy of Drs Rapeephan Rattanawongnara and Ekamol Tantisattamo.

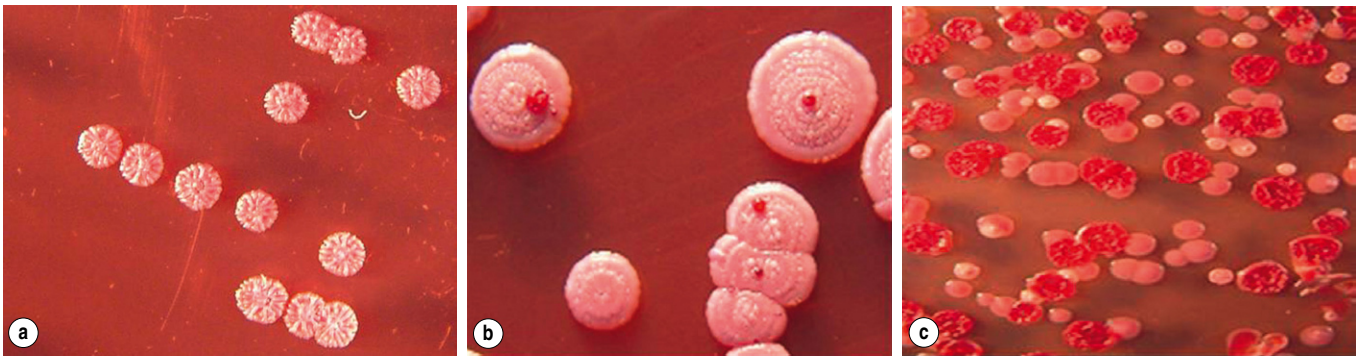


Fig. 119.5 Colony variation is commonly seen during culture of clinical isolates on Ashdown's agar. (a) Typical colony appearance of *B. pseudomallei* after incubation at 37°C in air for 3 days. (b) Colony variation within a single colony in which the parental colony (pink) has given rise to a second morphotype (red). (c) Variable colony morphology from a single sample; genotyping confirmed the presence of a single clonal type. Reproduced with permission from Wiersinga *et al*.[6] Original pictures courtesy of Mrs Vanaporn Wuthiekanun and Dr Narisara Chantratita.

diagnostic for melioidosis in patients presenting to hospital with a febrile illness, since the organism is not a member of the normal human flora. Because the organism can become disseminated in the host, the site of culture positivity may not necessarily reflect the major site of clinical infection. For example, a throat swab may be positive for *B. pseudomallei* in a patient who presents with a liver or splenic abscess. It is important to alert the laboratory to the suspicion of melioidosis because the organism can easily be overlooked as a contaminant (especially in cultures of samples taken from colonized sites) and because cultures should be handled in biosafety containment level 3 facilities. Preliminary culture results should be available within 48 hours, although identification may be delayed when microbiologists are not familiar with the organism. Speed to blood culture positivity has prognostic significance, and the bacterial count of *B. pseudomallei* in blood and urine are positively associated with outcome (reviewed in[12]).

Polymerase chain reaction (PCR) has been evaluated for the diagnosis of melioidosis but is not in routine use. Serologic assays such as the indirect hemagglutination (IHA) test have no role in the diagnosis of melioidosis where infection is endemic since anti-*B. pseudomallei* antibodies are common in the general population.[19] A single IHA titer of >1:40 or a rising titer in an individual who has not previously resided in areas where melioidosis is endemic may be diagnostically useful but requires careful interpretation based on a detailed travel and medical history.

MANAGEMENT

Appropriate parenteral antimicrobial agents should be commenced immediately on suspicion of the diagnosis of melioidosis. Ideally, culture should be performed prior to their administration but treatment should not be delayed if culture cannot be performed rapidly, as in the case of aspiration of pus under imaging or operative procedure. Choice and duration of therapy are shown in Table 119.1.

A retrospective study of the effect of granulocyte colony-stimulating factor (G-CSF) in patients with septic shock due to melioidosis at the Royal Darwin Hospital, Northern Australia demonstrated a dramatic decrease in mortality from 95% to 10% concurrent with its introduction.[20] However, a randomized placebo-controlled trial of G-CSF for severe melioidosis in northeast Thailand failed to show an effect on mortality.[21] No other adjunctive therapies have been shown to improve outcome from melioidosis. Patients with severe melioidosis associated with septic shock, respiratory failure associated with severe infection or acute respiratory distress syndrome, acute renal failure and other manifestations of a severe septic illness require intensive care management where this is available. The availability of intensive care facilities is an important contributor to the marked difference in outcome for high-income versus low- or lower-middle-income countries. Predictors of mortality from melioidosis include APACHE II and a scoring system based on a smaller number of readily available parameters.[22]

Table 119.1 Antimicrobial treatment of melioidosis

Drug	Dose	Duration
Initial parenteral treatment		
Ceftazidime Meropenem	50 mg/kg/dose (up to 2 g) q6–8h*, *or* 25 mg/kg/dose (up to 1 g) q8h*	Minimum 10–14 days, or longer (4–8 weeks) for deep-seated infection
Oral eradicative treatment		
Trimethoprim–sulfamethoxazole	Weight-based regimen for adults (q12h): • 2 × 160–800 mg (960 mg) tablets if >60 kg • 3 × 80–400 mg (480 mg) tablets if 40–60 kg • 1 × 160–800 mg (960 mg) *or* 2 × 80–400 mg (480 mg) tablets if <40 kg *with or without*	At least 3–6 months
Doxycycline	2.5 mg/kg/dose up to 100 mg po q12h†	

Notes:
1. Amoxicillin–clavulanate is an alternative treatment for pregnant women and children younger than 8 years and as an alternative to first-line therapy in other patient groups. Amoxicillin–clavulanate dosing for melioidosis is reviewed in[23]. For intravenous-phase treatment, the recommended dose is 20/5 mg/kg q4h. Parenteral amoxicillin–clavulanate is associated with higher rates of treatment failure, so avoid if first-line drugs can be given. For oral eradicative treatment, use amoxicillin–clavulanate at a dose of 20/5 mg/kg q8h. For adult patients <60 kg a dose of 1000/250 mg q8h is suggested. For patients >60 kg, the recommended maximum dose is 1500/375 mg q8h. Oral amoxicillin–clavulanate is associated with higher rates of relapse.
2. Adjust drug dosages as necessary in patients with renal failure, a common complication of melioidosis.

*Consider addition of trimethoprim–sulfamethoxazole 8/40 mg/kg (up to 320/1600 mg) q12h for treatment of patients with neurologic, prostatic, bone or joint melioidosis.
†Doxycycline is not used as part of oral treatment of melioidosis in Australia but is routinely used in Thailand. The equivalence of trimethoprim–sulfamethoxazole versus trimethoprim–sulfamethoxazole plus doxycycline is the subject of a randomized clinical trial in Thailand.

Hematologic and biochemical blood tests should be performed to detect the onset of acute renal failure, abnormal liver function tests and anemia, all of which are well recognized during severe melioidosis. Arterial blood gases should be taken in patients with lung involvement and/or any evidence of respiratory impairment. Admission C-reactive protein may be normal or only mildly elevated, including patients with severe sepsis, fatal cases and in relapsed melioidosis.

A chest radiograph should be taken in all patients with suspected melioidosis. Common radiographic patterns include localized patchy alveolar infiltrate, focal, multifocal or lobar consolidation, diffuse interstitial shadowing considered consistent with blood-borne spread of infection, pleural effusion, and upper-lobe involvement which may include cavitation. The radiographic pattern may mimic tuberculosis. The development of empyema and/or lung abscess(es) is well recognized, and repeat chest radiographs are indicated for patients with respiratory involvement.

Abdominal ultrasound or CT scan should be performed on all patients with melioidosis to exclude the presence of abscesses in liver and spleen. The finding of a 'Swiss cheese' appearance on ultrasound or a 'honeycomb' appearance on CT scan are characteristic for melioidosis. Clinical evidence of prostatic involvement requires appropriate imaging. Transrectal ultrasound findings include solitary and multiple abscesses. Early brain CT scan may be normal in patients with neurologic melioidosis, but MRI often shows dramatic changes.[15,16] The need for other imaging will depend on clinical features and organ involvement.

Parenteral antibiotics are required for a minimum of 10–14 days or until clear clinical improvement is observed (whichever is the longer). This is followed by oral antibiotics to complete 12–20 weeks of therapy (or more if clinically indicated), as described in Table 119.1. Melioidosis is characterized by difficulty in eradicating the causative organisms. Fever clearance is often slow (median fever clearance time of 9 days), and without evidence of clinical deterioration is not normally sufficient to indicate the need for a change in therapy. Sputum and draining abscess cultures may remain positive for several weeks in a patient who is otherwise responding to treatment. A patient who has clinical deterioration or persistently positive blood cultures should be viewed as failing treatment, at which stage the need for imaging, drainage of collections and change in antimicrobial therapy should be considered.

Death from melioidosis occurs in around 50% of cases in Thailand and 19% in Australia.[12,14] The majority of deaths are due to uncontrolled sepsis and/or organ dysfunction, including respiratory and renal failure. The single most important complication for patients who survive the first episode is recurrent melioidosis, which may occur despite prolonged appropriate oral antimicrobial treatment. This occurs in around 13% of patients followed for 10 years, half of which occurs within 12 months of the primary episode.[24,25] Recurrence represents either relapse following failure to eradicate bacteria responsible for the primary infection, or re-infection with a new strain. Three-quarters of recurrent cases are due to relapse and one-quarter to re-infection.[26] Time to recurrence is significantly shorter for episodes due to relapse compared with those due to re-infection.[24] Choice and duration of oral antimicrobial therapy are the most important determinants of relapse, followed by the presence of a positive blood culture and multifocal distribution.[24]

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References for this chapter can be found online at http://www.expertconsult.com

Chapter 120

Kenneth L Gage
C Ben Beard

Plague

Plague is an acute, life-threatening zoonosis caused by the bacterium *Yersinia pestis*.[1–3] The disease is best known for three devastating pandemics, including the Black Death of the Middle Ages. The last of these pandemics, which began in southwest China in the late 1800s, was largely over by the end of World War II but human cases of plague continue to occur in many regions. Plague is primarily a disease of rodents, and humans typically acquire infection as a result of being bitten by rodent fleas, less commonly by handling infected animals, and rarely by inhaling infectious respiratory particles. Historically, urban, rat-borne plague outbreaks have been responsible for most human plague cases and widespread epidemics. Over the past half century, however, most human plague outbreaks have occurred in remote, rural populations.[3,4] The three principal clinical forms of plague are bubonic plague, septicemic plague and pneumonic plague. The most common of these is bubonic plague, an acute illness characterized by fever and enlarged tender lymph nodes (buboes) that usually appear in the groin, axillary or cervical regions proximal to the site of inoculation. Bacteremia and sepsis occur when lymphatic defenses have been breached (septicemic plague) and plague pneumonia can arise from inhaling infectious respiratory droplets or secondarily as a result of blood-borne invasion of the lungs. Occasionally, pneumonic plague spreads from person to person, most often in crowded, substandard living conditions.[5]

Plague is often fatal when not diagnosed and treated appropriately early in the course of infection.[1,3] The aminoglycosides, tetracyclines and chloramphenicol are the antibiotics most commonly used to treat human plague.[1,6,7] Prevention and control of plague during outbreaks relies on prompt identification and treatment of human cases, isolation of pneumonic cases and identification of their contacts, and use of flea control measures.[5] Pneumonic plague patients should be isolated under respiratory droplet precautions until no longer infectious.[1,7] Persons who have been in close contact (<2 m) with pneumonic plague patients should be placed on antimicrobial prophylaxis. Others who have more distant contact should be monitored for fever. Human plague risks can be reduced further by environmental sanitation measures intended to reduce rodent populations.[5] Plague is considered to be an important potential weapon of bioterrorism.[2,7]

EPIDEMIOLOGY

Agent

Yersinia pestis is a Gram-negative, microaerophilic coccobacillus belonging to the Enterobacteriaceae. Molecular studies, including genomic sequencing of a few *Y. pestis* strains, indicate that this bacterium has recently evolved from the enteric pathogen, *Y. pseudotuberculosis*.[8] *Y. pestis* is nonmotile, nonsporulating, does not ferment lactose and exhibits bipolar staining with Wayson, Wright or Giemsa stain. Growth occurs in a variety of media at a wide range of temperatures (4–40°C; optimal 28–30°C) and pH values (5.0–9.6; optimal 7.2–7.6).[9]

The plague bacterium produces many factors that enable it to survive in and cause disease within its mammalian hosts (virulence factors) and be transmitted by flea vectors (transmission factors) (Table 120.1).[2,9] Some factors are expressed selectively at temperatures and environments encountered in fleas or mammals. For example, hemin storage locus (*hms*) products are expressed in the low temperature environment of the flea and produce a biofilm that enables plague bacteria to form blockages in the flea's gut. These blockages hamper the flea's ability to ingest blood and cause it to starve. Starving fleas repeatedly attempt to feed and often regurgitate in an effort to clear the blockage from their guts, a behavior that can flush *Y. pestis*-containing material from the blockage into the bite wound and result in infection of the host. At typical mammalian body temperatures biofilm production ceases, causing dissolution of blockages and occasionally clearance of *Y. pestis* infection from fleas. Murine toxin (*ymt*), which is highly toxic in mice and rats but has little effect on other mammals, also is required for survival of *Y. pestis* within flea midguts.

In mammals, plasminogen activator (*pla*) degrades blood clots and helps plague bacteria escape from the site of initial inoculation. YopM, which is resistant to *pla* activity, competes for thrombin and inhibits its activation of platelets, further promoting dissemination of *Y. pestis* within the host. Early in infection, *Y. pestis* behaves as a facultative intracellular pathogen, invading and multiplying within host phagocytes. Survival of plague bacteria within the host is made possible by a type III secretion system composed of various Yops that can interfere with the production of proinflammatory cytokines, impair the cytoskeletal dynamics of phagocytes and cause immune cell death by apoptosis. At mammalian host temperatures *Y. pestis* produces a capsular antigen (*caf1*) that renders it resistant to further phagocytosis and, along with the effects of various Yops, allows it to multiply extracellularly. Various iron uptake systems, including one that utilizes the siderophore yersiniabactin, enable the plague bacterium to compete with its hosts for this essential nutrient.

Three biovars (biotypes) of *Y. pestis* have been recognized according to the ability of the bacteria to ferment glycerol and reduce nitrate, distinctions that have also been supported by various molecular analyses.[8,9] These biotypes (Antiqua, Medievalis and Orientalis) originally were thought to have been correlated respectively with the three principal plague pandemics – Justinian's Plague, the Black Death, and the Modern (Third) Pandemic. More recent analyses have complicated this picture, calling into question these biovar–pandemic associations and establishing the presence of a previously unrecognized Microtus biovar and atypical isolates that have been termed pestoides group.[8]

Although plague is global in its distribution, the majority of its spread occurred during the Third Pandemic. As a consequence, strains

Table 120.1 Proposed virulence and transmission factors for *Yersinia pestis*

Genomic element	Virulence or transmission factor	Proposed role in virulence or transmission
9.5 kb plasmid (pesticin plasmid; a 19 kb dimer of this plasmid also exists)	Pesticin sensitivity (*pst*)	Loss of sensitivity to pesticin (a bacteriocin) is associated with reduced siderophore binding capability (affects iron uptake)
	Plasminogen activator (*pla*)	Fibrinolytic activity (important for dissemination)
70–75 kb plasmid (low calcium response plasmid)	*Yersinia* outer proteins (Yops – genes found in the Yop virulon, a type III secretion system; includes *lcrV* or V antigen)	Proposed functions vary among Yops and include: translocation of other Yops (effectors) across cell membranes; disturbance of phagocyte cytoskeleton dynamics (interferes with phagocytosis); blocking production of proinflammatory cytokines and interfering with ability of B and T cells to be activated by means of antigen receptors (immunosuppression); or binding thrombin (interferes with thrombin–platelet aggregation)
100–110 kb plasmid	Murine toxin (*ymt*)	Required for survival in fleas; also has β-adrenergic antagonist activity in rats and mice but not guinea pigs, rabbits, dogs or nonhuman primates
	F1 'capsular' antigen (*caf1*)	Resistance to phagocytosis by monocytes
Chromosomal	Pigmentation (*pgm* locus, includes genes of *hms* locus, high pathogenicity island and *ybt* operon)	Pigment-positive strains bind hemin and appear pigmented on culture media containing Congo Red; *pgm* locus contains genes of the high pathogenicity island (HPI), which is found in other Yersiniae and certain related enteric bacteria; the HPI contains the *ybt* (yersiniabactin) operon, which encodes genes of a siderophore-based iron uptake system; the *pgm* locus also contains the *hms* locus, which produces biofilm and must be functional for 'blocking' to occur in the flea vector (blocking increases efficiency of *Y. pestis* transmission by fleas)
	Endotoxin (lipopolysaccharide)	Lipopolysaccharide release associated with major pathogenic effects of plague sepsis, systemic inflammatory response syndrome and associated adult respiratory distress syndrome, cytokine activation, complement cascade, disseminated intravascular coagulation, bleeding, unresponsive shock and organ failure
	Serum resistance (lipopolysaccharide in part)	Resistance to complement-mediated lysis; proposed to be related in part to lipopolysaccharide structure
	pH 6 antigen (*psa*)	Entry into naive macrophages; assists in delivery of Yops into phagocytic cells

from the Americas demonstrate very little genetic diversity in comparison to those of central and eastern Asia which display rich biochemical, genetic and host diversities.[10,11]

Life cycle

Yersinia pestis is maintained in cycles involving transmission between rodents and their fleas and through long-term survival of infectious fleas in burrows or other off-host environments. (Fig. 120.1).[12] Other proposed means of maintenance include survival of *Y. pestis* in soils or in chronically infected rodents,[13] but evidence that either of these is important under natural conditions is limited. Humans and other incidental hosts of *Y. pestis* are not directly involved in maintaining its natural cycle.

Although most foci are maintained by transmission between wild rodents and their fleas, commensal rats (*Rattus rattus, R. norvegicus*, etc.) and their fleas (especially Oriental rat fleas, *Xenopsylla cheopis*) remain the principal sources of large epidemics. In a few areas, such as Madagascar, commensal rats also act as the primary rodent hosts of *Y. pestis*.[5] The most critical role played by susceptible rodents in the natural plague cycle is to serve as sources of infection for feeding fleas.[12] Although virtually any rodent can be infected with *Y. pestis*, not all succumb to plague, and the susceptibility of rodent populations and species can vary greatly. Only those fleas that feed on dying rodents with very high (>10^6 *Y. pestis*/ml blood) bacteremias are likely to become infected. Besides enabling infection of fleas by *Y. pestis*, fatal bacteremias in rodents force infected fleas to abandon their dead hosts and seek new ones, thus promoting spread of the disease among wild animals and humans.

Geographic distribution

Plague foci are widely distributed throughout the world, and human cases are typically reported from 10 or so countries each year.[14] Selected countries reporting significant human or animal plague outbreaks during the interval from 1988 to 2007 are shown in Figure 120.2.

Populations affected

Plague mostly affects impoverished populations of developing countries living in rural villages that are heavily infested with rodents

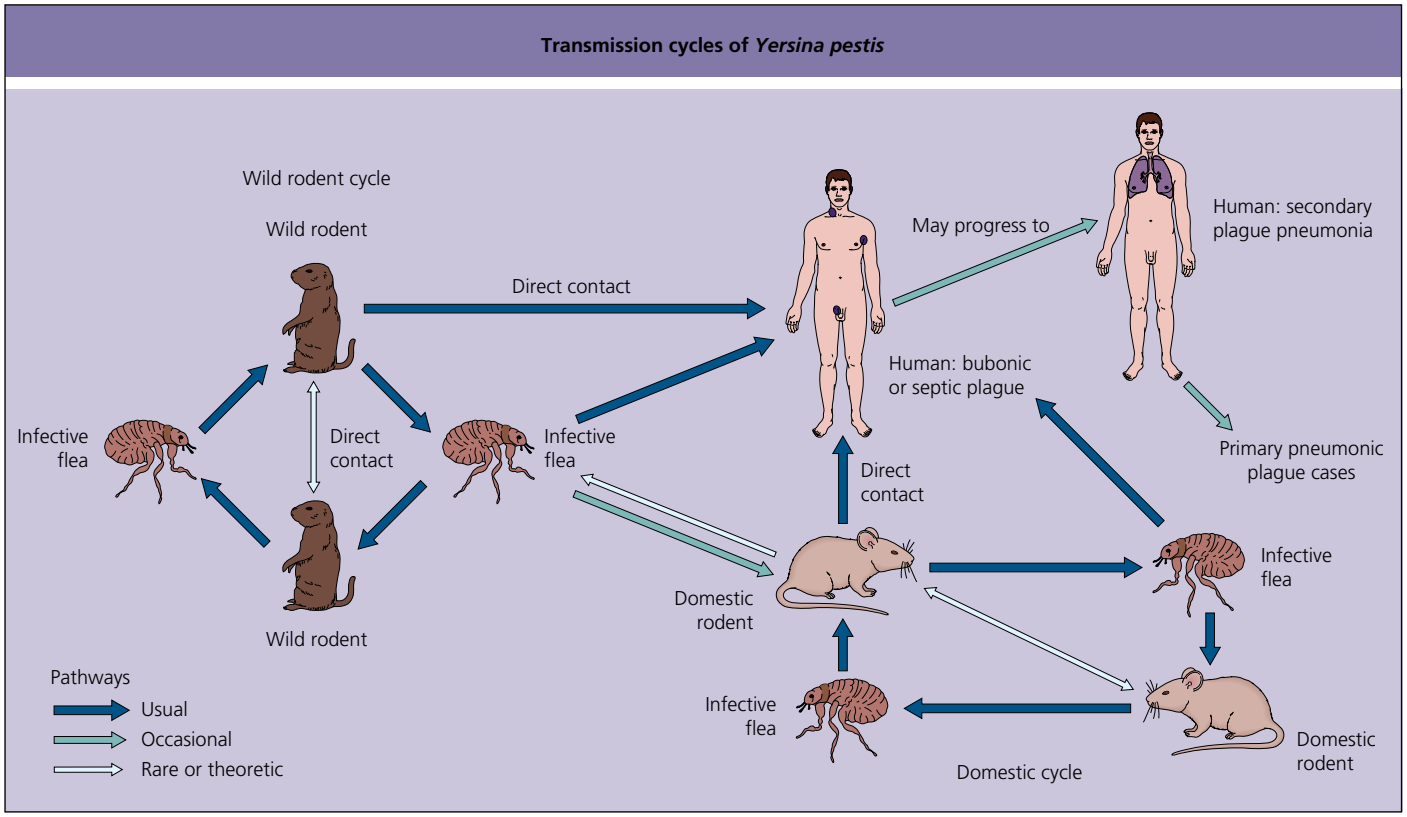


Fig. 120.1 Transmission cycles of *Yersinia pestis*.

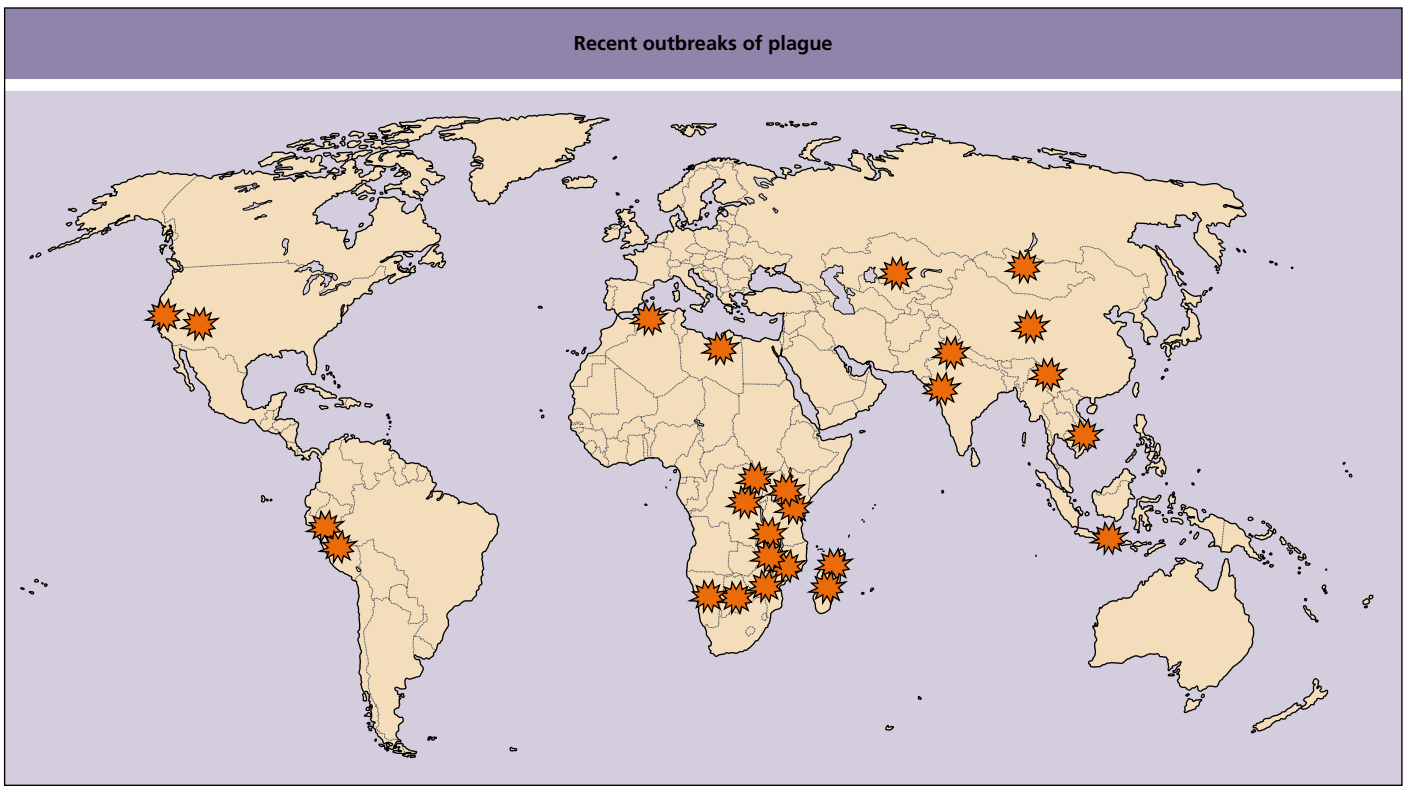


Fig. 120.2 Recent outbreaks of plague. Compiled from sources of the WHO, the Centers for Disease Control and Prevention, and the individual countries.

(particularly commensal rats) and their fleas. Urban rat-borne plague is now unusual, but still occasionally occurs in developing countries.[1,4] In the USA, most persons are exposed to infection around rural residential properties that are often poorly maintained and provide shelter and food for rodents.[4] Cases also occasionally occur among campers, hikers, hunters, pet owners, veterinary staff or others exposed to plague in natural settings or through handling infected animals.[5,15]

In the absence of control measures, plague can spread from rural areas to major cities and ports, either through unintended transport of rats or other animals, or by passage of infection between adjoining rodent populations.[4,5] Occasionally, persons who travel during the incubation phase later develop plague pneumonia and transmit the disease to others encountered along their way. Plague risks for persons visiting endemic areas for business or tourism are extremely low because these persons typically stay in relatively modern hotels and spend little time in the rural villages most likely to be affected by the disease.

Disease incidence

Virtually all plague cases reported to the World Health Organization (WHO) in recent decades have come from Asia, Africa and the Americas. While the last great pandemic originated in Asia, over the last 20 years there has been a striking shift in distribution of cases from that continent to Africa.[3,4,14] Based on the latest WHO statistics, 25 countries reported a total of 38 359 cases (mean of 2557 cases per year) and 2845 deaths (7.4% fatality rate) from 1989 to 2003 (Table 120.2).[14] During this 15-year period, nine countries in sub-Saharan Africa and nearby Madagascar reported 81% of the world's cases, with the remaining cases occurring in Asia (14.2%) and the Americas (4.3%). Madagascar alone reported over 34% of all cases and Tanzania accounted for 14%. In 2003, five African countries (Algeria, Democratic Republic of the Congo, Madagascar, Mozambique and Uganda) reported 98.7% of global plague cases and 98.8% of plague deaths.[14] At present, plague in Asia and the Americas occurs only sporadically, although large

Table 120.2 Reported cases of plague in humans by country, 1989–2003

Region	Country	Number of cases	Number of deaths
Africa	Algeria	11	1
	Botswana	173	12
	Democratic Republic of Congo	4935	616
	Kenya	44	8
	Madagascar	13148	1132
	Malawi	907	16
	Mozambique	2387	28
	Namibia	2012	80
	Tanzania	5249	335
	Uganda	821	130
	Zambia	1169	29
	Zimbabwe	417	34
	Total	**31 273**	**2421**
Americas	Bolivia	37	6
	Brazil	105	0
	Ecuador	14	14
	Peru	1380	60
	USA	101	10
	Total	**1637**	**90**
Asia	China	519	52
	India	892	58
	Indonesia	6	0
	Kazakhstan	24	7
	Laos	10	0
	Mongolia	115	36
	Myanmar	761	6
	Vietnam	3122	175
	Total	**5449**	**334**
World totals		**38 359**	**2845**

outbreaks remain a possibility, as demonstrated by an epidemic of more than 1200 cases that occurred in Peru from 1992 to 1995.

Although large outbreaks have not been reported in the USA since the early 1900s when the disease was first introduced, plague still represents an emerging disease in this country,[4] with more than 420 cases of human plague reported since 1950. The most recent outbreak occurred in 2006 when 17 cases were reported from seven states, equaling the highest number of reported cases in any year since 1984.[16] Although plague remains enzootic and occasionally causes epizootics in rural areas within the 17 western-most states, about 90% of all human cases reported in the past three decades have occurred in four states (New Mexico, Colorado, Arizona and California).[4,16]

Sources of infection and risks for humans

Flea bites are the most common source of human *Y. pestis* infection.[1] Plague also can be acquired through handling infected animals, which is an important source of exposure among persons who hunt and skin marmots (*Marmota* spp.) in central Asia and among similar groups in the USA who handle carcasses of prairie dogs, ground squirrels, rabbits and wild carnivores.[1,5] Pet owners and veterinary staff may become infected with *Y. pestis* while caring for infected domestic cats.[15] Outbreaks of plague have occurred in Saudi Arabia, Libya, Jordan and Kazakhstan as a result of persons handling or consuming infected camel or goat meat.[2,5]

Human risks of exposure to infectious fleas or animals are typically greatest during epizootics when *Y. pestis* spreads rapidly through rodent populations, causing high mortality among these animals and abandonment of dead hosts by fleas. Even in the presence of efficient flea vectors, epizootics probably occur only when rodent host densities exceed certain threshold levels, presumably because of the increased likelihood of host-to-host contact and transfer of infectious fleas between hosts. Among the most important factors affecting rodent populations and the occurrence of plague are those related to climate,[17] particularly precipitation and temperature, both of which can influence plague transmission through their impacts on rodent food availability, rodent host population dynamics, vector flea abundance, blockage of flea guts by biofilm and survival of infectious fleas.

Primary pneumonic plague occurs when persons inhale infectious respiratory secretions.[1,7] This usually arises during the course of an outbreak of bubonic plague in which persons develop secondary pulmonary infection and then spread plague to others through infectious respiratory droplets, starting a chain of airborne transmission. Persons living in the same home as a pneumonic plague patient or attending the sick are especially at risk. Unfortunately, plague can be spread intentionally as an aerosol, cause severe outbreaks (most likely pneumonic plague outbreaks) with many fatalities, create panic and require special preparations to ensure effective responses to bioterrorism attacks (see also Chapter 71). For these reasons *Y. pestis* is classified as a category A agent (highest bioterrorism risk), along with the agents of anthrax, smallpox, tularemia and viral hemorrhagic fevers.[2,7] *Yersinia pestis* strains could also be genetically modified so they would be resistant to the antibiotics commonly used to treat plague.

Under natural conditions, primary pneumonic plague comprises only a small fraction of the total number of cases in any plague-endemic region. In the USA primary pneumonic plague cases represent only about 2% of total cases, nearly all of which were associated with handling cats that had plague pneumonia and cough.[15] Human-to-human spread of plague through respiratory contact has not been documented in the USA since 1924,[7] but the likelihood of pneumonic plague outbreaks remains high in many developing countries.

Seasonality

Flea-borne cases of human plague in the northern hemisphere are most likely to occur between late spring and the end of summer when epizootic transmission peaks.[1] In tropical and semitropical plague foci, transmission may vary between a wet, relatively cool season and another season of hotter, drier weather, with the numbers of cases being lower during the latter period.[17] Occasional winter cases of human plague occur in temperate regions among hunters, trappers, pet owners or veterinary staff handling infected animals.

PATHOGENESIS AND PATHOLOGY

Yersinia pestis is among the most pathogenic bacteria known, using numerous chromosomal and plasmid-encoded gene products to cause severe disease and promote its establishment and spread within the host (see Table 120.1).[2,9]

Yersinia pestis organisms inoculated through the skin or mucous membranes travel to and multiply within regional lymph nodes.[1] In the early stages of infection, nodes are found to be edematous and congested with minimal inflammatory infiltrates and vascular injury. Fully developed buboes are surrounded by serous fluid, contain large numbers of plague bacteria and exhibit considerable vascular damage, hemorrhagic necrosis and neutrophil infiltrates. In later stages, abscess formation and spontaneous rupture of lymph nodes may infrequently occur.

Plague sepsis in the absence of signs of localized infection, particularly a bubo, is termed primary septicemic plague and can result from direct entry of *Y. pestis* through broken skin, mucous membranes or the bite of an infectious flea.[1] Septicemic plague can also occur secondarily to bubonic or pneumonic plague when lymphatic or pulmonary defenses are breached and *Y. pestis* enters the bloodstream and multiplies en masse. Bacteremia commonly occurs in all forms of plague, while septicemia occurs less commonly and can be immediately life threatening.

Yersinia pestis can cause serious disease in almost any organ. Fatal cases commonly experience diffuse interstitial myocarditis with cardiac dilatation, multifocal necrosis of the liver, diffuse hemorrhagic splenic necrosis and fibrin thrombi in renal glomeruli.[1] Disseminated intravascular coagulation (DIC) can occur, resulting in thrombosis within the microvasculature, necrosis and bleeding, and widespread cutaneous, mucosal and serosal petechiae and ecchymoses, as well as occasional gangrene of acral parts, such as the fingers and toes (see Fig. 120.4).

Primary plague pneumonia, which usually results from inhalation of infective respiratory particles,[1] often begins as a lobular process and then extends by confluence, becoming lobar and then multilobar. Typically, plague organisms are numerous in the alveoli and pulmonary secretions. Secondary plague pneumonia arising from hematogenous spread of *Y. pestis* typically begins as a diffuse interstitial process, with plague bacilli appearing most numerous in interstitial spaces. Pathologic findings in untreated cases of plague pneumonia usually include edema, diffuse pulmonary congestion, hemorrhagic necrosis and scant infiltration by neutrophils.[1]

PREVENTION AND CONTROL

Rapid identification and treatment of cases is of utmost importance to limit fatalities and prevent human-to-human spread of plague.[1,5] In endemic regions, human and animal-based plague surveillance should be done to identify areas at risk. Epidemiologic investigations of human cases and threatening epizootics must also be conducted to effectively target control actions. Rapid implementation of flea control measures to reduce risk of infectious flea bites is also critical. Other environmental measures include reducing sources of food and shelter for rodents, and occasionally selective use of rodenticides. Flea control should always be undertaken before the killing of rodents to reduce the chances that infected fleas will leave dying hosts and feed on humans.[5]

Health providers also need appropriate education and consultation so that they can help others protect themselves. Personal protective measures and preventive actions should be encouraged, including

avoidance of sick or dead animals, use of insect repellents (*N,N*-diethyl-M-toluamide, DEET), flea control for pets, use of rubber gloves by hunters, and provision of prompt veterinary care for cats that might be ill with plague. A plague vaccine is no longer available commercially, although clinical trials of recombinant vaccines containing F1 (*caf1*) and V antigens of *Y. pestis* are currently underway.[2]

CLINICAL FEATURES

Bubonic plague

The incubation period of bubonic plague is usually 2–6 days but occasionally longer. Typically, patients experience acute onset of fever, chills, myalgias, arthralgias, headache and lethargy.[1] Tenderness and pain often occur within 24 hours in a regional lymph node(s) proximal to the initial site of inoculation. The femoral and inguinal groups of nodes are most commonly affected, axillary and cervical nodes less so, depending on the site of inoculation and geographic location of the cases. Cervical lymph node involvement is more commonly observed in countries where individuals sleep on hut floors, presumably because this behavior results in a greater risk of being bitten in the neck region by infectious floor-dwelling fleas. Enlarging buboes become progressively swollen, painful and tender, sometimes exquisitely so. Typically, the patient avoids movement, stretching and pressure near the bubo and guards against palpation. Tissues surrounding the bubo often becomes edematous and the overlying skin may be reddened (Fig. 120.3). Although relatively uncommon, inspection of the skin around the bubo or distal to it may reveal a flea bite marked by a small scab, papule, pustule or ulcer. Larger furuncular lesions, sometimes with tularemia-like eschars, also can occur rarely. Plague buboes differ from lymphadenitis of most other causes by their rapid onset, extreme tenderness, surrounding edema, accompanying signs of toxemia and absence of cellulitis or readily apparent ascending lymphangitis.

When treated soon after illness onset with an appropriate antimicrobial agent, bubonic plague patients usually experience defervescence and improvement of other systemic manifestations over a 2- to 3-day period.[1] Buboes frequently remain enlarged and tender for a week or more after initiation of treatment and rarely become fluctuant. Without antimicrobial treatment, bubonic plague patients typically exhibit an increasingly toxic state of fever, tachycardia, lethargy followed by prostration, agitation, confusion and, in more severe cases, convulsions and delirium. Differential diagnostic options include staphylococcal or streptococcal adenitis, tularemia, cat-scratch disease, mycobacterial infection, acute filarial lymphadenitis, chancroid and strangulated inguinal hernia.

Septic plague

Septicemic plague typically presents as a rapidly progressive, overwhelming endotoxemia that is usually fatal unless treated promptly.[1] Primary sepsis occurs in the absence of regional lymphadenitis, and a diagnosis of plague is likely to go unsuspected until results are available from blood cultures. Patients with primary septicemic plague often present with gastrointestinal symptoms, including nausea, vomiting, diarrhea and abdominal pain, further complicating diagnosis. Petechiae, ecchymoses, bleeding from wounds or orifices, and ischemia of acral parts are manifestations of DIC (Fig. 120.4). Preterminal signs can include refractory hypotension, renal shutdown, obtundation and other signs of shock. Acute respiratory distress syndrome associated with septicemic plague may be confused with other conditions, including hantavirus pulmonary syndrome.

Differential diagnostic possibilities include septicemia caused by other bacterial infections, such as the agents of meningococcemia, bacterial endocarditis, tularemia or other Gram-negative bacterial infections.



Fig. 120.3 Left inguinal and femoral buboes, demonstrating surrounding edema and overlying desquamation.



Fig. 120.4 Septic plague patient who demonstrated disseminated intravascular coagulation, bleeding into the skin and acral gangrene as a late manifestation.

Pneumonic plague

Pneumonic plague is the most rapidly developing and life-threatening form of plague.[1,7] The incubation period for primary pneumonic plague is usually 2–5 days (range 1–6 days). Illness onset is most often sudden, with chills, fever, body pains, headache, weakness, dizziness and chest discomfort. Cough, sputum production, increasing chest pain, tachypnea and dyspnea typically predominate on the second day of illness; hemoptysis, increasing respiratory distress, cardiopulmonary insufficiency and circulatory collapse can also occur. The sputum of primary plague pneumonia patients is typically watery or mucoid, frothy and blood tinged, and can be bloody. Initially, chest signs may indicate localized pulmonary involvement and a rapidly developing segmental consolidation can appear before bronchopneumonia occurs elsewhere in both lungs (Fig. 120.5). Liquefaction necrosis and cavitation can develop at sites of consolidation and result in residual scarring.

Secondary pneumonic plague resulting from metastatic spread typically manifests as a diffuse interstitial pneumonitis with sputum production that is scant and more likely to appear tenacious and inspissated than the sputum of primary pneumonic plague patients.

Differential diagnostic possibilities include other bacterial conditions such as tularemia, mycoplasma pneumonia or other community-acquired bacterial pneumonias, Legionnaires' disease, Q fever and staphylococcal or streptococcal pneumonia. Viral pneumonias requiring differentiation include influenzal pneumonitis, hantaviral pulmonary syndrome and those caused by respiratory syncitial virus or cytomegalovirus infection.

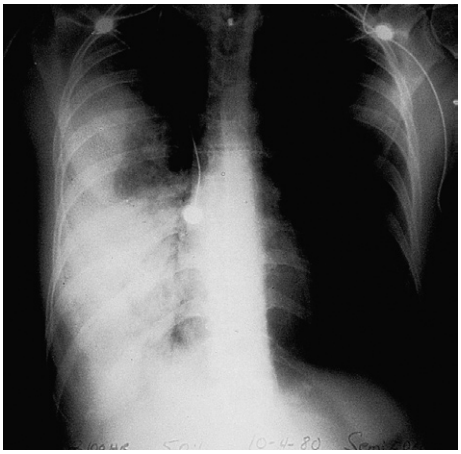


Fig. 120.5 Chest radiograph of a patient who has primary plague pneumonia, showing extensive infiltrates in the right middle and lower lung fields.

Other manifestations

Plague meningitis is unusual (3% of USA cases from 1950–1994) and typically arises as a complication among the treated survivors of bubonic plague.[1] Plague occasionally presents as pharyngitis accompanied by fever, sore throat and cervical lymphadenitis, and can be confused with other more common infectious causes of pharyngitis. Primary plague pharyngitis can result from respiratory exposures or ingestion of infected undercooked meat and is usually associated with marked cervical glandular enlargement. Oculoglandular plague can arise from inoculation of *Y. pestis* through the conjunctiva.

DIAGNOSIS

Except in outbreak situations, making a timely diagnosis of plague requires a high index of clinical suspicion, careful case history and physical examination. Treatment delays or misdiagnoses increase the likelihood of fatalities,[6] and infected travelers who seek medical care after returning from endemic areas are especially at risk. Laboratory tests for plague are highly reliable when conducted by experienced persons, but such expertise is usually limited to reference laboratories.

When plague is suspected, clinical specimens should be obtained promptly for microbiologic assays, chest radiographs taken and specific antimicrobial therapy initiated. Blood and other specimens, including bubo aspirates, sputum, tracheal washes, swabs of skin lesions or pharyngeal mucosa and cerebrospinal fluid, should be collected when appropriate and inoculated onto suitable media (e.g. brain–heart infusion broth, sheep blood agar, chocolate agar or MacConkey agar).[18] Bubo aspirates typically yield only small amounts of fluid and 1–2 ml of saline may need to be injected first to obtain adequate material. Smears of each specimen should be stained with Gram, Wayson or Giemsa stain. An acute-phase serum specimen should be collected for *Y. pestis* antibody testing, followed 3–4 weeks later by a convalescent-phase specimen. For fatal cases, samples from buboes, liver, spleen, lungs and bone marrow should be collected at autopsy for culture, fluorescent antibody or immunohistochemical staining, and histologic studies. *Yersinia pestis*-infected tissues can be transported in Cary Blair or similar holding medium. Presumptive identification of *Y. pestis* can be made by direct immunofluorescence assay, polymerase chain reaction or antigen capture enzyme-linked immunosorbent assay. A rapid immunogold dipstick assay that detects *Y. pestis* antigens in patient samples also appears promising,[19] providing staff are adequately trained in its application and use appropriate controls. In addition to providing rapid results, the dipstick-type assays can be used in remote village clinics, which typically lack refrigeration, extensive laboratory equipment and access to adequate microbiologic laboratories.

Laboratory confirmation of plague can be provided by isolation of *Y. pestis* from body fluids or tissues.[18] Plague bacteria are readily distinguished from other Gram-negative bacteria by polychromatic and immunofluorescence staining properties, growth characteristics, biochemical profiles and lysis by *Y. pestis*-specific bacteriophage. Laboratory mice are susceptible to *Y. pestis* and are used to make isolations from contaminated materials and for virulence testing. Alternatively, confirmation can be provided by demonstration of a fourfold change in serum antibodies to *Y. pestis*.[18] A titer of 128 or greater in a single serum sample from a patient who has a compatible illness and who has not received plague vaccine can provide presumptive evidence of infection. Less than 5% of patients fail to seroconvert and some will develop detectable antibodies within 5 days of illness onset, although most seroconvert 1–2 weeks later and a few require more than 3 weeks to seroconvert. Early antibiotic therapy may delay seroconversion by several weeks. Positive serologic titers decrease gradually over time but can be present for months to years. Passive hemagglutination assay (PHA) is used most frequently for serodiagnosis; however, enzyme-linked immunosorbent assays that detect IgM and IgG antibodies to *Y. pestis* are also useful as they can identify antibodies characteristic of early infection and differentiate these from antibodies resulting from previous vaccination.

White blood cell counts for plague patients typically range from 10 000 to 25 000/mm^3 and contain predominately early stage polymorphonuclear leukocytes.[1] In some instances leukemoid reactions occur with white cell counts in excess of 50 000/mm^3.

MANAGEMENT

Untreated, plague is fatal in over 50% of patients who have bubonic plague and in nearly all patients who have septicemic or pneumonic plague. Mortality rates in the USA were reportedly 14%, 22% and 57% for cases of bubonic, septicemic and pneumonic plague, respectively.[7] Fatalities are almost always due to delays in seeking treatment or misdiagnosis, emphasizing the need for rapid diagnosis and appropriate antimicrobial therapy (Table 120.3).[1,6]

Streptomycin has long been considered the drug of choice for treating plague, but another aminoglycoside (gentamicin) is increasingly being used because of its availability and ease of administration.[1,6] Tetracyclines or chloramphenicol are also effective, and chloramphenicol is indicated for conditions that require high tissue penetration, such as plague meningitis, pleuritis, endophthalmitis or myocarditis. Although doxycycline is typically the tetracycline of choice for treating plague, clinical trials of its efficacy have not been reported. However, a recent review of 75 human plague cases in New Mexico, USA indicated that gentamicin or tetracyclines were as efficacious as streptomycin in treating human plague.[6] Trimethoprim–sulfamethoxazole (co-trimoxazole) has been used successfully to treat bubonic plague, but it is considered a secondary choice. Although fluoroquinolones have yet to be evaluated for treating human plague, animal experiments suggest these agents would be effective and ciprofloxacin has been recommended as an antimicrobial for treating human cases during bioterrorism attacks.[7] Penicillins, macrolides and cephalosporins have a suboptimal effect and should not be used to treat plague. In general, antimicrobial treatment should be continued for either 7–10 days or for at least 3 days after the patient has become afebrile. Improvement is usually evident 2–3 days from the start of treatment, although fever may persist for several more days.

Complications of delayed treatment include DIC, adult respiratory distress syndrome and other consequences of bacterial sepsis; such patients require intensive monitoring and close physiologic support. Buboes may require surgical drainage and abscessed nodes can be a cause of recurrent fever in patients. Viable *Y. pestis* have been isolated from buboes 1–2 weeks after clinical recovery. Although isolated reports exist of *Y. pestis* strains resistant to streptomycin or tetracycline, the few antibiotic-resistant *Y. pestis* strains isolated from humans have not been associated with treatment failure and typically have exhibited only partial resistance to a single agent. However,

Table 120.3 Treatment guidelines for plague

Drug		Dosage	Route of administration
Streptomycin	Adults Children	1 g q12h 15 mg/kg q12h*	im im
Gentamicin	Adults Children Infants/neonates	1–1.5 mg/kg q8h† 2.0–2.5 mg/kg q8h 2.5 mg/kg q8h	im or iv im or iv im or iv
Tetracycline	Adults Children >8 years old	0.5 g q6h 6.25–12.5 mg/kg q6h	po po
Doxycycline	Adults Children >8 years old and >45 kg Children >8 years old and >45 kg	100 mg q12h 100 mg q12h 2.2 mg/kg q12h	po or iv po or iv po or iv
Chloramphenicol	Adults Children >1 year old	12.5 mg/kg q6h‡ 12.5 mg/kg q6h‡	po or iv po or iv

*Not to exceed 2 g/day.
†Daily dose should be reduced to 3 mg/kg as soon as clinically indicated.
‡Up to 100 mg/kg per day initially. Dosage should be adjusted to maintain plasma concentrations at 5–20 mg/ml. Hematologic values should be monitored closely.

a multidrug-resistant strain of *Y. pestis* isolated from a plague patient in Madagascar appeared to be highly resistant to the antibiotics typically recommended for treatment (tetracycline, streptomycin and chloramphenicol).[20] Resistance was plasmid-mediated and could be transferred to other strains of *Y. pestis* and to *Escherichia coli*. Surveillance for multidrug-resistant *Y. pestis* in Madagascar and elsewhere has not provided evidence for the spread of similar strains. Postexposure treatment for 7 days with a tetracycline, chloramphenicol or trimethoprim–sulfamethoxazole is recommended for persons who have had close contact with pneumonic plague patients in the previous 7 days.[7]

In addition to fever watch, antimicrobial prophylaxis is sometimes recommended for persons who have handled an infected animal or for household members of bubonic plague patients because of likely flea exposures.[1,6] Antimicrobial prophylaxis also might be recommended following a bioterrorism attack.[7] Isolation and respiratory droplet precautions are recommended for patients with pneumonic plague, including the use of masks for persons caring for these patients while they are infectious. The use of masks may interrupt person-to-person transmission during pneumonic plague outbreaks. Pneumonic plague patients are considered to be noncontagious following 48 hours of antibiotic treatment.

REFERENCES

References for this chapter can be found online at http://www.expertconsult.com

Chapter 121

C Ben Beard
David T Dennis

Tularemia

Tularemia is an uncommon, potentially severe bacterial zoonosis caused by *Francisella tularensis*. The natural cycle of the causative organism involves maintenance of infection in a wide diversity of animal hosts and in certain hard ticks. Transmission of *F. tularensis* to humans, which are incidental hosts, occurs by several modes, including bites by infective ticks and other arthropods, direct inoculation of *F. tularensis* through skin or mucous membranes from handling infectious materials, ingestion of contaminated water or food, or by inhalation of contaminated aerosols or dusts. The agent of tularemia is widely distributed in temperate and subarctic regions of North America and Eurasia. Human infection results in various clinical presentations of varying severity depending on the route of inoculation, the dose and virulence of the infecting strain, and the host defenses. The most common clinical form, ulceroglandular tularemia, presents as an illness with fever, an ulcer at the site of inoculation and regional lymphadenitis. Several other forms occur involving various organ systems. Tularemia is considered to be an important potential weapon of bioterrorism.

EPIDEMIOLOGY

Agent

Francisella tularensis is a small, facultatively intracellular, Gram-negative coccobacillus. The organism has a lipidated envelope and is able to survive under favorable conditions for several weeks in water, moist soil and decaying animal carcasses. Within the species *F. tularensis*, there are three recognized subspecies: *F. t. tularensis* (type A), *F. t. holarctica* (type B) and *F. t. mediasiatica*. Type A isolates are known almost entirely from North America, with the single exception of a collection of isolates made in the Danube region in Slovakia.[1] Type B isolates are found throughout the northern hemisphere, both in Eurasia and widely across North America. Subspecies *mediasiatica* is limited in its distribution to central Asia.[2]

Traditionally, type A isolates have been considered more virulent than type B isolates based on observations derived primarily from animal models. Recent studies, however, have revealed genetic differences in isolates evaluated from around the USA, indicating that type A strains from western USA are less virulent than type B strains in terms of clinical outcomes in humans.[3]

In addition to the three *F. tularensis* subspecies, there are two other closely related species in the genus, *Francisella novicida* and *Francisella philomiragia*. Disease caused by these agents is rare and generally less severe. *Francisella novicida* has been seen as a cause of illness in immunocompromised patients, and *F. philomiragia* has been seen in immunocompromised patients and near-drowning vicitims.[4]

Francisella tularensis is considered to be a potential agent of bioterrorism because it can be weaponized as an aerosol, could result in large numbers of casualties, and because it requires special actions for medical and public health preparedness.[5] (See also Chapter 71.)

Life cycle

Francisella tularensis is widespread in nature and has been recovered from more than 100 species of wild mammals, at least nine species of domestic animals (including cats, dogs and cattle), numerous species of birds, some amphibians and fish, and more than 50 species of arthropods.[6] The principal natural cycles of the agent involve maintenance of infection in wild mammalian hosts, such as lagomorphs (wild hares and rabbits), terrestrial rodents (especially voles and meadow mice) and aquatic rodents (water rats, muskrats, beaver). Certain species of hard ticks are able to maintain infection from one developmental stage to another. Transmission among animals is accomplished by the bites of blood-feeding arthropods or by direct exposure to contaminated materials in the environment (Fig. 121.1).[6] Predation and cannibalism may also contribute to the natural cycle.

Humans become infected:

- when they intrude into the arthropod-borne cycle and are bitten by ticks, which are true biologic vectors, or by blood-feeding flies or mosquitoes via mechanical transmission;
- by handling or ingesting infectious animal tissues or fluids;
- by ingestion of contaminated water or food; or
- by inhalation of infective aerosols or dusts.

Occasional cases occur following infective bites or scratches by cats[7] or other carnivores or predators with contaminated mouths or claws. Although the agent is highly infectious, requiring only 10–50 organisms to regularly cause experimental infections of humans, person-to-person transmission has not been documented.

Geographic distribution

Tularemia is endemic throughout much of the Nearctic and Palaearctic regions between latitudes 30° N and 71° N. This includes all of North America from the Arctic Circle to northern Mexico, much of Eurasia and some states of northern Africa along the Mediterranean coast.[6] In North America, the highest incidence of tularemia in humans occurs in south-central, south-eastern, Great Plains and Rocky Mountain regions of the USA (Fig. 121.2),[8] but cases have been reported throughout the continental USA, across Canada and in Mexico as far south as Guadalajara. In Eurasia, the disease occurs most frequently in Scandinavia and in states of the former Soviet Union. Tularemia also occurs sporadically throughout most of Europe, in some areas of the Near East and Middle East, in Central Asia and in Mongolia. It has not been documented in Central or South America, Australia or Africa outside the Mediterranean littoral.

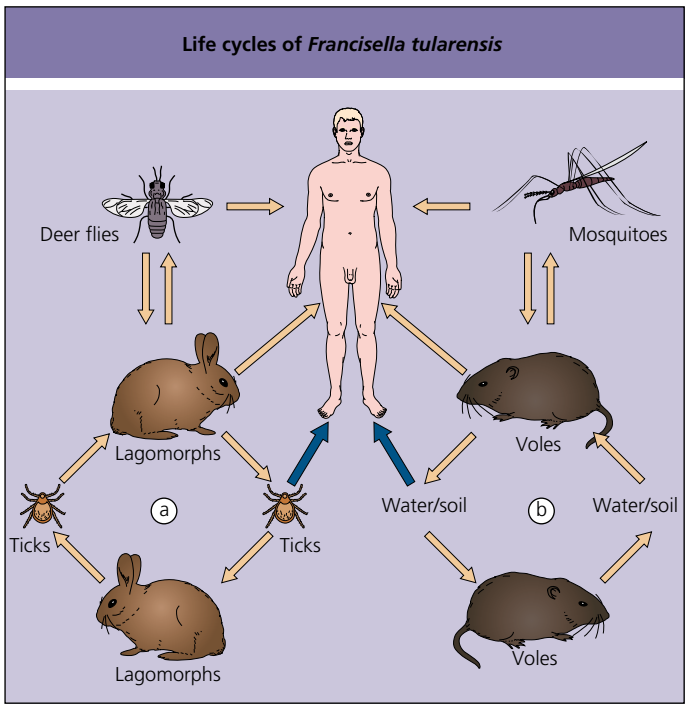


Fig. 121.1 Life cycles of *Francisella tularensis*. The two major life cycles in nature are shown. In cycle (a), which is dominant in North America, *F. tularensis* is maintained predominantly among lagomorphs and hard ticks. In cycle (b), which is dominant in Eurasia, *F. tularensis* is principally maintained among cricetine rodents, especially field voles and mice, water voles and other aquatic rodents. Humans are incidental hosts that are infected by tick vectors and by the bites of flies or mosquitoes that have contaminated mouthparts, by direct contact with infected animal carcasses or other contaminated materials, by ingestion of contaminated matter or by inhalation of infectious aerosols or dusts.

Populations affected

Tularemia is a rural disease. It affects persons of all ages and both sexes. Groups at highest risk include:[9–11]

- hunters and trappers, wildlife specialists, animal skinners and dressers, butchers and others who handle potentially infective animal carcasses;
- rural residents, especially farmers, who are exposed to water, soils and dusts contaminated by infected wild animals, such as meadow voles, lagomorphs and aquatic mammals; and
- persons exposed in enzootic areas to bites by certain hard ticks, tabanid flies or mosquitoes.

Disease incidence

Global incidence figures are not available. In Eurasia, recent outbreaks involving hundreds of cases each have been reported from Sweden,[12] Kosovo[13] and Spain,[14] and outbreaks involving thousands of persons have been reported in the past from the former Soviet Union. Tularemia incidence is relatively stable in the USA, where the disease has been in steady decline since 1945 (Fig 121.3).[8] In the period 2000–2005, a total of 778 cases were reported from 42 states, averaging 130 cases (range 90–154) per year. Four states accounted for 52% of all reported cases: Missouri (158 cases), Arkansas (117 cases), Oklahoma (76 cases) and Massachusetts (57 cases). The highest incidence among 5-year age groups was in 5–9 year olds and 75–79 year olds. Males accounted for 69% of reported cases during this period. Reported disease onset peaked in summer months, with 66% of cases occurring from May through August, although cases were reported from all months of the year (CDC, unpublished data).

Sources of human infection

Eurasia

Cricetine rodents (especially meadow voles, lemmings, water voles and muskrats), water and soil contaminated by these animals, hares (*Lepus* spp.) and bites by contaminated mosquitoes (especially *Aedes cinereus*) are the principal sources of human tularemia in Eurasia.[6] Mosquito-borne infection occurs in forested and marshy Scandinavian and Baltic regions. Sporadic cases also result in Eurasia from bites by infected ticks and by blood-feeding flies. Outbreaks of tularemia among farmers have been described in Europe following respiratory exposure to dusts from contaminated stored and fresh mown hay,[15,16] and among workers in agricultural processing plants exposed to contaminated water sprays. Ingestion of water and food contaminated by

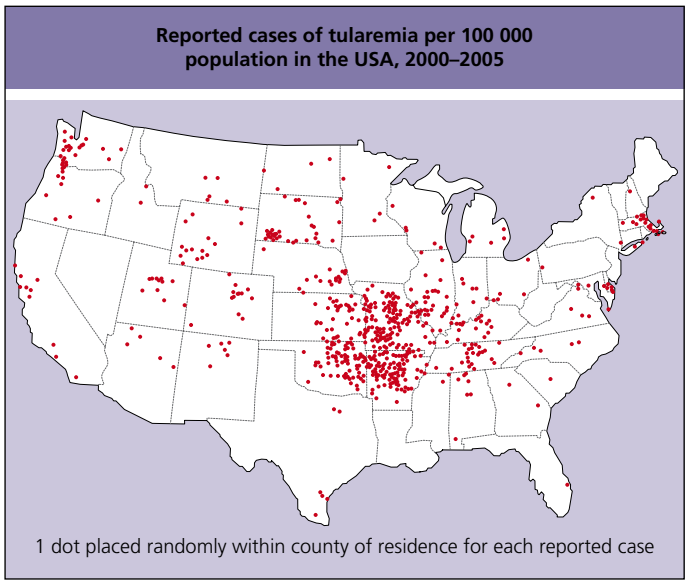


Fig. 121.2 Reported cases of tularemia per 100 000 population in the USA, 2000–2005.

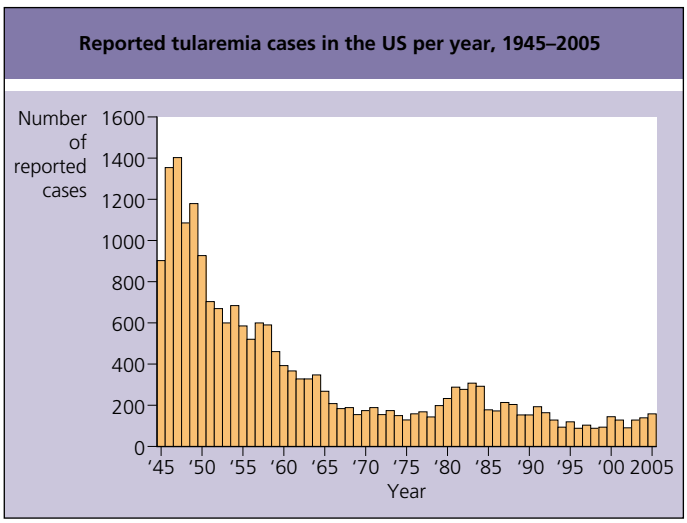


Fig. 121.3 Reported tularemia cases in the USA per year, 1945–2005.

infected rodents or hares has also resulted in outbreaks in the region, such as recently reported from Kosovo and Turkey.[13,17] In Japan, the disease has historically been associated with the trapping, handling and eating of wild hares.

North America

The principal animal sources of infection in North America are the cottontail rabbit (Fig. 121.4) (*Sylvilagus* spp.), wild hares and rodents (muskrats, beaver, voles, ground squirrels).[6,9] The agent is vectored by certain species of hard ticks, especially the dog tick, *Dermacentor variabilis* (Fig. 121.5), the lone star tick, *Amblyomma americanum*, and the Rocky Mountain wood tick, *Dermacentor andersoni*.[6] Biting tabanid flies, especially deer flies (*Chrysops* spp.) (Fig. 121.6), mechanically transmit the infection. The epidemiology of tularemia in North America has changed significantly since the 1930s and 1940s, when the disease most commonly called 'rabbit fever' had a much higher incidence and when cases were more likely to occur in the winter and be linked to the hunting, dressing and butchering of wild rabbits and hares than to arthropod bites[18,19] (CDC, unpublished data).

Seasonality

Mosquito-borne transmission in Eurasia peaks in the summer months. In North America, a peak of tularemia cases in the spring and summer months is associated mostly with bites by ticks and blood-feeding flies, and a second peak in the late autumn and winter is associated with handling infected animals, especially among hunters and trappers.[3,18]

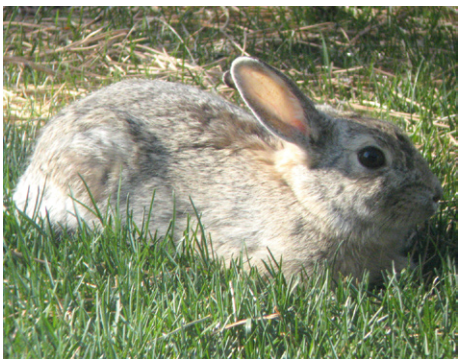


Fig. 121.4 Desert cottontail (*Syvilagus audubonii*). Important reservoir of tularemia in western USA.



Fig. 121.5 The dog tick (*Dermacentor variabilis*). Important vector of tularemia in eastern and central USA.



Fig. 121.6 The deer fly (*Chrysops discalis*). Primary vector of tularemia in western USA.

PATHOGENESIS AND PATHOLOGY

The principal pathologic changes in localized disease occur at the cutaneous site of inoculation and in the regional lymph nodes draining the site; when the disease is disseminated, the lungs, spleen, lymph nodes, liver and skin are most often involved.[20–22] The primary skin lesion begins as a papule several days following inoculation. The papule rapidly progresses to a vesicle that erodes and develops into an ulcer, which is typically 2–3 cm in diameter with an irregular, slightly raised and erythematous border. The base is necrotic, and frequently covered with a thick dark scab that can mimic the eschar of cutaneous anthrax (Fig. 121.7). Affected lymph nodes show hemorrhagic necrosis and may suppurate. Secondary skin lesions have also been described in tularemia, including papular and papulovesicular lesions, erythema nodosum and erythema multiforme.

Francisella tularensis is an obligate intracellular organism within the vertebrate host, and the response to infection has a prominent component of cell-mediated immunopathology.[23–25] Histologically, early disease is characterized by focal suppurative necrosis. The central area of necrosis is at first composed primarily of polymorphonuclear leukocytes and macrophages, which may be replaced by epithelioid cells in more advanced lesions. A wall of fibroblasts may surround the acute inflammatory reaction. Later, smaller lesions may be indistinguishable from miliary tubercles. A frequent finding on pathologic examination of affected lungs is small (3–12 mm), yellowish, necrotic subpleural nodules. Patchy interstitial infiltrates are common in pneumonic tularemia; bronchopneumonia is found in about 30% of cases, and lobar pneumonia with consolidation of an entire lobe in about 15% of pneumonic cases. Lung abscesses occasionally occur. Hilar lymph nodes may be inflamed and enlarged.

Prevention

Persons exposed in endemic areas to ticks, biting flies or mosquitoes should, when feasible, wear protective clothing and apply repellents containing DEET (*N*,*N*-diethyl-M-toluamide) to skin and clothing as directed by the manufacturer. Permethrin-based products can be applied to clothing to kill ticks and biting flies on contact. Frequent examinations should be made to identify and remove ticks on clothing and skin. Persons should always avoid direct contact with sick or dead animals, and hunters, trappers, dressers and butchers should wear impervious gloves when skinning and handling wild animal carcasses.

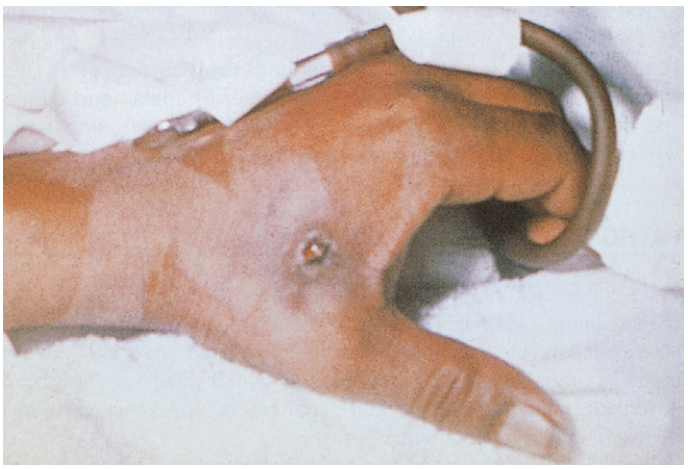


Fig. 121.7 Tularemic ulcer with eschar formation after percutaneous inoculation of *Francisella tularensis*.

The use of fine particle masks while engaged in landscaping activities has been suggested as a possible means of reducing infective inhalation exposures, such as those which can result when using power tools that generate environmental dusts. An outbreak of pneumonic tularemia occurred in Massachusetts, USA, among landscapers who were infected through this exposure route.[26]

Live attenuated vaccines have been used to protect laboratory personnel who routinely work with *F. tularensis*. Vaccines have also been used in an attempt to reduce the incidence of disease among rural residents of highly endemic areas of the former Soviet Union. The attenuated live vaccine strain (LVS) was developed by the US Department of Defense from an attenuated tularemia type B strain from the former Soviet Union and evaluated in the USA under a Food and Drug Administration investigational new drug protocol. Currently, no tularemia vaccine is licensed for general use, however, and this is an important area of active research.[25] Persons exposed to a laboratory accident possibly resulting in aerosolization or inoculation of *F. tularensis* should be considered for prophylactic antibiotic administration or placed on fever watch and closely monitored for early signs of illness.

CLINICAL FEATURES

The primary forms of tularemia include:[20–22]

- ulceroglandular tularemia (45–85% of cases);
- glandular tularemia (10–25% of cases);
- oculoglandular tularemia (<5% of cases);
- typhoidal (septic) tularemia (<5% of cases);
- oropharyngeal tularemia (<5% of cases); and
- pneumonic (inhalation) tularemia (<5% of cases).

The incubation period is usually 2–5 days (range 1–14 days). Onset is sudden; typically, the patient has fever of 100–104°F (38–40°C) and a constellation of nonspecific manifestations including chills, headache, generalized body aches (often prominent in the lumbosacral region), nausea, weakness, cough and chest pain.[20–22] Without treatment, nonspecific symptoms usually persist for several weeks. Sweats, chills, progressive weakness and weight loss characterize the continuing illness. Any of the principal forms of tularemia may be complicated by bacteremic spread that may lead to secondary sepsis, tularemic pneumonia, meningitis or other metastatic infection.

Before antibiotics became available, the overall mortality rate from infections with type A strains was in the range of 5–10%; however, a considerably higher fatality rate was reported for typhoidal and pneumonic forms of disease. A recent study was performed on 316 culture isolates that were obtained from human tularemia cases reported to the CDC from 1964 to 2004.[3] For the 235 patients for whom outcome was known, the overall case fatality rate was 9%. A molecular subtyping method that distinguished eastern and western clades of type A strains revealed that the case fatality rate for isolates that fit into the type-A-east subtype was 14%, while the case fatality rate observed in the type-A-west subtype was 0%. Type B isolates had an intermediate case fatality rate of 7%. This study suggests that significant variability exists among type A subtypes, and that the traditional view that type A is more virulent than type B is not always true.

Ulceroglandular tularemia

A local papule appears at the site of inoculation at the time of, or shortly after, the onset of fever and other generalized symptoms. This becomes vesiculated and pustular, and then ulcerates within a few days of its first appearance. Typically, the ulcer is tender, has an indolent character and may be covered by a scab (see Fig. 121.7). By the time of ulceration, painful lymphadenitis occurs in one or more adjacent nodes in the afferent pathway. In persons infected by handling contaminated materials, the epitrochlear nodes (8%) and the axillary nodes (65%) are the most commonly affected. In persons infected by arthropod bites, the femoral–inguinal nodes (64%), the axillary nodes (24%) and the cervical nodes (6%) are commonly involved.[22] In some cases, an abscessed node may suppurate, create a sinus tract and discharge purulent material to the outside.

Oculoglandular tularemia

Oculoglandular tularemia (Parinaud's syndrome) follows contamination of the conjunctival sac. Ulceration may occur on the conjunctiva, which becomes severely inflamed, with marked edema and vasculitis. Characteristically, there is painful swelling of nodes draining the periorbital tissues, such as the preauricular, submandibular and cervical chain nodes.

Glandular tularemia

Glandular tularemia differs from the ulceroglandular type only in not having the local cutaneous ulceration. It is more likely to follow arthropod-borne inoculation than direct percutaneous inoculation of the hands and fingers of persons handling infected animal tissues.

Typhoidal tularemia

Typhoidal tularemia presents as an acute illness without localizing signs.[22] The diagnosis is most often made by the identification of *F. tularensis* in blood cultures. Abdominal pain, diarrhea and vomiting may be prominent in early illness. Sepsis may occur and the systemic inflammatory response syndrome may ensue, rarely accompanied by complications such as disseminated intravascular coagulation and bleeding, acute respiratory distress syndrome, shock and organ failure. Typhoidal tularemia may result from inapparent inhalation exposures, that then progresses to pneumonia in more than 50% of cases; infection of the kidneys and the meninges may also occur. In some cases, the upper gastrointestinal tract may be the principal target organ in typhoidal tularemia.

Oropharyngeal tularemia

Oropharyngeal tularemia is acquired by ingesting inadequately cooked game, or contaminated water or food. The patient may develop a painful exudative pharyngitis or tonsillitis, or a stomatitis, sometimes with ulceration, and tender cervical lymphadenopathy. Suppuration, fistula formation and drainage of cervical nodes may occur.[13]

Pneumonic tularemia

Pneumonic tularemia is a common secondary complication of other forms of tularemia. Infrequently, primary pneumonia arises from inhalation of an infective aerosol or dust. In addition to fever, chills, fatigue and other generalized symptoms of infection, pneumonic manifestations include cough (usually with minimal sputum production), chest discomfort, sometimes with pleuritic pain, dyspnea, tachypnea and occasionally mild hemoptysis.

Francisella tularensis was weaponized for aerosol delivery by biowarfare programs during and after the Second World War, and it is assumed that this would be the most likely mode of delivery in a potential terrorism attack. The expected result would be an outbreak of pneumonic tularemia and nonspecific febrile illness (typhoidal tularemia) beginning 3–5 days after exposure; this might at first be difficult to distinguish from the many usual causes of community-acquired infection. It is possible that an aerosol exposure could also result in cases of oropharyngeal and oculoglandular disease. Since naturally acquired tularemia is most frequently a rural disease, bioterrorism might be suspected should a cluster of cases occur in an

urban population. The recognition, and medical and public health management of tularemia as a weapon of bioterrorism has recently been outlined.[5]

DIAGNOSIS

The presumptive diagnosis of tularemia is made by clinical examination combined with information on potentially infective exposures. Differential diagnostic possibilities are many, as follows:

- in persons who have glandular or ulceroglandular disease they include plague, cutaneous anthrax, sporotrichosis, cat-scratch fever, lymphogranuloma venereum, streptococcal or staphylococcal lymphadenitis, toxoplasmosis, mycobacterial infection, chancre and chancroid;
- in persons who have oropharyngeal tularemia, other bacterial and viral causes of stomatitis, pharyngitis and cervical adenitis must be considered, such as streptococcal infection, infectious mononucleosis, mycobacterial infection, adenoviral infection and diphtheria;
- in persons who have pneumonia, they include mycoplasmal pneumonia, chlamydial pneumonia, Legionnaires' disease, staphylococcal or streptococcal pneumonitis, *Haemophilus influenzae* pneumonia, plague, histoplasmosis and tuberculosis; and
- in persons who have typhoidal tularemia, they include bacterial endocarditis, disseminated mycobacterial or fungal infection, typhoid fever, brucellosis, listeriosis, leptospirosis, Q fever, plague and other causes of sepsis.

The clinical diagnosis of tularemia is confirmed by culture recovery of *F. tularensis*. Alternatively, acute and convalescent serum specimens that differ in titer by fourfold, with at least one serum positive, are also considered confirmatory. *Francisella tularensis* may be present in human lesion exudates, respiratory secretions, blood, and lymph node aspirates as well as animal tissues and fluids.

Francisella tularensis can be isolated from nutrient-enriched clinical specimens on sheep blood agar (SBA), but a cysteine-enriched medium is strongly recommended for subculture as the organism will usually fail to thrive with continued passage on SBA. *Francisella tularensis* grows well on several types of cysteine-supplemented agar, including chocolate agar (CA), cysteine heart agar with 9% chocolatized blood (CHAB), buffered charcoal yeast extract (BYCE), Thayer–Martin agar and IsoVitalex supplemented agars. For blood culture, the BACTEC system or an equivalent system is recommended. The agglutination reaction for combined IgM and IgG immunoglobulins is the routine serologic procedure used in most laboratories. Reference laboratories use microagglutination methods that are more sensitive than tube agglutination procedures. Antibody titers usually do not rise before 10 days or more of illness onset.

Direct fluorescent antibody staining, using a FITC-labeled antibody against *F. tularensis* cells, is a rapid assay for identification of *F. tularensis*. A variety of polymerase chain reaction (PCR) methods have also been described for molecular detection of *F. tularensis* and can be invaluable diagnostic tools when organisms are noncultivable. Once an isolate has been confirmed as *F. tularensis*, supplemental tests including biotyping (biochemical or molecular) and molecular subtyping can be used for additional characterization. These tests can be extremely useful for determining the source of infection, understanding transmission cycles or identifying species or subspecies.[27]

Personnel handling diagnostic cultures of *F. tularensis* are at considerable risk for infection, due to the low infectious dose for *F. tularensis*. Tularemia has been one of the most commonly reported laboratory-associated bacterial infections.[28] For diagnostic laboratories, biosafety level 2 practices, containment equipment, and facilities are recommended for activities involving clinical materials of human or animal origin suspected or known to contain *F. tularensis*. Biosafety level 3 practices, containment equipment and facilities are recommended, respectively, for all manipulations of suspect cultures, animal necropsies and for experimental animal studies.[29]

MANAGEMENT

Patients are best managed under hospital care until a full diagnostic evaluation and satisfactory treatment response has occurred. Streptomycin, which is bactericidal, has been the traditional drug of choice based on experience and efficacy.[5,30] It is given intramuscularly to adults in a dosage of 1 g and to children at 15 mg/kg (not to exceed 2 g/day), twice daily for 10 days. Gentamicin, an acceptable alternative, is given parenterally in an adult dosage of 5 mg/kg and to children at 2.5 mg/kg, per day for 10 days. A tetracycline (most commonly doxycycline) or chloramphenicol may be used in place of an aminoglycoside, especially in less severely ill patients, but use of these bacteriostatic agents occasionally results in primary treatment failures, and dosage schedules of at least 14 days are recommended to prevent relapses.

Oral or parenterally administered ciprofloxacin has been used to treat adults and children with good success in standard doses for 10 days.[31] Patients begun on parenterally administered antimicrobials can switch to oral administration when clinically indicated.

Francisella tularensis organisms routinely produce β-lactamase and are resistant to β-lactam antibiotics and azithromycin, but they are generally highly susceptible to aminoglycosides, tetracyclines, chloramphenicol and quinolones.[32,33] Penicillins and cephalosporins are not effective and should not be used.

Typically, fever and general symptoms of acute infection begin to regress within 24–48 hours of initiation of appropriate antibiotic administration. Factors associated with a poor outcome include delays in seeking medical care, or delays in diagnosis and treatment, and underlying medical disorders, such as diabetes or alcoholism.[34] Standard (universal) precautions only are required for purposes of hospital infection control.[5] Postexposure prophylactic antibiotic treatment of close contacts is not recommended because human-to-human transmission is not known to occur.

REFERENCES

References for this chapter can be found online at http://www.expertconsult.com

Chapter 122

Nicholas PJ Day
Paul Newton
Philippe Parola
Didier Raoult

Scrub typhus and other tropical rickettsioses

INTRODUCTION

Tropical rickettsioses are a diverse group of zoonotic infectious diseases caused by obligate intracellular bacteria grouped in the family Rickettsiaceae of α-proteobacteria. These organisms are nonflagellate, small coccobacilli occurring within the host cytoplasm or nucleus and are not bounded by a vacuole. They are usually transmitted to humans by arthropods, in which they may be maintained by transovarial transmission. The members of the family Rickettsiaceae responsible for human disease in the tropics are:

- *Orientia tsutsugamushi*, the causative organism of scrub typhus (tsutsugamushi disease in Japan);
- the typhus group (TG) of the genus *Rickettsia*, containing *R. prowazeckii*, the agent of classic epidemic or louse-borne typhus, and *R. typhi*, which causes murine or flea-borne typhus; and
- the spotted fever group (SFG) *Rickettsia*, containing a large and ever increasing number of species. These are mostly transmitted from rodents and other animals by ticks, except for *R. akari* (mites) and *R. felis* (fleas).

Q-fever has in the past been considered a rickettsiosis and will be covered in this chapter, though its causative agent *Coxiella burnetii* has been transferred to the α-proteobacteria order Legionellales.

The agents of rickettsioses are associated with arthropods including ticks, mites, fleas and lice, which may act as vectors, reservoirs and/or amplifiers of the organisms. Most of these vectors favor specific optimal environmental conditions, biotopes and hosts. These factors determine the geographic distribution of the vector and consequently the risk area for the rickettsioses. This is particularly true when vectors are also reservoirs of pathogens, as seen in the case of ticks for most spotted fever group rickettsioses.[1] Thus, although some rickettsioses are distributed worldwide (Q-fever, murine typhus), a specific area is usually associated with specific diseases, and most rickettsioses are therefore geographic diseases. Table 122.1 summarizes the rickettsioses occurring in tropical areas of Africa, Asia, America and Australia.

There are no vaccines currently available against any tropical rickettsioses. Prevention is mainly based on avoiding the arthropod bite. The best method to avoid tick, flea and chigger bites is to use topical DEET (N,N-diethyl-m-toluamide) repellent applied to exposed skin and treat clothing with permethrin, which kills arthropods on contact. Bites may also be limited by wearing long trousers that are tucked into boots. People staying in infested areas should be advised to check their bodies routinely for the presence of arthropods. Any tick found attached should be removed immediately using blunt, rounded forceps.

Scrub Typhus

INTRODUCTION

Scrub typhus, caused by *Orientia tsutsugamushi*, is an important and widespread cause of febrile illness in rural areas of Asia and northern Australia (Fig. 122.1). It is contracted via the bite of the larval stage of several species of trombiculid mite of the genus *Leptotrombidium* ('chiggers'), which live in a wide range of vegetation types from scrub and primary forest to gardens and beaches. Infected mites are characteristically found in discrete foci called mite islands, where the risk of contracting scrub typhus is high. The bacteria are maintained transovarially in the mite population, and often infect the rodents on which the larval stage of the mite normally feeds. Humans are accidental hosts (Fig. 122.2). Scrub typhus presents as a systemic vasculitis-like infection, though the target cell along with much about the pathogenesis remains unknown.

CLINICAL FEATURES

Symptoms usually occur between 6 and 10 days after the mite bite. The presenting features are typically fever, generalized or regional lymphadenopathy, a macular or maculopapular rash (Fig. 122.3), severe headache and myalgia. Muscle tenderness is minimal or absent. Nausea and vomiting, diarrhea, constipation, conjunctival suffusion and reversable sensorineural deafness can also occur. A painless papule occurs at the bite site that later ulcerates, forming a black crust or 'eschar' (Fig. 122.4) in a variable proportion of patients – this variability probably reflects, at least in part, the extent of the physical examination. However, the eschar and rash may be absent or unnoticed.

Complications include jaundice, meningoencephalitis, myocarditis, interstitial pneumonia leading on to adult respiratory distress syndrome (ARDS), and renal failure. Animal experiments suggest that disease severity may vary widely with the strain of bacteria. In the pre-antibiotic era mortality rates as high as 35% were reported, and the disease still carries a significant risk of death in rural areas where effective treatment is unavailable or delayed.

DIAGNOSIS

The gold standard diagnostic tests for scrub typhus are the indirect immunoperoxidase and the immunofluorescent assay (IFA), based on cell-culture-derived *O. tsutsugamushi* antigens, applied to paired

Table 122.1 Tropical rickettsioses throughout the world

Location by continent	Vectors	Disease	Agent	Specific areas	Risk of exposure
Africa/Middle East	**Ticks**				
	Rhipicephalus sanguineus	Mediterranean spotted fever	*Rickettsia conorii*	Mediterranean area (Algeria, Tunisia, Morocco, Libya, Egypt, Israel, Turkey), Kenya, Somalia, Central African Republic, Zimbabwe and South Africa	Urban (2/3) and rural (1/3)
	Amblyomma spp.	African tick-bite fever	*R. africae*	Sub-Saharan Africa	Rural area. Safari
	Hyalomma marginatum	Unnamed	*R. aeschlimmanii*	Morocco, Zimbabwe, South Africa	
	*Dermacentor marginatus**	Tick-borne lymphadenopathy	*R. slovaca*	Morocco*	
	*Dermacentor marginatus**	Unnamed	*R. raoultii*	Morocco*	
	*Rhipicephalus sanguineus**	Unnamed	*R. massiliae*	Morocco*,	
	*R. muhusamae**			Mali*, Central African Republic*	
	*H. truncatum**	Unnamed	*R. sibirica mongolotimonae*	South Africa, Algeria, Niger*	
	*Ixodes ricinus**	Unnamed	*'R. monacensis'*	Morocco*, Tunisia*	
	Fleas				
	Xenopsylla cheopis (rat flea)	Murine typhus	*R. typhi*	Ubiquitous. High prevalence in coastal areas	Contact with rats and rat fleas
	Pulex irritans (human flea)*	Flea-borne spotted fever	*R. felis*	Tunisia, Algeria	
	Lice				
	Pediculus humanus corporis	Epidemic typhus	*R. prowazekii*	Ethiopia, Burundi, Rwanda, Uganda, Algeria	Civil war, refugee camps, lack of hygiene in cold or mountainous areas
Americas	**Ticks**				
	Amblyomma cajennense	Rocky Mountain spotted fever	*R. rickettsii*	Central America (Mexico, Panama, Costa Rica), South America (Brazil, Colombia)	Rural areas
	Amblyomma triste	Unnamed	*R. parkeri*	Brazil	
	Amblyomma spp.	African tick-bite fever	*R. africae*	West Indies	Rural areas
	*Amlyomma triste**	Unnamed	*R. parkeri*	Brazil	
	Fleas				
	Xenopsylla cheopis (rat flea)	Murine typhus	*R. typhi*	Ubiquitous	Contact with rats and rat fleas
	Ctenocephalides felis (cat flea)*	Flea-borne spotted fever	*R. felis*	Mexico, Brazil, Peru	
	Lice				
	Pediculus humanus corporis	Epidemic typhus	*R. prowazekii*	Peru and Andes	Lack of hygiene in mountainous area
Asia	**Ticks**				
	Rhipicephalus sanguineus	Indian tick typhus	*R. conorii indica*	India. Suspected in Thailand, Korea, Laos and Sri Lanka	
	Ixodes granulatus	Flinders Island spotted fever	*R. honei*	Thailand	

	Ixodes ovatus *Dermacentor taiwanensis* *Haemaphysalis longicornis* *Haemaphysalis flava* *Ixodes ovatus*,* *I. persulcatus*,* *I. monospinus** *Hyalomma asiaticum** *Dermacentor nuttalli*, *D. marginatus* *Haemaphysalis concinna* *Dermacentor silvarum* Unknown	Oriental or Japanese spotted fever Unnamed Lymphangitis-associated rickettsiosis North-Asian tick typhus Far-Eastern tick-borne rickettsiosis Unnamed	*R. japonica* *R. helvetica* *R. sibirica mongolotimonae* *R. sibirica sibirica* *R. heilongjiangii* '*R. kellyi*'	Japan[†] Japan.[†] Suspected in Thailand, Laos China (Inner Mongolia)*,[†] Northern China, former USSR (Asian republics, Siberia), Armenia, Pakistan North-eastern China India	Agricultural activities, bamboo cutting
	Fleas *Xenopsylla cheopis* (rat flea) *Ctenocephalides felis* (cat flea)*	Murine typhus Flea-borne spotted fever	*R. typhi* *R. felis*	Ubiquitous Thailand, Laos	
	Trombiculid acarins *Leptotrombidium* spp.	Scrub typhus	*Orientia tsutsugamushi*	Asia–Pacific region from Korea to Papua New Guinea and Queensland, Australia, and from Japan to India and Afghanistan	Rural activities Agricultural activities Soldiers in the field
	Lice *Pediculus humanus corporis*	Epidemic typhus	*R. prowazekii*	China, India (Kashmir)	Civil war, refugee camps, lack of hygiene in cold or mountainous areas
	House mouse mite *Liponyssoides sanguineus*	Rickettsialpox	*R. akari*	Korea[‡]	
Australia	**Ticks** Unknown *Ixodes holocyclus*	Flinders Island spotted fever Queensland tick typhus	*R. honei* *R. australis*	Flinders Island, north-eastern Australia North-eastern Australia	
	Fleas *Xenopsylla cheopis* (rat flea)	Murine typhus	*R. typhi*	Ubiquitous	Contact with rats and rat fleas
	Trombiculid acarins *Leptotrombidium* spp.	Scrub typhus	*Orientia tsutsugamushi*	Northern Territory and Western Australia, Queensland, Australia	Rural activities Agricultural activities Soldiers in the field

*Suspected by detection of the pathogen in the relevant arthropod.
[†]Although not included in tropical areas, Japan and China are included as relevant to tropical travel medicine.
[‡]Isolated from voles (*Microtus fortis pelliceus*).
Note: Q-fever caused by *C. burnetii* is distributed worldwide (except in New Zealand).

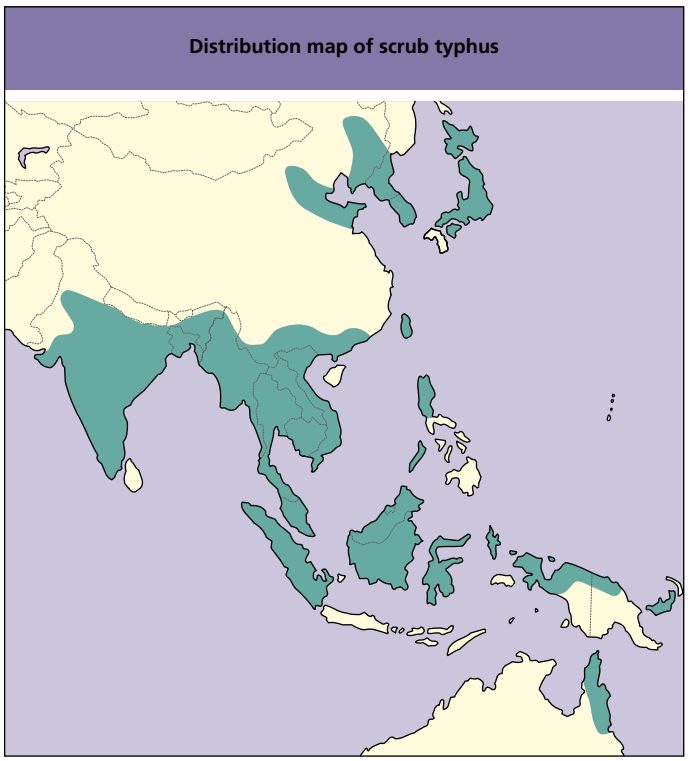


Fig. 122.1 Distribution map of scrub typhus. Scrub typhus or tsutsugamushi disease (from a Japanese word meaning 'noxious bug') is caused by infection with *Orientia tsutsugamushi* and is transmitted by larval trombiculid mites of the genus *Leptotrombidium*. The disease occurs in the Indian subcontinent, South East Asia, the Far East and islands of the south-west Pacific including the Republic of Palau. From Peters W, Pavsol G. Atlas of tropical medicine and parasitology, 6th ed. St Louis: Mosby; 2006. Copyright Elsevier 2006.

admission and convalescent samples. These tests are not standardized across laboratories and are usually unavailable in poor tropical areas. They are, however, superior to the old Weil–Felix test (based on detection of antibodies cross-reactive to antigens of the OX-K strain of the unrelated bacteria *Proteus mirabilis*). Polymerase chain reaction (PCR) methods for detection of the 47 kDa and 56 kDa protein genes of *O. tsutsugamushi* have been developed but are not standardized or in widespread use. Although *O. tsutsugamushi* can be cultured from blood, this takes several weeks and requires special tissue culture techniques and a biosafety level 3 facility. Anti-*O. tsutsugamushi* IgM- and IgG-based rapid diagnostic tests have been developed but have not been adequately assessed in the field.

TREATMENT

Scrub typhus is very responsive to treatment with appropriate antibiotics, which should be given empirically if the diagnosis is suspected. Unless contraindicated, doxycycline is the standard treatment (usual adult oral dose is 100 mg q12h for 7 days, following a 200 mg loading dose). Tetracycline 500 mg q6h for 7 days may also be used. Trials of shorter courses are underway. Azithromycin (500–1000 mg on the first day followed by 250–500 mg daily for 2 days) is an effective alternative[2] and has been shown to be effective in a single dose.[3] Azithromycin is particularly useful if doxycyclines are contraindicated, such as in pregnancy. Chloramphenicol is another alternative to the tetracyclines (500 mg q6h in adults or 50–75 mg/kg/day in children for 7 days). Roxithromycin, telithromycin and rifampin have also been used successfully.

Epidemic Typhus

INTRODUCTION

Epidemic typhus, caused by *R. prowazeckii*, is one of the most dangerous of the arthropod-borne diseases and remains a major public health threat. It is transmitted by the human body louse (*Pediculus humanus corporis*), which lives in human clothing and thrives in conditions such as poverty and war where hygiene levels are poor. The bacteria are not transmitted vertically in lice and humans are the major reservoir (sporadic cases have been reported through contact with the flying squirrel *Glaucomys volans* in the USA). *R. prowazekii* is transmitted to people by infected louse feces (in which *R. prowazekii* survives for weeks), through aerosols (thought to be the main route of infection for health-care workers attending patients) or by skin autoinoculation, following scratching. Recently epidemics of epidemic typhus have occurred in Ethiopia, Burundi, Russia and Peru.[4] Sporadic cases have been recently reported in North Africa and France. Epidemic typhus must therefore still be considered a potential major health risk in tropical countries, particularly in environments such as refugee camps in cooler mountainous areas.

CLINICAL FEATURES

The incubation period is about 10–14 days. Patients develop malaise and vague symptoms before the abrupt onset of signs including fever (100%), headache (100%) and myalgia (70–100%). Other common signs include nausea or vomiting, coughing and epistaxis. Meningoencephalitis is a common complication in severe cases, with meningism, tinnitus, deafness and altered consciousness, ranging from mild confusion through agitated delirium and coma. Diarrhea, pulmonary involvement, myocarditis, splenomegaly and conjunctivitis may also occur. Most patients (20–80%) develop a skin rash that classically begins on the trunk and spreads to the limbs. It may be macular, maculopapular or petechial and may be difficult to detect on darker skin tones. The overall mortality rate is around 20%, rising to 60% in the malnourished and aged, though with appropriate antibiotic treatment mortality may be as low as 4%. Recrudescence of epidemic typhus unrelated to louse infestation or Brill–Zinsser disease can appear many years after the acute disease and has milder symptoms.[4]

DIAGNOSIS

Epidemic typhus can be diagnosed serologically by IFA, though Western blot combined with cross-adsorption tests is required for differentiation from murine typhus in positives. The Weil–Felix test (based on cross-reactivity with the *Proteus vulgaris* OX-19 antigen) is even less specific and should not be used. *R. prowazeckii* can be cultured in L929 cells using blood or skin biopsy samples. Recently, a quantitative real-time PCR assay targeting the *glt*A gene specific for *R. prowazekii* has been developed.

TREATMENT AND PREVENTION

In suspected cases antibiotics should be given prior to confirmation of the diagnosis. The following antibiotic regimens are considered effective:

- doxycycline 100 mg daily (4 mg/kg daily for children), in two divided doses if possible, for 3 days;
- tetracycline 300–500 mg q6h (children 50 mg/kg q6h) orally or intravenously; and

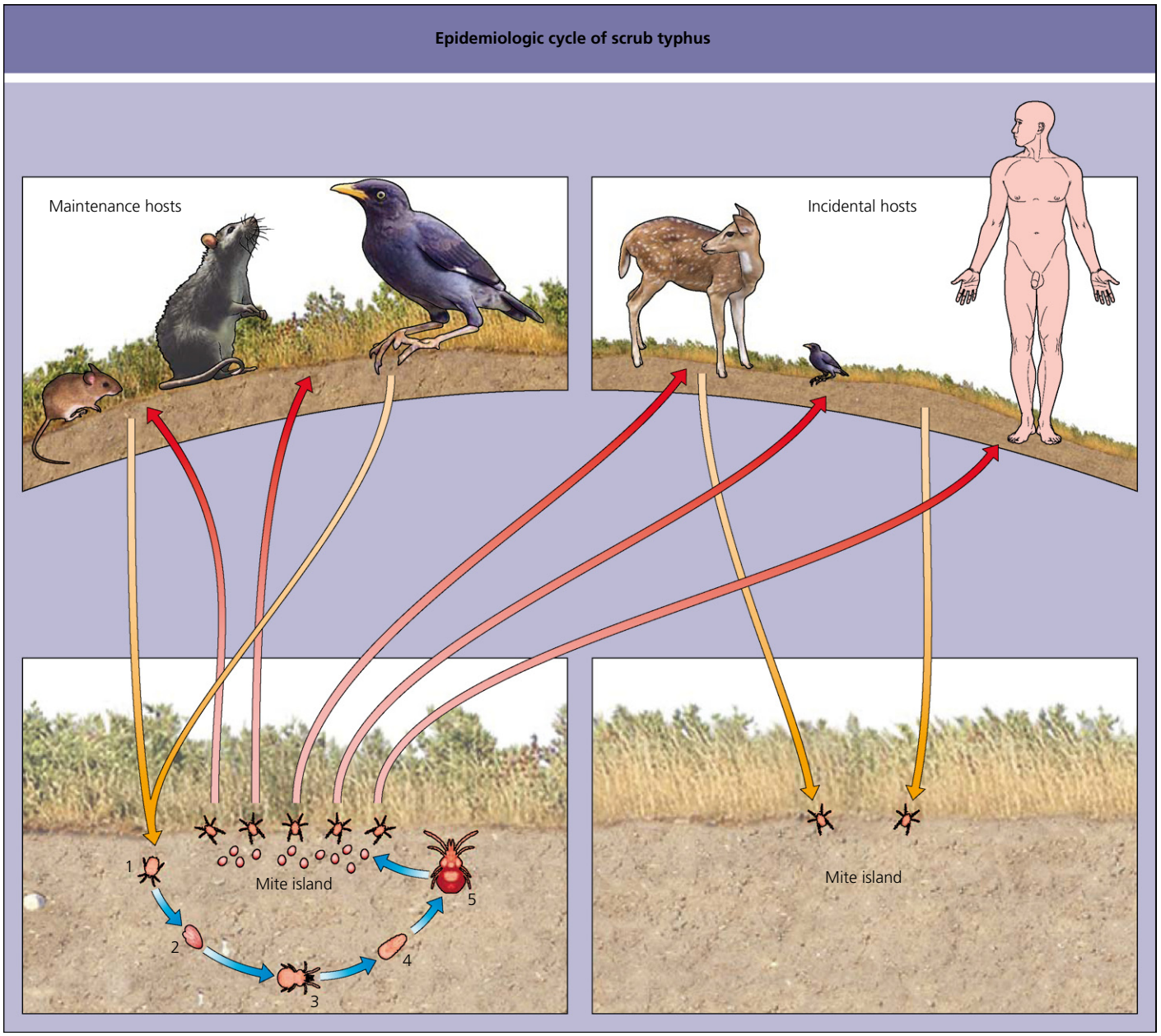


Fig. 122.2 Epidemiologic cycle of scrub typhus. *Orientia tsutsugamushi* is present in trombiculid-infested rodents. Not far from Kuala Lumpur, for example, *Leptotrombidium deliensis* is found in the forest and *Leptotrombidium akamushi* in the grass ('lalang'). The mites occur in colonies known as 'mite islands' that are associated with the nests of the rodents on which they normally feed. They pass through a cycle that includes a parasitic, blood-feeding, hexapod larva (1), a nymphochrysalis (2), followed by eight-legged, nonparasitic nymphal (3) and adult stages (5) between which is interposed an imagochrysalis (4). The cycle of infection is maintained in the focus, since engorged larvae are returned to the rodent nest, where they drop off and complete the rest of their cycle, with the adults finally laying eggs there. Vertical transmission of the Rickettsiae occurs so that the true reservoir of infection is the mite. Other mammals, humans and occasionally birds may be bitten by the larvae, which cause severe irritation. Thus, scrub typhus, although a zoonosis, can occur in small outbreaks if groups of people (e.g. soldiers in the jungle) rest in the vicinity of a mite island. IH, incidental hosts; MH, maintenance hosts; MI, mite island. adapted from Audy JR Red mites and typhus. London: Athlone Press; 1968.

- chloramphenicol 500–750 mg q6h (children 75 mg/kg q6h) orally or intravenously for 7 days.

In outbreak situations a single 200 mg dose of doxycycline is usually effective. Louse eradication (e.g. in refugee camps) is the most important preventive measure and is essential in the control of outbreaks. Since body lice live only in clothing, the simplest method of delousing is to remove and then destroy or wash and boil all clothing. Dusting of all clothing with 10% DDT, 1% malathion or 1% permethrin is also a rapid and effective method of killing body lice and reduces the risk of re-infestation.

Murine Typhus

INTRODUCTION

Murine typhus, also known as endemic typhus, is a flea-borne rickettsiosis caused by *R. typhi*. The rat flea *Xenopsylla cheopis* is the principal vector; rodents, mainly *Rattus norvegicus* and *R. rattus*, act as reservoirs. In suburban areas of the USA the cat flea, *Ctenocephalides felis,* has been



Fig. 122.3 Macular rash in a patient with scrub typhus.



Fig. 122.4 Eschar at the bite site, a hallmark of rickettsial diseases.

identified as the principal vector.[5] People become infected when flea feces containing *R. typhi* contaminate disrupted skin or are inhaled into the respiratory tract. Exposure to rat fleas is linked to exposure to rats, and high endemicity tends to occur in areas with large rat populations. Murine typhus has a worldwide distribution, occurring in urban as well as rural settings, though it probably has a higher incidence in warmer countries.

CLINICAL FEATURES

Murine typhus is usually a mild disease with nonspecific signs. The incubation period is 7–14 days and at presentation the classic triad of fever, headache and skin rash is observed in less than 15% of cases. Rash is present in less than 50% of patients and is often transient or difficult to observe. Nausea, vomiting, abdominal pain, diarrhea, jaundice, confusion, seizures and renal failure have also been reported. Peripheral lymphadenopathy is significantly less frequent in murine typhus than in scrub typhus, and eschars do not occur, useful distinguishing clinical features in areas of Asia where the two diseases coexist.[6] Mortality is low, around 1% with antibiotic treatment, and even without treatment the disease is usually a self-limiting illness lasting 7–14 days.

DIAGNOSIS

The IFA on paired admission and convalescent samples is the definitive diagnostic test, though false-positives can occur due to cross-reactivity with antigens from other bacteria. Cross-absorption of sera and Western blotting can be used to differentiate infections when cross-reactions occur. Other serologic tests exist but in general these have lower sensitivity than the IFA. PCR-based molecular methods have been devised for detecting TG organisms, though none is specific to murine typhus. Culture in vero or L929 cell lines may also be used but this is mainly a research tool as sensitivity is low and biosafety level 3 laboratory facilities are needed. Anti-*R. typhi* IgM- and IgG-based rapid diagnostic tests have been developed but have not been adequately assessed in the field.

TREATMENT

The drug of choice is doxycycline in both nonpregnant adults (100 mg q12h orally or intravenously) and children (4 mg/kg q12h up to a maximum of 100 mg per dose). The usual recommended duration of treatment is 7 days, but shorter courses may well be as effective. Chloramphenicol can be used as an alternative and may be useful in pregnancy. Whether azithromycin may be an efficacious alternative is currently unknown.

Rickettsial Spotted Fevers

INTRODUCTION

The spotted fevers are caused by a large and expanding number of rickettsial species, all of which are transmitted by ticks except for rickettsialpox caused by *R. akari*, which is mite borne, and the flea-borne infection due to *R. felis*. Ticks are not only vectors but also reservoirs of most of the currently known spotted fever group rickettsiae. Ecologic characteristics of the tick are keys to the epidemiology of tick-borne diseases. For example, the brown dog tick *Rhipicephalus sanguineus*, which is the vector of *R. conorii*, lives in dog environments (kennels and human houses) and has a low affinity for people. Cases of Mediterranean spotted fever are sporadic in endemic areas and most cases are encountered in urban areas. In contrast, *Amblyomma hebraeum*, which are heavily infected vectors of *R. africae* in southern Africa, emerge from their habitats and actively attack animals, particularly ruminants. Since they also feed readily on people that enter their biotopes, cases of African tick-bite fever often occur as grouped cases among subjects entering the bush (e.g. during a safari) and individuals can suffer several tick bites simultaneously.

CLINICAL FEATURES

The clinical symptoms of spotted fever group rickettsioses begin 6–10 days after the arthropod bite and typically include fever, headache, muscle pain, rash, local lymphadenopathy and a characteristic inoculation eschar ('tache noire') at the bite site. Despite the spotted fever appelation many patients with these infections confusingly have no rash – 'unspotted spotted fever'.

The main clinical signs vary depending on the rickettsial species involved and therefore may allow the clinician to distinguish between diseases. For example, African tick-bite fever is characterized by the occurrence of multiple inoculation eschars and grouped cases, as numerous highly infected *Amblyomma* may attack and bite many people in several places at the same time.[7] In contrast, in Mediterranean spotted fever due to *R. conorii*, a single eschar is usual because of the low likelihood of the tick biting people and a low rate of infection of the ticks. Details of each pathogen and clinical pictures are presented elsewhere (see Chapter 176).

Spotted fever group rickettsioses range from mild to severe and fatal diseases. For example, to date no mortalities or severe complications have been reported in patients with African tick-bite fever, whereas the mortality rate from Mediterranean spotted fever may be as high as 2.5%.

DIAGNOSIS

Investigations using a panoply of new molecular, immunologic and serologic tools have led to the recent dramatic expansion in the number of spotted fever rickettsial species now known to cause human disease. IFA is the most commonly used technique, though convalescent samples are usually needed. In specialist centers a panel of antigens is used for testing for a range of possible SFG pathogens. Cross-reactivity within the SFG, between the SFG and the TG, and with other unrelated pathogens is a problem, so cross adsorption assays and PCR-based techniques are often required to identify to the species level. Direct immunodiagnosis on blood and skin biopsies can be of value, as can culture of the pathogen. A real-time PCR has been developed based on 47 kDa, *glt*A and *omp*B gene targets which will distinguish scrub typhus, TG and SFG on admission blood samples.

TREATMENT

The treatment of choice for spotted fever rickettsioses is 200 mg doxycycline/day for 1–7 days depending on the severity of the disease. In children and pregnant women, macrolides including josamycin (50 mg/kg/day in children or 3 g/day in adults), roxithromycin, clarithromycin and azithromycin have all been used successfully for the treatment of Mediterranean spotted fever, as has chloramphenicol.

Q-Fever

INTRODUCTION

Q-fever is a zoonotic infection caused by *Coxiella burnetii*, usually acquired occupationally by the ingestion or inhalation of virulent organisms from infected mammals, mostly goats, sheep and cats, and their products. *Coxiella burnetii* has been found to infect more than 40 species of ticks throughout the world, but the role of ticks in human infections is not confirmed.

CLINICAL FEATURES

Acute Q-fever is usually mild, with asymptomatic seroconversion occurring in 50–60% of infected individuals.[8] A self-limited febrile syndrome occurs most frequently in symptomatic patients, but in more serious cases hepatitis, pneumonitis and prolonged fever may develop (see Chapter 176). Chronic Q-fever represents the development of the acute disease in predisposed hosts. It may present as endocarditis in patients with underlying heart valve lesions or, more rarely, as vascular aneurysms, graft infections, chronic bone infections or pseudotumors of the lung.

DIAGNOSIS

The diagnosis of Q-fever relies mainly on serology. *Coxiella burnetii* exhibits phase variation, and in acute Q-fever antibodies to phase II antigens predominate and their titer is higher than the phase I antibody titer. In chronic forms of the disease, however, elevated anti-phase I antibodies are uniformly detected. A phase I IgG titer of 800 or greater is one of the major modified Duke criteria for endocarditis. Q-fever can also be diagnosed by PCR, though this test is not widely available.

TREATMENT

The treatment of choice for acute Q-fever is doxycycline 100 mg 12 hourly for 14 days, though fluoroquinolones may also be used. The treatment of chronic disease is complex, involving long courses (≥18 months) of doxycycline + hydroxychloroquine, plus surgery if indicated.

REFERENCES

References for this chapter can be found online at http://www.expertconsult.com

Chapter 123

Finn T Black

Brucellosis

EPIDEMIOLOGY

Brucellosis, also known as undulant fever or Malta fever, is a zoonosis caused by bacteria of the genus *Brucella*. The disease exists worldwide, with the highest prevalence in the Mediterranean countries, Asia, Africa and Central and South America. Around 500 000 new cases are reported annually worldwide, of which only 100–200 are in the USA,[1] but brucellosis is clearly underreported.

Brucellosis is transmitted to humans by direct contact with infected animals, by ingestion of unpasteurized milk or milk products, through cuts and abrasions or by inhalation of aerosols. It is common though infrequently diagnosed in pastoral rural populations in Africa and the Middle East. In many European countries and in the USA it is mainly an occupational disease occurring in abattoir workers, butchers and farmers. Veterinary surgeons may become infected by accidental inoculation of live attenuated *Brucella* vaccine. Person–person transmission is extremely rare.

Four *Brucella* spp. can cause infection in humans:

- *Brucella melitensis*, which is found in goats, sheep and camels, is the most widespread and is the most virulent;
- *Brucella abortus*, which is found in cattle and camels, is less virulent;
- *Brucella suis*, which is found in pigs, is also less virulent; and
- *Brucella canis*, which is found in dogs, is the least common.

Other animals, including wildlife, may provide a reservoir for brucellae.[2]

PATHOGENESIS

Brucellae are facultative intracellular bacteria that are able to survive and multiply within mononuclear phagocytes. The mechanism is poorly understood but seems to include the suppression of degranulation of myeloperoxidase-containing granules, suppression of phagosome–lysosome fusion and production of protective enzymes.

The host reaction to brucellae is the formation of granulomas. *Brucella melitensis* and *B. suis* cause the most severe disease with caseating granulomas. Granulomas eventually heal with fibrosis and calcification.

Humoral antibodies seem to play some role in protection against re-infection. Control of the infection, however, depends on cell-mediated immunity.[2]

PREVENTION

The prevention of human brucellosis is dependent on the elimination of brucellosis in domestic animals. The use of veterinary vaccines for *B. abortus* and *B. melitensis* together with pasteurization of milk has resulted in a dramatic decrease in the incidence of human brucellosis. There is no effective vaccine available for *B. suis*. People at high risk of infection, such as veterinary surgeons and abattoir workers, should wear protective clothing. Laboratory-acquired brucellosis can be prevented by adherence to biosafety level 3 precautions. No effective vaccine is available for human use. Travelers to high endemic areas should be advised not to drink unpasteurized milk.

CLINICAL FEATURES

Brucellosis is a systemic infection that can involve any organ or organ system. The incubation period is normally between 2 and 4 weeks, but it may be months. The onset of clinical disease can be acute or insidious. Subclinical infection has been observed. Brucellosis is characterized by numerous somatic complaints in contrast to the few abnormal physical findings. Hepatosplenomegaly is present in 20–60%, depending on the species of *Brucella*, and mild lymphadenopathy is present in 10–20%. The nonspecific symptoms (e.g. fever, sweats, anorexia, fatigue, myalgia, malaise, headache and depression) are common and may mimic diseases such as tuberculosis, toxoplasmosis, mononucleosis, hepatitis, systemic lupus erythematosus, typhoid and many others.

When symptoms related to a single organ or organ system are dominant, it is often referred to as localized disease. The most common complications are listed in Table 123.1. The term 'chronic brucellosis' should be reserved for patients who have complaints of ill health for more than 12 months.[2] This includes patients who have relapsing illness or persisting focal infection and patients complaining of weakness, fatigue and depression, but with no objective signs of infection and no elevation of IgG antibody titer. This last group is believed to suffer from a psychoneurosis or a syndrome akin to chronic fatigue syndrome (see Chapter 70).

Table 123.1 Common complications of brucellosis

Organ system	Patients (%)
Cardiovascular	1–2
Endocarditis	0–2
Cutaneous	5–10
Gastrointestinal	50–70
Genitourinary	1–5
Orchitis	1–4
Neurologic	2–4
Osteoarticular	20–40
Sacroiliitis	10–15
Spondylitis	8–10
Pulmonary	15–25

Complications

Osteoarticular complications

Osteoarticular complications affect 20–40% of patients. Sacroiliitis is the most common reported complication, especially when *B. melitensis* predominates.[3] The characteristic radiographic findings are blurring of articular margins and widening of the sacroiliac space (Fig. 123.1). The clinical presentation is systemic symptoms and pain.

Spondylitis is most often seen in the lumbosacral region in elderly men, probably reflecting pre-existing anomalies in the spine, and it may be complicated by paraspinal abscesses (Fig. 123.2). The main symptoms are fever and vertebral pain. The typical radiographic findings are epiphysitis of vertebrae and narrowing of the intervertebral disc (Fig. 123.3). Bone scans and CT scans may detect infection earlier than radiography.[4]

Differential diagnosis includes tuberculosis, fungal and pyogenic osteomyelitis, multiple myeloma and metastatic carcinoma.

Arthritis especially involves the hips, knees and ankles.

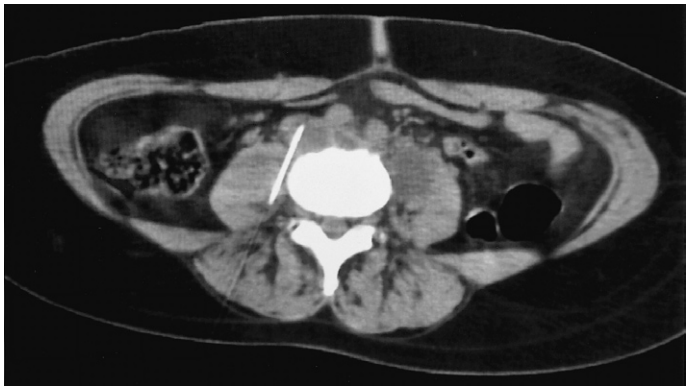


Fig. 123.2 CT scan of fine needle aspiration of paraspinal abscess (arrow) in a patient with brucellosis.

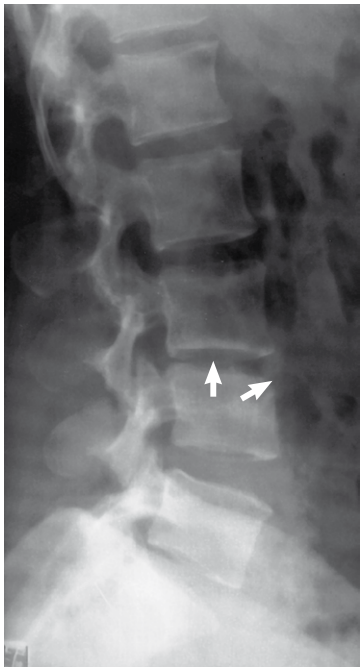


Fig. 123.3 Radiograph of the lumbar spine in a patient who has discitis and spondylitis of L3–4 caused by brucellosis. Note the reduced disc space and the destruction of the upper articular margins of L4 (arrows).

Gastrointestinal complications

Up to 70% of patients have intestinal complaints such as anorexia, nausea, vomiting, abdominal pain, diarrhea or constipation. The liver is probably always involved, but liver function tests are usually only mildly abnormal. Cirrhosis does not seem to follow *Brucella* infection.

Pulmonary complications

Respiratory symptoms are reported in 15–25% of patients. They range from flu-like symptoms to bronchitis, interstitial pneumonitis, lung abscesses, hilar lymphadenopathy and lung effusions.

Genitourinary complications

Complications from the genitourinary tract are rare. Acute orchitis or epididymo-orchitis with signs of systemic infection do occur and interstitial nephritis, glomerulonephritis and pyelonephritis resembling tuberculosis have been described. Brucellosis during pregnancy is rare but it can result in abortion like any other systemic infection.

Neurologic complications

Depression is a common complaint, but invasion of the central nervous system occurs in only 2–4% of cases. It usually presents as acute or chronic meningitis. Encephalitis, polyradiculopathy, psychosis and meningovascular complications have also been described.[5] Analysis of cerebrospinal fluid reveals elevated protein, lymphocytic pleocytosis, low to normal glucose and most often intraspinally produced specific antibodies. Brucellae are isolated from cerebrospinal fluid in less than 30% of patients.

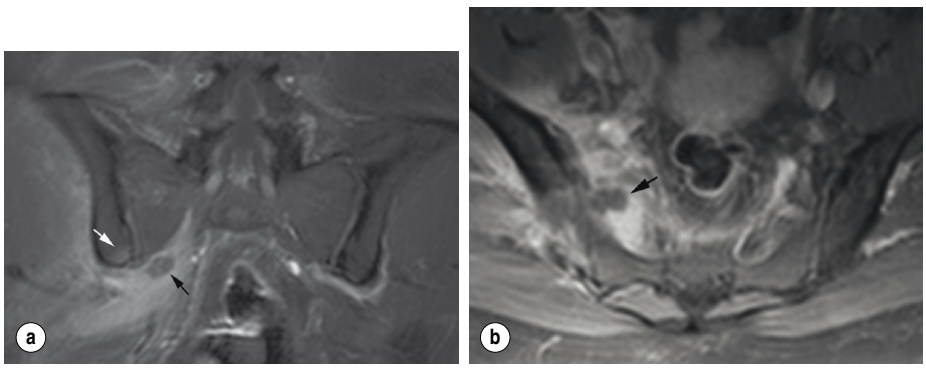


Fig. 123.1 MRI showing sacroiliitis: (a) coronal; (b) axial. T1 fat saturated images obtained after intravenous gadolinium contrast. There is osseous enhancement at the inferior part of the joint (white arrow) and huge soft tissue enhancement surrounding a non-enhancing abscess (black arrow).

Cardiovascular complications

Endocarditis, although rare, is the main cause of death related to brucellosis.[6] The aortic valve is involved more often than the mitral valve. Other complications include mycotic aneurysms, myocarditis and pericarditis.

Cutaneous involvement

Cutaneous manifestations of brucellosis consist mainly of transient nonspecific lesions including erythema nodosum, petechiae, vasculitis, papules and rashes.

DIAGNOSIS

Because the symptoms of brucellosis are nonspecific, it is crucial that the attending physician anticipate the probability of the disease. A certain diagnosis of brucellosis is made when brucellae are isolated from blood, bone marrow or other body fluids or tissues. Most laboratories employ rapid isolation methods for blood cultures. However, these cultures need to be maintained for up to 30–40 days to isolate *Brucella* spp. successfully. Bone marrow cultures are more sensitive than blood cultures in acute brucellosis and tend to remain positive later in the course of the infection, even during antimicrobial treatment.

The serum agglutination test is still the best standardized and most widely used serologic test.[7] It measures both IgG and IgM antibodies; antibodies to *Vibrio cholerae*, *Francisella tularensis* and *Yersinia enterocolitica* can give false-positive reactions. False-negative reactions due to blocking antibodies are seen and dilutions of 1:640 should be made. A titer of 1:160 is normally considered positive, as is a fourfold or greater rise in titer. Most patients who have acute infection develop IgM and IgG antibodies. The IgG antibodies persist as long as the infection is active and they increase with relapse and decrease with cure. The enzyme-linked immunosorbent assay (ELISA) appears to be more sensitive than and as specific as the serum agglutination test. It is rapid, easy to perform and can be automated.[7] Polymerase chain reaction (PCR) has also been shown to be very sensitive and specific, and appears to be a very useful test for initial diagnosis, post-treatment follow-up and early detection of relapses.[8,9]

MANAGEMENT

As single drug treatment results in a high relapse rate (5–40%), combination therapy is usually recommended. Duration of treatment is normally 6 weeks, but longer in complicated cases.[10]

Uncomplicated brucellosis is treated with oral doxycycline 100 mg q12h plus oral rifampin (rifampicin) 600–900 mg q24h, or oral doxycycline plus an intramuscular or intravenous aminoglycoside (e.g. streptomycin 1 g q24h or gentamicin 240 mg q24h) for 2–3 weeks. Children less than 8 years of age and pregnant women should not be treated with doxycycline. Instead, oral trimethoprim–sulfamethoxazole can be used (20 mg/kg sulfamethoxazole and 4 mg/kg trimethoprim q12h in children for 6 weeks, 800 mg sulfamethoxazole and 160 mg trimethoprim q12h in adults for 6 weeks) plus intramuscular gentamicin (5 mg/kg q24h in children and 240 mg q24h in adults for 1 week). Gentamicin can be replaced by oral rifampin (10–20 mg/kg q24h in children and 600 mg q24h in adults for 6 weeks).

Complications

Complications of brucellosis such as meningitis and endocarditis require prolonged courses of therapy, directed by the response.[2] In severe cases, a combination of three agents is often recommended (e.g. doxycycline or trimethoprim–sulfamethoxazole, plus an aminoglycoside and rifampin). Endocarditis often requires additional surgical intervention.[6]

Many other antimicrobial agents have shown *in-vitro* activity against *Brucella* spp., including fluoroquinolones, third-generation cephalosporins and azithromycin. However, these drugs should only be used in controlled trials or kept as second-line medication until further studies confirm their efficacy.

With chemotherapy the overall mortality rate is less than 2%.

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Chapter 124

Nicholas PJ Day
Charles N Edwards

Leptospirosis

INTRODUCTION

Leptospirosis, caused by pathogenic species of the bacterial genus *Leptospira*, is the most geographically widespread zoonotic disease, affecting humans in both rural and urban settings in temperate and tropical climes.[1] The disease is maintained in nature by chronic renal infection of mammals, leading to environmental contamination with infective organisms. Human infection follows exposure to infected animals, either directly or indirectly through contaminated soil and water. The majority of patients experience a mild febrile illness, while a minority develop a severe illness known as Weil's disease which is characterized by bleeding, jaundice and renal failure leading in the most severe cases to death from pulmonary hemorrhage or renal failure.

MICROBIOLOGY

Leptospires are highly motile, obligate aerobic spirochetal bacteria, about 0.25 × 6–25 µm in size (Fig 124.1).[2] Leptospires survive for days or weeks in warm, damp, slightly alkaline conditions, especially in still or slowly moving fresh water in the temperate summer and in damp soil and water in the tropics, particularly in the rainy season. Until recently the Leptospiraceae were classified mainly on the basis of gross agglutination between strains, with over 200 pathogenic and 60 nonpathogenic saprophytic serovars. More recently the genus *Leptospira* has been split into species on the basis of DNA–DNA hybridization. Eighteen species are now recognized, 12 pathogenic or probably pathogenic and six saprophytic. The two classification systems are currently used side by side, somewhat uncomfortably as a number of serovars have been found in multiple *Leptospira* spp.; further (hopefully simplifying) developments are likely in the future.



Fig. 124.1 Electron micrograph of a leptospire.

EPIDEMIOLOGY

In temperate climates, leptospirosis is acquired mainly through recreational or occupational exposure, or through living in slums, but in tropical regions exposure through vocational activities such as rice farming is more widespread.[2] The burden of disease is greatly underestimated in the tropical developing world. Leptospirosis is an important cause of febrile illness in tourists returning from the tropics, particularly those involved in adventure tourism. The reported incidence of leptospirosis in developed countries is declining and the disease is no longer reportable in the USA.

The serovar classification remains useful for epidemiologic purposes, as many geographically widespread serovar–reservoir associations exist. The most ubiquitous are *icterohaemorrhagiae* with rats *Rattus* species, *hardjo* with cattle and *pomona* with pigs. Other serovars are more restricted in their distribution, such as *lai*, which causes most cases of human infection in China and the Korean peninsula. The new, more discriminative technique of multilocus sequence typing confirmed that a large outbreak of human leptospirosis in northeast Thailand lasting several years was caused by a single ecologically successful clone (sequence type 34) of *L. interrogans* serovar *autumnalis*, which was associated with bandicoot rats.[3]

PATHOGENESIS AND PATHOLOGY

Leptospires gain access to the circulation through penetration of abraded skin or intact mucous membranes, disseminate and ultimately penetrate various tissues. This action results in a systemic illness with a wide spectrum of clinical features. A systemic vasculitis with endothelial injury is the basic microscopic finding, with damaged endothelial cells usually showing varying degrees of swelling, denudation and necrosis. Leptospires have been documented in large- and medium-sized blood vessels and capillaries in various organs. The major affected organs are:

- the kidneys, with a diffuse tubulointerstitial inflammation and tubular necrosis;
- the lungs, usually congested, with focal or massive intra-alveolar hemorrhage; and
- the liver, which shows cholestasis associated with mild degenerative changes in hepatocytes.

Other systems may also be affected, with myocarditis, meningoencephalitis and uveitis all occurring in severe disease. Whether a direct toxic effect of the leptospires or the resulting immune response causes the vascular injury is unclear. *Leptospira* outer membrane proteins (OMPs) and lipopolysaccharides may elicit inflammation through a Toll-like receptor 2-dependent pathway.[4] Thrombocytopenia and activation of the coagulation cascade are also commonly seen.[5] During recovery leptospires continue to be excreted in the urine for some days.

PREVENTION

Prevention measures are based upon an awareness of the epidemiology of disease occurring in a region.

Rodent control should be attempted where appropriate and feasible. Occupational protective clothing is effective in groups at risk. In some high-risk groups (e.g. soldiers on jungle maneuvers), oral chemoprophylaxis with doxycycline 200 mg weekly is effective,[6] but there have been few attempts to use this approach on a large scale, such as after massive flooding.

Vaccines are available for use in the livestock industry, and in some countries for human use, but these have been largely unsuccessful.[7] However the identification of new surface-exposed proteins and putative virulence determinants through the availability of whole genome sequences have provided new candidates for vaccine development.[8]

CLINICAL FEATURES

Humans become ill 7–12 days after exposure to leptospires. The disease course is classically described as being biphasic, with an acute bacteremic phase and a second 'immune' phase, though clinically the two phases merge, particularly in severe disease. The acute phase is characterized by a sudden onset of fever, chills, retro-orbital headache, anorexia, abdominal pain, nausea and vomiting, and conjunctival suffusion (Fig. 124.2). Myalgia with muscle tenderness in the calves, abdomen and paraspinal region is characteristic, and when affecting the neck and nuchal region may mimic meningitis. In mild disease defervescence of fever occurs after 3–9 days.

The second or 'immune' phase starts either immediately or 2–3 days later, and is characterized by leptospiruria and the appearance of IgM antibodies. Symptoms recur and signs of meningitis may develop in up to 50% of cases. Optic neuritis, peripheral neuropathy and prolonged uveitis can also occur. In severe cases, fever may persist and be associated with renal insufficiency or failure, varying levels of jaundice, and myocarditis. Coagulopathy may occur, leading to bleeding manifestations ranging from a petechial rash and conjunctival hemorrhage through to gastrointestinal bleeding and pulmonary hemorrhage. Death, occurring in 10–15% of these severe cases, is usually due to pulmonary hemorrhage, renal failure or cardiac failure and arrhythmias secondary to myocarditis. Chest radiographs may reveal numerous abnormalities including pulmonary infiltrates, pleural effusions or a diffuse pneumonitis.

Clinical laboratory investigations reveal red and white cells in the urine with proteinuria. An elevated white cell count, thrombocytopenia and a high creatinine phosphokinase are commonly observed. The bilirubin may be very elevated but the transaminases rarely exceed two to three times the upper limit of normal.

The differential diagnosis of leptospirosis includes dengue fever, malaria, rickettsioses, influenza and other acute febrile illnesses depending on the geographic location.

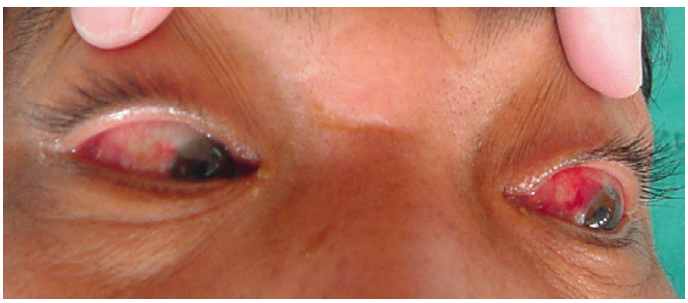


Fig. 124.2 Conjunctival suffusion and jaundice.

DIAGNOSIS

Because of the broad spectrum of symptoms and the wide differential diagnosis, a high index of clinical suspicion is required if appropriate diagnostic tests are to be made. Though leptospires can be isolated from blood and cerebrospinal fluid samples during the first 7–10 days of illness, and from urine during the second and third weeks, isolation of the organism requires special media and takes several weeks of incubation and thus does not contribute to individual patient care. A large number of methods for detection of leptospiral DNA by polymerase chain reaction have recently been described, and though more sensitive than culture, these have not yet been standardized and optimized for routine use.

A strong IgM antibody response, which appears about 5–7 days after onset of symptoms, can be detected using several commercial assays based on ELISA, latex agglutination and immunochromatographic rapid test technologies. Unfortunately, these have relatively poor sensitivity on admission samples. The microscopic agglutination test applied to paired acute and convalescent samples remains the gold standard, but this is a complex assay that detects antibodies against live antigen suspensions and is performed only in reference laboratories. This test yields information about the presumptive infecting serovar and thus has some epidemiologic value. In endemic areas, single elevated titers must be interpreted with caution because antibodies persist for years after acute infection.

Direct microscopic examination of clinical samples is of little value, but immunohistochemical staining of autopsy specimens is a valuable diagnostic tool.

MANAGEMENT

Patients with mild or anicteric disease usually get better without treatment. Doxycycline 100 mg/day has been shown to shorten the duration of the illness, and is as effective as cefotaxime or penicillin G while at the same time treating rickettsioses which may present in a clinically similar fashion.[9] Until the efficacy of antibiotic treatment in late-stage severe disease is resolved, patients should be treated with intravenous penicillin G (benzylpenicillin), cefotaxime or ceftazidime.[10] Hospitalization is recommended for severe cases. Excellent supportive care with particular attention to fluid and electrolyte balance and pulmonary and cardiac function is critical. Renal failure should be treated by hemodialysis or hemodiafiltration if available, though peritoneal dialysis can be used in resource-poor settings.

REFERENCES

References for this chapter can be found online at http://www.expertconsult.com

Chapter 125

David A Warrell

Relapsing fevers

INTRODUCTION

Repeated abrupt episodes of fever, separated by afebrile periods, give relapsing fever its name. There are two forms:

- tick-borne relapsing fever, caused by various *Borrelia* spp.; and
- louse-borne relapsing fever, caused by *Borrelia recurrentis*.

Both diseases are transmitted by arthropod ectoparasites and, rarely, by needlestick, blood transfusion or transplacentally, but they differ in their epidemiology, clinical features and management. Sometimes, they cause fever in returned travelers.

EPIDEMIOLOGY

Tick-borne relapsing fever

Tick-borne relapsing fever is endemic in most temperate and tropical countries except in Australasia and the Pacific region. In the West African savanna region, *B. crocidurae* is the most prevalent bacterial infection, creating a medical problem second only to malaria.[1] It has increased during the recent drought. In parts of East Africa, it is an important cause of abortion and perinatal mortality.[2]

Borrelia-tick complexes

Different species of *Borrelia* spirochetes and soft ticks of the genus *Ornithodoros* (Argasidae) are associated with different parts of the world (Table 125.1).

Tick vectors are predominantly soft ticks (Argasidae) of the genus *Ornithodoros*, found in dry savanna areas and scrub, particularly near rodent burrows, caves, piles of timber and dead trees, or in cracks and crevices in walls, roof spaces and beneath the floors of log cabins, anywhere inhabited by small rodents. Unlike louse-borne relapsing fever, tick-borne relapsing fevers are zoonoses with the possible exception of *B. duttonii* infection that was thought to be transmitted only between humans. However, new evidence suggests that in central Tanzania this species may infect domestic chickens and pigs.[3] Vertebrate reservoir species are rodents such as rats, gerbils, mice, squirrels and chipmunks and also dogs and birds. Ticks ingest spirochetes while sucking blood from infected animals or humans. They attack at night, remaining attached for less than 30 minutes before retreating back to their hiding places. Infection is either by a bite, through infected saliva, or by contaminating mucosal membranes with infected coxal fluid. Borreliae are not excreted in tick feces.

Ticks remain infected for life and while starved of blood for as long as 7 years. Spirochetes can be transmitted venereally from male to female ticks and by females (but perhaps not those of the *O. moubata* complex) transovarially to their progeny.

Table 125.1 Geographic distribution of *Borrelia* and *Ornithodoros* spp.

***Borrelia* spp.**	***Ornithodoros* spp.**	**Geographic distribution**
New World tick-borne relapsing fever borreliae		
B. hermsii	*O. hermsi*	Canada, Central and Western United States, Mexico
B. turicatae	*O. turicata*	Southwestern United States, Mexico
B. parkeri	*O. parkeri*	Western United States, Baja California
B. mazzotti	*O. talaje*	Mexico, Central America
B. venezuelensis	*O. (venezuelensis) rudis*	Colombia, Venezuela, Argentina, Bolivia, Paraguay
Old World tick-borne relapsing fever borreliae		
B. duttonii	*O. moubata*	Sub-Saharan Africa
B. crocidurae	*O. (erraticus) sonrai*	West, North, East Africa, Middle East
B. persica	*O. tholozani*	Middle East, Central Asia from Uzbekistan to western China
B. hispanica	*O. erraticus*	Iberian peninsula, Greece, Cyprus, North Africa
B. latyschewi	*O. tartakowskyi*	Eastern Europe, Iran, Iraq, Afghanistan
B. caucasia	*O. asperus*	Iraq, Eastern Europe

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Louse-borne relapsing fever

The incidence of this classic epidemic disease of armies, refugees and immigrants has fallen in the last decade, but louse-borne relapsing fever remains endemic and seasonally epidemic in the highlands of Ethiopia and adjacent areas of Sudan and Somalia, hilly areas of Yemen and perhaps some parts of the Peruvian and Bolivian Andes.[4] Serologic evidence of *B. recurrentis* infection was also found in homeless people in Marseille.[5] The human body louse, *Pediculus humanus corporis*, is an obligate blood-sucking human ectoparasite that ingests borreliae while feeding. Transmission is by scratching, which crushes lice so that their celomic fluid is inoculated through broken skin or intact mucous membranes such as the conjunctiva, or inoculates infected louse feces. Unlike ticks, lice cannot infect their progeny. Humans are the only reservoirs of this infection.

Louse-borne relapsing fever thrives in the highlands of Ethiopia during the rainy season when impoverished people, encouraged to wear clothes by the cold wet climate, are crowded together and infested with lice, but lack the facilities for controlling the infestation. More than 20 000 lice were recovered from the clothes of one infected person.

PATHOGENESIS

Relapsing fever spirochetes in the blood are lysed by specific bactericidal IgM antibodies, independent of complement and T cells. However, some spirochetes persist extracellularly in various organs: spleen, liver, kidneys, eye and especially in the brain and cerebrospinal fluid. Relapse of spirochetemia and symptoms is explained by antigenic variation, which has been investigated mainly in *B. hermsii*.[6] Transposition of silent gene sequences from an archive stored in extrachromosomal plasmids and their recombination and expression on a linear plasmid results in the synthesis of a new major outer membrane lipoprotein. The spirochete possesses virulence and protective factors. *Borrelia hermsii* surface protein CRASP-1 binds factor H (FH), FHR-1 and plasminogen. FH protects against opsonophagocytosis by inhibiting C3b. Plasminogen is bound and activated to plasmin, stimulating fibrinolysis that promotes spread. Erythrocyte rosetting shields spirochetes physically from host antibody.[7] Antigenic variation generates isogenic serotypes with properties such as enhanced invasiveness for cerebral vascular endothelium.

PREVENTION

Tick-borne relapsing fever

Tick infestation of dwellings can be reduced by improved house construction (e.g. rodent-proofing of cabins on the North Rim of the Grand Canyon), control of their peridomestic rodent hosts and use of residual insecticides (pyrethroids, benzene hexachloride, lambda-cyhalothrin, malathion or dichlorodiphenyltrichloroethane – DDT). Travelers should avoid sleeping in places where ticks and rodents are abundant, such as poorly maintained log cabins, and should apply repellents to their skin (*N*,*N*-diethyl-M-toluamide – DEET). Postexposure prophylaxis with doxycycline (200 mg followed by 100 mg on the next 4 days) has proved effective against *B. persica* in Israel.[8]

Louse-borne relapsing fever

Infested clothing should be de-loused using heat (>60°C), chlorine bleach or insecticide (10% DDT, 1% malathion, 2% temephos, 1% propoxur or 0.5% permethrin) and patients should be bathed with soap and 1% cresol (Lysol). Lice are abundant in hair, which should be washed or shaved off. Breaking transmission from lice to the susceptible population is essential for the control of an epidemic.

CLINICAL FEATURES

Tick-borne relapsing fever

Ticks bite painlessly and detach after only a short feed and infection produces no eschar, so exposure usually passes unnoticed. After an incubation period of 3–18 days, the illness starts with sudden fever, chills, headache, muscle and joint pains, extreme fatigue, prostration and drenching sweats.[4] Epistaxis, abdominal pain, diarrhea, cough and erythematous or petechial rashes may follow. Depending on the *Borrelia* spp. involved, up to 40% of patients may develop neurologic disturbances reminiscent of those in Lyme disease: paresthesiae, visual symptoms, lymphocytic meningitis, cranial nerve palsies (especially VII), encephalitis, myelitis, sciatica and radiculitis. Several cases of adult respiratory distress syndrome (ARDS) have been described in the United States.

The density of spirochetemia governs clinical severity. Symptoms abate after a few days, only to recur about 7–15 days later. As many as eight relapses may follow, becoming sequentially less severe. Abortion and perinatal mortality is common. In one study in Tabora, Tanzania, parturition was precipitated in 58% of infected pregnant women. Perinatal mortality was 436/1000 births; its risk related to low birth weight and gestational age, and the total loss of pregnancies including abortions was 475/1000. Spirochetemia was higher in pregnant than nonpregnant women.[2]

Louse-borne relapsing fever[4,9]

After an incubation period of 4–17 (average 7) days, the first symptoms are fever, chills, headache, muscle and body aches, fatigue, dizziness, anorexia and nightmares. At least 50% of patients develop bleeding: epistaxis, subconjunctival hemorrhage (Fig. 125.1) or petechial hemorrhages, especially on the trunk (Fig. 125.2). An enlarged and tender liver, often with an enlarged spleen, is palpable in as many as 50% of cases in some outbreaks, and about half of these are jaundiced. The respiratory system is affected in about 15% of cases. Cough may indicate pneumonia or acute pulmonary edema. Transient myocardial damage may lead to acute left ventricular failure with pulmonary edema. Neurologic signs are less common than in tick-borne relapsing fever. Confusion or coma suggests circulatory failure, cerebral hemorrhage (Fig. 125.3) or hepatic failure. Fetal loss is very common in pregnant women.

Spontaneous crisis and Jarisch–Herxheimer reaction

The clinical course of relapsing fever, especially louse-borne, usually ends either with a 'spontaneous crisis' on about day 5 of the untreated illness or by a Jarisch–Herxheimer reaction (J-HR).[9] About 1–3 hours

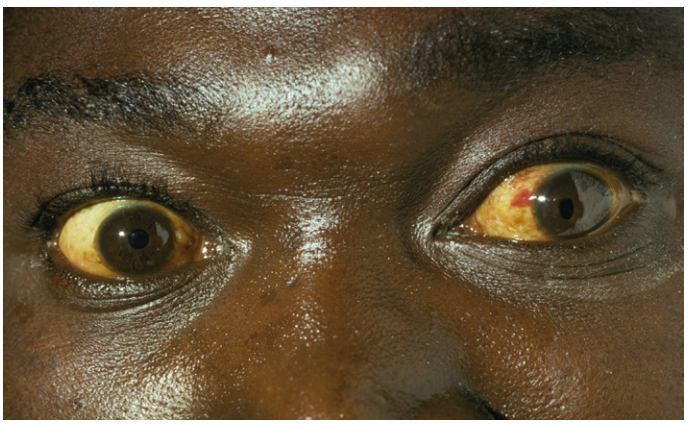


Fig. 125.1 Subconjunctival hemorrhages and jaundice in an Ethiopian patient with louse-borne relapsing fever. Copyright D.A. Warrell.



Fig. 125.2 Petechial hemorrhages in an Ethiopian patient suffering from louse-borne relapsing fever complicated by typhoid. Copyright D.A. Warrell.

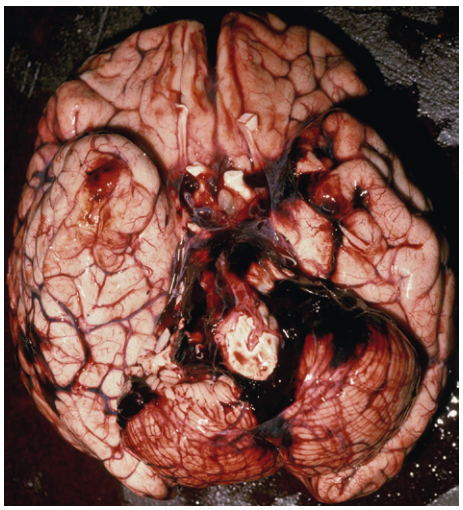


Fig. 125.3 Fatal cerebral hemorrhage in louse-borne relapsing fever. Copyright D.A. Warrell.

after antibiotic treatment, the patient becomes restless. Violent rigors herald rapid increases in temperature, respiratory rate, pulse rate and blood pressure. There may be associated vomiting, diarrhea, coughing, musculoskeletal pains and delirium. Some patients die of hyperpyrexia at the height of the fever. During the ensuing flush phase of this endotoxin-like reaction, there is profuse sweating, intense vasodilatation and a fall in mean arterial pressure, and a slow decline in temperature over the next 6–12 hours. Fatalities during this phase are due to hypovolemic shock or acute pulmonary edema resulting from myocarditis, sometimes precipitated by the patient's getting out of bed and standing up. The incidence of Jarisch–Herxheimer reactions varies from 30% to almost 100% in different published reports of patients who were carefully observed.

A borrelial pyrogen, an outer membrane variable major lipoprotein[10] released by the action of antimicrobial agents, stimulates an explosive release of cytokines from macrophages through nuclear factor kappa B (NF-κB),[11] principally tumor necrosis factor (TNF), interleukin (IL)-6, IL-8 and IL-1β, just before the start of the clinical manifestations of the Jarisch–Herxheimer reaction (Fig. 125.4).[12] The reaction is unaffected by corticosteroids, but is delayed by the opiate agonist–antagonist meptazinol and can be prevented by a polyclonal antibody against TNF if this is given just before antibiotic treatment.[13]

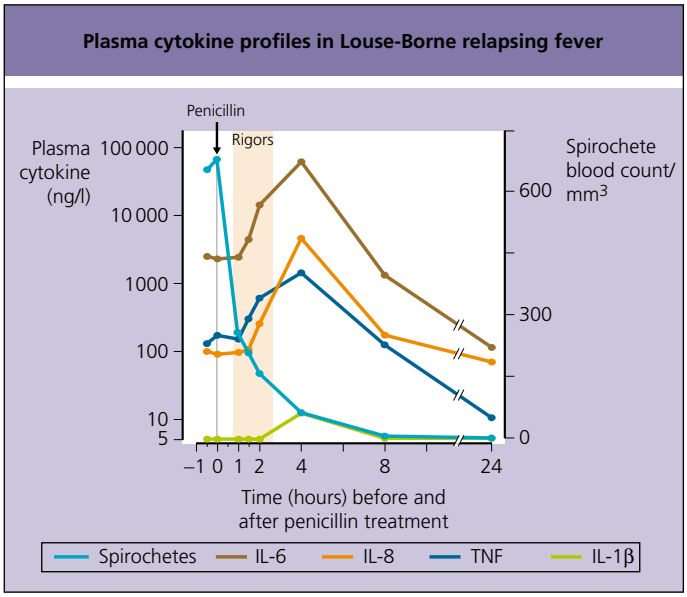


Fig. 125.4 Plasma cytokine profile in a patient with louse-borne relapsing fever during the Jarisch–Herxheimer reaction to penicillin treatment. Copyright D.A. Warrell.

LABORATORY FINDINGS

In blood films, spirochetes are best demonstrated with Giemsa or Wright's stain, counterstained with 1% crystal violet (Fig. 125.5). Thick films have a 20-fold higher sensitivity than thin films. In louse-borne relapsing fever, spirochete density in peripheral blood may exceed 500 000/mm^3. There is a peripheral neutrophil leukocytosis but, during the Jarisch–Herxheimer reaction or spontaneous crisis, leukocytes almost disappear transiently from the peripheral blood. Thrombocytopenia is common and there is a coagulopathy attributable partly to hepatic dysfunction and partly to disseminated intravascular coagulation with increased fibrinolytic activity. Biochemical evidence of hepatocellular damage is found in most patients.[4] A few patients show transient, mild renal impairment. A mild neutrophil–lymphocyte pleocytosis has been described.

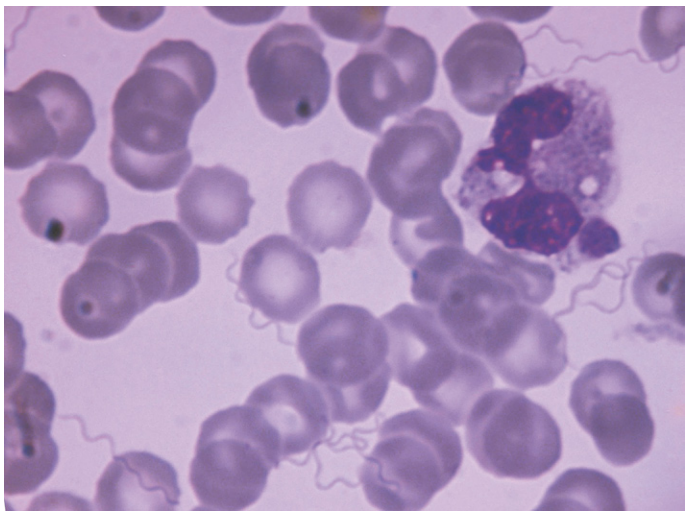


Fig. 125.5 Spirochetes of *Borrelia recurrentis* in a thin blood film. Copyright D.A. Warrell.

DIAGNOSIS

In patients with acute fevers after travel to tropical countries, it is essential to examine thick and thin blood films for malarial parasites. This may reveal relapsing fever spirochetes. Misidentification of microgametes of *Plasmodium vivax* has led to the diagnosis of 'pseudo-borreliosis'.[14] In tick-borne relapsing fever, spirochetes may be difficult or impossible to find, even at the height of a relapse and, increasingly, polymerase chain reaction (PCR) and serology are being used. However, Lyme disease borreliae may produce cross-reacting antibodies, due to expression of conserved antigenic epitopes. ELISA, using the GlpQ gene product, can distinguish relapsing fever from Lyme disease.[15] In louse-borne relapsing fever, the higher and more persistent spirochetemia is more easily detected.

In vitro culture of borreliae, including *B. recurrentis, B. duttonii* and *B. crocidurae,* is now possible, using Barbour–Stoenner–Kelly medium.[16]

Differential diagnosis

In a febrile patient with jaundice, petechial rash, spontaneous systemic bleeding and hepatosplenomegaly, the following differential diagnoses should be considered, depending on the geographic location: falciparum malaria, yellow fever and other viral hemorrhagic fevers (such as Rift Valley fever in the Horn of Africa), viral hepatitis, rickettsial infections (especially louse-borne typhus which has the same epidemiologic predispositions as louse-borne relapsing fever) and leptospirosis. Secondary infections, known to complicate louse-borne relapsing fever, include bacillary dysentery, salmonellosis, typhoid (see Fig. 125.2), typhus, malaria and tuberculosis.

In the differential diagnosis of travelers at risk, two less common causes of episodic recurrent fever are trench fever (caused by *Bartonella quintana*) if there was possible contact with lice, or rat-bite fever (caused by *Spirillum minus*).

PROGNOSIS

Reported case fatalities during *B. recurrentis* epidemics have exceeded 40% but this can be reduced to less than 5% with antimicrobial treatment, provided that appropriate ancillary treatment is given during the life-threatening Jarisch–Herxheimer reaction. Tick-borne relapsing fever is less dangerous. Deaths during relapses are most unusual but have been reported in tick-borne relapsing fever.

MANAGEMENT

The principles of treatment are:

- to eliminate spirochetemia and prevent relapses, using antibiotics;
- to monitor the patient very carefully through the Jarisch–Herxheimer reaction; and
- to restore and maintain circulating volume during the 24 hours after starting antibiotic treatment.

Antibiotic agents

The choice of agents is based on clinical experience.[17]

Tick-borne relapsing fever

Doxycycline 100 mg per day for 10 days is recommended for adults. For pregnant women and young children, erythromycin can be used.

Louse-borne relapsing fever

A single 500 mg oral dose of tetracycline or erythromycin stearate is effective. In severely ill patients who are likely to vomit, effective parenteral treatment consists of either a single intravenous dose of tetracycline hydrochloride (250 mg)[18] or, for pregnant women and children, a single intravenous dose of erythromycin lactobionate (300 mg for adults, 10 mg/kg for children). In mixed epidemics of louse-borne relapsing fever and louse-borne typhus, a single oral dose of doxycycline 100 mg has proved effective.[19] Penicillins and chloramphenicol are also effective.[17,18] Some experienced clinicians prefer to use a low dose of penicillin (adult dose, 100 000–400 000 units by intramuscular injection) in severe cases and pregnant women because they believe that the incidence and severity of the Jarisch–Herxheimer reaction will be less although the risk of relapse is greater.[4,20]

Supportive treatment

Postural hypotension and cardiac arrhythmias are prevented by nursing the patient flat in bed for 24 hours after antibiotic treatment. Hyperpyrexia and hypovolemia must be prevented. Acute heart failure with pulmonary edema responds to intravenous furosemide and digoxin. Bleeding and clotting problems are treated with vitamin K, platelets and clotting factor concentrates. Complicating infections, notably typhoid, salmonellosis, bacillary dysentery, tuberculosis, malaria and typhus in some endemic situations, must be treated appropriately.

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Chapter 126

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Viral hemorrhagic fevers

INTRODUCTION

Viral hemorrhagic fever (VHF) describes a syndrome which can be caused by multiple unrelated viruses. The clinical disease is often characterized by an acute onset, high fever and, in some cases, a high mortality rate. Although not fully defined for all VHFs, the bleeding manifestations are thought to be the result of capillary leakage. Death is usually attributable to multiorgan system failure and hypovolemic shock, with or without acute respiratory distress syndrome (ARDS).

VIROLOGY AND NATURAL HISTORY

To date, all known VHFs are caused by a range of single-stranded enveloped RNA viruses including arenaviruses, bunyaviruses, filoviruses, paramyxoviruses, flaviviruses and henipahviruses (Fig. 126.1; Table 126.1). Almost all of the VHFs can be described as zoonoses; these agents rely on an animal or insect reservoir for their maintenance and transmission. Therefore initial infections with these viruses are usually geographically restricted to areas where the reservoir and/or vector resides. However, the possibility of subsequent person-to-person transmission and importation outside the endemic or epidemic area(s) does exist.

VHF occurs primarily in rural or developing countries where there is substantial contact with rodents, ticks or mosquitoes and poor facilities for medical care. Only dengue, and occasionally yellow fever, are seen in populous areas. Distribution of individual VHF is often limited by reservoir/vector presence. However, as a result of the increasing mobility of populations, infected patients can and do appear almost anywhere in the world. This was highlighted recently with an imported case of Marburg hemorrhagic fever from Uganda to the Netherlands in 2008. Large epidemics of VHF can kill thousands of people, such as yellow fever outbreaks, or several hundred people, as has been the case with recent Marburg and Ebola outbreaks. However, there are often sporadic cases that go undiagnosed or fail to capture the attention of the local medical community; thus the incidence as well as the distribution of VHFs is likely underreported. Details pertaining to each family of VHF viruses are presented below.

Arenaviruses are rodent-borne pathogens that have potential to cause hemorrhagic fever (HF) infections in humans.[1,2] The viruses are classified into two categories: Old World and New World, based on geographic location. Geographic distribution of these viruses appears to be dependent on the presence of the rodent host. In West Africa, Lassa virus is the sole Old World arenavirus reported to be responsible for human infection. However, recent reports suggest that there may be additional or divergent arenaviruses circulating and potentially responsible for clinical disease.[3] Of the New World arenaviruses, Junin virus, Machupo virus, Guanarito virus and Sabia virus are the etiologic agents of Argentine HF, Bolivian HF, Venezuelan HF and Brazilian HF, respectively.

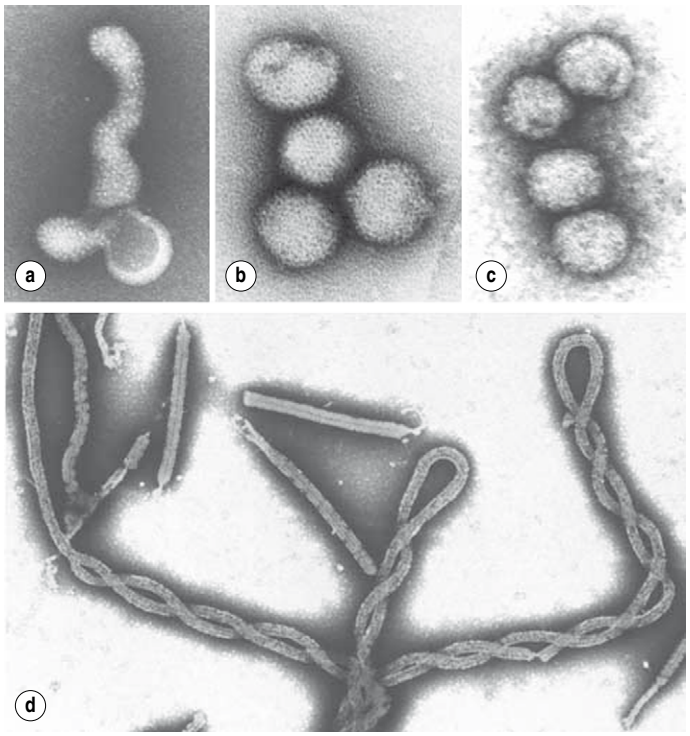


Fig. 126.1 Transmission electron micrographs of negatively stained hemorrhagic fever viral particles. (a) Junin virus. Arenavirus particles range in morphology from highly pleomorphic as shown in this field to mainly spherical. Virion sizes range from 50 to 300 nm with a mean of 100–130 nm. (b) Rift Valley fever virus. Bunyaviral particles are roughly spherical and range in diameter from 90 to 120 nm. (c) Yellow fever virus. Flaviviral particles are essentially isometric and consistent in size, ranging from 40 to 50 nm in diameter. (d) Ebola virus. Filoviral particles are mostly filamentous and vary in length up to 14 000 nm with a uniform diameter of 80 nm. Mean unit length is about 1000 nm. Other forms of filoviral particles included U shaped, '6' shaped or circular configurations; branching of filamentous particles can also occur. From Jahrling PB, Marty AM, Geisbert TW. Viral hemorrhagic fevers. In: Dembek ZF, ed. Medical aspects of biological warfare. In: Lenhart MK, Lounsbury DE, Martin JW, eds. Textbooks of military medicine. Washington, DC: Department of the Army, Office of The Surgeon General, Borden Institute; 2007:Ch. 13.

In the Bunyaviridae family,[4] Crimean–Congo hemorrhagic fever virus (CCHF),[5] hantaviruses[6] – particularly Andes virus (ANDV), Hantaan virus (HTNV), and Sin Nombre virus (SNV) – and Rift Valley fever virus[6,7] cause VHF in humans. CCHF is spread by ticks and occurs widely across Africa, south-eastern Europe, the Middle East and Asia.

Table 126.1 Epidemiologic characteristics of the most important viral hemorrhagic fevers

	ARENAVIRUSES*		FILOVIRUSES		BUNYAVIRUSES			FLAVIVIRUSES	HENIPAVIRUSES	
	Lassa virus	**Junin, Machupo, Guanarito, Sabia**	**Ebola**	**Marburg**	**Hantavirus**	**Crimean–Congo**	**Rift Valley fever**	**Yellow fever**	**Nipah**	**Hendra**
Geography	West Africa	South America, North America	Central and West Africa	East and Central Africa	Worldwide	Europe, Asia, Africa	Africa	South America, Africa	Malaysia, Singapore	Australia
Primary source of infection	Rodent (*Mastomys natalensis*)	Rodent (*Calomys, Zygodontomys* sp.)	Unknown; possibly bats		Rodents; species depends on location	Tick-borne (>27 species)	Mosquito	Mosquito-borne (*Aedes* and *Hemogogus* spp.)	Unknown; possibly bats	
Transmission	Nosocomial Rodent to human/person to person	Rodent to human	Nosocomial; possibly reservoir to human; person to person; also infected great apes and possibly monkeys can transmit to humans		Rodent to human; human to human for Andes virus	Nosocomial tick to human; possibly animal to human; possibly person to person	Mosquito to human; possibly animal to human via blood	Mosquito to human (human reservoir in outbreaks)	Contact with infected pigs, bats, or materials contaminated with secretions from infected bats	Contact with infected horses
Risk factors	Close contact *Mastomys* rural West Africa; close contact infected persons	Contact rodents in circumscribed agricultural areas South America, possibly North America	Close contact with infected blood or secretions from infected persons; environmental risk unknown (possibly caves for Marburg)		Contact with rodent or rodent urine in dust; laboratory	Tick bites; contact with infected livestock; contact with blood or secretions from infected persons	Mosquito bites rural Africa	Mosquito bites rural West Africa and South American rainforests	Close contact infected blood or secretions from infected animals or persons	
Treatment	Responds to ribavirin when treated early	Junin responds to immune plasma given early; all may respond to ribavirin	No currently approved treatment; does not respond to ribavirin; several therapeutics under efficacy testing in animal models		Ribavirin may be beneficial early; trial and current use in China; open-label trial in USA for acute pulmonary disease was inconclusive	Sensitive to ribavirin; several published reports of successful case treatment	Sensitive to ribavarin; limited data in monkeys; no human data	No therapy; does not respond to ribavirin	No currently approved treatment	
Vaccine	DNA and recombinant Vesicular Stomatis Virus (rVSV) vaccines under evaluation in animal models. Development of human vaccine possible	Attenuated vaccine to Junin virus has greatly reduced cases in Argentina; no vaccine available for others	rVSV and adenovirus vaccine platforms have demonstrated efficacy in nonhuman primates	rVSV adenovirus vaccine, VEE replicon and virus-like particle vaccine platforms have demonstrated efficacy in nonhuman primates	M gene DNA vaccine expressing glycoproteins 1 and 2 protects small animals from challenge and elicits neutralizing antibody in Rhesus monkeys; also formalin-inactivated vaccine but no trial data	Killed vaccine available in Eastern Europe and China, but no published trials	Attenuated vaccine for animals, none for humans	Live attenuated vaccine since1940s; one of the most effective vaccines ever	No currenly approved vaccine	

*Arenaviruses have been isolated from wood rats in the south-western USA – their capacity to cause human infection is uncertain.

Hantaviruses are found throughout the world and infection of the natural rodent host results in a chronic carrier state without pronounced pathology or signs of disease. Diseases caused by hantaviruses generally manifest as either hemorrhagic fever with renal syndrome (HFRS) or hantavirus pulmonary syndrome (HPS). Although reported worldwide, HFRS is widely distributed in eastern Asia, with particularly high prevalences observed in China, Russia and Korea. HPS has been reported in the USA as well as areas of South and Central America. Rift Valley fever (RVF) virus is mosquito borne and causes an acute illness in livestock and wild animals. RVF is described as being both endemic and epidemic in areas of Africa and the Middle East.

The filoviruses (Ebola and Marburg viruses) are responsible for some of the most lethal VHFs.[8] The Ebola viruses are comprised of four distinct species with mortality rates up to 90% while Marburg virus is a single species with different isolates having varying mortality rates (20–90%). Natural outbreaks of filoviruses in humans have been reported in the Democratic Republic of the Congo, Republic of the Congo, Sudan, Uganda, Angola and Gabon.[9] Filoviruses have been implicated in large scale die-offs of great ape populations. There is growing evidence that bats are the likely reservoir and infection of nonhuman primates represents inadvertent transmission to these animals.

The Flaviviridae which cause HF include yellow fever virus (see Chapter 164) and dengue virus (see Chapter 127), both of which are spread by mosquitoes.[10] Yellow fever is endemic in sub-Saharan Africa and tropical regions of South America with fatality rates of 5–7%. Dengue fever (DF) and dengue hemorrhagic fever (DHF) are caused by one of four closely related, but antigenically distinct, virus serotypes. Infection with one of these serotypes provides immunity to only that serotype for life, so individuals living in a dengue-endemic area can have more than one dengue infection during their lifetime. The average case fatality rate associated with DHF is 1%. Other flaviviruses can cause hemorrhagic fever, including Kyasanur Forest disease virus, Omsk hemorrhagic fever virus and Alkhumra virus, but these are confined to very local areas and are not discussed in detail here.

In the Paramyxoviridae, the genus Henipavirus composed of Nipah virus and the closely related Hendra virus (formerly called equine morbillivirus) have recently been identified as producing VHF in humans.[11] Nipah virus was first identified in an outbreak of respiratory disease and encephalitis in pigs and humans in Malaysia and Singapore. Hendra virus was isolated during an outbreak of respiratory illness and neurologic disease among horses and a small number of people in Australia. Transmission to humans is typically due to exposure to bodily fluids and excretions from infected horses for Hendra virus and to close contact with infected pigs, bats or materials contaminated with saliva or waste from infected bats for Nipah virus. Bat species, particularly flying foxes, have been implicated as potential reservoirs in the transmission of Nipah virus. Current distribution of Nipah virus includes India, Bangladesh and Singapore.

DIAGNOSIS AND CLINICAL FEATURES

The most critical element for the clinical diagnosis in nonendemic areas is a thorough case history spanning the incubation period (3 days to 4 weeks before the onset of fever). The case history must include:

- an extensive review of recent travel, particularly to endemic areas;
- contact with severely ill febrile individuals;
- contact with potential vectors (e.g. ticks or mosquitoes);
- contact with animals or animal products (e.g. animal blood or carcasses, rodent droppings, etc.);
- any entry into environments where a reservoir may be or may have been present (e.g. caves housing large bat populations or houses with large rodent infestations); and
- participation in activities that may have placed the patient in close contact with infected or diseased individuals such as traditional funeral practices or preparations.

A medical care provider or other worker who might have had contact with blood from a primary case should also alert the physician to possible VHF (see also PP60).

The essential clinical features (Fig. 126.2) are highlighted in Table 126.2. Often clinical presentations are nonspecific and include a history of sudden or rapid onset of fever, severe body pains and headache that is often described as prominent or excruciating. Other features may include confusion, severe pharyngitis, nausea and vomiting, petechiae, oozing from the gums and bradycardia (Figs 126.3, 126.4). Proteinuria is common. Peripheral white blood cell counts are often low very early in disease, but may dramatically increase later, and therefore the presence of neutrophilia may falsely indicate a bacterial disease and be misleading. Thrombocytopenia is common and platelet function is often impaired, even in the presence of low-normal platelet counts. Partial thromboplastin times may be prolonged, but prothrombin times may be relatively unaffected depending on the agent, the severity or stage of the disease (early or late).

As the disease progresses, hypovolemic shock, pulmonary edema and frank bleeding may ensue. Aspartate aminotransferase (AST) is usually raised and virtually all VHFs distinguish themselves from viral hepatitis by the disproportionately high AST levels compared with alanine aminotransferase (ALT). Ratios of AST to ALT may be as high as 11:1; the level of AST also typically reflects prognosis. Patients are rarely jaundiced (except in yellow fever) and the bilirubin is usually normal.

The viruses are pantropic. These agents typically have a period of uncontrolled viral replication with high titers of infectious virus in the blood and tissues (Fig. 126.5). Encephalopathy and neurologic sequelae such as ataxia and deafness can occur, particularly in the early convalescent phase. This phenomenon is most prominent in patients who



Fig. 126.2 Clinical manifestations of viral hemorrhagic fever. (a) Soft and hard palate erythema in Lassa fever. (b) Maculopapular skin rash in Lassa fever. (c) Mild oral and nasal mucosal bleeding in Lassa fever. From Schlossberg D, ed. Clinical infectious disease. New York: Cambridge University Press; 2008:1319–32.

Table 126.2 Key clinical features of viral hemorrhagic fevers

	ARENAVIRUS		FILOVIRUS		BUNYAVIRUS			FLAVIVIRUS		HENIPAVIRUSES	
	Lassa virus	South American	Ebola*	Marburg	Old World hantavirus	New World hantavirus	Crimean–Congo	Rift Valley fever	Yellow fever	Nipah virus	Hendra virus
Untreated case fatality (%)	2–20	16	25–90	20–90	1–15	45–50	10–50	1–2	20–50	40%	66%†
Cardiovascular system											
Thrombocytopenia	+	+++	+++	+++	++	+	+++	++	++		
Oozing	++	++	+++	+++	++	–	+++	++	+++		
Petechiae	–	+++	+	+	+++		+				
Ecchymoses			++	++		–	++				
Circulatory shock	+++	+++	+++	+++	+++	+++	+++	+++	+++		
Tissue edema	++		+	+	+	+					
Major hemorrhage	+	+	++	++	+	–	++	+	++		
Central nervous system											
Encephalopathy	+++	+	+	+	–	–	+			+++	+++
Ataxia	+	++			+						
Deafness	++										
Blindness			+								
Mood alteration	+	+	+	+			++			+++	+++
Intracranial bleeding					+						
Other major systems											
Renal	–	–	+/–	+/–	+++	+			+		
Pulmonary (ARDS)	+++	+		+	+	+++			+		
Hepatic	+	+	+	+	+	+	+	+	+++		

ARDS, adult respiratory distress syndrome.
+ Denotes present to differing degrees indicated by number of marks; – denotes absence; no mark indicates no reliable data.
*Reston is not a human pathogen.
†There have only been three reported cases of Hendra virus.



Fig. 126.3 Petechial rash in a patient with Crimean–Congo hemorrhagic fever. From Peters W, Pasvol G. Atlas of tropical medicine and parasitology, 6th ed. Philadelphia: Mosby; 2006. Copyright Elsevier 2006.



Fig. 126.4 Ecchymoses in a patient with Crimean–Congo hemorrhagic fever. From Peters W, Pasvol G. Atlas of tropical medicine and parasitology, 6th ed. Philadelphia: Mosby; 2006. Copyright Elsevier 2006.

recover from Lassa fever infections. The etiology of the central nervous system sequelae remains unclear and will likely have to be determined on an agent-by-agent basis.

Care must be taken in collecting, handling and transporting specimens, and consultation with the laboratory is essential. At a minimum, standard precautions should be implemented at all times and all potentially infectious specimens must be clearly labeled as hazardous. Blood samples should be drawn into a vacuum tube system. Specimens for transport should be transferred to a leakproof plastic container and double wrapped in leakproof containers in which they can be transported to a suitable reference laboratory (see Chapter 164).

Laboratory diagnosis utilizes several methods, including:

- presence of virus-specific IgM or IgG in the serum;
- presence of viral RNA, usually in serum or white cells, demonstrated by a reverse transcriptase polymerase chain reaction (RT-PCR) or genomic chip-based method;
- presence of viral antigen through enzyme-linked immunosorbent assay (ELISA) for specific viral antigens in serum or blood;
- isolating the virus from blood, serum, oral swab or other clinical sample;

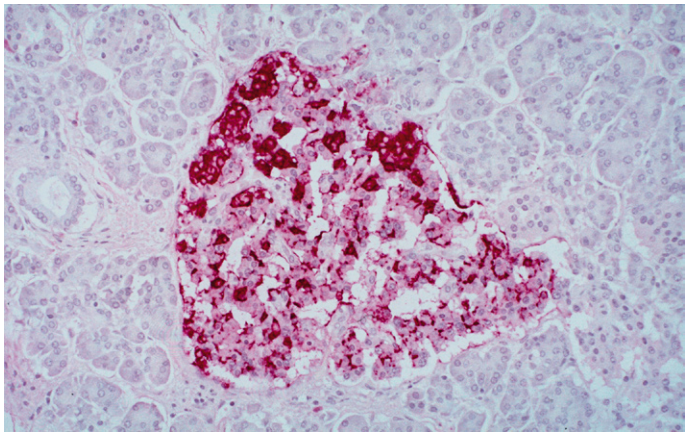


Fig. 126.5 Immunohistochemical staining for hemorrhagic fever viral antigens. Pancreas from a fatal human case of Marburg hemorrhagic fever (Ravn strain). Note that Marburg virus-positive staining (red) is limited to the pancreatic islet with multifocal distribution within the islet (streptavidin-alkaline phosphatase method, section counterstained with hematoxylin). From Jahrling PB, Marty AM, Geisbert TW. Viral hemorrhagic fevers. In: Dembek ZF, ed. Medical aspects of biological warfare. In: Lenhart MK, Lounsbury DE, Martin JW, eds. Textbooks of military medicine. Washington, DC: Department of the Army, Office of The Surgeon General, Borden Institute; 2007:Ch. 13.

- visualization using electron microscopy; and
- demonstrating a fourfold rise in antibody titer.

Sera may be inactivated for use in serologic assays by gamma irradiation; if this is unavailable, heating at 140°F (60°C) for 30 minutes is usually sufficient. Immunofluorescence assays and ELISAs may detect both virus antibody and antigen. More recently, molecular techniques such as PCR or direct sequencing performed on serum or tissues to detect viral RNA have been found to be rapid, reliable and safe. However, while these techniques or these assays are widely available in the developed world, they are not always available in the VHF endemic areas, and a combination of both traditional antigen/antibody detection methods as well as newer molecular techniques can be used. Lastly, available facilities or infrastructure may also dictate which detection methods are utilized. Recent advances in the development of field portable equipment will likely continue to increase capabilities for rapid detection and diagnosis even in remote areas.[12]

MANAGEMENT

For many of the VHFs it is likely that aggressive and appropriate clinical management can reduce reported mortality and morbidity rates. Patient travel and additional trauma should be avoided. Any surgery should be carried out using the most stringent of precautions to protect medical staff and the risk of uncontrolled bleeding in severe cases must be considered. Gentle sedation and pain relief should be implemented. Standard precautions for management of patients with bleeding diatheses should be used. Management of dehydration and bleeding such as the use of intravenous fluids, blood replacement, platelet transfusions, factor replacements or the use of anticoagulants should be evaluated on a disease-by-disease basis and use of these interventions should be closely monitored as fluid balance will likely prove to be the main challenge of acute disease management. Although patients often present with a high hematocrit due to dehydration, pulmonary edema is a real risk for many VHFs and patients should be infused with caution. Full intensive care support, where available, may include mechanical ventilation, extracorporeal membrane oxygenation, monitoring of central venous pressure and dialysis as well as equipment and medications for management of seizures

and arrhythmias. Use of vasopressors as well as low-dose steroids have been suggested but to date there are no clinical data to evaluate their efficacy in these diseases.

The development of disseminated intravascular coagulation (DIC) remains a controversial observation for many of the VHFs and it may be that DIC is observed for only certain VHFs or in the most severe presentations. However, when present, effective clinical management may improve survival. For filoviruses the development of a consumptive coagulopathy, with fibrin deposition and significantly elevated levels of D-dimers and the procoagulant protein tissue factor have been documented. Recently, significant improvement in survival was observed in nonhuman primate models of filoviruses using strategies that target the activation of the coagulation pathway.[13,14]

The availability of effective antivirals is limited. Some utility has been reported for the use of ribavirin for the treatment of Lassa fever, Argentine hemorrhagic fever and CCHF (see Table 126.1). Therapy with immune plasma has also been advocated for VHFs, but efficacy has not been fully demonstrated. Recent data indicate that postexposure vaccination in filoviruses models could also reduce or prevent morbidity and mortality. Regardless of the therapeutic intervention, early implementation will likely increase efficacy. Appropriate treatment should be started as soon as possible when cases or potential cases are identified.

Pregnant patients often present with absent fetal movements and abortion is a common complication. For Lassa fever aggressive obstetric intervention to remove the deceased fetus has been shown to increase the survival of the mother. Transportation of VHF patients in rural or remote settings is contraindicated. In severe cases the cardiovascular system is often unstable and any trauma is likely to induce bleeding. Furthermore, transportation without adequate precautions has the potential to expand the epidemic area and expose a greater number of people to secondary infection. Patients may be managed quite successfully in standard hospital isolation rooms with rigorous barrier nursing techniques.

PREVENTION

Education, surveillance and early identification and management of infected patients is critical to disease control and prevention of spread. Community outreach and education should include not only the medical staff for the proper handling of patients and materials from infected patients but also the community as a whole on how these viruses are transmitted and what steps may be take to prevent infection and spread. The key to the prevention of nosocomial transmission in both endemic and epidemic areas has consistently been good hospital and laboratory practice, with strict isolation of febrile patients and rigorous use of gloves and disinfection.[15] Disinfection can be accomplished by washing/soaking with fresh solutions of 0.5% phenol in detergent, 0.5% hypochlorite solution, formaldehyde, glutaraldehyde or paracetic acid.

The 1995 interim update on the 1988 US Centers for Disease Control and Prevention guidelines for the management of patients with VHF recommend patient isolation in a single room, preferably but not necessarily with a negative air pressure gradient from the hallway through an anteroom to the patient room.[16] Past recommendations for strict isolation of patients in a plastic isolator have been abandoned in favor of straightforward strict barrier nursing. This practice presents no excess risk to hospital personnel and allows substantially better care of the patient.

REFERENCES

References for this chapter can be found online at http://www.expertconsult.com

Chapter 127

Cameron P Simmons

Dengue

INTRODUCTION

Dengue is a vector-borne viral infection and a globally important public health problem.[1,2] The dengue viruses (serotypes 1, 2, 3 and 4) are enveloped, single-stranded RNA viruses of the Flaviviridae family. Transmission from human to human is predominantly by the mosquito *Aedes aegypti*, which bites in the daytime, is adapted to human habitats and has a preference for human blood meals. More than 1 billion people are at risk of dengue infection in over 100 countries (Fig. 127.1).

Any of the four serotypes of dengue virus can result in dengue, which is a systemic febrile illness lasting 3–7 days and characterized by viremia, fever, rash, headache, muscle and joint ache. Occasionally, dengue manifests as dengue hemorrhagic fever (DHF), a potentially life-threatening illness associated with capillary leakage, hemorrhagic manifestations and, in severe cases, hypovolemic shock.

TRANSMISSION

Human-to-human transmission of dengue viruses occurs through the bites of infected female *Aedes aegypti* mosquitoes and less commonly *Aedes albopictus*. The virus circulates in the blood of infected humans for 2–7 days. Mosquitoes become infected after feeding on the blood of a viremic individual. Once infected, a mosquito is capable of transmitting the virus to susceptible individuals for the rest of its life during probing and blood feeding. Transovarial transmission of dengue virus to the next mosquito generation has been observed under laboratory conditions, but the relevance of this to disease epidemiology is yet to be determined. A sylvatic (jungle) cycle of transmission occurs amongst primates in some parts of the world, but in the vast majority of endemic regions transmission is from human-to-human via the mosquito vector.

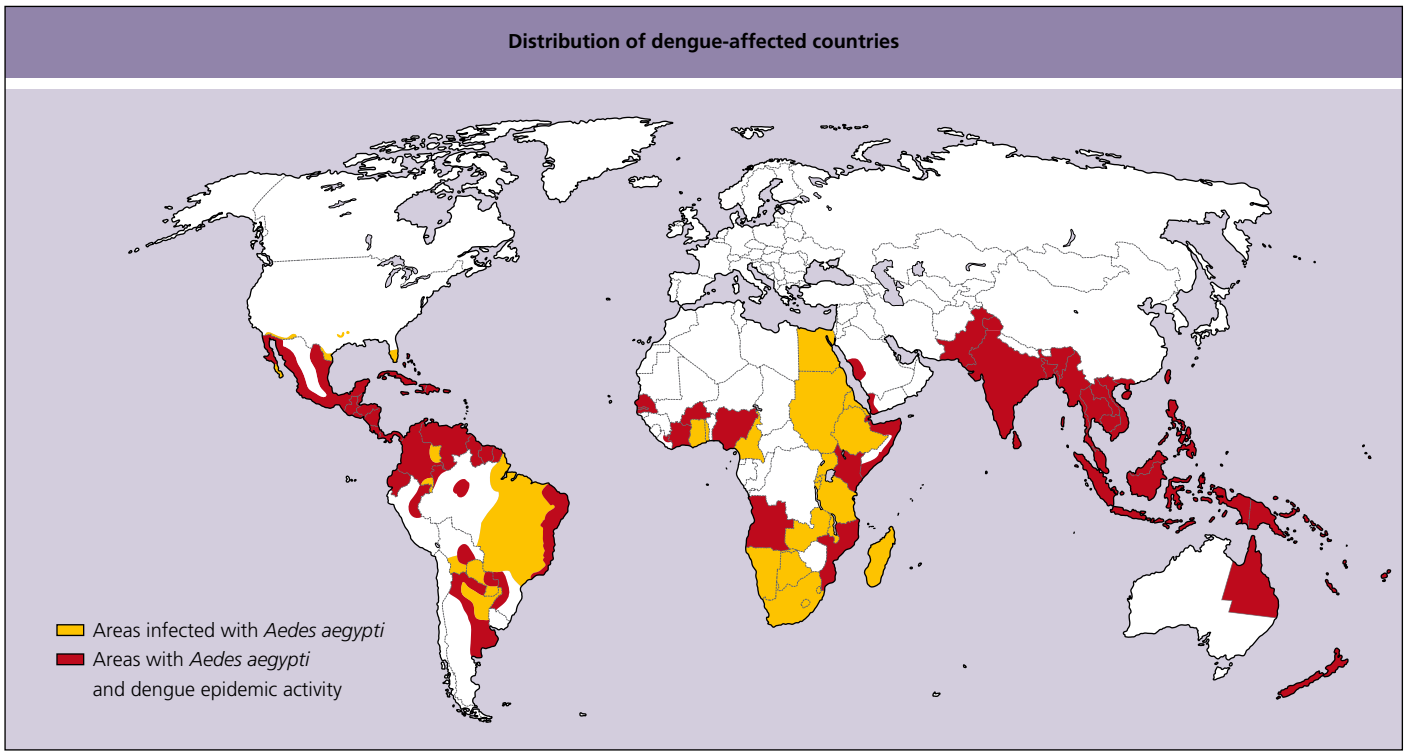


Fig. 127.1 Distribution of dengue-affected countries.

PATHOGENESIS

Dengue hemorrhagic fever is strongly associated with secondary, so-called heterotypic infections (two sequential infections caused by different serotypes). DHF is rare given the total number of secondary infections.[3–6] In secondary infections, disease severity is linked to high initial viral loads[7,8] and robust humoral and cellular anamnestic immune responses driven by antigenic cross-reactivity between dengue viruses (DENVs).[9–13] DHF also occurs during primary DENV infection of infants born to DENV-immune mothers.[14–17] Halstead has proposed that infants born to DENV-immune mothers and previously infected children or adults have in common a single immune risk factor – DENV-reactive IgG antibodies.[18] The capacity of subneutralizing concentrations of DENV-reactive IgG antibodies to enhance DENV infections in Fc receptor-bearing cells,[18,19] a phenomenon called antibody-dependent enhancement (ADE), provides a unifying basis to explain severe dengue in secondary infections and in infants with primary infections. ADE could result in higher viral burdens *in vivo* and a host inflammatory response that might account for the capillary leakage syndrome.

Pathogenesis has also been linked to the antigenic characteristics or virulence of certain viral lineages. For example, dengue serotype 2 viruses from South East Asia may be more likely to elicit DHF. Host genetic factors may also be relevant and predispose some individuals to DHF, although definitive data in this area remain scant. There are animal models of dengue virus infection, but no model reliably replicates the clinical syndrome of DHF in such a way that it could serve to fully define its pathogenesis. The current understanding of DHF pathogenesis is summarized in Figure 127.2.

PREVENTION

Several tetravalent live-attenuated dengue vaccines are in clinical development, though none is yet close to licensure.[20] Specific antiviral therapies are also in development and these could conceivably be used for prophylaxis in the future. For travelers to tropical countries, the only practical method of prevention is to avoid mosquito bites by wearing protective clothing and using appropriate repellents.

Reduction of mosquito populations currently remains the best method of preventing dengue transmission. Vector control consists of scrupulous destruction and control of breeding sites. All unwanted containers should be discarded, buried or filled with sand. Larvivorous small fish or a crustacean called Mesocyclops can provide control of mosquito larvae. Adult mosquitoes can be destroyed by pyrethrin knock-down sprays or organophosphate sprays delivered in microdroplets. Nevertheless, the relentless increase in the geographic footprint of dengue, and the total number of affected people, indicates most vector control strategies in endemic areas are inadequate, not sustainable and/or poorly targeted.

CLINICAL FEATURES

Dengue fever

The differential diagnosis of classic dengue fever includes other arboviral infections as well as measles, rubella, enterovirus infections, adenovirus infections and influenza. Other differential diagnoses that should be considered depending on local disease prevalence include typhoid, malaria, leptospirosis, hepatitis A, rickettsial diseases and bacterial sepsis.

Most dengue infections are clinically silent or result in only mild, undifferentiated febrile illness, particularly in children. Classic dengue fever is typically seen in adolescents and adults and characterized by a sudden onset of fever with severe headache, retro-orbital pain, gastrointestinal disturbances and generalized pains in the muscles and bones ('breakbone fever'). A maculopapular rash may appear around the time of defervescence, usually 3–7 days after fever onset (Fig. 127.3). Fever is accompanied by leukopenia and moderate thrombocytopenia. Manifestations of vascular permeability are usually not present.

Dengue hemorrhagic fever/dengue shock syndrome

Dengue hemorrhagic fever is an acute vascular permeability syndrome accompanied by abnormal hemostasis. The early clinical presentation is similar to dengue fever, and includes abrupt onset of fever, malaise, vomiting, headache and anorexia. Capillary leakage begins during the febrile phase but typically manifests around

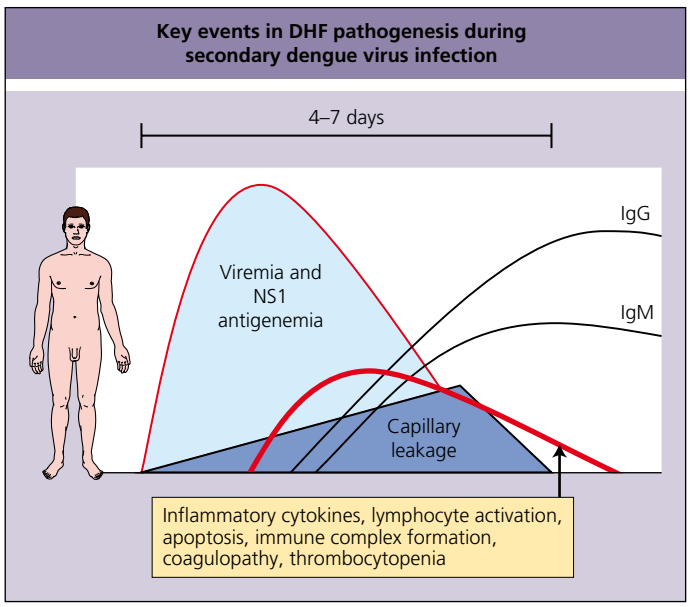


Fig. 127.2 Key events in dengue hemorrhagic fever pathogenesis during secondary dengue virus infection.



Fig. 127.3 Diffuse macular recovery rash in an adult patient with dengue. The rash may appear between 3 and 6 days after fever onset. Note the 'islands' of normal skin surrounded by erythematous skin.

the time of defervescence, the 'critical period'. Capillary leakage can be indicated by hypoproteinemia, elevated hematocrit, pleural effusions, ascites, gallbladder wall thickening and perivesicular edema. Scattered spontaneous petechiae and easy bruising at venepuncture sites are relatively common findings. Frank bleeding from mucosal sites (e.g. gastrointestinal, nose, gums, vagina) can occur in severe cases. Modest hepatic involvement is typical and the liver can be palpable and tender and accompanied by abnormal liver transaminase levels. Common laboratory abnormalities include thrombocytopenia and leukopenia with a relative lymphocytosis. Convalescence is fairly rapid and uneventful after the 1- to 2-day critical period around the time of defervescence.

Dengue shock syndrome (DSS), the most common life-threatening complication of acute dengue, occurs when excessive capillary leakage precipitates circulatory dysfunction or collapse. Circulatory dysfunction is seen as a narrowing of pulse pressure (≤20 mmHg) or hypotension for age accompanied by one or more of the following: cold clammy extremities, delayed capillary refill time, flushed face, circumoral cyanosis, diaphoresis, restlessness, irritability and midepigastric pain. Respirations and pulse are rapid. The pathophysiologic presentation of DSS (thrombocytopenia and relatively slow development of hypovolemia) is distinct from other infectious causes of cardiovascular shock.

MANAGEMENT

World Health Organization guidelines for dengue management have been published[21] and are currently being revised. There are no specific treatments for dengue. The mainstay of treatment remains good supportive care, with a particular focus on careful fluid management. Oral rehydration is usually sufficient for patients with little or no evidence of capillary permeability. Acetaminophen (paracetamol) can be used to reduce fever; however, aspirin and nonsteroidal anti-inflammatory drugs are contraindicated.

Patients with a rapidly rising hematocrit, profound thrombocytopenia, persistent vomiting, severe abdominal pain, ecchymoses or mucosal bleeding require very careful and frequent monitoring of vital signs and hematocrit (Fig. 127.4). A rapidly rising hematocrit should prompt conservative parenteral fluid therapy. Rapid but careful restoration of circulating plasma volume is the mainstay of therapy for patients with established DSS. Fluid volumes for resuscitation should be kept to a minimum but sufficient to maintain cardiovascular stability and adequate urine output during the phase of active leakage. As soon as re-absorption begins, intravenous fluids should be stopped. Fluid overload with respiratory compromise is a recognized complication and a contributor to mortality (Fig. 127.5). Isotonic crystalloid solutions should be the first choice for resuscitation, with colloid solutions reserved for patients presenting with severe DSS and those with repeated or prolonged cardiovascular compromise (Fig. 127.6).

In the event of significant bleeding, transfusion is indicated but with due care to the risks of fluid overload. There is no evidence from randomized controlled trials to support the use of platelet concentrates, even for profound thrombocytopenia. Similarly, there is no evidence that corticosteroids improve clinical outcome in children with established shock.

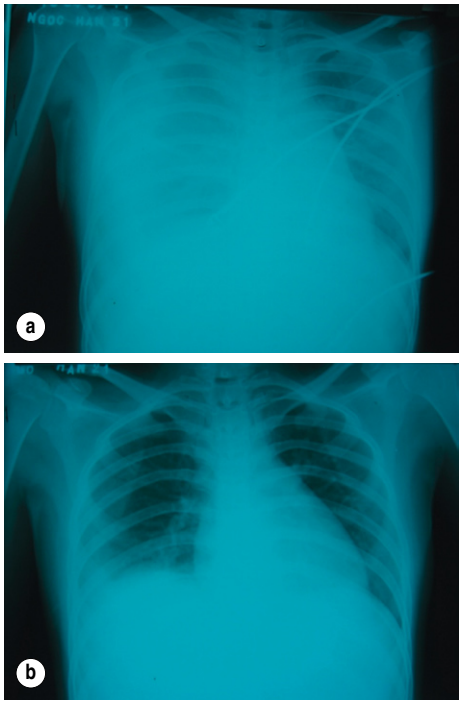


Fig. 127.5 (a) Antero-posterior chest X-ray showing a large pleural effusion on the right side in an adult patient with dengue shock syndrome. (b) The same patient at hospital discharge. Such severe radiologic findings occur in dengue patients with capillary leakage and who have received an excessive volume of intravenous fluid.



Fig. 127.4 Hematoma in a patient with severe dengue. The combination of increased vascular fragility, platelet dysfunction/thrombocytopenia and coagulation disorders is believed to explain hemorrhagic manifestations in dengue. Frank hemorrhage, as pictured here, is relatively uncommon given the overall disease burden.



Fig. 127.6 A patient with dengue shock syndrome. As shown in this image, patients with severe dengue require fluids (or blood products in cases with severe bleeding) to maintain hemodynamic stability. Respiratory support can be required in cases with severe pleural effusion and respiratory compromise.

LABORATORY DIAGNOSIS

Laboratory confirmation of dengue is helpful to clinical management and for epidemiologic surveillance. In many settings, a presumptive diagnosis of dengue is made by detection of high levels of DENV-reactive IgM in a single acute plasma/serum sample. The interpretation of dengue serology requires an understanding that antibody responses elicited by flaviviruses can be cross-reactive. Therefore adequate controls should be included in serologic assays in settings where other flaviviruses co-circulate with dengue viruses (e.g. Japanese encephalitis virus). A definitive diagnosis of dengue can be made by reverse transcriptase polymerase chain reaction (RT-PCR), virus isolation or detection of the dengue viral antigen NS1 in plasma.

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References for this chapter can be found online at http://www.expertconsult.com

Chapter 128

Mehmet Doganay

Anthrax

INTRODUCTION

Anthrax is primarily a disease of herbivores which can be transmitted to humans. It was one of the first zoonoses described. Currently, anthrax has assumed greater importance as a result of the potential use of *Bacillus anthracis* spores as an agent of bioterrorism and biological warfare.

EPIDEMIOLOGY

Anthrax occurs worldwide. This infection is still endemic or hyperendemic in both animals and humans in some parts of the Middle East, in West Africa, Central Asia, South America and Haiti. Soil is the reservoir for the infectious agent. Figure 128.1 shows the cycle of infection in nature. Infection in humans is correlated with the incidence of disease in domestic animals. In economically advanced countries, where animal anthrax has been controlled, it occurs only occasionally among humans. Human anthrax is most common in enzootic areas in developing countries, among people who work with livestock, eat undercooked meat from infected animals, or work in establishments where wool, goatskins and pelts are stored or processed.[1,2]

The main route of transmission is contact with or inhalation of *B. anthracis* spores. Human cases may occur in an agricultural or an industrial environment. Agricultural cases have occurred in individuals who came into contact with sick or dead animals in rural areas. In certain impoverished communities, livestock owners are forced to slaughter animals at the first sign of infection in order to salvage the meat, hair and hides because of economic problems. Farmers,

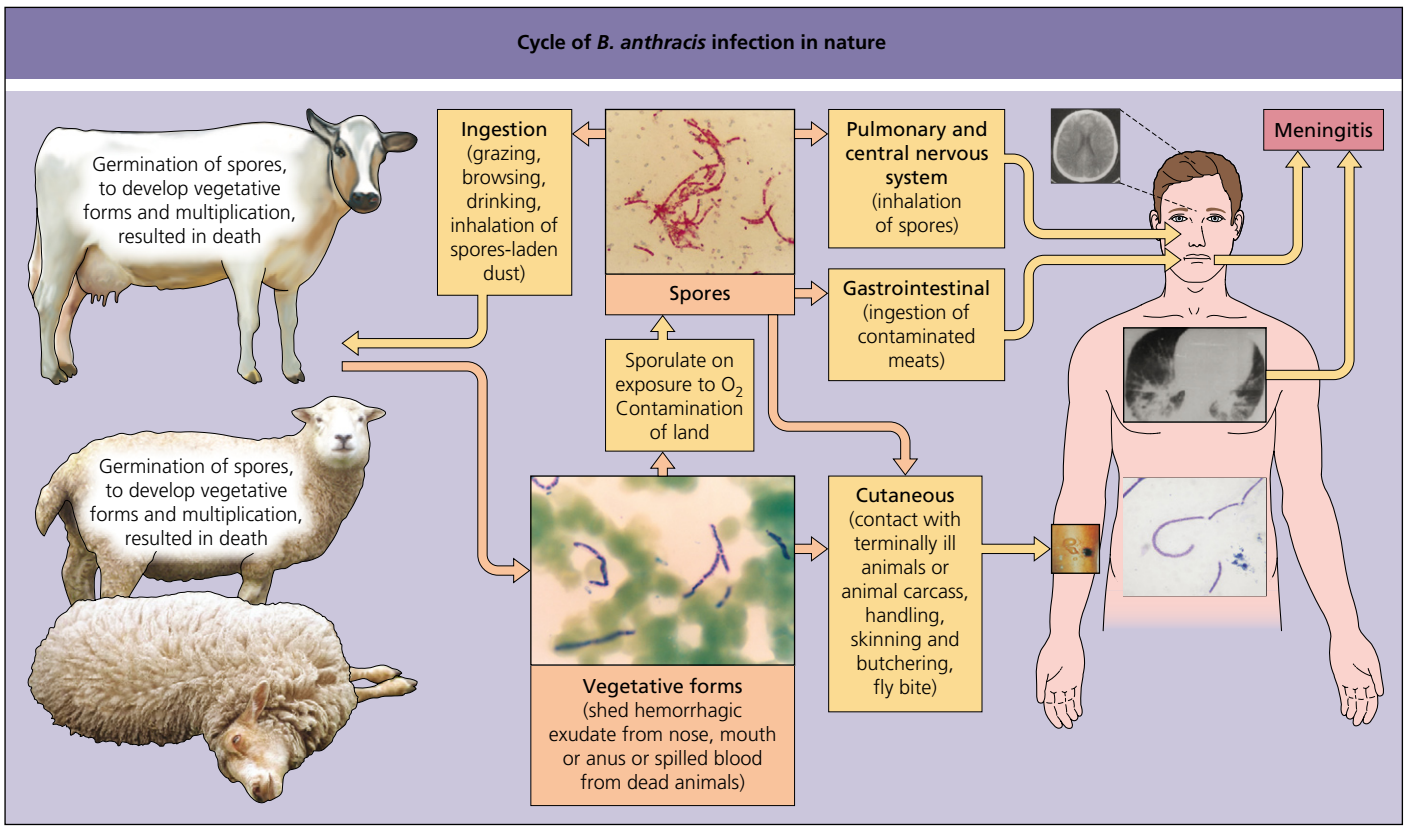


Fig. 128.1 Cycle of *Bacillus anthracis* infection in nature.

butchers, knackers, shepherds and veterinarians are therefore the most frequently infected.[1–4] Anthrax is also reported in women spinning wool with hand spindles and in carpet weavers.[5] Another route of infection is by ingestion of raw or undercooked meat from an infected carcass.[6–8] Travelers should be aware that, in certain societies, some traditional meals are made of raw meat and are consumed without any cooking or preservation methods.

There is also an infection risk after contact with a commercial product prepared from inadequately treated wool or leather. Products made from contaminated hair (e.g. shaving brushes, wool coats), skins (e.g. drums, drumheads made of animal skin) and bone meal (e.g. fertilizer) may continue to be sources of infection for many years.[1,2]

Insect vectors, such as horseflies, have been reported to transmit *B. anthracis* from an infected animal to a second animal. They could also theoretically infect humans by mechanical transfer but this has not been well documented.[1,2]

Industrial anthrax occurs as a result of the inhalation of spore-laden dust or other aerosols, or contact with spores. In the industrial environment, spores in dust clouds created from the handling of dry hides, skins, sheep wool, goat hair, bone meal and the like are inhaled or spread through contact with the skin of workers. Most cases in industrialized countries are associated with exposure to animal products, particularly goat hair imported from countries in which anthrax is endemic.[1,2,9]

The disease is generally accepted as noncontagious; records of person-to-person spread exist but are very rare. All cases were cutaneous anthrax; there are no reports of inhalation or ingestion anthrax. Occasionally, laboratory-acquired infections have occurred.[1,2,10]

In 2001 there was an outbreak of anthrax in the USA that apparently resulted from the deliberate distribution of spores in the postal system, resulting in a number of cases of both cutaneous and inhalational anthrax.[11]

PATHOGENESIS

Bacillus anthracis, the causative agent of anthrax, is a Gram-positive, aerobic or facultatively anaerobic, endospore-forming, rod-shaped bacterium. The spores lodging in a cut or abrasion in the skin germinate there and the emergent vegetative bacilli spread to the regional lymph nodes. Ingestion anthrax results if spores lodge similarly in lesions in the gastrointestinal tract or achieve access to Peyer's patches. Inhaled spores may, on occasion, lead to infection of nasal-associated lymphoid tissues; those reaching the lungs do not germinate in the alveoli but remain dormant until carried by macrophages to the tracheobronchial lymph nodes. The spores germinate inside the macrophages and the emergent vegetative forms are released from the macrophages, multiply in the lymphatic system and enter the bloodstream, causing toxemia and sepsis.[2,12]

Bacillus anthracis has two principal virulence factors: the toxin complex and the polypeptide capsule. Both are plasmid mediated. The genes for the toxin components and virulence gene regulators AtxA and PagR are located on a large (182 kb) plasmid designated pX01 and the genes for capsule synthesis and their regulators AcpA and AcpB are located on a smaller (95 kb) plasmid, pX02. Loss of either plasmid results in considerable reduction in virulence. Possibly also contributing to virulence are other proteins encoded on the chromosome or plasmids, such as cell surface S-layer extractable antigen 1 (EA1) and surface array protein (Sap), anthrolysin O, siderophores and metal cation transport proteins.[2,13]

The toxin complex consists of three synergistically acting proteins: protective antigen (PA, 83 kDa), lethal factor (LF, 90 kDa) and edema factor (EF, 89 kDa). LF in combination with PA (lethal toxin, LeTx) and EF in combination with PA (edema toxin, EdTx) are secreted during multiplication of the vegetative cells and are accepted as responsible for the characteristic signs and symptoms of anthrax. EdTx is a calmodulin-dependent adenylate cyclase that increases intracellular levels of cyclic AMP (cAMP) on entry into most types of cell, leading to impaired maintenance of water homeostasis and characteristic edema. EdTx also inhibits macrophage activity by altering their cytokine production, decreases the circulating lymphocyte population and diminishes neutrophil function. LF is a zinc-dependent metalloprotease that cleaves the amino termini from mitogen-activated protein kinase kinases (MAPKKs), thereby disrupting signaling pathways with range of resulting pathologic effects. LeTx kills or disrupts production and function of macrophages, dendritic cells, neutrophils, and some epithelial and endothelial cells, downregulates cytokine production (preventing induction of chemokines integral to responses to viral or bacterial infection), inhibits B-cell proliferation and reduces B-cell production of immunoglobulin. Inflammatory mediators released in response to LeTX may also contribute to the sudden death characteristic of systemic anthrax and its lethal effect on microvascular endothelial cells is presumed to be responsible for the also characteristic terminal hemorrhages.[2,12–14]

CLINICAL FEATURES

The disease occurs primarily in three forms: cutaneous, gastrointestinal and respiratory. Sepsis and meningitis can rarely develop after the lymphohematogenous spread of *B. anthracis* from a primary lesion (cutaneous, gastrointestinal or pulmonary).

Cutaneous anthrax

Cutaneous anthrax accounts for 95% of human cases. The spore is introduced into the skin via a cut, abrasion or insect bite. The incubation period ranges from 1 to 19 days, usually 2–7 days. The lesion begins as a pruritic papule. The papule enlarges and a ring of vesicles develops around the papule at day 2–4 of the disease. Vesicular fluid may be a hemorrhagic exudate (Fig. 128.2). This area is surrounded by a small ring of erythema and marked edema develops. Unless there is secondary infection, there is no pus and the lesion is not painful, although painful lymphadenitis may occur in the regional lymph nodes. Eventually, the vesicle or vesicular ring ruptures, discharging a clear fluid, and a central depressed black necrotic lesion known as an eschar is formed (Fig. 128.3). Edema extends some distance from the lesion. The eschar begins to resolve about 10 days after the appearance of the initial papule. Resolution is slow (2–6 weeks), regardless of treatment (Fig. 128.4).[2,12]

The lesion is usually 1–3 cm diameter and remains round and regular. Rarely, a lesion may be larger and irregularly shaped. Systemic symptoms, including low-grade fever, malaise and headache, may be present. The cutaneous reaction may be severe in some patients and is characterized by significant local and spreading edema associated with blebs, bullae, induration, chills and fever (see Fig. 128.3b). Clinical symptoms may be more severe if the lesion is located in the face, neck



Fig. 128.2 A cutaneous anthrax lesion with extensive erythema and hemorrhagic bullae on the wrist.



Fig. 128.3 Cutaneous anthrax. (a) A well-developed lesion on the right forearm (third day of disease). (b) The extension of the skin lesion in the same patient on the sixth day. Extensive edema, induration and bullous changes have occurred over the last 3 days despite antibiotic therapy. Antibiotic therapy does not prevent inflammatory reactions.

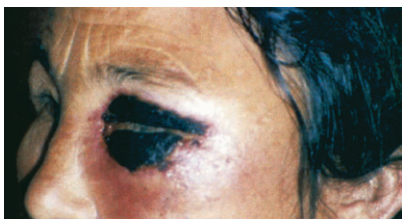


Fig. 128.4 A dried black anthrax eschar on the eyelids on the 15th day of therapy (third week of the disease). The lesion healed leaving a deep scar. Courtesy of Professor O Ural, Konya, Turkey.



Fig. 128.5 An anthrax lesion of the eyelids surrounded by erythema and massive edema extending from the left eye to the right and down to and beyond the neck. Such extensive edema is characteristic of anthrax. This lesion healed with therapy and left a deep scar.

or chest. In these more severe forms, clinical findings are high fever, toxemia, regional painful adenomegaly and extensive edema; shock and death may ensue (Fig. 128.5).[2–4,12,15]

More than 90% of lesions occur in exposed areas such as the face, neck, arms or hand (Table 128.1). The site of infection often reflects the occupation of the patient. Workers who carry hides or carcasses on their shoulders are prone to infection on the back of the neck. Handlers of contaminated animal products tend to be infected on the arms, wrists and hands. The patients generally have a single cutaneous lesion but sometimes they have two or more. Atypical localization can also be seen.[2–4,12,15]

Gastrointestinal anthrax

Ingestion of *B. anthracis* in contaminated food or drink can cause gastrointestinal anthrax. The incubation period is commonly 3–7 days. There are two clinical forms of gastrointestinal anthrax: intestinal and oropharyngeal.[2,6–8,12]

Table 128.1 Distribution of lesions in 107 cases with cutaneous anthrax

Site of lesion	No. of cases	%
Hands and fingers	79	73.8
Wrists and arms	10	9.3
Eyelids and face	11	10.3
Neck	2	1.9
Feet and legs	5	4.7
Total	107	100

Data from Doganay.[15]

The symptoms of intestinal anthrax are initially nonspecific and include nausea, vomiting, anorexia and fever. With progression of the illness, abdominal pain, hematemesis, bloody diarrhea and massive ascites occur, and signs suggestive of acute abdomen appear. Toxemia and shock then develop, followed by death. The lesions occur most commonly on the wall of the terminal ileum or cecum. The stomach, duodenum, upper ileum and large bowel are occasionally affected.[2,7,8,12]

Oropharyngeal anthrax is less common than the gastrointestinal form. The lesion is generally localized in the oral cavity, especially on the buccal mucosa or tongue, or the tonsils, and the posterior wall of the pharynx. In some cases, the lesion may be present in two or more places in the gastrointestinal system, oropharynx and intestine. The oral lesion is generally 2–3 cm in diameter and covered with a gray pseudomembrane surrounded by extensive edema. When infection is localized on the tonsils, the affected tonsil is also intensely edematous and covered with pseudomembrane. The main clinical features are sore throat, dysphagia, fever and painful regional lymphadenopathy in the neck. The illness progresses rapidly and edema develops around the lymph node and may extend to the upper anterior chest wall. Bacteremia may develop. The infection leads to toxemia and acute respiratory distress syndrome. Shock and coma ensue. In some cases, toxemia leads to sudden death. Despite intensive medical therapy, the mortality is about 50%.[2,6,7,12]

Inhalation anthrax

This natural form of anthrax was previously almost always caused by industrial exposure to spores; however, the most serious outbreaks of anthrax in 1979 in Sverdlovsk and in October 2001 in America are new and notable exceptions.[2,11,16]

Inhalation anthrax shows a biphasic clinic pattern with a mild initial phase followed by an acute and severe second phase. After an incubation period of 1–6 days (up to 43 days in the event at Sverdlovsk), the illness begins with mild fever, fatigue, malaise, myalgia, nonproductive cough and some chest or abdominal pain. The disease progresses to the second phase within 2–3 days. The second phase is characterized by high fever, toxemia, dyspnea and cyanosis. Hypothermia and shock develop, resulting in death. In up to half of patients, meningitis develops as a complication.[2,9,12]

Anthrax meningitis

The meningeal form of anthrax is very rare. The world's literature contains approximately 100 cases of anthrax meningitis, with a mortality rate of over 90%.[2,14] Meningitis may be a complication of the three forms of primary anthrax. The most common portal of entry is skin (52%) and then the lungs (22.9%). Anthrax meningitis also occurs in cases of gastrointestinal anthrax. The organisms can spread to the central nervous system by hematogenous or lymphatic routes. The primary focus of infection cannot be determined in about

10% of cases and it is called primary anthrax meningitis. Blood cultures are positive for *B. anthracis* in 70% of patients who have meningitis.[2,12,17,18]

The clinical presentation includes sudden onset of fever, fatigue, myalgia, headache, nausea, vomiting, agitation, seizures, delirium and meningeal symptoms. The initial signs are followed by rapid neurologic deterioration and death. The cerebrospinal fluid is often bloody and contains many Gram-positive bacilli.[2,12,17,18]

Anthrax sepsis

Sepsis may occur by spreading of *B. anthracis* via the lymphohematogenous route from a primary lesion. Sepsis is rarely seen in patients who have cutaneous anthrax; it is more commonly seen in patients who have inhalation and gastrointestinal anthrax. Clinical features include fever, respiratory distress and changing mental status. Severe toxemia and shock may lead to death in a short time.[2]

COMPLICATIONS

Some 10–20% of untreated cases of cutaneous anthrax might be expected to result in death. With treatment, the mortality rate is less than 1%. Toxemic shock due to massive edema, airway obstruction by compression on the trachea from edematous swelling around the neck, temporal artery inflammation, deep tissue necrosis and secondary infection, deep scar tissue and eyelid deformity are all recorded as complications in cases of cutaneous anthrax.[2–4,15]

Serious complications such as sepsis and meningitis can be seen in inhalation anthrax and gastrointestinal anthrax. These complications are less frequently seen in cutaneous anthrax. The mortality rate in industrial-related inhalation anthrax is over 80%, despite treatment. Gastrointestinal anthrax is also a potentially fatal disease. Mortality is greater than 50%. If an early diagnosis is made and appropriate treatment given, the disease can be cured.[2,6–8,17]

DIFFERENTIAL DIAGNOSIS

A history of exposure to contaminated animal materials, occupational exposure and living in an endemic area are all important clues for the suspicion of anthrax. The ulcerative eschar of cutaneous anthrax must be differentiated from other papular and ulcerative lesions that present with regional lymphadenopathy. If regional lymphadenopathy together with a purulent lesion is present, a cutaneous anthrax lesion may be superinfected with pyogenic bacteria such as staphylococci. The differential diagnosis should include ecthyma gangrenosum, rat-bite fever, ulceroglandular tularemia, plague, glanders, orf, rickettsialpox, erysipelas, staphylococcal skin and lymph node infection, syphilitic chancre and cutaneous tuberculosis. A severe cutaneous anthrax lesion involving the face, neck and anterior chest wall must be differentiated from orbital cellulitis, dacryocystitis and deep tissue infection of the neck. Necrotizing soft tissue infections – particularly group A streptococcal infections and gas gangrene – and severe cellulitis due to staphylococci should also be considered in the differential diagnosis of severe forms of cutaneous anthrax. Gas and abscess formation are not observed in patients who have cutaneous anthrax.[2–4,12]

Intestinal anthrax mimics food poisoning (in the early stages), acute abdomen of other causes and hemorrhagic gastroenteritis, particularly necrotizing enteritis due to *Clostridium perfringens*. In the differential diagnosis of oropharyngeal anthrax, streptococcal pharyngitis, Vincent's angina, Ludwig's angina, parapharyngeal abscess and deep tissue infection of the neck should be considered.[2,6,7]

The differential diagnosis of anthrax meningitis should include acute meningitis of other bacterial etiology and subarachnoid hemorrhage.[2,12,18] Sepsis due to other bacteria should be considered in the differential diagnosis of anthrax sepsis.[2,12]

The initial symptoms are nonspecific and clinical presentation of inhalation anthrax is similar to those of atypical pneumonia from other causes and cardiovascular collapse with noninfectious causes.[2,12]

INVESTIGATIONS

The investigation of a potential exposure to the infectious agent is very important for suspicion of anthrax. However, the source of infection cannot be determined in some cases. In cutaneous lesions, swabs are appropriate for collecting vesicular exudates for microscopy and bacterial culture. In a well-formed eschar, in which vesicular exudate is absent, the edge of the eschar can be lifted up with forceps and fluid obtained by a capillary tube. A smear is made from the material and is stained with polychrome methylene blue and examined microscopically for the presence of the pink-staining encapsulated bacilli (McFadyean reaction). The samples are also inoculated on blood agar.[2]

For the isolation of *B. anthracis* in patients who have suspected gastrointestinal anthrax, swabs from oropharyngeal lesions and samples of vomit, feces, blood and ascites should be obtained. Specimens likely to be contaminated with commensal flora should be cultured on polymyxin-lysozyme-EDTA-thallous acetate (PLET) agar as a selective medium for *B. anthracis*.[2]

Radiographic examination of the chest usually reveals widening of the mediastinum in inhalation anthrax. Parenchymal infiltration and pleural effusion can also be seen. Direct examination of a smear of pleural fluid or blood, stained with polychrome methylene blue or Gram stain, may show encapsulated bacilli. *Bacillus anthracis* can be isolated from the cultures of these specimens.[2,9,12]

Blood or cerebrospinal fluid smear stained with polychrome methylene blue should be examined for the encapsulated bacilli, and blood and/or cerebrospinal fluid cultures should be taken for isolation of *B. anthracis* in cases of sepsis or meningitis. Blood culture is also positive in many cases with systemic anthrax.[2,12,17]

Serologic tests are also useful for diagnosis of anthrax. New diagnostic techniques include immunohistochemical testing of clinical specimens by using *B. anthracis* capsule and cell wall antibody and, most recently, by *B. anthracis*-specific polymerase chain reaction. These new rapid methods may become useful in early diagnosis and in culture-negative patients.[2,12]

MANAGEMENT

In-vitro human clinical isolates of *B. anthracis* are sensitive to many antibiotics: penicillins, aminoglycosides, macrolides, quinolones, carbapenems, tetracyclines, vancomycin, clindamycin, rifampin (rifampicin), cefazolin and linezolid.[2,19] Penicillin G is still the drug of choice in the therapy of naturally occurring anthrax. In mild uncomplicated cutaneous anthrax, intramuscular procaine penicillin (800 000 to 1 million units q12–24h) or amoxicillin (500 mg orally q6–8h) is suggested for 3–5 days. *B. anthracis* cannot be isolated from cutaneous lesions 24–48 hours after antibiotic administration. Early treatment will limit the size of the lesion but will not alter the evolutionary stages (Table 128.2).[2,12]

In patients showing signs of systemic involvement, such as inhalation anthrax or gastrointestinal anthrax, meningoencephalitis, sepsis and cutaneous anthrax with extensive edema, antibiotics should be given intravenously. Penicillin G is the first choice, given at a dose of 4 million units q4–6h (total daily dose should be 20–24 million units) until the patient's symptoms resolve and fever returns to normal. At this point, the intravenous antibiotic can be switched to

Table 128.2 Suggested antibiotic therapy for anthrax in adults

Category	Antibiotic	Duration of therapy
Naturally occurring anthrax	First choice* • Penicillin G procain 0.6–1.2 mU im q12–24h • Penicillin G, sodium or potassium 4 mU iv q4–6h • Amoxicillin 500 mg po q6–8h Alternative* • Doxycycline 100 mg po q12h • Ciprofloxacin 500–750 mg po q12h, 200–400 mg iv q12h	Uncomplicated cutaneous anthrax: 3–5 days Systemic anthrax†: 10–14 days
Biological weapon or bioterrorism-related anthrax	Ciprofloxacin 500–750 mg po q12h, 200–400 mg iv q12h Doxycycline 100 mg iv/po q12h	60 days

*Antibiotic therapy should be given orally in mild cutaneous anthrax. In cases of severe cutaneous anthrax or systemic anthrax, initial antibiotic therapy should be given intravenously; when fever has subsided to normal, antibiotic therapy may be switch to oral.
†In systemic anthrax, initial antibiotic choice should be combined with one or two of the following antibiotics: penicillin, ampicillin, ciprofloxacin, imipenem, meropenem, vancomycin, rifampin (rifampicin), clindamycin, streptomycin or other aminoglycoside.
Adapted from[2,12,20].

intramuscular penicillin G or to an oral antibiotic. In the treatment of systemic anthrax, penicillin should be combined with one or two other antibiotics to which *B. anthracis* is susceptible. Penicillin G may be combined with clindamycin, clarithromycin or ciprofloxacin in treating inhalation anthrax or with an aminoglycoside (streptomycin is suggested) in gastrointestinal anthrax.[2,12]

Anthrax meningoencephalitis is a life-threatening infection. Currently, a combination of penicillin G and rifampin is the first choice of treatment. Other possible regimens would be penicillin G in combination with vancomycin, or vancomycin and rifampin.[2,12,18]

In the event of allergy to penicillin, doxycycline or ciprofloxacin is accepted as best alternative. Tetracyclines and erythromycin are also effective alternatives in mild cutaneous anthrax in developing countries. In uncomplicated cutaneous anthrax, doxycycline or ciprofloxacin is given orally. In systemic anthrax, doxycycline or ciprofloxacin should be given intravenously and may be combined with another suitable antibiotic, as with penicillin.[2,12,18–20]

Duration of treatment in uncomplicated cutaneous anthrax is suggested as 3–5 days (may be 3–7 days). Therapy in cases of systemic anthrax should be continued for 10–14 days.[2]

Supportive treatment for shock, intubation, tracheotomy or ventilatory support in respiratory problems and steroid administration or other anti-brain edema therapies for anthrax meningoencephalitis are life saving in some cases. It is noted that surgical resection of intestinal lesions with medical treatment has shown a beneficial effect.[2,8,12]

PREVENTION

The prevention of anthrax in human is based on:

- control of the infection in animals;
- prevention of contact with infected animals and contaminated animal products;
- environmental and personal hygiene;
- medical care of cutaneous anthrax; and
- disinfection of fur and wool.

Occupational groups at risk may be vaccinated. An acellular vaccine is available in the USA and the UK, and is used mostly in occupational and military settings. This vaccine is not recommended for normal travelers unless there is likely to be occupational exposure. A live spore vaccine has been produced and used in China and Russia. Accelerated development is underway to produce safer and more effective vaccines.[1,2]

REFERENCES

References for this chapter can be found online at http://www.expertconsult.com

Practice point | 59 |

Rose McGready
François Nosten

What are the treatment options for a pregnant patient with malaria?

DEFINITION OF THE PROBLEM

Malaria is the most important parasitic infection in humans and in pregnancy is detrimental to the mother and fetus. In pregnancy the adverse effects of malaria infection result from:

- systemic infection, comparable to the effects of any severe febrile illness in pregnancy, i.e. maternal/fetal mortality, abortion, stillbirth and premature delivery; and
- parasitization, i.e. birth weight reduction, maternal/fetal anemia, interaction with HIV and susceptibility of the infant to malaria.

The hallmark of malaria in pregnancy is parasites sequestered in the placenta. Sequestered parasites evade host defense mechanisms including splenic processing and filtration. Sequestration is not known to occur in the benign malarias due to *Plasmodium vivax*, *Plasmodium ovale* and *Plasmodium malariae*. *Plasmodium falciparum* causes greater morbidity (principally low birth weight and anemia) and mortality than non-*falciparum* infections. The clinical manifestations in pregnancy depend on premunition, i.e. the degree of natural immunity to malaria (Table PP59.1).

Premunition appears slowly following continuous and numerous re-infections by malaria parasites to prevent it waning. Premunition acquired in childhood is impaired during the first pregnancy but recovers during subsequent gestations. *Plasmodium falciparum* is unique in its ability to sequester in large numbers in the placenta and placental parasites have been shown to bind preferentially to chondroitin sulfate A. During the course of pregnancy or over successive pregnancies women develop variant specific immunity to chondroitin sulfate A binding infected red blood cells (IRBC). Thus, in areas of high transmission (conferring high premunition), severe anemia is common and is associated with mortality, predominantly affecting primigravidae; on the other hand, in areas of low transmission or in epidemics, pregnant women of any gravida are at risk of severe and cerebral malaria and death.

Ideally malaria infection during pregnancy should be prevented. Failing this (an increasing problem due to drug resistance) prompt diagnosis and effective treatment will prevent fatal outcomes and help reduce morbidity in both mother and baby. Clinicians who assess and manage pregnant women with malaria in nonendemic countries will be unfamiliar with the condition. Delay and misdiagnosis, with subsequent failure to provide prompt effective treatment, are the main recurring themes in death from malaria.

CLINICAL CASES

Case 1 – severe malaria

A 37-year-old West African woman presented for the first time in pregnancy to a London maternity department at 29 weeks gestation in her first pregnancy, with a 5-day history of fever. She had recently returned to her home in London (resident 2 years) after a 1-month visit to West Africa to take care of her mother (no prophylaxis). On admission her temperature was 103.1°F (39.5°C), pulse rate 130 beats/min (bpm) and blood pressure 108/56 mmHg. She was conscious and responsive. Her urine contained protein on ward testing and was sent for culture (subsequently negative). Her uterus was soft and fetal size was appropriate for 29 weeks. There was a fetal tachycardia (180 bpm) with a suspicious cardiac rhythm: a flat trace with reduced short-term variability (<5 bpm). Her initial investigation results were as follows: hemoglobin (Hb) 8.2 g/dl, white blood cell count 18.3 × 10^9/l and platelets 50 × 10^9/1. Apart from her thrombocytopenia, her coagulation screen was normal. Two hours after admission her malaria film was reported to be positive with 10% *Plasmodium falciparum* IRBC including schizonts and malaria pigment. Her blood sugar was 2.6 mmol/l.

The patient was transferred to the intensive care unit and commenced on an infusion of 10% dextrose after a bolus of 50 ml of 50% dextrose. She received a loading dose of intravenous quinine 20 mg/kg over 4 hours and clindamycin 5 mg/kg. In view of the hyperparasitemia, she was transferred to a tertiary referral unit for consideration of exchange transfusion. During the transfer she became unconscious and her blood sugar was 1.4 mmol/l. She regained consciousness with 0.5 g/kg glucose. She had repeated episodes of hypoglycemia over the next few days. She required two units of blood for severe symptomatic anemia. After an initial rise in parasitemia (6-hourly blood smear) in the first 24 hours her parasitemia was down to 1% by the third day of treatment. On the fourth day she developed acute respiratory distress syndrome and despite ventilation died the next day. Fetal heart beat was not detectable before she died.

Case 2 – uncomplicated malaria

A 26-year-old Burmese woman in her third pregnancy presented at 24 weeks gestation for antenatal booking. She had arrived in the UK 1 week previously, as part of the resettlement of Karen refugees from the Thai–Burmese border. She reported having had fever in the last 24 hours with headache, muscle and joint pain for 2 days. Much to the chagrin of the physician on duty she requested a malaria smear. This was agreed to and was positive for *P. falciparum*, 1.2% IRBC, and *P. vivax*, 0.2% IRBC. Routine booking investigations were carried out. All results were unremarkable apart from the hemoglobin (9.5 g/dl with iron-deficient indices). The patient was diagnosed as having uncomplicated malaria and was treated with coartemether 4 tablets with 200 ml of milk. She vomited all the tablets 15 minutes after treatment initiation and said it was because of the milk. The physician ordered metoclopramide and repeated the dose with soya milk and without further vomiting. The patient was admitted to the ward, spiked a fever that evening of 104°F (40.0°C), treated with paracetamol and oral fluids. There were no further febrile episodes, the remaining doses of coartemether were well tolerated and

Table PP59.1 Main consequences of malaria in pregnancy in different transmission situations

	Nonimmune*	Low premunition	High premunition
Susceptibility	++++	+++	++
Risk of illness	++++	+++	+
Severe anemia	?	+++	+++
Severe/cerebral malaria	++++	+++	-
Maternal/fetal mortality	++++	+++	+
Birth weight reduction	?	++	++
Fetal wastage	++++	++++	+
Gravida at risk	All	All	Primigravidae
Placental parasitemia	?	+	+++

*Includes women never exposed to malaria and women who come from endemic areas but who have lived for a prolonged period outside a malaria-endemic area.
+, Level of severity.
?, No data available. (There are actually no collective data on the effects of malaria in nonimmune women, but the severity of the infections caused by *Plasmodium falciparum* in this group would probably mask the effect on maternal anemia and birth weight.)

the patient was blood smear negative on the second day of treatment. Ultrasound assessment revealed an appropriately grown fetus with normal amniotic fluid volume. There was bilateral notching of the uterine artery Doppler waveforms with raised resistance indices, suggesting a degree of placental dysfunction. The umbilical artery and fetal arterial Doppler studies were normal. By the next antenatal visit the uterine artery Doppler waveforms had normalized.

Six weeks later the woman complained again of headache, muscle and joint pain, no fever and again requested a malaria smear. *P. vivax* trophozoites 0.5% IRBC, schizonts and gametocytes were all reported. The physician on duty diagnosed *P. vivax* and treated the woman with chloroquine 10, 10 and 5 mg/kg once daily on days 0, 1 and 2, respectively. At 32 weeks gestation the fundal height was 27 cm. By 37 weeks gestation fundal height measurement and serial scans for growth demonstrated intrauterine growth retardation (IUGR), despite normal Dopplers. The woman had spontaneous onset of labor at 37.2 weeks gestation and a normal vaginal delivery of a term 2.3 kg normal infant. At 18 hours postpartum the midwife on the labor ward recorded the temperature of the patient as 100.4°F (38°C). As everyone was aware of the woman's history of malaria a blood smear was ordered for the mother and baby (normal vital signs) and both were positive for *P. falciparum*. Attempts to salvage the placenta to do a malaria smear were made too late and were not done. The mother and the baby were successfully treated with 3 days of atovaquone–proguanil (Malarone).

DIAGNOSIS

Failure to consider malaria as a possible diagnosis can result in death. The diagnosis of malaria in pregnancy, as in nonpregnant patients, relies on microscopic (the current gold standard) examination of thick and thin blood films for parasites, or the use of rapid diagnostic tests that detect specific parasite antigen (see Chapter 111). Microscopic diagnosis allows species identification and estimation of parasitemia, so that appropriate antimalarials can be prescribed. Always consider malaria in the febrile patient and never forget to ask for a history of travel. Thrombocytopenia, metabolic acidosis and elevated lactate levels are also highly suggestive of severe malaria. The investigation of a patient with suspected falciparum malaria in pregnancy should be treated with great urgency. Blood and urine should be cultured to exclude other infections. In the comatose patient, lumbar puncture should be considered to exclude meningitis and encephalitis, providing there is no evidence of raised intracranial pressure.

In a woman from an endemic country with severe anemia and suspected asymptomatic disease, presumptive treatment should be given even if a blood smear is negative.

MANAGEMENT

Prompt treatment is vital in pregnancy as the disease is more severe and mortality is 2–10 times higher than in nonpregnant patients. The disease in pregnancy is associated with higher parasitemia and is dangerous for both mother and fetus. The aim of treatment of uncomplicated malaria is to cure the patient and in severe malaria to save the mother's life. Severity of malaria is determined by the symptoms and microscopic findings (Table PP59.2).

Management of malaria in pregnancy depends on the infecting species, severity of the disease, the stage of pregnancy (first trimester or other), prophylaxis or intermittent preventive therapy, and drug availability. The fast expansion of resistant isolates has compromised many of the standard antimalarials, including chloroquine, sulfadoxine–pyrimethamine and quinine monotherapy.[2] Patients may deteriorate under treatment, at which point management should be upgraded to account for severity. Drugs for treatment are summarized in Table PP59.3. Specific points related to treatment of malaria in pregnant women have been summarized in Table PP59.4.

CONCLUSION

There is a paucity of data on antimalarials used for treatment and prevention in pregnancy. Most of what we know about chloroquine in pregnancy comes from research on autoimmune disease in pregnancy and for quinine from its historical use. Sulfadoxine–pyrimethamine has been widely advocated as a preventive antimalarial in pregnancy without clear evidence of the efficacy or pharmacokinetic properties in pregnant women. There have only been 10 randomized controlled trials (RCTs) specifically involving treatment for pregnant women with acute uncomplicated falciparum malaria involving a total of 1914 patients. Much larger numbers of women must be treated and followed prospectively until the data on efficacy, outcome and drug safety

Table PP59.2 Classification of severity of malaria in pregnancy

Grade of malaria	Microscopic findings	Symptoms	Case fatality rate
Uncomplicated	Positive blood smear with parasite count <4% IRBC or parasitemia of 150 000/µl	First symptoms are usually nonspecific and similar to a minor systemic viral illness Headache, lassitude, fatigue, abdominal discomfort, dizziness and muscle and joint aches, followed by fever, chills, sweating, anorexia, vomiting and worsening malaise are the typical features of uncomplicated malaria No signs of severity* or organ dysfunction	Low: ~0.1% for *P. falciparum* infections Non-*falciparum* species are rarely fatal
Uncomplicated hyperparasitemia	*P. falciparum* asexual parasitemia with ≥4% IRBC 2% should be used in the nonimmunes from nonendemic countries	As for uncomplicated	As for uncomplicated if treated adequately
Severe (rare reports with *P. vivax* monoinfection)	*P. falciparum* asexual parasitemia (or positive rapid diagnostic test)	No other obvious cause of symptoms and with signs of severity* and/or organ dysfunction	High: 15–20% in nonpregnant patients compared with 50% in pregnancy Untreated severe malaria is always fatal

IRBC, infected red blood cells.
*Signs of severity: *Clinical manifestations*: prostration, impaired consciousness, respiratory distress (acidotic breathing), multiple convulsions, circulatory collapse, pulmonary edema (radiologic), abnormal bleeding, jaundice, hemoglobinuria; *Laboratory tests*: severe anemia, hypoglycemia, acidosis, renal impairment, hyperlactatemia, hyperparasitemia.
Adapted from WHO.[1]

Table PP59.3 Drugs for treatment of malaria in pregnancy

Species and trimester	Severity	Treatment
P. vivax, P. ovale and *P. malariae*, regardless of trimester	Not usually severe If severe, see *P. falciparum* (severe)	Chloroquine phosphate (1 tablet contains 250 mg salt equivalent to 155.3 mg base) Dose: 10 mg/kg base q24h on days 1 and 2, followed by 5 mg/kg base on day 3 For chloroquine-resistant *P. vivax,* amodiaquine, quinine or artemisinin derivatives can be used
First trimester: *P. falciparum* or mixed species, i.e. *P. falciparum* and non-*falciparum* (usually *P. vivax*) co-infection	Uncomplicated	1st episode: quinine 10 mg/kg q8h for 7 days, preferably with clindamycin* 5 mg/kg q8h for 7 days Subsequent episodes: repeat treatment with quinine and clindamycin* as above ACT that is locally effective or artesunate 2 mg/kg q24h for 7 days with clindamycin* as above
Second and third trimesters	Uncomplicated	1st episode: ACT that is locally effective such as artemether–lumefantrine§ or artesunate plus clindamycin* as above Malarone (atovaquone–proguanil)† alone can be used for treatment in pregnancy but it is highly recommended to use it with artesunate to maximize fever and parasite clearance times and ensure cure Subsequent episodes: artesunate plus clindamycin* as above Artesunate–atovaquone–proguanil and dihydroartemisinin–piperaquine§ can be used for *P. falciparum* recurrence in the same pregnancy
Regardless of trimester	Uncomplicated hyperparasitemia	Artesunate: 4 mg/kg po loading dose then 2 mg/kg q24h for 7 days with clindamycin* as above, *or* Quinine iv (see below)
Regardless of trimester	Severe	Artesunate‡: 2.4 mg/kg iv at 0, 12 and 24h, then q24h until the patient can tolerate oral artesunate 2 mg/kg q24h for a total of 7 days, and clindamycin* 5 mg/kg q8h for 7 days, *or* Quinine: 20 mg/kg iv loading dose given over 4h, then 10 mg/kg 8h after the loading dose was started, followed by 10 mg/kg q8h for 7 days Once the patient has recovered sufficiently to tolerate oral medication both quinine 10 mg/kg and clindamycin 5 mg/kg q8h should be continued for/until 7 days

ACT, artemisin-based combination therapy.
*When clindamycin is not available, give artesunate monotherapy.
†Malarone use in pregnancy.[2]
‡Intravenous artesunate is the best treatment[3] but unfortunately is not available routinely in the UK. Some hospitals may have their own supply.
§Prescribe artemether–lumefantrine and dihydroartemisinin–piperaquine with fat.[4]
Dosages for pregnant women are likely to be revised when all the pharmacokinetic antimalarial pregnancy data become available.[4]
Adapted from WHO.[1]

Table PP59.4 Problems pertinent to treatment of malaria in pregnant women

Adjunctive treatment	Relationship to pregnancy	Details of treatment
Antiemetics	There are no trials specifically in pregnancy but pregnant women have a higher tendency to vomit than nonpregnant women	If vomiting occurs within 30 mins, the full dose should be repeated. If vomiting occurs from >30 to 60 mins, repeat half the dose. An antiemetic (metoclopramide) can be given im or iv; wait 20 mins before repeating the dose Antiemetics are widely used for treatment in malaria although there have been no studies of their efficacy. There is no evidence to indicate harm. If a patient vomits twice they will need parenteral treatment. Oral medication can be recommenced when oral fluids are well tolerated. Although these patients may show no signs of severity, the treatment regimen should be switched to that used for severe cases
Antipyretics	Fever is a cardinal feature of malaria. In pregnant women it has been associated with premature labor	Paracetamol 1 g q4–6h (maximum 4 g q24h) is safe and effective
Intravenous infusions	Due to greater circulating volume in pregnancy and increased losses, hydration is essential Quinine is still widely used in pregnancy but can be a particularly offensive drug to take due to cinchonism (tinnitus, hearing loss, nausea, uneasiness, restlessness and blurring of vision)	Febrile pregnant women with nausea and vomiting may be dehydrated and oral fluids need to be encouraged and adequate urine output confirmed. Intravenous fluids may be required, particularly on quinine
Glucose	Pregnancy can predispose to hypoglycemia and the nausea associated with malaria prevents some women from taking an adequate oral intake, thus promoting hypoglycemia Hypoglycemia in pregnancy may be profound and recurrent, particularly on quinine	Hypoglycemia may manifest as fetal brady- or tachycardia. In the most severely ill patients it is associated with lactic acidosis and high mortality In patients who have been given quinine, abnormal behavior, sweating and sudden loss of consciousness are the usual manifestations
Tocolytic therapy	Pre-term labor appears to be associated with the fever from malaria	Usual pre-term labor protocols apply Monitor uterine contractions
Fetal distress	Exclude maternal hypoglycemia, particularly if the patient is being treated with quinine and hyperthermia is the cause	Normal labor protocols should be implemented but fetal heart rate must be monitored. This may reveal fetal tachycardia, bradycardia or late decelerations in relation to uterine contractions, indicating fetal distress
Antibiotics	Secondary bacterial infection, principally Gram-negative septicemia, has been reported in pregnancy	The patient is collapsed with a systolic blood pressure <80 mmHg in the supine position Blood cultures should be taken if the patient shows signs of shock or fever returns after apparent fever clearance Hypovolaemia should be corrected and broad-spectrum antibiotics started immediately (e.g. ceftriaxone). Once the results of blood culture and sensitivity testing are available, give the appropriate antibiotic
Assisted ventilation	Pulmonary edema is a grave complication of severe malaria, with a high mortality in pregnancy (over 50%)	May be present on admission or develop suddenly and unexpectedly, or develop immediately after childbirth The first indication of impending pulmonary edema is an increase in the respiratory rate which precedes the development of other chest signs Hypoxia may cause convulsions and deterioration in the level of consciousness and the patient may die within a few hours Ensure the pulmonary edema has not resulted from iatrogenic fluid overload
Blood transfusion	Severe anemia is associated with perinatal mortality, maternal morbidity and an increased risk of postpartum hemorrhage	Women who go into labor when severely anemic or fluid overloaded may develop acute pulmonary edema after separation of the placenta Transfuse pregnant women slowly, preferably with packed red blood cells, and furosemide 20 mg iv; alternatively exchange transfusion may be given

in pregnancy (including developmental assessment of the infant) are robust, and clear guidelines can be issued.

For first trimester antimalarials, there is not a single RCT published. Given the impact of malaria in pregnancy and the huge number of women affected, this is a scandal. The collective data on epidemiology, prevention and treatment of malaria in pregnancy clearly demonstrate how dangerous malaria in pregnancy is to both the mother and the baby. Malaria in pregnancy is a medical emergency. It can be prevented and treated with reasonable confidence using Tables PP59.3 and PP59.4.

REFERENCES

References for this chapter can be found online at http://www.expertconsult.com

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Steven M Opal

Management of a patient from Gabon with fever, malaise, sore throat and a negative malaria smear

Systemic illness accompanied by high fever should always prompt a search for malaria in any patient from a malaria-endemic region such as Gabon in equatorial West Africa. Once malaria has been confidently excluded, a more detailed differential diagnosis should be considered. Routine respiratory viruses, infectious mononucleosis or streptococcal pharyngitis would be the most common illnesses to account for an acute febrile illness with sore throat and malaise. Other infections such as typhoid, meningococcal meningitis, leptospirosis, dengue or plague are possible in this setting. The presence of severe prostration, myalgia, unrelenting headache followed by pharyngitis and any evidence of hemorrhagic complications should bring to mind the possibility of a hemorrhagic viral infection in this patient. Sporadic or epidemic occurrences of viral hemorrhagic fever (VHF) from arenaviruses, flaviviruses, bunyaviruses, filoviruses and hantaviruses are increasingly recognized by clinicians in specific geographic regions worldwide. Global markets for international trade in combination with improved access to remote areas by expanded transportation systems now make it feasible that these highly contagious viral illnesses could disseminate widely. Even in nonendemic regions physicians need to be cognizant of the risk of hemorrhagic viral fevers in international travelers or immigrants from tropical regions. Animal handlers in primate research laboratories throughout the world are also at risk. It is essential that health-care facilities develop a strategy to manage VHF even if the chances of seeing such patients seem remote.

It is important that physicians be aware of the potential risk of viral hemorrhagic fevers for several reasons:

- to ensure appropriate diagnosis and management of index cases;
- to provide advice, counseling and possible prophylaxis to close contacts;
- to minimize the risk of nosocomial transmission among health-care workers (HCWs) caring for such patients; and
- to alert public health authorities as soon as possible in the event of an outbreak of VHF.

Strict adherence to basic infection control techniques and some advance planning will minimize the risk to HCWs and allow rapid, compassionate and safe care of affected patients.

MICROBIOLOGY AND PATHOGENESIS

Hemorrhagic viruses in which person-to-person transmission has occurred in health-care settings include Lassa fever, Ebola virus and Marburg virus, new world arenaviruses and the tick-transmitted virus of Crimean–Congo hemorrhagic fever. These hemorrhagic fever viruses are particularly important to recognize because nosocomial transmission to HCWs is a real possibility. The animal reservoir and mode of transmission to humans is reasonably well understood for Lassa fever and other arenaviruses (contact with the excreta from rodents such as the *Mastomys* rat), Crimean–Congo hemorrhagic fever (tick transmitted from wild and domestic mammalian reservoirs) and Marburg virus (contact with African green monkeys). Recently the animal reservoir for Ebola virus has been determined to be bat species in endemic regions in Africa and possibly chimpanzees (although they may be as much a victim of Ebola virus as humans, rather than a reservoir). In a patient from Gabon, Ebola would be the most likely VHF in this region of the world although yellow fever, Marburg virus, Lassa fever or even Rift Valley fever could be possible.

These viral syndromes share many overlapping clinical features in humans. After an incubation period of between 3 and 21 days, patients develop the abrupt onset of fever, headache, myalgia, sore throat, respiratory symptoms, abdominal pain, nausea, vomiting, diarrhea and conjunctivitis with associated pharyngitis and cervical lymphadenitis. A macular skin eruption may occur in infections with Ebola and Marburg viruses. Various degrees of mucosal and cutaneous hemorrhage occur associated with thrombocytopenia and disseminated intravascular coagulation. The geographic location or travel history of the patient is most useful in the initial clinical distinction between these different types of VHF before virologic confirmation.

These viruses share rapid growth potential and the ability to invade a variety of cell types, resulting in high-grade viremia. The patient's blood and body fluids become contagious to others who come into direct contact with them. Transmission may also occur through handling of bodies during burial rituals, as demonstrated in recent Ebola outbreaks in Central Africa. Although many other nontransmissible febrile illnesses present in a similar fashion, strict infection control measures need to be instituted to guard against potential transmission of hemorrhagic fever viruses until the diagnosis is established. Health-care workers caring for patients with VHF are at risk owing to high viral concentrations present in body fluids and secretions.

DIAGNOSIS AND MANAGEMENT

Recent improvements in the serologic diagnosis of VHF now make it possible to make a specific diagnosis in the early phases of acute illness in the majority of patients. An antigen-capture enzyme-linked immunosorbent assay has been developed for Ebola virus and may allow rapid diagnosis in acutely ill patients. Specific IgM and IgG capture enzyme-linked immunosorbent assay antibody studies are available for serologic diagnosis in convalescent samples. Virus isolation from the blood and body secretions of acutely ill patients is the definitive diagnostic method. This is an extreme biohazard and should only be attempted in laboratories with

biosafety level IV facilities. Reverse transcription and polymerase chain reaction for specific viral RNA is also a useful diagnostic method in patients who have VHF. Many of these methodologies are unavailable in regions of the world where these diseases are endemic. For this reason, the recent development of an immunochemical staining method for skin biopsy samples is particularly valuable. This method allows for fixation of tissues at the site of diagnosis and eliminates the biohazard of transportation of infected human tissues.

Routine diagnostic methods to evaluate other common febrile illnesses should not be delayed because of suspected VHF. In particular, care must be taken to exclude falciparum malaria which may be fatal if unrecognized and left untreated. In practice, most cases of suspected VHF turn out to have malaria. Universal precautions when handling blood and body secretions should suffice to protect HCWs from hemorrhagic viruses.

INFECTION CONTROL METHODS

These viruses are transmitted through direct contact with the patient or the patient's secretions. There is a remote risk of air-borne transmission of some strains of Ebola virus based on studies with nonhuman primates, but this has not been observed as a significant hazard in actual human outbreaks of Ebola virus infections. The primary infection control strategy is strict contact isolation, universal blood and body substance precautions, use of disposable sterile medical devices and needles, and enhanced preventive measures in the handling of blood and body fluids. Body substances are contagious during the acute febrile illness, but there is no evidence of transmission during the incubation phase of the illness. The guidelines listed in Table PP60.1 should be instituted in patients who have suspected VHF.

TREATMENT OPTIONS AND POSTEXPOSURE PROPHYLAXIS

The arenaviruses, including Lassa fever, are susceptible to ribavirin and this antiviral agent has proven to be efficacious in patients and in HCWs occupationally exposed to blood or body fluids (e.g. by percutaneous needlestick accident). Regrettably, ribavirin has been studied extensively in the laboratory and in previous outbreaks of Ebola virus and has proven ineffective in patients. New antiviral agents are desperately needed to treat Ebola and are a current area of intense research interest (Table PP60.2).

Passive immunotherapy from plasma or antibody preparations from surviving patients with high-titer antibody or experimental animals has variable and generally disappointing activity as a salvage therapy in severely ill patients with Ebola infection, but might be a consideration in a HCW following accidental exposure to the virus. Inhibitors of the tissue factor:factor VII coagulation pathway appear to be of some therapeutic benefit in experimental trials in primate studies, and some immune-based strategies against major viral glycoprotein antigens found on the surface of Ebola viruses appear promising as a preventive and even as a possible treatment option against Ebola virus.

REPORTING

All patients who have suspected viral hemorrhagic fever should be reported to public health authorities as soon as possible. This allows for a co-ordinated response to an epidemic situation and ensures that diagnostic and therapeutic efforts will be handled appropriately. Expert international assistance is necessary should a viral hemorrhagic fever occur in an international traveler.

Table PP60.1 Infection control methods for suspected viral hemorrhagic fever

Isolation method	Comments
Isolation room	Use a negative pressure room if available. Use strict contact isolation, universal blood and body substance precautions and enhanced prevention measures in the handling of blood and body fluids
Personnel and visitors	Restrict traffic flow into the patient's room. A daily record should be kept of all those who enter and leave the patient's room. Only essential personnel should be exposed to the patient and the patient's body fluids
Personal protection	Fluid-impervious gowns, gloves, face shields or surgical masks with eye protection (goggles); if the patient has cough, vomiting or extensive hemorrhage, respirators with filters (high-efficiency particulate air respirators) and shoe coverings should be worn.
Clinical samples	Clinical samples should be placed in plastic sealed bags and transported in a leak-proof container without contaminating the external surfaces. Samples should be handled in a biologic safety cabinet (biosafety level III). Serum should be pretreated with a polyethylene glycol phenolic for 1 h before handling. Automated analyzers should be disinfected with 1:100 dilution of bleach after use. Fixation of blood smears and tissue samples will inactivate the virus and can be handled in a routine manner
Decontamination of the environment and of linen	Disinfect contaminated environmental surfaces using a registered hospital disinfectant or 1:100 dilution of bleach. Soiled linens can either be decontaminated by autoclaving or incineration. Hot cycle laundering with bleach may be acceptable
Human excrement and blood and body fluids	Human excreta, blood and body fluids should be decontaminated by 1:100 dilution of bleach for 5 min before disposal
Surgical procedures and autopsy	If a surgical procedure is essential, extreme caution should be exercised to avoid blood contamination. Double gloves, full-face shields with high-efficiency particulate air filtration, water-impervious gowns and shoe covers should be worn. Avoid generation of an aerosol. Deceased persons should not be embalmed. The body should be placed in leak-proof, sealed material and cremated or buried in a sealed casket

Table PP60.2 Treatment options and postexposure prophylaxis for Ebola virus

Agent	Level of evidence	Comment
Ribavirin	No activity in animal models or uncontrolled clinical trials	Not effective against Ebola virus
S-adenosyl-L-homocysteine inhibitor	Experimental studies only	Some activity in mouse models
Interferon-α 2b	Experimental studies only	Delays illness and viremia but no survival benefit in experimental animals
Immune plasma or hyperimmune antibody preparations targeting viral glycoproteins	Variable experimental data, case reports	Variable, generally disappointing results; might decrease viremia
Small inhibitory RNAs against Ebola virus polymerase	Experimental studies only	Early promising results in guinea pig studies
Tissue factor inhibitors	Primate laboratory animals, no clinical studies	33% survival rates in primate models
Attenuated recombinant vesicular stomatitis virus (VSV) vectors expressing Ebola virus glycoproteins	Primate laboratory animals, no clinical studies	Protection when administered up to 24 h after Ebola virus challenge

FURTHER READING

Further reading for this chapter can be found online at http://www.expertconsult.com

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Nick J Beeching
Gail Thomson

Follow-up of the returned traveler who has swum in Lake Malawi

INTRODUCTION

Lake Malawi is a huge freshwater resource 630 km long and 50 km wide in Central Africa, providing essential food and income for a large proportion of Malawians and people of the other nations that border its shores. Over the past two decades it has become a major attraction for both 'local' tourists and for backpackers and overlanders from outside Africa, with the associated development of hotels and facilities for water sports such as scuba diving and windsurfing. These are especially found in the south around Cape Maclear and Monkey Bay, where the shores are relatively shallow and the snail vectors for *Schistosoma haematobium* have become established. Further north and centrally, the ecology of the lake differs, with deep water close to the land. Bilharzia was recognized by early European visitors to Malawi and was noted in 50% of lakeshore inhabitants in the early 1900s. It has become more widely established since then, particularly with the development of irrigation schemes. The predominant species is *S. haematobium*, but small pockets of *S. mansoni* also exist.

Schistosomiasis has become a common diagnosis in expatriates and short-term tourists in Malawi and similar risks are also recognized for travelers to Zimbabwe, the Dogon area of Mali and other freshwater lakes in Africa. A large case-control study showed that 33% of foreigners in Malawi had serologic evidence of exposure to infection, the highest risk being associated with repeated exposure to the lake, especially around Cape Maclear.[1] Our own experience of screening groups of returned travelers is that 75% of those who spend a week scuba diving at Cape Maclear will have clinical or asymptomatic laboratory evidence of infection. People are at risk from showers and swimming pools that are fed directly with unchlorinated lake water as well as from paddling and swimming. Brief exposure is sufficient; we recently screened students from the same school who had spent 48 hours only at two locations around the lake. Of those camping near Cape Maclear, 90% (19/21) had been infected, compared with none of 17 students camping on Likoma Island, another popular tourist spot.

GENERAL APPROACH TO THE TRAVELER WHO HAS SWUM IN LAKE MALAWI

The general approach to the traveler who has swum in Lake Malawi is the same as that for any other traveler, including the need to exclude malaria in febrile patients (Chapter 111). A detailed travel and exposure history must be taken, including details of travel dates; business and leisure activities undertaken; consumption of unsafe food, water and drinks; history of insect bites or animal exposures; specific infection exposures; use of health care facilities while overseas; illness in fellow travelers and sexual activities. The precise timing, frequency, type and locations of any freshwater contact should be recorded. Previous travel and possible schistosomal risk activity should be checked, along with pre-existing illnesses including atopy and urologic or gastrointestinal problems. Enquiries should routinely review adherence to antimalarial chemoprophylaxis and mosquito avoidance measures, and the use of measures that might reduce schistosomal load such as vigorous rubbing of the skin immediately after immersion, or pre- or postexposure use of antischistosomal soaps, *N,N*-diethyl-M-toluamide (DEET) or permethrin on the skin.

Only a minority of people will remember experiencing 'swimmer's itch', lasting from a few to 48 hours after swimming, caused by penetration of the skin by cercariae. This does not reliably predict later symptoms, which first appear in a substantial minority of patients 3–8 weeks after exposure, as the 'Katayama syndrome', which is related to an immunologic reaction to final migration of schistosomules around the body and the onset of oviposition by the maturing flukes (see Chapter 112). Typical symptoms include fever, headache, malaise, wheezing, dry cough and dyspnea. Transient urticarial rashes are common (Fig. PP61.1), and lymphadenopathy and hepatosplenomegaly may be found on examination.[2] Usually this is a diagnosis of exclusion, and the supportive laboratory finding of eosinophilia exceeding 440–500 × 10^6/l (depending on local definitions) is not always present. We have seen cases misdiagnosed as glandular fever due to coincident lymphocytosis, false-positive slide tests for infectious mononucleosis and mild disturbance of liver function tests. Transient shadows may be seen on chest radiographs.

Whether or not patients have experienced earlier symptoms, continued oviposition from 3 to 6 months after exposure may then cause symptoms related to the organs involved. *Schistosoma haematobium* principally affects bladder, prostate and seminal vesicles, and typical complaints are of terminal hematuria, perineal discomfort and, in males, alteration in the consistency (thin or lumpy) or color (yellow or frank blood) of semen. Women occasionally notice a wart-like genital granuloma. *Schistosoma mansoni* primarily affects the large bowel, leading to blood in feces and alteration in bowel habit, but the anatomic location of both species has considerable overlap. Symptoms are more common with *S. haematobium* infections and may be associated with nonspecific fatigue.[3]

The long-term outcome of untreated, relatively light infections of visitors is unknown, but is likely to be benign and not to lead to the complications caused by chronic and repeated infections of inhabitants of endemic areas, such as bladder cancer and portal or pulmonary hypertension (see Chapter 112). However, a small minority of travelers develop central nervous system complications such as epilepsy or spinal cord damage due to ectopic deposition of ova, and the general expert consensus is that all exposed travelers should be screened and treated.



Fig. PP61.1 Giant urticaria associated with Katayama syndrome after swimming in Lake Malawi. Courtesy of Dr ME Jones, Edinburgh.

SCREENING

The minimum investigations, whether or not symptoms are present, should include an absolute eosinophil count, testing of urine for blood, and microscopy for ova in feces and on a filter of a whole urine sample collected over a period of 4 hours straddling the middle of the day (4-hour mid-day urine specimen).[3,4] If such a sample cannot be obtained, a 'terminal urine' sample should be obtained for microscopy, i.e. the last few drops of urine at the end of micturition are most likely to contain ova. 'Routine' urine microscopy is insufficiently sensitive, as egg output can vary during the day such that greater yield is found around noon. Semen microscopy may become positive earlier than urine or feces, and males can be asked to provide a sample for microscopy, particularly if being screened in the first few months after water exposure. If the index of clinical suspicion remains high and other tests are negative, a squash preparation of a fresh rectal biopsy can be examined for ova. Viability of ova, hence current infection, can be inferred from the observation of active flame cells within the miracidium developing in the ovum. Eosinophilia is only found in about 50% of patients in the chronic phase of infection.

The rather crude serologic tests available from European reference centers include an enzyme-linked immunosorbent assay (ELISA) using circumoval protein as antigen to detect circulating antischistosomal antibodies. These become positive from about the time of oviposition, but seroconversion may take up to 6 months and late follow-up screening is essential for those who are asymptomatic and who have negative tests before this time. The serologic tests available from the US Centers for Disease Control and Prevention use an ELISA based on microsomal fractions of adult *S. mansoni*, which become positive soon after exposure to infection, but a species-specific immunoblot may also be required to detect *S. haematobium* or other species. Once positive, serology remains so for years and titers may even increase during the first year after treatment. Follow-up serologic tests after treatment are not, therefore, useful as a test of cure or even for distinguishing re-infection after subsequent re-exposure of the patient to infected water. The disappearance of any symptoms or other positive tests, including eosinophilia, is more reliable for follow-up after treatment.

TREATMENT

Treatment is relatively easy and harmless, using oral praziquantel in a dose of 40 mg/kg, sometimes split into two doses 6 hours apart to reduce any associated nausea, which is usually short lived. Many scuba divers and other visitors treat themselves with locally purchased praziquantel immediately after diving, but this is useless as postexposure prophylaxis. There has been interest in the use of dimeticone or DEET-based preparations for pre- and postexposure prevention of cercarial penetration of skin, but this has not gained wide acceptance. Prophylaxis and chemosuppression with artemisinin compounds have shown more promise in field trials against *S. japonicum* and these deserve further study in African settings.

As praziquantel has limited efficacy during the incubation and invasive phases of *S. haematobium* infection,[5] patients treated at the stage of Katayama syndrome symptomatology need to be re-treated 3 months later. Patients with moderate-to-severe Katayama syndrome may also benefit from a short course of prednisone (e.g. 40 mg daily for several days), preferably started a few hours before anthelmintic treatment. However, this anecdotal recommendation merits a prospective randomized trial to establish both short- and long-term benefit in reducing chronic fatigue, which is quite common after Katayama symptomatology. Corticosteroids reduce the efficacy of praziquantel, so increased dosing of praziquantel is therefore recommended by some.

Patients who present later with symptoms such as hematuria or positive laboratory tests should also be treated and should be followed up 6 months later to establish disappearance of any previous positive findings. Asymptomatic travelers with negative screening tests 6 months or more after lake exposure do not require treatment or further follow-up. It is debatable whether every patient who presents with hematuria and ova in the urine should have further investigations such as ultrasonography or cystoscopy to exclude coincident tumors or other problems. We have a low threshold for urologic referral, which is essential if local symptoms do not resolve after antischistosomal drugs.

SUMMARY

Overall, the risk of acquiring schistosomiasis from swimming in the south of Lake Malawi is so high that screening of returned expatriates, travelers and immigrants is essential and is unlikely to yield false-positive results. Many expatriates regard the risk as so common that they bypass the screening process and treat themselves and their families as a routine every 6 months. We suggest that this approach is inappropriate for the occasional traveler who has swum in Lake Malawi (or similar freshwater bodies), who should be fully assessed before possible treatment with an anthelmintic that is only available on a named-patient basis outside the tropics.

REFERENCES

References for this chapter can be found online at http://www.expertconsult.com

Chapter 129

Roger G Finch

Principles of anti-infective therapy

The discovery, development and widespread use of antimicrobial drugs are counted among the major achievements of modern medicine. Not only has the outcome from life-threatening infections, such as bacterial meningitis, pneumococcal pneumonia and postpartum sepsis, been dramatically improved, but much minor morbidity arising from community-acquired infections has been controlled with benefit to the individual, society and the economic well-being of nation states. Furthermore, the infectious complications of many surgical procedures have been reduced while organ transplantation and cancer chemotherapy have become much safer procedures.

However, the very success of antimicrobial agents has led to their worldwide acceptance by health-care professionals and the public alike, with the result that much prescribing is excessive, inappropriate and unnecessary.[1] Indeed, in many countries, antimicrobial drugs can be purchased by the public without prescription, leading to so-called 'over-the-counter' selling, which is largely unregulated and poorly defined in terms of impact on patient safety and public health, as well as the inevitable pressure it places on the development of antibiotic resistance (see also Chapter 131).

Unique among therapeutic agents, antimicrobial drugs are directed not at any host-derived disease or pathophysiologic process but at an invading micro-organism. In turn, many pathogens have developed the ability to evade inhibition as a consequence of genetic modification. Resistance to antimicrobial drugs now threatens the ability to effectively treat a number of infectious diseases.[2–4] The gradual erosion of efficacy has meant that recommendations for treating specific infections need to be regularly reviewed and revised as resistance rates increase. For these reasons there is a continuous need to develop new agents, which is a costly and time-consuming process and subject to the vagaries of industrial economic priorities. In the case of antibacterial drugs there is significant concern over the current shortfall in new agents to treat an increasing number of multidrug-resistant pathogens.[5,6]

ANTI-INFECTIVE DRUGS

Anti-infective drugs are generally classified according to their major microbial targets rather than by target disease or infection. Pharmacopoeias and formularies categorize these as antibacterial, antiviral, antifungal and antiparasitic (antiprotozoal or anthelmintic) agents. Within these categories some specific indications can be found such as drugs used in the treatment of HIV/AIDS.

Among anti-infective drugs the largest proportion is for the treatment of bacterial infections. For example, in the UK more than 100 antibacterial agents are available. However, many of these are derivatives of a limited number of classes of agents. As a result, antibacterial activity is dependent upon approximately a dozen distinct targets. This in part explains the ease with which resistance can emerge, disseminate and, in turn, often affect several agents within a single class of drug.

The burden of human infectious disease varies internationally. In tropical and subtropical countries parasitic disease is more common. However, the range of drugs available to treat these infections is limited and includes many agents that have been in clinical use for decades. The World Health Organization regularly publishes a list of 'essential' drugs.[7] The majority of such drugs are off-patent and generically available and therefore of low cost. However, this emphasis on generic well-established agents also reflects the low priority given to developing agents for diseases in resource-poor countries relative to the major markets of the world. However, more recently as a consequence of greater awareness through international travel and population migration, and the attention being paid to the HIV pandemic in the developing world, greater investment is being targeted at new drugs for diseases such as malaria and tuberculosis.

In contrast to the relative dearth of new agents to treat parasitic disease, drugs available to treat viral infections (Table 129.1), and in particular HIV/AIDS (Table 129.2), have increased remarkably in the past quarter century. There are now more than 20 agents available for managing HIV/AIDS, although none is curative. Agents effective against herpesviruses, notably herpes simplex, varicella-zoster and cytomegalovirus, have had a major impact on both primary and recurrent infections with these viruses and have been of particular benefit in controlling these infections as they occur in the context of HIV disease, transplantation and malignant disease.

GENERAL PRINCIPLES OF PRESCRIBING

The widespread availability and use of antibacterial agents to treat and prevent infectious disease needs to be balanced against the ever-present threat of drug resistance either among target pathogens or in micro-organisms making up the host normal flora. This can have impact beyond the individual patient since dissemination of either a resistant organism or a resistance mechanism can have serious consequences within hospitals and nursing homes or as a result of spread within the community at large.[6]

As a consequence a set of principles has emerged with the objective of not only ensuring safe and effective prescribing but also sustaining the efficacy of anti-infective drugs by reducing the risk of resistance emerging.[8] These principles govern such issues as when to prescribe and which agent(s) to select, as well as issues of dose, frequency and duration of therapy. The requirement for therapeutic drug monitoring applies to selected agents to ensure effective or nontoxic concentrations, or both. In addition, it is important to identify prior

Table 129.1 Antiviral drugs: non-HIV

Agent	Mechanism of action	Antiviral activity	Mechanism of resistance	Toxicity/side-effects
Aciclovir	Inhibits DNA polymerase, chain terminator. Requires viral thymidine kinase and cellular enzymes	HSV-1, HSV-2, VZV, CMV (much less activity), EBV (*in vitro*)	Mutations in thymidine kinase (more common) and mutations in DNA polymerase	Intravenous: phlebitis, crystalline nephropathy. Confusion, delirium, lethargy, tremors, nausea, vomiting, lightheadedness, diaphoresis, rash
Adefovir	Nucleotide analogue	HBV	Mutational resistance	Renal impairment at >30 mg q24h
Amantadine	Inhibits transmembrane protein M2, reduced uncoating of viral genome	Influenza A	Point mutation in gene encoding transmembrane domain M2 protein	Nervousness, anxiety, lightheadedness, confusion, insomnia
Cidofovir	Acyclic nucleoside phosphonate (does not require a virus-specific thymidine kinase)	HSV-1, HSV-2, VZV, EBV, CMV	Mutations in DNA polymerase	Severe nephrotoxicity, neutropenia, ocular hypotony, metabolic acidosis. Carcinogenic, teratogenic
Entecavir	Nucleoside reverse transcriptase inhibitor	HBV	Mutational resistance	Hepatotoxicity, headache, hyperglycemia, raised lipase
Famciclovir	Prodrug to penciclovir	See penciclovir	See penciclovir	Headache, nausea, diarrhea, vomiting, pruritus, LFT abnormalities
Fomivirsen (intravitreal)	Antisense oligonucleotide	CMV	*In-vivo* resistance not seen	Iritis, vitreitis, increased ocular pressures, visual changes
Foscarnet	Noncompetitive inhibitor of viral DNA polymerase (does not require thymidine kinase)	HSV-1, HSV-2, VZV, CMV, EBV, influenza A, influenza B, HBV, HIV	In CMV, single mutation in conserved region of DNA polymerase	Renal impairment, electrolyte disturbances, seizures, anemia, neutropenia, fever, nausea, vomiting, diarrhea, headache
Ganciclovir	Inhibitor of DNA polymerase, also competitive inhibitor of deoxyguanosine triphosphate (monophosphorylation by infection-induced kinases in HSV and VZV, and viral-encoded phosphotransferase in CMV-infected cells)	HSV-1, HSV-2, VZV, CMV, EBV, HHV-6	One or more point mutations in UL97, mutations in CMV DNA polymerase	Bone marrow suppression, fever, rash, increased LFTs, nausea, vomiting, eosinophilia, seizures, confusion, encephalopathy
Interferon-α (including pegylated interferon)	Induces changes in infected/exposed cells to promote resistance to infecting virus. Produces proteins that inhibit RNA synthesis, cleaves cellular and viral DNA, inhibits messenger RNA, alters cell membranes, inhibits release of replicated virions	Papillomavirus, HCV (pegylated), HBV, HDV, HIV	Fever, headache, chills, arthralgias, myalgias, fatigue, dizziness, neutropenia, thrombocytopenia, somnolence, depression, cognitive changes, suicidal ideation, increased LFTs, altered thyroid function, nausea, vomiting, diarrhea	
Lamivudine	Competitively inhibits viral reverse transcriptase, terminates proviral DNA chain extension	HBV, HIV	Mutations at YMDD locus (conserved domain reverse transcriptase)	Low-dose equivalent to placebo. High dose: headache, fatigue, insomnia, myalgias, arthralgias, diarrhea, rash, lactic acidosis, hepatomegaly

Oseltamivir	Neuraminidase inhibitor	Influenza A, influenza B	Mutations in viral neuraminidase and viral hemagglutinin	Nausea, vomiting
Penciclovir (topical)	Incorporated into DNA molecule	HSV-1, HSV-2, VZV, EBV (less so), CMV (less so), HBV (*in vitro*)	Mutations in thymidine kinase and mutations in DNA polymerase	Topical same as placebo
Ribavirin	Guanosine analogue, three possible mechanisms: competitive inhibition of host enzymes, inhibition of viral RNA polymerase complex, inhibition of messenger RNA formation	RSV, HCV (clinically); but also influenza A, influenza B, mumps, measles, parainfluenza, herpesviruses, togavirus, bunyavirus, adenovirus, Coxsackie virus, hemorrhagic fever virus, HAV, HBC, Lassa fever virus, Hantavirus	Anemia, hyperbilirubinemia, elevated uric acid, nausea, headache, lethargy. Teratogenic, mutagenic, embryotoxic, gonadotoxic	
Rimantadine	Inhibits transmembrane protein M2, reduced uncoating of viral genome	Influenza A	Point mutation in gene encoding transmembrane domain M2 protein	Nervousness, anxiety, lightheadedness, confusion, insomnia (much less so than amantadine)
Trifluridine (topical)	Pyrimidine nucleoside	HSV-1, HSV-2, CMV, vaccinia, some adenoviruses		
Valaciclovir	Valine ester of aciclovir	See aciclovir	See aciclovir	Headache, nausea, abdominal pain
Valganciclovir	Metabolized to ganciclovir	See ganciclovir	See ganciclovir	Bone marrow suppression, fever, nausea, headache, vomiting, insomnia, abdominal pain, peripheral neuropathy, paresthesias, potential carcinogen
Zanamivir (aerosolized/ intranasal)	Neuraminidase inhibitor	Influenza A, influenza B	Mutations in viral neuraminidase and viral hemagglutinin	Nasal, throat discomfort, bronchospasm in asthmatics

CMV, cytomegalovirus; EBV, Epstein–Barr virus; HAV, hepatitis A virus; HBV, hepatitis B virus; HCV, hepatitis C virus; HDV, hepatitis D virus; HHV, human herpesvirus; HSV, herpes simplex virus; LFT, liver function test; RSV, respiratory syncytial virus; VZV, varicella-zoster virus.

Table 129.2 Antiviral drugs: HIV

Agent	Mechanism of action	Toxicity/side-effects
Abacavir	Nucleoside reverse transcriptase inhibitor	Hypersensitivity syndrome, rash, fever, nausea, vomiting, diarrhea, abdominal pain, elevated LFTs
Atazanavir	Protease inhibitor	Increased unconjugated bilirubin, gastrointestinal symptoms
DAPD	Nucleoside reverse transcriptase inhibitor	Nausea, vomiting, diarrhea, abdominal pain, hepatitis
Darunavir	Protease inhibitor	Hepatoxicity, rash, nausea, diarrhea, erythema multiforme, lipodystrophy
Delavirdine	Non-nucleoside reverse transcriptase inhibitor	Rash, headache, Stevens–Johnson syndrome, nausea, depression
Didanosine	Nucleoside reverse transcriptase inhibitor	Nausea, vomiting, diarrhea, abdominal pain, peripheral neuropathy, pancreatitis
Efavirenz	Non-nucleoside reverse transcriptase inhibitor	Dizziness, difficulty concentrating, nausea, vomiting, diarrhea, rash, flu-like symptoms
Emtricitabine	Nucleoside reverse transcriptase inhibitor	Nausea, diarrhea, headache
Enfurvirtide	Fusion inhibitor	Injection site inflammation
Etravirine	Non-nucleoside reverse transcriptase inhibitor	Rash, hypersensitivity reaction, nausea
Fosamprenavir	Protease inhibitor	Rash, hyperlipidemia, diarrhea, lipodystrophy, headache
Indinavir	Protease inhibitor	Nephrolithiasis, nausea, dysgeusia, benign hyperbilirubinemia
Interleukin-2	Peripheral expansion of existing $CD4^+$ lymphocytes	Nausea, vomiting, diarrhea, fever, asthenia, pruritus
Lamivudine	Nucleoside reverse transcriptase inhibitor	No significant toxicity, peripheral neuropathy, pancreatitis
Lopinavir	Protease inhibitor	Nausea, asthenia, diarrhea
Maraviroc	Co-receptor blocker	Hepatoxicity, fever, rash, abdominal pain
Nelfinavir	Protease inhibitor	Diarrhea
Nevirapine	Non-nucleoside reverse transcriptase inhibitor	Dizziness, rash, difficulty concentrating, nausea, vomiting, diarrhea, flu-like symptoms
Raltegravir	Integrase inhibitor	Rash, hyperlipidemia, injection site pain
Ritonavir	Protease inhibitor	Dysgeusia, nausea, vomiting, diarrhea, circumoral paresthesias, increased triglycerides, LFTs, CPK and uric acid
Saquinavir	Protease inhibitor	Nausea, vomiting, diarrhea, headache
Stavudine	Nucleoside reverse transcriptase inhibitor	Peripheral neuropathy
Tenofovir	Nucleoside reverse transcriptase inhibitor	Nausea, vomiting, diarrhea, headache, elevated LFTs and CPK
Tipranovir	Protease inhibitor	Nausea, vomiting, diarrhea
Zidovudine	Nucleoside reverse transcriptase inhibitor	Anemia, neutropenia, nausea, vomiting, myositis, neuropathy

CPK, creatine phosphokinase; LFTs, liver function tests.

experience of adverse drug reactions in an individual as well as the potential future risks of toxicity from hypersensitivity or other effects. Here the importance of drug interactions is stressed. One additional issue specific to antibacterial drugs is the ability of some to select for superinfecting organisms such as *Clostridium difficile* or *Candida* spp. These pathogens also have the ability to disseminate within the hospital to cause epidemic disease.

Rationale for therapeutic prescribing

Antimicrobial prescribing should be based on clear evidence of infection established by either laboratory investigation or sound clinical criteria. In general, laboratory confirmation of infection, although desirable, is only possible in a minority of treated infections and is largely confined to hospitalized patients where access to laboratory investigation is readily available.

The majority of prescribing is based on a clinical assessment of infection where symptoms and signs point to the likely source and nature of the disease. The need to distinguish infectious from noninfectious causes of systemic inflammation is of fundamental clinical importance and has led to clear criteria which support the identification of the 'sepsis syndrome'. These have particular application in managing severe sepsis.[9] Table 129.3 summarizes the differences between the systemic inflammatory response, sepsis, severe sepsis and septic shock (see also Chapter 44).

Once a definitive or presumptive clinical diagnosis has been made, such as pneumonia or urosepsis, a putative microbiologic diagnosis can be inferred based on knowledge, preferably locally derived, of the usual microbiologic causes of a particular infection. There are only a few clinical diagnoses that can confidently predict the exact microbiologic cause of an infection; examples include erysipelas (*Streptococcus pyogenes*), cutaneous anthrax (*Bacillus anthracis*) and herpesvirus infections such as herpes simplex and varicella-zoster.

Table 129.3 Definitions of systemic inflammatory response, sepsis, severe sepsis and septic shock

Systemic inflammatory response
• Temperature >38 °C or <35 °C • Heart rate >90 beats/min • Respiratory rate >20 breaths/min or $Paco_2$ <32 mmHg • WBC >12 000 cells/mm^3 or <4000 cells/mm^3 or >10% immature (band) forms
Sepsis
Clinical features of SIRS plus culture-proven infection *or* Infection identified by visual inspection
Severe sepsis
Sepsis plus one or more signs of organ hypoperfusion or dysfunction: • Areas of mottled skin • Capillary refilling requires 3 seconds or longer • Urine output <0.5 ml/kg for at least 1h, or renal replacement therapy • Lactate >2 mmol/l • Abrupt change in mental status • Abnormal electroencephalographic findings • Platelet count <100 000 platelets/ml • Disseminated intravascular coagulation • Acute lung injury or acute respiratory distress syndrome • Cardiac dysfunction, as defined by echocardiography or direct measurement of the cardiac index
Septic shock
Severe sepsis plus one or more of the following: • Systemic mean blood pressure is <60 mmHg (or <80 mmHg if the patient has baseline hypertension) despite adequate fluid resuscitation • Maintaining the systemic mean blood pressure >60 mmHg (or >80 mmHg if the patient has baseline hypertension) requires dopamine >5 µg/kg per min, noradrenaline (norepinephrine) <0.25 µg/kg per min or adrenaline (epinephrine) <0.25 µg/kg per min despite adequate fluid resuscitation

The results of microbiologic sampling may confirm the nature of an infection and support the continued use of initial empiric treatment or indicate alternative therapy. However, most prescribing is empiric, particularly in community practice, which accounts for about 80–90% of antimicrobial prescribing. Many such infections are managed satisfactorily in the absence of microbiologic investigation.

Prophylactic prescribing

In contrast to therapeutic prescribing for an established infection, antimicrobial agents are prescribed for a variety of medical and surgical indications in order to prevent infection arising as a complication of that condition or surgery. Such prophylactic use must recognize that the risk:benefit ratio must be justified since prophylactic prescribing means that a large number of patients, of whom only a proportion will be at risk of infection, will be exposed to antimicrobial agents. The selected regimen must therefore have been demonstrated to be safe as well as effective in the particular indication.

Surgical prophylaxis

Many surgical procedures such as large bowel surgery and cholecystectomy were formerly associated with high rates of infectious complications. However, short-course (single-dose) perioperative prophylaxis given at the time of induction of anesthesia substantially reduces this risk.[10] The antimicrobial spectrum of the drug(s) selected must be appropriate for the micro-organisms most commonly responsible for complicating infections.[11] In the case of large bowel surgery an extended spectrum cephalosporin combined with metronidazole, both administered as a single dose, are effective. Amoxicillin–clavulanate, ampicillin–sulbactam, cefoxitin and cefotetan provide alternative choices.

Joint arthroplasty and cardiac valve implant surgery present a particular risk. Micro-organisms introduced at the time of surgery can have serious consequences. Coagulase-negative staphylococci and *Staphylococcus aureus* (including methicillin-resistant *Staph. aureus*, MRSA) are particularly frequent pathogens. Perioperative prophylaxis using an antistaphylococcal penicillin such as flucloxacillin or oxacillin is widely used; however, where there are concerns over the possibility of drug-resistant strains, vancomycin is preferred.[12]

Medical prophylaxis

Medical prophylaxis refers to the use of antimicrobial prophylaxis in a variety of nonsurgical conditions and procedures known to be at risk of serious infectious complications. Anatomic or functional asplenia predisposes to severe sepsis, particularly in childhood where *Strep. pneumoniae, Haemophilus influenzae* or *Neisseria meningitidis* is seen. Prophylaxis using long-term daily administration of low-dose oral penicillin (erythromycin in the penicillin allergic) is commonly recommended during childhood (see Chapter 82).[13,14]

Infective endocarditis complicates bloodstream infection in selected, very-high-risk patients with pre-existing valvulopathy; this risk justifies perioperative antibiotic prophylaxis for certain procedures in the oral cavity, although the efficacy of this approach has not been validated and remains controversial (see Chapter 47 for detailed discussion).[15–17] The choice of antibiotic is determined by the nature of the operative procedure and recent history of antibiotic use, as well as the potential for drug intolerance in the recipient.

Malaria is a risk to those traveling to the tropics and subtropics. The use of antimalarial agents for the period immediately prior to departure, during residence in a malaria risk country and for 4 weeks after return, is an effective and well established practice in preventing this potentially life-threatening disease.[18] The choice of regimen is determined by the itinerary of the traveler, the risk of exposure to chloroquine-sensitive or chloroquine-resistant infection, resistance to other antimalarial agents and the potential for adverse drug reactions. These regimens are discussed in Chapters 111 and 150.

A further example of medical prophylaxis is the use of postexposure prophylaxis in those exposed to HIV infection as a result of a needlestick injury. This primarily affects health-care workers who have sustained a percutaneous hollow needle injury from a known or presumptive HIV-infected source. Prompt administration of combined antiretroviral drugs (e.g. zidovudine, lamivudine and lopinavir/ritonavir) for a period of 4 weeks is currently recommended after a careful assessment of the risks (see Chapter 86).[19] Such a regimen is effective but may give rise to significant toxicity in some individuals.

Choice of agent or agents

Fundamental to the treatment of infectious diseases is the selection of an antimicrobial regimen with established efficacy against the target infection based on clinical trials and supported by widespread clinical use. The antimicrobial spectrum of activity should be based on susceptibility data from epidemiologic studies and ideally confirmed by laboratory testing of antimicrobial samples. The most frequently used methods are disc diffusion tests – such as the Kirby–Bauer method in North America or the British Society for Antimicrobial Chemotherapy (BSAC) method in Europe – and the determination of the minimum inhibitory concentration (MIC) of the antibiotic. Disc susceptibility testing involves the agar inoculation of an approximated number of bacteria as a 'lawn', with the overlaying of antibiotic-impregnated discs. After overnight incubation, zones of inhibition appear around the discs

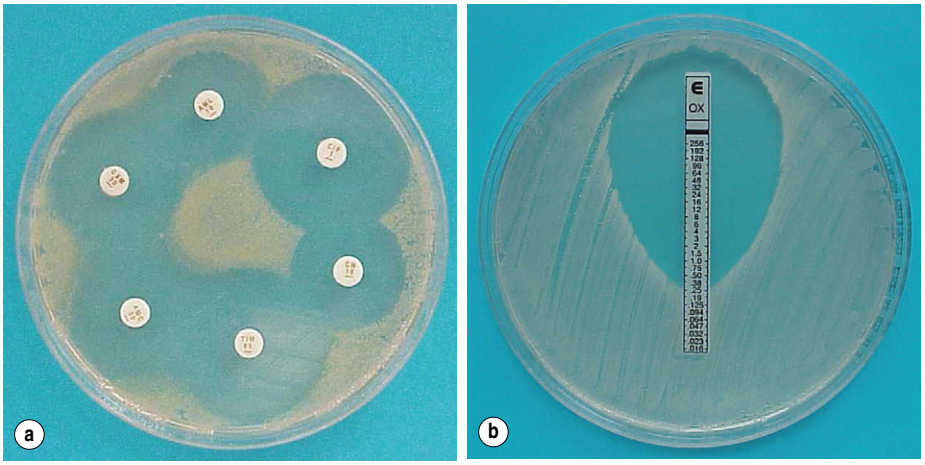


Fig. 129.1 (a) A disc diffusion sensitivity plate showing a fully sensitive coliform tested against a typical range of first-line antibiotics. Courtesy of Dr M Cubbon, Brighton, UK. (b) An E-test showing a methicillin-sensitive *S. aureus*. The MIC of oxacillin for this strain is 0.25 mg/l, and is obtained by noting the point at which the zone of inhibition intersects with the test strip. Courtesy of Dr M Cubbon, Brighton, UK.

impregnated with antibiotics to which the organism is susceptible (Fig. 129.1a). MIC testing used to be done by agar or broth dilution, but now most laboratories use an ingenious antibiotic-impregnated strip, the E-test (Fig. 129.1b) or other automated techniques. In the USA, the National Committee for Clinical and Laboratory Standards Institute (CLSI) and the European Committee on Antimicrobial Susceptibility Testing (EUCAST) set 'breakpoints' for the MIC based on the integration of MICs with achievable antibiotic levels in serum and tissues, clinical pharmacology and data from *in-vitro* and animal models. Typically these results are presented to the clinician as a report of 'sensitive', 'intermediate' or 'resistant'.

For example, amoxicillin is widely used to treat mild to moderate community respiratory infections such as otitis media which is caused by *Strep. pneumoniae, Strep. pyogenes* or *H. influenzae* infection to which amoxicillin is active against the majority of isolates. In contrast, acute lower urinary tract infection (cystitis) is usually caused by enteric pathogens and requires agents such as trimethoprim or nitrofurantoin for its management. However, resistance to amoxicillin by *H. influenzae* and to trimethoprim among *Escherichia coli* is threatening the efficacy of these agents.

Broad-spectrum versus narrow-spectrum antimicrobial agents

The terms 'broad spectrum' and 'narrow spectrum' are widely used to distinguish between agents that target a limited range of pathogens in contrast to broad-spectrum agents to which many pathogens are susceptible. Historically, the terms have been applied to developments in the penicillin class of drugs. Penicillin G (benzylpenicillin) targeted a limited number of pathogens – for example, *Strep. pneumoniae*, hemolytic streptococci and *Neisseria* spp. (meningococci and gonococci). With the development of the aminopenicillins (e.g. amoxicillin), the spectrum increased to also include *H. influenzae* and *E. coli* and were thus considered as broad-spectrum agents; however, as stated above, with time many of these pathogens have become resistant as a result of β-lactamase production.

Among currently available agents the carbapenems (e.g. meropenem) and the ureidopenicillins (e.g. piperacillin) are truly broad-spectrum agents with activity against many Gram-positive and Gram-negative pathogens and, in the case of meropenem, many anaerobic bacteria as well. At the present time vancomycin, other glycopeptides and linezolid are considered narrow-spectrum agents. This contrasts with many other classes and individual drugs. Figure 129.2 provides a summary of the common susceptibility patterns for many commonly prescribed antibacterial drugs.[20]

The antimicrobial spectrum of an antibiotic has important practical implications. Narrow-spectrum agents have a more limited range of indications and in general require greater diagnostic precision to ensure they are used appropriately. They also require greater emphasis on documenting the microbiologic nature of an infection. In contrast, broad-spectrum agents usually capture a greater range of micro-organisms and indications. They are also widely used as initial empiric therapy of respiratory tract and intra-abdominal infections. Microbiologic sampling, although desirable, is not essential for their successful clinical use.

One of the consequences of broad-spectrum agents is their impact on the normal flora, particularly that of the gastrointestinal tract. As a consequence, microbial overgrowth by resistant organisms such as *Candida* spp. and *C. difficile* may occur and give rise to superinfection or *C. difficile*-associated diarrhea, respectively.[21]

Bactericidal versus bacteristatic activity

Antibacterial drugs act by either killing or inhibiting the growth of micro-organisms. These bactericidal or bacteristatic effects are determined by the mode of action of a drug against a particular target organism. Table 129.4 identifies the usual mode of action of commonly prescribed agents.

Cell-wall active agents, such as the penicillins, cephalosporins and glycopeptides, are largely bactericidal as are many (but not all) agents that interfere with protein synthesis. Examples of the latter include the tetracyclines. Metabolic inhibitors of folic acid synthesis, namely the sulfonamides and trimethoprim, are also bacteristatic. Cidality can be affected by drug concentration and *in-vitro* conditions of testing, and may also vary by target pathogen. Nonetheless, it is a useful distinguishing feature with clinical implications in selected circumstances.

In general, bactericidal and bacteristatic agents are equally effective in the management of many mild to moderate infections. However, bacteristatic agents are dependent upon an effective host immune response, notably the ability to phagocytose drug-exposed bacteria. There are a number of clinical conditions in which host immunity is either deficient or suppressed and where bacteristatic agents should be avoided in favor of bactericidal agents. Such immunocompromised patients may be either immunodeficient or immunosuppressed.

Immunodeficiency captures a variety of congenital and acquired defects of immunity, of which many are associated with an increased frequency and severity of infection. While immunosuppression can also arise from impaired host defenses as a result of underlying disease, it is more often a complication of cytotoxic chemotherapy, immunosuppressant treatment or radiotherapy. Patients undergoing organ or bone marrow transplantation or cancer therapy are particularly at risk of infection when profoundly neutropenic.[22] The period of immunosuppression is usually limited as the target disease responds to treatment.

For the above reasons bactericidal agents are preferred when managing infection in immunocompromised patients, especially those with neutropenia. Other circumstances where bactericidal agents are recommended are in the management of infective endocarditis.[23–25] The

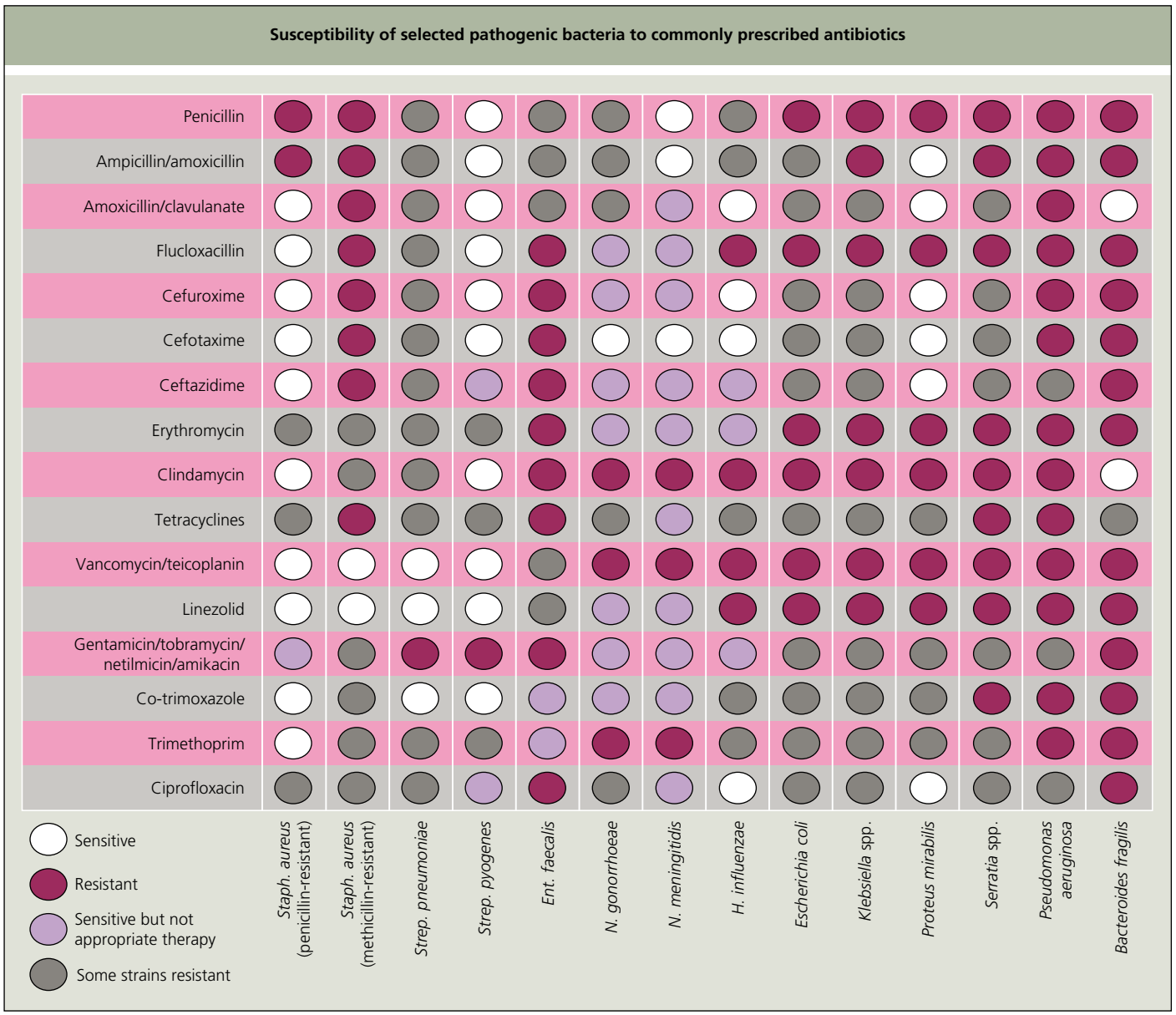


Fig. 129.2 Susceptibility of selected pathogenic bacteria to commonly prescribed antibiotics. Adapted with permission from Warrell DA, Cox TM, Firth JD, eds. *Oxford Textbook of Medicine*, 5th ed. Oxford: Oxford University Press; 2010.

infected vegetations are impermeable to phagocytic cells and require high plasma concentrations of bactericidal drug(s) to sterilize them.

Dose selections and frequency of administration

In an ideal world the correct dose and frequency of administration of an antimicrobial should be accurately defined and confirmed by clinical trials that focus on efficacy and safety in the management of most common infections. However, in the treatment of infection there are many biologic variables that are either host or pathogen derived. Furthermore, the pathophysiologic response to infection adds additional variables. Despite these caveats the selection of antimicrobial chemotherapy is supported by a matrix of information that includes *in-vitro* evaluation of antimicrobial activity, animal models of infections, pharmacokinetic studies of the drug in health and disease, and clinical trial data developed to support the approved indications. Table 129.5 identifies some common bacterial infections affecting adults and the agents used in their treatment.

Pharmacokinetics

The pharmacokinetic profile of a drug is a reflection of its absorption, distribution, metabolism and excretion. The bioavailability of a drug indicates the degree of absorption from the gastrointestinal tract. Parenterally administered agents, such as the aminoglycosides, which are not absorbed from the gut achieve 100% bioavailability. The bioavailability of orally administered antibiotics ranges from 20% for sulfasalazine to up to 100% for ciprofloxacin, nitrofurantoin and cefaclor, for example. To be effective at the site of infection, antibiotics must be well distributed in the tissues. In general, lipophilic agents (e.g. chloramphenicol) and uncharged or nonpolar drugs (e.g. fluoroquinolones) are able to cross biologic membranes and achieve effective concentrations in tissues. The apparent volume of distribution of an antibiotic (V_d) is calculated by dose/plasma concentration and may reflect the ability of the drug to enter inflammatory cells and tissues.

Drugs once absorbed are generally distributed in the blood and tissues bound to plasma proteins, most notably albumin. The degree of protein binding varies from drug to drug. The unbound or 'free' drug is the biologically active moiety which is in equilibrium with the

Table 129.4 Examples of microbicidal and microbistatic antibacterial agents

Antibacterial agent or class	Target	Action
	Peptidoglycan synthesis	
β-Lactams	Transpeptidases	Cidal
Glycopeptides	D-alanyl-D-alanine ligase	Cidal
Daptomycin	Dephosphorylation of lipids	Cidal
Isoniazid, ethionamide, ethambutol	*Mycolic acid synthesis*	Cidal
	Protein synthesis	
Aminoglycosides, tetracyclines, oxazolidinones	Ribosomal 30S subunit	Static
Chloramphenicol, macrolides, lincosamides, streptogramins, oxazolidinones	Ribosomal 50S subunit	Static
Mupirocin	tRNA synthetase	Cidal
Fusidic acid	Elongation factor G	Cidal
	Nucleic acid synthesis	
Metronidazole, nitrofurantoin	DNA disruption	Cidal
Quinolones	DNA gyrase A and topoisomerase IV	Cidal
Novobiocin	DNA gyrase B	Cidal
Rifampin (rifampicin)	RNA	Cidal
Flucytosine	RNA and DNA synthesis	Cidal
	Folic acid metabolism	
Trimethoprim, *para*-aminosalicylic acid	Dihydrofolate reductase	Static
Sulfonamides, diaminodiphenyl sulfone	Dihydropterate synthase	Static

Table 129.5 Common clinical sites for bacterial infections in adults, frequently encountered organisms and appropriate antibiotics*

Infection site	Common bacterial etiology	Appropriate antibiotics†
Oropharynx, tonsil	*Streptococcus pyogenes*	Penicillin V × 10 days, macrolides, second-generation cephalosporin, clindamycin
Acute bacterial sinusitis	*Strep. pneumoniae, Haemophilus influenzae, Moraxella catarrhalis*, group A streptococcus	Amoxicillin, ampicillin–clavulanate, cefpodoxime, cefuroxime axetil, oral first- or second-generation cephalosporin
Acute exacerbation of chronic bronchitis	*Strep. pneumoniae, H. influenzae, M. catarrhalis*	Mild: amoxicillin, doxycycline, trimethoprim–sulfamethoxazole, oral cephalosporin Severe: ampicillin–clavulanate, azithromycin, oral cephalosporin, levofloxacin, gatifloxacin, moxifloxacin
Pneumonia, community-acquired, smoker	*Strep. pneumoniae, H. influenzae, M. catarrhalis*	Outpatient: azithromycin, fluoroquinolone, second-generation cephalosporin
Pneumonia, community-acquired, non-smoker	*M. pneumoniae, Chlamydia pneumoniae, Strep. pneumoniae*	As above
Pneumonia, community-acquired	As above	Inpatient: third-generation cephalosporin, plus erythromycin or azithromycin, fluoroquinolone
Urinary tract infection, uncomplicated cystitis	Enterobacteriaceae	Trimethoprim–sulfamethoxazole, trimethoprim, nitrofurantoin, fluoroquinolone
Pyelonephritis	Enterobacteriaceae, *Enterococcus* spp.	Fluoroquinolone, ampicillin plus gentamicin, third-generation cephalosporin, ticarcillin–clavulanate, ampicillin–sulbactam, piperacillin–tazobactam
Urethritis, gonococcal	*Neisseria gonorrhoeae*	Ceftriaxone, cefixime, ciprofloxacin, ofloxacin
Urethritis, nongonococcal	*Chlamydia* spp., *Mycoplasma hominis, Ureaplasma* spp.	Doxycycline, azithromycin
Pelvic inflammatory disease	*N. gonorrhoeae, Chlamydia* spp., *Bacteroides* spp., Enterobacteriaceae, *Streptococcus* spp.	Ofloxacin or levofloxacin plus metronidazole, ceftriaxone plus doxycycline, cefotetan or cefoxitin plus doxycycline, ampicillin–sulbactam plus doxycycline
Prostatitis, <35 years old	*N. gonorrhoeae, Chlamydia* spp.	Ofloxacin, ceftriaxone plus doxycycline
Prostatitis, >35 years old	Enterobacteriaceae	Fluoroquinolone, trimethoprim–sulfamethoxazole, aminoglycoside

Table 129.5 Common clinical sites for bacterial infections in adults, frequently encountered organisms and appropriate antibiotics*—cont'd

Infection site	Common bacterial etiology	Appropriate antibiotics†
Gastroenteritis	*Shigella* spp., *Salmonella* spp., *Campylobacter* spp., *Escherichia coli* O157 H7	Fluoroquinolone
Cholecystitis	Enterobacteriaceae, enterococci, anaerobes	Ampicillin–sulbactam, piperacillin–tazobactam, imipenem, meropenem
Diverticulitis	Enterobacteriaceae, anaerobes, enterococci	Ampicillin–sulbactam, piperacillin–tazobactam, imipenem, meropenem, metronidazole plus fluoroquinolone
Spontaneous bacterial peritonitis	Enterobacteriaceae, *Strep. pneumoniae*	Cefotaxime, ceftriaxone, ticarcillin–clavulanate, piperacillin–tazobactam, ampicillin–sulbactam
Cellulitis	Group A streptococcus, *Staphylococcus aureus*	Nafcillin, oxacillin, flucloxacillin first-generation cephalosporin, erythromycin, ampicillin–clavulanate
Septic arthritis, monoarticular, sexually active	*N. gonorrhoeae*	Ceftriaxone, cefotaxime, ceftizoxime
Septic arthritis, monoarticular, not sexually active	*Staph. aureus, Streptococcus* spp., Gram-negative *L. bacillus*	Nafcillin, oxacillin or flucloxacillin or clindamycin or vancomycin plus third-generation cephalosporin or ciprofloxacin
Septic arthritis, prosthetic	*Staph. epidermidis, Staph. aureus*, Enterobacteriaceae, *Pseudomonas* spp.	Vancomycin ± rifampin (rifampicin) plus ciprofloxacin or aztreonam, or ceftazidime or cefepime or aminoglycoside
Osteomyelitis	*Staph. aureus*	Nafcillin, oxacillin, first-generation cephalosporin, vancomycin
Meningitis	*Strep. pneumoniae, N. meningitidis*	Ceftriaxone ± vancomycin
Endocarditis, native valve	Viridans streptococcus, other streptococcal species, enterococci, staphylococci	Penicillin or ampicillin, plus nafcillin–oxacillin, plus gentamicin; vancomycin plus gentamicin
Endocarditis, prosthetic valve	*Staph. epidermidis, Staph. aureus*	Vancomycin plus gentamicin ± rifampin (rifampicin)

*This table is not intended to be all-inclusive. Specific infections are considered in detail in other chapters. Note that some agents have restricted geographic availability.
†This is a general overview; the choice of antibiotics must consider the resistance pattern in any given geographic area (e.g. penicillin and macrolide resistance in *Strep. pneumoniae*; ampicillin and trimethoprim–sulfamethoxazole resistance among *E. coli*), as well as adjustments based on the identified etiologic agent and susceptibility testing.

bound fraction. In general, the degree of protein binding has a limited impact on the outcome of most infections. However, highly bound agents (>95%) may have difficulty crossing some biologic membranes which may have clinical importance when treating infections of the joints, abscesses and the meninges.

Drugs may be excreted unchanged but in general undergo metabolism, especially in the liver for subsequent excretion in the bile. Typical metabolites are the result of glucuronidation, conjugation and acetylation. Drugs metabolized by the cytochrome P450 system may interact with other drugs which share this route. Most metabolites are microbiologically inactive. One exception is the desacetyl metabolite of cefotaxime, which contributes in part to the antimicrobial activity of the parent drug.

In those with impaired liver function, drugs excreted through the liver should be used with caution or avoided. Dose adjustment may be appropriate following careful assessment of liver function tests. The Child–Pugh assessment is often used as an indicator of the severity of liver impairment in clinical trials of new agents in order to guide dosaging.[26,27]

Drug excretion is also largely the result of renal excretion. The latter may be either a metabolically active or passive process, and involve the glomeruli or renal tubules or both. Impairment of renal function often leads to a prolonged drug half-life for renally excreted drugs. Dose modification may be required to avoid drug accumulation and drug toxicity.

A clear understanding of the elimination half-life and excretion of antibiotics also influences dosage selection. Drugs with a short half-life are rapidly eliminated and need more frequent administration to produce satisfactory antibiotic concentrations at the site of infection. Drugs with a longer half-life are often more convenient, especially those where single daily dosing is effective.

Pharmacokinetic and pharmacodynamic parameters

The pharmacokinetic (PK) variables that are most useful in antibiotic chemotherapy include bioavailability (F), maximal serum concentration (C_{max}), time to reach C_{max} (t_{max}), elimination half-life ($t_{½}$) and area under the drug concentration curve following a dose (AUC).

Recently the pharmacodynamic (PD) characteristics of antimicrobials have become important in determining dosage schedules. In addition, they can be used as predictors of safety and the risks of selecting drug-resistant organisms. Antibiotic pharmacodynamics can be described as the interrelation between pharmacokinetics (drug concentrations) and the antimicrobial effects that result from these concentrations in, for instance, serum, tissues and body fluids. The most useful pharmacokinetic and pharmacodynamic variables are summarized in Table 129.6. These include the ratio of the area under the 24-hour concentration–time curve to the MIC (AUC_{24}/MIC) the ratio of C_{max} to MIC

Table 129.6 Pharmacokinetic and pharmacodynamic parameters that affect antibiotic therapy

Parameters		Details
Pharmacokinetic	*F*	Bioavailability, fraction of the administered dose absorbed intact; intravenous drugs have 100% bioavailability; oral drugs vary with absorption and are usually less bioavailable than intravenous forms
	C_{max}	Maximal serum concentration after single or multiple doses
	t_{max}	Time after drug administration to reach C_{max}
	$t_{½\ elim}$	Elimination half-life; time to reduce peak serum concentration by 50%
	AUC	Area under the concentration–time curve (relates to total drug exposure following a dose)
Pharmacodynamic	C_{max}/MIC, peak/MIC	Ratio of the maximum serum concentration to the MIC (predicts activity of concentration-dependent bactericidal antibiotics)
	AUC/MIC	Ratio of the area under the concentration–time curve to the MIC (also predicts activity of concentration-dependent bactericidal antibiotics)
	t >MIC, t_{eff}	Time above the MIC; the duration of time during a dosing interval that serum concentration remains above the MIC (predicts activity of time-dependent bactericidal antibiotics)

(peak/MIC) and the time during a given dosing interval that the serum concentration remains above the MIC (time above MIC, *t* >MIC).[8]

Data based on PK:PD parameters and timed plasma samples from volunteers and patients may be employed in mathematical models using approaches such as Monte Carlo simulations. This has greatly contributed to defining effective and safe dosage schedules for many recent antibacterial[28] and antiviral agents,[29] and most recently to define optimum schedules for antifungal drugs.[30]

Using this basic pharmacokinetic data and *in-vitro* susceptibility profiles of micro-organisms, the relationship between PK and PD variables can be characterized graphically.[31] Figure 129.3 illustrates these key parameters in relation to the MIC of a target pathogen. The relationship of C_{max} and AUC to MIC as well as *t* (time) above MIC can distinguish drugs which exhibit concentration-dependent killing from those characterized by time-dependent killing.[28] Examples of the former include the fluoroquinolones and aminoglycosides and, in the case of the latter, the penicillins and cephalosporins.

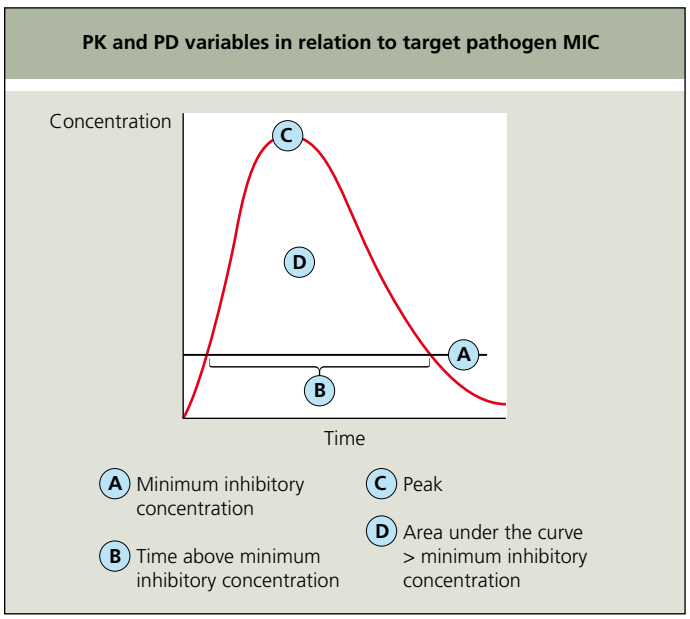


Fig. 129.3 The relationship between the pharmacokinetic profile of an antibiotic and the minimum inhibitory concentration (MIC) against a hypothetical target organism. Reproduced with permission from Finch.[8]

Such PK:PD data have supported single daily dosage regimens for the aminoglycosides[32] and dosage revisions for ciprofloxacin and more recently levofloxacin.[33] PK:PD data are also important in determining the dose and frequency of administration of new drugs and as an indicator of the emergence of resistance in marginally susceptible pathogens.

Postantibiotic effects

Bacterial growth may be inhibited following exposure to an antibiotic even after the drug concentration has fallen below the MIC. This is known as the postantibiotic effect (PAE) and is determined *in vitro* by observing bacterial growth after drug removal.[34] Animal models have been described which measure PAE *in vivo*. Postantibiotic effects can vary by drugs and micro-organism. For example, prolonged PAEs have been reported after aminoglycoside or fluoroquinolone exposure of Gram-negative *L. bacilli*, whereas most β-lactam antibiotics exhibit shorter PAEs. The prolonged PAE for gentamicin against Gram-negative bacilli has provided additional support for current once-daily dosage schedules.

Mutant preventing concentrations

Target pathogens with MICs at or below clinically achievable concentrations of drug may develop resistance. This was recognized in relation to *Pseudomonas aeruginosa* and *Strep. pneumoniae* exposed to borderline plasma concentrations of ciprofloxacin. Dosage schedules are now better designed and selected to minimize this risk by defining the mutant preventing concentration (MPC) of a drug against selected target pathogens.[35]

Drug safety

No drug is free from side-effects. Adverse reactions range from drug hypersensitivity to dose-related side-effects and unpredictable or idiosyncratic phenomena. They may be trivial and readily reversible, have more serious consequences or be life-threatening and occasionally fatal. They should be constantly under consideration by the prescriber and reported to the regulatory authorities, particularly in relation to new agents and in the case of unusual or serious side-effects. They should also be prominently and readily identified within the patient's medical record.

With regard to antibiotics, the penicillins and cephalosporins share the β-lactam ring structure and in turn have many adverse effects in common. These include hypersensitivity rashes and other reactions and toxicity to hematopoietic cells. However, there are differences between these two classes. Hypersensitivity reactions occur less commonly to the cephalosporins. This applies to anaphylaxis which is the

most severe and feared hypersensitivity reaction. Any severe hypersensitivity reaction to a β-lactam is a bar to its future use and of the β-lactam agents in general. However, milder forms of hypersensitivity, such as skin eruption to a penicillin, do not necessarily preclude the use of a cephalosporin.

Many adverse drug events can be minimized in persons with known excretory organ malfunction by adjusting the dose. The aminoglycosides, such as gentamicin, have been extensively studied and dosage schedules developed which are adjusted for age, weight, gender and renal function.

Prescribing of antimicrobial drugs, as in other areas of medicine, must be based on a careful assessment of the patient, the nature and severity of the disease being treated and any identifiable predispositions to drug toxicity. Such a risk assessment may lead to the decision to use a drug with a higher adverse reactions profile in the severely ill patient which contrasts to the management of mild, often self-limiting community infections where safety has a higher priority. In relation to prophylactic use, only agents with the highest safety profiles are appropriate, since in only a proportion of recipients will clear benefit ensue.

Therapeutic drug monitoring

Therapeutic drug monitoring (TDM) aims to ensure safe and effective prescribing for drugs with a narrow therapeutic index. The latter reflects the limited margin between the therapeutic and toxic concentrations of a particular drug. In contrast, drugs such as penicillins have a wide therapeutic index and even when given in high concentration are generally safe.

In practice much TDM is directed at ensuring therapeutic nontoxic concentrations of the aminoglycosides. Other examples include TDM for antifungals such as itraconazole and most recently voriconazole[36] in the treatment of severe fungal infections as well as, in some circumstances, antiretroviral agents such as the protease inhibitors and non-nucleoside reverse transcriptase inhibitors.[37]

Combined drug regimens

In general and whenever possible it is preferable to manage an infection with a single drug. Provided the drug is effective this reduces the cost of treatment and limits the risk of adverse reactions. However, this demands that the agent selected is not only appropriate to the nature of the infection but also that there is either microbiologic documentation or strong clinical evidence as to the likely microbiologic cause and that it will be susceptible to the agent selected.

There are an increasing number of diseases and circumstances where a combination of drugs is appropriate or necessary to control the infection. Examples include tuberculosis,[38] HIV/AIDS infection[39] and, more recently, malaria.[40] However, in practice, drugs are frequently combined where there is uncertainty about the microbiologic nature of a diagnosis or where the spectrum of a single agent is inadequate, particularly in situations such as intra-abdominal sepsis. Here infection is often polymicrobial with both aerobic and anaerobic pathogens present. Combining an extended-spectrum cephalosporin, such as cefuroxime or cefotaxime, with metronidazole provides an appropriate 'broad-spectrum' regimen. Another example is in the treatment of severe community-acquired pneumonia when both atypical and conventional pathogens need to be 'covered'.[41,42]

Another important reason for combined drug regimens is to prevent resistant organisms emerging on therapy. This was first demonstrated in the treatment of tuberculosis where minority populations of organisms can become resistant to isoniazid or rifampin (rifampicin). These occur at low frequency, being of the order of 1 in 10^8 to rifampin and 1 in 10^9 to isoniazid. Single-drug therapy would eliminate the susceptible organisms and initially be accompanied by clinical improvement. However, the minority resistant populations subsequently multiply and eventually cause relapse of disease which is much harder to treat. For these reasons the standard regimen for treating pulmonary tuberculosis includes isoniazid and rifampin combined with a third (pyrazinamide) and fourth (ethambutol) agent for the first 2 months, by which time the results of susceptibility testing are generally available. It is then usually safe to continue with a two-drug regimen of isoniazid and rifampin for the remaining 4 months of treatment (see Chapter 143).[38]

Similar principles are increasingly being applied to the treatment of severe malaria, particularly in regions of the world where chloroquine-resistant malaria is widespread. Examples include combinations of artemisinin with tetracycline or mefloquine.[40] It is surprising that it has taken so long to move from single-agent treatment of malaria to combined regimens in an era when very few antimalarial drugs are being developed and drug-resistant disease has become so widespread.

Other circumstances where combined regimens have become the norm are in the management of HIV/AIDS[39] and chronic hepatitis C.[43] In both diseases clinical trials have shown greater efficacy with combined regimens. In the case of HIV/AIDS, viral load is more rapidly controlled and sustained with faster immune reconstitution and resultant clinical well-being. By selecting two or more agents from different classes of antiretrovirals, drug resistance is delayed in addition to providing a more efficacious regimen.

In the case of hepatitis C, pegylated interferon combined with ribavirin is not only more effective than single-drug therapy in terms of disease control but also in the case of genotype 2 and 3 infection offers a prompt and sustained viral response with high cure rates.[44]

Another rationale for combined drug regimens is to achieve synergistic inhibition of a target pathogen. The combining of a penicillin (for susceptible strains) with an aminoglycoside to treat enterococcal endocarditis is now standard therapy since *in vitro* and *in vivo* the combination is more effective than a penicillin alone.

Other synergistic combinations include β-lactam/aminoglycoside regimens to treat serious *P. aeruginosa* infections of the bloodstream and lower respiratory tract infections, especially in the immunocompromised patient.[45] Likewise, amphotericin B and flucytosine are synergistic in the treatment of cryptococcal meningitis.[46]

Economic considerations

Antibiotics account for approximately 20% of all prescribed drugs. Primary care is responsible for around 90% of this prescribing, the remainder being used in the treatment of hospitalized patients. However, community prescribing relies largely on oral and, to a lesser degree, topical agents. In contrast, in hospital, parenteral administration is the dominant route. Parenteral agents are more expensive, more costly to prepare, require infusion systems and account for much staff time in their preparation and administration. With the improved oral bioavailability of agents such as the fluoroquinolones, there is an economic advantage to place less reliance on parenteral regimens, unless this is clinically unavoidable. Switching from intravenous to oral treatment is increasingly used in managing a variety of infections and can result in significant cost savings without impairing outcomes.[47]

Another factor affecting the cost of treatment is the increasing use of generic agents which account for more than three-quarters of prescribing in primary care. Generics are generally substantially cheaper than patented preparations and many hospitals permit generic substitution of a proprietary drug by pharmacists dispensing these drugs.

In addition to the cost of treatment, many health-care systems are looking critically at the total cost of managing specific infectious diseases. For example, this has led to a careful assessment of which patients justify hospital management according to diagnosis, disease severity and age. Furthermore, the choice, route and duration of treatment are also undergoing critical review. No longer can the unit cost of a medicine be seen as the sole determinant that dictates choice of therapy.

Increasingly health-care systems require an economic assessment of the performance and use of new agents at the time of licensing. In the UK the National Institute for Health and Clinical Excellence assesses the benefit of a particular drug in managing a disease in the context of health-care gain using indices such as number required to

treat to achieve observed benefit. Such a macroeconomic approach has advantages and disadvantages. Approval in the context of socialized medicine means that specific treatments can be adopted. However, the purchaser is rarely the individual who remains dependent upon competing demands on health-care budgets to gain access to new medicines. Furthermore, products which are approved by the regulatory authority may not necessarily be approved for widespread use despite passing the hurdle of documented efficacy, safety and pharmaceutical quality. In other countries the availability of a new drug post-approval may be linked to an agreement on reimbursement charges from government. In general, these systems have been created to control the ever-spiraling costs of medicines and health care. However, they are also proving a disincentive to industry to develop new drugs such as antibacterials which are generally used in short courses, unlike drugs used to manage chronic disease.

Failure of antimicrobial therapy

Despite adhering to the above prescribing principles in the selection and use of antimicrobial agents, some infections still fail to respond to treatment. It is therefore important to review the response to therapy. This applies particularly to the seriously ill hospitalized patient where clinical response and laboratory monitoring, such as declining neutrophil count and other inflammatory markers provide supportive evidence of improvement. In primary care the patient should be advised about the anticipated course of their illness and to seek further medical assessment should any complications develop or the symptoms fail to respond.

Failure to respond to antimicrobial treatment may have several explanations. These include the accuracy of the primary diagnosis, the choice of initial therapy, the selection of dose and route of administration, the duration of treatment and, in the case of trauma or implant surgery, an assessment of the possible presence of deep-seated infection. Surgical drainage of pus with abscess formation is often overlooked and needs emphasis.

Failure to make a correct diagnosis may be the result of an inaccurate clinical assessment and, in turn, presumptive microbiologic assessment to guide initial empiric therapy – for example, the incorrect diagnosis of superficial cellulitis instead of more deep-seated necrotizing fasciitis. Here the selection of oxacillin or flucloxacillin alone will be ineffective, particularly against MRSA or anaerobic pathogens.[48] A careful review and further investigations, where indicated, are important in reassessing the unresponsive patient.

The dose and route of medication are also of importance. For example, pneumococcal pneumonia can be effectively managed in the community with oral agents for patients with mild to moderate infection. However, in those with bacteremic infection, parenteral therapy is preferred, especially when complications such as meningitis arise and where the choice of drug, route and dose of therapy differ.

Another example is selection of an incorrect formulation of a drug. Vancomycin is the preferred agent for managing severe *C. difficile*-associated diarrhea (CDAD) but needs to be prescribed orally and not intravenously as in the case of systemic staphylococcal infection. Furthermore, the oral dose of vancomycin for CDAD differs from that used to treat bloodstream staphylococcal disease.

Most mild to moderate infections respond to 5–10 days of antibiotic therapy. However, selected infections require more protracted therapy to avoid treatment failure and relapse. Severe *Legionella pneumophila* pneumonia may require up to 3 weeks of treatment,[42] as does meningitis caused by *Listeria monocytogenes*. This emphasizes the value of establishing a microbiologic diagnosis, particularly when treating severe infection.

The place of bactericidal regimens in selected patients has been emphasized, particularly in those with known defects in immune function, notably the profoundly neutropenic patient. Likewise infections at body sites relatively protected from the host immune response require bactericidal regimens for optimum control. Examples include infective endocarditis and bacterial meningitis.

Infection as a complication of the presence of foreign materials, notably vascular and articular prosthetic devices, is increasing. Infection is often caused by skin micro-organisms, notably coagulase-negative staphylococci. These are not only of variable sensitivity to many commonly used agents but also adhere to the foreign material as biofilms in which they are protected against host defenses and are less responsive to treatment. Prolonged therapy or surgical revision, sometimes with removal of the infected device, may be necessary to eliminate the infection.

Formularies, guidelines and medicines management

The complexity and ever-changing nature of disease management has given rise to a variety of prescribing support systems and initiatives which are grouped under the title of antibiotic stewardship.[49] These are now recognized as essential in health care where advances in medicine, together with education and training, often go hand in hand with patient care. Furthermore, the fiscal burden of the cost of medicines and their administration has added to the need for formularies and prescribing support systems. In the case of antimicrobial therapy the situation is further compounded by the occurrence and threat of drug-resistant pathogens to the individual patient and the wider community.

The variety of prescribing support systems varies from country to country and even within different hospitals and administrative units of health-care delivery. They include formularies, treatment guidelines, online prescribing systems, care pathways, care bundles and audit, all of which influence antimicrobial prescribing.[49]

Formularies

Formularies, in their original form, were simply a listing of drugs stocked by the pharmacy. Formularies would include agents to cover topical, oral and parenteral use. A decision to include an agent in a formulary is currently, but not universally, based on recommendations by an institutional Drug and Therapeutics Committee or its equivalent. Such decisions are influenced by medical need, cost and increasingly clear evidence of efficacy and safety for the proposed indication.[50] Many institutions distinguish between drugs that may be freely prescribed being appropriate for many common indications from those for specialist use such as antibiotics used in regimens for managing infections in neutropenic patients. In addition, some agents have restricted uses and can only be prescribed following discussion and approval by a microbiologist or infectious disease specialist.

Increasingly, national considerations are being applied to the approval and availability of treatments. These economic analyses influence prescribing, reimbursement costs and, in some countries, whether or not a drug is made nationally available or not. In the UK, the National Institute for Health and Clinical Excellence increasingly assesses medicines in the context of disease management and makes judgments on the availability of new products, notably those which carry a high cost.

Treatment guidelines

Treatment guidelines are increasingly linked to formularies.[51] Local guidelines make recommendations with regard to the preferred and alternative agents relevant to specific diseases and conditions. At institutional level such decisions require evidence based on a review of the published literature in addition to the manufacturer's dossier. Such evidence includes controlled clinical studies, meta-analyses and, whenever possible, nationally or internationally published evidence-based guidelines.

Treatment guidelines have a number of advantages. They reflect current best therapy whenever possible and identify issues relevant to cost containment such as when generics or generic substitution is appropriate. Standardization of management also supports purchasing arrange-

ments and in particular provides an important resource for education and training of prescribing practitioners. In addition, audits of compliance by prescriber, unit and institution are possible and increasingly provide a means for measuring patient outcome according to treatment. The latter is challenging to achieve but important in order to continuously update treatment recommendations.

Implementation of guidelines has been shown to improve management, reduce inappropriate prescribing, lower costs and reduce bacterial resistance for selective indications, such as ventilator-associated pneumonia.[52] However, compliance with guidelines is often poor.[53]

Electronic prescribing support is becoming more widely available and permits an accurate recording of prescribing practice by patient and prescriber.[54] More sophisticated systems influence choice through prompts to approved drugs for given diagnoses. Others have taken this one stage further and include microbiologic data to further refine prescribing selection, or have targeted specific classes of antibiotic to improve usage and reduce adverse effects.[55]

The appointment of clinical pharmacists to support good prescribing practice is a more recent phenomenon.[56] Their knowledge of medicines and skills can improve prescribing standards, minimize prescribing errors and encourage audits. Their appointment also permits cost saving exercises such as automatic stop orders and intravenous to oral switching algorithms. Invariably they also influence decisions by Drug and Therapeutics Committees. Antimicrobial pharmacists have emerged in the UK as a further refinement of this role with particular emphasis on hospital prescribing practice. The adoption of antimicrobial management teams is increasing worldwide but does require institutional support and the correct professional expertise.[57]

SUMMARY

In conclusion, safe and effective prescribing is a professional and lifelong commitment by all prescribing professionals. The ever-changing nature of therapeutics inevitably requires a major commitment to continuous education and professional development.

The principles of good prescribing practice as described above are particularly important in relation to antimicrobial agents where the dynamics of infectious disease with regularly emerging novel pathogens, new expressions of virulence and new resistance mechanisms add further complexity to prescribing.

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Chapter 130

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Mechanisms of action

ANTIBIOTICS THAT ACT ON THE CELL WALL

The basis of the bacterial cell wall is peptidoglycan, containing alternating residues of *N*-acetyl-glucosamine and muramic acid in β-1→4 linkage. The carboxyl groups of muramyl residues are substituted by short peptides (usually pentapeptides) terminated by a D-Asp-D-Ala-D-Ala sequence. Cell-wall active antibiotics act by inhibiting the activity of enzymes involved in the synthesis of the precursors or in the reticulation of peptidoglycan.

β-Lactams

The β-lactam nucleus is the basic building block of an exceptionally large class of antibiotics that all share a common mode of action but have quite distinct properties in terms of spectrum, pharmacokinetics and, to some extent, activity against resistant strains.

Chemical structure

All antibiotics in this class contain a cyclic amide called β-lactam. With the exception of the monobactams, this cycle is fused with a five- or six-membered cycle. According to the nature of the four-membered latter and/or of the heteroatom included, the following classes have been described (Fig. 130.1):

- penams – β-lactams with a five-membered ring containing a sulfur atom (penicillins);
- clavams – β-lactamase inhibitors that contain a five-membered ring with an oxygen as the heteroatom (e.g. clavulanic acid; sulfur analogues have also been reported);
- carbapenems – five-membered rings without heteroatom and with a double bond (e.g. thienamycin, imipenem);
- cephems – six-membered unsaturated rings with a sulfur atom (cephalosporins);
- oxacephems – the oxygen analogues of cephems (latamoxef);
- monobactams – cyclic amides in a four-membered ring (azetidine) with a methylcarboxylate function in the case of nocardicins and a sulfonate in the case of the other monobactams (e.g. aztreonam).

Other β-lactams include thiacephems, dethiacephems, dethiacephams, heterocephems and cephams, as well as diverse bicyclic systems.

Mode of action

β-Lactams act primarily as inhibitors of transpeptidases (specialized acyl serine transferases), thereby impairing the synthesis of the cell wall (Fig. 130.2).[1] β-Lactams mimic the D-Ala-D-Ala sequence in that the distance between the carboxylate or the sulfonate (monobactams) and the cyclic amide is similar, and act as a false substrate for D-alanyl-D-alanyl transpeptidases. The carbonyl of the beta-lactam ring reacts with a serine residue of the transpeptidases (also called penicillin-binding proteins, PBPs) to give an inactive acyl enzyme ('suicide inhibition' by formation of a covalent bond; Fig. 130.3).[2] Transpeptidases, located in the periplasmic space, are directly accessible in Gram-positive bacteria but protected by the outer membrane in Gram-negative bacteria, which β-lactams must cross (mainly via porin channels).

The impairment of cell wall formation by β-lactams explains the inhibition of the growth of bacteria, but the bactericidal effect results from indirect mechanisms (mostly the activation of autolytic enzymes). β-Lactams are usually active against rapidly dividing bacteria only.

Resistance

Resistance to β-lactams occurs by three main different mechanisms.

- First, access to the PBPs in Gram-negative bacteria might be abolished by alteration of porin channels, which affects highly water-soluble β-lactams.
- Second, resistance can be conferred through modification of PBPs, in particular PBP2 which is essential for the 'shaping' of bacteria. The most typical example is found in methicillin-resistant *Staphylococcus aureus* (MRSA) that produces an altered protein (called PBP2' or PBP2a) with a very low affinity for β-lactams. This makes the bacteria resistant to all conventional β-lactams. Recent research has shown that PBP2a is insensitive to β-lactams because the protein is in 'closed' conformation, preventing access of the drug to the reactive serine. This is circumvented if bacteria are exposed to acidic pH, which causes an opening of the enzymatic cavity.[3] This can also occur if β-lactams have a bulky hydrophobic substituent attached to the molecule in the vicinity of the carboxylate (see Fig. 130.4 for cephalosporins). These molecules typically show a loss of activity towards MRSA of only one to eight dilutions (corresponding to the energy needed to open the PBP2a) compared to fully susceptible *S. aureus* (in contrast with often more than 256-fold for conventional β-lactams). Two anti-MRSA β-lactam antibiotics (ceftobiprole and ceftaroline)[4] have been clinically developed. Penicillin-binding proteins in staphylococci or in other organisms (e.g. streptococci) can also show decreased affinity; in such cases resistance is more specific to some β-lactams but can also be only partial (decreased susceptibility).
- The third and for many years the most frequent mechanism of resistance is the production of hydrolyzing enzymes called β-lactamases.[5] The corresponding genes may be carried either on chromosomes (where their expression may be constitutive or inducible) or on plasmids, and their products are secreted out of the cell wall in Gram-positive bacteria and in the periplasmic space in Gram-negative bacteria. β-Lactamases are serine proteases that have a high affinity for β-lactams and cleave the amide bond. Although most β-lactamases open the β-lactam ring in the same way

Diversity of β-lactam antibiotics

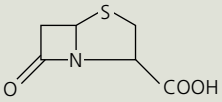
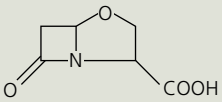
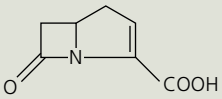
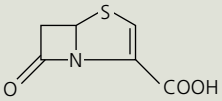
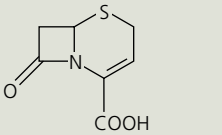
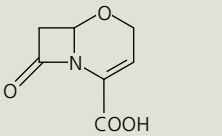
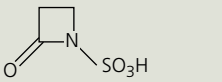
Structure	Group	Examples
	Penam	Penicillins
	Clavam	β-lactamase inhibitors (clavulanic acid)
	Carbapenem	(Thienamycin, imipenem)
	Penem	(Faropenem)
	Cephem	Cephalosporins
	Oxacephem	(Latamoxef)
	Monobactam	(Aztreonam)

Fig. 130.1 Diversity of β-lactam antibiotics: main ring structures names and representative antibiotics. The figure highlights the fact that all drugs share a common four-piece β-lactam ring and a carboxylate (or sulfonate) function at an appropriate, similar distance. (Clavams are weak antibiotics but efficient β-lactamase inhibitors.)

as transpeptidases, the hydrolysis rate is far quicker than in PBPs (see Fig. 130.3), resulting in the regeneration of the enzyme and the production of an irreversibly inactivated antibiotic. While PBPs turn slowly (one β-lactam per hour), the β-lactamases can turn on good substrates over 1000 times per second. X-ray data and genetic studies of β-lactamases and PBPs show a high level of structural homology, suggesting that both derive from a common ancestor.

A number of β-lactams have been made to resist to β-lactamases by appropriate steric hindrance or change in conformation (Fig. 130.5), giving rise to the large number of successive generations of so-called β-lactamase-resistant penicillins and cephalosporins. β-Lactamases, however, have an extraordinary plasticity and inevitably develop activity against all new derivatives at a rapid pace (Table 130.1).

Thanks to their specific structure, clavams are poor antibiotics but bind tightly to β-lactamases and inactivate them. Given in combination with β-lactams, they provide protection unless the bacteria overproduce β-lactamases. Some β-lactamases can also hydrolyze the clavams.

Pharmacodynamics

β-Lactams are relatively slow-acting antibiotics that must be present at a concentration above the minimum inhibitory concentration (MIC) for as long as possible. Conversely, concentration above four to five times the MIC provides little gain in activity, so that frequent dosing is more appropriate than infrequent administration of large doses. Administration of β-lactams by continuous infusion is gaining increasing popularity,[6] but care must be taken regarding the stability of the drugs when exposed to room temperature for prolonged periods of time (carbapenems are unstable and cannot be used by continuous infusion; a 'prolonged' infusion time of 3–4 hours has been proposed for doripenem as part of the drug labeling). In general, β-lactams show only a moderate postantibiotic effect.

Future developments

Efforts continue to develop β-lactams active against MRSA in order to obtain compounds with increased intrinsic activity (applying the approach used with cephalosporins to penems). In parallel, efforts are being made to develop more effective β-lactamase inhibitors (especially for Gram-negative bacteria). Faropenem, an oral penem, was halted in its development in spite of excellent activity against pneumococci, largely because of fear of triggering resistance to the whole class of penems if used indiscriminately in the community.

Hybrid molecules combining a β-lactam part and an active antibiotic part from a different class (e.g. glycopeptides, fluoroquinolones) have also been described, with some potentially promising compounds (because of markedly improved activity) in development.

Glycopeptides and lipoglycopeptides

Chemical structure

Currently available glycopeptide antibiotics (vancomycin, teicoplanin) contain two sugars and an aglycone moiety made of a relatively highly conserved heptapeptide core, bearing two chloride substituents. The aglycone fraction is responsible for the pharmacologic activity of the molecule, whereas the sugars are thought to modulate its hydrophilicity and its propensity to form dimers (see below). As a result of their large size, glycopeptides are not only unable to cross the outer membrane of Gram-negative bacteria, explaining why they are inactive against these organisms, but are also unable to penetrate inside bacteria, limiting them to an extracellular target. Lipoglycopeptides are semisynthetic derivatives characterized by the addition of a hydrophobic moiety, which confers additional properties to the drugs.

Mode of action

Glycopeptides inhibit the late stages of cell wall peptidoglycan synthesis (see Fig. 130.2) by binding to D-Ala-D-Ala termini of the pentapeptide-ending precursors localized at the outer surface of the cytoplasmic membrane. At the molecular level, glycopeptides form a high affinity complex with D-Ala-D-Ala by establishing hydrogen binding via their aglycone moiety.[7] The strength of this binding is greatly enhanced either by the dimerization of the glycopeptide molecules mediated by their sugars and the chloride atom on the aglycone (vancomycin) or by their anchoring in the membrane by a fatty acyl chain substituent (teicoplanin).[8] The subsequent steric hindrance around the pentapeptide termini blocks the reticulation of peptidoglycan by inhibiting the activity of transglycosylases (responsible for the fixation of a new disaccharide–pentapeptide subunit on the nascent peptidoglycan) and of transpeptidases (catalyzing the formation of interpeptide bridges).

Lipoglycopeptides (telavancin, oritavancin) add membrane-destabilization effects and stronger inhibition of the transglycosidase activities to the basic mode of action of the older molecules (resulting in the leakage of ions and other small molecules, causing rapid bacterial death). This is due to their membrane-anchoring properties (favored by the abundance of acidic phospholipids in bacterial membranes) and to dimerization of molecules[9] (Fig. 130.6) and, for oritavancin and enterococci, the possibility to bind an additional site in the petidoglycan.[9a] Dalbavancin (currently under development) also has a lipophilic side chain but does not appear to destabilize bacterial membranes, and results in exceptionally low MICs and a very prolonged half-life.

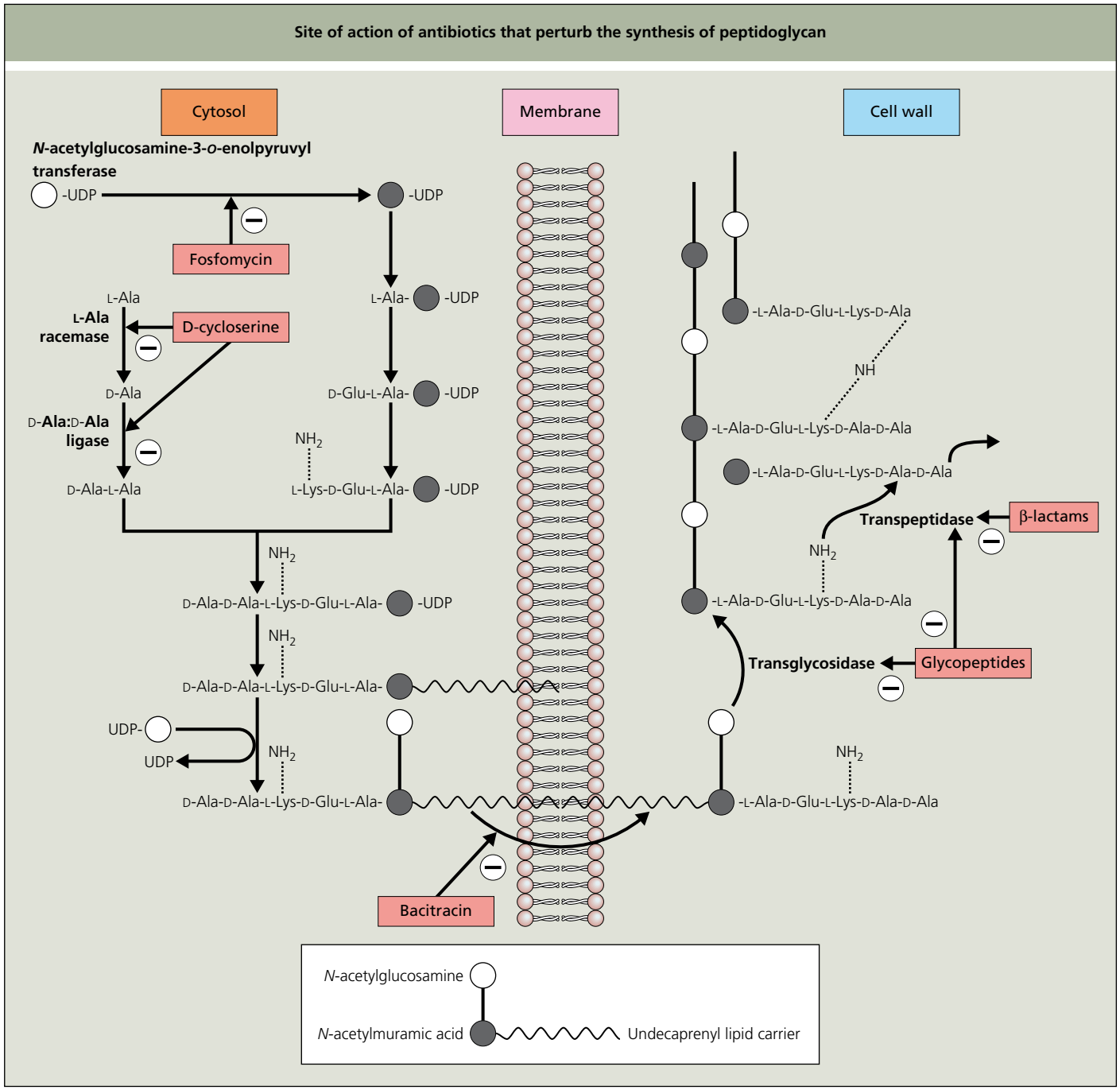


Fig. 130.2 Site of action of antibiotics that perturb the synthesis of peptidoglycan. The peptidoglycan unit is formed in the cytosol of bacteria by binding to uridine diphosphate (UDP) *N*-acetylmuramic acid of a short peptide (the nature of which differs between bacteria). This precursor is then attached to a lipid carrier and added to *N*-acetylglucosamine before crossing the bacterial membrane. At the cell surface peptidoglycan units are reticulated by the action of transglycosylases (catalyzing the polymerization between sugars) and of transpeptidases (catalyzing the polymerization between peptidic chains). The antibiotics act as follows: fosfomycin is an analogue of phosphoenolpyruvate, the substrate of the *N*-acetylglucosamine-3-*O*-enolpyruvyl transferase synthesizing *N*-acetylmuramic acid from *N*-acetylglucosamine and phosphoenolpyruvate; cycloserine is an analogue of D-Ala and blocks the action of D-Ala racemase and D-Ala: D-Ala ligase; bacitracin inhibits the transmembrane transport of the precursor; vancomycin binds to D-Ala-D-Ala termini and thus inhibits the action of transglycosylases and transpeptidases; and β-lactams are analogs of D-Ala-D-Ala and suicide substrates for transpeptidases.

Resistance

Resistance to glycopeptides results from substituting a D-lactic acid or a D-serine in place of terminal D-Ala of the pentapeptide. While this does not prevent the action of the transpeptidase, it ruins the binding of the glycopeptides because of the loss of one crucial hydrogen bond.[7] This mode of resistance is most prevalent in enterococci, but rarely in *S. aureus*. In the latter, resistance is more commonly acquired as a result of thickening of the cell wall associated with an increased abundance of free D-Ala-D-Ala termini. As a result, the MICs of the organisms increase modestly by about two- to eightfold, producing the so-called vancomycin- or glycopeptide-intermediate phenotype (VISA and GISA), eventually reaching a value of 4 mg/l or more at which clinically achievable serum concentrations of vancomycin can no longer inhibit bacterial growth (current CLSI and EUCAST breakpoints set a limit of 2 mg/l for susceptible strains). A further difficulty is the fact that the expression of

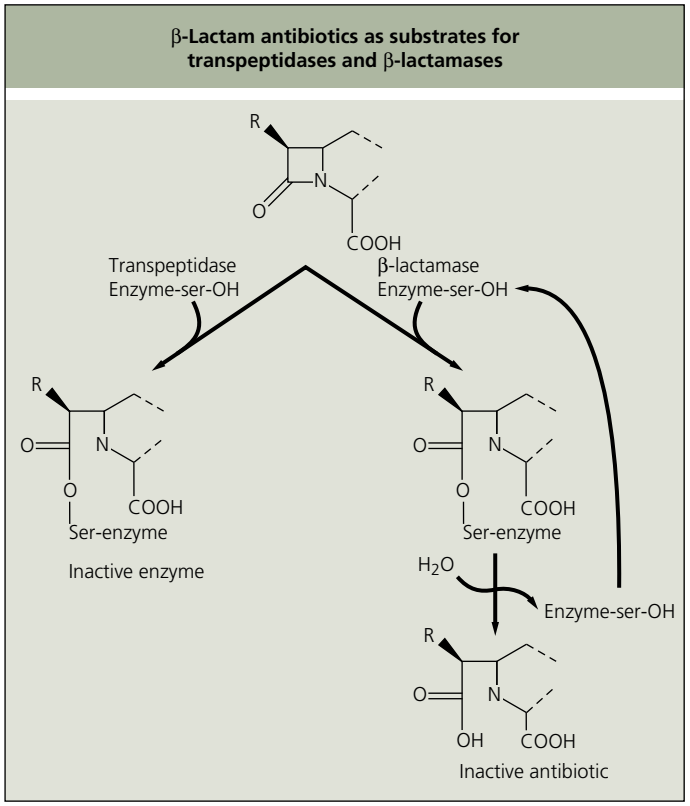


Fig. 130.3 β-Lactam antibiotics as substrates for transpeptidases and β-lactamases. The left part of the illustration shows how a β-lactam covalently binds to the transpeptidases. Hydrolysis of this acylated enzyme is very slow (one β-lactam per hour), making the enzyme inactive. The right part of the illustration shows that the same reaction occurs in the case of a β-lactamase. Hydrolysis of the acylated enzyme is, however, very rapid (1000 β-lactams per second), making the antibiotic inactive and regenerating the enzyme for a new cycle of hydrolysis.

this resistance is variable and may only affect a small proportion of a given inoculum, giving rise to a heteroresistance phenotype that makes detection by automated systems fairly unreliable.[10]

Lipoglycopeptides (telavancin, oritavancin) are partially immune to both resistance mechanisms thanks to their dual mode of action. It remains uncertain, however, whether keeping only the membrane-destabilizing effects (typically expressed only at higher drug concentrations than are needed to bind to D-Ala-D-Ala) will be sufficient to maintain clinically useful activity. Dalbavancin has variable activity against VISA strains and no useful activity against vancomycin-resistant organisms.

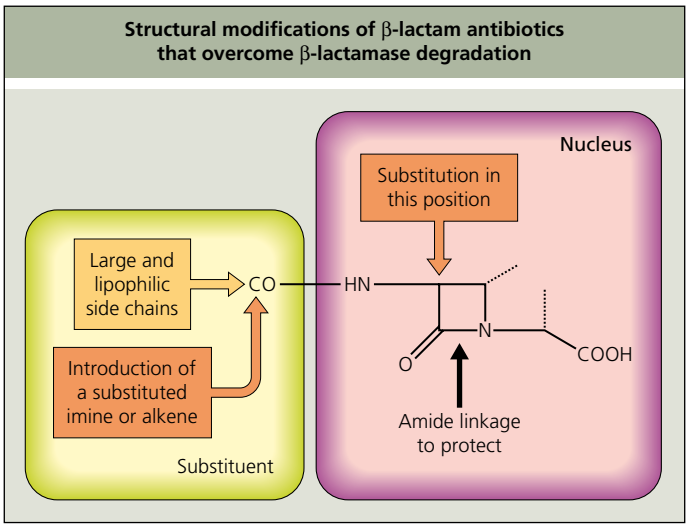


Fig. 130.5 Structural modifications of β-lactam antibiotics that overcome β-lactamase degradation. A first strategy, applied in penicillins, cephalosporins, oxacephems and monobactams consists of the introduction of a large side chain on the nucleus, possibly containing a substituted imine or alkene. A second strategy, applied in oxacephems and cefoxitin as well as in temocillin, consists of the introduction of a methoxy group on the β-lactam ring.

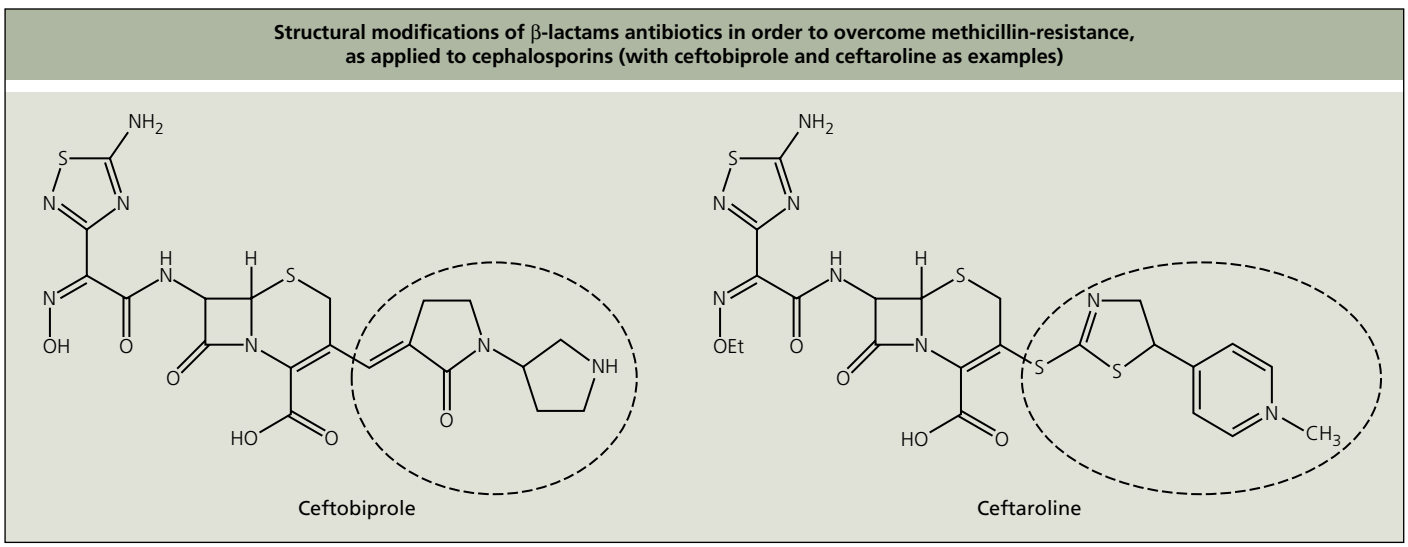


Fig. 130.4 Structural modifications of β-lactam antibiotics in order to overcome methicillin resistance, as applied to cephalosporins (with ceftobiprole and ceftaroline as examples). The bulky hydrophobic moieties (dotted-lined ellipse) added to the molecules forces a conformational change in PBP2a resulting in the opening of the active site and allowing acylation (inactivation) by the antibiotic. Although activity is largely restored towards methicillin-resistant organisms, MICs remain still typically one to four dilutions higher than for susceptible ones. The increase in lipophilicity also makes it necessary to administer the molecules as prodrugs – medocaril for ceftobiprole and fosamyl for ceftaroline (not shown).

Pharmacodynamics

Conventional glycopeptide antibiotics exhibit slow bactericidal activity, which is concentration independent. Because of their half-life (about 8 hours for vancomycin and much longer for teicoplanin), conventional glycopeptides display a 24-hour area under the serum concentration–time curve/MIC (AUC_{24h}/MIC) rather than a time above MIC phamacokinetic/pharmacodynamic pattern of activity.[11] This means that their mode of administration – whether discontinuous (q12h for vancomycin) or continuous – is similar. There is an increasing tendency to use vancomycin by continuous infusion (favored by its excellent aqueous stability) and to rely on trough rather than peak levels for optimum therapy. This contrasts with lipoglycopeptides, for which C_{max}/MIC ratios are probably more important. Lipoglycopeptides accumulate in macrophages and act on phagocytosed *S. aureus*.

Glycopeptides exhibit a synergistic effect with aminoglycosides, probably by facilitating the penetration of the latter drugs in bacteria.

Future developments

Talavancin has approved in 2009 for clinical use in the US, while oritavancin is still under development due to safety issues. Future developments should be geared towards clinical demonstration of superiority of these highly bactericidal molecules in comparison with vancomycin for 'difficult' indications.

Other agents acting on the cell membrane and cell wall synthesis

Daptomycin is a cyclic peptide flanked by an oxodecyl side chain conferring a strong amphiphilic character to the molecule (molecules in this group are often referred to as lipopeptides or peptolides; this erroneously suggests high lipophilicity, which is not true for daptomycin since the octanol–water partition is actually quite negative).

Table 130.1 Functional classification of β-lactamases

Group	Molecular class	Preferred substrates	Active β-lactams	Typical examples
Group 1: serine cephalosporinases not inhibited by clavulanic acid	C	Cephalosporins I, II, and III (>> cephalosporins IV, monobactams, penicillins)	Carbapenems Temocillin (cephalosporins III and IV; variable upon level of expression)	AmpC from Gram-negatives; variable upon the species
Group 2: serine β-lactamases				
2a: penicillinases inhibited by clavulanic acid	A	Penicillins (penicillin, ampicillin >> carbenicillin >> oxacillins)	Amoxicillin + clavulanic acid Cephalosporins Carbapenems	Penicillinases from Gram-positives
2b: broad-spectrum β-lactamases inhibited by clavulanic acid	A	Penicillins (penicillin, ampicillin >> carbenicillin >> oxacillins) Cephalosporins I and II	Cephalosporins III and IV Monobactams* Carbapenems Amoxicillin + clavulanic acid	TEM-1,TEM-2, SHV-1 from Enterobacteriaceae, *Haemophilus* spp., *Neisseria gonorrhoeae*
2be: extended spectrum β-lactamases inhibited by clavulanic acid (ESBL)	A	Penicillins Cephalosporins I II III (IV) Monobactams	Carbapenems Temocillin	TEM-3 to -29, 42, 43, 46–49, 52–58, 60, 61, 63, 65, 66, 72, 92 from Enterobacteriaceae SHV-2 to -9, 11–14, 18–22, 24 from *Klebsiella* spp. CTX-M-1 to CTX-M-54 (five phylogenetic groups) in Enterobacteriaceae K1-OXY from *Klebsiella oxytoca*
2br: broad spectrum β-lactamases with reduced binding to clavulanic acid	A	Penicillins	Most cephalosporins Monobactams* Carbapenems	TEM-30 to -41 (= IRT-1 to IRT-12), 44, 45, 50, 51, 59, 68, 73, 74, 76–79, 81–84 from *Escherichia coli* SHV-10 from *Klebsiella* spp.
2c: carbenicillin-hydrolyzing β-lactamases inhibited by clavulanic acid	A	Penicillins Carbenicillin (Cephalosporins I and II)	Piperacillin + tazobactam Cephalosporins III and IV Monobactams* Carbapenems	PSE-1, PSE-3, PSE-4 from *Pseudomonas aeruginosa*
2d: cloxacillin-hydrolyzing β-lactamases generally inhibited by clavulanic acid	D	Penicillins Cloxacillin Cephalosporins I and II	Carbapenems Cephalosporin III Monobactams* Piperacillin + tazobactam	OXA-1 to OXA-4 in Enterobacteriaceae OXA-2, OXA-10 (PSE-2) in *Pseudomonas aeruginosa* (pencillins, cefpirome, cefepime >> cephalosporins III) OXA-11 to -19, 28, 32, 45 are ESBLs in *P. aeruginosa* (R to Ceph 3, Ceph 4 and aztreonam) OXA-23, -24, -58 are carbapenemases in *Acinetobacter baumannii*

Table 130.1 Functional classification of β-lactamases—cont'd

Group	Molecular class	Preferred substrates	Active β-lactams	Typical examples
2e: cephalosporinases inhibited by clavulanic acid	A	Cephalosporins I and II	Cephalosporins III and IV Monobactams* Penems	FPM-1 from *Proteus vulgaris* Cep-A from *Bacteroides fragilis*†
2f: carbapenem-hydrolyzing-nonmetallo β-lactamases	B	Most β-lactams, including carbapenems (low or high resistance level depending on enzyme, species and genetic environment)	Carbapenems Monobactams and β-lactam inhibitors (variable activity depending on type of enzyme, bacterial host and genetic environment)	SME-1 to -3 from *Serratia* spp. IMI-1/2 and NMC-A from *Enterobacter cloacae* KPC-1 to -4 from *Klebsiella* spp. other Enterobacteriaceae and *Pseudomonas* GES-1 to -11 in Enterobacteriaceae, *P. aeruginosa* and *A. baumannii*
Group 3: metallo-β-lactamases inhibited by EDTA	B	Most β-lactams, including carbapenems	Monobactams*‡	L-1, XM-A from *Stenotrophomonas maltophilia* CcrA from *B. fragilis* A2h, CphA from *Aeromonas hydrophila* IMP-1 to -23, VIM-1 to -18 in *Pseudomonas*, other Gram-negative nonfermenters and Enterobacteriaceae SPM-1, GIM-1, SIM-1, DIN-1 in *P. aeruginosa* and *A. baumannii*
Group 4: penicillinases not inhibited by clavulanic acid		Penicillins, including carbenicillin and oxacillins	Monobactams*‡ and generally carbapenems	SAR-2 from *B. cepacia*

*Monobactams are not active on Gram-positive bacteria.
†Penems are the only molecules active in this case.
‡Remain active for most of the rare published studies.
EDTA, ethylenediaminetetraacetic acid.
Compiled from [62, 63, 64, 65]. The number of enzymes as well as their spectrum of activity is continually evolving.

Daptomycin is used against vancomycin-resistant enterococci for staphylococcal infections. It has a novel mode of action, in that the molecule binds to Ca^{2+} to form an oligomeric assembly with the lipid tails pointing inwards. The loose micelles serve to deliver daptomycin to the bacterial membrane in a 'detergent-like' form, causing leakage of cytosolic contents and a rapid bactericidal effect (Fig. 130.7).[12] Daptomycin is only active against Gram-positive bacteria since it cannot cross the outer membrane of Gram-negative organisms. Daptomycin shows preferential interaction with the phospholipid, phosphatidylglycerol, which is abundant in prokaryotic cell membranes and largely absent from eukaryotic cell membranes, except in lung surfactant where it forms aggregates, thereby explaining the failure of daptomycin in treating pulmonary infections.

Resistance to daptomycin has already been described, resulting from mutations in genes that encode enzymes involved in the synthesis of phosphatidylglycerol. *Staphylococcus aureus* with a VISA phenotype (see glycopeptides section) are less susceptible to daptomycin due to impaired access through the thickened cell wall.[9]

Daptomycin activity is concentration dependent, whereas its toxicity (mainly for skeletal muscle) is more related to the frequency of exposure. As a result, daptomycin should be administered once daily. The original dose during development was 4 mg/kg. Whether this will be sufficient for difficult-to-treat staphylococcal infections and can be increased without eliciting unacceptable toxic reactions is presently investigated.

D-Cycloserine is a broad-spectrum antibiotic which has structural similarities to D-Ala (see Fig. 130.2; Fig. 130.8) and inhibits the conversion of L-Ala into D-Ala (catalyzed by a racemase) and the dimerization of D-Ala (catalyzed by the D-Ala:D-Ala ligase).[13]

Fosfomycin, which bears structural similarities to phospho-*enol*-pyruvate, inhibits very early stage synthesis of peptidoglycan by impairing the formation of uridine diphosphate (UDP)-*N*-acetylglucosamine-*enol*-pyruvate, a precursor of UDP-*N*-acetylmuramic acid (see Figs 130.2, 130.8).[14]

Bacitracin is a polypeptide of complex structure. It acts as an inhibitor of peptidoglycan synthesis at the level of translocation of the precursor across the bacterial membrane (see Fig. 130.2).[15]

ANTIBIOTICS THAT ACT ON PROTEIN SYNTHESIS

Bacterial ribosomes comprise:

- a 30S subunit that binds mRNA and initiates protein synthesis; and
- a 50S subunit that binds aminoacyl tRNA, catalyzes peptide bond formation and controls the elongation process.

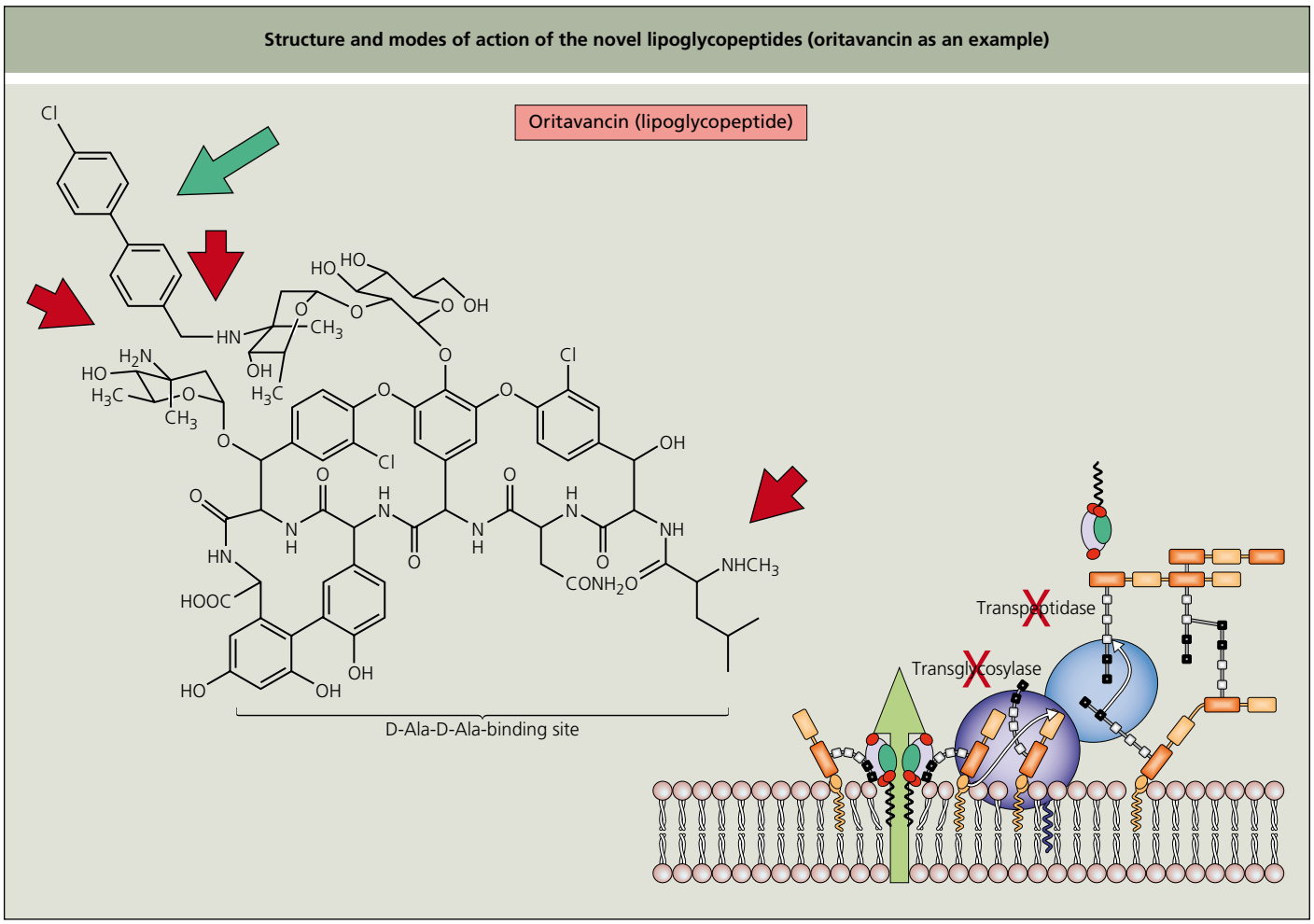


Fig. 130.6 Structure and proposed modes of action of the novel lipoglycopeptides (oritavancin as an example). Inset: the drugs are characterized by the presence of a bulky hydrophobic moiety (chorodiphenyl in this example; dark green arrow) and either additional amino groups (red arrows) or other polar groups (phosphonate for telavancin; not shown), resulting in a global amphiphilic character for the whole molecule. Note that the D-Ala-D-Ala binding site of the molecule remains unchanged. Main figure: the molecule interacts with the D-Ala-D-Ala termini of the pentapeptide (closed squares) as vancomycin, inhibiting both the transpeptidase and the transglycosidase activities, but also inserts itself into the membrane through its lipophilic side chain (this is favored, in the case of oritavancin, by its strong capacity to dimerize). As a result, lipoglycopeptides induce membrane leakage and thereby display a marked, concentration-dependent bactericidal effect. This second mode of action is exerted even if bacteria display a D-Ala-D-Lac or D-Ala-D-Ser in place of the D-Ala-D-Ala moiety, in as in vancomycin-resistant organisms. Specificity towards bacterial membranes probably stems from the fact that membrane-anchoring is enhanced by the presence of phospatidylglycerol, an acidic phospholipid abundant in bacterial but not in eukaryotic cell membranes. Adapted from Van Bambeke *et al*.[9]

The main sites identified in the 50S unit are the donor peptidyl site (P-site), where the growing peptide chain is fixed, and the acceptor aminoacyl site (A-site), where peptide bond formation occurs.

Aminoglycosides

Chemical structure

Streptomycin, discovered in 1944, has a limited spectrum of activity. Other aminoglycosides with a broader spectrum (kanamycins or gentamicins) have subsequently been obtained from natural sources. In the 1970s, the development of netilmicin and amikacin demonstrated the possibility of obtaining compounds active against strains resistant to earlier aminoglycosides.

Aminoglycosides comprise several aminohexoses joined by glycosidic linkages to a dibasic cyclitol.[16] The latter is streptidine in streptomycin and its derivatives, fortamine in the fortimicin series and 2-deoxystreptamine in most clinically used aminoglycosides. This 2-deoxystreptamine moiety links to cyclic sugars either at positions 4 and 5 (neomycin and paromomycin) or 4 and 6 (kanamycin, tobramycin, amikacin and dibekacin for the kanamycin family; gentamicin C_1, C_{1a}, C_2, sisomicin, netilmicin and isepamicin for the gentamicin family; Fig. 130.9). All compounds are positively charged at physiologic pH.

Bacterial targeting

Aminoglycosides selectively inhibit bacterial protein synthesis by binding to the 30S ribosomal subunit. However, molecules displaying a hydroxyl function in C6′ in place of an amino function (G-418, also known as geneticin) can also affect protein synthesis of eukaryotes.

Mode of action

Although the highly polar nature of aminoglycosides prevents diffusion through membranes, they do cross the outer membrane of

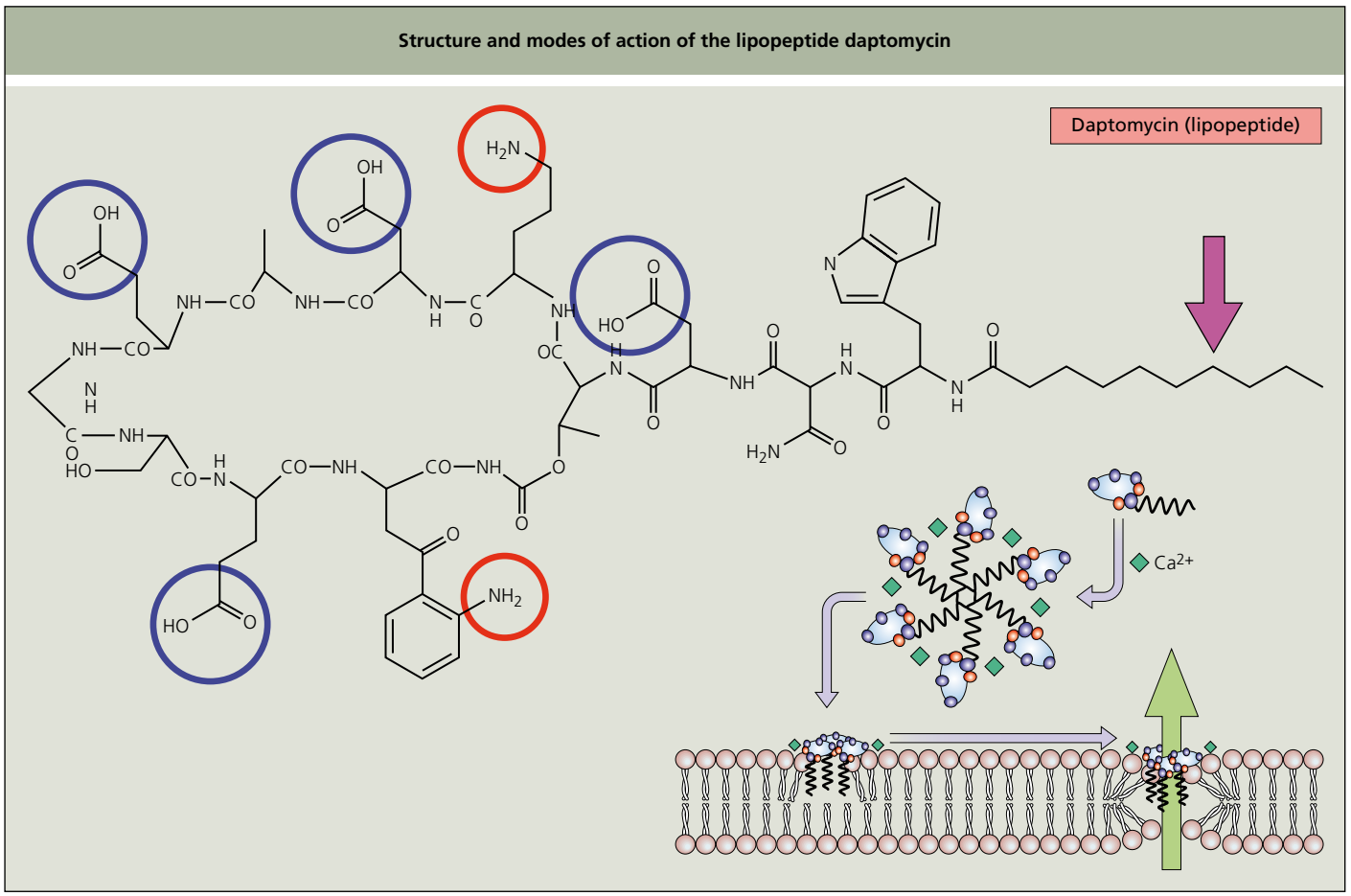


Fig. 130.7 Structure and mode of action of the lipopeptide daptomycin. The drug is a cyclic, polar depsipeptide (the ionizable residues are circled) flanked with a lipophilic oxodecyl side chain (purple arrow) conferring to the molecule a marked amphiphilic character. In the presence of Ca^{2+}, daptomycin forms loose micelles that serve as a delivery system to the bacterial membrane where the drug lipophilic side chain can then interact with the fatty acid chains of the phospholipids causing permeabilization and rapid bacterial death. As for lipoglycopeptides, specificity towards bacterial membranes stems from the fact that daptomycin–membrane interactions are favored by the presence of phosphatidylglycerol, an acidic phospholipid abundant in bacterial but not in eukaryotic cell membranes (it is, however, present in lung surfactant, causing daptomycin inactivation in this environment and explaining clinical failures in pulmonary infections). Modified from Van Bambeke *et al*.[9]

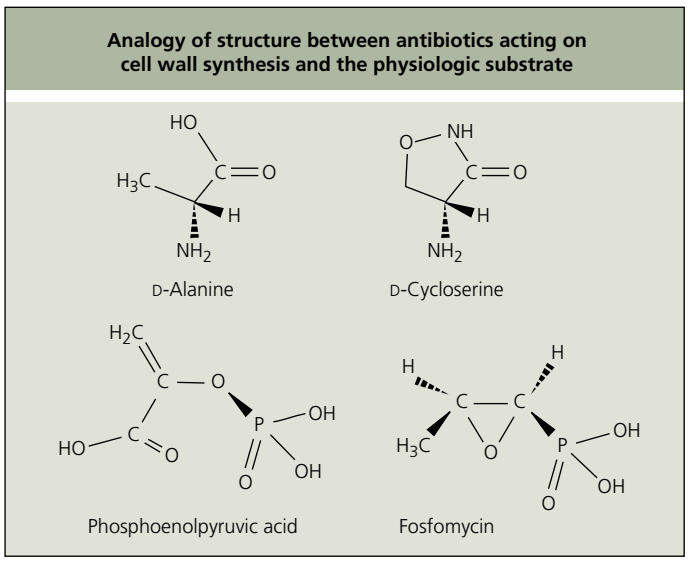


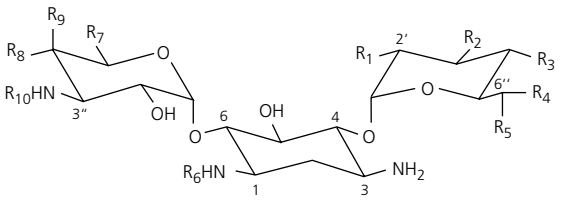
Fig. 130.8 Analogy of structure between cycloserine and fosfomycin and the corresponding physiologic substrates involved in peptidoglycan synthesis.

Gram-negative bacteria through a non-energy-dependent process involving drug-induced disruption of Mg^{2+} bridges between adjacent lipopolysaccharide molecules. Their transport across the cytoplasmic (inner) membrane of both Gram-positive and Gram-negative bacteria is dependent upon electron transport and is therefore termed energy-dependent phase I (EDP-I). Being related to the capacity of the bacteria to maintain a transmembrane electrical potential, this transport is impaired in anaerobic environments, at low external pH or in high osmolar culture media, which explains the corresponding reductions of antibacterial activity.

Once in the bacterial cytosol, aminoglycosides bind largely to the aminoacyl site of the 30S subunit of ribosomes and, to a lesser extent, to specific sites of the 50S subunit, again through an energy-dependent process (energy-dependent phase II, EDP-II), disturbing the elongation of the nascent peptide. Their mechanism of action is complex, involving inhibition of the transfer of peptidyl tRNA from the A-site to the P-site and impairment of the proofreading process that controls translational accuracy.[17] The aberrant proteins may be inserted into the cell membrane, leading to altered permeability and further increasing aminoglycoside transport, which contributes to and explains the highly bactericidal, concentration-dependent activity of aminoglycosides. (Binding of aminoglycosides to RNA has also been related to antiviral effects and impairment of eukaryotic protein synthesis, which, however, are not observed under conditions of clinical usage of presently developed aminoglycosides.)

Structural formulae of the 2-deoxystreptamine-containing aminoglycosides

4, 6-Disubstituted deoxystreptamine



(a)

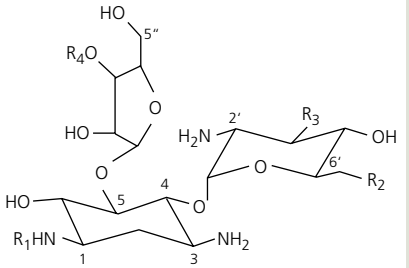
	Aminoglycoside	R_1	R_2	R_3	R_4	R_5	R_6	R_7	R_8	R_9	R_{10}
Kanamycins	Kanamycin A	OH	OH	OH	H	NH_2	H	CH_2OH	OH	H	H
	Kanamycin B	NH_2	OH	OH	H	NH_2	H	CH_2OH	OH	H	H
	Kanamycin C	NH_2	OH	OH	H	OH	H	CH_2OH	OH	H	H
	Amikacin	OH	OH	OH	H	NH_2	COR′	CH_2OH	OH	H	H
	Tobramycin	NH_2	H	OH	H	NH_2	H	CH_2OH	OH	H	H
	Dibekacin	NH_2	H	H	H	NH_2	H	CH_2OH	OH	H	H
	Arbekacin*	NH_2	H	H	H	NH_2	COR′	CH_2OH	OH	H	H
Gentamicins	Gentamicin C_1†	NH_2	H	H	CH_3	$NHCH_3$	H	H	CH_3	OH	CH_3
	Gentamicin C_{1a}†	NH_2	H	H	H	NH_2	H	H	CH_3	OH	CH_3
	Gentamicin C_2†	NH_2	H	H	CH_3	NH_2	H	H	CH_3	OH	CH_3
	Gentamicin C_{2b}†	NH_2	H	H	H	$NHCH_3$	H	H	CH_3	OH	CH_3
	Gentamicin B†	OH	OH	OH	H	NH_2	H	H	CH_3	OH	CH_3
	Isepamicin†	OH	OH	OH	H	NH_2	COR	H	CH_3	OH	CH_3
	Sisomicin	–	–	–	–	–	H	H	CH_3	OH	CH_3
	Netilmicin	–	–	–	–	–	R″	H	CH_3	OH	CH_3

R = $CHOHCH_2NH_2$; R′ = $CHOH(CH_2)_2NH_2$; R″ = CH_2CH_3 (a) = primed sugar for sisomicin and netilmicin

*In Japan

†Commercially availiable gentamicin is a mixture of C1, C1a and C2 in a ratio of 30, 30 and 40%, respectively

4, 5-Disubstituted deoxystreptamine



Aminoglycoside	R_1	R_2	R_3	R_4	R_5
Neomycin B	H	NH_2	OH	X	H
Paromomycin I	H	OH	OH	X	H
Lividomycin A	H	OH	H	X	Mannose
Ribostamycin	H	NH_2	OH	H	
Butirosin B	Y	NH_2	OH	H	

X= Y=

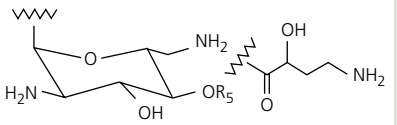


Fig. 130.9 Structural formulae of the 2-deoxystreptamine-containing aminoglycosides. The numbering of the atoms shown is as in Mingeot-Leclercq *et al*.[16] with the primed numbers (′) being ascribed to the sugar attached to C4 of the 2-deoxystreptamine (as this C is of the R configuration) and the doubly primed numbers (″) being ascribed to the sugar attached to either the C6 (S configuration) for the 4,6-disubstituted 2-deoxystreptamine or the C5 (R configuration) for the 4,5-disubstituted 2-deoxystreptamine. (i) Eventually no molecule is shown in bold; (ii) the choice of one or another molecule is highly variable from one country to another (for non truly scientific reasons).

Resistance

Resistance occurs mainly from the production of aminoglycoside-modifying enzymes (Fig. 130.10). The semisynthetic derivatives (e.g. netilmicin, amikacin, isepamicin) were specifically designed to protect against the principal enzymes. However, multienzyme-producing bacteria have become increasingly common, causing multidrug resistance.[16] These enzymes may have physiologic functions against natural substrates and only target aminoglycosides opportunistically. However, point mutations and selection have quickly increased specificity and efficacy.[18]

A second mechanism of resistance causes membrane impermeabilization, the underlying mechanism of which is mainly active drug efflux, with at least five distinct systems described in bacteria of medical interest such as *Escherichia coli* and *Pseudomonas aeruginosa*.[19] Because efflux proteins seem to capture their substrate from the inner, hydrophobic core of membranes and since aminoglycosides are polar, it is believed that it is actually a combination of aminoglycosides and phospholipids that serves as a substrate for efflux.

A third mechanism involves post-transcriptional methylation of 16S rRNA occurring in Enterobacteriaceae and nonfermenters. This affects all currently used 2-deoxystreptamine-containing aminoglycosides, with at least six distinct genes reported worldwide.[20]

Pharmacodynamics

Aminoglycosides demonstrate rapid, concentration-dependent killing as well as an important postantibiotic effect, probably due to a largely irreversible binding to the ribosomes. Once-a-day regimens provide the optimal mode of administration, producing high peak serum concentrations. Simultaneously, toxicity (renal and auditory) is delayed as uptake of the drug into the target tissues is saturable. As a result, a once-daily schedule is preferred.

Aminoglycosides are synergistic with antibiotics acting on cell wall synthesis by facilitating bacterial penetration of the aminoglycoside. In contrast, their activity is antagonized by bacteriostatic agents such as chloramphenicol and tetracyclines, probably by inhibition of their energy-dependent uptake and interference with the movement of the ribosome along mRNA.

Future developments

Efforts have been directed at:

- increasing the binding affinity while retaining binding selectivity; and
- developing derivatives resistant to aminoglycoside-inactivating enzymes, with one novel molecule (ACHN-490 [6′ (hydroxylethyl)-1-(haba)-sisomicin]) currently in clinical development.

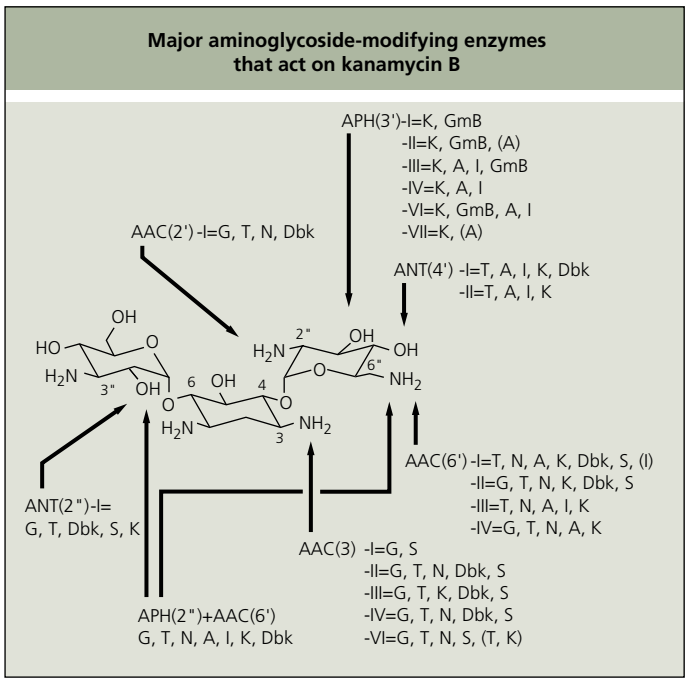


Fig. 130.10 Major aminoglycoside-modifying enzymes that act on kanamycin C. This aminoglycoside is susceptible to the largest number of enzymes. The *N*-acetyltransferases (AACs) affect amino functions and the *o*-nucleotidyltransferases affect hydroxyl functions. Each group of enzymes inactivates specific sites, but each of these sites can be acted upon by distinct isoenzymes (Roman numerals) with different substrate specificities (phenotypic classification). At least one enzyme is bifunctional and affects both positions 2″ (*o*-phosphorylation) and 6′ (*N*-acetylation). The main aminoglycosides used clinically on which these enzymes act are amikacin (A), dibekacin (DbK), commercial gentamicin (G), gentamicin B (Gmb), kanamycin A (K), isepamicin (I), netilmicin (N), sisomicin (S) and tobramycin (T). The drug abbreviations that appear in parentheses are those for which resistance was detectable *in vitro* although clinical resistance was not conferred. Data from Shaw *et al*.[18]

Another approach has been to reduce aminoglycoside toxicity based on the underlying cellular and molecular mechanisms uncovered over the last 20 years.[21]

Tetracyclines and alkylaminocyclines

Chemical structure

The early tetracyclines were derived from *Streptomyces* spp. (tetracycline, oxytetracycline), in contrast to more recent semisynthetic compounds (doxycycline, minocycline). All are characterized by four hydrophobic fused rings, which are diversely substituted, but principally by oxygenated hydrophilic groups. Alkylaminocyclines possess an additional substituent with a bulky hydrophobic moiety and an ionizable amino function (Fig. 130.11). Tigecycline is often referred to as a glycylcycline based on the presence of a glycyl moiety as a spacer between the main part of the molecule and the ter-butyl-amino group. Because other derivatives with similar properties have different spacers but a similar hydrophobic amino group, a better name for the class is alkylaminocyclines.

Bacterial targeting

Tetracyclines penetrate the outer membrane of Gram-negative organisms through porins. Intracellular accumulation inside the bacteria depends on the pH gradient between the cytosol and the external medium, which may occur by diffusion or a transmembrane proton-

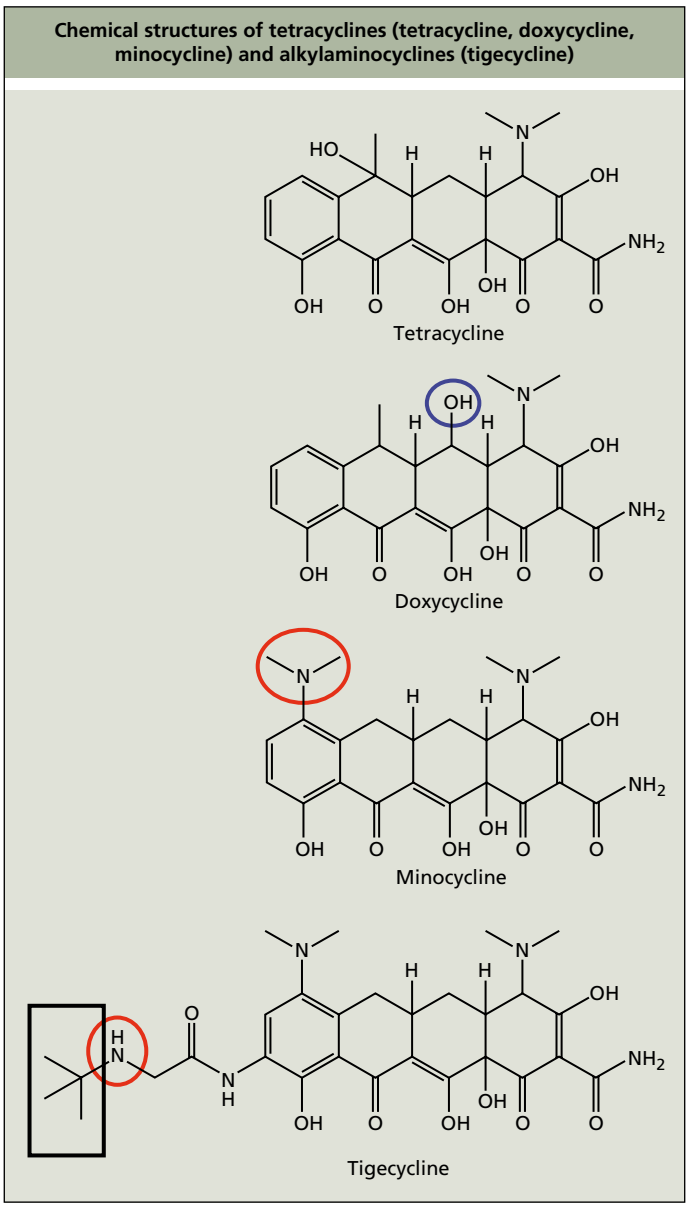


Fig. 130.11 Chemical structures of tetracyclines (tetracycline, doxycycline, minocycline) and alkylaminocyclines (tigecycline). The figure highlights the displacement of a hydroxyl group between tetracycline and doxycycline, and the additional ionizable aminofunction in minocycline which confer a more prolonged half-life and improved activity (minocycline). Tigecycline is the first of a new group of derivatives with a bulky hydrophobic moiety (square; *ter*-butyl in the case of tigecycline) and an additional amino group (circled) that make the molecule less susceptible to efflux by tetracycline–specific transporters as well as to ribosomal protection, the two main mechanisms of bacterial resistance towards all other tetracyclines.

driven carrier. The main argument in favoring the latter is that it could explain the selective action of tetracyclines (Fig. 130.12).

Mode of action

Tetracyclines interfere with the initiation step of protein synthesis (Fig. 130.12). More precisely, they inhibit the binding of aminoacyl tRNA to the A-site of the ribosome.[22] The 7S protein and the 16S RNA show the greatest affinity for tetracyclines and are therefore the main targets. This binding inhibits the fixation of a new aminoacyl tRNA on the ribosome. In addition, tetracyclines bind, or at least protrude, in the P-site by

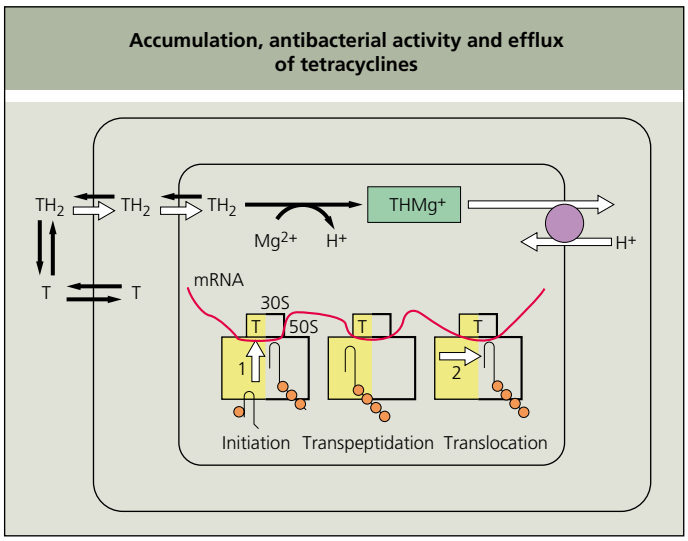


Fig. 130.12 Accumulation, antibacterial activity and efflux of tetracyclines. Tetracyclines freely diffuse through the extracellular membrane of Gram-negative organisms. Penetration inside bacteria is an energy-dependent process, depending on the pH and Mg^{2+} gradient between the extracellular medium of Gram-positive organisms or the periplasmic medium of Gram-negative organisms and the intracellular medium. Only the protonated form is highly diffusible, so that accumulation is favored by a lowering of the extracellular pH. Once inside the cytosol, the tetracycline molecule forms a nondiffusible complex with Mg^{2+}. This type of complex with a bivalent cation is also the substrate of the efflux pumps present in the membrane of resistant bacteria and acting as H^+ antiports (purple circle). The antibacterial action of the tetracyclines (T) is due to their binding to the 30S subunit of the ribosomes. In the pretranslocational state, tetracyclines inhibit the binding of aminoacyl tRNA (arrow 1) to the A-site (yellow part of the ribosome). In the post-translocational state, tetracyclines protrude in the P-site (white part of the ribosome) and inhibit the binding of the peptidyl-tRNA (arrow 2).

alteration in ribosome conformation in the post-translocational state, and may modify the ribosome conformation at the level of the head of the 30S subunit and the interface side of the 50S subunit.

Resistance

Resistance to tetracyclines is widespread. Some 29 genes on mobile elements have been identified in the so-called 'tetracycline' (tet) family and three in the 'oxytetracycline resistance' gene family (otr).[22] Among the tet and otr genes, two main mechanisms have been described, namely efflux (which is an important and widespread mechanism of bacterial resistance, and was actually discovered with tetracyclines) and ribosomal protection. Resistance by enzymatic inactivation has been described but remains uncommon. Low levels of resistance can also result from mutations or a decrease in porin content of the outer membrane, with consequent decreased tetracycline uptake in Gram-negative bacteria.

As tigecycline increases the number of bonds to the target 16S RNA, the drug is unaffected by the ribosome protection mechanism.[23] Together with the vicinal hydrophobic moiety, it makes the molecule less susceptible to efflux, and is therefore active against many Gram-negative organisms with the exception of *P. aeruginosa* and Proteae because of a resistance nodulation cell division (RND)-type efflux pump (MexXY-OprM) constitutively expressed by these bacteria.[24]

Pharmacodynamics

Tetracyclines are essentially bacteriostatic and need to be administered at intervals determined by the drug half-life in order to maintain their serum level above the MIC of the infecting organism for as long as possible. Because of their prolonged half-life, however, AUC_{24h}/MIC emerges as the main parameter driving the activity of tetracyclines (including tigecycline) *in vivo*. In contrast to conventional tetracyclines, tigecycline has no useful oral bioavailability and must be given by the intravenous route.

Future developments

Mutants resistant to tigecycline (mostly related to efflux) have already been reported in *Acinetobacter* spp. The development of efflux pump inhibitors would also be useful not only in this context but also to extend the activity of tigecycline and similar compounds against *P. aeruginosa*. Orally bioavailable alkylaminocyclines are also in development.

Fusidic acid

Chemical structure

Fusidic acid is a steroid-like structure, belonging to the fusidane class. It is used in its sodium salt form.

Mode of action and resistance

Fusidic acid prevents the dissociation of the complex formed between guanosine diphosphate, elongation factor 2 and the ribosome, thereby inhibiting the translocation step of the peptidyl tRNA from the P-site to the A-site of the ribosome.[25] Because of a lack of cross-resistance with other antistaphylococcal agents, fusidic acid is enjoying a revival in several countries in the treatment of multiresistant *S. aureus*, but always in combination because of the high rate of emergence of resistance when used as monotherapy (mainly based on data from drug usage as a topical application; this could be much less if the drug is given orally).[26] The latter seems to result from the acquisition of the *fusB* gene (located on a transposon-like element of which homologues exist in many clinically important and environmental Gram-positive bacterial species), which protects ribosomal protein synthesis inhibition from fusidic acid in a dose-dependent fashion.[27]

Pharmacodynamics

Fusidic acid is bacteriostatic but may be bactericidal at high concentrations. Although fusidic acid has been used in many countries for years, it has never been approved in the US so far. In view of the mounting epidemic of community-acquired MRSA, a clinical development of fusidic acid in the US is presently under way.

Mupirocin

Chemical structure

Mupirocin contains a short fatty acid side chain (9-hydroxynonanoic acid) linked to monic acid by an ester linkage. Mupirocin is also called pseudomonic acid because its major metabolite is derived from submerged fermentation by *Pseudomonas fluorescens*. Pseudomonic acid A is responsible for most of the antibacterial activity; three other minor metabolites of similar chemical structure and antimicrobial spectrum have been called pseudomonic acids B, C and D.

Mode of action and resistance

Mupirocin inhibits bacterial RNA and protein synthesis by binding to bacterial isoleucyl tRNA synthetase, which catalyzes the formation of isoleucyl tRNA from isoleucine and tRNA. This prevents incorporation of isoleucine into protein chains, leading to arrest of protein synthesis. Resistance to mupirocin develops through the production of a modified target enzyme.[28]

Because of its unique mechanism of action, there is no cross-resistance between mupirocin and other antimicrobial agents.[29]

Pharmacodynamics

Mupirocin is bacteriostatic at low concentration but becomes bactericidal at concentrations achieved locally by topical administration. The *in vitro* antibacterial activity is greatest at acidic pH, which is advantageous in the treatment of cutaneous infections because of the low pH of the skin.

Future developments

The peculiar mode of action of mupirocin has triggered genomic-based research to identify similar targets at the level of the other amino acids, which could lead to new compounds.

Retapamulin

Retapamulin is a semisynthetic derivative of pleuromutilin, a naturally occurring tricyclic antibiotic, diterpene, discovered in the early 1950s, and out of which only veterinary antibiotics had been developed until now. Retapamulin inhibits bacterial protein synthesis by binding to domain V of 23S rRNA, thereby blocking peptide formation directly by interfering with substrate binding. Resistance occurs through mutations in the genes encoding 23S rRNA, but is not crossed with other antibiotics as the binding site is unique compared with those of other antibiotics acting on ribosomes.[30] This makes retapamulin appealing for the treatment of a variety of susceptible Gram-positive pathogens. Retapamulin has been developed as a topical antibiotic for the management of impetigo and uncomplicated secondarily infected traumatic skin lesions.

Macrolides

Chemical structure

The main active macrolides are 14-, 15- or 16-membered lactone rings, substituted by two sugars, one of which bears an aminated function. In 15-membered macrolides (azithromycin), an additional aminated function is inserted in the lactone ring, conferring to this subclass of molecule the name of 'azalides'. Ketolides are 14-membered macrolides in which the cladinose is replaced by a keto function and which possess in their macrocycle a carbamate linked to an alkyl-aryl extension, represented by telithromycin as the only registered antibiotic (Fig. 130.13).[31]

Erythromycin, the first clinically developed macrolide, is a natural product. Most of the more recent molecules are semisynthetic derivatives designed to be stable in acidic milieu, and are therefore characterized by an improved oral bioavailability. Both 16-membered macrolides and ketolides are intrinsically acid stable.

Bacterial targeting

Macrolides specifically bind to the 50S subunit of the ribosomes (more precisely, to the 23S rRNA), which does not exist in eukaryotic cells.

Mode of action

Macrolides reversibly bind to the peptidyl transferase center, located at the 50S surface, causing multiple alterations of the 50S subunit functions. While macrolides only bind to domain V of the 23S rRNA, ketolides also bind to domain II of 23S rRNA as a result of their carbamate extension, and thus are double anchored to their target (Fig. 130.14).[32] Macrolides are classically thought to block the peptide bond formation or the peptidyl tRNA translocation from the A-site to the P-site. However, additional consequences of their binding to ribosomes have been reported. It has been proposed that they could also favor the premature dissociation of peptidyl tRNA from the ribosome during the elongation process, leading to the synthesis of incomplete peptides.[33] It has also been suggested that erythromycin prevents the assembly of the 50S subunit, a property not generalizable to other macrolides.

Resistance

Clinically meaningful resistance occurs primarily by modification of the bacterial target and therefore affects all macrolides; it can also occur by efflux.[34] Target modification also affects lincosamides and streptogramins because of the common binding site for these three classes of antibiotic and also explains why macrolides, streptogramins, lincosamides and chloramphenicol have antagonist pharmacologic activity. Resistance to macrolides may be inducible or constitutive; however, if inducible it will not affect streptogramins and lincosamides since these are not inducers.[34] Ketolides (due to their lack of cladinose) and 16-membered macrolides are not inducers and therefore show activity on a subset of resistant strains. Moreover, the double ribosomal anchoring of ketolides confers a higher affinity not only for wild-type ribosomes, but also for ribosomes of strains resistant by methylation of domain V, with consequent improved activity against resistant strains.[31]

Although efflux mechanisms are being reported, 16-membered macrolides are again spared this effect. The frequency of strains susceptible to 16-membered macrolides and resistant to 14- and 15-membered macrolides remains low, however.

Pharmacodynamics

Macrolides are essentially bacteriostatic antibiotics, except at high concentrations. Their concentration at the infected site therefore needs to be durably maintained above the MIC of the pathogen. Because of their prolonged half-life, clarithromycin, azithromycin and telithromycin have been shown to primarily reflect the AUC_{24h}/MIC parameter *in vivo*.[31]

Future developments

Efforts to develop new ketolides continue, with the aim of selecting compounds with improved intrinsic activity (especially against multiresistant pneumococci and community-acquired MRSA) and a better safety profile, as telithromycin has been severely restricted in its indications because of liver toxicity.[31]

Lincosamides

Chemical structure

Lincomycin and its 7-chloro-7-deoxy derivative, clindamycin, comprise a propylhygrinic acid linked to an aminosugar.

Mode of action

Lincosamides bind to the 50S ribosomal subunit and have a mode of action similar to that of macrolides.

Resistance

The main mechanism of resistance to lincosamides is similar to that of macrolides and streptogramins and consists of an alteration of the 50S subunit. Rare cases of enzymatic inactivation (adenylation reaction) of the antibiotic have also been described for clindamycin. Resistance dissociation is, however, observed for those strains for which macrolide resistance is inducible, as clindamycin is not an inducer.[35] Likewise, efflux-mediated resistance to macrolides does not affect lincosamides, also leading to resistance dissociation.[34]

Pharmacodynamics

Lincosamides are bacteriostatic and are antagonists of macrolides and streptogramins, which bind at the same site on the ribosomes.

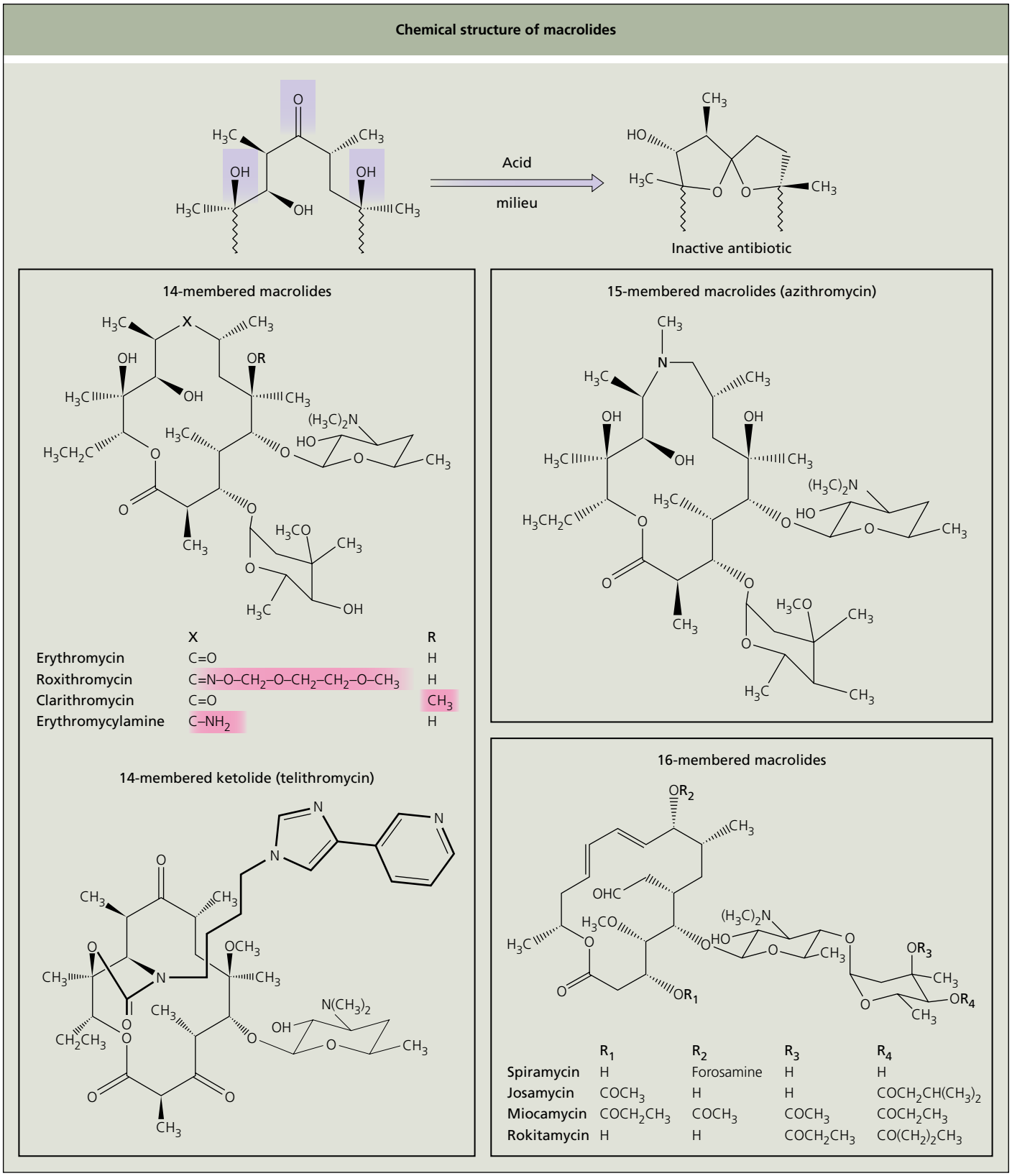


Fig. 130.13 Chemical structure of the macrolides. The upper panel shows the degradation of erythromycin in the gastric milieu (substituents responsible for the instability of the miolecule are shown in purple). 16-Membered macrolides and ketolides are intrinsically stable. The structural modifications conferring stability in acidic milieu to 14- and 15-membered macrolides are highlighted in pink in the middle panel.

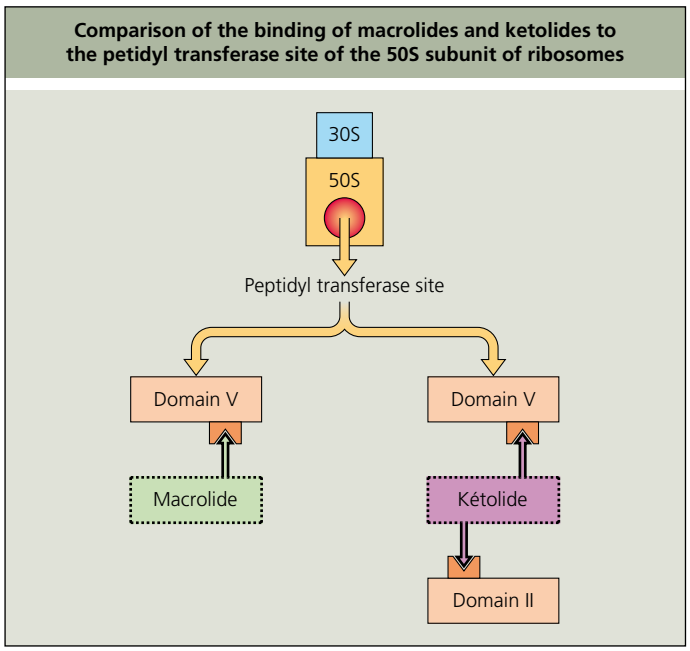


Fig. 130.14 Comparison of the binding of macrolides and ketolides to the peptidyl transferase site of the 50S subunit of ribosomes. Macrolides are characterized by a single anchoring point and ketolides by a double anchoring point, which increases the affinity of ketolides for wild type and methylated ribosomes.

Streptogramins

Chemical structure

Streptogramins are antibiotics that comprise a pair of synergistic constituents, namely a depsipeptide (group I) and a lactonic macrocycle (group II). Quinupristin–dalfopristin is the only combination used in the clinic so far.[36]

Mode of action

Streptogramins bind to the 50S subunit of the bacterial ribosome. They interfere with protein synthesis by a double mechanism, involving inhibition of the incorporation of aminoacyl tRNA in the ribosome and the translation of mRNA. The synergistic effects of the two components could be due to a modification of the conformation of the ribosome caused by binding of the group I component, which exposes a site of fixation for the group II component.[33]

Resistance

Resistance is by mutation of the ribosomal target and results in cross-resistance with macrolides and lincosamides. Because of the presence of two components, however, synergistins remain active against many macrolide and lincosaminide-resistant isolates. Resistance to streptogramins alone is rare and occurs by enzymatic inactivation (hydrolase and acetylase).

Pharmacodynamics

Streptogramin constituents are highly synergistic and exhibit dose-dependent bactericidal activity in combination. In addition, they also increase the antibiotic activity of aminoglycosides and rifamycins. Streptogramins also exhibit a prolonged postantibiotic effect with delayed regrowth when the antibiotic concentration falls under its MIC. This may result from persistent binding of the drug to its target.

Future developments

Quinupristin/dalfopristin currently has limited use only. There is, however, a potential interest in novel streptogramins for the treatment of infections caused by bacteria resistant to other antibiotics, mainly multiresistant *S. aureus* and vancomycin-resistant enterococci. At least one compound for oral administration (NX-103) is under development.

Chloramphenicol and thiamphenicol

Chemical structure

These antibiotics are constructed on a dichloroacetamide bearing a diversely substituted phenyl group.

Bacterial targeting

Chloramphenicol acts principally by binding to the 50S subunit of the bacterial ribosomes. However, it can also interact with mitochondrial ribosomes of eukaryotic cells, which results in its toxicity.

Mode of action

Chloramphenicol enters the bacteria by an energy-dependent process. Its antibiotic activity is due to competitive inhibition for the binding of aminoacyl tRNA to the peptidyltransferase domain of the 50S subunit. This induces conformational change in the ribosome, which slows or even inhibits the incorporation of the aminoacyl tRNA and in turn the transpeptidation reaction.[37]

Resistance

Resistance to chloramphenicol is mainly due to the production of a specific inactivating acetyltransferase.[38] The encoding gene is often located on plasmids that also confer resistance to other antibiotic classes. Another mechanism of resistance results in reduced drug entry into the bacterium.

Pharmacodynamics

Chloramphenicol is bacteriostatic. It competes in binding to the ribosomes with macrolides and lincosamides, making its combination with these drugs useless.

Oxazolidinones

Chemical structure

Like fluoroquinolones, oxazolidinones are synthetic molecules. The first derivatives were described in the 1970s, although linezolid, the first clinically available molecule, only became available in the 1990s (Fig. 130.15). The 5-(S)-configuration of the oxazolidinone ring is essential for activity, which is further improved by its substitution by an *N*-fluorinated aryl group and a C5 acylaminomethyl group.[39]

Mode of action

Oxazolidinones inhibit protein synthesis at an earlier step than other antibiotics acting on the ribosome. Their binding site is located in the vicinity of the peptidyl transferase with the A-site of the bacterial ribosome where they seem to interfere with the placement of the aminoacyl tRNA.[40] This interaction prevents the formation of the initiation ternary complex which associates $tRNA^{met}$, mRNA and the 50S subunit of the ribosome, and therefore the binding to the ribosome as well as the synthesis of peptide bonds, and the translocation of $tRNA^{met}$ into the P-site. They can compete for binding to the 50S subunit with other antibiotics (e.g. lincosamides, chloramphenicol) without being antagonistic.

Linezolid, the only available oxazolidinone, is mainly active against Gram-positive cocci, with minimal activity against most Gram-negative bacteria as a result of drug efflux in these organisms.[41]

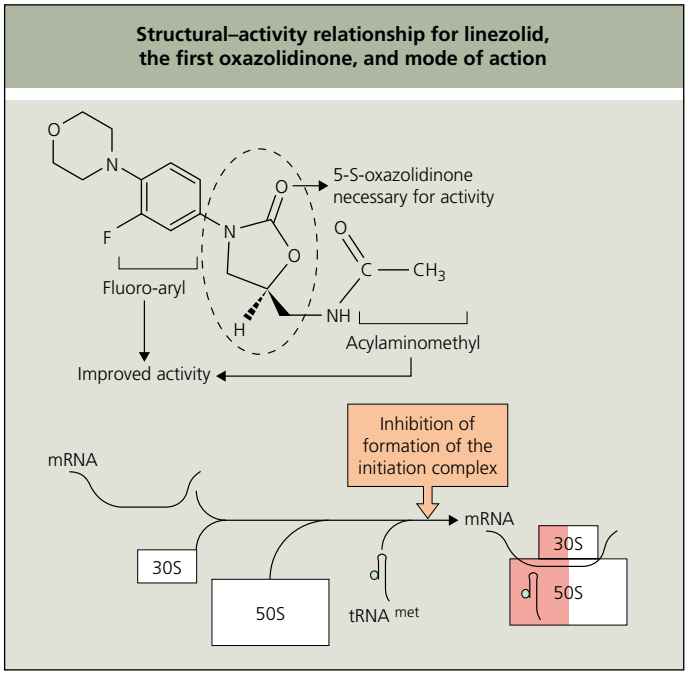


Fig. 130.15 Structure–activity relationship for linezolid, the first oxazolidinone, and mode of action. The drug prevents the formation of the ternary complex between mRNA, ribosome subunits and $tRNA^{met}$ necessary for protein synthesis.

Oxazolidinones also interact with mitochondrial ribosomes to inhibit protein synthesis, which is probably the basis for the undesirable myelosuppressive effects of linezolid.[42]

Resistance

Because of the unique mode of action of oxazolidinones, there is no cross-resistance with other antibiotics acting on protein synthesis. Resistance develops following point mutations of the 23S rRNA[43] or deletions in the gene encoding riboprotein L4.[44]

Pharmacodynamics

Oxazolidinones are bacteriostatic against enterococci and staphylococci with a short postantibiotic effect. For streptococci, linezolid is bactericidal for the majority of strains. Because of its prolonged half-life, linezolid activity is primarily dependent on the AUC_{24h}/MIC parameter *in vivo*.[11]

Future developments

New oxazolidinones being developed have a broadened spectrum, increasing intrinsic activity and, potentially, reduced side-effects, and no new compounds (torezolid and radezolid) are currently in clinical development.

DRUGS THAT AFFECT NUCLEIC ACIDS (DNA/RNA)

Fluoroquinolones

Chemical structure

Fluoroquinolones are totally synthetic products originally derived from nalidixic acid. All current compounds have a dual ring structure, with a nitrogen at position 1, a free carboxylate at position 3 and a carbonyl at position 4. A fluor substituent at position 6 usually greatly enhances activity, whereas the substituents at positions 7, 8 and N1 modulate the spectrum, the pharmacokinetics and the side-effects of the drugs (Fig. 130.16).[45] In this respect, more recent molecules (moxifloxacin, gemifloxacin, gatifloxacin) have been designed to better cover Gram-positive organisms, while retaining activity against Gram-negative organisms and, in the case of moxifloxacin, against anaerobes. They all present a small hydrophobic substituent at N1 and a diaminated small-sized ring substituent at position 7.

Bacterial targeting

Fluoroquinolones cross the outer membrane of Gram-negative bacteria via porins. Their affinity for their bacterial target is 1000 times greater than for the corresponding eukaryotic enzyme, which ensures their specificity.

Mode of action

Fluoroquinolones inhibit the activity of topoisomerases. These enzymes are responsible for the supercoiling of DNA (DNA gyrase) and the relaxation of supercoiled DNA (topoisomerase IV). Both enzymes have a similar mode of action, implying:

- the binding of DNA to the enzyme;
- the cleavage of the DNA;
- the passage of the DNA segment through the DNA gate; and
- the resealing of the DNA break and release from the enzyme.

Gyrase and topoisomerase IV are tetramers composed of two types of subunit, namely two GyrA or ParC catalyzing the DNA cutting and resealing, and two GyrB or ParE responsible for the transduction and binding of ATP. The main target of fluoroquinolones is DNA gyrase in Gram-negative bacteria and topoisomerase IV in Gram-positive bacteria.[46]

Fluoroquinolones form a ternary complex with DNA and the enzyme (Fig. 130.17).[47] This binding site for fluoroquinolones is formed during the gate-opening step of the double-stranded DNA. Co-operatively, four fluoroquinolone molecules are fixed to single-stranded DNA. Their stacking is favored by the presence of the co-planar aromatic rings in their structure and by the tail-to-tail interactions between the substituents at N1. Interaction with DNA occurs by hydrogen bonds or via Mg^{2+} bridges established with the carbonyl and carboxylate groups. Interaction with the enzyme is mediated by fluoride at position 6 and the substituents at position 7. The binding of the fluoroquinolones stabilizes the cleavable complex (formed by the cut DNA and the enzyme) and leads to the dissociation of the enzyme subunits. The latter action is, however, observed only for potent molecules or at higher concentrations.

Fluoroquinolones are highly bactericidal, which is not explained by the above mechanisms alone and requires RNA and protein synthesis to be observed, suggesting the formation of abnormal proteins as a consequence of DNA cleavage. Quinolones also induce an SOS (DNA repair) response, which involves three proteins (RecA, LexA and RecBCD). Induced RecA cleaves the repressor of the SOS regulon (LexA), stimulating repair of damage caused by fluoroquinolones to DNA. Induced RecBCD binds to the chromosome at the double-strand break created by the ternary complex of topoisomerase–DNA–quinolone, leading to mutagenesis as well as increased cell survival in the presence of quinolones. This system therefore protects against the antibacterial activity of fluoroquinolones and could be a basis for emergence of resistance.[48]

Resistance

Resistance was long considered to be only chromosomally mediated from mutation of the topoisomerases (reducing drug-binding ability), porin impermeabilization (for Gram-negative bacteria) or

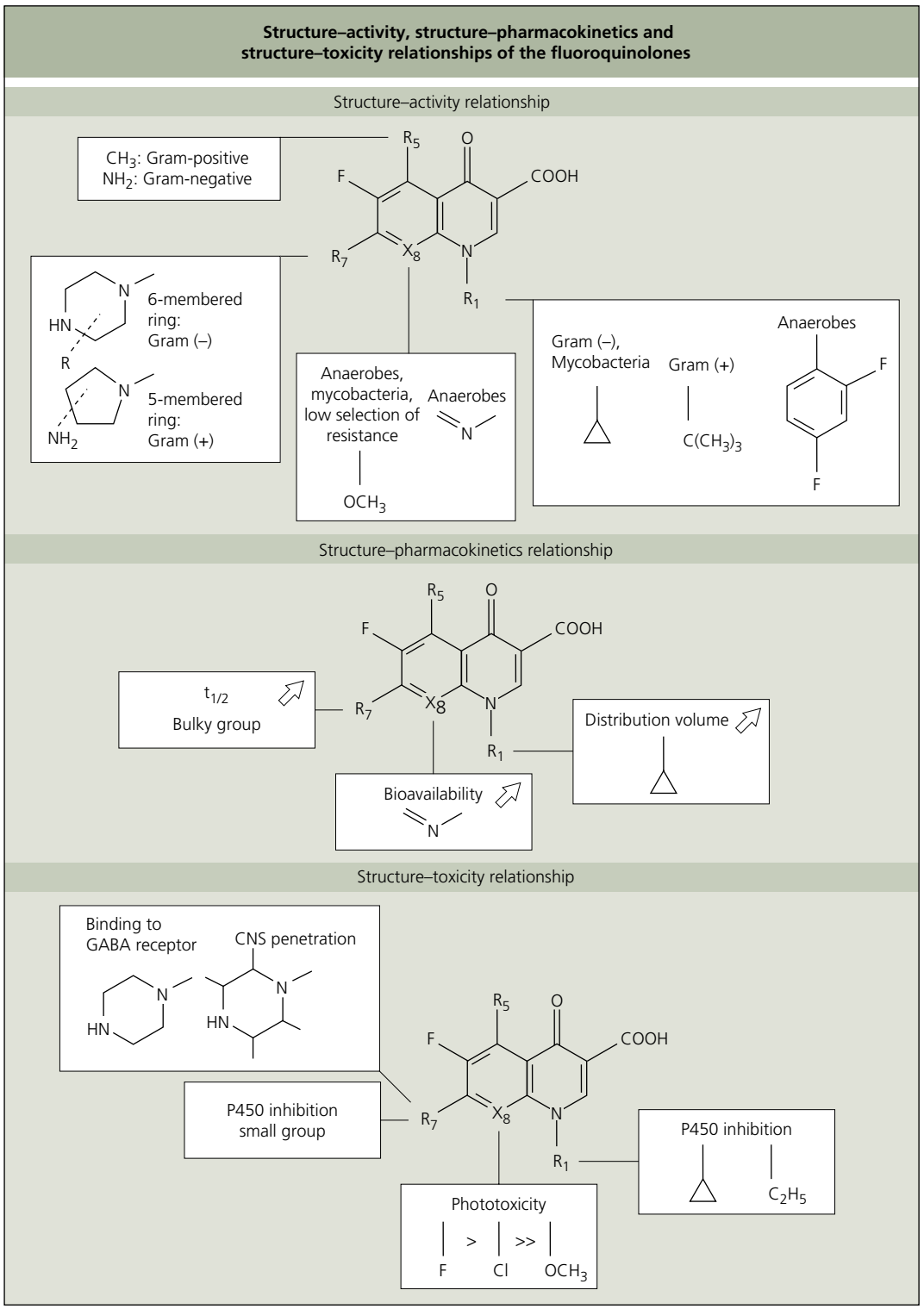


Fig. 130.16 Structure–activity, structure–pharmacokinetics and structure–toxicity relationships of the fluoroquinolones. These considerations form the basis of the rational development of the new molecules of this class, which have a very extended spectrum (including Gram-positive bacteria and anaerobes), a long half-life and minimal phototoxicity and metabolic interactions.

efflux.[45] A methoxy substituent at position 8 (see Fig. 130.16) lowers the ratio of the MIC in gyrase mutant strains to the corresponding MIC in the wild type, thereby reducing the risk of selecting resistant mutants during therapy. Plasmid-mediated resistance, however, is now increasingly observed,[49] related to the production of:

- Qnr proteins capable of protecting DNA gyrase from quinolones (which have homologues in water-dwelling bacteria where they probably serve as chaperones); and
- AAC(6′)-Ib-cr, a variant aminoglycoside acetyltransferase capable of acetylating ciprofloxacin.

Pharmacodynamics

The activity of fluoroquinolones is largely both concentration dependent (which drives the bactericidal effect) and proportional to the amount of drug administered (which drives the global efficacy *in vivo*), making these drugs C_{max}/MIC and AUC_{24h}/MIC dependent for their activity. For older fluoroquinolones that have a short half-life (e.g. ciprofloxacin), this imposes the use of repeated doses per day (combining all doses in a single administration would favor bactericidal effects but would risk toxic reactions). For newer fluoroquinolones with more prolonged

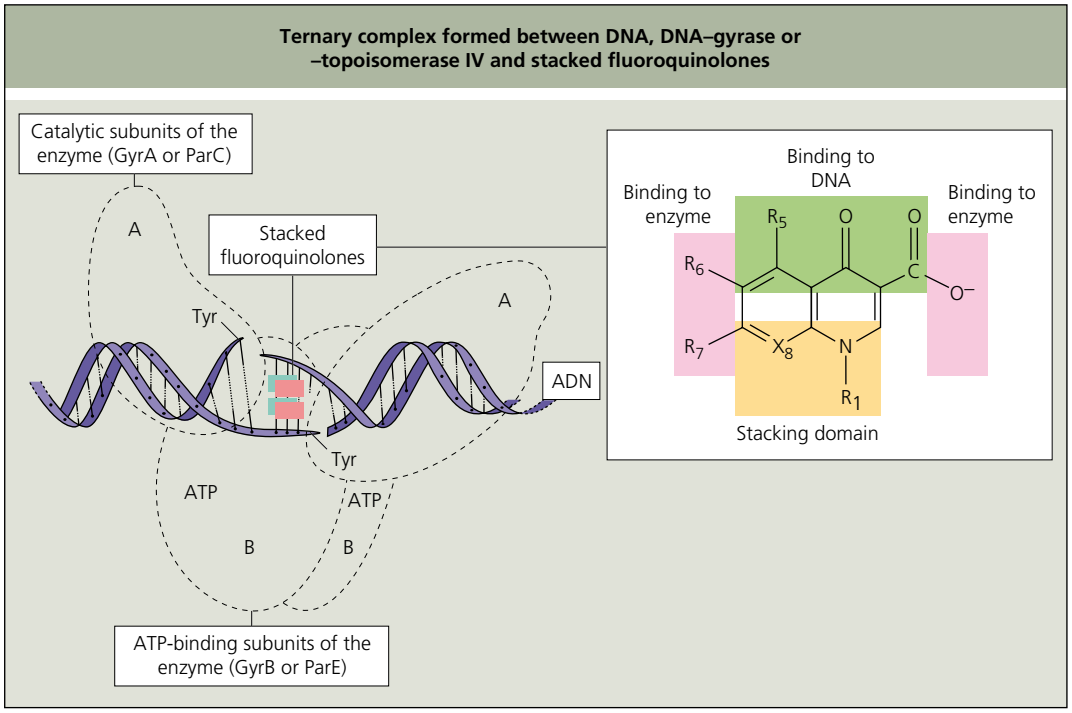


Fig. 130.17 Ternary complex formed between DNA, DNA–gyrase or –topoisomerase IV and stacked fluoroquinolones. Subunits A form covalent bonds via Tyr122 with the 5′ end of the DNA chain. The binding site for fluoroquinolones is located in the bubble formed during the local opening of the DNA molecule. The right panel shows the parts of the antibiotic molecules interacting with DNA, with the enzyme, or favoring the stacking of the fluoroquinolone molecules. Adapted from Shen *et al*.[47]

half-lives (levofloxacin, moxifloxacin, gemifloxacin), once-daily administration is possible, with the aim of obtaining both C_{max}/MIC (>8) and AUC_{24h}/MIC (from 30 to 125 or more) ratios, offering optimal efficacy and minimizing selection of resistant subpopulations.[45,50] The European breakpoints (usually ≤2 mg/l) have taken this into account.

Future developments

New molecules with extended spectra, still greater intrinsic activity and resistance to efflux transporters are being sought, although a major difficulty is to avoid increases in toxicity. Molecules with enhanced activity at acid pH (for urinary tract, intracellular or stomach infections) or much lower MICs than registered fluroquinolones are currently being developed.

Nitroimidazoles and nitrofurans

Chemical structure

The nitroheterocyclic drugs include nitrofuran and nitroimidazole compounds (Fig. 130.18).

Mode and spectrum of action

The activity of the nitroheterocyclic drugs requires activation of the nitro group attached to the imidazole or furan ring, which must undergo single- or two-electron enzymatic reduction in the bacteria.[51] Although the nitro radicals generated by reduction of the parent drugs are similar for the nitroimidazoles and the nitrofurans, these drugs differ by their reduction potential, and therefore their spectrum of activity. Thus nitroimidazoles must be fully reduced to generate the highly reactive species (hydroxylamines) that cause damage, whereas singly reduced nitrofurans may directly inhibit the activity of enzymes involved in the degradation of glucose and pyruvate and covalently bind to proteins and DNA by an alkylation reaction. Nitroimidazoles will, therefore, express activity only towards truly anaerobic and microaerophilic bacteria, and to other parasitic organisms such as *Trichomonas vaginalis*, capable of generating a sufficiently low redox potential thanks to the presence of an H_2-generating organelle (hydrogenosome), whereas nitrofuranes are equally active against anaerobic and aerobic bacteria.

Resistance

Resistance to nitroimidazoles in true anaerobic bacteria is rare, but has been described in *Bacteroides fragilis* (combination of decreased antibiotic uptake, reduced nitroreductase and pyruvate:ferredoxin oxidoreductase activity and increased lactate dehydrogenase activity).[52] It has become significant in *Helicobacter pylori* (null mutations in *rdxA* encoding an oxygen-insensitive nitroreductase that normally prevents reoxidization of metronidazole in the microaerophilic environment of this bacterium).[53]

Pharmacodynamics

Nitroimidazoles show concentration-dependent killing, which is consistent with the current clinical pattern of dispensing a large dose in a single administration (although more frequent administration of lower dose is also recommended).

Future developments

The variety of substitutions that can be attached to the ring structures may allow new drug development. This has not been explored so far for antibacterial therapy, although finding new derivatives for amebiasis and giardiasis is being actively pursued.

Ansamycins

Chemical structure

These macrocyclic antibiotics are lipophilic and therefore easily diffuse through membranes. They comprise two aromatic rings (containing a quinone), connected by a long chain (or 'ansa' – hence the name given to this class of antibiotics), which confers a rigid character to the whole molecule. The first clinically developed and major antibiotic in this class is rifampin (rifampicin). Successful successors have been rifapentin, rifamixin and rifabutin.

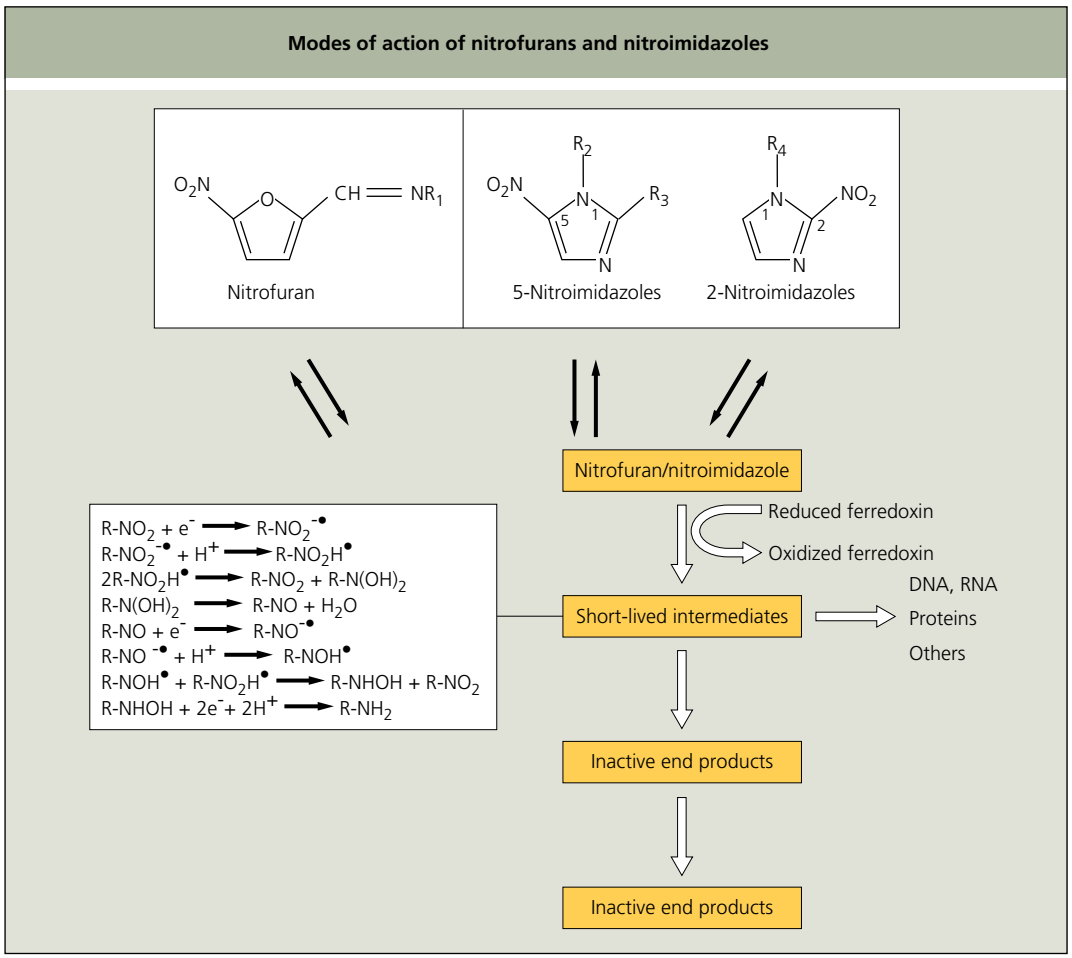


Fig. 130.18 Structures and modes of action of nitrofurans and nitroimidazoles. The molecules must be reduced to form highly reactive products that interact with intracellular targets.

Mode of action

Ansamycins inhibit the initiation of the transcription of DNA in mRNA and therefore subsequent protein synthesis. The RNA polymerase is made up of five subunits ($\alpha_2\beta\beta'\sigma$):

- α subunits establish contact with transcription factors;
- β′ subunit is a basic polypeptide that binds DNA;
- β subunit is an acidic polypeptide and is part of the active site; and
- σ initiates transcription and then leaves the polymerase nucleus.

The core polymerase ($\alpha_2\beta\beta'$) therefore retains the capacity to synthesize RNA but is defective in its ability to bind and initiate DNA transcription.

Inhibition by rifamycins follows binding of the antibiotic to the β subunit of the RNA polymerase or, to a lesser extent, of the DNA–RNA complex. This binding is mediated by hydrophobic interactions between the aliphatic ansa chain and the β subunit. The precise site of binding has been identified only partly, by studying mutants in RNA polymerase that have acquired resistance to rifampin. All the mutations affecting drug binding belong to three clusters of amino acids in the central domain of the β subunit. Specificity of action depends on the fact that ansamycins alter mammalian cell metabolism only at concentrations 10 000 times those necessary to cause bacterial cell death (Fig. 130.19).

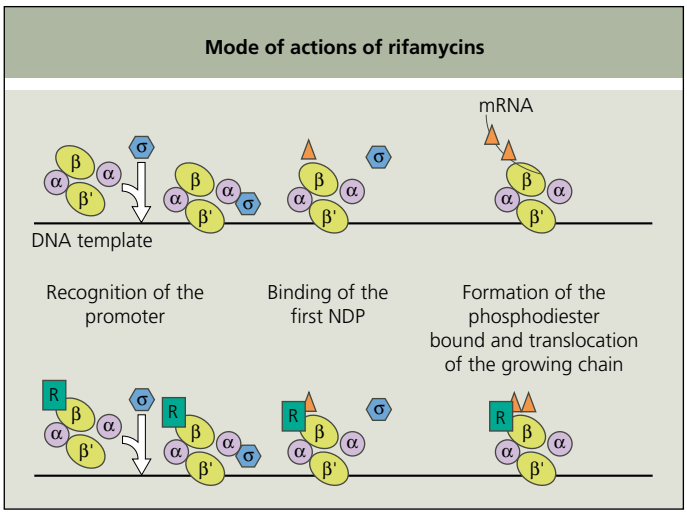


Fig. 130.19 Mode of action of rifamycins. Synthesis of mRNA by RNA polymerase is shown in the upper panel and inhibition by rifamycins (R in the green squares) is shown in the lower panel. The RNA polymerase core is made up of four subunits, of which the β′ subunit binds to the DNA template and the β subunit binds the ribonucleotide diphosphate (NDP; triangle). The σ factor only participates to the initiation step by allowing for the recognition by the enzyme core of promoter sequences on the DNA template. Rifamycins bind to the β subunit. They do not interfere with the binding of the nucleotide diphosphate, but rather inhibit the transcription initiation either by impairing the formation of the first phosphodiester bond or the translocation reaction of the newly synthesized dinucleotide.

Pharmacodynamics

Rifamycins are bactericidal. This effect could be due to either the high stability of the complex formed between rifampin and the enzyme or the formation of superoxide ions of the quinone ring of the antibiotic molecule. Because their action is to hinder bacterial multiplication,

they are, at least *in vitro*, antagonists to antibiotics requiring active bacterial growth to exert their activity (β-lactams) or to other antibiotics acting on protein synthesis (macrolides and aminoglycosides). This antagonism is, however, not observed *in vivo* because of the different distribution of these antibiotics (intracellular for rifamycins vs extracellular for β-lactams and aminoglycosides).

Rifamycins show a long postantibiotic effect because of the irreversible character of their binding. The excellent penetration of rifamycins in eukaryotic cells has often been a major argument for supporting their use against intracellular organisms, including mycobacteria (exposure to critical concentrations of rifampin may be sufficient to kill intermittently metabolizing mycobacterial populations).[54]

Future developments

Benzoxazinorifamycins constitute a new group of semisynthetic molecules with development oriented towards *Chlamydia* infections and other intracellular organisms.[55] Of note, diarylquinolines, inhibitors of mycobacterial ATP synthase,[56] have now emerged as novel tuberculostatic agents acting even on dormant organisms, with TMC207 (R207910) having reached a clinical stage.

ANTIMETABOLITES

Sulfonamides and diaminopyrimidines

Prontosil (sulfamidochrysoidine) – a synthetic compound with antibacterial activity found by Domagk in 1932 – was a prodrug which led to the development of the sulfonamides. With diaminopyrimidines, they inhibit the folate pathway in bacteria. For many years, diaminopyrimidines were used in combination with sulfonamides except for specific indications (parasitic diseases) or for the treatment of uncomplicated cystitis. A recent revival of diaminopyrimidines has been the discovery and development of iclaprim (not yet approved).

Chemical structure

Sulfonamides are derived from *p*-aminobenzenesulfonamide which is a structural analogue of *p*-aminobenzoic acid, a factor required by bacteria for folic acid synthesis (Fig. 130.20). A free amino group at position 4 and a sulfonamide group at position 1 are required for antibacterial activity. Heterocyclic or aromatic rings substituting the sulfonamide enhance this activity by modifying absorption and gastrointestinal tolerance.

Diaminopyrimidines such as trimethoprim (Fig. 130.20) and pyrimethamine are pyrimidines substituted at position 5 by an aromatic group. Pyrimethamine has an additional substituent at position 6. Iclaprim (Fig. 130.20) is the result of pharmacochemical research aimed at selecting compounds not only more active than trimethoprim but also expanding activity against trimethoprim-resistant strains. It is a racemic mixture of two enantiomers, both of which show equipotent activity.

Mode of action

Sulfonamides inhibit tetrahydrofolic acid synthesis, acting at the level of dihydropteroate synthetase as analogues of *p*-aminobenzoic acid, and as alternative substrates to become incorporated into a product with pteridine.[57,58]

Diaminopyrimidines are specific competitive inhibitors of bacterial dihydrofolate reductase.[57,58] Selectivity of action is achieved as trimethoprim in establishing more binding interactions with the bacterial than with the corresponding eukaryotic enzymes. In addition, the NADPH co-factor may stabilize the enzyme–trimethoprim complex in bacteria. Iclaprim shows additional increased binding and the ability to inhibit the trimethoprim-resistant enzyme, based on the lipophilic interactions of the cyclopropyl ring with two hydrophobic amino acids (Leu54 and Ile50) of the target enzyme.[59]

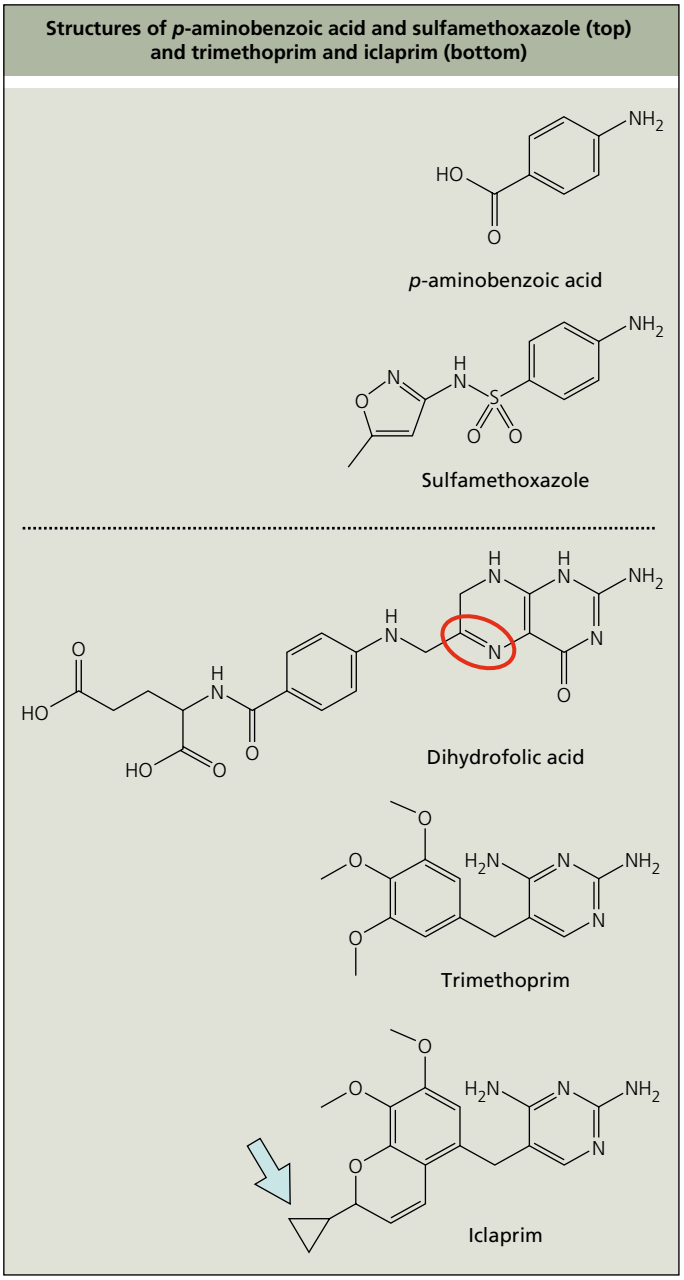


Fig. 130.20 Structures of *p*-aminobenzoic acid and sulfamethoxazole, and trimethoprim and iclaprim. The figure highlights the similarity between *p*-aminobenzoic acid (a sulfonamide) and dihydrofolic acid and trimethoprim, which are the basis for the activity of these drugs (in dihydrofolic acid, the red circle outlines the positions that are reduced for conversion into tetrahydrofolic acid); this part of the figure also shows the differences between trimethoprim and iclaprim, for which the bulky cyclopropyl moiety (block arrow) strengthens the interactions with the target enzyme and helps to overcome resistance to trimethoprim.

Resistance

For sulfonamides, resistance mainly occurs from hyperproduction of *p*-aminobenzoic acid or a reduced affinity of dihydrofolate reductase for the antibiotic, causing resistance to the whole class. For diaminopyrimidines, resistance mostly occurs via enzyme mutations which prevent binding, but this can be reversed by suitable modification such as that made with iclaprim.[59]

Pharmacodynamics

Sulfonamides are bacteriostatic; however, in combination with diaminopyrimidines they are bactericidal. The pharmacodynamics of iclaprim are poorly defined, mainly because of low blood levels and interference by thymidine on its activity.

ANTIBIOTICS ACTING ON THE MEMBRANE

Cyclic polypeptides (polymyxins/colistins)

Chemical structure

These are a collection of cyclic, branched polypeptides (molecular masses about 1000 Da) containing both cationic and hydrophobic amino acids. Some of these are of the D configuration or are non-DNA coded, which confers resistance to mammalian peptide-degrading enzymes. Polymyxins are obtained from *Bacillus polymyxa* and colistins from *Aerobacillus colistinus*. Only polymyxin B and colistin (identical to polymyxin E) are used in clinical practice. Commercial colistin contains at least two components (E1 and E2, also called colistin A and colistin B) differing by the length of the fatty acid chain.

Mode of action

Because of their amphipathic character, polymyxins and colistins act as detergents and alter the permeability of the cytoplasmic membrane.[60] They therefore act at all stages of bacterial development. However, they cannot diffuse easily through the thick peptidoglycan layer of Gram-positive bacteria. In contrast, they bind easily to the outer membrane of Gram-negative bacteria (interacting with the lipopolysaccharide (LPS) and triggering a 'self-promoted uptake' process) from where they reach the cytoplasmic membrane through polar as well as nonpolar channels. These properties explain their strong and fast bactericidal activity through disruption of the inner membrane and their essentially Gram-negative spectrum.

Resistance

Acquired resistance to polymyxins and colistins is chromosomal and results from decreased permeability of the outer membrane secondary to changes in its biochemical composition. Bacteria with decreased sensitivity are characterized by a decreased phospholipid/lipid ratio and a higher content of divalent cations (Ca^{2+}, Mg^{2+}). Protein H1 from *P. aeruginosa* (OprH) prevents binding of polymyxins and colistins to lipopolysaccharide, and its overproduction correlates with reduced sensitivity. However, this change is not sufficient per se and must be combined with other modifications of the membrane; two genes downstream to OprH (PhoP and PhoQ) co-regulate OprH and polymyxin B resistance. Resistance to polymyxins and colistins was previously uncommon but is now increasingly described in strains exhibiting multiple resistance to β-lactams and aminoglycosides. A puzzling observation is that the regrowth is easily observed *in vitro*, suggesting the occurrence of a so-called 'adaptive resistance', the mechanism of which is still uncertain.

Pharmacodynamics

Colistin A and polymyxin B show concentration-dependent activity but little or no postantibiotic effect, justifying the administration of repeated daily doses.[61] The pharmacodynamic parameters governing the activity of colistin are still undefined as both C_{max}/MIC and time above MIC are critical not only for efficacy but also to prevent regrowth.

Nonantibiotic pharmacologic and toxicologic properties related to chemical structure

As membrane-disrupting and lipid-binding agents, polymyxins and colistins display a number of non-antibiotic effects. Some of them are potentially useful, such as inactivation of endotoxins and synergy with serum bactericidal activities. Others, however, are highly detrimental to the host and include activation of the alternate complement pathway, mastocyte degranulation with histamine release, decreased production of cytokines (but increased tumor necrosis factor release), increased membrane conductance in epithelia, and apoptosis.

Future developments

Because of the widespread emergence of resistance to other antimicrobials, colistin B has become more frequently used in chronic, difficult-to-treat infections (e.g. pulmonary infections in cystic fibrosis patients). Polymyxin B has also been investigated as an anti-endotoxin agent.[54] New, potentially less-toxic derivatives of these molecules and other membrane-destabilizing peptides have also been isolated or synthesized recently with some of them being under clinical development.[53]

REFERENCES

References for this chapter can be found online at http://www.expertconsult.com

Chapter 131

Franz-Josef Schmitz
Ad C Fluit

Mechanisms of antibacterial resistance

INTRODUCTION

Although treatment of infections is often initiated empirically, the laboratory determination of bacterial susceptibility to an antimicrobial agent is essential because of widespread resistance to all classes of antimicrobial agents. Standardized methods for determining susceptibility and resistance have been formulated.[1] Bacterial isolates considered resistant to an antibiotic by these methods usually cannot be treated by this antibiotic, although the successful clinical outcome for isolates deemed susceptible likewise cannot be guaranteed.

There is a strong correlation between the presence of some determinants of bacterial resistance and the outcome of antimicrobial therapy. The presence of a β-lactamase in *Neisseria gonorrhoeae* strongly correlates with penicillin treatment failure. The presence of the *mecA* gene in *Staphylococcus aureus* is highly predictive of treatment failure with oxacillin, and in fact oxacillin-resistant *Staph. aureus* (usually called methicillin-resistant *Staph. aureus*, MRSA) is by definition also considered resistant to all β-lactam antibiotics.

However, the presence of a resistance gene is not equivalent to treatment failure. The gene should also be expressed in sufficient amounts to lead to phenotypic resistance. Expression may differ depending on culture conditions or site of infection. For example, β-lactamase production is common among Enterobacteriaceae but resistance to penicillins depends on the mode and degree of expression.

Antibiotic resistance can be divided into eight basic groups depending on the mechanism involved:

- the presence of an enzyme that inactivates the antibiotic;
- the presence of an alternative enzyme to that inhibited by the antibiotic;
- target mutation, which reduces binding of the antibiotic to the target;
- target modification, which reduces binding of the antibiotic to the target;
- reduced uptake of the antibiotic,
- active efflux of the antibiotic,
- removal by binding of the antibiotic, and
- protection of the target enzyme by binding another protein.

The genetic determinants for resistance against antimicrobial agents can be located on the bacterial chromosome or on plasmids. The analysis of resistance genes and their distribution through the use of modern DNA techniques has provided new insights into the mechanisms of resistance and the spread of resistance genes in hospitals and the community.

RESISTANCE TO β-LACTAM ANTIBIOTICS

Penicillin is the oldest β-lactam antibiotic; subsequent developments have included a whole array of β-lactam-based antibiotics, such as the first-generation through to the fourth-generation cephalosporins, monobactams and carbapenems. Almost immediately after the introduction of penicillin, resistance was observed in staphylococci. The β-lactam antibiotics interfere with cell wall synthesis by binding enzymes called penicillin-binding proteins (PBPs). Resistance to β-lactams is mainly caused by either the presence of β-lactamases, which destroy the lactam ring, or the presence of altered PBPs, which are not inhibited by these antibiotics. In Gram-positive bacteria the β-lactamase is excreted into the extracellular environment, and in Gram-negative bacteria it is excreted into the periplastic space. Whether the β-lactam antibiotic is effective against the bacterium depends on a number of factors (Fig. 131.1), including:[2]

- the concentration of the antibiotic in the environment;
- the rate of entry through the outer membrane (in the case of Gram-negative bacteria);
- the amount of β-lactamase;
- the hydrolysis rate for the antibiotic by the β-lactamase; and
- the affinity of the PBPs for the antibiotic.

The number of β-lactamases has risen steadily since the introduction of penicillin. The β-lactamases have been classified according to their

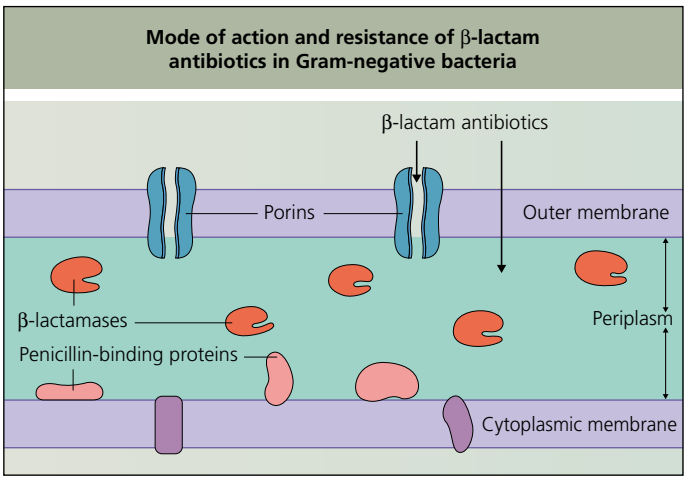


Fig. 131.1 Mode of action and resistance of β-lactam antibiotics in Gram-negative bacteria.

functional aspects.[3] This system is based on hydrolysis rates for a number of substrates and the level of inhibition by clavulanic acid, but simple point mutations may alter the classification. They have also been classified according to the nucleotide sequences that encode β-lactamases.[4] Classes A, C and D have a serine at their active site, whereas class B has a zinc atom at the active site. Class A enzymes are encoded mostly on plasmids, whereas class C enzymes are generally chromosomally encoded, although the genes for these β-lactamases are also found increasingly on plasmids. Class A enzymes are generally constitutively expressed. Class C enzyme genes are present in almost all Gram-negative bacilli, except *Salmonella* spp., but their presence does not necessarily lead to resistance. Class C enzymes are usually inducible and a total of four genes are required for expression of β-lactamase activity. *Escherichia coli* possesses the *ampC*, *ampD* and *ampG* genes, but lacks the *ampR* gene. Why *E. coli* possesses three of these genes and lacks the fourth is unclear. Class D is a limited group of enzymes able to hydrolyze oxacillin; they are related to class C enzymes. Class B is of increasing importance because many act as carbapenemases. The β-lactamases range between 30 and 40 kDa in size.

The commonest β-lactamases in Enterobacteriaceae are TEM-1, TEM-2 and SHV-1 (TEM are the first three letters of the name of the patient from whom the isolate was obtained; SHV stands for sulfhydryl variable). These are simple penicillinases and their activity can be inhibited by compounds such as clavulanic acid and tazobactam, thereby rendering penicillin derivatives active again. However, TEM and SHV enzymes can easily obtain a broader spectrum through mutations, which may lead to resistance against third-generation cephalosporins. Inactivation of aztreonam, ceftazidime, cefotaxime or ceftriaxone is considered an indicator for the presence of such an extended-spectrum β-lactamase (ESBL). However, these antibiotics can also be inactivated by the overproduction of ampC.

Resistance to third-generation cephalosporins was first described in 1983 and was mediated by a plasmid encoding for a TEM-related β-lactamase. The majority of ESBLs are found in *Klebsiella pneumoniae*, but also increasingly in other Enterobacteriaceae. Extended-spectrum β-lactamases are encoded by plasmids and are highly transmissible. More than 160 TEM-type and 100 SHV-type β-lactamases have been described; nearly half are ESBLs, but at least 10 members of the TEM family are no longer inhibited by clavulanic acid (inhibitor-resistant TEM). In addition, 60 CTX-M-type, and 10 OXA-type ESBLs are known. Besides these commonly occurring ESBL families, other ESBLs have been described.[5]

Some plasmid-encoded cephalosporinases, which are also called cephamycinases, are derived from plasmid-encoded ampC β-lactamases, but are produced constitutively. The prevalence of plasmid-encoded ampC β-lactamases among Enterobacteriaceae is increasing. Further spread of these genes through the hospital or the community may further endanger the use of cephalosporins.

Another group comprises the carbapenemases. These enzymes inactivate the highly active carbapenems such as imipenem and new carbapenems such as ertapenem and doripenem. In the past these enzymes were only encoded chromosomally, but increasingly the genes encoding these β-lactamases are also found on plasmids. This will lead to an increase in resistance against carbapenems. However, resistance is still rare with the exception of *Pseudomonas aeruginosa* and *Acinetobacter* spp. Many carbapenemases belong to Class B. However, an OXA carbapenemase, which is a Class A enzyme, has been reported. In addition, three *K. pneumoniae* carbapenemases (KPC) are known. These enzymes have also been reported in other Enterobacteriaceae, including *Salmonella*, and have caused several outbreaks in the eastern USA.

Altered PBPs are also a major reason for resistance against β-lactam antibiotics. An altered PBP is involved in methicillin resistance in staphylococci. Both MRSA and methicillin-resistant *Staphylococcus epidermidis* (MRSE) are important causes of nosocomial infections. These infections are difficult to treat because both MRSA and MRSE are generally multiresistant and susceptible to only a limited number of antibiotics.

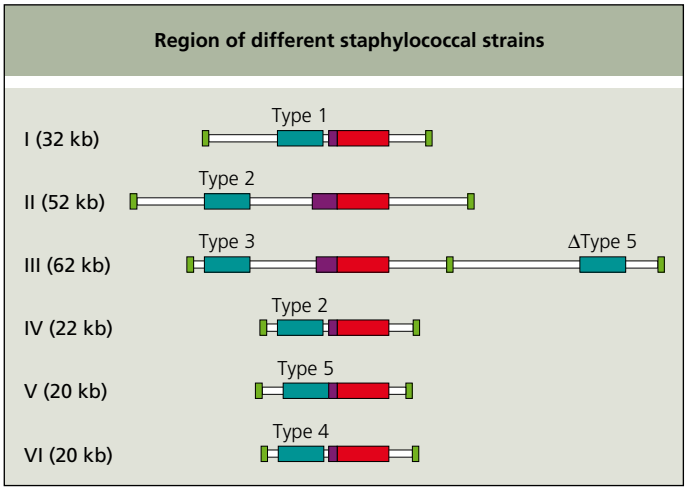


Fig. 131.2 *MecA* region of different staphylococcal strains. Red: *mecA*, methicillin-resistance gene; purple: regulatory genes *mecI/R*; gray: *ccr* genes with indication of type; green: direct repeats; white: variable sequences.

This PBP2a is encoded by the *mecA* gene. Regulation of methicillin resistance is complex. Expression can be heterogeneous and only a few cells express the phenotype, although all cells are genotypically identical and possess the *mecA* gene. Through mutation the resistance phenotype may become homogeneous, which is the case in the majority of isolates. Expression is also influenced by the plasmid-encoded β-lactamase regulatory (blaR) systems blaR1 and blaI inducer-repressor system, which interacts with the mec-associated mecR1 and mecI system.[6] The *mec* determinant appears to originate in coagulase-negative staphylococci, which have a much higher prevalence of the gene, and horizontal transfer appears to take place on a regular basis.

The *mecA* gene is located on a staphylococcal chromosome cassette (SCC*mec*). Six basic types of SCC*mec* have been described based on the type of *ccr* genes and the presence or absence of the complete *mecI* and *mecR* genes (mec complex A and B, respectively) (Fig. 131.2). Within these basic types considerable variation has been observed. This is in part due to the insertion of resistance plasmids and/or transposons into some SCC*mec*. The *ccr* genes are site-specific recombinases which recognize the direct repeats and are involved in the mobilization of SCC elements and thereby their transfer between different staphylococci. SCC*mec* is always integrated in the *orfX* gene of staphylococci.[7] Besides the presence of *mecA*, some methicillin-resistant strains are overproducers of β-lactamases.[8,9]

Resistance to penicillin in *Streptococcus pneumoniae* is also due to the presence of altered PBPs, and this mechanism can be responsible for chromosomally mediated penicillin resistance in *N. gonorrhoeae*. In *Enterococcus faecium* mutations in PBP5 are responsible for resistance to ampicillin. Ampicillin resistance is frequently found in hospital-associated strains. Vancomycin-resistant clinical isolates are often also ampicillin resistant.

A reduction in porins in Gram-negative bacteria such as Enterobacteriaceae, and in particular in *P. aeruginosa* and *Acinetobacter* spp., contributes to β-lactam resistance, but by itself is insufficient to account for resistance.

RESISTANCE TO AMINOGLYCOSIDES

The first clinically effective aminoglycoside, streptomycin, was found in *Streptomyces griseus* in 1944. Numerous aminoglycosides have since been isolated from species belonging to the genera *Streptomyces*, *Micromonospora*, *Bacillus* and *Pseudomonas*. Synthetic derivatives, such as amikacin, were also produced. Clinically, the most important aminoglycoside antibiotics are gentamicin, tobramycin, amikacin and

streptomycin. They have a broad antimicrobial spectrum and are effective against both Gram-positive and Gram-negative organisms; however, they are ineffective against anaerobes. Aminoglycosides bind to ribosomes and thus interfere with protein synthesis.

Resistance to aminoglycoside antibiotics is widespread and of clinical importance. It is observed in both Gram-negative and Gram-positive bacteria. Aminoglycosides enter the bacterial cell in several phases. In the first phase the aminoglycosides bind to anionic sites on the cell and, after binding, diffuse through outer membrane proteins. In *P. aeruginosa* the entry is enhanced; the lipopolysaccharides of *P. aeruginosa* are rich in phosphate groups to which the aminoglycosides bind. This binding displaces magnesium ions, thus allowing entry of the aminoglycoside. The second and third phases are energy dependent and transport the aminoglycoside molecules across the cytoplasmic membrane. The lack of activity of aminoglycosides against anaerobes is explained by the fact that their transport across the cytoplasmic membrane depends on aerobic respiration.

Inactivation of aminoglycosides is the major mechanism of resistance against these antibiotics; others include ribosomal modification and reduced permeability. The enzymes responsible for aminoglycoside inactivation belong to three classes, depending on the type of modification that causes inactivation: phosphotransferases (APH), acetyltransferases (AAC) and nucleotidyltransferases (ANT). Each class is subdivided on the basis of the site of modification on the substrate and substrate specificity (Table 131.1). Often these enzymes are able to modify several structurally related aminoglycosides. The enzymes have to inactivate their targets before they reach the ribosomes and they appear to be either located inside the cell or associated with the cytoplasmic membrane.[10]

Studies concerning the evolution of resistance using nucleotide sequencing clearly show that, at least within enzyme classes, the genes are related. For example, immunologic and hybridization tests have shown that the APH(3′) enzymes from streptococci and staphylococci are closely related, as are those enzymes on the Gram-negative transposons (Tn), Tn*5* and Tn*903*. However, there is far less homology between enzymes from Gram-negative bacteria compared to enzymes from Gram-positive bacteria. Nevertheless, the genes from Gram-negative bacteria appear to be more closely related to a gene of *Streptomyces fradiae*. It is likely that gene transfer has taken place between these species. Aminoglycoside resistance genes are believed to originate from genes involved in the production of aminoglycosides in aminoglycoside-producing species.[11]

Table 131.1 Characteristics of a selected set of aminoglycoside-modifying enzymes. Only the clinically relevant antibiotics are listed

Enzyme subclass	Number of different genes described that encode enzymes capable of the same aminoglycoside modification	Modification	Distribution
AAC(1)	1		
AAC(3)-I	2	Gentamicin	Gram-negative bacteria
AAC(3)-II	3	Gentamicin, tobramycin	Gram-negative bacteria
AAC(3)-III	3	Gentamicin, tobramycin	Gram-negative bacteria
AAC(3)-IV	1	Gentamicin, tobramycin	Gram-negative bacteria
AAC(3)-VI	1	Gentamicin	Gram-negative bacteria
AAC(3)-VII	1		Fungi
AAC(3)-VIII	1		Fungi
AAC(3)-IX	1		Fungi
AAC(3)-X	1		Fungi
AAC(6′)-I	9	Amikacin	Gram-negative and Gram-positive bacteria
AAC(6′)-II	2	Gentamicin, tobramycin	Gram-negative bacteria
AAC(6′)-III	1		Gram-negative bacteria
AAC(6′)-APH(2″)	1	Amikacin, gentamicin, tobramycin	Gram-positive bacteria
AAC(2′)I	1	Gentamicin, tobramycin	Gram-negative bacteria
ANT(2″)-I	3	Gentamicin, tobramycin	Gram-negative bacteria
ANT(3″)-I	1	Streptomycin	Gram-negative bacteria
ANT(4′)-I	1	Amikacin, tobramycin	Gram-positive bacteria
ANT(4″)-I	1	Amikacin, tobramycin	Gram-negative bacteria
ANT(6)-I	1	Streptomycin	Gram-positive bacteria
ANT(9)-I	1		Gram-positive bacteria
APH(3′)-I	3		Gram-negative bacteria
APH(3′)-II	1		Gram-negative bacteria
APH(3′)-III	1	Amikacin	Gram-positive bacteria
APH(3′)-IV	1		Gram-positive bacteria
APH(3′)-V	3		Fungi
APH(3′)-VI	2	Amikacin	Gram-negative bacteria
APH(3′)-VII	1		Gram-negative bacteria
APH(3″)-I	2	Streptomycin	Fungi
APH(6)-I	4	Streptomycin	Fungi
APH(4)-I	2		Gram-negative bacteria, fungi

AAC, acetyltransferase; ANT, nucleotidyltransferase; APH, phosphotransferase.

Besides the transfer of genes between bacteria and aminoglycoside-producing species, transfer between Gram-positive and Gram-negative species has been documented.[12] It should be noted, however, that transfer of a functional resistance gene from one species to another does not assure the expression of resistance.

Aminoglycoside resistance in staphylococci is well documented[13] and up to six genes have been identified. Often more than one resistance gene is present. One of the most remarkable aminoglycoside enzymes is the bifunctional AAC(6′)-APH(2″) enzyme, which is found on Tn*4001* of *Staph. aureus* and in *Enterococcus faecalis* isolates. Nucleotide sequencing data suggest that the enzyme arose through the fusion of two genes, each encoding one of the partners. The epidemiology of Tn*4001* is well studied and illustrates that substitutions, insertions and deletions play an important role in the adaptation of these elements to new hosts.

Hybridization studies in Australia showed that Tn*4001* could be present on the bacterial chromosome as well as on a number of structurally related plasmids, and this was linked to the rapid spread of gentamicin- and tobramycin-resistant *Staph. aureus* in Australia. The same transposon was also observed in the USA but with shorter inverted repeats and insertion sequences flanking the resistance gene.

Initially, only resistance against the naturally occurring aminoglycosides was observed and only APH(3′)-II and APH(3′)-III were capable of modifying amikacin *in vitro*, but the introduction of this antibiotic quickly changed this. In the San Juan Veterans Administration Medical Center, the prevalence of amikacin resistance increased from 0.2% to 3.6% among aerobic Gram-negative bacilli in 4 years through the use of amikacin as a first-line antibiotic. Analysis of plasmids obtained from amikacin-resistant *Serratia marcescens* and *K. pneumoniae* showed that these were almost identical. This suggested that the plasmid disseminated through the hospital and that this dissemination occurred quickly.[14]

Besides antibiotic modification, alteration of the binding site for aminoglycosides can play a role in Gram-negative bacteria. 16S rRNA methylation as protection against aminoglycosides has been reported, but is not widespread. Resistance to streptomycin is often caused by mutation of the S12 protein in the small ribosomal subunit (30S subunit).

RESISTANCE TO MACROLIDES, LINCOSAMIDES AND STREPTOGRAMINS

Macrolide, lincosamide and streptogramin (MLS) antibiotics are chemically distinct inhibitors of bacterial protein synthesis. Macrolides are composed of a minimum of two amino sugars or neutral sugars attached to a lactone ring of variable size. Macrolides can be subdivided according to the chemical structure of their lactone ring into 14-membered, 15-membered or 16-membered lactone ring macrolides. These classes differ in their pharmacokinetic properties and in their responses to bacterial resistance mechanisms. Lincosamides are alkyl derivatives of proline and are devoid of a lactone ring. Streptogramin antibiotics are mixtures of naturally occurring cyclic peptide compounds. They are composed of two factors, A and B (e.g. pristinamycin II and I, virginiamycin M and S), with synergistic inhibitory and bactericidal activities[15,16] (see Chapter 135).

Intrinsic resistance to macrolide, lincosamide and streptogramin B (MLS_B) antibiotics in Gram-negative bacilli is due to low permeability of the outer membrane to these hydrophobic compounds.

Three different mechanisms of acquired MLS resistance have been described in Gram-positive bacteria. First, target modification alters a site in 23S rRNA that is common to the binding of MLS_B antibiotics. Modification of the ribosomal target confers cross-resistance to MLS_B antibiotics (MLS_B-resistant phenotype) and remains the most frequent mechanism of resistance, although enzymatic modification of the antibiotics and active efflux appear to be increasing.[15,17,18]

Target modifications

Macrolide, lincosamide and streptogramin antibiotics bind to 50S ribosomal subunits and inhibit elongation of peptide chains. Resistance to MLS_B antibiotics is mostly due to acquisition of erythromycin resistance methylase (*erm*) genes that encode enzymes that N^6-dimethylate an adenine residue of 23S rRNA. The precise site of methylation has been located in a highly conserved region of the rRNA. Nucleotide alterations in 23S rRNA, both mutational and post-transcriptional, cluster in the peptidyltransferase region in 23S rRNA domain V, providing a physical basis and a common location for MLS_B antibiotic sites of action. Methylation of rRNA probably leads to a conformational change in the ribosome that results in decreased affinity and leads to co-resistance to all MLS_B antibiotics. This suggests that the binding sites for these drugs overlap or at least functionally interact. Streptogramin A-type antibiotics are unaffected, and synergy between the two components of streptogramin against MLS-resistant strains is maintained.

A sequence comparison of *erm* genes from various bacterial species and results of hybridization experiments under stringent conditions led to the recognition of at least nine classes of resistance determinants (Table 131.2). Because a number of these genes cross-hybridize, clinical isolates can be assigned to one of four hybridization classes: *ermA*, *ermC*, *ermAC*, and *ermF*. This gene distribution is relatively species specific. The amino acid sequences of the methylases encoded by these determinants are related, indicating that the *erm* genes are derived from a common ancestor, possibly belonging to an antibiotic producer.

Table 131.2 Distribution of *erm* genes in clinically important bacterial species

Hybridization class	Gene	Host
ermA	*ermA*	*Staphylococcus aureus*
		Coagulase-negative staphylococci
		Enterococcus faecium
		Streptococcus pyogenes
	ermTR	Streptococci (including *S. pneumoniae* and *S. pyogenes*)
ermAM	*ermP*	*Clostridium perfringens*
	ermZ	*Clostridium difficile*
		Enterococcus faecalis
	ermBC	*Escherichia coli*
		Lactobacillus reuteri
	ermAM	*Streptococcus sanguis*
		Streptococcus pneumoniae
		Streptococcus agalactiae
		Streptococcus pyogenes
		Streptococci (including *S. pneumoniae* and *S. pyogenes*)
		Enterococci
ermC	*ermB*	*Staphylococcus aureus*
		Bacillus subtilis
		Lactobacillus spp.
		Enterococcus faecium
	ermC	*Staphylococcus aureus*
		Coagulase-negative staphylococci
	ermM	*Staphylococcus epidermidis*
ermF	*ermF*	*Bacteroides fragilis*
		Bacteroides ovatus

Data from Leclercq & Courvalin.[15]

Expression of MLS_B resistance can be constitutive or inducible. The character of resistance is not related to the class of *erm* determinant but, rather, depends on the sequence of the regulatory region upstream from the structural gene for the methylase. Regulation by these regions occurs by a translational attenuation mechanism in which mRNA secondary structure influences the level of translation. In laboratory mutants and clinical isolates, single nucleotide changes, deletions or duplications in the regulatory region convert inducibly resistant strains to constitutively resistant ones that are cross-resistant to MLS_B antibiotics.

Expression of MLS resistance in staphylococci may be constitutive or inducible. When expression is constitutive, the strains are resistant to all MLS_B antibiotics. Streptogramin A-type antibiotics escape resistance, and synergy with streptogramin B-type antibiotics is retained. When expression is inducible, the strains are resistant to 14- and 15-membered macrolides only. The 16-membered macrolides, the commercially available lincosamides and the streptogramin antibiotics remain active. This dissociated resistance is due to differences in the inducing abilities of MLS antibiotics; only 14- and 15-membered macrolides are effective inducers of methylase synthesis in staphylococci.

Resistance to MLS antibiotics in streptococci can also be expressed constitutively or inducibly. However, unlike the situation with staphylococci, various macrolides or lincosamides may act as inducers to various degrees. Thus, in streptococci, whether inducible or constitutive, ribosomal methylation leads to cross-resistance among macrolides, lincosamides and streptogramin B antibiotics.

In addition, alterations in ribosomal protein L4 account for resistance in pneumococcal strains selected *in vitro* by macrolide passage. The presence of alterations in the L4 ribosomal protein is consistent with the interpretation that this protein is in contact with or near the peptidyltransferase region in domain V of 23S rRNA. Thus, this alteration may act indirectly to alter 23S rRNA conformation.[19] In some cases, these modifications also reduce the *in vitro* activities of ketolides, derivatives of macrolides, which were designed in order to act against macrolide-resistant micro-organisms.

Mutations in domain V of the 23S rRNA genes resulting in resistance to erythromycin have been reported for *Campylobacter* spp. For *Helicobacter pylori* three point mutations in the same domain have been associated with clarithromycin resistance: A2142G, A2143G, and A2142C. In addition, a T2183C mutation has been described, but its influence on the susceptibility to clarithromycin is unknown.

Antibiotic inactivation

Unlike target modification, which causes resistance to structurally distinct antibiotics, enzymatic inactivation confers resistance only to structurally related drugs.[15,17–20]

Enzymes (ErmA and ErmB) that hydrolyze the lactone ring of the macrocyclic nucleus and phosphotransferases (type I (*mphA*) and type II) that inactivate macrolides by introducing a phosphate on the 2′-hydroxyl group of the amino sugar have been reported in members of the family Enterobacteriaceae and in *Staph. aureus*. The gene *linA* mediates resistance to lincosamides. The product of *linA* has been partially purified and demonstrated to act as a lincosamide O-nucleotidyltransferase. Lactonases that are capable of cleaving the macrocyclic lactone ring structure of type B streptogramins have been identified in staphylococci (*vgb* and *vgbB* genes). Two staphylococcal-related determinants, *vat*, *vatB*, *vatC*, and *satA* encoding an acetyltransferase that inactivates type A streptogramins, have been characterized. In addition, the *vga* and *vgaB* genes encode resistance to type A streptogramins. The *vat* and *vgb* genes are adjacent to each other on plasmid pIP630. This vat–vgb region is flanked by inverted copies of the insertion sequence IS*257*, suggesting a role for this element in dissemination of these determinants. In enterococci the *vatD* gene has been described. A *vatE* gene has also been reported. Both *vatD* and *vatE* confer resistance to streptogramin A compounds.

Active efflux

The presence of multicomponent macrolide efflux pumps in staphylococci (*msrA*, *msrB*) and *N. gonorrhoeae* (*mtr*), as well as an efflux system in streptococci (*mefA*, *mefE*), has also been documented.[21–24] *msr* genes confer resistance only to 14- and 15-membered ring macrolides. Recent epidemiologic surveys have shown that some erythromycin-resistant strains of pneumococci and group A streptococci have been shown to have the M phenotype, namely resistance to macrolides but susceptibility to lincosamide and streptogramin B antibiotics. These strains contain the *mefA* or *mefE* gene coding for an efflux pump for 14- and 15-membered macrolides. The presence of a plasmid-mediated gene, *vga*, encoding for a putative ATP-binding protein, has been associated with an active efflux of streptogramin A group compounds. An overview on macrolide resistance genes was published in 2001.[19]

RESISTANCE TO FLUOROQUINOLONES

Fluoroquinolone antibiotics exert their antibacterial effects by inhibition of certain bacterial topoisomerase enzymes, namely DNA gyrase (bacterial topoisomerase II) and topoisomerase IV.[13,24–27] These essential bacterial enzymes alter the topology of double-stranded (ds) DNA within the cell. In most bacteria, the chromosome exists as a single circle of dsDNA, which is maintained in a highly negatively supercoiled state. This energetically activated form is required for critical cellular processes such as replication and transcription.

Deoxyribonucleic acid gyrase and topoisomerase IV are heterotetrameric proteins composed of two subunits, designated A and B. The genes encoding the A and B subunits are referred to as *gyrA* and *gyrB* (DNA gyrase) or *parC* and *parE* (DNA topoisomerase IV; *grlA* and *grlB* in *Staph. aureus*).

Deoxyribonucleic acid gyrase is the only enzyme that can affect supercoiling of DNA. Inhibition of this activity by fluoroquinolones is associated with rapid killing of the bacterial cell. Topoisomerase IV also modifies the topology of dsDNA, but whereas DNA gyrase seems to be important for maintenance of supercoiling, topoisomerase IV is predominantly responsible for separation of daughter DNA strands during cell division.

In Gram-negative organisms, DNA gyrase is the primary target for quinolones, whereas topoisomerase IV appears to be the primary target in *Staph. aureus* and *Strep. pneumoniae*. In Gram-positive species, mutations in genes encoding topoisomerase IV appear to precede mutations in DNA gyrase. Nevertheless, in *Strep. pneumoniae* it has been shown that different quinolones can have different primary targets in the same bacterial species (i.e. quinolone structure determines the mode of antibacterial action). Thus, the primary target seems to be dependent on the bacterial species as well as on the quinolone structure.

Target modification

Alterations of the target enzymes appear to be the most dominant factors in expression of resistance to quinolones.[13,24–28] Many Gram-negative fluoroquinolone-resistant organisms contain a *gyrA* mutation, resulting in inhibition of supercoiling of DNA and elevated minimum inhibitory concentrations (MICs; Table 131.3). The first molecular characterization of a quinolone resistance mutation in *gyrA* was reported in 1988 from *E. coli*. The mutations found were situated in a relatively hydrophilic region of the polypeptide and close to a tyrosine residue at amino acid 122 at the active site, which has been shown to be the site covalently bound to the DNA. The small region from codon 67 to 106 was designated the quinolone resistance-determining region (QRDR). In almost all instances, amino acid substitutions within the QRDR involve the replacement of a hydroxyl group with a bulky hydrophobic residue. This suggests that mutations in *gyrA* induce changes in the binding site conformation or charge (or both) that may be important for interactions between quinolones and DNA gyrase.

Although quinolones are thought to interact primarily with the A subunit of DNA gyrase, there are mutations in the B subunit that also confer quinolone resistance in some species, such as *E. coli*. However, the frequency of *gyrB* mutations has been shown to be relatively low compared with the frequency of *gyrA* mutations in clinical isolates of *E. coli* and other Gram-negative organisms. No *gyrB* mutations have been reported as resulting in cross-resistance between quinolones and the B subunit inhibitors coumermycin and novobiocin. This is consistent with evidence which suggests that the GyrB protein comprises two distinct domains: an *N*-terminal domain containing the sites for hydrolysis of adenosine triphosphate and binding of coumermycin, and a *C*-terminal domain containing the QRDR of GyrB.

Table 131.3 Alterations in DNA gyrase subunit A conferring quinolone resistance

Organism	Amino acid substitution
Acinetobacter baumanni	Gly 81 → Val Ser 83 → Leu
Aeromonas salmonicida	Ser 83 → Ile Ala 67 → Gly
Coxiella burnetti	Glu 87 → Gly
Campylobacter jejunii	Ala 70 → Thr Thr 86 → Ile
Campylobacter lari	Ser 83 → Arg Thr 86 → Ile Glu 87 → Lys, Gly Thr 86 → Ile Asp 90 → Ala, Asn Pro 104 → Ser
Enterobacter cloacae	Ser 83 → Leu
Escherichia coli	Ala 51 → Val Ala 67 → Ser Gly 81 → Cys, Asp Asp 82 → Gly Ser 83 → Leu, Trp, Ala, Val Ala 84 → Pro Asp 87 → Asn, Val, Thr, Gly, His Gln 106 → Arg, His Ala 196 → Glu
Enterococcus faecalis	Ser 83 → Ile Gln 106 → His, Arg
Enterococcus faecium	Glu 87 → Lys
Helicobacter pylori	Asp 86 → Asn Asn 87 → Lys, Ile, Tyr Ala 88 → Val Asp 91 → Gly, Asn, Tyr Ala 97 → Val
Mycobacterium avium	Ala 90 → Val
Mycobacterium smegmatis	Ala 90 → Val Asp 94 → Gly
Mycobacterium tuberculosis	Gly 88 → Ala, Cys Ala 90 → Val Ser 91 → Pro Asp 94 → Asn, His, Gly, Tyr, Ala, Val Ser 95 → Thr
*Neisseria gonorrhoeae**	Ser 83 → Phe Asp 87 → Asn
Pseudomonas aeruginosa	Thr 83 → Ile Asp 87 → Tyr, Asn, Gly, His
Shigella dysenteriae	Ser 83 → Leu Asp 87 → Asn, Gly
Salmonella	Ala 67 → Pro Asp 72 → Gly Val 73 → Ile Gly 81 → Asp, Cys, His, Ser Ser 83 → Ala, Asp, Phe, Tyr Asp 87 → Gly, Tyr, Asn Leu 98 → Val Ala 119 → Glu, Ser, Val Glu 139 → Ala
Staphylococcus aureus	Ser 84 → Leu, Ala, Phe Ser 85 → Pro Glu 88 → Lys, Gly
Staphylococcus epidermidis	Ser 84 → Phe

*The substitutions for *Neisseria gonorrhoeae* are based on *Escherichia coli* sequence.
Adapted from Everett & Piddock.[25]

Topoisomerase IV is a secondary target for fluoroquinolone action in *E. coli* in the absence of a sensitive DNA gyrase. Mutations in *parC* result in further decreased susceptibility. These mutations in *parC* have been shown to occur at Ser80 and Glu84, which are analogous to codons Ser83 and Asp87 of *E. coli* gyrA, and to be common in fluoroquinolone-resistant clinical isolates of *E. coli*. A mutation has also been reported in the *parE* gene that results in decreased fluoroquinolone susceptibility. However, as in the case with *gyrB*, such mutations appear to be rare in clinical isolates.

In *Staph. aureus*, topoisomerase IV is the primary target of fluoroquinolones. Strains with mutations in *gyrA* and *gyrB* without *grlA* mutations resulting in high-level fluoroquinolone resistance can be isolated by single-step selection with fluoroquinolones in *E. coli* but not in *Staph. aureus*. Previously, 116 clonally unrelated *Staph. aureus* isolates originating from nine different countries were screened for mutations in the *gyr* and *grl* gene loci.[28] In correlating the characterized mutations to the resulting MIC of ciprofloxacin, it is clear that all studied isolates without the *grlA* mutation at position Ser80 were susceptible to ciprofloxacin. All ciprofloxacin-resistant isolates had the *grlA* mutation Ser80 in combination with either a Ser84 mutation or a Glu88 mutation within the *gyrA* gene. In two isolates a Ser80 to Phe mutation was combined with no mutations in the *gyrA* gene, resulting in a MIC value for ciprofloxacin of 2 μg/ml, which, although elevated from a wild-type level, is still below the breakpoint for resistance. These data support the finding that in *Staph. aureus*, *grlA* mutations precede *gyrA* mutations in developing resistance to ciprofloxacin.

Combinations of single point mutations within the *gyrA* gene from various species have been shown to be associated with higher MIC values for ciprofloxacin than single point mutations. Similarly, two combinations of single point mutations within *grlA*, of a Glu84 to Val mutation or an Ala48 to Thr mutation in combination with a Ser80 to Phe mutation, were associated with relatively higher ciprofloxacin MIC values (64–256 μg/ml) than only a single Ser80 to Phe mutation

(8–64 μg/ml). Sequence data show that some *grlA* and *gyrA* mutations are conserved in both MRSA and methicillin-sensitive *Staph. aureus* from unrelated clones of *Staph. aureus* isolated from different countries.

Topoisomerase IV mutations have now been characterized in several other organisms. In *Strep. pneumoniae* topoisomerase IV also appears to be the primary target for fluoroquinolone action. Ciprofloxacin-resistant mutants of *Strep. pneumoniae* were generated by stepwise selection at increasing drug concentrations. First-step mutants exhibiting low-level resistance had no detectable changes in their topoisomerase QRDR, suggesting altered permeation or another novel resistance mechanism. Second-step mutants exhibited an alteration in ParC at Ser79 to Tyr or Ser79 to Phe or at Ala84 to Thr. Third- and fourth-step mutants displaying high-level ciprofloxacin resistance were found to have, in addition to ParC alteration, a change in GyrA at residues equivalent to *E. coli* GyrA resistance hotspots Ser83 and Asp87 or in GyrB at Asp435 to Asn, equivalent to *E. coli* Asp426. ParC mutations preceded those in GyrA, suggesting that topoisomerase IV is the primary topoisomerase target and gyrase the secondary target for ciprofloxacin in *Strep. pneumoniae*. Additionally, it has been shown that in *Strep. pneumoniae* different quinolones can have different primary targets. The targeting of DNA gyrase by sparfloxacin in *Strep. pneumoniae* but of topoisomerase IV by ciprofloxacin indicates that target preference can be altered by change in quinolone structure.

Decreased uptake

Deoxyribose nucleic acid gyrase and topoisomerase IV are both located in the cytoplasm of the bacterial cell. In order to reach their targets, fluoroquinolone antibiotics must traverse the cell envelope. In Gram-positive bacteria this consists of the cell wall and a single membrane, whereas in Gram-negative bacteria the fluoroquinolone must first cross the outer membrane. Changes in the outer membrane of Gram-negative bacteria have been associated with decreased uptake and increased resistance to fluoroquinolones.[24–27] Some of these changes may be due to the effect of quinolones or *gyrA* mutations, or both, on differential expression of outer membrane proteins, because it has been shown that *gyrA*-mediated changes in supercoiling of DNA can affect the expression of porin genes.

Active efflux

Increased efflux as a mechanism of fluoroquinolone resistance has been reported in fluoroquinolone-resistant *Staph. aureus*. The *norA* gene encodes the multidrug efflux pump NorA.[29] The NorA protein has a hydropathic amino acid profile consistent with a location in the cytoplasmic membrane and it exhibits a low level of fluoroquinolone efflux, with a preference for hydrophilic fluoroquinolones. Efflux is an active process and can be inhibited by protonophores. NorA-mediated fluoroquinolone resistance is due to overexpression of the wild-type gene *norA*. In *P. aeruginosa*, resistance to fluoroquinolones as well as to a number of other antimicrobial agents has often been associated with decreased accumulation and increased expression of outer membrane proteins, often with concomitant increase in cytoplasmic membrane proteins. Resistance is due to overexpression of one or more efflux systems (i.e. OprK) capable of removing fluoroquinolones and other antibiotic compounds. *E. coli* has also been shown to possess efflux systems, notably EmrAB and AcrAB.[25]

Protection of gyrase and topoisomerase IV

Plasmid-encoded quinolone resistance was discovered in the late 1990s. The responsible gene, *qnrA1*, encodes a pentapeptide repeat protein that binds to gyrase and protects it from the action of quinolones. Remarkably, another member of the pentapeptide-repeat family, encoding a microcin, kills bacteria by inhibiting gyrase, which is also the mode of action for quinolones. Organisms that produce this microcin make another pentapeptide repeat protein that protects gyrase from the microcin and also some quinolones. The *qnr* gene product leads to a 10- to 100-fold increase in the MIC for quinolones. Because MIC values for quinolones are often extremely low, the expression of the *qnr* gene may be insufficient to reach the breakpoint for resistance or even intermediate resistance. Nevertheless, it has been speculated that this may offer the bacterium a larger time window to develop mutations in gyrase or topoisomerase and thereby higher levels of resistance.

A number of variants of *qnrA1* have been reported, as well as more distant relatives called *qnrB* and *qnrS*. Variants of the latter types are also known. Isolates harboring *qnr* genes have been reported worldwide and the mechanism seems of growing importance. At least one large national outbreak with an *Enterobacter* strain has been reported.[30]

MAR operon

Escherichia coli and a number of other organisms possess mechanisms that provide intrinsic protection against a wide range of chemically unrelated toxic substances, including quinolone antibiotics. Multiple antibiotic resistance (MAR) in *E. coli* has been shown to be the result of mutations in the *mar* locus of the *E. coli* chromosome. A homologue of the *mar* locus exists in other members of the Enterobacteriaceae as well as in other bacteria. In *E. coli*, the *mar* locus consists of two divergently expressed operons, *marC* and *marRAB*, both of which are required for full expression of the MAR phenotype. Expression of the MAR phenotype, whether by induction or mutation, protects the cells from fluoroquinolone killing at up to four times the MIC. This may be more clinically important than the relatively modest increases in MIC associated with MAR because cells that escape death would have the potential to mutate to higher levels of fluoroquinolone resistance.[24,25,31]

RESISTANCE TO TETRACYCLINES

Tetracyclines probably penetrate bacterial cells by passive diffusion. Tetracycline acts by reducing the affinity of the A and P sites of the 30S ribosomal subunit for aminoacyl transfer RNA, resulting in the inhibition of protein synthesis.[13,24,32–35]

A growing number of bacterial species are acquiring resistance to the bacteriostatic activity of tetracycline. To date, at least 38 tetracycline-resistance (Tet) determinants and three oxytetracycline-resistance (Otr) determinants, first found in oxytetracycline-producing *Streptomyces* spp., have been described and characterized, with new Tet determinants being identified regularly. Most are associated with plasmids, whereas others are on the chromosome. Resistance to tetracyclines is primarily due to acquisition of Tet determinants rather than to mutation of existing chromosomal genes.[36]

The two most widespread mechanisms of bacterial resistance are:

- energy-dependent efflux pumps; and
- an elongation-factor G-like protein that confers ribosomal protection.

Both mechanisms are widespread among Gram-negative and Gram-positive bacteria (Table 131.4). Oxidative destruction of tetracycline has been found in a few species. Nevertheless, the enzymatic inactivation of the antibiotic is not thought to be important in nature.

The classification of Tet determinants according to their mechanism of resistance is shown in Table 131.5. Resistance to tetracycline may result in cross-resistance to the second-generation tetracyclines doxycycline and minocycline. Resistance to tigecycline, the newest generation of tetracyclines (i.e. glycylcycline), has been reported.

Reduced intracellular concentration of tetracycline

Because the ribosome is the target, antibiotic activity of tetracycline depends on the presence of the drug in the cytoplasm. A reduced

Table 131.4 Distribution of tetracycline-resistance determinants

Genus	Tet determinant
Acinetobacter	Tet A, B, M, 39
Actinobacillus	TetB, H, L
Actinomyces	TetL, W
Aerococcus	TetM, O
Aeromonas	TetA, B, C, D, E, 34
Afipia	TetM
Alteromonas	TetD
Arcanobacterium	TetW
Bacillus	TetK, L, W
Bacteroides	TetM, Q, X, 36
Brevundimonsa	TetB
Butyrivibrio	TetW
Campylobacter	TetO
Chlamydia	TetC
Citrobacter	TetA, B, C, D
Clostridium	TetK, L, M, P, W, 32, 36
Corynebacterium	TetM, 33
Edwardsiella	TetA, D
Eikenella	TetM
Enterobacter	TetB, C, D, M
Enterococcus	TetK, L, M, O
Erypsipelothrix	TetM
Escherichia	TetA, B, C, D, E, G, M
Eubacterium	TetK, M
Fusobacterium	TetM
Gardnerella	TetM
Gernella	TetM
Haemophilus	TetA, B, M
Kingella	TetM
Klebsiella	TetA, D, M
Lactobacillus	TetK, M, O, S, W, 36
Lactococcus	TetM, S
Listeria	TetK, L, M, S
Megasphaera	TetO, W
Microbacterium	TetM
Mitsuokella	TetM, W
Mobiluncus	TetO
Moraxella	TetB, H
Morganella	TetL
Mycobacterium	TetK, L, OtrA, B
Mycoplasma	TetMa
Neisseria	TetB, M, O, Q, W
Nocardia	TetK, L
Pasteurella	TetB, D, H
Peptostreptococcus	TetK, L, M, O
Photobacterium	TetB, M
Plesiomonas	TetA, B, D
Porphyromonas	TetM, W
Prevotella	TetM, Q, W
Proteus	TetA, B, C
Providencia	TetG
Pseudomonas	TetA, B, C, M, 34
Ralstonia	TetM
Roseburia	TetW
Salmonella	TetA, B, C, D, E, L
Selenomonas	TetM, W
Serratia	TetA, B, C, 34
Shigella	TetA, B, C, D
Staphylococcus	TetK, L, M, O, U, W, 38
Stenotrophomonas	Tet35
Streptococcus	TetK, L, M, O, W
Streptomyces	TetK, L, M, W, OtrA, B, C
Ureaplasma	TetMa
Veillonella	TetA, L, M, S, W
Vibrio	TetA, B, C, D, E, G, M, 34, 35
Yersinia	TetB

Data from Roberts.[32]

Table 131.5 The mechanism of resistance for different tetracycline determinants

Efflux	*tet*(A), *tet*(B), *tet*(C), *tet*(D), *tet*(E), *tet*(G), *tet*(H), *tet*(J), *tet*(K), *tet*(L), *tet*(V), *tet*(Y), *tet*(Z), *tet*(30), *tet*(31), *tet*(33), *tet*(35), *tet*(38), *tet*(39), *tetA*(P), *otr*(B), *otr*(C), *tcr3*
Ribosomal protection	*tet*(M), *tet*(O), *tet*(Q), *tet*(S), *tet*(T), *tet*(W), *tet*(32), *tet*(36), *tetB*(P), *otr*(A)
Enzymatic	*tet*(X), *tet*(34), *tet*(37)
Unknown	*tet*(U)

Data from Roberts.[36]

tetracycline concentration in the cytoplasm can be achieved by two mechanisms:[13,24,32–35]

- the permeability of the cell envelope may be lowered; or
- tetracycline may be pumped out of the cytoplasm in an energy-dependent fashion.

Bacteria differ in their cell wall composition, causing differences in permeability and hence insensitivity to antibiotics. The peptidoglycan layer surrounding most Gram-positive bacteria does not reduce cytoplasmic accumulation of low molecular weight antibiotics such as tetracycline. In contrast, the outer membrane of Gram-negative bacteria is an effective permeability barrier for hydrophobic compounds. The effects of permeability barriers are usually supported by additional resistance mechanisms to achieve high-level resistance. Energy-dependent efflux of tetracycline causes high-level resistance in bacteria by itself.

Two different types of efflux pump are involved in tetracycline resistance: multidrug-resistance pumps and tetracycline-specific transporters. A multidrug-resistance pump belonging to the Acr family is responsible for the reduced tetracycline accumulation in *P. aeruginosa*. Furthermore, *E. coli* has a chromosomal multidrug-resistance efflux system that is associated with the *mar* locus. Multidrug efflux pumps transport their substrate straight out of the cell into the surrounding medium. In contrast to the broad substrate range of multidrug transporters, many of the efflux pumps identified in Gram-positive and Gram-negative bacteria specifically transport tetracycline. The efflux proteins exchange a proton for a tetracycline–cation complex and are antiporter systems. Efflux determinants from Gram-negative bacteria (TetA–E, TetG–H) share a common genetic organization, which is different from the one in Gram-positive bacteria. Both Gram-negative and Gram-positive bacteria contain a structural and a repressor gene that are expressed in opposite directions from overlapping operator regions. The Gram-positive *tetK* and *tetL* genes encoding tetracycline efflux proteins are regulated by mRNA attenuation in a similar way to that described for Gram-positive *erm* genes encoding rRNA

methylase and *cat* genes encoding chloramphenicol acetyltransferases. Tetracycline-specific exporters pump their substrate into the periplasm and not across the outer membrane, as found for the multidrug efflux pumps.

Protection of the ribosome

Protection of the ribosome from the action of tetracycline as a mechanism of tetracycline resistance was discovered in streptococci. Tetracycline resistance can result from production of a protein that interacts with the ribosome such that protein synthesis is unaffected by the presence of the antibiotic. To date, six classes of Tet determinants that confer tetracycline resistance on the level of protein synthesis have been identified. Most of the work on the mechanism of ribosomal protection has been done on TetM. The ribosomal protection proteins encoded by the other classes have an amino acid sequence similarity of at least 40% to TetM. Therefore, the mechanism of action may be similar for all ribosomal protection proteins. TetM ribosomal protection protein resembles elongation factors (EFs) in three properties:

- it has amino acid sequence similarity to EF-G (which translocates the peptidyl transfer RNA during protein synthesis) and EF-Tu;
- it has a ribosome-dependent guanosine triphosphatase activity; and
- it seems to confer resistance by reversible binding to the ribosome.

However, to date, the biochemical basis of tetracycline resistance mediated by TetM remains unclear. One possibility is that TetM stabilizes the ribosome–tRNA interaction in the presence of tetracycline.[13,24,32–34,36]

RESISTANCE TO CHLORAMPHENICOL

Chloramphenicol is a bacteriostatic antibiotic that binds to the 50S ribosomal subunit and inhibits the peptidyltransferase step in protein synthesis. Resistance to chloramphenicol is mostly due to inactivation of the antibiotic by a chloramphenicol acetyltransferase (CAT) enzyme that acetylates the antibiotic. Chloramphenicol resistance most commonly results from the acquisition of plasmids that encode CAT; however, in certain Gram-negative bacteria, decreased outer membrane permeability can confer resistance to chloramphenicol and structurally unrelated compounds.[13,24]

Enzyme inactivation

Chloramphenicol contains two hydroxyl groups that are acetylated in a reaction catalyzed by CAT. Monoacetylated and diacetylated derivatives are unable to bind to the 50S ribosomal subunit to inhibit prokaryotic peptidyltransferase. Expression of the *cat* genes in *Staph. aureus*, *Strep. pneumoniae* and *Enterococcus faecalis* is typically inducible, and expression appears to be regulated by translational attenuation in a similar manner to the *erm* genes conferring resistance to macrolides. The *cat* gene is preceded by a nine amino acid leader peptide, and the leader mRNA can form a stable stem-loop structure, which masks the ribosome binding site of the *cat* gene. Chloramphenicol appears to cause the ribosome to stall on the leader sequence, opening the stem-loop structure, thereby exposing the *cat* ribosome binding site and allowing *cat* expression. In Gram-negative bacteria, resistance to chloramphenicol is usually mediated by plasmid-mediated or transposon-mediated genes that are generally expressed constitutively.

Decreased permeability

In Gram-negative bacteria, resistance may also be due to chromosomal mutations that result in decreased outer membrane permeability. In *P. aeruginosa*, nonenzymatic chloramphenicol resistance is associated with the presence of the *clmA* gene. The ClmA protein appears to effect reduced expression of the outer membrane porins OmpA and OmpC and decreased chloramphenicol uptake. In *E. coli*, resistance to chloramphenicol and structurally unrelated antibiotics is part of the MAR phenotype.[37]

RESISTANCE TO GLYCOPEPTIDES

The glycopeptide antibiotics vancomycin and teicoplanin inhibit cell wall synthesis in Gram-positive bacteria by interacting with the terminal D-alanyl-D-alanine (D-Ala-D-Ala) group of the pentapeptide side chains of peptidoglycan precursors. This interaction prevents the transglycosylation and transpeptidation reactions required for polymerization of peptidoglycan. Almost all bacteria synthesize peptidoglycan terminating in D-Ala-D-Ala, but the exclusion limits of the porin proteins of Gram-negative outer membranes prevent transport of the glycopeptides, and so only Gram-positive species are susceptible to clinically achievable concentrations of this class of antibiotics.

Glycopeptide resistance in enterococci

The vancomycin-resistant enterococci (VRE) can be divided into six different phenotypic groups: A, B, C, D, E and H (Table 131.6).[13,24,38–43]

The origin of the *van* genes in enterococci is not known. The *vanA* gene has 52% amino acid sequence identity to the D-Ala-D-Ala ligase of *Salmonella* spp. and can complement a temperature-sensitive ligase mutant of *E. coli*. The D-Ala-D-Ala ligase is responsible for the production of the D-Ala-D-Ala dipeptide, which in Gram-positive bacteria is the target for glycopeptide antibiotics. The *vanB* and *vanC* genes are also both highly comparable in sequence to D-Ala ligases.

Table 131.6 Resistance to enterococcal glycopeptides

Resistance	ACQUIRED					Intrinsic
Phenotype	**VanA**	**VanB**	**VanD**	**VanG**	**VanE**	**VanC**
MIC (mg/l)						
Vancomycin	64–1000	4–1000	64–128	8–16	16	2–32
Teicoplanin	16–512	0.5–1	4–64	0.5	0.5	0.5–1
Expression	Inducible	Constitutive	Constitutive	Inducible	Constitutive	Inducible
Location	Plasmid Chromosome		Chromosome	Chromosome	Chromosome	Chromosome
Modified target	D-Ala–D-Lac	D-Ala–D-Lac	D-Ala–D-Lac	D-Ala–D-Ser	D-Ala–D-Ser	D-Ala–D-Ser

Adapted from Shlaes & Rice[38] and Courvalin.[44]

VanA resistance phenotype

The *vanA* gene is carried within a transposon together with several other genes, many but not all of which are required for the expression of resistance to vancomycin. The VanA product is a D-Ala-D-lactate (D-Lac) ligase. The *vanH* gene apparently encodes an enzyme that catalyzes the conversion of pyruvate to D-lactic acid. The VanA ligase uses this as a substrate to form the depsipeptide D-Ala-D-Lac, which is then incorporated into an alternative, vancomycin-resistant peptidoglycan precursor (Fig. 131.3). The VanX protein appears to cleave the D-Ala-D-Ala dipeptide, decreasing the amount of substrate that is available for the formation of the normal pentapeptide. The VanX protein does not hydrolyze the D-Ala-D-lactate pentapeptide or pentadepsipeptide.

Most vancomycin-resistant strains of enterococci also produce a carboxypeptidase. The structural gene for this carboxypeptidase in VanA-harboring strains is *vanY*. The carboxypeptidase may reduce the levels of the normal precursor so that the alternative precursor predominates. The *vanY* gene, however, is not required for resistance to cyclic glycopeptides. The inducible nature of glycopeptide resistance in most VanA enterococci suggests that expression is regulated at the genetic level. The genes *vanR* and *vanS* are involved in the regulation of VanA resistance, and the analysis of the amino acid sequences of the gene products has indicated similarity with two-component signal transducing regulatory systems that sense and respond to environmental stimuli. VanR appears to act as a transcriptional activator and seems to be stimulated by VanS. The phosphorylated VanR peptide acts on a promoter that lies between *vanS* and *vanH* and from which the *vanH*, *vanA* and *vanX* genes are co-transcribed. The environmental stimulus that triggers the initial phosphorylation of VanS has not been identified, but it is probably related to the presence of vancomycin and its interaction with the D-Ala-D-Ala target site, which inhibits transglycosylation.

In addition to the high-level glycopeptide resistance mediated by the *vanH*, *vanA*, *vanX* and *vanY* genes, a second mechanism of resistance exists. The *vanZ* gene mediates resistance to teicoplanin while vancomycin MICs are unaffected. The mechanism by which the VanZ peptide confers this low-level teicoplanin resistance has yet to be established.

Other Van resistance phenotypes

The drug resistance of VanB-harboring strains appears to be similar to that of VanA-harboring strains, except that the VanB-harboring strains originally described remained susceptible to teicoplanin. However, it has become clear that glycopeptide-resistant enterococci containing the *vanB* gene are phenotypically diverse, exhibiting a wide range of vancomycin MICs, including high-level resistance. In addition, the emergence of mutants that express *vanB* constitutively has been described. Resistance mediated by *vanB* may also be transferable, with the gene located either on the chromosome or on plasmids.

The *vanC* resistance determinants are present on the chromosome in *Enterococcus casseliflavus* and *Enterococcus gallinarum* and are intrinsic characteristics of these species. VanC-harboring enterococci have low-level resistance to vancomycin and remain susceptible to teicoplanin. The pentapeptide that results from the action of the VanC ligase terminates in D-Ala-D-Ser. This substitution probably reduces vancomycin binding, albeit not to the same degree as the depsipeptide found in VanA and VanB enterococci. Insertional inactivation of *vanC* caused reversion to vancomycin susceptibility, suggesting the existence of a second chromosomal ligase that synthesizes vancomycin-susceptible precursors.

VanC-harboring strains with high-level resistance to glycopeptides as a result of the acquisition of the *vanA* gene cluster have also been isolated. The biochemical basis for the VanC phenotype displayed by

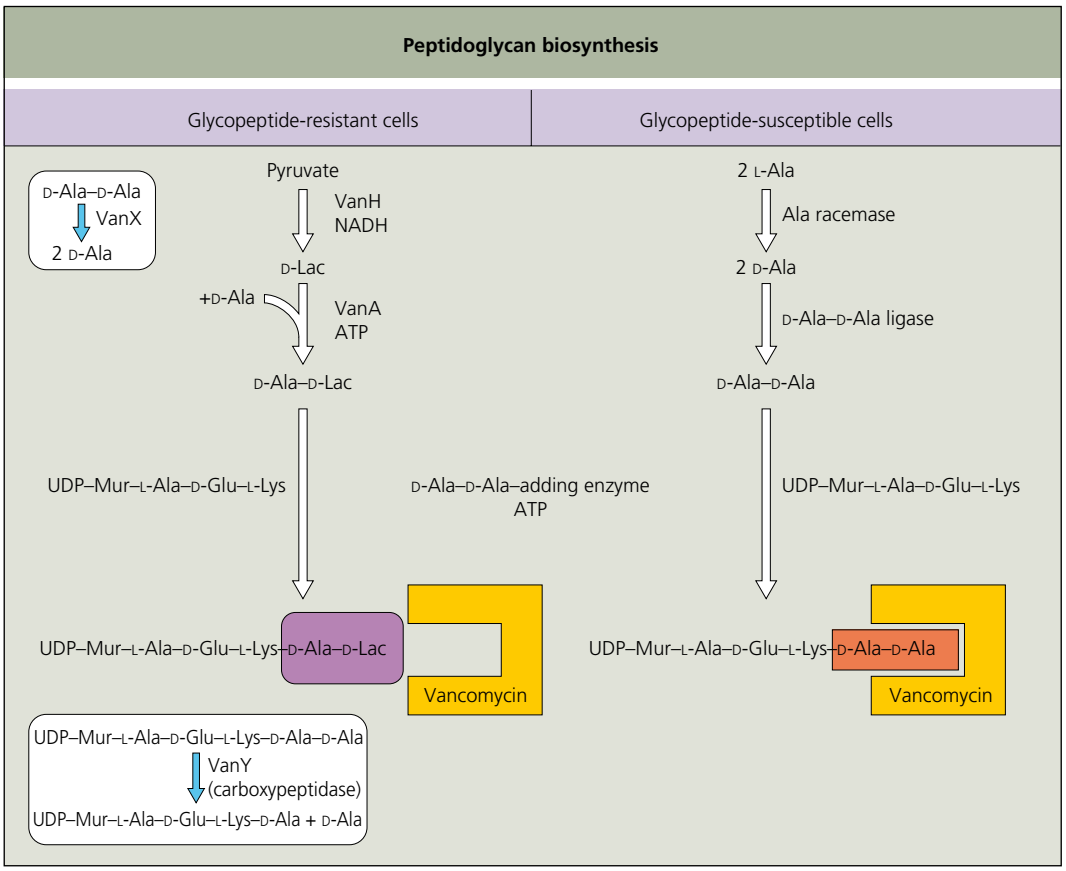


Fig. 131.3 Peptidoglycan biosynthesis. ATP, adenosine triphosphate, Lac, lactate; UDP, uridine diphosphate. Adapted from Shlaes & Rice.[38]

most isolates of *Enterococcus casseliflavus* and *Enterococcus flavescens* remains to be clarified in detail. Two genes, designated *vanC-2* and *vanC-3*, have been identified in these species. There is extensive similarity between the *vanC-2* or *vanC-3* gene and the *vanC* gene, now designated *vanC-1*, from *Enterococcus gallinarum*, although they do not cross-hybridize.

A third mechanism of vancomycin resistance is VanD. Resistance levels for vancomycin and teicoplanin are 64–128 and 4–64 μg/ml, respectively. The genes are located chromosomally and are constitutively expressed. Conjugative transfer has not been demonstrated. VanE and VanG are two mechanisms that yield low-level vancomycin resistance and teicoplanin is unaffected. Both mechanisms are inducible and located on the chromosome. The VanG cluster can be transferred by conjugation.[44]

Glycopeptide resistance in staphylococci

Resistance to glycopeptides among staphylococci is phenotypically diverse.[43] *Staph. aureus* strains of intermediate resistance to glycopeptides have been obtained *in vitro* and *in vivo* by the selection of resistant mutants. Vancomycin-intermediate *Staph. aureus* (VISA) or glycopeptide-intermediate *Staph. aureus* (GISA) isolates show a thicker cell wall which presumably binds the vancomycin before it reaches its target.[45] Furthermore, most GISA isolates show reduced cross linking when compared with isogenic revertants. Interestingly, only some of them (Michigan, New Jersey, Düsseldorf) showed a reduction in D-glutamic acid amidation.[43] The genetic mechanism for this cell-wall thickening has not been resolved. Comparison of a *Staph. aureus* isolate before vancomycin treatment and its vancomycin intermediate descendent after treatment by whole genome sequencing showed 37 differences between the isolates. However, the relevance of these changes has not been demonstrated.[46]

It is likely that the following mechanisms are associated with the appearance of the GISA strain:[43]

- accelerated cell wall synthesis, which leads to a thickened cell wall capable of affinity trapping large amounts of vancomycin and shielding the membrane-associated lipid II target molecules;
- in addition, impaired cross linking, most probably caused by the accelerated cell wall synthesis, will be reduced due to the fact that nonamidated precursors are poor substrates for the staphylococcal transpeptidation reaction; and
- since lower nonamidation and lower cross linking both lead to even higher consumption of vancomycin per unit cell wall weight, they also contribute positively to the resistance phenotype.

High-level vancomycin resistance has also been reported and is caused by acquisition of the VanA transposon by MRSA, almost certainly from vancomycin-resistant enterococci.[45]

Glycopeptide resistance in other Gram-positive species

Glycopeptide resistance is intrinsic in some Gram-positive species such as *Lactobacillus* spp., *Leuconostoc* spp., *Pediococcus* spp. and *Erysipelothrix rhusiopathiae*; the mechanism has yet to be clarified.

RESISTANCE TO TRIMETHOPRIM AND SULFONAMIDES

Trimethoprim and sulfonamides are synthetic agents that affect the biosynthesis of tetrahydrofolic acid, an essential derivative used in amino acid and nucleotide synthesis.[13,24,47–49] Sulfonamides are analogues of *para*-aminobenzoic acid. They competitively inhibit the enzyme dihydropteroate synthase (DHPS), which catalyzes the condensation of dihydropteridine with *p*-aminobenzoic acid synthesis. Trimethoprim is an analogue of dihydrofolic acid. It competitively inhibits the enzyme dihydrofolate reductase (DHFR). Dihydrofolate reductase catalyzes the reduction of dihydrofolic acid to tetrahydrofolic acid, the final step in tetrahydrofolic acid synthesis. Trimethoprim–sulfamethoxazole (co-trimoxazole) is a combination of trimethoprim with a sulfonamide.

Intrinsic resistance to trimethoprim and sulfonamides

Outer membrane impermeability results in trimethoprim and sulfonamide resistance in *P. aeruginosa*. Intrinsic resistance to trimethoprim in a number of species is due to host DHFR enzymes with low affinity for the drug. Folate auxotrophs such as *Enterococcus* spp. and *Lactobacillus* spp. that are able to use exogenous preformed folates exhibit reduced susceptibilities to sulfonamides and trimethoprim.

Resistance to trimethoprim

Both high- and low-level resistance has been reported in several species. In some cases, chromosomally encoded trimethoprim resistance may be due to:

- overproduction of the host DHFR;
- mutations in the DHFR structural gene *folA*; or
- mutations that inactivate thymidylate synthetase, an enzyme that converts deoxyuridylate to thymidylate.

The thymidylate mutants require exogenous thymine or thymidine for DNA synthesis and are thus resistant to folate pathway antagonists.

High-level resistance to trimethoprim in enterobacteria is almost always caused by the acquisition of DNA that specifies a trimethoprim-resistant DHFR with an altered active site. At least 11 modified DHFRs have been characterized in Gram-negative organisms. In staphylococci, trimethoprim resistance is encoded by the *dfrA* gene, which encodes a trimethoprim-resistant type S1 DHFR. The transposon-encoded *dfrA* gene appears to be responsible for both high- and low-level trimethoprim resistance in *Staph. aureus* and coagulase-negative staphylococci. The differences in resistance level correlate with differences in transcription caused by deletions adjacent to a copy of IS*257* in Tn*4003*, which affects the promoter used by *dfrA*. The *Staph. epidermidis* trimethoprim-sensitive chromosomal DHFR gene *dfrC* differs from *dfrA* by only four base pairs, strongly suggesting that *dfrA* originated from the *Staph. epidermidis* chromosomal gene. Site-directed mutagenesis of *dfrA* and kinetic analyses of the purified DHFRs indicate that a single alteration (Phe98 to Tyr) is responsible for trimethoprim resistance of type S1 DHFR.

Resistance to sulfonamides

Chromosomally encoded sulfonamide resistance has been described and resistance seems to be due to increased production of *para*-aminobenzoic acid. Furthermore, alterations of DHPS could lead to low affinity for sulfonamides. Acquired sulfonamide resistance can result from the acquisition of plasmids that encode a drug-resistant DHPS.

RESISTANCE TO OTHER ANTIBIOTICS

Resistance to linezolid, a recently licensed oxazolidinone, has already been reported. Linezolid is used to treat infections with enterococci and staphylococci, and in both genera resistance has been reported. In enterococci, a G2505A or G2576T mutation in the 23S rRNA gene has been described. The latter mutation has also been reported for staphylococci and streptococci. In addition, G2447T and C2534T mutation have been reported for the latter species.[50–52] Plasmid-mediated linezolid resistance has also been described. The plasmid encodes a Cfr methyltransferase which modifies 23S rRNA.

Fusidic acid inhibits elongation factor G (EF-G), a protein of the ribosome, and thereby protein synthesis. Many resistant isolates have mutations in the *fusA* gene, which encodes EF-G. Reduced uptake and efflux have been reported, but details are largely unknown.

Mupirocin inhibits isoleucyl RNA synthetase to inhibit protein synthesis. Low-level resistance is caused by mutations in the gene of this enzyme on the chromosome. Acquisition of a novel isoleucyl tRNA synthetase encoded by *mupA* leads to high-level resistance.

Metronidazole resistance in *H. pylori* is the result of the disruption of the *rdxA* gene that encodes NADPH nitroreductase. This enzyme converts metronidazole in a metabolite that is toxic for the bacterial cell. Only a small percentage of isolates lack mutations in the *rdxA* gene; it is speculated that mutations affecting its expression are involved.[53]

RESISTANCE IN *MYCOBACTERIUM TUBERCULOSIS*

Mycobacterial resistance to first-line antimicrobial agents is a major concern. The slow growth rate of *M. tuberculosis* and the serious consequences of inappropriate therapy make the study of the mechanisms of antibiotic resistance of particular importance.

The main antibiotics for the treatment of mycobacterial infections are isoniazid and rifampin (rifampicin). Resistance was reported soon after the introduction of isoniazid in 1952. Isoniazid acts by inhibiting an oxygen-sensitive pathway in the mycolic acid biosynthesis of the cell wall. The mechanism of resistance appears to be multifactorial, and genetic modifications in a number of genes may have an effect. Some of these effects have only been observed in *in-vitro* generated mutants. The genes involved include the *katG* gene-encoded catalase-peroxidase, the isoniazid target-encoding *inhA* gene and the upstream region of the neighboring *mabA* gene, upstream of the *aphC* gene and the *kas* gene.[54] Mutations in *katG* are the most important for clinical resistance. The product of the *katG* gene is essential to convert isoniazid to its active form.

The molecular basis of rifampin resistance is understood better. Rifampin interferes with RNA synthesis. At least eight amino acid substitutions in the *rpoB* subunit of RNA polymerase have been described as conferring resistance.[55]

Resistance to aminoglycosides has been reported for *M. tuberculosis* and is caused by an rRNA gene mutation. Resistance to pyrazinamide is most commonly caused by mutations in the *pncA* gene or its upstream region. The *pncA* product is required to convert pyrazinamide into its active form. Tolerance to isoniazid and ethambutol can probably be caused by an efflux mechanism, but details are lacking.

MULTIPLE RESISTANCE

Bacteria are often resistant to more than one antimicrobial agent. Multiple resistance is conferred by three mechanisms:

- reduced permeability;
- active efflux; and
- multiple resistance genes.

Reduced permeability is generally caused by alteration in the cell wall of the bacterium, especially the reduced expression of porins. The best known is the reduction of the outer membrane protein (omp) ompF, from *E. coli*. In general, it can be said that reduction in porin numbers leads to a decreased uptake of antibiotics. The resulting lower concentration of antibiotics can be frequently dealt with by a resistance mechanism that, without a reduced number of porin proteins, only results in a slight increase in MIC.

Efflux of antibiotics is a common resistance mechanism. Often an efflux pump is only able to pump out a single antibiotic and its close structural homologues (e.g. pumps dedicated to tetracycline efflux). However, more general-purpose efflux systems are also known. These pumps can handle a wide variety of different compounds including many antibiotics. The efflux systems belong to a number of families:

- ATP-binding cassette (ABC) superfamily of transporters;
- major facilitator (MSF) superfamily;
- resistance-nodulation-division (RND) superfamily;
- small multidrug resistance (SMR) family;
- drug metabolite transporter (MDT) superfamily; and
- multidrug and toxic compound extrusion (MATE) family.

ABC and MSF transporters are not only broadly distributed among prokaryotes, but also among eukaryotes. The transporters can be composed of either a single polypeptide or multiple polypeptides depending on the family (Fig. 131.4). Gram-negative bacteria as well as mycobacteria have an inner and outer membrane and the compounds must be transported across both. The ABC transporters are dependent on ATP for their activity, whereas the proton-motive force is used by other transporters. Mutations are usually required for constitutive expression of these pumps and, in turn, high-level resistance. However, mutations may also affect the substrate specificity facilitating the efflux of novel substrates. The AcrAB of the MAR system in *E. coli* and the MexAB pumps of *P. aeruginosa* belong to the RND superfamily. Multidrug resistance among *P. aeruginosa* is caused in large part by the latter system, but a significant contribution is made by acquired resistance genes. Resistance to disinfectants is usually also mediated by efflux pumps.[56]

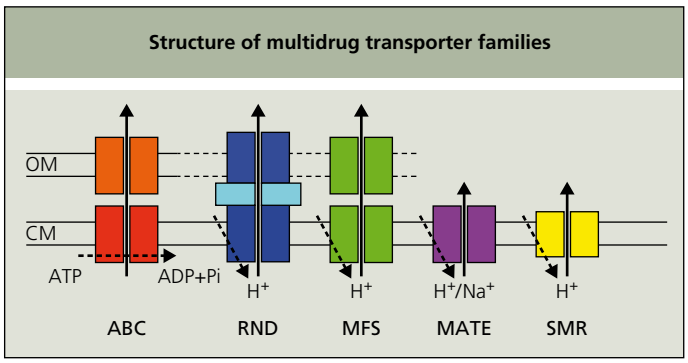


Fig. 131.4 Schematic structure of multidrug transporter families. Different subunits are indicated by different colors. Efflux is indicated by arrow. Energy source for efflux is indicated by broken arrow. The cellular membrane (CM) is present in all cells, but Gram-positive bacteria lack an outer membrane (OM). Some multidrug transporter families are present in both Gram-positive and Gram-negative bacteria as indicated by dotted lines for the OM. (See text for details.)

The MAR system in *E. coli* is one of the best studied systems and reduced uptake and active efflux are combined in a single regulatory system. The MAR system was discovered by Levy and coworkers, who observed that resistant mutants were obtained at a frequency of 10^{-7} when *E. coli* was plated on agar media containing either tetracycline or chloramphenicol. Usually, mutants obtained with one antibiotic were also resistant to the other, but cross-resistance to β-lactams, puromycin, rifampin and nalidixic acid was also observed. A three-gene operon containing *marRAB* is essential for drug resistance by this mechanism (Fig. 131.5). Sequencing studies revealed that resistant mutants had mutations either in the putative operator–promoter region of the operon or in the *marR* gene. The MarR product acts as a negative regulator for the *mar* operon. The MarA product is required for resistance. The MarA product is supposed to act on at least two different promoter regions. The first is involved in the expression of OmpF protein. Expression of OmpF is regulated by the *micF* gene. Transcription of this gene leads to the production of an antisense RNA for the *ompF* mRNA. Stimulation of *micF* RNA production by the MarA product causes reduced translation of the OmpF mRNA owing to its blockage by the *micF* antisense RNA and thereby reduced permeability through OmpF.

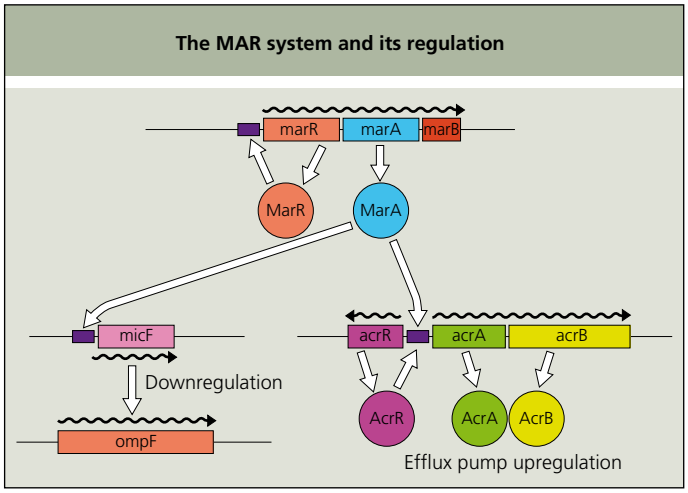


Fig. 131.5 The MAR system and its regulation.

The MarA product also interacts with the *acr* operon. This operon encodes two subunits of an efflux pump. Inactivation of this efflux system results in hypersensitivity to a wide variety of antimicrobial agents. The expression of the *acr* operon appears to be regulated by AcrR, the product of the third gene of this operon. MarA apparently interacts with the binding of AcrR to its operator, resulting in increased expression of the two subunits of the efflux pump. However, the expression of the pump is also increased by a variety of environmental stimuli, such as ethanol and high concentrations of salt, but these stimuli apparently do not operate through either MarA or AcrR. Despite the detailed molecular knowledge about this mechanism of multiple resistance, its clinical impact is not clear.[57]

Multiple resistance caused by the presence of a number of different resistance genes can originate from the sequential acquisition of mutations or resistance genes, but often these genes are transferred as part of complete units. These units are located either on transposons or multiresistance plasmids that have acquired these genes over time. Transposons, easily mobilizable genetic elements that range in size from a few to more than 150 kilobases, are an important source for the spread of antibiotic resistance. Numerous different transposons have been described and many of them carry one or more antibiotic-resistance determinants. Resistance to any class of antimicrobial agent may be encoded on a transposon. Transposons may integrate either in plasmids or the bacterial chromosome and may be present in multiple copies, thereby enhancing their effectiveness in the expression of resistance.

A number of transposons have been well studied. These include Tn*5*, Tn*7*, Tn*10* and Tn*21* from Gram-negative bacteria and Tn*554*, Tn*916* and Tn*4001* from Gram-positive bacteria.[58] Transposition may be very efficient and the spread of Tn*4001* described above is an example. Tn*916* and its derivatives, found in enterococci, lactococci and staphylococci, was one of the first conjugative transposons discovered. It encodes resistance against tetracycline and chloramphenicol. Tn*916* is a member of a large family of related conjugative transposons, but other families have also been described. Conjugative transposons are not limited to Gram-positive bacteria; they are also present in Gram-negative bacteria. Tn*916* has also been found in *N. gonorrhoeae* for example. Conjugative transposons have a somewhat different mechanism of transfer from the more common transposons such as Tn*5* and Tn*10*, but they are well equipped for dissemination between species, although transfer is regulated. For some conjugative transposons, this transfer may be upregulated (by up to 10 000 times) by antibiotics, which enhances the dissemination of antibiotic-resistance determinants. In addition, these conjugative transposons may mobilize co-resident plasmids, which also may carry antibiotic-resistance determinants, resulting in the transfer of multiple antibiotic resistance.[59]

Integrons are a special group of genetic elements. There are two groups of integrons: resistance integrons and superintegrons. Superintegrons are found in many Gram-negative species and are located on the chromosome. These integrons contain tens to hundreds of gene cassettes, which encode for a large variety of different functions. Resistance integrons contain up to 10 gene cassettes. Nearly all known gene cassettes in these integrons encode for resistance to antibiotics or disinfectants. The most common resistance integrons belong to class 1, but two other classes are known as well. The class 1 integrons are characterized by two conserved sequences (CSs). The 5′-CS contains the *int* gene, which encodes a protein homologous to other members of the integrase family. The 3′-CS consists of the *qacEΔ1* and *sulI* genes and an open reading frame, *orf5*.[60] The *qacEΔ1* and *sulI* genes define resistance against quaternary ammonium compounds and sulfonamide, respectively (Fig. 131.6).

Integration of gene cassettes by the integrase takes places between the conserved segments, preferentially at a position at the end of the 3′-CS called the *attI* site. Cassettes can also be excised by the integrase and can exist as free circular DNA molecules. This process can also lead to rearrangement of the order of cassettes in an integron. Each gene cassette has an imperfect inverted repeat element. This so-called 59-base pair element, which may vary in length between 57 and 141 base pairs, is unique for each gene cassette.[61] At least 100 gene cassettes have been described, including genes defining resistance against β-lactam antibiotics, aminoglycosides, trimethoprim, chloramphenicol and antiseptics and disinfectants (Table 131.7).[60] Generally the

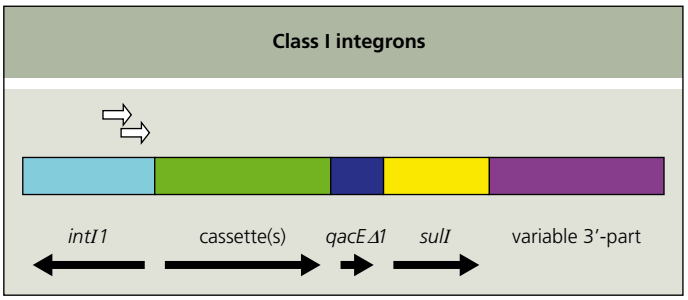


Fig. 131.6 Class 1 integrons. Open arrows indicate the promotors P1 and P2 and point in the direction of transcription from each promotor site; black arrows indicate the direction of open reading frames (orf) (see text for details).

Table 131.7 Resistance and mechanism of resistance for gene cassettes

Resistance	Enzyme
β-Lactams	Class A β-lactamases Class B β-lactamase Class D β-lactamases
Aminoglycosides	Aminoglycoside adenylyltransferases Aminoglycoside acetyltransferases
Chloramphenicol	Chloramphenicol acetyltransferases Chloramphenicol exporter
Trimethoprim	Class A dihydrofolate reductases Class 8 dihydrofolate reductases
Rifampin (rifampicin)	ADP ribosylation
Streptothricin	Streptothricin acetyltransferase
Antiseptics and disinfectants	Quaternary compound exporter
Unknown function	Not applicable

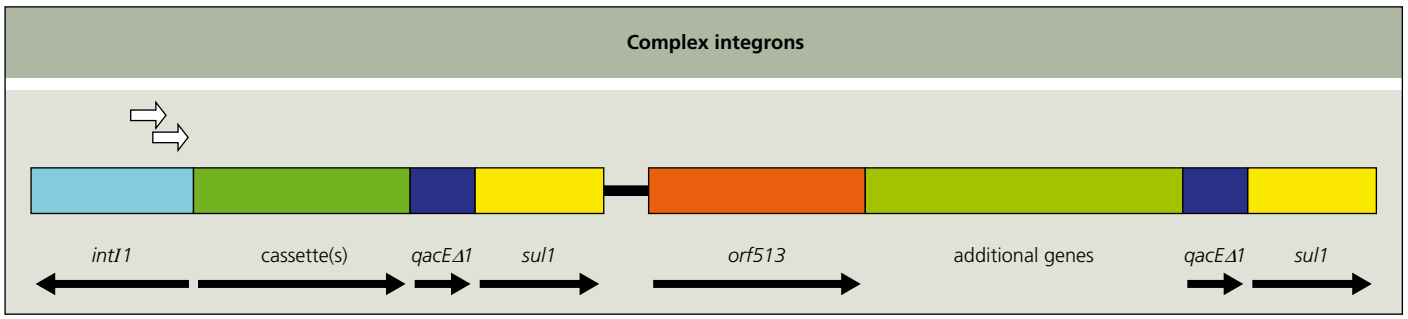


Fig. 131.7 Complex integrons. Open arrows indicate the promotors P1 and P2 and point in the direction of transcription from each promotor site; black arrows indicate the direction of open reading frames (orf) (see text for details).

cassettes do not have promoters, but transcription occurs from one of two promoter sequences present in the 5′-CS. Integrons are widespread in Enterobacteriaceae but are also found in pseudomonads. Isolates may carry more than one integron.[60]

Class 1 integrons can also form complex integrons. In complex integrons an integron is normally followed by *orf513*, a number of additional genes and a second copy of the 3′-CS (Fig. 131.7). The additional genes are not gene cassettes, but have their own promoters and often encode resistance to antibiotics including third-generation cephalosporins. The first complex integron was described in 2000.[62] Subsequently, numerous complex integrons have been found worldwide. Their prevalence seems to be increasing, probably through

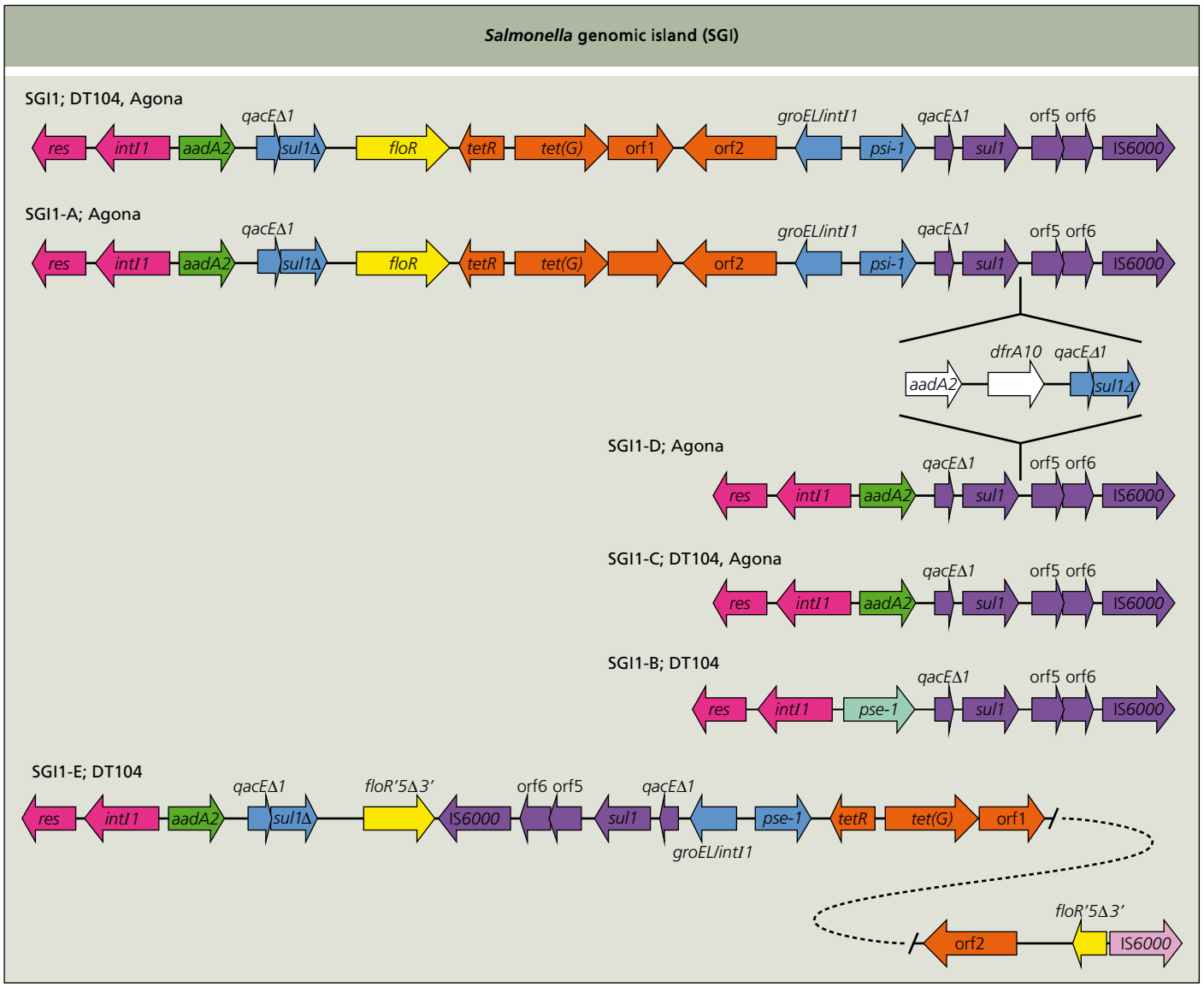


Fig. 131.8 *Salmonella* genomic island (SGI).

selection by cephalosporin use. Complex integrons appear to be frequently linked to an atypical class of insertion elements. These elements are mobile and may also help to explain the mobility of complex integrons between different bacterial strains and species.

Class 1 integrons can also be found on the chromosome of *Salmonella*, where they are present on *Salmonella* genomic island 1 (SGI1). SGI1 is formed by a variable stretch of DNA, which may be up to 43 kb in length. It is located on the *Salmonella* chromosome between the *thdf* and the *yidY* genes, which are present in all *Salmonella enterica* serovars. SGI1 can contain a variety of different resistance genes. All resistance genes are grouped in one region called the multidrug resistance (MDR) region. Seen from the *thdf* gene, the MDR region is located in the distal part of SGI1. In *Salmonella enterica* serovar Typhimurium DT104 SGI1 is frequently present. The MDR region consists of two integrons and a number of intervening genes, including resistance genes. It can be considered as an archetype for SGI1 and is designated SGI1 without further additions. Variations on this theme are found in other serovars. Only one integron may be present and variation in gene cassettes is observed (Fig. 131.8).[63] More than a dozen different SGI1 variants have been described. The MDR region of SGI1 seems to be an important hotspot for the collection of resistance genes. Although resistance to mostly older antibiotics is encoded, some of these antibiotics (e.g. ampicillin and tetracycline) are commonly used in animal husbandry and humans. SGI1 is mobile when helper functions are provided by plasmids.

CONCLUSION

Antibiotic resistance is ubiquitous and increasing. The best-known examples are MRSA, vancomycin-resistant enterococci, penicillin-resistant *Strep. pneumoniae* and ESBL-carrying Enterobacteriaceae. Studies into the molecular mechanisms of antimicrobial resistance help us to understand the problem and to monitor outbreaks, but other measures are required to quell the spread of resistance genes. Local, national and international antimicrobial surveillance studies are required to gain insight into trends in antimicrobial resistance on which empiric treatment of patients can be based. However, only the prudent use of antibiotics and infection prevention measures will limit or even prevent the spread of antibiotic resistance.

It is not only the use of antimicrobial agents for the treatment of humans that plays a role in the spread of resistance; their use for the treatment of animals also plays a role. The practice in animal husbandry of using antibiotics in subtherapeutic concentrations as growth enhancers is a particular cause for concern, although now banned in the European Union and other countries. This practice started in the 1950s. In the 1960s it became controversial and experts questioned the wisdom of adding antibiotics to feed owing to the emergence of multidrug-resistant Enterobacteriaceae. (In fact, the first multidrug-resistant Enterobacteriaceae had been observed in the 1950s.) In 1966 a multidrug-resistant *Salmonella* strain ingested via food caused an outbreak that resulted in six deaths. Many studies were issued, but on a political level little action was taken and the controversy continues today. An example is avoparcin, which gives rise to cross-resistance to vancomycin. Evidence was recently provided that transfer of vancomycin-resistant enterococci from animals to humans may be possible.[64] Although transfer of resistant strains from animals to humans has been demonstrated, it is often contended that strains of bacteria living in animals are not able to survive in humans because they are not well adapted. However, animal strains of at least some multiresistant strains are able to survive for weeks in humans. They may not cause disease directly, but they provide a reservoir of resistance determinants, which can spread easily between strains and species.[65]

Interestingly, in Europe the use of avoparcin has been high and the use of vancomycin in hospitals low, but the levels of vancomycin-resistant enterococci causing infections in patients are low. In the USA, although avoparcin is not used in feed, the amount of vancomycin used in hospitals is high and vancomycin-resistant enterococci are often isolated from patients. This suggests that the use of antibiotics in hospitals may pose a greater threat for the spread of resistance than use of subtherapeutic concentrations of antibiotics in feed. Inappropriate use of antibiotics in both veterinary and medical practice thus contributes to the spread of antibiotic resistance, leading to potentially untreatable infections.

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Chapter 132

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Antibiotic prophylaxis

INTRODUCTION

Surgical site infections (SSIs) are a major source of morbidity and mortality and represent the second most common cause of health-care-associated infections (HAI) after urinary tract infections.[1] Depending on the type of surgery, 2–20% of patients develop SSIs, with risk being highest for cardiac and intra-abdominal surgeries.[2] SSI is the most common postoperative complication, and there are published data to show that postoperative complications can have a significant impact on the patient and on hospital resources, with reported increases in length of stay, hospital costs and mortality. Length of stay can increase by 4–11 days compared to patients without complications after surgery.[3,4] Recent studies from the US National Surgical Quality Improvement Program (NSQIP) have shown that postoperative complications can increase hospital costs by US$1398 (for infectious complications) and mortality may increase by 69%.[3,5]

Moreover, an increasing proportion of SSIs are now being caused by multidrug-resistant organisms such as methicillin-resistant *Staphylococcus aureus* (MRSA) and fungi such as *Candida albicans*.[6] Consequently, the selection of appropriate antimicrobial prophylaxis is becoming an increasingly complex issue. In this chapter we highlight the general principles of antibiotic prophylaxis and summarize the recommendations for major types of surgery and other invasive procedures.

PATHOGENESIS

Surgical site infections develop due to microbial contamination of the surgical wound, most commonly from patients' endogenous flora or from an exogenous source, such as personnel in the operating room or the environment. The role of wound contamination during the postoperative period, from direct inoculation or from hematogenous seeding, is less clear but is thought to be involved in late infections of prosthetic devices.

The risk of SSI is due to a combination of factors as follows:[7]

$$\frac{\text{Dose of bacterial contamination} \times \text{virulence}}{\text{Resistance of the host patient}} = \text{Risk of surgical site infection}$$

Preoperative antisepsis and antimicrobial prophylaxis are performed to decrease the bacterial load in the wound; however, it is not possible to completely sterilize it, as up to 20% of skin flora resides beneath the surface in sebaceous glands and hair follicles. Regarding the bacterial 'dose', studies demonstrate that a concentration of $>10^5$ organisms per gram of tissue is the critical threshold above which risk of SSI increases significantly.[8] As shown in the above equation, however, this dose could be lower if the virulence of the organism is high, host immunity is low or prosthetic material (including suture material) is in place.

Since the microbiology of SSIs is usually a function of the patient's skin flora, the causative organisms are most often staphylococci: *S. aureus* and coagulase-negative staphylococci. However, some patients can also be colonized with a variety of other organisms including Gram-negative bacilli and *Candida* species which can then lead to infection of the wound. Finally, in intra-abdominal surgeries, gastrointestinal flora such as enteric bacteria and anaerobes can contaminate the wound and cause infection.

SSI RISK CLASSIFICATION SYSTEMS AND SSI RISK FACTORS (TABLE 132.1)

Since 1964, surgical wounds have been categorized by the traditional wound classification system, which stratifies wounds into four categories based on the expected bacterial dose that would contaminate the wound: clean, clean–contaminated, contaminated and dirty–infected. Data show that these wound categories have different associated infection rates, ranging from 5% for clean surgeries and up to 22% for the contaminated category, if antimicrobial prophylaxis is not administered (down to 0.8% and 10% respectively if antibiotics are administered).[10]

However, surveillance systems that track SSI rates have moved away from stratifying wounds based on this classification, as it fails to account for patient- and procedure-related factors. The system that is most widely used at present is the National Nosocomial Infection Surveillance (NNIS) risk index; this index was itself based on an index developed during the Study on the Efficacy of Nosocomial Infection Control (SENIC) project which was the first study to demonstrate efficacy of SSI surveillance.[11] The NNIS risk index counts the number of risk factors present among the following:

- an American Society of Anesthesiologists (ASA) preoperative assessment score of 3, 4 or 5;
- an operation classified as either contaminated or dirty–infected; and
- an operation with duration of >T hours, where T depends on the type of operative procedure.

After a recent assessment by the Centers for Disease Control and Prevention (CDC) in patients across the US,[12] the latest addition to this score has been the subtraction of a point if the procedure was performed laparoscopically (as more and more procedures are). This index has been shown to be useful in performing risk adjustment when benchmarking SSI rates. The NNIS risk index is used by the CDC's National Healthcare Safety Network (NHSN) for surveillance in the US, as well as by national surveillance systems in most countries in Europe and elsewhere around the world. Finally, in addition to the risk factors present in the NNIS risk index, there are additional host factors that may place a patient at higher risk for developing SSIs, in particular diabetes mellitus and malnutrition.[13]

Table 132.1 Surgical site infection risk classification systems

Wound classification system[9]	
I. Clean	An uninfected operative wound in which no inflammation is encountered and the respiratory, alimentary, genital or uninfected urinary tract is not entered. In addition, clean wounds are primarily closed and, if necessary, drained with closed drainage. Operative incisional wounds that follow nonpenetrating (blunt) trauma should be included in this category if they meet the criteria
II. Clean–contaminated	An operative wound in which the respiratory, alimentary, genital or urinary tract is entered under controlled conditions and without unusual contamination. Specifically, operations involving the biliary tract, appendix, vagina and oropharynx are included in this category, provided no evidence of infection or major break in technique is encountered
III. Contaminated	Open, fresh, accidental wounds. In addition, operations with major breaks in sterile technique (e.g. open cardiac massage) or gross spillage from the gastrointestinal tract, and incisions in which acute nonpurulent inflammation is encountered are included in this category
IV. Dirty–infected	Old traumatic wounds with retained devitalized tissue and those that involve existing clinical infection or perforated viscera. This definition suggests that the organisms causing postoperative infection were present in the operative field before the operation
SENIC (Study on the Efficacy of Nosocomial Infection Control) index[11]	
1 point	Operative time >2h
1 point	Abdominal procedure
1 point	Contaminated or dirty procedure
1 point	≥Three discharge diagnoses
NNIS (National Nosocomial Infection Surveillance) risk index[12]	
1 point	ASA score of 3, 4 or 5
1 point	Contaminated or dirty procedure
1 point	Length of procedure >T hours (75th percentile duration of specific procedure)
Minus 1 point	Use of laparoscope

ASA, American Society of Anesthesiologists.

SURVEILLANCE FOR SSI

Given the potential consequences of SSIs and other HAIs, substantial resources have been put toward surveillance and prevention programs in the US and other countries in order to monitor and reduce infection rates. In the US, the CDC developed the National Nosocomial Infection Surveillance (NNIS) system as early as the 1970s as a voluntary reporting system. This system has since been renamed the National Healthcare Safety Network (NHSN) and enables confidential data exchange and analysis within its information technology architecture. In the 1990s, a number of European countries established national or regional HAI surveillance systems, largely modeled on the NNIS system. More recently, an initiative known as Hospitals in Europe Link for Infection Control through Surveillance (HELICS) was created in order to establish a surveillance system supported by the European Union.[14]

There is some suggestion that surveillance can help improve practice by promoting awareness of HAI rates and potential problems. According to data from the NNIS system, SSI rates in most patient groups have declined since the 1990s as surveillance has progressed.[15]

One key component of good SSI surveillance is having a case definition that is applied accurately and consistently. Comparing data across different institutions, regions or countries is complicated by potentially variable application of case definitions, along with fundamental differences in health-care systems. Postdischarge surveillance has represented one of the more problematic aspects of SSI surveillance, as it is difficult to achieve consistent case identification methods among postoperative patients who have been discharged and found to have an SSI at an outpatient visit.

INDICATIONS FOR ANTIBIOTIC PROPHYLAXIS

In most instances, antibiotic prophylaxis is administered to prevent an SSI by decreasing the microbial burden at the surgical site. While there is fairly good agreement between consensus statements regarding which surgery types require prophylaxis, the increase in less invasive procedures, including laparoscopic procedures and procedures performed by interventional radiology, has led to some controversy regarding the necessity of prophylaxis. We will address these less invasive procedures in detail. Finally, in some instances, antibiotic prophylaxis is administered not to prevent an SSI, but rather to prevent endocarditis from procedure-induced transient bacteremia in patients with underlying cardiac disease.

GENERAL PRINCIPLES OF ANTIBIOTIC PROPHYLAXIS

Choice

The choice of antibiotic should ideally be one which is 'safe, inexpensive, and bactericidal with an *in vitro* spectrum that covers the most probable intraoperative contaminants for the operation' as noted by the CDC's SSI guidelines.[7] Thus the antibiotic choice will vary depending on the type of procedure. Cephalosporins appear to be effective in a wide variety of surgery types, and they fit the criteria listed above. Additional reasons for their attractiveness are their relatively long duration of action and comparatively low cost. First-generation cephalosporins, such as cefazolin,

are frequently used for clean procedures (and some clean–contaminated procedures) due to their adequate coverage of Gram-positive organisms, as well as some Gram-negative organisms. For surgeries involving the distal intestinal tract, second-generation cephalosporins, such as cefoxitin, are frequently used for their additional anti-anaerobic spectrum of activity.

Dose

It is crucial to achieve and maintain levels of the antibiotic in the serum and at the surgical site that exceed the minimum inhibitory concentration (MIC) for likely pathogens, from the time of incision until the incision is closed. Therefore, both the dose and timing of administration of the antibiotic are important. The dose may need to be individualized based on a given patient's weight; this is particularly important in the setting of rising rates of obesity worldwide. Based on pharmacokinetic principles, lean body weight and volume of distribution are related, suggesting that larger doses are necessary for larger patients. For example, it is currently recommended to increase the cefazolin dose from 1 g to 2 g for patients weighing >80 kg to increase the likelihood of serum and adipose tissue concentration above the MIC of likely organisms.[16] Moreover, cefazolin concentrations in patients undergoing gastric bypass surgery after a 2 g preoperative dose were found to be insufficient and decreased as patient body mass index increased.[17] Redosing of the antibiotic is key if the procedure extends beyond 4 hours and/or major blood loss occurs. The goal is to redose every 1–2 half-lives of the drug being used; assuming normal renal function, this is approximately every 4 hours for most cephalosporins.

Timing

In order to allow the antibiotic adequate time to distribute to the site of action and to maintain adequate levels throughout the procedure, guidelines recommend that most intravenous antibiotics should begin infusing within 1 hour of surgical incision time. The exceptions to this recommendation are vancomycin and fluoroquinolones, which should begin infusing within 2 hours prior to surgical incision to minimize the potential for adverse reactions associated with rapid infusion and to ensure adequate tissue levels. However, a more precise or optimal time within this 1-hour window remains somewhat controversial. Guidelines, including the US National Surgical Infection Prevention Project, recommend administering antibiotics 'as near to the incision time as possible to achieve low SSI rates'.[18,19]

Earlier studies determined that antibiotics given 1–2 hours preoperatively were more effective at preventing SSIs than were antibiotics given 2–24 hours preoperatively or postoperatively (in fact, antibiotics given after surgery resulted in SSI rates no different than if no antibiotics were given at all).[20,21] It is unclear, however, whether data support administering antibiotics within 5–10 minutes of incision time. As mentioned above, antibiotics must have time to achieve adequate levels in serum and local tissues; whether 5 minutes is sufficient is uncertain and this optimal time may vary among different antibiotics.

In a recent study, Garey *et al.* found that, with regard to vancomycin prophylaxis for cardiac surgery, administration within 16–60 minutes before incision was associated with the lowest SSI rate, and administration within 0–15 minutes prior was associated with a relative risk of 7.8 by comparison.[22] Weber *et al.* retrospectively reviewed 3836 consecutive surgical procedures, including gastrointestinal, vascular and trauma surgeries, and found that SSI risk was lowest when cefuroxime was administered 59–30 minutes prior to incision (adjusted odds ratio=1.95 when cefuroxime was administered less than 30 minutes prior).[23] Hence, administration of prophylactic antibiotics 'as near to the incision time as possible' may not be optimal; instead, administration 30 minutes to an hour prior may be more ideal. Further studies are needed to clarify this issue.

Duration

Antibiotic prophylaxis continued for more than 24–48 hours after surgery does not further decrease SSI rates, but rather increases the emergence of antibiotic-resistant bacteria.[7,18,24,25] In fact, it is unclear whether any antibiotic is necessary beyond the time of wound closure. Nonetheless, it is variable from surgery to surgery as to whether guidelines recommend single dose or continuation for 24 hours postoperatively based on the methods in published literature. The exception is cardiac surgery, where most guidelines recommend prophylaxis for 48 hours, although it is very possible that 24 hours would be just as effective in preventing SSIs.[26,27]

β-Lactam allergy

Because β-lactams, such as cephalosporins, are often the drugs of choice for surgical prophylaxis, it is important to elucidate the type of reaction that is being labeled as a 'penicillin' or 'β-lactam' allergy. Unless there has been a true, major allergic reaction (e.g. urticaria, angioedema, anaphylaxis, toxic epidermal necrolysis, Stevens–Johnson syndrome), the patient may be able to safely tolerate a cephalosporin. It is now recognized that the cited cross-reactivity to cephalosporins in penicillin-allergic patients of 10% is an overestimate and primarily based on data from the 1960s and 1970s.[28]

The incidence of true adverse reactions to cephalosporins is low in patients with reported penicillin allergies and this cross-allergenicity is now thought to be related to side chain similarity to penicillin or amoxicillin.[28] This similarity is diminished in second- and third-generation cephalosporins. Moreover, the lack of allergic reactions after receiving cefazolin preoperatively in patients who report a non-severe penicillin allergy has been documented.[29] Usual alternatives to β-lactams in patients with severe allergies are vancomycin and clindamycin. If Gram-negative pathogens are also of concern, however, an additional agent such as an aminoglycoside (e.g. gentamicin) may need to be added.

Methicillin-resistant *Staphylococcus aureus*

In recent years, given the increase in methicillin-resistant *Staphylococcus aureus* (MRSA) in both the hospital and the community, the issue of MRSA coverage for surgical prophylaxis has arisen, particularly in the cardiac surgery population. It is well known that hospitalized patients colonized with *S. aureus* (methicillin-susceptible or -resistant) are at higher risk of developing subsequent *S. aureus* infections than are noncolonized patients, usually with their colonizing strains.[30] This has been demonstrated for SSIs as well.[31] Well-described risk factors for MRSA carriage include recent hospitalization or surgery, dialysis, the presence of chronic open wounds and antibiotic use.[32]

In general, however, glycopeptides such as vancomycin are not recommended as first-line prophylaxis. For patients known to be colonized with MRSA, consideration should be given towards using vancomycin for prophylaxis as opposed to a β-lactam such as cefazolin. Guidelines and the literature, however, remain unclear regarding when vancomycin should be used as a preferred first-line agent for prophylaxis. In 1999, the CDC's SSI prevention guidelines stated that 'a threshold has not been scientifically defined that can support the decision to use vancomycin' for surgical antibiotic prophylaxis.[7]

In the intervening decade, no new studies have been published to delineate this threshold. However, there have been several studies directly comparing the efficacy of vancomycin versus a β-lactam in preventing SSI. Finkelstein *et al.* conducted a study in an Israeli hospital with a 'high' prevalence of MRSA infections (rate was not specified) in which patients were randomly assigned to receive vancomycin or cefazolin prior to cardiac surgery.[33] They found that overall SSI rates were similar in the two groups and that infections due to methicillin-susceptible staphylococci were more common in the vancomycin group (there was a nonsignificant trend towards more infections caused by methicillin-resistant organisms in the cefazolin group). A meta-analysis

by Bolon *et al.* included this study as well as six other randomized trials (total 5761 procedures) and similarly found that neither antibiotic class (glycopeptides or β-lactams) was superior in preventing cardiac SSIs.[34] In fact, in subgroup analysis β-lactams appeared to be superior to glycopeptides in preventing chest SSIs. This may be due to the fact that, while glycopeptides are more broad-spectrum for Gram-positive coverage than β-lactams, they appear to have poorer tissue penetration and slower bacterial killing.[35]

More recently, Garey *et al.* reported that when their hospital switched from cefuroxime to vancomycin, their monthly coronary artery bypass graft (CABG) SSI incidence decreased significantly (as compared to their valve replacement SSI rates).[36] However, this was not a prospective, randomized study. Hence, while further research is needed on this topic, currently cephalosporins (usually cefazolin) remain the first-line agents for prophylaxis in cardiac surgery.

Non-systemic agents

Topical *Staphylococcus aureus* decolonization

Because *S. aureus* is one of the most significant pathogens causing SSIs, much attention has been paid to strategies for the prevention of *S. aureus* infections, both methicillin-susceptible and methicillin-resistant. These strategies have mainly focused on topical *S. aureus* decolonization using chlorhexidine gluconate or mupirocin.

Chlorhexidine gluconate

Chlorhexidine gluconate is an antiseptic agent that has been shown to decrease microbial flora on the skin and prevent infection risk in various settings, including as a skin preparatory agent for surgical procedures and for insertion of vascular access devices, as a surgical hand scrub, and for oral hygiene. Numerous studies have been published regarding its use in preoperative bathing, the hypothesis being that by decreasing overall microbial skin colonization there is less opportunity for the surgical wound to become contaminated by the patient's endogenous flora. A recent Cochrane Collaboration review evaluated data from six randomized controlled trials with a total of >10 000 participants; pooled data did not show any significant decrease in SSI rates with antiseptic bathing or showering.[23] Although the studies included in this review varied in their methodologic quality, the two largest trials (with >6000 subjects) were deemed to be of high quality.

Nevertheless, many institutions continue to practice this intervention for their patients preoperatively, particularly in light of numerous recent studies indicating chlorhexidine bathing's efficacy in other settings (e.g. prevention of catheter-associated bloodstream infections and of transmission of vancomycin-resistant enterococci in the intensive care unit[37,38]). Currently there is interest in the use of chlorhexidine-impregnated wipes as an alternative to bathing or showering with chlorhexidine soap. Although the wipes only contain 2% chlorhexidine, they have been shown to achieve higher chlorhexidine concentrations on the skin than the 4% chlorhexidine soap,[39] and may represent an attractive alternative for patients who are unable to shower or bathe thoroughly. Preoperative chlorhexidine bathing may be considered if other measures have been instituted and SSI rates remain high.

Finally, Segers *et al.* studied the effects of preoperative decontamination of the nasopharynx and oropharynx using 0.12% chlorhexidine gluconate.[40] Patients received an oropharyngeal rinse and nasal ointment containing either chlorhexidine or placebo prior to cardiac surgery and continuing until the nasogastric tube was removed (usually the day after surgery). The intervention decreased the overall nosocomial infection rate, but did not significantly decrease the SSI rate. More data are needed to make conclusive recommendations regarding nasopharyngeal and oropharyngeal decontamination with chlorhexidine.

Mupirocin

Mupirocin is a topical antibiotic that can be administered intranasally in an attempt to eradicate a significant reservoir for *S. aureus* (patients who develop *S. aureus* infections are often colonized in their nares at the time of infection). As the incidence of infections due to MRSA has increased, there has been growing interest in the use of mupirocin preoperatively for the purposes of preventing postoperative *S. aureus* and MRSA infections. One of the largest studies looking at this issue was published by Perl and colleagues in 2002, in which >4000 preoperative patients were enrolled in a randomized, placebo-controlled trial.[41] They found that intranasal mupirocin did not significantly reduce the rate of *S. aureus* SSIs overall, but that there was a significant reduction in *S. aureus* SSIs specifically for *S. aureus* carriers. Since then, many other smaller studies have been published on this theme, and recently two systematic reviews were performed on some of these studies. In one review by Trautmann *et al.*, the authors concluded that mupirocin prophylaxis did not reduce the overall *S. aureus* SSI rate in patients undergoing orthopedic, gastrointestinal and cardiothoracic surgery, although the MRSA SSI rate was reduced.[42] However, this review did not look at the effect of mupirocin on *S. aureus* carriers specifically and excluded non-English language papers. By contrast, the review by van Rijen *et al.* focused solely on patients who were *S. aureus* carriers and found that mupirocin significantly reduced the overall rate of postoperative *S. aureus* infections.[43] Interestingly, however, there was no significant decrease in SSIs due to *S. aureus*. The main limitation of this analysis was the fact that its outcome was mostly determined by the results of the study by Perl *et al.*,[41] as they had by far the largest study group. The use of prophylactic intranasal mupirocin has been widely adopted for preoperative patients and is recommended by the Society of Thoracic Surgeons' practice guidelines for all patients undergoing cardiac surgery 'in the absence of a documented negative test for staphylococcal colonization'.[27]

RECOMMENDATIONS FOR SPECIFIC PROCEDURES (TABLES 132.2, 132.3)

One of the most important principles in the selection of an antimicrobial for prophylaxis is to effectively provide coverage for the most common pathogens associated with wound infections following the specific surgical procedure. Basic principles of pharmacokinetics and pharmacodynamics dictate that the agent chosen should have effective tissue penetration, a relatively long half-life, minimal toxicity and minimal side-effects. Published literature supports the use of many different regimens for the prophylaxis of any one surgical procedure (see Tables 132.2 and 132.3). Other factors such as cost and antimicrobial resistance, however, tilt the pendulum towards more narrow-spectrum, older antimicrobial agents.

Cardiothoracic surgery

Antimicrobial prophylaxis for cardiac procedures is addressed in all standard guidelines as well as specifically in a statement from the Society of Thoracic Surgeons.[27,44] Antimicrobial prophylaxis should be administered for all cardiac procedures with median sternotomy. The incidence of deep sternal wound infections is reported to be between 0.25% and 4%,[44] and the consequences of these infections can be devastating. Two recent reviews of deep sternal wound infections, one during 1990–2003 and the other during 2001–2005, reported in-hospital mortality rates of 6.9% and 9.2%, respectively.[45,46]

Prophylaxis should target staphylococcal species, namely *S. aureus* and coagulase-negative staphylococci. As mentioned above, the increasing prevalence of MRSA complicates antimicrobial choices and some guidelines have recommended the use of vancomycin in institutions with a 'high rate' of infection due to MRSA, but the threshold for a 'high rate' has not been established (see section on MRSA above).

Table 132.2 Antibiotic prophylaxis recommendations for selected surgical procedures (superscripts denote source of recommendations; see footnote below)

Type of surgery	Recommended drugs	Alternative drugs	Duration
Cardiothoracic	Cefazolin*†‡ *or* cefuroxime†‡ *or* cefamandole* *or* vancomycin (if high risk for MRSA) †‡	*β-Lactam allergy:* Vancomycin†‡ ± gentamicin* *or* clindamycin†	Single-dose‡, ≤48h†, ≤72h*
Gastrointestinal			
Gastroduodenal	Cefazolin* (high risk only‡)	*β-Lactam allergy:* Clindamycin + gentamicin‡ *or* ciprofloxacin‡ *or* levofloxacin‡ *or* aztreonam‡	Single dose*‡
Biliary			
Open procedures	Cefazolin*	*β-Lactam allergy:* Clindamycin + gentamicin *or* ciprofloxacin *or* levofloxacin or aztreonam	Single dose*
Laparoscopic procedures	Cefazolin (high risk only)‡	*β-Lactam allergy:* Clindamycin + gentamicin‡ *or* ciprofloxacin‡ *or* levofloxacin‡ *or* aztreonam‡	Single dose‡
Appendectomy for uncomplicated appendicitis	Cefoxitin*‡ *or* cefotetan* *or* cefmetazole* *or* cefazolin + metronidazole‡ *or* ampicillin–sulbactam‡	*β-Lactam allergy:* Clindamycin + gentamicin‡ *or* ciprofloxacin‡ *or* levofloxacin‡ *or* aztreonam‡ *or* gentamicin + metronidazole*	Single dose*‡
Colorectal	*Oral:* Neomycin + erythromycin base*†‡ *or* neomycin + metronidazole†‡ *Parenteral:* Cefotetan*† *or* cefoxitin*†‡ *or* cefmetazole* *or* cefazolin + metronidazole†‡ *or* ampicillin–sulbactam†‡	*β-Lactam allergy:* Clindamycin + gentamicin†‡ *or* fluoroquinolone†‡ *or* aztreonam†‡ *or* metronidazole + gentamicin† *or* fluoroquinolone†	*Parenteral:* Single dose (≤24 h)*†‡
Head and neck			
Clean with prosthesis placement	Cefazolin*	Clindamycin	Single dose*
Incisions through oral or pharyngeal mucosa	Cefazolin*‡ *or* clindamycin*	Cefazolin + metronidazole* *or* clindamycin + gentamicin*‡	Single dose*‡
Neurosurgery			
Elective craniotomy or cerebrospinal fluid shunting	Cefazolin*‡ *or* vancomycin (if high risk for MRSA *or* β-lactam allergy)*‡	Oxacillin* *or* nafcillin*	Single dose*‡
Obstetrics/gynecology			
Caesarian delivery	Cefazolin*‡	*β-Lactam allergy:* Clindamycin + gentamicin‡ *or* ciprofloxacin‡ *or* levofloxacin‡ *or* aztreonam‡	Single dose*‡
Hysterectomy	Cefotetan*† *or* cefazolin*†‡§ *or* cefoxitin†‡§ *or* ampicillin–sulbactam†‡ *or* metronidazole§ *or* tinidazole§	*β-Lactam allergy:* Clindamycin + gentamicin *or* fluoroquinolone *or* aztreonam or metronidazole + gentamicin *or* fluoroquinolone *or* clindamycin monotherapy	Single dose (≤24 h)*†‡§
Ophthalmic	Topical gentamicin*‡ *or* tobramycin*‡ *or* ciprofloxacin‡ *or* gatifloxacin‡ *or* levofloxacin‡ *or* moxifloxacin‡ *or* ofloxacin‡ *or* neomycin–gramicidin–polymyxin B*‡ *or* cefazolin by subconjunctival injection‡	Addition of tobramycin by subconjunctival injection*	Prior to procedure*, 2–24 h‡
Orthopedic			
Hip fracture repair	Cefazolin*	Vancomycin*	24 h*
Implantation of internal fixation devices	Cefazolin*	Vancomycin*	24 h*
Total joint replacement	Cefazolin*†‡ *or* cefuroxime†‡ *or* vancomycin (if high risk for MRSA)*†‡	*β-Lactam allergy:* Vancomycin*†‡ *or* clindamycin†	Single dose (≤24 h)*†‡

(Continued)

Table 132.2 Antibiotic prophylaxis recommendations for selected surgical procedures—cont'd

Type of surgery	Recommended drugs	Alternative drugs	Duration
Urologic (high risk patients only)	*Oral:* Trimethoprim–sulfamethoxazole* *or* ciprofloxacin‡ *Parenteral:* Cefazolin* *or* ciprofloxacin‡	*Oral or parenteral:* Levofloxacin	Single dose*‡
Vascular	Cefazolin*†‡ *or* cefuroxime†‡ *or* vancomycin (if high risk for MRSA)†‡	Vancomycin ± gentamicin* *β-Lactam allergy:* Vancomycin†‡ *or* clindamycin†	Single dose (≤24 h)*†‡
Transplantation			
Heart	Cefazolin*	Cefuroxime* *or* cefamandole* *or* vancomycin ± gentamicin*	48–72 h*
Lung and heart–lung	Cefazolin*	Cefuroxime* *or* cefamandole* *or* vancomycin*	48–72 h*
Liver	Cefotaxime + ampicillin*	*Beta-lactam allergy:* clindamycin + gentamicin *or* ciprofloxacin *or* levofloxacin *or* aztreonam	48 h*
Pancreas and pancreas–kidney	Cefazolin*	*β-Lactam allergy:* Clindamycin + gentamicin *or* ciprofloxacin *or* levofloxacin *or* aztreonam	Single dose*
Kidney	Cefazolin*	*β-Lactam allergy:* Clindamycin + gentamicin *or* ciprofloxacin *or* levofloxacin *or* aztreonam	Single dose*

MRSA, methicillin-resistant *Staphylococcus aureus*.
*As recommended by the American Society of Health-System Pharmacists (ASHP) Therapeutic Guidelines on Antimicrobial Prophylaxis in Surgery.
†As recommended by the National Surgical Infection Prevention Project[18,19]
‡As recommended by The Medical Letter.
§As recommended by the American College of Obstetrics and Gynecology[49]

Table 132.3 Recommended initial dose, half-life and time to redosing intraoperatively[18,19]

Antimicrobial	Usual half-life with normal renal function (hrs)	Infusion time (min)	Usual intravenous dose (weight-based dosing)	Redosing interval (hrs)
Ampicillin	1–1.8	10–30	1–2 g (50 mg/kg/dose)	4
Ampicillin–sulbactam	1–1.8	10–30	1.5–3 g (50 mg/kg/dose ampicillin component)	4
Aztreonam	1.5–2	3–5 (slow iv push) 15–60 (intermittent infusion)	1–2 g (30 mg/kg/dose)	3–5
Cefamandole	0.5–2.1	3–5 (slow iv push) 15–60 (intermittent infusion)	1 g (20–40 mg/kg/dose)	3–4
Cefazolin	1.2–2.5	3–5 (slow iv push) 15–60 (intermittent infusion)	1–2 grams (20–30 mg/kg/dose)	2–5
Cefotaxime	1.5–6	3–5 (slow iv push) 15–60 (intermittent infusion)	1–2 g (50 mg/kg/dose)	4
Cefuroxime	1–2	3–5 (slow iv push) 15–60 (intermittent infusion)	1.5 g (50 mg/kg/dose)	3–4
Clindamycin	2–5.1	10–60 (do not exceed 30 mg/min)	600–900 mg (3–6 mg/kg/dose)	3–6
Ciprofloxacin	3.5–5	60	400 mg (10–15 mg/kg/dose)	4–10
Gentamicin	2–3	30–60	1.5 mg/kg/dose*	3–6
Levofloxacin	6–8	60	500 mg (10 mg/kg/dose)	12–18
Metronidazole	6–14	30–60	500 mg–1 g (15 mg/kg/dose)	6–8
Nafcillin	0.5–1.9	30–60	1–2 g (12–50 mg/kg/dose)	2–4
Oxacillin	0.5–1.8	10–30	1–2 g (12–50 mg/kg/dose)	2–4
Vancomycin	4–6	60 (≥60 min if >1 g)	1 g (10–15 mg/kg/dose)	6–12

Recommendations from the US Surgical Infection Prevention and Surgical Care Improvement Projects prefer cefazolin or cefuroxime, but suggest the use of vancomycin in patients at high risk for MRSA. Similarly, the Society of Thoracic Surgeons recommends consideration for the use of vancomycin combined with a β-lactam for patients with either presumed or known staphylococcal colonization, institutions with a 'high incidence' of MRSA, patients susceptible to colonization (hospitalized >3 days, transfer from another inpatient facility, already receiving antibiotics) or a procedure involving insertion of a prosthetic valve or vascular graft. This recommendation, however, is less well established.

Duration of prophylactic antibiotics beyond 48 hours does not decrease the potential for SSIs but rather increase the potential for antimicrobial resistance, and should be avoided. Continuation of antimicrobial prophylaxis until chest tubes or other indwelling catheters are removed is not warranted.[44]

Gastrointestinal surgery

Prophylaxis is recommended for most gastrointestinal procedures. The biliary tract is considered a sterile site, and the stomach contains few bacteria due to the highly acidic environment. However, the density of bacteria progressively increases from the small bowel to the colon, as does the proportion of anaerobic to aerobic bacteria. Prophylaxis is recommended for all procedures that enter the small bowel and colon. The intrinsic risk of infection associated with entry into other areas of the gastrointestinal tract is considered low and does not support routine antibiotic prophylaxis except in high-risk patients (see Less invasive procedures, discussed later in this chapter). In gastroduodenal surgeries, patients at a higher risk of infection include those with achlorhydria, decreased gastric motility, gastric outlet obstruction, morbid obesity, gastric bleeding or cancer.[47] For biliary procedures, high-risk patients may include the elderly, those with recent symptoms of inflammation, common duct stones, jaundice or those with a history of previous biliary surgery. Colorectal procedures have a high risk of infection and warrant routine antibiotic prophylaxis in all patients.

In gastroduodenal procedures, first- and second-generation cephalosporins have demonstrated effectiveness and are the most widely used, but the number of studies is limited compared to other types of surgery. In these cases, SSIs caused by *Bacteroides* species are rare. The need to cover anaerobic bacteria increases in distal areas of the gastrointestinal tract, and antibiotics for prophylaxis of colonic procedures should be directed at both Gram-negative aerobes and anaerobic bacteria.

Head/neck surgery

Antimicrobial prophylaxis is recommended for all head and neck procedures that involve entry into the oropharynx (clean–contaminated procedures). Antimicrobial prophylaxis is not warranted for patients undergoing clean procedures of the head and neck.[48] Antimicrobial choice should target the normal flora of the mouth and oropharynx, especially streptococci and oropharyngeal anaerobes, as postoperative wound infections are frequently polymicrobial.

Neurosurgery

Clean neurosurgical procedures are associated with a relatively low risk of SSI, usually less than 5%. Nonetheless, antimicrobial prophylaxis is recommended for clean procedures, which include craniotomies, laminectomies and ventricular fluid-shunt placements. Surgical site infections in this population are primarily associated with Gram-positive bacteria, especially *S. aureus* and coagulase-negative staphylococci.

Obstetric/gynecologic surgery

Antimicrobial prophylaxis is recommended for hysterectomies (vaginal, abdominal) and Caesarian deliveries. For most SSIs following gynecologic procedures, the bacterial source is the skin or the vagina. Aerobic Gram-positive cocci (e.g. staphylococci) predominate from the skin, but the potential for exposure to Gram-negative aerobes and anaerobic bacteria can be expected when the vagina is opened or incisions are made near the perineum or groin.[7,49] Postoperative infections associated with hysterectomies are often polymicrobial. Cephalosporins have been the most widely studied in these procedures and studies comparing different cephalosporins have not shown differences in rates of infection. Cefazolin, cefotetan, cefoxitin and ampicillin–sulbactam have been recommended.

For Caesarian sections, a first-generation cephalosporin is the drug of choice. Unlike in other surgical procedures, current guidelines recommend that the timing of antimicrobial administration for Caesarian deliveries should be delayed until after cord clamping to avoid effects on the child's normal bacterial flora. This delay in administration is supported by a few studies including a large, randomized trial in 642 women that demonstrated no differences in infectious morbidity between women administered antimicrobials preoperatively compared to after cord clamping.[50] However, based on newer studies that have shown otherwise,[51,52] a recent joint publication by the American College of Obstetricians and Gynecologists and the American Academy of Pediatrics stated that administering antibiotics pre-incision appears to be more effective in preventing SSIs than post-cord clamping.[53]

Orthopedic surgery

Clean orthopedic procedures not involving implantation of foreign materials do not require routine antimicrobial prophylaxis as the risk of an SSI is low. Any possible benefit is not considered significant enough to outweigh antimicrobial cost and potential for toxicity and antibiotic resistance. There are additional orthopedic procedures, however, where the use of antimicrobial prophylaxis significantly decreases the incidence of SSIs. These procedures include the insertion of a prosthetic joint, ankle fusion, revision of a prosthetic joint, reduction of hip fractures, reduction of high-energy closed fractures and reduction of open fractures. Such procedures are associated with a risk of infection of 5–15%, which is reduced to less than 3% by the use of prophylactic antimicrobials.[54]

Skin organisms account for most SSIs in orthopedic surgery, with *S. epidermidis* and *S. aureus* accounting for >70% of infections following total joint replacements. First-generation cephalosporins are the most widely studied antimicrobials for prophylaxis in this population. While second-generation cephalosporins offer no clear advantage, either cefazolin or cefuroxime is recommended as the preferred antimicrobial for prophylaxis in patients undergoing hip or knee arthroplasty without a β-lactam allergy. Similar to cardiac procedures, vancomycin is an alternative to consider in institutions with a 'high rate' of MRSA.

The duration of antimicrobial prophylaxis following orthopedic procedures has been debated. There is no evidence that extension of antimicrobial prophylaxis duration beyond 24 hours or until drains or catheters are removed is beneficial in reducing infections.[55]

Urologic surgery

The most common infectious complication following urologic surgery is bacteriuria. More importantly, however, antimicrobial prophylaxis prevents bacteremia and SSIs with the secondary goal of preventing bacteriuria. Most urologic procedures do not require prophylaxis in patients with sterile urine. The notable exceptions are in patients undergoing transurethral prostatectomy, transrectal prostatic biopsies and insertion of urologic prostheses.[56–58]

A wide range of antimicrobials has been studied as prophylaxis in urologic procedures. Current recommendations include the use of trimethoprim–sulfamethoxazole, cefazolin or a fluoroquinolone as the preferred antimicrobials in high-risk patients only (i.e. positive or unavailable urine culture, preoperative urinary catheter, transrectal prostatic biopsy or insertion of prosthetic material) and in patients undergoing a transurethral prostatic biopsy. *Escherichia coli* is the most common organism complicating these procedures, but other Gram-negative bacilli and enterococci also cause infection. The choice of agent for urologic procedures must be dictated by patient-specific and local susceptibility data, given that significant increases in trimethoprim–sulfamethoxazole and fluoroquinolone resistance in *E. coli* have been noted.

Vascular surgery

Antimicrobial prophylaxis is indicated for abdominal and lower extremity vascular procedures including aneurysm repair, thromboendarterectomy and vein bypass. Infection involving vascular graft material can be particularly devastating. It appears that patients undergoing vascular-access surgery for hemodialysis also have a decreased rate of infection following the administration of antimicrobial prophylaxis compared to placebo.[59] The most common organisms associated with infections following vascular procedures include *S. aureus*, *S. epidermidis* and enteric Gram-negative bacilli. Cefazolin and cefuroxime remain the drugs of choice, with the option to use vancomycin in institutions with a 'high rate' of infections caused by MRSA.

LESS INVASIVE PROCEDURES

The purpose in administering antibiotic prophylaxis for most of the procedures listed above is to prevent the development of an infection at the surgical site. One additional purpose for antibiotic prophylaxis is to prevent bacteremias that could lead to endocarditis. Guidelines on endocarditis prevention in dental and other procedures have recently been revised and are described here in detail.

Furthermore, as technology advances, the ability to perform procedures in a minimally invasive way is progressively increasing. Many procedures that previously could only be performed openly in an operating room setting can now be performed laparoscopically or by a nonsurgical interventionalist. These procedures are associated with their own, usually lower, risk of infection. Because most of these procedures involve newer technology, there is a smaller body of literature associated with their infection risk and antibiotic prophylaxis. Here we discuss what is available for selected procedure types.

Prevention of infective endocarditis in dental and other procedures

Previous guidelines from the American Heart Association (AHA) published in 1997 were somewhat complex and recommended antibiotic prophylaxis for the prevention of infective endocarditis (IE) in numerous settings.[60] In recent years, however, it has been acknowledged that IE is much more likely to result from randomly occurring bacteremias (such as from brushing teeth) than from dental, gastrointestinal or genitourinary tract procedures, and that ongoing maintenance of good oral hygiene is likely to have a greater beneficial effect than antibiotic prophylaxis pre-procedure. Moreover, as prophylaxis is likely to prevent very few cases of IE, the risks associated with prophylaxis probably outweigh the benefits.

In 2006 the British Society for Antimicrobial Chemotherapy issued guidelines recommending prophylaxis only for patients with a history of previous IE or who have had cardiac valve replacement or surgically constructed pulmonary shunts or conduits.[61] Similarly, in 2007 the AHA released revised and simplified guidelines which have greatly narrowed the settings in which antibiotic prophylaxis is recommended for IE prevention[62] (see Tables 132.4 and 132.5 for specific recommendations). Prophylaxis is indicated for all dental

Table 132.4 Cardiac conditions for which prophylaxis is reasonable with dental procedures[62]

- Prosthetic cardiac valve or prosthetic material used for valve repair
- Previous infective endocarditis
- Congenital heart disease (CHD). Only patients with the following conditions are candidates for prophylaxis:
 - Unrepaired cyanotic CHD, including palliative shunts and conduits
 - Completely repaired congenital heart defect with prosthetic material or device, whether placed by surgery or by catheter intervention, during the first 6 months after the procedure. After 6 months, prophylaxis is *not* recommended because endothelialization of prosthetic material occurs within this time
 - Repaired CHD with residual defects at the site or adjacent to the site of a prosthetic patch or prosthetic device (which inhibit endothelialization)
- Cardiac transplantation recipients who develop cardiac valvulopathy

Table 132.5 Prophylactic regimens for dental, oral, respiratory tract or esophageal procedures

Situation	Agent	DOSING REGIMEN: SINGLE DOSE 30–60 MIN BEFORE PROCEDURE	
		Adults	Children
Oral prophylaxis			
Standard	Amoxicillin	2 g	50 mg/kg (max 2 g)
Penicillin allergy	Clindamycin *or*	600 mg	20 mg/kg (max 600 mg)
	Cephalexin*† *or*	2 g	50 mg/kg (max 2 g)
	Azithromycin *or* clarithromycin	500 mg	15 mg/kg (max 500 mg)
Parenteral prophylaxis			
Standard	Ampicillin	2 g iv or im	50 mg/kg (max 2 g) iv or im
Penicillin allergy	Clindamycin *or*	600 mg iv	20 mg/kg (max 600 mg) iv
	Cefazolin†	1 g iv or im	50 mg/kg (max 1 g) iv or im
	Ceftriaxone†	1 g iv or im	50 mg/kg (max 1 g) iv or im

*Or other first- or second- generation oral cephalosporin in appropriate equivalent dosage.
†Cephalosporins should not be used in individuals with immediate-type hypersensitivity reaction (urticaria, angioedema or anaphylaxis) to penicillins.
Adapted from Wilson *et al.*[62]

procedures that involve manipulation of gingival tissue or the periapical region of teeth or perforation of the oral mucosa (including standard dental cleanings) in patients at risk. Viridans group streptococci are the most common cause of endocarditis following these procedures. Prophylaxis is not recommended, however, for routine anesthetic injections through noninfected tissue, taking dental radiographs, placement of removable prosthodontic or orthodontic appliances, placement of orthodontic brackets, bleeding from trauma to the lips or oral mucosa, orthodontic appliance adjustment or shedding of deciduous teeth.[62]

Pacemaker/cardioverter-defibrillator implantation

Along with a worldwide aging population, the incidence of cardiac device implantation is increasing, along with an increasing risk of infection. The estimated rate of infection after implantation of permanent endocardial leads is 1–2%, although published rates vary.[63] While there are few large-scale randomized controlled trials evaluating the efficacy of antibiotic prophylaxis in this setting, there is a meta-analysis pooling data from >2000 patients in seven trials which suggested a protective effect of antibiotic prophylaxis in patients undergoing permanent pacemaker implantation.[64] Antibiotics used in the trials were either a penicillin with activity against *S. aureus* or a first-generation cephalosporin, but duration of therapy varied considerably, ranging from 6 hours to 8 days. In most studies the antibiotic was administered within the 2 hours preceding the incision.

More recently, a large prospective survey (the PEOPLE study) in patients with implantation of pacemakers and cardioverter-defibrillators reported that antibiotic prophylaxis appeared to have a protective effect (adjusted odds ratio 0.4) against the development of a cardiac device-related infection.[63] Nonetheless, there was variability in type and duration of antibiotic prophylaxis (antibiotics were administered according to local guidelines). Hence, while it remains unclear as to the optimal duration and choice of antibiotics, there does appear to be a body of literature suggesting that antibiotic prophylaxis is beneficial in preventing cardiac device-related infections. A single dose of a β-lactam antibiotic such as cefazolin is probably appropriate to cover skin flora.

Gastrointestinal endoscopy

Antibiotic prophylaxis has been used in the setting of gastrointestinal endoscopy for two purposes:

- to prevent endocarditis from bacteremia; and
- to prevent gastrointestinal or intra-abdominal infections from local seeding.

It is no longer recommended for the prevention of IE (see Prevention of infective endocarditis in dental and other procedures, above). Here we will discuss settings in which prophylaxis has been used to prevent local infections.

Endoscopic retrograde cholangiopancreatography

Cholangitis and pancreatitis can occur as a result of endoscopic retrograde cholangiopancreatography (ERCP), particularly when biliary drainage is incomplete.[65] Antibiotic prophylaxis is a common practice for some of these patients, but practices and guidelines vary widely as to which specific subsets of patients should receive antibiotics. Most randomized controlled trials investigating this issue are relatively small, and since infection rates are low it is probable that they were not adequately powered to detect a difference in infection rates. A meta-analysis by Harris *et al.* summarized the results of five randomized controlled trials and found no significant difference in infection rates between the group that received prophylaxis and the group that did not.[66] A recent retrospective review followed outcomes over an 11-year period during which the use of prophylaxis was sequentially scaled back. Initially all patients with biliary or pancreatic obstruction, those likely to need therapeutic intervention and those with immunosuppression received prophylaxis. By the end of the study period, the only patients who received prophylaxis were those in whom drainage was suspected to be incomplete, or were considered immunosuppressed. The authors found no significant difference in infection outcomes during this time.[67] Finally, a review by Subhani *et al.* examined the evidence for antibiotic prophylaxis in ERCP and stated that prophylaxis for all patients was not beneficial. However, they suggested that if prophylaxis were given to patients with incomplete drainage, that 80% of cholangitis episodes would be prevented.[68]

The American Society for Gastrointestinal Endoscopy (ASGE),[69] the British Society of Gastroenterology Endoscopy Committee[70] and the European Society of Gastrointestinal Endoscopy[71] all recommend antibiotic prophylaxis when ERCP is performed for pancreatic pseudocyst drainage or biliary obstruction (ASGE narrows the latter criterion to those in whom incomplete drainage is anticipated, as is recommended by Subhani *et al.*[68]). The antibiotic chosen should cover biliary organisms, including enterococci and enteric Gram-negative bacteria. Fluoroquinolones or β-lactam/β-lactamase inhibitor combinations, such as ampicillin–sulbactam, are sometimes used.

Endoscopic ultrasound-fine needle aspiration

With regard to endoscopic ultrasound-fine needle aspiration (EUS-FNA) procedures, antibiotic prophylaxis is often administered when FNA of cystic lesions along the GI tract is performed so as to prevent cyst infection; however, these infections are rare and there are no randomized studies evaluating the efficacy of antibiotic prophylaxis in these patients, despite the fact that expert opinion frequently recommends it.

Percutaneous endoscopic gastrostomy

Antibiotic prophylaxis is recommended for percutaneous endoscopic gastrostomy (PEG) tube placements, typically with single-dose cefazolin. A recent Cochrane Review did find a significant decrease in peristomal infections with antibiotic prophylaxis.[72]

Interventional radiology

There are no randomized controlled trials in the interventional radiology (IR) arena proving the efficacy of antibiotic prophylaxis. Nonetheless, it has become a standard of care in many institutions to administer antibiotics in this setting. In a recent review by Ryan *et al.*,[73] it is acknowledged that the benefit of antibiotic prophylaxis for central venous access is unproven, and antibiotics are not recommended for routine vascular access procedures such as angiography, angioplasty, stent placement and peripheral access (the authors leave open the option of single-dose cefazolin for tunneled central venous access although there are no specific data to support this recommendation). Nonetheless, Ryan *et al.* do recommend antibiotic prophylaxis for other IR procedures such as embolization procedures (uterine fibroid, hepatic chemoembolization, splenic, renal) and tube placements (cholecystostomy, gastrostomy, nephrostomy). Again, however, few studies exist in the IR realm to support these recommendations. When prophylaxis is given, a single-dose antibiotic covering the local organisms is sufficient (frequently used options include cefazolin for skin flora and ampicillin–sulbactam or ceftriaxone for gastrointestinal or genitourinary organisms).

Laparoscopic cholecystectomy

Most randomized controlled trials looking at the utility of antibiotic prophylaxis for laparoscopic cholecystectomy have included only a small number of patients. However, a meta-analysis combining results from five studies with a total of 899 patients found no significant difference in wound infection rates between those that received antibiotic prophylaxis and those that did not.[74] Hence, most experts do not recommend routine prophylaxis for laparoscopic cholecystectomy.

REFERENCES


References for this chapter can be found online at http://www.expertconsult.com

Chapter 133

Dilip Nathwani

Non-inpatient parenteral antimicrobial therapy

INTRODUCTION

Most antibiotics given at home are administered orally. However, over the past 20 years, intravenous antimicrobial therapy delivered either at home or in the non-inpatient setting has developed as an important component of health-care delivery. This type of service has been termed outpatient parenteral antimicrobial therapy (OPAT), but the more general term of non-inpatient parenteral antimicrobial therapy (NIPAT) will be used in this chapter.

Over a quarter of a million patients are treated in the USA annually in this manner.[1] Indeed, it is estimated that in the 1990s up to 1 in 1000 North Americans each year received outpatient parenteral therapy. The use of home intravenous antimicrobial therapy was first reported in 1974 for children who had cystic fibrosis-associated pneumonia and subsequently for patients who had osteomyelitis.[2,3] In addition to the USA, NIPAT is now a common treatment modality in many regions, including Europe[4] and Australia.[5] These programs are useful both for patients who have infections requiring prolonged intravenous antibiotic therapy (e.g. osteomyelitis, septic arthritis or endocarditis) and for patients who have common acute infections such as skin and soft tissue infections (SSTIs) or pyelonephritis, in whom in-hospital admission may be avoided entirely. Increasingly, patients with device or prosthesis infections, wound infections and line-related bacteremia are amenable to non-inpatient therapy.

In two large recent US studies of the experience of infectious disease consultants and geriatricians with NIPAT, the four commonest infections treated, in descending order, were bone and joint infections, endocarditis, SSTIs and bacteremia. Prosthetic or orthopedic device infections, line infections and intra-abdominal infections are also important emerging disorders treated in the outpatient setting.[6,7] The potential advantages and disadvantages of NIPAT are summarized in Table 133.1. A crucial component for successful and safe treatment is that patients are clinically stable and have appropriate home circumstances and support.

This chapter focuses on the core aspects of delivering parenteral antimicrobials in the non-inpatient setting and summarizes current guidance on good practice. Key areas include patient evaluation and selection, including typical models of care, the technology that is needed to deliver this, different indications for non-inpatient therapy and attributes of key antimicrobials for such therapy, monitoring treatment and measuring outcomes. However, related topics such as duration of therapy, when to switch from intravenous to oral therapy, and the pharmacologic details of the infusions are not addressed here although are available in published guidelines.

Table 133.1 Potential advantages and disadvantages of outpatient parenteral antimicrobial therapy

Potential advantages	Potential disadvantages
Patient at home with family Continue work, school Decreased nosocomial infections Fewer cannula-associated infections Improved utilization of hospital beds Patient sense of empowerment Reduced health care costs (possible)	Increased patient/family stress Nonadherence with therapy Misuse of intravenous access Decreased supervision Feeling of abandonment Inappropriate antibiotic selection Nonadherence to bed rest, leg elevation Potential for unnecessarily prolonged duration of OPAT because of less medical incentive to stop treatment

Adapted from Howden & Grayson.[8]

EVIDENCE BASE AND CLINICAL PRACTICE GUIDELINES

Although there have been very few randomized trials of non-inpatient therapy, those that have been done have primarily compared inpatient treatment to home therapy; these and many published case series in diverse settings have generally reported good treatment outcomes.[8–10] A recent primary care randomized controlled trial of intravenous antibiotic treatment of cellulitis confirmed that intravenous antibiotic therapy can be safely and effectively delivered at home and is preferred by patients. Up to one-third of the patients in this study were deemed unsuitable because of co-morbidities or lack of home support.[11] While the primary driver for non-inpatient treatment has been cost containment, increasingly there is widespread acceptance by patients, caregivers, clinicians and payers that it is a safe, successful and cost-effective option with enhanced quality of life compared to inpatient hospital treatment. The other benefits include a reduced risk of health-care-acquired infections.

While experience over nearly 25 years has demonstrated the benefits of non-inpatient parenteral therapy for a variety of infections, high-grade evidence-based guidelines are lacking. However, there are a number of guidelines or good practice statements to assist physicians and other health-care professionals with various aspects of the administration of NIPAT. These are outlined in Table 133.2 [4,8,10,12,13]

Table 133.2 Guidelines for the administration of NIPAT

Guideline	Country of origin	Year	Reference	Comment
Practice guidelines for outpatient parenteral antimicrobial therapy	USA	2004	Tice *et al.*[10]	The most complete updated guideline on all aspects of NIPAT but primarily aimed at the US health-care system
Hospital-in-the home treatment of infectious diseases	Australia	2002	Howden & Grayson[8]	Good practice statement written by two Australian experts; provides good practical advice. Emphasis on home therapy and infusion options
Outpatient and home parenteral antibiotic therapy (OHPAT) in the UK: a consensus statement by a working party	UK expert panel	1998	Nathwani & Conlon[12]	Good practice statement with UK focus, written by multidisciplinary team of authors. Broad applicability
Advisory group on home-based and outpatient care (AdHOC): an international consensus statement on non-inpatient parenteral therapy.	International (non-US)	2000	Nathwani *et al.*[4]	Useful perspective on models of care and how to overcome clinical, logistic, fiscal and political barriers to setting up non-inpatient services globally
Managing skin and soft tissue infections: expert panel recommendations on key decision points	USA and UK	2003	Eron *et al.*[13]	Skin and soft tissue infection focus on management with particular emphasis on severity classification to determine site of care

NIPAT, non-inpatient parenteral antimicrobial therapy.

and provide a resource for good practice in a variety of clinical settings; they can also be adapted for different geographic regions and health-care systems. A good resource for updated guidance, presentation and outcomes research, as well as access to the OPAT handbook, is http://www.opat.com. The updated US guidelines[10] provide an excellent summary of reports that support the effectiveness of non-inpatient parenteral therapy for a variety of conditions. Many more such studies or reports can be added to this literature, particularly with newer antibiotics such as ertapenem, daptomycin and caspofungin.

MODELS FOR NON-INPATIENT INTRAVENOUS ANTIMICROBIAL THERAPY AND PATIENT SAFETY

The delivery of high-quality, safe home therapy is best achieved by a NIPAT team consisting of physicians, nurses and pharmacists who use clearly delineated treatment protocols.[6,10,12] Physicians should be experienced in the treatment of infection and have a good understanding of antimicrobial dynamics and pharmacokinetics to allow appropriate decisions regarding the selection and duration of therapy, as well as drug monitoring. For more complex, life-threatening infections such as endocarditis it is important to select patients carefully to minimize the risk of harm. Strict criteria[14] for defining the most appropriate patients are therefore evolving, thereby giving greater reassurance about safety to infection specialists, cardiologists, patients and their caregivers.

Since nursing staff usually administer therapy, they have regular contact with the patient and caregivers and are often the initial contact when problems arise. NIPAT pharmacists assist in the choice and mode of therapy, drug supply and the stability of the drug in the ambulatory setting.

This multidisciplinary team approach is essential to ensure safe and effective functioning of an NIPAT program. Key generic considerations that govern this include the following.

- Is parenteral therapy needed and, if so, is this decision based on medical need and appropriateness and not driven by bureaucratic or economic factors?
- Are appropriate resources available for delivering the service?
- Is the type of ambulatory setting safe, adequate and supported?
- Are the patient and caregivers willing to participate safely, effectively and reliably?
- Is there an effective communication system between all members of the team and the patient?
- Is there an understanding by the patient and caregivers of the risks and benefits of the treatment?
- Is there a clear, unequivocal, 24-hours-a-day mechanism of support for dealing with patient or caregiver queries and emergencies?
- Is there a care pathway where all key decisions around treatment and monitoring are documented confidentially and are easily accessible?
- Is there a quality assurance system[15] in place with specific program outcomes to evaluate the effectiveness and safety of the program?

Many NIPAT units have an infusion center (generally located within a hospital or clinic) where patients can be medically reviewed and receive directly observed therapy. NIPAT can be administered by a nurse visiting the patient at home, the patient receiving treatment in an infusion center or patients (or their relatives) self-administering therapy. Self-administration requires a well-motivated patient and caregiver who are capable of being educated regarding safe drug administration. It can be particularly useful for patients requiring prolonged or multi-dose therapy, or for those who require repeated courses of intravenous therapy (e.g. patients who have cystic fibrosis). In some countries (e.g. Italy) general practitioners often deliver therapy in the patient's home, particularly for lower respiratory tract infections.[16] The use of the intramuscular route is not uncommon in this country.

TECHNOLOGY USED IN NON-INPATIENT PARENTERAL ANTIMICROBIAL THERAPY

Recent advances in medical technology have allowed development of new venous access devices and drug delivery systems that have improved the safety of NIPAT.

Venous access devices

The optimal choice of vascular access is generally based on a number of factors, including the proposed treatment duration, the medication to be infused and the type of delivery system to be used.

Peripherally inserted central catheters

Peripherally inserted central catheters (PICCs) are a convenient form of intravenous access for NIPAT. They are made of flexible silicone, are introduced into the antecubital vein and advanced into the superior vena cava, and are easily held in position with an adhesive dressing. Advantages of PICCs include the fact that they can be inserted and removed in the outpatient setting, are very durable, can be kept patent with an infrequent saline flush and have a relatively low infection rate.[17] Because of their central positioning, they are suitable for administration of concentrated antibiotic solutions such as used in continuous-infusion dosing.

Peripheral intravenous cannulae

Peripheral intravenous cannulae are generally used for short-duration therapy, but to minimize the risk of phlebitis they should be changed every 2–3 days. Thus, nursing staff need to be skilled in cannula insertion and care.

Long-term central venous catheters

These catheters (e.g. Hickman's, Port-A-Cath) are occasionally used in patients who have few other options for intravenous access, or who require them for administration of parenteral nutrition or cancer chemotherapy. In-hospital admission and anesthesia are generally required for insertion; however, they have a low infection rate and provide effective access for patients who require prolonged or repeated intravenous therapy.

Drug delivery systems

Like the choice of intravenous access, the optimal NIPAT drug delivery system is influenced by the agent to be delivered and the proposed treatment duration.[8,18]

Direct push

Intravenous injection over 5–10 minutes is useful for antibiotics such as cephalosporins, penicillins and teicoplanin. Spring-loaded devices are available that can deliver an intravenous push using small (e.g. 10–20 ml) syringes.

Gravity

Drug administration by gravity is usually used for agents that require dilution in larger volume solutions (e.g. 100–1000 ml) before infusion, or where infusions require administration over an extended period of time (e.g. vancomycin, amphotericin B, ertapenem, daptomycin).

Controlled-rate infusion devices

A number of compact, battery-operated, computerized infusion pumps are available that can be programmed to deliver the antibiotic by either continuous infusion or intermittent bolus. They can be readily carried in a small bag around the waist or neck, and allow the patient to continue with normal activities while receiving therapy. These pumps are generally expensive to purchase but are reusable and most models will alarm if the intravenous line becomes blocked or develops in-line air bubbles. Nonprogrammable continuous-infusion devices are also available that are either spring loaded or elastomeric – in these the tension in either the spring or the elastomeric 'bladder' propels the infusion. Although these pumps are cheaper, they are generally not reusable and will not alarm if the infusion is interrupted. Both devices are ideal for the continuous infusion of antimicrobials that are stable in solution over a 24-hour period and that have optimal activity when stable high serum concentrations are maintained (e.g. antistaphylococcal penicillins).[19–22]

INTRAVENOUS ANTIBIOTIC REGIMENS

Ideally, the principles used for appropriate antibiotic prescribing should be similar to those applied to patients managed in hospital. Appropriate antimicrobial agent(s) include those with the narrowest antibacterial spectrum appropriate for the responsible pathogen, most practical dosing regimen and lowest purchase and delivery costs. Patient-specific factors are also important, such as avoiding aminoglycosides in patients who have significant renal impairment or β-lactams in penicillin hypersensitive patients. However, in the non-inpatient setting it is important to consider a number of pragmatic considerations which may determine the ultimate antimicrobial choice. Although most antibiotics, like the semisynthetic penicillins, can be administered three or four times a day or through continuous infusions by using controlled-infusion devices, long-acting agents such as ceftriaxone or the glycopeptides make once daily or less frequent (e.g. teicoplanin) dosing possible, and are often favored as they are convenient and reduce overall resource use such as the need for high technology infusion devices. Such agents are also preferred as they do not require an infusion but rather can be given easily by a bolus (direct push).

Other important considerations include using agents that have a proven track record of safety, stability and effectiveness in the ambulatory setting. While many currently used antimicrobials in the ambulatory setting often have an antimicrobial spectrum that is broader than necessary for many indications, there is presently no evidence that such agents (e.g. cephalosporins) are promoting antimicrobial resistance, increasing *Clostridium difficile* diarrhea or are less effective than their narrow-spectrum counterparts.[16,23] In many countries where the non-inpatient infrastructure is less well developed or resources are scarce, drugs that do not require technologic support, infusion pumps or close therapeutic monitoring, and can be given simply, are deemed attractive. Indeed, the cost-effectiveness of NIPAT is an important consideration for drug choice in many health-care facilities.[24]

A core group of antimicrobials have been used in the NIPAT setting, with more antibiotics being added to the core list with broadening worldwide experience and the emerging clinical application of new agents. These are summarized in Table 133.3. The antibiotic groups below summarize key features of some of the agents in used in NIPAT.

β-Lactams

The clinical efficacy of β-lactams against many pathogens is related to the proportion of the dosing interval during which the serum drug concentrations are maintained above the minimum inhibitory concentration (MIC) of the infecting pathogen(s).[19] Thus, β-lactams with a short half-life (e.g. penicillin, ampicillin, antistaphylococcal penicillins) should either be dosed frequently (e.g. q4–6h), which is generally impractical for NIPAT, or administered by continuous infusion. There is increasing experience with the successful use of continuous-infusion antistaphylococcal penicillins (e.g. oxacillin, flucloxacillin) and some cephalosporins (e.g. ceftazidime) for the treatment of a range of conditions, including osteomyelitis, endocarditis and pneumonia.[19,20,21,25] Limiting factors with continuous-infusion administration include the availability and cost of accurate drug delivery devices and the instability in solution of some agents (e.g. ampicillin) after compounding.

Cephalosporins such as ceftriaxone, which allow for once-daily dosing, can be extremely useful for NIPAT.[23] Similarly, recent studies in which oral probenecid used to prolong the half-life of

Table 133.3 Parenteral antibiotics commonly used in the non-inpatient setting

Antibiotic	$t_{½}$ (h)	Stability (90% at 20°C)	Usual dosing	Monitor	Phlebitis risk* and main side-effects	Main clinical indications	Comment
Aminoglycosides	2–3	30 days	q24h	Renal levels	1; Nephrotoxicity, vestibular toxicity	Cystic fibrosis, pyelonephritis; endocarditis (q8–12h)	Once daily; increasingly used except for endocarditis
Amphotericin B (deoxycholate)	24–360	5 days	Three times weekly	Renal, electrolytes	3; Nausea, chills, nephrotoxicity	Invasive fungal infections	Still commonly used but superseded by lipid-based amphotericin or caspofungin
Caspofungin	>48	1 day	q24h (higher first loading dose)	Renal, electrolytes	1; Mainly GI, sometimes electrolyte imbalance	Invasive fungal infections, *Candida* spp. endocarditis	Limited experience in NIPAT
Cefazolin	1–2	1 day	q8–12h q24h with probenecid	WBC	1; Similar to ceftriaxone	SSTIs, osteomyelitis, pyelonephritis	Narrower spectrum than ceftriaxone Longer half-life/less frequent dosing when added to probenecid
Ceftriaxone	5–11	3 days	q24h	WBC	1; Mainly GI Monitor for *Clostridium difficile* diarrhea	SSTIs, osteomyelitis, Lyme disease, pyelonephritis, meningitis, pneumonia, endocarditis	Extensive experience of safety and effectiveness in non-inpatient setting
Ertapenem	4	6 hours	q24h	WBC	2; Mainly GI Monitor for *C. difficile* diarrhea	SSTIs, intra-abdominal infections, diabetic foot ulcer infections, some complicated UTIs	Expanding experience for a variety of infections in the non-inpatient setting
Ganciclovir	3	5 days	q24h (maintenance therapy)	WBC	1; Mainly GI Marrow toxicity (e.g. neutropenia)	CMV invasive infections, e.g. retinitis	Less used due to the availability of oral valganciclovir
Teicoplanin	40	48 hours	q24h iv, or higher dose two to three times a week parenterally	Renal, WBC	1; Fevers, anemia and rash	SSTIs, osteomyelitis, bacteremia, endocarditis	Extensive experience in bacteremia, endocarditis, non-inpatient setting outside USA
Vancomycin	6	7 days	q12–24h	Renal, WBC	2; Nephrotoxicity, rash	SSTIs, bacteremia, osteomyelitis, endocarditis	Extensive experience in treating Gram-positive infections in the non-inpatient setting
Cidofovir	40	24 hours	Once weekly (induction); once every 2 weeks (maintenance)	Renal, WBC	1; Nephrotoxicity, neutropenia, fever	CMV retinitis	Limited experience but alternative to ganciclovir in the non-inpatient setting, infrequent dosing
Daptomycin	38–48	2 hours	q24h	Renal, WBC, weekly CPK levels	2; GI, myopathy	SSTIs, right-sided endocarditis, bacteremia	Emerging agent, limited experience in non-inpatient setting Off label use in bone and joint infections
Dalbavancin (not yet licensed for clinical use)	6–10 days	Not known	Two doses, 1 week apart	WBC	1; GI, fever, endocarditis	SSTIs, catheter-related bacteremia	New agent, very limited experience of use in non-inpatient setting High potential for use, e.g. in emergency departments

Phlebitis risk: 1 = mild; 2 = moderate; 3 = severe.
CMV, cytomegalovirus; CPK, creatinine phosphokinase; GI, gastrointestinal; NIPAT, non-inpatient parenteral antimicrobial therapy; SSTIs, skin and soft tissue infections; UTIs, urinary tract infections WBC, white blood cell count.

the first-generation cephalosporin cefazolin have established that this combination given once daily is effective in the treatment of conditions such as cellulitis.[24]

More recently, ertapenem, a long-acting carbapenem that can be given once daily by infusion, has been used to treat skin and soft tissue infections, intra-abdominal and pelvic infections and diabetic foot ulcer infections caused by polymicrobial infections or extended-spectrum β-lactamase (ESBL)-producing coliforms.[26,27]

Aminoglycosides

A number of clinical studies suggest that administration of aminoglycosides (e.g. 4–5 mg/kg/day or fixed 7 mg/kg/day gentamicin) as a once-daily dose rather than as two or three divided doses is associated with similar efficacy and probably reduced toxicity as compared with multidosing regimens when treating Gram-negative infections.[28,29] Once-daily gentamicin is preferred when treating infections such as pyelonephritis, cholangitis and moderate–severe Gram-negative pneumonia. However, they remain unpopular with elderly patients,[7] and data regarding their efficacy – including settings such as pregnancy, neonates, burns patients, cystic fibrosis and some cases of endocarditis – are limited or lacking.

Glycopeptides

Glycopeptides (e.g. vancomycin, teicoplanin) are effective against many Gram-positive pathogens. Vancomycin generally needs to be administered twice daily over at least 1–2 hours, while teicoplanin, after initial loading, may be given rapidly once daily or in some cases less frequently.

Vancomycin can often be given once daily in elderly patients whose renal function has declined with age. Although glycopeptides have been used in NIPAT, because of their infrequent dosing requirements they are usually only an appropriate choice when used to treat resistant bacteria such as methicillin-resistant *Staphylococcus aureus* (MRSA), or in patients who are allergic to β-lactams. The efficacy of teicoplanin in some situations has been questioned although the published experience of this agent in the non-inpatient setting is highly effective.[30]

The emergence of resistant pathogens such as vancomycin-resistant enterococci reinforces the view that glycopeptides should only be used when clearly indicated although in our experience this resistance is primarily seen in critical care facilities in hospitalized patients rather than the ambulatory setting and the exact role of glycopeptide use is as yet unclear.[31]

Dalbavancin, an investigational, long-acting glycopeptide analogue that can be administered in two single doses a week apart for SSTIs, appears to exhibit many ideal properties for a NIPAT agent.[32,33] However, its cost-effectiveness, potential for promoting resistance due to long-acting, subinhibitory tissue concentrations and the possibility of protracted adverse reactions due to the very long half-life remain concerns. Other long-acting, investigational glycopeptides such as oritavancin and telavancin may be potential candidates for use in the ambulatory setting.

More recently daptomycin, a new once-daily cyclic lipopeptide, has been used to treat complicated SSTIs.[34] and bacteremia due to endocarditis. It requires monitoring with weekly creatine phosphokinase to predict rare myopathy. Its potential for use as an alternative to the glycopeptides in the ambulatory setting for SSTIs, right-sided endocarditis[35] and other infections (e.g. bone and joint infections) is likely to expand.

Antiviral agents

Ganciclovir is effective for both acute treatment and long-term suppression of serious cytomegalovirus disease and is administered once or twice daily. Such regimens are suitable for NIPAT, although the availability of oral ganciclovir and the highly bioavailable valganciclovir have reduced the need for long-term intravenous suppressive therapy and may avoid the need for intravenous induction therapy in some patients.[36] Other intravenous antiviral agents (e.g. foscarnet and cidofovir) may occasionally be used in NIPAT.

Antifungal therapy

While parenteral amphotericin B deoxycholate has been the main agent used to treat serious fungal infections in the non-inpatient setting,[37] concerns about nephrotoxicity remain. Once daily caspofungin is increasingly used in the treatment of serious invasive candidal infections such as fungemia and endocarditis.[38]

INDICATIONS FOR HOME INTRAVENOUS ANTIMICROBIAL THERAPY

A wide variety of infections can be safely and conveniently treated with NIPAT, should such treatment be appropriate. Commonly used antibiotics and their likely indications are outlined in Table 133.3 although many of these patients may be treated effectively with oral antibiotics with high bioavailability where indicated. Infections that are relatively common and generally require only brief NIPAT include skin and soft tissue infections, pyelonephritis, pneumonia, bacterial meningitis, infective exacerbations associated with chronic lung disease and, increasingly, line-related bacteremia and late-stage Lyme disease. Such patients do not generally need long-term venous access and ideally can be treated with agents that require only infrequent dosing (e.g. once or twice daily). Serious diseases that are often suitable for NIPAT include endocarditis, osteomyelitis, septic arthritis, prosthetic device infections, deep-seated diabetic foot infections, deep abscesses (e.g. brain, psoas, liver – generally after initial drainage), invasive fungal and cytomegalovirus disease in transplant recipients and HIV-infected patients. Since these conditions usually need prolonged intravenous antibiotic therapy, long-term venous access (e.g. PICC) is often required and innovative treatment regimens (e.g. continuous-infusion agents or twice or thrice weekly agents) may be appropriate, depending on the responsible pathogen(s). Although these conditions are not common, they are often large consumers of in-hospital bed days; hence, NIPAT may substantially improve in-hospital bed utilization by treating this relatively small number of patients.

Patient selection

Appropriate patient selection is a crucial component in ensuring a safe and successful NIPAT program. Both patient-specific and disease-specific factors are important in the decision to accept a patient for NIPAT. In particular, special care should be taken when assessing elderly or isolated patients for NIPAT, who often do not cope well with medical illness, and patients who have serious diseases such as endocarditis, where potentially catastrophic complications can occur.

Patient-specific factors

Factors that may be potential contraindications to NIPAT include:

- patients who live alone or in isolated areas, or who do not have a telephone or other means of rapid communication;
- active substance abuse;
- aggressive patients, relatives or pets (these generally argue against patient suitability for NIPAT care, since the safety of members of the NIPAT team is crucial); and
- the presence of a language barrier between patient and staff that cannot be overcome with the assistance of interpreters or family members (this suggests that safety at home cannot be assured and that in-hospital therapy may therefore be more appropriate).

Disease-specific factors

A clearly defined diagnosis is important before embarking on NIPAT. Patients who have common, less serious conditions such as cellulitis

can often be transferred directly from the emergency department or general practitioner to the NIPAT program, avoiding in-hospital admission. Patients who have more serious conditions such as endocarditis, osteomyelitis and meningitis generally require a period of inpatient assessment, treatment and stabilization before transfer to a NIPAT program to complete their treatment course.

Disease-specific indications

Specific common and emerging infections that can be treated with NIPAT are discussed in more detail below.

Skin and soft tissue infections

Bacterial skin and soft tissue infections collectively refer to a variety of microbial invasions of the skin layers (epidermis, dermis and subcutaneous tissues), inducing a host response. Infections may present either as cutaneous abscesses with a collection of pus surrounded by an area of erythema and swelling or as diffuse, spreading infections as in cellulitis or erysipelas. Bacterial skin and soft tissue infections occur frequently and range in severity from superficial infections of mild to moderate severity to deeper, occasionally necrotizing, infections. Although SSTIs represent a common indication for antibiotic therapy, an etiologic diagnosis is often difficult and most patients are treated empirically. As the description implies, complicated SSTIs are usually more severe, progress rapidly, involve deeper tissues and possess a greater risk of limb loss than uncomplicated SSTIs. Complicated cellulitis, complex abscesses, post-traumatic or surgical site infections, and infected diabetic and ischemic ulcers are commonly encountered examples. Hospitalization along with surgical debridement or drainage, parenteral antibiotic therapy, and management of co-morbid conditions such as peripheral vascular disease or diabetes mellitus is usually necessary.

Many SSTIs may be amenable to NIPAT and the key decision issues in these cases are around the need for hospitalization and supportive therapy and the most appropriate antibiotic therapy. The Eron classification for SSTIs based on severity assessment and the presence of stable co-morbidities provides a useful practical guide to determining the most appropriate site of care and antibiotic choice.[39]

Numerous US studies have demonstrated the efficacy of once-daily ceftriaxone 1–2 g for cellulitis, although the appropriateness of this relatively broad-spectrum agent for this indication has been questioned.[10] The first-generation cephalosporin, cefazolin, when given in a dose of 2 g twice daily,[40] or 2 g once daily together with oral probenecid 1 g once daily, is effective.[41] Both regimens have comparable clinical efficacy to that of once-daily ceftriaxone and represent practical, appropriate NIPAT options. Subsequent switching to oral agents such as dicloxacillin (500 mg q6h), cephalexin (500 mg q6h) or clindamycin (300 mg q6h) after initial improvement usually results in cure.

Pyelonephritis and complicated urinary tract infections

Gentamicin (4–7 mg/kg intravenously q24h), ceftriaxone (1 g intravenously q24h) or ciprofloxacin (500–750 mg orally q12h) are appropriate empiric single agents for pyelonephritis, since the usual pathogens are often Gram-negative bacilli. Ampicillin or penicillin may also be given empirically to treat possible enterococcal infections, although this is an uncommon pathogen in young patients. Antibiotic selection should be reviewed once the results of urine and blood cultures are available.

Studies have demonstrated that oral fluoroquinolones are highly effective in treating pyelonephritis, and they may be considered as an alternative to parenteral therapy for patients in whom adherence is assured.[42] Although ciprofloxacin would not usually be the first-line choice for in-hospital care of pyelonephritis, the fact that its use may avoid the need for intravenous access offers a significant practical advantage.

Recently, outbreaks of multiresistant ESBL-producing *Escherichia coli* infections, or sometimes *Klebsiella* spp., have caused urinary tract infections in the community.[43] Many of these patients have been effectively treated with parenteral once daily ertapenem as resistance to aminoglycosides and quinolones has increased.

Community-acquired pneumonia

Ceftriaxone 1–2 g once daily is widely used for the home intravenous treatment of pneumonia. However, in many regions ceftriaxone would not be the drug of first choice for in-hospital care of community-acquired pneumonia and the drug's broad spectrum of activity may be unnecessary. Furthermore, the availability of highly effective oral therapy, the need for oxygen support and close monitoring in patients who are deemed of need for parenteral drugs makes this a less attractive therapeutic indication for NIPAT.

Endocarditis

Increasingly, a large and significant number of patients with endocarditis are treated in the non-inpatient setting and account for a higher proportion of treatment days.[44] Indeed, the ambulatory intravenous therapy option is now included in some key guidelines for the treatment of endocarditis.[45] Although endocarditis can be successfully treated with NIPAT it can pose problems because of the risk of life-threatening complications and for this reason strict selection criteria for patients who have endocarditis have been proposed.[14,45] They suggest that most patients who have endocarditis should generally be managed in hospital for the initial 2 weeks. One exception may be patients with uncomplicated viridans streptococcal endocarditis who rapidly become afebrile and clear their bacteremia; such patients may be suitable for transfer home after 1 week. Patients who have complicated endocarditis (heart failure, conduction abnormality, perivalvular abscess), aortic valve disease, prosthetic valve endocarditis, acute endocarditis or infection caused by virulent organisms such as *Staphylococcus aureus* should generally be managed primarily as inpatients.

Ceftriaxone (2 g/day) has been most commonly used for the home treatment of uncomplicated viridans streptococcal endocarditis; it appears to be effective when given either alone for 4 weeks or together with an aminoglycoside for 2 weeks.[14,44,45] Monitoring of renal and auditory function is particularly important if aminoglycosides are being used for prolonged periods. There are also infrequent case reports of treatment with intermittent-dose (and occasionally continuous-infusion) penicillin via a computerized pump for viridans streptococcal endocarditis in the NIPAT setting.

There are also a number of reports of successful NIPAT for staphylococcal endocarditis. Treatment options include the use of antistaphylococcal penicillins given by computerized pump as a continuous infusion or by intermittent bolus.[20,21,25] Vancomycin may be used in β-lactam-allergic patients but appears to be less effective than β-lactams for susceptible staphylococcal strains.[46]

Daptomycin is another potential option for methicillin-resistant or methicillin-susceptible *S. aureus* bacteremia secondary to right-sided endocarditis.[35] There are very limited NIPAT data for endocarditis caused by other organisms such as enterococci, HACEK organisms and fungi. Outpatient parenteral antimicrobial therapy for endocarditis generally requires central venous access (e.g. PICC) and close weekly clinical and drug monitoring.

Osteomyelitis

Most forms of osteomyelitis require 4–6 weeks of parenteral antimicrobial therapy, although the treatment choice, duration and need for surgery may differ depending on the responsible pathogen(s), host factors and the bone involved. *Staphylococcus aureus* is the most common cause of osteomyelitis, but other pathogens such as coagulase-negative staphylococci, *Pseudomonas* spp. and Enterobacteriaceae may be involved when osteomyelitis is nosocomial in origin or associated with foreign bodies or intravenous drug abuse.[47] Thus, various treatment options may need to be considered.[48] Long-term intravenous

access is generally required unless oral fluoroquinolones are considered appropriate.[47–49]

Prosthetic device infections

Prosthetic orthopedic infections and other device-related (e.g. endovascular) infections, primarily due to *S. aureus* or coagulase-negative staphylococci, continue to increase and often require prolonged antibiotic therapy. They represent a significant clinical and health-care resource challenge.[50] Oral options are often limited; oral linezolid is less desirable because of potential hematologic, metabolic and neurologic side-effects with protracted therapy. Therefore, glycopeptides or lipopeptides are attractive options, usually in combination with oral rifampin (rifampicin). These infections represent a major future opportunity for NIPAT.

MONITORING PATIENTS RECEIVING NON-INPATIENT PARENTERAL ANTIMICROBIAL THERAPY

Patients require careful monitoring while receiving both NIPAT and oral therapy. Although the home environment has many advantages for the patient, the careful regular monitoring that generally occurs in hospital is not present at home. In general, patients should be reviewed by the NIPAT physician at least once a week, usually in the outpatient department or office. Specific factors to assess on review include:

- patient's reaction to NIPAT;
- response to therapy;
- drug side-effects; and
- other complications (e.g. venous cannula infection).

Routine hematology and biochemistry, as well as serum drug levels, are also often monitored weekly.

Since in some situations more frequent reviews, or even emergency assessments, may be necessary, all good NIPAT programs should have a system to manage these.

SUMMARY

Given recent trends worldwide, an increasing proportion of medical care that previously would have been administered in hospital is likely to be delivered at home or in other ambulatory care settings. Patient selection should be based primarily on medical suitability rather than economic considerations. However, ensuring appropriate antibiotic selection and providing safe delivery systems and continuity of care by a multidisciplinary team are key challenges. The importance of evaluating key patient and program outcomes cannot be overemphasized if NIPAT programs are to remain a safe, effective and high quality standard of care.

REFERENCES


References for this chapter can be found online at http://www.expertconsult.com

Chapter 134

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β-Lactam antibiotics

INTRODUCTION

Although Alexander Fleming observed that a *Penicillium* mold inhibited the growth of bacteria in culture in 1928, it was not until 1941 that Florey, Chain and Abraham used penicillin for the first time to treat patients with staphylococcal and streptococcal infections.[1] More than 60 years later, the β-lactam antibiotics remain the mainstay of treatment for a variety of bacterial infections (Table 134.1) and now include:

- penicillins;
- cephalosporins;
- monobactams;
- carbapenems; and
- β-lactamase inhibitor combinations.

Table 134.1 Clinical use of β-lactam antibiotics by site of infection

Infection site	β-Lactam used
Skin/soft tissue infections	Cephalosporins, penicillins, carbapenem (ertapenem)
Head and neck infections	
Dental infections	Penicillins
Pharyngitis	Cephalosporins, penicillins
Sinusitis	Penicillins, cephalosporins
Meningitis	Cephalosporins (third- and fourth-generation agents), carbapenem (meropenem)
Lower respiratory tract infections	Penicillins, cephalosporins, carbapenems (especially for hospital-acquired infections)
Urinary tract infections	Penicillins, cephalosporins, monobactam, carbapenems (especially for infections due to multidrug-resistant Gram-negative bacilli)
Intra-abdominal infections	Cephalosporins (in combination with agent anaerobic activity), carbapenems, ureidopenicillin with β-lactamase inhibitor
Bone and joint infections	Penicillins, cephalosporins

PENICILLINS

The natural penicillins such as penicillin G are used primarily for the treatment of selected Gram-positive as well as selected Gram-negative infections. The penicillinase-resistant penicillins, including nafcillin and oxacillin, had been used for the treatment of infections due to staphylococci prior to the emergence of widespread resistance among staphylococci due to the acquisition of low-affinity penicillin-binding proteins. These agents are active against other Gram-positive organisms and continue to remain agents of choice in treating methicillin-susceptible staphylococci. The aminopenicillins such as ampicillin and amoxicillin have a similar spectrum of activity as the natural penicillins, but have additional activity against Gram-negative organisms including many Enterobacteriaceae. When used together with β-lactamase inhibitors, they have good activity against Gram-positive, Gram-negative and anaerobic organisms that produce β-lactamases, enzymes which can hydrolyze these agents. The carboxypenicillins (ticarcillin) and ureidopenicillins (piperacillin) have activity against aminopenicillin-resistant Gram-negative bacilli, especially *Pseudomonas aeruginosa*, and can also be used in conjunction with β-lactamase inhibitors for extended activity against β-lactamase-producing organisms.

Cephalosporins

Cephalosporins are frequently designated as belonging to a generation, first through fourth, to suggest a general spectrum of activity of the agents. In this categorization, first-generation cephalosporins had activity against Gram-positive cocci, but had limited activity against Gram-negative pathogens. The second-generation cephalosporins had improved Gram-negative activity compared with that of the first-generation cephalosporins. Selected second-generation cephalosporins (i.e. cefoxitin and cefotetan) also had activity against anaerobes. Third-generation cephalosporins had further improved Gram-negative activity, but their activity against Gram-positive bacteria was variable (e.g. cefotaxime, ceftazidime and ceftriaxone). Cefepime (primarily USA) and cefpirome (Europe) are known as fourth-generation cephalosporins with demonstrated efficacy against most clinically important Gram-positive and Gram-negative bacteria.

Another useful categorization of cephalosporins is based on chemical structure[2,3] and could become more widely accepted with the emergence of new agents having a unique microbiologic spectrum, such as ceftobiprole and ceftaroline with anti-methicillin-resistant *Staphylococcus aureus* (MRSA) activity. As cephalosporin resistance increases due to extended spectrum β-lactamases and carbapenemases, the antimicrobial spectrum of agents categorized within these generations will need to be redefined, especially for Gram-negative bacteria.

Monobactams

Monobactams, with aztreonam as the only commercially available agent, are effective only against aerobic Gram-negative organisms and have no activity against Gram-positive organisms or anaerobes.

Carbapenems

The carbapenems (imipenem, meropenem, ertapenem and doripenem) have the broadest bacterial coverage of the β-lactam antibiotics. These agents have been used to treat patients with infections caused by Gram-positive, Gram-negative and anaerobic bacteria.

Mechanism of action

β-Lactam antibiotics inhibit bacterial cell wall synthesis, a bactericidal mechanism of action. These agents bind tightly to proteins known as penicillin-binding proteins (PBPs) on the inner surface of the bacterial cell membrane, thereby interrupting the terminal transpeptidation process in bacterial cell wall biosynthesis. Ultimately, loss of viability and, in some bacteria, lysis, occurs as the result of the activation of autolytic enzymes.

Bacterial resistance

Four major mechanisms lead to bacterial resistance to β-lactam antibiotics:

- failure of the antibiotic to penetrate the bacterial cell membrane;
- efflux of the antibiotic from the periplasmic space by specific pumping mechanisms;
- alterations in PBPs that reduce the binding affinities of the β-lactams (intrinsic resistance);[4] and
- bacterial production of β-lactamases, which hydrolyze the β-lactam ring and render the antibiotics microbiologically inactive. This is the most important and most common cause of resistance, especially in Gram-negative bacteria.[5]

Strategies to combat resistance, including the use of β-lactamase inhibitors, are discussed later in this chapter.

PHARMACOKINETICS AND DISTRIBUTION

Absorption

The β-lactams have variable absorption from the gastrointestinal tract. Some agents, such as the antipseudomonal penicillins and methicillin, are acid-labile and cannot be taken orally. The absorption characteristics and pharmacokinetics of the β-lactams are shown in Table 134.2. Of note, amoxicillin is almost totally absorbed when administered orally whereas ampicillin is only partially absorbed. The presence of food in the stomach can delay absorption and can lower the peak serum concentration for some β-lactams, such as ampicillin, cefaclor, cephalexin and ceftibuten, but can increase the absorption of cefuroxime and cefpodoxime.

Distribution

Following absorption, β-lactams are variably and reversibly bound to serum proteins, mostly albumin. Protein-bound drug does not exert antimicrobial activity. Excretion of the β-lactams is primarily renal (glomerular filtration and tubular secretion) and, in general, the serum half-life of these drugs is short, often 1 hour or less.

Table 134.2 Pharmacokinetics of selected β-lactam antibiotics

Generic name	Oral absorption (%)	Effect of food on absorption	Protein binding (%)	Serum half-life (h)	Biliary excretion (% of dose)
Penicillins					
Amoxicillin–clavulanate	75/–	Minimal/increases	20/30	1.3/1.0	2–3/<1
Ampicillin–sulbactam	40/–	Decreases/–	28/38	1.1/1.0	3/0.24
Azlocillin	NA		30	0.8–1.5	5.3
Bacampicillin	87–95	None	28	1.1	0.1
Carbenicillin (Indanyl)	30–40		50–60	1.0	Minimal
Cloxacillin	50	Decreases	90–98	0.5	2–10
Dicloxacillin	35–76	Decreases	95–97	0.7	Some penetration into bile
Methicillin	NA		17–45	0.5–1.0	2–3
Mezlocillin	NA		16–42	1.1	2.5
Nafcillin	10–20	Decreases	90	0.5	8
Oxacillin	30	Decreases	94	0.5	2–10
Penicillin G	15	Decreases	65	0.5	5
Penicillin V	60	Lowers and delays peak	80	0.5	
Piperacillin–tazobactam	NA		26–33/31–32	1.0/0.7–0.9	20
Ticarcillin–clavulanate	NA		45/30	1.2/1.0	4/<1

Table 134.2 Pharmacokinetics of selected β-lactam antibiotics—cont'd

Generic name	Oral absorption (%)	Effect of food on absorption	Protein binding (%)	Serum half-life (h)	Biliary excretion (% of dose)
Cephalosporins					
Cefaclor	52–95	Lowers and delays peak	22–25	0.6–0.9	Some penetration into bile
Cefadroxil	90	None	20	1.5	High conc. in bile
Cefazolin	NA		73–87	1.4–2.0	0.03
Cefditoren (pivoxil)	14–16	Increased with fatty meal	88	1.6	Some penetration into bile
Cefdinir	16–25	Reduced after a fatty meal	60–70	1.7	
Cefepime	NA		20	2.0	Some penetration into bile
Cefixime	40–50		65	3–4	5
Cefoperazone	NA		82–93	1.9	20–30
Cefotaxime	NA		30–51	1.0–1.5	0.1
Cefotetan	NA		78–91	3–4.6	13
Cefoxitin	NA		65–79	0.7–1.1	High conc. in bile
Cefpodoxime (proxetil)	46–50	Increases	18–40	2.1–2.8	Some penetration into bile
Cefprozil	95	None	35–45	1.3–1.8	
Ceftazidime	NA		10–20	1.5–1.8	0.21
Ceftibuten	>90	Lowers peak	65–77	2–2.4	
Ceftizoxime	NA		30	1.3–1.9	0.2–7.8
Ceftobiprole (medocaril)	NA		16	3.0	<1%
Ceftriaxone	NA		85–95	5–11	10–65
Cefuroxime (axetil)	52	Increases	33–50	1.5	0.13
Cephalexin	90	Lowers and delays peak	5–15	0.7–1.1	0.29
Cephalothin	NA		65–80	0.5–1.0	High conc. in bile
Loracarbef	~90	Decreases	25	1.2	
Monobactam					
Aztreonam	NA		56	1.7–2.9	<1
Carbapenems					
Doripenem	NA		5–15	1.0	<1%
Ertapenem	NA		85–95	4	Some penetration into bile
Imipenem–cilastatin	NA		15–25	1.0	<0.3
Meropenem	NA		2	1.0–1.5	Some penetration into bile

Pharmacokinetic data as per references 6–32.
NA, not applicable because agent is administered by parenteral route only.
Absence of information is due to lack of published data.

Procaine penicillin G and benzathine penicillin G are intramuscular preparations that are absorbed slowly, allowing for longer dosing intervals. Nafcillin, the ureidopenicillins (20–30%), cefoperazone (20%), ceftriaxone (10–65%) and cefotetan (13%) have significant excretion in bile.[13]

Imipenem, a carbapenem, is inactivated by dehydropeptidase I, an enzyme present on the renal brush border and other tissues. Cilastatin, a dehydropeptidase inhibitor and nephroprotectant, is administered along with imipenem to prevent subtherapeutic levels of the antibiotic. Cilastatin is not microbiologically active nor does it alter the pharmacokinetics of other drugs.[33]

The β-lactam antibiotics achieve therapeutic concentrations in most tissues including lung, kidney, bone, muscle and liver, and in secretions such as synovial fluid, pleural fluid, pericardial fluid,

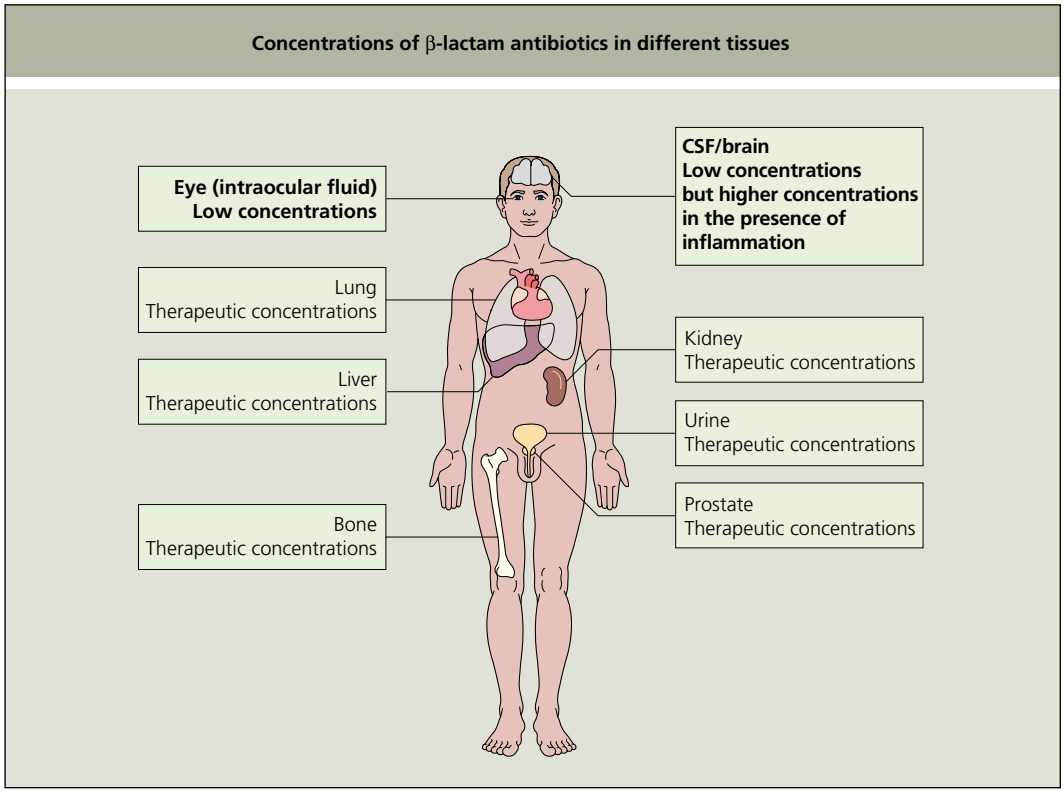


Fig. 134.1 Concentration of β-lactam antibiotics in different tissues.

peritoneal fluid and bile. The microenvironment that may be found in an abscess, including a low pH, the presence of neutrophils and associated proteins, and low oxygen tension, does not inhibit the function of β-lactam antibiotics. In general, β-lactams are considered to be unable to penetrate host cells and are therefore ineffective against intracellular organisms. However, these agents have been shown to be effective in infections due to *Listeria* and *Salmonella* that are considered to be successful intracellular pathogens. Low concentrations of β-lactams are found in prostatic secretions, brain tissue, intraocular fluid and cerebrospinal fluid (CSF; Fig. 134.1). In the presence of inflammation, however, concentrations in the CSF are much higher, accounting for the efficacy of some β-lactams in the treatment of meningitis.[34] The penicillins and cephalosporins can penetrate the aqueous humor of the eye, but do not reach therapeutic levels in the posterior chamber.

ROUTE OF ADMINISTRATION AND DOSAGE

β-Lactams are available for oral, intravenous and intramuscular use. Generic names, routes of administration and standard dosages for adult and pediatric patients with normal renal function are listed in Table 134.3. In dosing the β-lactam antibiotics, it is important to remember that:

- food can have an effect on oral absorption; and
- absorption of both cefuroxime and cefpodoxime are decreased by H_2 blockers or nonabsorbable antacids.[35]

Clinical experience has supported the general concept that high doses of β-lactam antibiotics should be used in patients who are neutropenic and for severe infections such as bacteremia and meningitis. Dosing of β-lactams is based on achieving plasma drug concentrations above the minimum inhibitory concentration (MIC) of the infecting bacteria for substantial periods of the dosing interval; this is the critical pharmacodynamic relationship to consider in choosing a dose regimen. Many of these agents have short half-lives after intravenous dosing and therefore could be more optimally dosed if infused over several hours.

INDICATIONS

The β-lactam antibiotics can be effectively used for the treatment of a variety of infections. These agents are widely distributed following administration and are routinely used in the treatment of sinusitis, otitis, pharyngitis, epiglottitis, dental infections, bronchitis, pneumonia, meningitis, infections of the genitourinary tract (including cervicitis and urethritis caused by *Neisseria gonorrhoeae*), peritonitis, biliary and gastrointestinal infections, skin and soft tissue infections, osteomyelitis, septic arthritis and infection of prosthetic devices, including venous access catheters. The choice of antibiotic and recommended duration of therapy for these infections is discussed in the chapters on the specific diseases. The remainder of this section focuses on the use of the β-lactam antibiotics in special circumstances. Table 134.4 summarizes the relative susceptibilities of various micro-organisms to the β-lactam antibiotics.

Prophylaxis

Antimicrobial prophylaxis in surgery (see also Chapter 132)

β-Lactam antibiotics are commonly used to decrease the incidence of infection for selected surgical procedures.[38] Prior to widespread methicillin-resistance in *Staph. aureus* infections acquired in the hospital setting, a single dose of cefazolin had been shown to decrease the incidence of wound infection for selected 'clean' procedures. This approach is used

Table 134.3 β-Lactam antibiotics – spectrum of activity, generic names, routes of administration and dosages used in patients with normal renal function

Agent	Antimicrobial spectrum	Generic name	Route	Adult dose (pediatric dose)
Penicillins				
Natural penicillins	Gram-positives, anaerobes and some Gram-negatives	Penicillin V	po	250–500 mg q6–12h (6.25–12.5 mg/kg q8h)
		Penicillin G (benzathine)	im	1.2 million U every 3–4 weeks (25 000–50 000 U/kg every 3–4 weeks; or if <27 kg, 300 000–600 000 U and if >27 kg, 900 000 U)
		Penicillin G (procaine)	im	300 000–600 000 U q12h (25 000–50 000 U/kg q24h or 12 500–25 000 U/kg q12h)
		Penicillin G, sodium or potassium	iv	1–4 million U q4–6h (6250–100 000 U/kg q6h or 4166.6–66 666 U/kg q4h)
Penicillinase-resistant penicillins	Penicillin-resistant, methicillin-susceptible staphylococci and some streptococci	Cloxacillin	po	250–500 mg q6h
		Dicloxacillin	po	125–500 mg q8h (3.125–6.25 mg/kg q6h)
		Methicillin	im/iv	1–2 g q6h
		Nafcillin	im/iv	1–2 g q4h (12.5–25 mg/kg q6h or 8.3–33.3 mg/kg q4h)
		Oxacillin	iv	1–2 g q4h (12.5–25 mg/kg q6h or 8.3–33.3 mg/kg q4h)
Aminopenicillins	Same as penicillin G plus β-lactamase-negative Gram-negative bacilli and some Enterobacteriaceae	Amoxicillin	po	250–500 mg q8h or 875 mg q12h (12.5–25 mg/kg q8h or 7–13 mg/kg q8h)
		Ampicillin	im/iv	500 mg–2 g q4–6h (25–50 mg/kg q6h)
			po	250–500 mg q6h
		Bacampicillin	po	400–800 mg q12h (12.5 mg/kg q12h)
with a β-lactamase inhibitor	Expanded activity against β-lactamase-producing bacteria	Amoxicillin–clavulanic acid	po	250–500 mg q8h or 875 mg q12h (if >40 kg, dose as adult)
		Ampicillin–sulbactam	im/iv	1.5–3 g q6h (25–50 mg/kg q6h)
Carboxypenicillins	Some ampicillin-resistant Gram-negatives including *Pseudomonas aeruginosa* (at highest dose regimens)	Ticarcillin	im/iv	3 g q4–6h (if <60 kg, 50 mg/kg q4–6h)
with a β-lactamase inhibitor	Expands activity against β-lactamase-producing bacteria	Ticarcillin–clavulanic acid	iv	3.1 g q4–6h (if <60 kg, 50 mg/kg [based on ticarcillin component] q4–6h)
Ureidopenicillins	Similar to carboxypenicillins	Mezlocillin	im/iv	3–4 g q4–6h (50 mg/kg q4h)
		Piperacillin	im/iv	3 g q4–6h (50–75 mg/kg q6h or 33.3–50 mg/kg q4h)
with a β-lacatamase inhibitor	Expands activity against β-lactamase-producing bacteria	Piperacillin–tazobactam	iv	3.375 g q4–6h (80 mg/kg q8h) 4.5 g q6h (for nosomomial pneumonia)
Cephalosporins				
Referred to as 'first generation'	Methicillin-susceptible staphylococci, streptococci and some Gram-negative bacilli	Cefadroxil	po	500 mg–1 g q12h (15 mg/kg q12h)
		Cefazolin	im/iv	1–2 g q8h (16.6–33.3 mg/kg q8h)
		Cephalexin	po	250–500 mg q6h (6.25–12.5 mg/kg q6h or 8.0–16 mg/kg q8h)
		Cephalothin	im/iv	500 mg–2 g q4–6h (25 mg/kg q6h or 16.6 mg/kg q4h)
Referred to as 'second generation'	Greater activity than earlier agents against Gram-negative bacilli. Some anaerobes for specific agents (especially cefotetan, cefoxitin)	Cefaclor	po	250–500 mg q8h (10–20 mg/kg q12h or 6.6–13.3 mg/kg q8h)
		Cefotetan	im/iv	1–3 g q12h (20–40 mg/kg q12h)
		Cefoxitin	im/iv	1 g q8h to 2 g q8h (27–33 mg/kg q8h)
		Cefprozil	po	250 mg q4h or 500 mg q12–24h (15 mg/kg q12h)

Table 134.3 β-Lactam antibiotics – spectrum of activity, generic names, routes of administration and dosages used in patients with normal renal function—cont'd

Agent	Antimicrobial spectrum	Generic name	Route	Adult dose (pediatric dose)
		Cefuroxime (axetil) Cefuroxime Loracarbef	po im/iv po	125–500 mg q12h (10–15 mg/kg q12h) 750 mg–1.5 g q6–8h (25–50 mg/kg q8h) 200–400 mg q12h (7.5–15 mg/kg q12h)
Referred to as 'third generation'	Many β-lactamase positive Gram-negatives, some *P. aeruginosa* (especially cefoperazone, ceftazidime)	Cefditoren (pivoxil) Cefdinir Cefixime Cefoperazone Cefotaxime Cefpodoxime (proxetil) Ceftazidime Ceftibuten Ceftizoxime Ceftriaxone	po po po im/iv im/iv po im/iv po im/iv im/iv	200–400 mg q12h 300 mg q12h or 600 mg q24h (7 mg/kg q12h or 14 mg/kg q24h) 200 mg q12h or 400 mg q24h (8 mg/kg q24h or 4 mg/kg q12h) 1 g q12h to 2 g q4h (25–100 mg/kg q12h) 1 g q12h to 2 g q4h (8.3–33.3 mg/kg q4h or 16.6–66.6 mg/kg q6h) 100–400 mg q12h (5 mg/kg q12h) 1 g q12h to 2 g q8h (25–50 mg/kg q8h) 400 mg q24h (9 mg/kg q24h) 500 mg q12h to 4 g q8h (50 mg/kg q6–8h) 1–2 g q24h (50–75 mg/kg q24h or 25–37.5 mg/kg q12h)
Referred to as 'fourth generation'	Similar to above Gram-positives including MRSA, and many β-lactamase-positive Gram-negatives, some *P. aeruginosa*	Cefepime Ceftobiprole (medocaril)	im/iv iv	1–2 g q8–12h (50 mg/kg q8–12h) 500 mg q8h
Monobactam				
	Gram-negatives	Aztreonam	im/iv	1–2 g q6–12h (30–40 mg/kg q6–8h)
Carbapenems				
	Gram-positives (except MRSA), many Gram-negatives (including ESBL-producers) and many *P. aeruginosa* (except ertapenem)	Doripenem Ertapenem Imipenem–cilastin Meropenem	iv im/iv im/iv iv	500 mg q8h 1 g q24h 500 mg–1 g q6h (15–25 mg/kg q6h) 1 g q8h (20–40 mg/kg q8h)

Dosing guidance as per references 9–12,18,19.

commonly for cardiac, noncardiac thoracic, vascular, orthopedic, ophthalmic and neurosurgical procedures. With the emergence of MRSA, concern has been raised about the effectiveness of using β-lactams with no activity against MRSA, especially in centers where MRSA prevalence is high.

For 'clean–contaminated' procedures in which colonized mucosa is violated, such as head and neck surgery, abdominal surgery and gynecologic surgery, antibiotic prophylaxis may also be used. Cefazolin can be used before head and neck surgery. Similarly, patients undergoing biliary tract surgery who are at high risk of infection due to advanced age, acute cholecystitis, a nonfunctioning gallbladder, obstructive jaundice, or choledocholithiasis may benefit from preoperative cefazolin. In the setting of acute appendicitis, cefoxitin or ampicillin–sulbactam has been recommended. Women undergoing vaginal or abdominal hysterectomy, emergency Caesarian section or first trimester abortion may be candidates for antibiotic prophylaxis with cefazolin or other agents.

Antibiotics should be used not only as prophylaxis but also as treatment for 'dirty' surgical procedures in which the surgical site is obviously contaminated by bacteria (e.g. a perforated viscus). Antimicrobial prophylaxis in surgery is summarized periodically in the publication *The Medical Letter*.[38]

Endocarditis prophylaxis

Patients who have underlying cardiac or congenital valvular abnormalities are candidates for antibiotic prophylaxis when they undergo procedures that can cause transient bacteremia. Cardiac conditions that place a patient at increased risk of endocarditis include prosthetic valves, a previous history of endocarditis, most congenital cardiac abnormalities (except an isolated secundum atrial septal defect), rheumatic and other acquired valvular dysfunction, hypertrophic cardiomyopathy and mitral valve prolapse when accompanied by regurgitation.

Procedures that can cause transient bacteremia and may place a patient at risk of endocarditis include:

- dental procedures (including professional cleaning);
- tonsillectomy and/or adenoidectomy;
- surgical procedures involving intestinal or respiratory mucosa;
- rigid bronchoscopy;
- sclerotherapy for esophageal varices;
- esophageal dilatation;
- gallbladder surgery;
- cystoscopy;

Table 134.4 Susceptibilities of selected bacteria to β-lactam antibiotics

Generic name	Streptococci*	Oxacillin-susceptible, penicillinase-producing *S. aureus*†	Enterococci	Enteric Gram-negative bacilli‡	*Pseudomonas aeruginosa*	Anaerobes
Penicillins						
Amoxicillin	++	0	++	0	0	0
Amoxicillin–clavulanate	++	++	++	++	0	++
Ampicillin	++	0	++	0	0	0
Ampicillin–sulbactam	++	++	++	++	0	++
Carbenicillin	+	0	0	0	+	0
Cloxacillin	+	++	0	0	0	0
Dicloxacillin	+	++	0	0	0	0
Methicillin	+	++	0	0	0	0
Mezlocillin	+	0	+	+	+	+
Nafcillin	+	++	0	0	0	0
Oxacillin	+	++	0	0	0	0
Penicillin	++	0	++	0	0	0
Piperacillin	++	0	+	+	++	+
Piperacillin–tazobactam	++	++	+	++	++	++
Ticarcillin	++	0	0	+	+	+
Ticarcillin–clavulanate	++	++	0	++	+	++
Cephalosporins						
Cefaclor	+	+	0	+	0	+
Cefadroxil	+	+	0	0	0	0
Cefazolin	++	++	0	0	0	0
Cefdinir	++	+	0	+	0	0
Cefditoren	++	+	0	+	0	0
Cefepime	++	+	0	++	++	0
Cefixime	++	0	0	++	0	0
Cefoperazone	++	+	0	+	+	+
Cefotaxime	++	+	0	++	0	0
Cefotetan	+	0	0	+	0	+
Cefoxitin	+	0	0	+	0	++
Cefpodoxime	++	++	0	+	0	0
Cefprozil	++	+	0	+	0	0
Ceftazidime	+	0	0	++	++	0
Ceftibuten	+	0	0	+	0	0
Ceftizoxime	++	+	0	+	0	+
Ceftobiprole	++	++[b]	++	++	+	0
Ceftriaxone	++	+	0	++	0	0
Cefuroxime	++	+	0	+	0	+
Cephalexin	++	+	0	+	0	+
Cephalothin	++	++	0	+	0	+
Loracarbef	+	+	0	+	0	+
Monobactam						
Aztreonam	0	0	0	++	+	0
Carbapenems						
Doripenem	++	++	+	++	++	++
Ertapenem	++	++	0	++	0	++
Imipenem	++	++	++	++	++	++
Meropenem	++	++	+	++	++	++

Interpretations: 0, not active, 90% of the minimum inhibitory concentrations (MICs) are greater than resistance breakpoint; +, intermediate activity, approximately 90% of the MICs are greater than susceptible, but less than the resistant breakpoint; ++, clinically useful activity, generally with 90% of the MICs within the susceptible range, using interpretive criteria as defined by the Clinical and Laboratory Standards Institute (CLSI) or the Food and Drug Administration (FDA). Susceptibility data based on references 17,36,37.

*Non-meningitis isolates.

†Methicillin-susceptible *Staphylococcus aureus* and *S. epidermidis*. All methicillin-resistant *S. aureus* and *S. epidermidis* are resistant to all β-lactams, with the exception of ceftobiprole.

‡Primarily *Escherichia coli, Klebsiella pneumoniae, Enterobacter cloacae, Enterobacter aerogenes* and *Proteus mirabilis*.

- urethral dilatation;
- urethral catheterization and/or urinary tract surgery if there is infection;
- prostatic surgery; and
- incision and drainage of infected tissue.

Recommendations for using β-lactams to prophylax patients at risk for infective endocarditis have recently been updated by an American College of Cardiology/American Heart Association Task Force on Practice Guidelines.[39] In this update, prophylaxis is not recommended for patients undergoing nondental procedures and is no longer recommended for many patients that had been considered candidates for prophylaxis in the past (specifically, patients with mitral valve prolapse and mitral regurgitation). For at-risk patients undergoing dental procedures, the antibiotic of choice has remained constant for over two decades and is a single dose of amoxicillin 2 g (50 mg/kg for children) taken orally 30–60 minutes before the procedure. Alternatives exist for patients who are allergic to penicillins.[39] Guidelines posted by the National Institute for Health and Clinical Excellence (NICE)[40] and published by the British Society for Antimicrobial Chemotherapy[41] further underscore that past practices of using antibiotic prophylaxis in patients at risk for infective endocarditis need to be reconsidered (see Chapter 132 for detailed discussion on antimicrobial prophylaxis).

Rheumatic fever prophylaxis

Because patients who have had acute rheumatic fever are at risk of recurrent attacks if they have group A streptococcal infections, the American Heart Association (AHA) recommends prophylaxis with penicillin for these patients. The dose is either a single injection of benzathine penicillin G 1.2 million U intramuscularly every 4 weeks or penicillin V 250 mg orally q12h. It seems that prophylaxis can be safely discontinued in patients with a history of carditis after 10 years or at age 25. In patients without a history of carditis, prophylaxis can be stopped after 5 years or at age 18. The decision to stop prophylaxis, however, must be individualized because a patient who is at continued risk of streptococcal infection (e.g. teacher or pediatrician) may benefit from continued antibiotic prophylaxis (see also Chapter 48).[42]

Intrapartum prophylaxis

Penicillin G is the agent of choice for use in intrapartum prophylaxis for early onset group B streptococcal disease in newborns. Ampicillin is an acceptable alternative agent. These agents should be given to women in labor as soon as risk for intrapartum transmission of group B streptococcus is identified. Initial intravenous doses (5 million U penicillin G or 2 g ampicillin) should be followed with half doses every 4 hours until the newborn is delivered.

Pneumococcal infections

Penicillin and amoxicillin are the antibiotics of choice for infections (such as community-acquired pneumonia, bacteremia or meningitis) caused by penicillin-susceptible strains of *Streptococcus pneumoniae*. However, an increasing proportion of isolates of this pathogen are not susceptible to penicillin. For meningitic isolates and epidemiologic purposes, an interpretation of intermediate is defined as a strain with a penicillin MIC of 0.1–1 μg/ml and resistance is defined by a penicillin MIC of ≥2 μg/ml. However, recent revisions to the interpretive criteria define penicillin MICs >2 μg/ml for non-meningitis-related *Strep. pneumoniae* isolates as nonsusceptible. Although only about 5% of clinical isolates in the USA were not susceptible to penicillin in the early 1990s,[43] a study of isolates collected from hospitalized patients throughout the USA in 2007 indicated that 25.4% were intermediate and 11.1% were resistant to penicillin.[44] Because of the emergence of resistance, some suggest that suspected cases of pneumococcal pneumonia and meningitis should be treated with vancomycin and/or a third-generation cephalosporin or meropenem until susceptibilities are known. There have been reports of failure of third-generation cephalosporins such as cefotaxime or ceftriaxone in the treatment of penicillin-resistant pneumococcal meningitis, again suggesting that vancomycin or meropenem should be included until susceptibilities are known.[45]

Staphylococcal infections

Soon after the introduction of penicillin for the treatment of staphylococcal infections, penicillinase-producing strains became so common that this agent was no longer effective. Penicillinase-resistant penicillins (nafcillin, oxacillin, methicillin, dicloxacillin, cloxacillin, flucloxacillin) are often agents of choice for methicillin-susceptible strains of *Staph. aureus*. Other β-lactam antibiotics that are effective in the treatment of susceptible staphylococcal infections are:

- the aminopenicillins in combination with a β-lactamase inhibitor (ampicillin–sulbactam or amoxicillin–clavulanate);
- the antipseudomonal penicillins in combination with a β-lactamase inhibitor (ticarcillin–clavulanate, piperacillin–tazobactam); and
- the carbapenems (doripenem, ertapenem, imipenem and meropenem).

The first-generation cephalosporins, which are as effective as the penicillinase-resistant penicillins in the treatment of staphylococcal infections, require less frequent dosing and may be used in patients with a history of mild penicillin allergy.

Isolates of *Staph. aureus* or *Staph. epidermidis* that are resistant to methicillin should be considered to be resistant to all other β-lactams,[46] with the exception of ceftobiprole.[36] Ceftobiprole has received regulatory approval in Canada and some European countries for treating patients with complicated skin and skin-structure infections, including those caused by MRSA.[47,48]

Gram-positive bacilli

Penicillin G is the treatment of choice for:

- infections (oral–cervicofacial, thoracic, abdominal) due to actinomycosis;
- elimination of the carrier state of diphtheria;
- infections (pulmonary, cutaneous, gastrointestinal) due to anthrax, except for β-lactamase-producing strains;
- gas gangrene caused by species of *Clostridium* spp.; and
- erysipeloid caused by *Erysipelothrix rhusiopathiae*.

Either penicillin G or ampicillin may be used for infections caused by *Listeria monocytogenes*. No currently available cephalosporin has useful activity against *L. monocytogenes*.

Infections caused by Gram-negative organisms including *Pseudomonas aeruginosa*

β-Lactam antibiotics with *in-vitro* activity against *P. aeruginosa* are ticarcillin, carbenicillin, piperacillin, ceftazidime, cefoperazone, cefepime, ceftobiprole, aztreonam, imipenem, meropenem and doripenem. Of these agents, the carbapenems doripenem, imipenem and meropenem have the most consistent activity against pseudomonads. Because resistance to carbapenems, most evident with imipenem, has emerged in many areas, it is important to be aware of *P. aeruginosa* resistance patterns for a particular health-care facility. During treatment of pseudomonal infections, resistance to all β-lactam agents used as sole therapy has been observed.[49] For this reason, a suitable β-lactam antibiotic is often used in conjunction with a second agent, such as a fluoroquinolone or an aminoglycoside.

The antipseudomonal penicillins piperacillin and ticarcillin are often used in conjunction with a β-lactamase inhibitor. However, the inhibitor does not confer improved activity against many β-lactam-resistant *Pseudomonas* spp., because the mechanism of resistance is typically not due to β-lactamase production.[50]

The development of drug-resistant isolates of *P. aeruginosa* and other Gram-negative bacilli has emerged as a clinically important challenge, especially in considering treatment of patients with nosocomial infections. These pathogens are commonly found in intensive care units where patients have been exposed to broad-spectrum antibiotics for prolonged periods of time. Many organisms produce chromosomal β-lactamases that are intrinsic to that species, including *Citrobacter* spp., *Enterobacter* spp., *Serratia* spp., *Proteus* spp., *Providencia* spp., *P. aeruginosa* and *Acinetobacter* spp. Many Gram-negative bacteria, especially *Escherichia coli* and *Klebsiella* spp., may acquire extra DNA (e.g. a plasmid) that encodes for other types of β-lactamase, such as extended-spectrum β-lactamases (ESBLs). These bacteria may develop resistance to most β-lactams during therapy if they acquire an ESBL or hyperproduce chromosomal β-lactamases.[51] Older recommendations of concurrent use of an aminoglycoside in conjunction with a cephalosporin for the treatment of infections caused by these bacteria to prevent therapeutic failures have been challenged due to the realization that cross-resistance in ESBL-producing bacteria can occur due to the co-acquisition of modifying enzymes for both antibiotic classes.[52]

Among β-lactams, carbapenems have the broadest spectrum of activity against Gram-negative organisms resistant to other antibiotics. Given the emergence of resistance, especially resistance due to ESBL-producing bacteria, carbapenems have been used more frequently as empiric therapy in seriously ill patients, especially those at risk for being infected with Gram-negative pathogens. Although imipenem resistance in *P. aeruginosa* has risen in some centers to 10–15%, some of these isolates continue to be susceptible to meropenem and doripenem, carbapenems with more potent *in-vitro* activity against most Gram-negative bacteria.[53] Resistance to carbapenems is increasing in some centers due to the production of acquired carbapenem-hydrolyzing β-lactamases, including enzymes that require metal ions for hydrolysis. The metallo-β-lactamases confer resistance to all β-lactams except aztreonam, in contrast to other carbapenemases that generally are associated with resistance to all β-lactams.[54]

Among the cephalosporins, cefepime is effective in the treatment of severe infections due to a broad spectrum of pathogens that involve the lower respiratory and urinary tracts, the skin and soft tissue, and the female reproductive tract. It has been shown to be more effective than ceftazidime in the treatment of pneumonia in patients with cystic fibrosis where *P. aeruginosa* is a common pathogen.[55] In addition to having activity against strains of *P. aeruginosa* resistant to ceftazidime, cefepime has also shown activity against many Enterobacteriaceae, including *Enterobacter* spp., that are resistant to other cephalosporins.[56] However, cefepime is inactivated by many ESBLs. Although a recent meta-analysis suggested that a subset of seriously ill patients had higher mortality rates after treatment with cefepime compared with alternative agents,[57] a 2009 FDA analysis did not show a statistically significant increase in mortality in cefepime-treated patients.

Anaerobic infections

Anaerobic bacteria may play a significant role in brain abscess, dental infection, sinusitis, lung abscess, intra-abdominal abscess and bone and soft tissue infection. Although β-lactam antibiotics have been used extensively in the treatment of anaerobic infections, there is a trend for increased resistance of anaerobes to some β-lactam antibiotics due to production of β-lactamases.[58]

Most *Clostridium* strains, with the exception of some strains of *C. ramosum*, *C. clostridiforme* and *C. innocuum*, remain susceptible to penicillin. Penicillin resistance is increasingly seen in the genus *Fusobacterium*, most commonly in *F. varium* and *F. mortiferum*, and although generally still sensitive to penicillin, the MICs for *F. nucleatum* have increased. Penicillin resistance is a major problem encountered in the treatment of infections caused by β-lactamase-producing *B. fragilis* and other *Bacteroides* spp.[59]

Penicillin is slightly more active than nafcillin against anaerobes, although neither is considered to be a potent anti-anaerobic agent. Ticarcillin, mezlocillin and piperacillin also have excellent activity against anaerobes, although there has been an increase in *B. fragilis* strains resistant to ticarcillin. Of the β-lactamase-stable cephalosporins, cefoxitin, cefotetan and ceftizoxime all show activity against anaerobes. Cefoxitin remains the most active cephalosporin against *B. fragilis*. Resistance to these cephalosporins is seen with some species of *Clostridium*, *Fusobacterium* and non-spore-forming Gram-positive rods. Cephalosporins such as ceftazidime and the first-generation agents cefazolin and cephalothin have poor activity against Gram-negative anaerobes whereas the broader spectrum cephalosporins cefotaxime, cefoperazone and ceftriaxone have modest activity (resistance seen in 30–60% of strains) and are therefore not the agents of choice for the empiric treatment of anaerobic infections. Cefotaxime generates a desacetyl metabolite that works synergistically with the parent compound in the treatment of some anaerobic species *in vitro*, but is still not a primary agent for anaerobic infections *in vivo*.

β-Lactamases in anaerobes include the typical cephalosporinases in the *B. fragilis* group and the penicillinases in *Clostridium* spp. and *F. nucleatum*. Virtually all *B. fragilis* isolates produce β-lactamases, including a subset of isolates that produce a broad-spectrum metallo-β-lactamase. β-Lactamase production has not been reported in strains of *Clostridium perfringens*.[59]

The addition of a β-lactamase inhibitor increases the activity of some penicillins against β-lactamase producing anaerobes, in particular *Bacteroides* spp., resulting in efficacy for the combinations of ticarcillin–clavulanate, piperacillin–tazobactam, amoxicillin–clavulanate and ampicillin–sulbactam. The most active β-lactam agents against anaerobic isolates in the USA are the carbapenems, imipenem, meropenem, ertapenem and doripenem. Aztreonam has no activity against anaerobes and must be used with other agents when treating mixed aerobic and anaerobic infections.[60]

Central nervous system infections (meningitis)

Certain β-lactam antibiotics are able to penetrate inflamed meninges and are commonly used to treat meningitis (e.g. penicillin G, ampicillin, nafcillin, oxacillin, cefotaxime, ceftizoxime, ceftriaxone, ceftazidime and meropenem). The most common pathogens in a series of adult patients with meningitis in descending order were *Strep. pneumoniae*, *Neisseria meningitidis* and *L. monocytogenes*.[61] With the widespread use of conjugate *Haemophilus influenzae* and *Strep. pneumoniae* vaccines, *N. meningitidis* has become the most important cause of bacterial meningitis in children in the USA. These bacteria have remained consistently susceptible to β-lactam antibiotics.

Because of the severity of infections in the central nervous system there is a need to start effective therapy before the identity and susceptibility of the bacteria causing infection is known. The activity of β-lactams against the leading causes of these infections makes them appropriate choices for initial therapy. Although penicillin G at a dose of 20–24 million U/day intravenously q4h is a treatment of choice for susceptible strains of *Strep. pneumoniae* and for nearly all *N. meningitidis*, due to the possibility of infection by penicillin-resistant pneumococci, β-lactamase producing *H. influenzae* and the rare β-lactamase-producing *N. meningitidis*, the use of ceftriaxone with vancomycin is generally considered the most appropriate β-lactam-containing regimen for use as empiric therapy for bacterial meningitis. β-Lactams are not sufficient to eliminate the carrier state of *N. meningitidis* that often occurs in patients recovering from bacterial meningitis. For this reason rifampin should be given at the completion of therapy. The agent of choice for the treatment of meningitis due to *L. monocytogenes* is ampicillin or penicillin alone or in combination with gentamicin.

In children, the preferred agents for the treatment of *H. influenzae* meningitis are the third-generation cephalosporins cefotaxime or ceftriaxone.[62] Cefuroxime is not considered an appropriate alternative to these newer agents because of failures of treatment as well as the development of meningitis during cefuroxime treatment. A randomized trial found that ceftriaxone resulted in less hearing impairment and sterilized the CSF earlier than cefuroxime when used as treatment for meningitis in children.[63]

Patients with staphylococcal meningitis (which is usually seen after trauma or neurosurgical procedures) are best treated with high doses of nafcillin or oxacillin if the organism is susceptible. *Pseudomonas aeruginosa* meningitis has been effectively managed with ceftazidime, although meropenem may prove to be an alternative.[64]

Corticosteroids have been shown to reduce the neurologic sequelae of meningitis. Clinical trial experience has shown that early adjunctive therapy with dexamethasone can improve the outcome of bacterial meningitis.[65]

Biliary system infections (cholangitis)

Infection of the biliary tract generally occurs if there is an abnormality such as gallstones, strictures or a stent. Infection rarely complicates malignant obstruction of the biliary tree. In the obstructed biliary tract, there is very little excretion of any antibiotic. The β-lactams that achieve significantly higher biliary than serum levels are nafcillin, mezlocillin, piperacillin, cefoperazone and ceftriaxone. Ampicillin achieves concentrations in the bile equal to or greater than those in serum. Interestingly, biliary levels are higher after oral amoxicillin or ampicillin than they are after intravenous administration. Biliary concentrations of ticarcillin, cefazolin, cefotaxime, ceftazidime and cefuroxime are all less than serum concentrations.[66]

Ureidopenicillins mezlocillin and piperacillin have been used in biliary tract infections as has cefoperazone. Cefoxitin, cefuroxime and ceftriaxone are also commonly used in conjunction with an aminoglycoside in patients with cholangitis.

In patients undergoing biliary surgery, adequate serum levels of antibiotic have been shown to be more important than biliary levels when the goal is to reduce postoperative infection.

Intra-abdominal infections (see Chapter 37)

Intra-abdominal infections, such as acute appendicitis, penetrating abdominal trauma and bowel perforation, are generally polymicrobial in nature and caused by a combination of aerobic, anaerobic and facultative anaerobic organisms. Clinical trials have confirmed the efficacy of β-lactam antibiotics alone or in combination with other agents for various intra-abdominal infections.[67] Cefoxitin, imipenem, cefotetan, piperacillin–tazobactam and ticarcillin–clavulanic acid have all been shown to be effective in treating intra-abdominal infections when used as monotherapy. Meropenem has been shown to have efficacy similar to that of imipenem for the treatment of intra-abdominal sepsis.[68] Ertapenem has similar efficacy in treating intra-abdominal infections as piperacillin–tazobactam.[69] Doripenem has been shown to have similar efficacy to meropenem in treating patients with complicated intra-abdominal infections.[70] The combination of clindamycin with either ceftazidime or aztreonam has been successful in the treatment of intra-abdominal infections. Failures of ampicillin–sulbactam have occurred when pseudomonal infections occur.

Although enterococci are commonly isolated from intra-abdominal infections (14–33%), many physicians do not include anti-enterococcal therapy in the initial treatment of these infections. 'Breakthrough' enterococcal infections occur in patients who have been hospitalized for long periods of time with persistent or recurrent intra-abdominal sepsis or who are immunosuppressed.[71]

Spontaneous bacterial peritonitis

Few studies have evaluated the efficacies of different antibiotics in the treatment of spontaneous bacterial peritonitis (SBP). The organisms that typically cause SBP are the Gram-negative bacilli (especially *E. coli* and *Klebsiella* spp.), Gram-positive cocci (including pneumococci, other streptococci, enterococci and staphylococci) and anaerobes. When used in conjunction with an aminoglycoside, ampicillin had a cure rate of 76% in one study. Cefotaxime was shown to be more effective (cure rate 85%) than ampicillin and tobramycin (cure rate 56%) in another study of severe infections in patients with cirrhosis, of which approximately 75% were SBP. In another study, amoxicillin and clavulanic acid had a cure rate of 80% for SBP. Aztreonam monotherapy has been associated with Gram-positive superinfection. Therefore, if aztreonam is to be used for SBP, then an additional antibiotic providing Gram-positive coverage is needed.[72]

Pancreatitis and its complications (see Chapter 37)

The prophylactic use of antibiotics in uncomplicated acute pancreatitis is controversial. Early studies that used ampicillin, an antibiotic that does not achieve therapeutic levels in pancreatic tissue, showed no benefit. However, several recent studies have shown a potential benefit. Patients with acute necrotizing pancreatitis treated with imipenem for 14 days had a lower incidence of pancreatic sepsis than those not treated; however, a trend toward a decreased mortality rate was not statistically significant.[73] A study that used cefuroxime in patients with acute necrotizing pancreatitis found that rates of bacteremia and mortality were both lower than those of controls.[74]

It is clear that β-lactam antibiotics have a role in the management of infectious complications of pancreatitis such as abscess or infected pseudocyst. The agents commonly used in addition to cefuroxime and imipenem include ticarcillin–clavulanic acid, piperacillin–tazobactam, ampicillin–sulbactam and meropenem.

Endovascular infections (endocarditis) (see Chapter 47)

Updated statements regarding the treatment of endocarditis were published by the AHA in 2005[75] and the British Society for Antimicrobial Chemotherapy (BSAC) in 2004.[76] The drug of choice for the treatment of endocarditis caused by viridans streptococci is penicillin G. Depending upon the drug susceptibility of the organism, gentamicin can be added for part or all of a 2–6 week course. A 2-week course of combination ceftriaxone with an aminoglycoside may be sufficient as therapy for patients with uncomplicated endocarditis due to viridans streptococci. Alternatively, a 4-week course of ceftriaxone can be used.[77] Enterococcal endocarditis is best treated with ampicillin or penicillin in combination with an aminoglycoside for 4–6 weeks.

The treatment of choice for native-valve endocarditis caused by methicillin-susceptible *Staph. aureus* is nafcillin, flucloxacillin or oxacillin for 6 weeks. Gentamicin has been used for the first 3–5 days to decrease the number of days of bacteremia, but has not been shown to change the outcome.[78]

Intravenous drug users with right-sided staphylococcal endocarditis have been successfully treated with 2 weeks of nafcillin and tobramycin.[79] Prosthetic valve endocarditis with methicillin-susceptible *Staph. aureus* is optimally treated with nafcillin or oxacillin (if the organism is sensitive) in combination with rifampin and gentamicin (for the first 2 weeks) for at least 6 weeks.

Endocarditis caused by the slow-growing fastidious Gram-negative organisms *Haemophilus parainfluenzae*, *H. aphrophilus*, *Actinobacillus actinomycetemcomitans*, *Cardiobacterium hominis*, *Eikenella corrodens* and *Kingella kingae* (the HACEK group) can be treated with ampicillin and gentamicin for 4 weeks or ceftriaxone alone for 4 weeks.

Neutropenic fever

β-Lactam antibiotics have been used for many years in managing febrile patients with cancer and treatment-induced neutropenia. Because life-threatening infections can occur due to Gram-negative bacteria, especially *P. aeruginosa*, and resistance to β-lactams has continued to become more prevalent among Gram-negative enteric pathogens, using these agents as monotherapy may not be as effective as has been previously reported.[80] Combining a β-lactam with a second agent that has activity against a broad-spectrum of Gram-negative bacteria, typically an aminoglycoside, has been recommended in patients who

present with high risk for rapidly progressing disease.[81] Most recently, the emergence of methicillin-resistant staphylococci as an important cause of disease in this patient population has further complicated using a β-lactam to provide reliable activity against Gram-positive bacteria in these patients.

Lyme disease

Early infection caused by *Borrelia burgdorferi* can be managed with either amoxicillin or doxycycline. Since co-infection with *Ehrlichia* or *Anaplasma* spp. is known to occur in many areas, many clinicians now prefer to use doxycycline. For later manifestations of Lyme disease, however, the β-lactams are the agents of choice. Lyme carditis can be successfully treated with either a 2-week course of ceftriaxone or intravenous penicillin G. Lyme meningitis and Lyme arthritis can also be treated with ceftriaxone or penicillin G, but for 2–4 weeks. A 30-day treatment course of amoxicillin and probenecid has been used for the treatment of Lyme arthritis. In pregnant women, doxycycline cannot be used, amoxicillin is used in early Lyme disease and intravenous penicillin G is used for disseminated early Lyme disease or any manifestation of late disease (see Chapter 43).[82]

Syphilis

Parenteral penicillin G is the preferred agent for treating all stages of syphilis and is the only therapy that has proved effective for neurosyphilis, syphilis in pregnancy and congenital syphilis.[83] Primary and secondary syphilis can be treated with a single dose of benzathine penicillin G (2.4 million U in adults, 50 000 U/kg intramuscularly in children up to the adult dose). Late latent syphilis is treated with benzathine penicillin G (2.4 million U intramuscularly every week for 3 weeks). Procaine penicillin can be used where benzathine penicillin is unavailable, and there are alternatives for patients who are allergic to penicillin (see Chapter 57).

It is important to remember that patients being treated for any of the spirochete diseases – syphilis, Lyme disease or borreliosis – may develop a Jarisch–Herxheimer reaction, which may produce fever, tachycardia, chills, headaches, sore throat, malaise, myalgias, arthralgias, rash and, rarely, hypotension. This reaction has been observed in approximately 50% of patients treated for primary syphilis and 75% of patients with secondary syphilis.[84] Generally, it occurs a few hours after the first dose of penicillin, lasts for only a few hours and does not occur with subsequent doses of the antibiotic. Pre-treatment of patients with louse-borne relapsing fever (caused by *Borrelia recurrentis*) with hydrocortisone or acetaminophen does not prevent the Jarisch–Herxheimer reaction.[85] It is important to distinguish the reaction from penicillin allergy so that appropriate treatment is not discontinued.

DOSAGE IN SPECIAL CIRCUMSTANCES

Renal impairment

The majority of the β-lactam antibiotics are excreted almost entirely via the renal route, and so dose adjustments are necessary in the presence of kidney disease. Failure to reduce the dose of penicillin in uremic patients has resulted in toxicity, most notably encephalopathy.[86] In patients with especially high glomerular filtration rates, rapid clearance of renally excreted antibiotics may result in a need to increase dosages in order to achieve therapeutic exposures.[87] As biliary secretion plays a major role in the excretion of ceftriaxone, cefoperazone, nafcillin and oxacillin, the doses of these antibiotics do not need to be adjusted in renal failure. Because biliary secretion plays a lesser, although significant role in the excretion of the ureidopenicillins, the dosages of these drugs do not have to be reduced as much as for the other penicillins. Some β-lactams must be re-dosed after peritoneal dialysis, which removes variable amounts of the drug. With the exception of ceftriaxone, mezlocillin, nafcillin, dicloxacillin and oxacillin, the β-lactams must be re-dosed following hemodialysis. Specific dose adjustments are needed for patients with renal impairment and for patients on hemodialysis or peritoneal dialysis (Table 134.5).

Hepatic impairment

The dosages of some β-lactams must be adjusted in patients with severe hepatic disease. As a result of reduced desacetylation in patients with liver disease, the half-life of cefotaxime may increase slightly, but the half-life of cefoperazone may increase significantly and dosage reductions are required. Although biliary excretion plays a role in the excretion of ceftriaxone, no dose adjustment is needed in patients with liver disease.

Extremes of age

Dose reductions of the β-lactam antibiotics should be made in the elderly in the presence of renal dysfunction (see Table 134.5). Otherwise, elderly patients tolerate standard doses of the β-lactam antibiotics.

Because neonates do not have fully developed renal function, special modifications in dosage are necessary. In addition, because children have a high risk of cholestatic complications with ceftriaxone, another agent should be used when possible (see Adverse reactions and interactions, below). Ceftriaxone should not be used in neonates with hyperbilirubinemia.

β-Lactams in pregnancy

The penicillins, the β-lactamase inhibitors and the cephalosporins, aztreonam, meropenem, ertapenem and doripenem are considered category B in pregnancy (Table 134.6). This means that animal studies have shown no risk to the fetus, but adequate human studies have not been performed, or that animal studies have shown risk and human studies have shown no risk. When they are indicated, these antibiotics are commonly used in clinical practice in pregnant women.

Imipenem–cilastatin is pregnancy category C, meaning that animal studies show toxicity to the fetus and human studies are inadequate. However, the benefit of using these drugs may exceed the risk of not treating a serious infection in a pregnant woman when no alternatives exist.[93] Meropenem or doripenem, category B, may be a suitable alternative to imipenem, category C, for infections with resistant Gram-negative aerobic organisms.

β-Lactam antibiotics that are not protein bound are transported across the placenta and reach the drug levels that are present in maternal serum. β-Lactams that are highly protein-bound reach only low concentrations in amniotic fluid and the fetus.[94]

As a general principle, the β-lactam antibiotics have accelerated elimination and lowered plasma concentrations in pregnant women as compared with nonpregnant women. As a result, the dose or frequency of administration should be increased in pregnant women.[93]

ADVERSE REACTIONS AND INTERACTIONS

Adverse reactions that occur with the β-lactam antibiotics are summarized in Table 134.7.

Allergic reactions

The most common adverse event associated with the use of β-lactam antibiotics is an allergic reaction. The reported frequency of allergic reaction to penicillin varies from 0.7% to 10%. Anaphylaxis historically was documented to occur in 0.004–0.015% of patients.[95] A maculopapular rash occurs late in the treatment course of 2–3% of patients receiving a course of penicillin. Ampicillin and amoxicillin induce rashes in a higher percentage of patients treated (5.2–9.5%) than other β-lactams and almost invariably cause a rash when given

Table 134.5 Drug dosages in patients with renal failure

Generic name	Dose in normal renal function	Max. daily dose with normal renal function	Adjustment in dose (D) or interval (I)	GFR (ml/min) >50	GFR (ml/min) = 10-50	GFR (ml/min) <10	Supplement after HD	Supplement with PD	CVVHD
Penicillins									
Amoxicillin	250–500 mg q8h	2–3 g/q24h	I	q8h	q8–12h	q24h	Yes, 250–500 mg	250 mg q12h	500 mg q8–12h
Amoxicillin–clavulanate	250–500 mg q8h		I	q8h	q8–12h	q24h	Yes, 250–500 mg q24h	Usual regimen	
Ampicillin	250 mg–2 g q6h	2–4 g/q24h	I	q6h	q6–12h	q12–24h	Yes	250 mg q12h	1–2 g q6–12h
Ampicillin–sulbactam (AM–SB)	1–2 g AM–500 mg–1 g SB q6–8h	8 g AM–4 g SB/q24h	I	q6h	q8–12h	q24h	Yes	2 g AM–1 g SB/24h	3 g q8h
Dicloxacillin	125–500 mg q8h	2 g/q24h	D	100%	100%	100%	No	No	
Mezlocillin	1.5–4.0 g q4–6h	24 g/q24h	I	q4–6h	q6–8h	q8h	No	No	
Nafcillin	1–2 g q4–6h	12 g/q24h	D	100%	100%	100%	No	No	2 g q4–6h
Oxacillin	1–2 g q4–6h	12 g/q24h	D	100%	100%	100%	No	No	2 g q4–6h
Penicillin G	0.5–4 million U q4–6h	24 million U	D	100%	75%	20–50%	Yes	Dose for GFR <10	0.5–3 million U q6h
Penicillin V	250–500 mg q6h	3 g/q24h	D	100%	100%	100%	Yes	Dose for GFR <10	300 mg q6h
Piperacillin	3–4 g q4–6h	24 g/q24h	I	q4–6h	q6–8h	q8h	Yes	Dose for GFR <10	
Piperacillin–tazobactam	3.375 g q6h	13.5 g/q24h	D&I	3.375 g q6h	2.25 g q6h	2.25 g q8h	Dose for GFR <10 + 0.75 g	Dose for GFR <10	2.25–3.375 g q6h
Ticarcillin	3 g q4h	24 g/q24h	D&I	1–2 g q4h	1–2 g q8h	1–2 g q12h	Yes, extra 3 g	Dose for GFR <10	
Ticarcillin–clavulanate	3.1 g q4h	24 g/q24h	D&I	3.1 g q4h	3.1 g q8–12h	2 g q12h	Yes, extra 3.1 g	3.1 g q12h	3.1 g q6h
Cephalosporins									
Cefaclor	250–500 mg q8h	1.5 g/q24h	D	100%	50–100%	50%	Yes	Usual regimen	
Cefadroxil	500 mg–1 g q12h	2 g/q24h	I	q12h	q12–24h	q36h	Yes, extra 500 mg–1 g	Usual regimen	
Cefazolin	1–2 g q8h	12 g/q24h	I	q8h	q12h	q24–48h	Yes, extra 500 mg–1 g	500 mg q12h	2 g q12h
Cefditoren (pivoxil)	200–400 mg q12h	800 mg/q24h	D&I	100%	200 mg q12h	200 mg q24h	Dose for GFR <10	No data	
Cefdinir	300 mg q12h	600 mg/q24h	I	q12h	q24h	q48h	Yes	Dose for GFR <10	
Cefepime	250 mg–2 g q8–12h	6 g/q24h	D&I	100%	50–100% q24h	25–50% q24h	Yes, dose for GFR <10	Dose for GFR <10	2 g q12h
Cefixime	200 mg q12h or 400 mg q24h	400 mg/q24h	D	100%	75%	50%	Yes	Usual dose	
Cefoperazone	1–2 g q12h	12 g/q24h	D	100%	100%	100%	Yes, extra 1 g	No	1 g q12h
Cefotaxime	1–2 g q6–12h	12 g/q24h	I	q6h	q6–12h	q24h or 50%	Yes, extra 500 mg–2 g	1 g q24h	2 g q12h
Cefotetan	1–2g q12h	6g/q24h	I	100%	q24h	q48h	Yes, extra 1g	1g q24h	

(Continued)

Table 134.5 Drug dosages in patients with renal failure—cont'd

Generic name	Dose in normal renal function	Max. daily dose with normal renal function	Adjustment in dose (D) or interval (I)	GFR (ml/min) >50	GFR (ml/min) = 10-50	GFR (ml/min) <10	Supplement after HD	Supplement with PD	CVVHD
Cefoxitin	1–2 g q6–8h	12 g/q24h	I	q6–8h	q8–12h	q24–48h	Yes, extra 1 g	1 g q24h	
Cefpodoxime (proxetil)	100–400 mg q12h	800 mg/q24h	I	q12h	q24h	q24h	Yes	Dose for GFR <10	
Cefprozil	250–500 mg q12h	1 g/q24h	D	100%	50%	50%	Yes, extra 250 mg	Dose for GFR <10	
Ceftazidime	1–2 g q8h	8 g/q24h	I	q8–12h	q12–24h	q24–48h	Yes, extra 1 g	500 mg q24h	2 g q12h
Ceftibuten	400 mg q24h	400 mg	D	100%	25–50%	25–50%	Yes, extra 400 mg	100–200 mg q24h	
Ceftizoxime	1–2 g q8–12h	12 g/q24h	I	q8–12h	q12–24h	q24h	Yes, extra 1 g	500 mg–1 g q24h	
Ceftobiprole (medocaril)	500 mg q8h	1.5 g/q24h	D&I	100%	500 mg q12h (30–50 ml/min)	250 mg q12h (<30 ml/min)	No data	No data	
Ceftriaxone	1–2 g q24h	4 g/q24h	D	100%	100%	100%	No	Usual regimen	2 g q12–24h
Cefuroxime (axetil)	250–500 mg q12h	1 g/q24h	D	100%	100%	100%	Yes	Dose for GFR <10	250–500 mg q12h
Cefuroxime	750 mg–1.5 g q8h	6 g/q24h	I	q8h	q8–12h	q24h	Yes	Dose for GFR <10	
Cephalexin	250–500 mg q6h	4 g/q24h	I	q6–8h	q8–12h	q12–24h	Yes	Dose for GFR <10	250–500 mg q12h
Loracarbef	200–400 mg q12h	800 mg/q24h	I	q12h	q24h	q3–5d	Yes	Dose for GFR <10	
Monobactam									
Aztreonam	500 mg–2 g q8–12h	8 g/q24h	D	100%	50%	25%	Yes, extra 500 mg	Dose for GFR <10	2 g q12h
Carbapenems									
Doripenem	500 mg q8h	1.5 g/q24h	D&I	100%	250 mg q8h (30–50 ml/min)	250 mg q12h (<30 ml/min)	No data	No data	
Ertapenem	1 g q24h	1 g/q24h	D	100%	100%	50%	Yes, extra 150 mg If dose given within 6h of HD	Dose for GFR <10	
Imipenem–cilastatin	250 mg–1 g q6h	4 g/q24h	D	100%	50%	25%	Yes	Dose for GFR <10	250 mg q6h, 500 mg q8h or 500 mg q6h
Meropenem	1 g q8h	4 g/q24h	D&I	100%	50% q12h	50% q24h	Yes	Dose for GFR <10	1 g q12h

Dosing guidance as per references 9,10,18,88–92.
CVVHD, continuous venovenous hemodialysis. GFR, glomerular filtration rate; HD, hemodialysis; PD, peritoneal dialysis.
Absence of information is due to lack of published data.

Table 134.6 Pregnancy categories of the β-lactam antibiotics

Class of β-lactam	Pregnancy category
Penicillins – all	B
β-Lactamase inhibitors – all	B
Cephalosporins – all	B
Aztreonam	B
Doripenem	B
Ertapenem	B
Imipenem–cilastatin	C
Meropenem	B

Categories as of August 2008. Category B means that animal studies have shown no risk to the fetus, but adequate human studies have not been performed, or animal studies have shown risk and human studies have shown no risk. Category C means that animal studies show toxicity to the fetus and human studies are inadequate.

Table 134.7 Adverse reactions associated with β-lactam antibiotics

Reaction site/system	Examples
Local	Pain, induration, tenderness at site of intramuscular injection; burning during intravenous administration; phlebitis
Hypersensitivity	Rash, pruritus, urticaria, fever, chills, Stevens–Johnson syndrome, anaphylaxis
Gastrointestinal	Diarrhea, nausea, vomiting, abdominal pain, *Clostridium difficile*-associated diarrhea
Hematologic	Eosinophilia, leukopenia, anemia, positive Coombs' test, hemolytic anemia, neutropenia, lymphopenia; thrombocytosis, thrombocytopenia, elevated prothrombin time, bleeding, abnormal clotting time, abnormal platelet aggregation
Hepatic	Elevated serum transaminases (aspartate transaminase, alanine transaminase), hepatitis, elevated alkaline phosphatase, elevated bilirubin
Renal	Elevated blood urea nitrogen and creatinine, falsely elevated creatinine, casts in urine
Central nervous system	Headache, dizziness, somnolence, confusion, tremor, myoclonus, seizures, encephalopathy
Genitourinary	Vaginitis
Superinfection	Thrush, vaginal candidiasis, infection with resistant bacteria

during acute infectious mononucleosis (Epstein–Barr virus). Reactions to penicillins are characterized according to the time of onset following administration of the drug:

- immediate reactions occur in the first hour;
- accelerated reactions occur 1–72 hours after drug administration; and
- late reactions occur 72 hours or more after starting a course of the antibiotic.

Both immediate and accelerated reactions may result in urticaria and anaphylaxis.

Previous exposure to penicillin does not seem to increase the risk of penicillin allergy. However, it is clear that people who have had allergic reactions to penicillin have a higher risk of allergic reactions than people who have tolerated therapy in the past. In patients with a history of penicillin allergy, rechallenge with penicillin results in acute reactions in an estimated 65% of patients, anaphylaxis in 5–10% and fatal anaphylaxis in 0.2–0.5%.

Skin testing is a useful technique in the evaluation of patients with a history of penicillin allergy, but is not useful as a screening test for the general population because many skin test positive patients without a history of penicillin allergy can tolerate penicillin therapy. Skin testing is not useful for identifying non-IgE-mediated adverse drug reactions such as drug fever.

Although fatalities have occurred as a result of the skin test itself, the procedure is generally regarded as safe. A wide range in the incidence of positive skin tests in patients with a previous history of penicillin allergy has been noted (8.75–63%), and therefore a significant proportion of patients who give a history of penicillin allergy can tolerate the drug.[96] In one large study, penicillin skin testing allowed the safe use of penicillin in 90% of patients who gave a history of penicillin allergy.[97] Skin test reactivity declines with time in patients with a history of penicillin allergy. People with dermatitis and allergic rhinitis do not have an increased risk of penicillin allergy, but the risk may be increased for atopic individuals.[98]

Patients with a history of penicillin allergy are four times as likely to have a reaction to first-generation cephalosporins than patients without a history of allergy (8.1 vs 1.9%). Second- and third-generation cephalosporins have an incidence of skin reaction (rash) ranging from 1% to 3%, similar to the incidence of rash with penicillin. Anaphylaxis, however, is uncommon with cephalosporins. There seems to be a lower incidence of allergy to second- and third-generation cephalosporins in patients with a history of penicillin allergy. Patients with allergy to penicillins should be considered allergic to the carbapenems, but there seems to be no cross-reactivity with aztreonam. No major allergic reactions to aztreonam have been reported, but rarely patients will develop a rash. There is more allergic cross-reactivity among penicillin derivatives than among cephalosporin derivatives. However, allergic cross-reactivity between cephalosporin derivatives is greater than cross-reactivity between cephalosporins and penicillins.[99]

At times, it may be necessary to administer penicillin to patients with a previous severe reaction to the drug. For instance, penicillin is the only acceptable treatment for a pregnant woman with syphilis. Effective methods of desensitization have been described,[100] but adverse reactions are common and the patient should be in an intensive care unit for close monitoring.

Hematologic effects

Hematologic toxicity is rare, but leukopenia (occurring in 0.2% of patients on mezlocillin in one study) has been observed when the penicillins or cephalosporins are used at high doses,[101] and also rarely with imipenem. Counts return when the drug is discontinued, and lower dosages can often be tolerated without neutropenia. Isolated eosinophilia can occur in patients on cephalosporins (1–7%). A Coombs' positive hemolytic anemia is rarely observed with the penicillins and cephalosporins.[102]

Dose-dependent defects of platelet aggregation and a prolongation of the bleeding time can be seen with carbenicillin and ticarcillin, and can occur with all of the penicillins at high doses. Clinically significant bleeding can occur but is uncommon.[103]

Hypoprothrombinemia occurred frequently with cephalosporins that are no longer widely used. These agents all had a methylthiotetrazole (MTT) group (i.e. cefoperazone, cefotetan, cefmenoxime), which may interfere with the activation of factors II, VII, IX and X, and may also prevent the activation of vitamin K.[104] Patients with renal failure, malnutrition, intra-abdominal infection or recent gastrointestinal

surgery seem to be at the highest risk and may benefit from weekly prophylaxis with vitamin K when being given one of these antibiotics. Isolated thrombocytopenia rarely complicates the use of the β-lactam antibiotics. An immune mechanism has been documented. Thrombocytopenia can occur as soon as 5 days after the initiation of the antibiotic and generally resolves when the agent is withdrawn.[105]

Renal effects

Interstitial nephritis, characterized by fever, rash, eosinophilia, proteinuria, hematuria, eosinophiluria and occasionally renal insufficiency can be seen with the penicillins.[106] β-Lactam antibiotics that exist as sodium salts, particularly carbenicillin and ticarcillin, can induce hypokalemia.[106] Cephalothin can cause renal damage that histopathologically resembles that of nafcillin.[107] The concurrent use of aminoglycosides may add to the nephrotoxicity of cephalosporins such as cephalothin. About 1% of patients on ceftazidime have elevated blood urea nitrogen or creatinine, but these are generally not clinically significant.[108] The sodium load of some penicillins can be high, most notably with ticarcillin (4.7 mEq/g), but also with ampicillin, methicillin, penicillin G, azlocillin, mezlocillin and piperacillin, thereby posing a problem for patients with congestive heart failure.

Neurologic effects

Many of the β-lactams can cause neurotoxicity and, in particular, seizures. Seizures have been reported following the use of penicillin, ampicillin, amoxicillin, oxacillin, nafcillin, carbenicillin, ticarcillin, piperacillin, cefazolin, cefonicid, cephalexin, ceftazidime, imipenem, meropenem, ertapenem and doripenem. Benzylpenicillin, cefazolin and imipenem have the highest neurotoxic potential of the β-lactam antibiotics. Seizures have been reported to occur in 0.4–1.5% of patients taking imipenem.

Several risk factors that may predispose a patient to neurotoxicity have been identified, for example:

- high doses;
- renal insufficiency;
- disruption of the blood–brain barrier,
- pre-existing central nervous system (CNS) disease;
- advanced age;
- concurrent administration of nephrotoxic drugs;
- concurrent drugs that may reduce the seizure threshold; and
- concurrent administration of other β-lactam antibiotics.[109]

Neurotoxicity of penicillins is clearly related to elevated CSF antibiotic levels, such as may occur when high doses are being used in patients with impaired renal function. Penicillin levels in CSF should not exceed 5 mg/l.

Gastrointestinal effects

Gastrointestinal upset and diarrhea are common side-effects of the β-lactams. Enterocolitis caused by *Clostridium difficile* may result from use of any of the β-lactams.

Hepatitis is a rare side-effect of penicillins such as mezlocillin and nafcillin, and resolves after discontinuation of therapy. Hepatitis as a result of intravenous oxacillin can occur as early as 2 days into treatment, is thought to result from a hypersensitivity reaction, does not appear to be dose related and is reversible on discontinuation of the drug.[110] Mild elevations in transaminases and alkaline phosphatase also occur with the cephalosporins and carbapenems, but the drug can usually be continued.[111] Serum transaminases become elevated in 2–4% of patients receiving aztreonam.

Gallbladder sludge formation[112] and cholelithiasis[113] have occurred in patients on ceftriaxone. Children, patients receiving prolonged or high doses, and patients on total parenteral nutrition appear to be at risk of this complication.

A disulfuram-like reaction has been associated with the cephalosporins with an MTT group.[114] Patients taking these agents and then ingesting alcohol have developed flushing, tachycardia, diaphoresis, headache, nausea, vomiting and dizziness.

Other reactions

Local side-effects of the β-lactam antibiotics are not uncommon. At the intramuscular injection site, patients may experience pain, tenderness and edema. Thrombophlebitis can occur in up to 5% of patients receiving parenteral therapy with some agents.

Other reactions to penicillin are less common. Serum sickness, consisting of fever, urticaria, joint pains and angioneurotic edema, can occur and, rarely, exfoliative dermatitis, the Stevens–Johnson syndrome and allergic vasculitis. Late-onset morbilliform rashes can develop as a result of penicillin therapy and may disappear, even if the penicillin is continued, but desquamation can occur.

Drug interactions

The most clinically important drug interaction with the β-lactam antibiotics occurs with probenecid, a uricosuric and renal tubular blocking agent. Probenecid causes a two- to fourfold increase in the peak serum concentration of the β-lactam antibiotics. It also prolongs serum levels for these antibiotics. It is used most often with penicillin (e.g. in the treatment of gonococcal infections), but can also be used with ampicillin, methicillin, oxacillin, cloxacillin and nafcillin. The recommended dose in adults is 2 g/day in divided doses, and in children a 25 mg/kg initial dose is followed by 40 mg/kg/day in four divided doses (adult dose used for children who weigh over 50 kg). The mechanism of action involves not only inhibition of renal tubular secretion of the β-lactams, but also a decrease in the apparent volume of distribution of the drug.[115] Probenecid has little effect on the serum levels of imipenem and aztreonam and has no effect on drug levels of ceftazidime. Adverse reactions, including anaphylaxis, can occur with probenecid and the clinician must also be aware that toxicity can result from supratherapeutic levels of the β-lactam antibiotics when used with probenecid.

As cephalosporins with the MTT side chain can interfere with hemostasis, in the rare circumstance where these agents might be used in modern clinical practice, care must be taken when using these antibiotics in patients taking warfarin.

Synergistic activity against various bacteria occurs when the penicillins, cephalosporins, carbapenems and monobactams are used in conjunction with aminoglycosides.[116] However, using two β-lactam antibiotics together may result in either synergy or antagonism.

The bactericidal effect of ampicillin may be reduced when other antibiotics (chloramphenicol, erythromycin, sulfa drugs and tetracycline) are used simultaneously. The clinical significance of this is unclear.

When ampicillin is used in patients who are taking oral contraceptive agents, breakthrough bleeding may occur and the contraceptive may be less effective.

Piperacillin and ticarcillin must be used cautiously in any patient on vecuronium because the neuromuscular blockade can be further prolonged. Piperacillin can also lower serum levels of tobramycin if the drugs are used together.

Fatal reactions associated with the appearance of precipitates of calcium and ceftriaxone have occurred in neonates; this has led to recommendations not to administer ceftriaxone within 48 hours of giving intravenous calcium-containing solutions.

REFERENCES


References for this chapter can be found online at http://www.expertconsult.com

Chapter 135

André Bryskier

Macrolides, ketolides, lincosamides and streptogramins

Macrolides and Ketolides

INTRODUCTION

Shortly after the beginning of the antimicrobial era, it became increasingly apparent that bacteria could rapidly evolve resistance to existing antibacterial agents. New agents would need to be developed to keep pace with the remarkable capacity of bacterial pathogens to genetically adapt to antimicrobial chemotherapy. Systematic screening was performed by many researchers for new compounds possessing antibacterial activity in general and antistaphylococcal activity in particular. Researchers at Eli Lilly first discovered the erythromycin complex in 1952.

Erythromycin A, the most active component of the erythromycin complex, was initially intended to provide an alternative therapy in response to the emergence of penicillinase-producing strains of *Staphylococcus aureus*. The discovery of vancomycin did not supplant the use of erythromycin because of its poor tolerance, but the semisynthetic penicillins (methicillin and oxacillin) and the first injectable cephalosporins (cephalothin and cephaloridine) led to a decline in the use of erythromycin A.

Two circumstances led to a revival of interest in the macrolides (the second wave of macrolides in early 1980s – roxithromycin, clarithromycin, azithromycin, dirithromycin, flurithromycin):

- the recognition of the pathogenic potential of *Chlamydia trachomatis*; and
- the outbreak of 'Legionnaires' disease'.

Legionella pneumophila is an intracellular pathogen: β-lactams do not accumulate in phagocytic cells, in contrast to the excellent activity of the macrolides, tetracyclines, fluoroquinolones and ansamycins against this pulmonary pathogen.

The third wave of macrolides (the ketolides – telithromycin, cethromycin, CEM 101, modithromycin) was in response to the emergence of erythromycin A-resistant strains among Gram-positive cocci and *Campylobacter* spp., *Helicobacter pylori*, *Mycobacterium avium* complex, etc.

DEFINITIONS

The macrolides are hydrophobic molecules having a central 14- to 16-membered-ring lactone with few or no double bonds and no nitrogen atom. Several amino or neutral sugars may be attached to the lactone nucleus.

The azalides, such as azithromycin or tulathromycin (a veterinary drug), are not included as they have an endolactone nitrogen atom. Nevertheless, they are semisynthetic derivatives of erythromycin A and due to their antibacterial spectrum belong to the macrolide family. Semisynthetic ketolides (such as telithromycin, cethromycin, CEM 101) were obtained by the removal of the neutral sugar (L-cladinose). The 3-OH group obtained was oxidized yielding a 3-carbonyl group, which characterizes the ketolides.

CLASSIFICATION

There are several classifications of macrolides: a chemical classification and a simplified classification that considers the lactone structure and the natural or semisynthetic origin of the molecule. The natural macrolides of importance in human pathology are 14- or 16-membered-ring macrolides and, in addition in the semisynthetic derivatives, the 15-membered-ring macrolides (azalides).

The 14-membered-ring macrolides are divided into two groups: those of natural origin such as erythromycin A (Fig. 135.1) and olean-

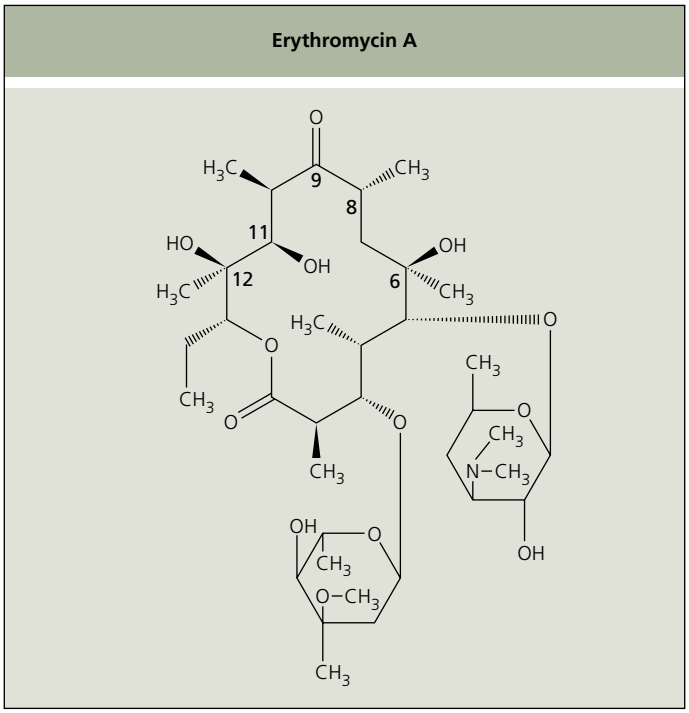


Fig. 135.1 Erythromycin A.

domycin, and the semisynthetic derivatives of erythromycin A. The latter may be separated into three subgroups:

- those with a modified substituent of the lactone nucleus, such as roxithromycin, clarithromycin, dirithromycin, flurithromycin and davercin (group II_A);
- those whose lactone nucleus has undergone modification, involving two subgroups, azalides (azithromycin, tulathromycin, gamithromycin) and oxolides (group II_{B1} and II_{B2}); and
- those with a modification of the neutral sugar – ketolides (3-keto group) and acylide derivatives (alkyl or alkyl aryl 3-substituent) (group II_C) (Fig. 135.2).

Groups III and IV comprise, respectively, the 16-membered-ring natural molecules, such as josamycin, spiramycin and midecamycin, and the synthetic molecules such as miocamycin or rokitamycin.

Few specific macrolides are used in veterinary medicine but include tulathromycin (15-membered-ring derivative), tylosin and desmycosin (16-membered-ring macrolides). Some macrolides, such as spiramycin, are used in both animals and humans.

PHYSICOCHEMICAL CHARACTERISTICS

The main physicochemical properties of macrolides and ketolides are presented in Table 135.1.

Stability in acidic medium

Erythromycin A and, to a lesser extent, spiramycin and josamycin are unstable in an acidic medium. Erythromycin A is degraded by the formation of a hemiketal or a spiroketal form of erythronolide A (Fig. 135.3).

Josamycin (leucomycin A_3) may be converted in an acidic medium to isojosamycin, which has a hydroxyl group at C-13 because of the rearrangement of the dienole system of josamycin.

Spiramycin is a complex formed by three major components (I–III), which differ in the substituent at position 3 of the lactone nucleus: 3-OH (spiramycin I), 3-O-acetyl (spiramycin II) and 3-O-propionyl (spiramycin III). The spiramycins are much more stable than erythromycin A in an acidic medium.

Roxithromycin, clarithromycin and azithromycin are much more stable in an acidic medium than erythromycin A.

Roxithromycin is highly stable at pH 4.2 (Fig. 135.4) compared to erythromycin base and erythromycin A, 2′-propionate. Clarithromycin is 6-O-methylerythromycin A, and the hydroxyl at position 11 remains free. A degradation product devoid of antibacterial activity occurs under conditions of extreme acidity and is termed pseudoclarithromycin (Fig. 135.5).

Azithromycin and roxithromycin are hydrolyzed at the β-O-glycoside bond at position 3 in a highly acidic medium (pH 1.2). The resultant descladinose degradation product has a 3-hydroxyl group on the lactone nucleus. These compounds are highly stable in an acidic medium (Fig. 135.6).

The different ketolides are very stable in an acidic medium, even if the pH is 1.2 depending on the substituent fixed on aglycone. This stability is different from that obtained with clarithromycin, azithromycin or roxithromycin.

Ketolides such as telithromycin are not destroyed at pH 1.2 after more than 6 hours of exposure.

IN-VITRO ACTIVITY (SEE ALSO TABLE 135.2)

The natural antibacterial spectrum of the macrolides, irrespective of their structure, includes Gram-positive bacteria (e.g. *Staphylococcus* spp., *Streptococcus* spp., *Listeria* spp., *Erysipelothrix insidiosa*, *Corynebacterium* spp., *Lactobacilli* spp., *Leuconostoc* spp., *Pediococcus* spp.) and Gram-negative cocci along with fastidious Gram-negative bacilli (e.g. *Haemophilus*

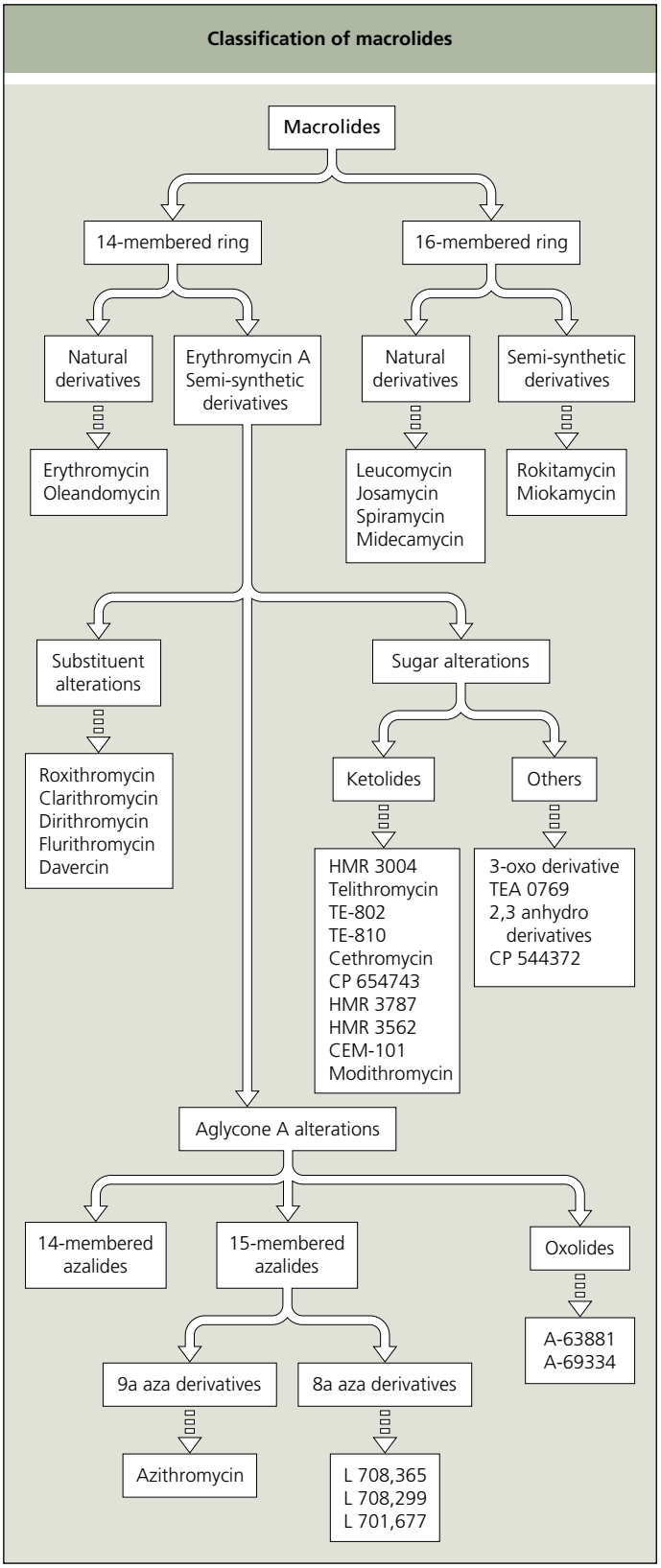


Fig. 135.2 Classification of macrolides.

influenzae, *Moraxella catarrhalis*, *Pasteurella* spp. and *Bordetella* spp.). Some anaerobic bacteria, intracellular bacteria, atypical bacteria such as mycoplasmas and related species are also susceptible to the macrolides. Some atypical mycobacteria, spirochetes, bacteria responsible for gastrointestinal infections (*Vibrio cholerae* and other *Vibrio* spp.,

Table 135.1 Main characteristic of macrolides and ketolides

	pK	Molecular weight
Erythromycin A	8.6	733.94
Oleandomycin	8.5	687.89
Clarithromycin	8.3	747.96
Roxithromycin	9.2	837.04
Dirithromycin	9.2	835
Erythromycylamine	8.9	734
Flurithromycin	–	751.95
Azithromycin	8.9–9.1	748.9
Josamycin	7.1	828.02
Midecamycin	6.9	813.99
Spiramycin I	7.7	843.06
Miocamycin	6.5	898.05
Rokitamycin	6.3	828
Telithromycin	8.7	812

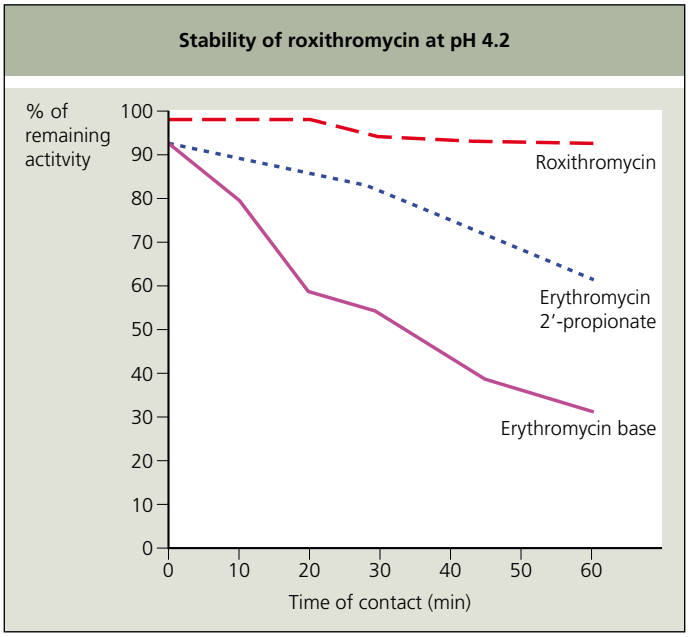


Fig. 135.4 Stability of roxithromycin at pH 4.2.

Campylobacter spp. and *H. pylori*) and *Treponema pallidum* remain susceptible to macrolides. They also display activity against *Leptospira*, *Borrelia* and *Bartonella* spp. However, these molecules are weakly active against enterococci and are moderately active against the viridans group of streptococci.

Erythromycin A overall is less active than clarithromycin but more active than azithromycin, roxithromycin and dirithromycin. Erythromycin A is the reference macrolide of natural origin. It is 10 times more active than oleandomycin, 2–10 times more active than josamycin, 2–12 times more active than spiramycin and up to 10 times more active than midecamycin.

The antibacterial spectrum of telithromycin, cethromycin and others encompasses that of the macrolides.

Telithromycin is more active *in vitro* than clarithromycin against Gram-positive and Gram-negative cocci, and also against erythromycin A-susceptible strains of Gram-positive bacilli. It is active against strains of Gram-positive cocci resistant to erythromycin A by an efflux or (inducible) MLS_B mechanism of resistance.

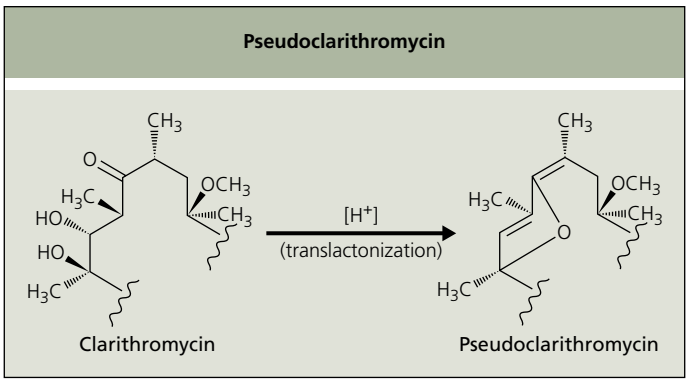


Fig. 135.5 Pseudoclarithromycin.

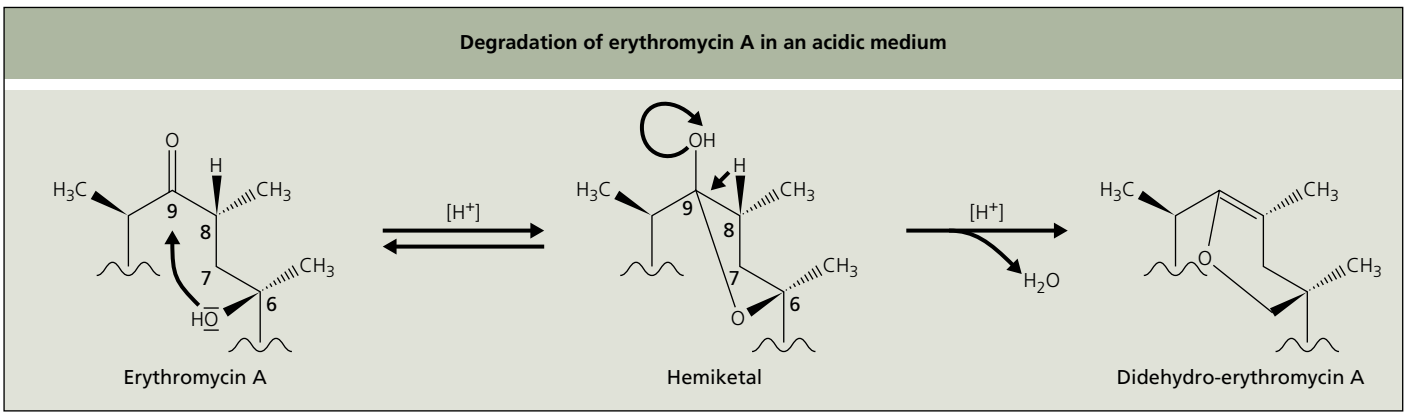


Fig. 135.3 Degradation of erythromycin A in an acidic medium.

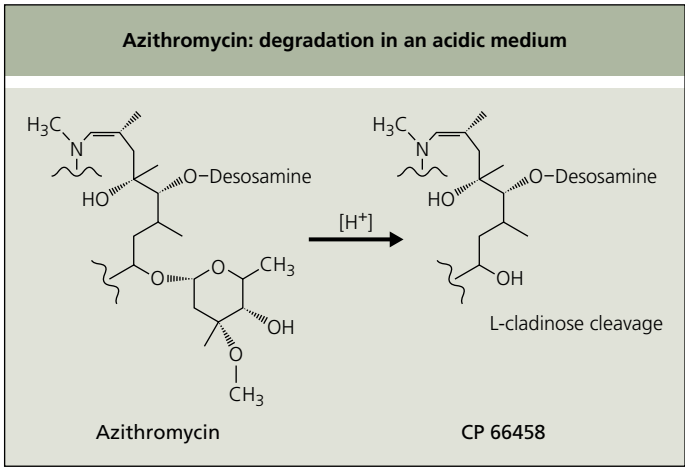


Fig. 135.6 Azithromycin: degradation in an acidic medium.

Telithromycin, like erythromycin A, is very active against β-hemolytic streptococci of Lancefield groups A, B, C and G when the strains are susceptible to erythromycin A. The *in-vitro* activity of telithromycin remains excellent against erythromycin A-resistant strains, particularly where an efflux mechanism is involved.

Ketolides remain highly susceptible against isolates of *Strep. pneumoniae* whatever their phenotype of resistance to erythromycin A.

Telithromycin displays a good activity against viridans group streptococci, irrespective of the resistance phenotype (erythromycin A-resistant, penicillin G-resistant). The Gram-positive bacilli comprise several bacterial genera and species, including *Listeria* spp., *Bacillus* spp., *Erysipelothrix rhusiopathiae*, coryneforms and lactic bacilli.

Diphtheria antibiotic treatment is based on penicillin G or erythromycin A. Rare erythromycin A-resistant strains have been described. Telithromycin is very active against *Corynebacterium diphtheriae*, whether or not the strain is toxigenic.

The *in-vitro* activity of macrolides and ketolides against *Listeria monocytogenes* and *Erysipelothrix rhusiopathiae* is good, and some reports have shown good *in-vitro* activity against *Bacillus anthracis*.

Table 135.2 *In-vitro* activity of macrolides and ketolides

	MIC_{50} (mg/l)				
	ERY	**CLA**	**AZI**	**ROX**	**TEL**
Staph. aureus (methicillin sensitive)	0.25	0.25	1.0	0.5	0.06
Strep. pyogenes	0.06	0.015	0.06	0.12	≤0.008
Strep. agalactiae	0.06	0.015	0.06	0.12	0.008–0.12
Streptococcus groups C and G	0.06	0.03	0.12	0.12	≤0.008
Strep. pneumoniae	0.06	0.015	0.06	0.06	0.015
Enterococcus faecalis	4.0	4.0	8	8	0.12
Enterococcus faecium	>64	>64	>64	>64	2.0
Viridans streptococci	2.0	1.0	4.0	4.0	≤0.03–0.25
Listeria monocytogenes	0.25	0.06	0.5	0.25	0.03
Erysipelothrix rhusiopathiae	0.03	0.06	0.5	0.06	≤0.015
Corynebacterium diphtheriae	0.008	0.004	0.015	0.008	0.004
Nocardia spp.	16	16	>128	64	–
Pediococcus spp.	0.125	0.06	0.125	0.06	0.008
Lactobacillus spp.	0.06	0.015	0.06	0.06	≤0.03
Leuconostoc spp.	0.06	0.03	0.125	0.125	0.25
Corynebacterium jeikeium	>128	>128	>128	>128	0.06
Corynebacterium urealyticum	>64	>64	>64	>64	≤0.015
Rhodococcus equi	0.5	0.06	1.0	0.25	0.25
Gardnerella vaginalis	0.06	0.06	0.06	0.03	≤0.002
Neisseria meningitidis	0.12	0.015	0.015	0.06	0.0015–0.12
Neisseria gonorrhoeae	0.25	0.25	0.12	0.5	0.12
Moraxella catarrhalis	0.12	0.06	0.03	0.12	0.06
Haemophilus influenzae	4.0	4.0	1.0	8.0	1.0
Pasteurella multocida	4.0	2.0	1.0	4.0	1.0
Bordetella pertussis	0.06	0.015	0.03	0.12	0.01
Campylobacter jejunii	1.0	1.0	0.12	4.0	0.5–1.0
Haemophilus ducreyi	0.06	0.008	0.015	0.03	0.004

Table 135.2 *In-vitro* activity of macrolides and ketolides—cont'd

	MIC_{50} (mg/l)				
	ERY	**CLA**	**AZI**	**ROX**	**TEL**
Helicobacter pylori	0.25	0.015	0.25	0.12	0.25
Mycoplasma pneumoniae	0.0039	0.019	0.0024	0.0039	0.00097
Legionella pneumophila	0.25	0.03	0.12	0.12	0.03
Chlamydophila pneumoniae	0.03	0.125	0.125	0.125	0.125
Borrelia spp.	0.015–0.03	0.0006–0.0025	0.002–0.008	0.0025–0.006	–
Bartonella spp.	0.06	0.03	0.03	–	0.006

ERY, erythromycin A; CLA, clarithromycin; AZI, azithromycin; ROX, roxithromycin; TEL, telithromycin.

The lactic bacilli form a complex group that includes *Lactobacillus*, *Leuconostoc*, *Pediococcus* and *Lactococcus* spp. against which telithromycin has shown a good *in-vitro* activity.

A number of coryneforms are involved in nosocomial infections, such as *Corynebacterium jeikeium* and *Corynebacterium urealyticum*. Macrolides are inactive against *C. jeikeium* and *C. urealyticum*; in contrast, telithromycin is very active against the majority of corynebacteria. Telithromycin activity against *C. jeikeium* is distributed in a bimodal population. There are discrepancies among authors with respect to the activity against *C. urealyticum*.

Macrolides are weakly active or inactive against enterococci; in contrast, telithromycin exhibits good activity against *Enterococcus faecalis*.

Neisseria meningitidis strains of reduced susceptibility to penicillin G have been described while rifampin (rifampicin)-resistant strains are increasingly common. Some erythromycin A or spiramycin-resistant strains have been observed which harbor an efflux mechanism of resistance mediated by Mtr (encoded by the *mtr* gene). The activity of telithromycin remains good.

Macrolides and ketolides are inactive against Enterobacteriaceae with few minor exceptions. Azithromycin exhibits a certain *in-vitro* activity against *Salmonella*, *Shigella* spp. and *Escherichia coli*.

Macrolides and ketolides are highly active against *Leptospira interrogans* whatever the serovar (minimum inhibitory concentrations (MICs) range from 0.01 to 0.04 mg/l) and *Bartonella* spp. (MICs 0.03–0.125 mg/l).

Lyme disease is caused by *Borrelia burgdorferi* which is divided into at least three subspecies which are responsible for different syndromes: rheumatismal (*B. burgdorferi sensu lato*), neurologic (*B. garinii*) and dermatologic (*B. afzelii*). Activity of macrolides/ketolides is species dependent.

Bacteria responsible for lower respiratory tract infections

Lower respiratory tract infections, whether parenchymatous (pneumonia) or nonparenchymatous (acute bronchitis or acute exacerbation of chronic bronchitis), are due to common respiratory pathogens or to intracellular or atypical bacteria.

The pathogens most commonly involved in lower respiratory tract infections are *Strep. pneumoniae*, *H. influenzae*, *Staph. aureus* and, more marginally, *Moraxella catarrhalis*.

The determination of the *in-vitro* activity of an antibacterial agent against *H. influenzae* is poorly standardized. Telithromycin has *in-vitro* activity similar to that of azithromycin against *H. influenzae* and greater than that of clarithromycin and roxithromycin which are poorly active. *Bordetella pertussis* is responsible for whooping cough. Macrolides remain the standard antibiotic treatment. Erythromycin A-resistant strains are of rare occurrence. Activities of telithromycin and other macrolides are good.

Mycoplasma pneumoniae is susceptible to macrolides and ketolides and is also responsible for a so-called atypical pneumonia. Pneumonia due to *Chlamydophila pneumoniae* and *L. pneumophila* is highly susceptible to macrolides-ketolides.

In-vitro activities of macrolides and telithromycin against unusual bacteria involved in a pathologic process have been investigated.

Parachlamydia and *Simkania*

Trophozoites of *Acanthamoeba* hosting *Chlamydia*-like bacteria have been isolated from patients with fever associated with the use of humidifiers in Vermont (Hall's coccus strain) and from the nasal mucosa (strain Bn9). The use of antibiotics directed against *Parachlamydia* has been reported from patients suffering from community-acquired pneumonia. *Parachlamydia* does not grow on an acellular medium.

Tropheryma whipplei

T. whipplei is responsible for Whipple's disease, which is characterized by arthralgia and diarrhea.

The growth of *T. whipplei* in MRC5 cells is inhibited by macrolides/ketolides according to the method of quantification of the copies of DNA.

Bacteria involved in sexually transmitted diseases

Macrolides are highly active *in vitro* against *Neisseria gonorrhoeae*, irrespective of the resistance profile of the strain isolated (resistance to penicillin G, spectinomycin, tetracyclines, etc.), but the therapeutic activity is often disappointing.

Strains of *Treponema pallidum* resistant to erythromycin A and azithromycin have been reported.

Chancroid is caused by *Haemophilus ducreyi*. Macrolides represent the standard treatment; telithromycin is highly active against this bacterial species.

Chlamydia trachomatis is the causative agent of trachoma (ophthalmic disease) in endemic regions, nongonococcal urethritis and much pelvic inflammatory disease. The activity of macrolides/ketolides is good, with MICs ranging from 0.008 mg/l to 0.06 mg/l.

The *in-vitro* activity of macrolides/ketolides against *Ureaplasma urealyticum* is good, with MICs of 0.06 µg/ml.

Fourteen- and 15-membered-ring macrolides are inactive against *Mycoplasma hominis*, but 16-membered-ring macrolides exert good *in-vitro* activity; activity of ketolides is structure dependent.

Anaerobic bacteria

Macrolides exhibit variable activity against Gram-positive anaerobes and are poorly active against Gram-negative anaerobic bacilli. Telithromycin possesses excellent *in-vitro* activity against Gram-positive bacteria, moderate activity against Gram-negative bacilli of the *Bacteroides* group, good activity against *Porphyromonas* and *Prevotella* spp. and variable activity against *Fusobacterium* spp.

MECHANISM OF ACTION

The macrolides/ketolides inhibit protein synthesis by binding to the 50S subunit of the bacterial ribosomes. In the presence of macrolides/ketolides, peptidyl tRNA accumulates in the bacterial cell, causing depletion of the free transfer tRNA needed for activation of the α-amino acids. The presence of erythromycin A causes the release of a basic amino acid-rich peptide chain by transient inhibition of transpeptidation. Translocation is impaired by the nascent peptide, which adheres to the surface of the ribosome, generating friction forces. This phenomenon is optimal at certain chain lengths when it becomes a floating strip. What appears certain is that erythromycin A modifies the interaction between peptidyl tRNA and the peptidyltransferase donor site.

Intrabacterial penetration

Before reaching ribosomes, the molecule has to cross one (Gram-positive) or two (Gram-negative) barriers. Erythromycin A and its derivatives penetrate Gram-positive bacteria and also *H. influenzae* passively. The affinity for ribosomes is higher for ketolides than for erythromycin A.

Exit tunnel

The exit tunnel passes and spans through the middle of the 50S ribosomal subunit and is paved mainly by RNA loop. The tunnel accommodates a peptide of 25–40 amino acids of any sequence and any conformation, and is formed with proteins L22 and L4.

The binding site of macrolide, lincosamide and streptogramin B (MLSB) antibacterials is located in the entrance of the exit tunnel before the ribosomal proteins L4 and L22 constrict it, causing destabilization of peptidyl tRNA during translocation and inducing dissociation of peptidyl tRNA from the ribosomes during the elongation phase of protein synthesis.

Fourteen- and 15-membered-ring macrolides reduce the diameter of the tunnel entrance blocking the progression of the nascent peptide by steric hindrance.

Lincosamides and 16-membered-ring macrolides which possess an L-mycarose sugar inhibit the peptidyltransferase enzymatic activity. However, 14- and 15-membered-ring macrolides as well as streptogramin B analogues block the entrance of the exit channel of the 50S ribosomal subunit.

The translation reaction contains a peptidyl-tRNA hydrolase. This enzyme hydrolyzes peptidyl tRNAs that dissociate from the ribosome by drop off, but not those remaining bound to the ribosome. The length of the drop off nascent peptide is compound-dependent.

Peptidyltransferase center

The peptidyltransferase center consists of domains II and V, which formed a pocket with 5S rRNA. Macrolides and ketolides are fixed on domain V; ketolides are also fixed on domain II.

Cethromycin and telithromycin interact like erythromycin A with domain V through desosamine sugar and aglycone.

Ketolides are composed of two additional parts: C11–C12 carbamate residue and a long side chain either on the carbamate moiety for telithromycin or through the 6-hydroxyl group for cethromycin.

INTRACELLULAR CONCENTRATIONS

The macrolides penetrate and accumulate in phagocytic cells, thereby allowing the treatment of infections due to intracellular bacteria, such as *Chlamydia*, *Legionella*, *Rickettsia* spp. and *Francisella tularensis*.

The mean ratios of cell concentrations to those present in the extracellular medium are presented in Table 135.3.

Like azithromycin, telithromycin gradually accumulates in polymorphonuclear neutrophils, particularly in the granules (60%), and is eliminated progressively from the cell (efflux). Telithromycin uses a transport system to penetrate the phagocytic cell.

MECHANISM OF RESISTANCE

Epidemiology

Since the first report in the mid-1970s of *Strep. pneumoniae* multidrug-resistant strains in South Africa, the prevalence rate of erythromycin A resistance has increased worldwide. It is the same for *Strep. pyogenes* and other Gram-positive cocci. Surveillance programs such as Protek also include detection of phenotypes and genotypes of resistance. For 2004–2005, 116 centers were enrolled in 35 countries. The prevalence rate of erythromycin A resistance worldwide was 35%. The main mechanism of resistance was methylation (*erm B* gene) and represented about 66% of the resistant isolates. In the USA, the main mechanism of resistance was efflux (*mef A* gene) and represented about 80% of the resistant isolates.

In Europe, the highest rate of resistance was recorded in France, Spain and Italy where resistance rates were 52.5%, 32.2% and 33.5%, respectively, for *Strep. pneumoniae*. In Northern Europe except Belgium (31.8%), resistance rates were low.

Molecular mechanisms of resistance

There are currently four recognized mechanisms of resistance to erythromycin A:

- modifications in the ribosomal target;
- inactivation of the molecule;
- efflux; and
- mutations in the 23S ribosomal RNA and ribosomal proteins L4 and L22.

These resistance mechanisms are described in detail in Chapter 131.

Table 135.3 Accumulation ratio of the main macrolides (*in vitro*)

	Cells	C/E
Erythromycin A	PMN MA	4.4–18 18–38
Roxithromycin	PMN MA	14–100 61
Clarithromycin	PMN	9–16
Dirithromycin	PMN MA	7–60 668
Spiramycin	MA	20–35
Josamycin	PMN	16
Rokitamycin	PMN	30
Azithromycin	PMN	80–300
Telithromycin	PMN	250–300

C/E, accumulation ratio; MA, alveolar macrophages; PMN, neutrophils.

PHARMACODYNAMIC PROPERTIES

The thigh-infection models in neutropenic and normal mice have shown that macrolides/ketolides can be separated in two subgroups: those that are time dependent (T > MIC) such as erythromycin, roxithromycin, clarithromycin, and those that are concentration dependent such as azithromycin and dirithromycin.

The pharmacodynamics of telithromycin data indicates that there is a good correlation between efficacy and concentration, particularly the area under the curve (AUC).

PHARMACOKINETIC PROPERTIES

Adult volunteers

The absorption and bioavailability vary according to the molecule, partly explaining the difference in unit dosage and frequency of administration of the different macrolide or ketolide drugs.

Absorption can be rapid, with a lag time of 0.25 hours (roxithromycin, clarithromycin). The average peak serum concentration ranges from 0.4 mg/l (azithromycin 500 mg) to 11 mg/l (roxithromycin 300 mg). It is reached after a period of between 0.6 hour (rokitamycin) and 4.0 hours (dirithromycin) and is dose dependent. The relative bioavailability (AUC/F) ranges from 3.7 mg.h/l (rokitamycin) to 132 mg.h/l (roxithromycin 300 mg) and is dependent on the administered dose, but is not proportional to the dose for certain molecules with nonlinear pharmacokinetics. The apparent elimination half-life ranges from 0.1 hour (miocamycin) to 44 hours (dirithromycin). The absolute bioavailability of the different molecules ranges from 10% (dirithromycin) to about 50–60% for roxithromycin and clarithromycin. It is of the order of 37% for azithromycin and 14-hydroxyclarithromycin, the main metabolite of clarithromycin.

Plasma protein binding is mainly to the α_1-glycoproteins for macrolides, ranging from 15% (josamycin) to more than 90% (roxithromycin).

For telithromycin and cethromycin, peak plasma concentrations varied from 1.9 mg/l (telithromycin 800 mg) to 0.14–1.7 mg/l (cethromycin 100 mg and 1200 mg, respectively) and were reached 1.0 hour after administration and the apparent elimination half-life from 3.5–6.6 hours (cethromycin) to 7.16 hours (telithromycin 800 mg) (see Table 135.4).

Elderly subjects

In elderly patients with no liver abnormalities the pharmacokinetic profiles were similar to those of adults (Table 135.5). As macrolides are not eliminated via the kidney, there is no need to modify the daily dosing in renal insufficiency.

Table 135.4 Pharmacokinetics of macrolides and ketolides

	Dose (mg)	Compound or metabolite	C_{max} (μg/ml)	T_{max} (h)	AUC/F (μg/h/ml)	T½ β (h)
Azithromycin	500 500	– –	0.35 ± 0.1 0.59 ± 0.2	0.9 ± 0.9 2.2 ± 0.02	3.58 ± 1.2 3.35 ± 0.4	– 41.2 ± 4.3
Clarithromycin	100 200 – 400 – 600 – 800 – 1200 – 250 – 500 –	C C OH-C C OH-C C OH-C C OH-C C OH C OH C OH	0.35 0.6 0.41 0.13 0.78 2.03 1.06 2.63 1.02 3.97 1.54 0.7–0.8 0.6–0.7 1.77–1.89 0.7–0.8	1.46 1.56 2.29 1.86 2.11 1.83 2.45 2.67 2.72 2.2 2.61 1.7 ± 1.9 2.2–2.3 2.3 2.3–2.8	1.67 2.99 3.4 8.55 7.52 15.44 9.95 24.73 10.72 44.6 23.9 4–4.2 4.6–4.9 11.1–11.7 6.1–6.9	2.27 2.3 3.37 3.6 3.89 3.64 3.84 4.25 5.09 5.98 9.19 2.6–2.8 3.9–5.1 3.3–3.5 6.4–6.6
Dirithromycin	250 500 750 1000	– – – –	ND 0.29 ± 0.2 0.64 ± 0.4 0.41 ± 0.2	– 4 4 4	– 0.86 ± 0.6 1.84 ± 1.2 1.61 ± 1.1	– 32.5 30.6 31.9
Roxithromycin	150 300 450	– – –	7.9 ± 0.6 10.8 ± 0.6 12.2 ± 0.7	1.9 1.5 1.3	81 ± 10 132 ± 17 170 ± 20	10.5 ± 1.4 11.9 ± 0.5 13.8 ± 0.9
Flurithromycin	500 750 375	– – –	1 ± 0.7 1.58 ± 0.3 1.41 ± 0.5	0.92 0.72 1	0.4 3.52 4.41	3.54 2.8 3.94

(Continued)

Table 135.4 Pharmacokinetics of macrolides and ketolides—cont'd

	Dose (mg)	Compound or metabolite	C_{max} (μg/ml)	T_{max} (h)	AUC/F (μg/h/ml)	T½ β (h)
Oleandomycin	1000	–	2.8	2.6	10.8	3.4
Spiramycin	1000 1500 2000 500	– – – –	0.96 1.53 1.65 2.14	3 3 4 –	5.43 9.8 11.26 6.19	5.37 5.52 6.23 4.51
Josamycin	1000	–	2.74	0.75	4.2	1.5
Josamycin	400 1000	– –	0.33 2.41	0.77 0.75	0.91 4.9	1.51 2
Oleandomycin	1000	–	4	1	14	4.2
Midecamycin	400	–	0.25	0.5	0.34	1
Midecamycin	600 1200	– –	0.8 1.9	1 2	– –	– –
Rokitamycin	600	–	1.9	0.6	3.7	2
Miocamycin	600 400 – – 800 – – 800 – –	– Mb12 Mb6 Mb9a Mb12 Mb6 Mb9a Mb12 Mb6 Mb9a	3.01 0.6 0.17 0.16 1.37 0.44 0.4 1.73 0.66 0.57	2 0.48 0.6 0.73 0.52 0.65 0.83 0.79 0.83 1.06	3 0.73 0.28 0.27 1.72 0.76 1.46 2.59 1.46 1.33	1 1.24 1.47 1.45 1.32 1.71 2.34 1.31 2.34 2.09
Telithromycin	800	–	1.90	1.0	8.25	7.16

C, parent compound; Mb, metabolite; OH-hydroxyl metabolite.

Table 135.5 Pharmacokinetics of macrolides in elderly patients

		Dose (mg)	Route	C_{max} (μg/ml)	T_{max} (h)	$AUC_{0-\infty}$ (μg/h/ml)	T½ (h)
Spiramycin adipate	Young Elderly	500 500	iv iv	2.14 2.51		6.7 16.7	4.5 9.8
Josamycin	Young Elderly	1000 1000	po po			6.25 6.25	1.69 3.4
Miocamycin (Mb12)	Young Elderly	800 800	po po	1.34 1.15	0.67 0.58	1.92 2.52	1.24 2.36
Roxithromycin	Young Elderly	300 300	po po	9.7 10.8	1.6 2.06	122.6 197.3	11.2 15.5
Dirithromycin	Young Elderly	500 500	po po	0.34 0.52	3.90 4.80	0.91 2.42	
Clarithromycin	Young Elderly	500 500	po po	2.41 3.28		18.9 30.8	4.9 7.7
14-OH clarithromycin	Young Elderly	500 500	po po	0.66 1.33			7.2 14.0
Azithromycin	Young Elderly	500 500	po po	0.41 0.38	2.5 3.8	2.5 3.0	
Telithromycin	Young Elderly	800 800	po po	3.0 1.9	0.5 1.0	11.56 7.25	11.46 10.64

CLINICAL STUDIES

High concentrations in the tissues and biologic fluids of the main respiratory tract sites can be detected following single or multiple doses of macrolides (Table 135.6). For telithromycin, the concentrations in different part of the lung tissue after a single dose of 800 mg are summarized in Table 135.7.

TOLERANCE

Macrolides have the capacity to interfere with the metabolism and pharmacokinetics of other drugs (Table 135.8) and other drugs may produce adverse events when administered with macrolides (Table 135.9).

MACROLIDES/KETOLIDES VERSUS OTHER ANTIBACTERIALS: ADVANTAGES

Macrolides are considered safe drugs. They are mainly an alternative to β-lactam antibiotics when the latter are ineffective against main common pathogens or the patient is hypersensitive to β-lactams. However, some primary indications remain for macrolides such as diphtheria, chancroid, *Helicobacter pylori and Mycobacterium avium*.

One of the major uses for macrolides is atypical and intracellular pathogenic infections such as *Chlamydia* and *Legionella* spp. in parenchymal lower respiratory tract infections or nongonococcal urethritis.

Oral antibacterials are mainly composed of β-lactams (penicillins, cephems), macrolides/ketolides, fluoroquinolones, cyclines, ansamycins and, where available, oral streptogramins.

One of the main advantages of macrolides compared to other compounds is their ability to be used during pregnancy, in the pediatric setting and in adult and elderly patients. Fluoroquinolones and tetracyclines are contraindicated during pregnancy and in pediatrics. However, since fluoroquinolones possess an oral and an intravenous formulation they may be useful in those patients in whom intravenous formulations of macrolides are not well tolerated.

Lincosamides

INTRODUCTION

Lincomycin was isolated in 1962 from fermentation of *Streptomyces lincolnensis* subsp. *lincolnensis*. Numerous analogues have been prepared from lincomycin by semisynthesis to enlarge the anti-

Table 135.6 Tissue distribution of macrolides (peak concentrations mg/kg and µg/ml)

	Dose (mg)	Serum (µg/ml)	RESPIRATORY TRACT		
			Bronchial mucosa	Bronchial secretions	Sinus
Erythromycin	500**	3.08	7.20	1.05	0.9–1.8
Oleandomycin	200*	6.26		3.77	2.68
Roxithromycin	150** 500**	2.51 2.4		3.10	4.15 2.18
Spiramycin	2000**	0.39	13–36	7.3	2–8.8
Josamycin	1000**	2.3		1.60	2.8
Miocamycin	600**			5.16	
Azithromycin	500*		3.89	0.23	1.34
Dirithromycin	500**	0.22	1.9	1.3	

*Single dose;
**Multiple doses.

Table 135.7 Telithromycin concentration in lung tissues

Sampling time (h)	No.	Plasma concentration (mg/l)	CONCENTRATION OF TELITHROMYCIN		
			Bronchial mucosa (mg/kg)	ELF (mg/l)	Alveolar macrophages (mg/l)
1–3	5	1.07	0.68	5.4	65
6–8	6	0.61	2.2	4.2	100
24	6	0.07	3.5	1.17	41
48	6	ND	ND	ND	2.15

ELF, epithelial lining fluid; ND, not detectable.

Table 135.8 A selection of drugs whose metabolism may interfere with that of macrolides

- Alfentanil, sufentanil
- Antipyrine
- Astemizol
- Bromocriptine
- Carbamazepine
- Ciclosporin A
- Cimetidine
- Cisapride
- Clozapine
- Diltiazem
- Digoxin
- Dysopyramide
- Ergotamine
- Ethylestradiol
- Felodipine
- Glibenclamide
- Levodopa, carbidopa
- Lovastatin
- Methylprednisolone
- Phenytoin
- Rifampin (rifampicin), rifabutin
- Sodium valproate
- Theophylline
- Triazolam, midazolam
- Verapamil
- Warfarin
- Zidovudine

bacterial spectrum and enhance antibacterial activity. A series of 7-chlorolincomycin derivatives has been synthesized, yielding clindamycin. Two human drugs have been introduced in clinical practice, lincomycin and clindamycin; pirlimycin is used in veterinary medicine.

CHEMICAL STRUCTURES

The lincosamides consist of three components: an amino acid, a sugar and an amide bond connecting these two moieties to one another. The amino acid residue is a substituted L-proline. The sugar is lincosamine.

Clindamycin is the 7(*S*)-chlorodeoxylincomycin derivative. Pirlimycin is the 4-*cis*-ethyl-L-pipecolic derivative of clindamycin. For oral use, a palmitate salt of clindamycin hydrochlorine is available; for injection, clindamycin phosphate (1200 mg equivalent to 1000 mg clindamycin base) is available.

A number of compounds with which lincomycin is incompatible have been reported, including β-lactams (penicillin G, cloxacillin, methicillin, ampicillin, ticarcillin, cephalothin), novobiocin, hydrocortisone, streptomycin, vitamin B and potassium canrenoate. Compounds reported as being incompatible with clindamycin phosphate include ampicillin, calcium gluconate, magnesium sulfate, phenytoin, group B vitamins and barbiturates.

Solutions containing tobramycin sulfate and clindamycin phosphate are unstable.

ANTIBACTERIAL ACTIVITY

Lincosamides are antibiotics whose antibacterial spectrum includes Gram-positive cocci, anaerobic bacteria and certain so-called atypical bacteria. Enterobacteriaceae, *Pseudomonas* spp. and *Acinetobacter* spp. are excluded from the antibacterial spectrum of the lincosamides.

The lincosamides possess good antistaphylococcal activity. They are active against methicillin-susceptible *Staph. aureus* strains but not against methicillin-resistant strains (MIC >64 mg/l). They are active against coagulase-negative staphylococci, with the exception of *Staph. cohnii*, *Staph. sciuri* and *Staph. xylosus*, which have low-level resistance to lincomycin and to streptogramin B. The MICs of lincomycin are 4–8 mg/l, as opposed to 0.5–1 mg/l for susceptible strains. They are inactive against enterococci. They possess good activity against group A and B streptococci, viridans group streptococci and *Strep. pneumoniae*. These strains appear to be inhibited by concentrations of 0.4 mg/l. The lincosamides are active against *Corynebacterium diphtheriae*, *Nocardia* spp. and *Bacillus anthracis* but are inactive against *Listeria monocytogenes*.

Clindamycin is active against *Erysipelothrix rhusiopathiae*. They are inactive or weakly active against *Neisseria meningitidis* and *Neisseria gonorrhoeae*, *H. influenzae*, *Moraxella catarrhalis* and *Pasteurella* spp. Clindamycin is active against *Bordetella pertussis*, but lincomycin is inactive. Clindamycin is inactive against *Bordetella bronchiseptica*. Against *Campylobacter jejuni*, clindamycin possesses good activity (MIC_{50} 0.4 mg/l), whereas lincomycin is inactive. The activity of clindamycin against *Helicobacter pylori* is good (MIC 1 mg/l).

The lincosamides are inactive against *Legionella pneumophila*. Clindamycin has activity against *Chlamydia trachomatis* (MIC 1.0 mg/l), whereas lincomycin is inactive (MIC 256 mg/l). In contrast to the 14-membered-ring macrolides, apart from certain ketolides, clindamycin is active against *Mycoplasma hominis* but is weakly active against *Mycoplasma pneumoniae* and *Ureaplasma urealyticum*. Clindamycin

Table 135.9 Drug interference by macrolides

	Theophylline	Carbamazepine	Ciclosporin A	Midazolam	Terfenadine
Erythromycin	+	+	+	+	+
Oleandomycin	+	+	+	+	NT
Azithromycin	–	–	NT	–	–
Clarithromycin	(+)	+	+	+	+
Dirithromycin	(+)	NT	+	NT	
Roxithromycin	(+)	–	(+)	+	+
Josamycin	+	–	+	NT	NT
Spiramycin	–	NT	–	NT	NT
Rokitamycin	(+)	NT	+	NT	NT
Midecamycin	–	NT	NT	NT	NT
Miocamycin	–	+	+	NT	NT
Flurithromycin	NT	+	NT	NT	NT

NT, not tested.

Table 135.10 *In-vitro* activity of clindamycin

Micro-organism(s)	MIC (mg/l)		
	50%	90%	Range
Staph. aureus Met^s	0.12	0.125	
Staph. aureus Met^r	1.0	>16	
Coagulase-negative staphylococci	0.25	>16	
Strep. pyogenes	0.03	0.06	
Strep. agalactiae	0.06	0.5	
Strep. pneumoniae Pen^s	0.125	0.25	
Strep. pneumoniae Pen^r	1.0	16	
Enterococcus spp.	>16	>16	
Viridans group streptococci	0.5	0.5	
Neisseria meningitidis			>5
Neisseria gonorrhoeae			2.0
Bordetella pertussis			0.25–>8
Bordetella bronchiseptica			≤32
Haemophilus influenzae			8.0
Mycoplasma pneumoniae			1.0
Mycoplasma hominis			0.03
Ureaplasma urealyticum			2.0
Chlamydia trachomatis			1.0
Campylobacter jejunii	0.4	0.8	0.1–>50
Helicobacter pylori	0.5	2.0	0.25–2.0
Gardnerella vaginalis	0.003	0.06	0.2–2.0
Nocardia spp.			0.78–0.25
Actinomyces spp.			0.03–0.25
Legionella pneumophila			12.5
Cornebacterium diphtheriae			≤0.2

Met^r, methicillin resistant; Met^s, methicillin sensitive; Pen^r, penicillin resistant; Pen^s, penicillin sensitive.

is active against *Gardnerella vaginalis*. Lincomycin is active against *Leptospira* spp. Clindamycin has good activity against anaerobes.

Among Gram-positive anaerobes, clindamycin exhibits good activity against *Clostridium perfringens*, *Clostridium tetani* and *Clostridium difficile*. Its activity against *Bifidobacterium*, *Propionibacterium* spp. and Gram-positive cocci is moderate. It exhibits good activity against *Eubacterium* spp.

Among Gram-negative anaerobes, clindamycin displays good activity against the *Bacteroides fragilis* group, with the exception of *Bacteroides thetaiotaomicron*, *Porphyromonas* spp. and *Prevotella* spp. Activity against *Veillonella* spp. is good (MIC >0.05 mg/l) (Table 135.10).

MODE OF ACTION AND RESISTANCE

The lincosamides act on the 50S ribosomal subunit of the bacterial ribosome. The binding sites are similar to those for erythromycin A. The lincosamides prevent transpeptidation during the formation of the nascent peptide chain by inhibiting peptidyltransferase.

The intrinsic resistance of Enterobacteriaceae, *Pseudomonas* spp. and *Acinetobacter* spp. is due to the relative impermeability of the bacterial outer membrane. In Gram-positive cocci, penetration is a passive phenomenon. The enterococci are resistant to the lincosamides. Acquired resistance to lincosamides may be due to at least three mechanisms: modification of the bacterial target, modification of the antibiotics and reduced permeability. The modification of the target is due to methylation of an adenine residue on the 23S rRNA of the 50S subunit by a bacterial methylase.

In the constitutive form of resistance in *Staph. aureus*, all macrolides, lincosamides and streptogramin B are also resistant (*erm* gene); conversely, when it is an inducible resistance, there is dissociation between the three families and the lincosamides remain active, even if the activity is reduced. In streptococci, in contrast to *Staph. aureus*, the lincosamides can induce resistance. Inducible resistance may be highlighted by the D-test in *Staph. aureus*.

A plasmid-mediated 3-lincomycin or 4-clindamycin-*O*-nucleotidyltransferase has been detected in *Staph. aureus*. There is high-level resistance to lincomycin and a reduction in susceptibility to clindamycin has been noted. In coagulase-negative staphylococci, the main mechanism of resistance is adenine methylation (MLS_B type) and is identical to that of *Staph. aureus*. These species possess more specific resistance mechanisms, such as a reduction in permeability to 14-membered-ring macrolides as has been described for *Staph. epidermidis*. The 14-membered-ring macrolides may also be inactivated enzymatically; this resistance phenotype has been described for *Staph. hominis*, *Staph. haemolyticus* and *Staph. saprophyticus*.

Like *Staph. aureus*, certain species may inactivate lincomycin or clindamycin by a plasmid-mediated enzyme: *O*-nucleotidyltransferase.

It has been shown that strains of *B. fragilis* are divided into three groups:

- those which are very susceptible to clindamycin (MIC ≤0.25 mg/l);
- those which are moderately susceptible (MIC 0.25–8 mg/l); and
- those which are resistant (MIC >16 mg/l).

Strains of *Strep. pneumoniae* and *Strep. pyogenes* susceptible to clindamycin and resistant to erythromycin A derivatives have been described. This dissociation is due to an efflux resistance mechanism (M phenotype).

Antiparasitic activity

In 1967, it was shown that clindamycin and its derivatives possessed good antiplasmodial activity. Several clinical studies have been conducted with clindamycin administered at a dose of 450 mg every 6 hours for 14 days to four patients to treat a *Plasmodium vivax* infection. A relapse occurred between 41 and 51 days after the end of treatment.

In nonimmune patients clindamycin (450 mg q6h for 3 days) was combined with quinine in the treatment of chloroquine-susceptible or chloroquine-resistant *P. falciparum* infections. A curative effect was obtained with the combination, but major gastrointestinal disorders were noted. Other studies have also been undertaken with various results obtained.

If clindamycin is used, it is recommended that a fast-acting schizonticide, such as quinine or amodiaquine, be administered first, followed by clindamycin, to avoid problems of gastrointestinal intolerance.

Activity against *Toxoplasma gondii*

Clindamycin has been proposed as alternative therapy in the treatment of encephalic toxoplasmosis in patients intolerant to sulfonamides.

There are contradictory studies on the *in-vitro* activity of clindamycin. Clindamycin exerts an inhibitory effect at 0.5 ng/ml and above, but the 90% inhibitory concentration is 100 mg/l. The discrepancy in the results between the different studies is probably due to slow inhibition of the growth of the parasite, which occurs after 24 hours.

It has been shown that clindamycin alone or in combination with pyrimethamine possesses curative activity in acute murine toxoplasmosis. Clindamycin exhibits protective activity against murine cere-

bral toxoplasmosis. Multicenter studies have shown beneficial effects of clindamycin combined with pyrimethamine in the treatment of acute cerebral toxoplasmosis. Clindamycin has also been proposed as treatment for toxoplasmic chorioretinitis.

PHARMACOKINETICS

Lincomycin

Lincomycin is presented in several pharmaceutical forms: oral, intravenous, intramuscular and rectal. *Micrococcus luteus* is used in the microbiologic assay and chromatographic methods have been described.

Oral form

After administration of 500 mg and 1000 mg, peak serum concentrations are reached in 2–4 hours and are 1.8–5.3 mg/l and 2.5–6.7 mg/l, respectively. The consumption of food affects the oral bioavailability. Concentrations in plasma are detectable for about 12 hours. Absorption of lincomycin represents about 20–35% of the administered dose. Fecal elimination is 30–40% of the administered dose over a period of 72 hours. The bioavailability of lincomycin after oral administration is not affected by celiac disease or colonic diverticulosis, but a reduction in the concentrations in plasma has been demonstrated in patients with Crohn's disease.

Intravenous form

After infusion over 1 hour, the concentrations obtained range from 9.1 mg/l (300 mg) to 36.2 mg/l (500 mg). The AUCs are proportional to the administered doses (300–1500 mg). The mean apparent elimination half-life is 5 hours and about 40% of the administered dose is eliminated in the urine.

Plasma protein binding, albumin, is dose dependent and ranges from 28% to 86%, depending on the dose and the method of determination. On average it is 75%.

Plasma clearance is dose dependent because of protein saturation and is 13.32 l/h. The clearance of the free fraction is between 28.6 l/h for 2400 mg intravenously and 36.4 l/h for 1200 mg intravenously. Renal clearance is weak – 43 ml/min (2.6 l/h). Urinary elimination ranged between 30% and 40%.

After an intravenous dose of 500 mg, fecal elimination of lincomycin is about 14% over 96 hours.

Intramuscular form

After a single intramuscular administration of 600 mg, the peak concentration in serum is between 7.2 and 18.5 mg/l and is reached after between 0.5 and 5 hours. After repeated doses of 600 mg, the residual concentration (8 hours) is 6.4 mg/l. After a daily dose of 500 mg, the residual concentration at 24 hours is 1.1 mg/l. The apparent elimination half-life range is 4–5 hours. The plasma clearance is dose dependent and is between 0.098 and 0.139 l/kg.

Urinary elimination is between 10% and 47%, depending on the study. Fecal elimination after a dose of 500 mg is 4–6% over a period of 48–72 hours.

The bioavailability after intramuscular administration appears to be absolute.

Rectal form

After administration of a 500 mg suppository of lincomycin, a peak concentration in serum of 1.1 mg/l is reached in 1.5 hours, with an AUC of 8.9 mg.h/l, compared to a peak of 2.8 mg/l and an AUC of 25.7 mg.h/l after administration of a rectal solution of lincomycin.

Specific underlying diseases

Renal impaired patients

After intramuscular administration of 600 mg of lincomycin, the apparent elimination half-life, the peak concentration in serum and the AUC are increased. Lincomycin is not dialyzable.

Hepatic impaired patients

After a single dose of 600 mg intramuscularly to patients with hepatic insufficiency, the apparent elimination half-life is increased (8.90 vs 4.85 hours) and the peak concentration in serum is decreased (9.2 vs 12.8 mg/l).

Metabolism

A small amount of lincomycin is inactivated in the liver. The metabolites have not been fully characterized and are devoid of antibacterial activity. Biliary elimination is extensive, ranging from 30% to 40% of the administered dose.

Clindamycin

Clindamycin is presented in the form of clindamycin phosphate for parenteral use and clindamycin hydrochloride for oral use. The assays are performed using a high-performance liquid chromatographic method or a microbiologic method using *Micrococcus luteus* as the test strain.

Intravenous form

Clindamycin phosphate is soluble in water. This solubility is pH dependent: at pH 7 the solubility is between 200 and 300 mg/ml, whereas at pH 4 it is 25 mg/ml. Clindamycin phosphate is administered in the form of an infusion over 10–45 minutes, depending on the dose to be administered. Clindamycin phosphate is slowly hydrolyzed to clindamycin, with a hydrolysis half-life of between 3 minutes (1200 mg) and 1 minute (300 mg). About 10% of the drug is present in the serum after 8 hours in the form of clindamycin phosphate. The peak concentration in serum of clindamycin appears about 3 hours after the end of the infusion and is between 5.4 mg/l (300 mg) and 15.87 mg/l (1200 mg). The apparent elimination half-life of clindamycin is 2–3 hours, whereas that of clindamycin phosphate is 0.04–0.16 hour. Plasma clearance is of the order of 0.18 l/h/kg for clindamycin and 0.40 l/h/kg for clindamycin phosphate. Clindamycin binding to α_1-glycoprotein ranges from 92% to 93%.

Clindamycin is mainly eliminated by the hepatobiliary route. The metabolites of clindamycin are found in the urine and stools, but about 28% of the parent compound is found in the urine.

Intramuscular form

After administration of single doses of 300, 450 or 600 mg of clindamycin phosphate, the peak concentrations in serum are between 3.17 and 6.56 mg/l and are reached in 1.5–3 hours. It is possible to detect clindamycin in the serum after 20 minutes. About 75% of the drug reaches the plasma in the form of clindamycin.

Oral form

After absorption, clindamycin palmitate (hydrochloride) is rapidly hydrolyzed in the intestinal lumen before being absorbed in the form of the hydrochloride and the palmitate is no longer detectable in the serum. The bioavailability is of the order of 80%. The degrees of absorption are identical after single and repeated doses. The bioavailability of clindamycin does not appear to be affected by the ingestion of food, although absorption is delayed by food consumption.

Clindamycin hydrochloride is poorly soluble in water and the solubility is pH dependent: when the pH is less than 6, the solubility is greater than 160 mg/ml, whereas between 7 and 8 the solubility is weak, of the order of 2.7 ± 1.0 mg/l.

After administration of a single dose of 150, 300 or 450 mg of clindamycin hydrochloride, the peak concentrations in serum are between 2.56 and 5.58 mg/l and are reached within 1 hour.

The dose eliminated in the urine ranges from 8% to 26%. A number of metabolites are eliminated in the urine. Traces of clindamycin hydrochloride are found there, in contrast to clindamycin phosphate, for which 1–2% of the administered dose is eliminated in this form.

Radiolabeled clindamycin hydrochloride (300 mg) was administered and the radioactivity was 61% and 28% over a period of 168 hours in feces and urine, respectively.

Metabolism

The free form of clindamycin is hydrolyzed in the liver. Seven metabolites have been described. Fecal and urinary elimination of the metabolites differ.

After a single dose of 300 mg of clindamycin hydrochloride, the compounds eliminated in the urine are distributed as follows: clindamycin 27%, clindamycin sulfoxide 37%, *N*-demethyl clindamycin 6% and clindamycin-*N*-demethyl sulfoxide 2%. A hydroxyl derivative and a carboxyl derivative, both incompletely identified, have been detected, and each accounts for about 15% of the compounds eliminated in the urine.

Patients with renal impairment

Clindamycin is mainly eliminated by the hepatobiliary route and metabolized in the liver. It is not significantly eliminated after hemodialysis or peritoneal dialysis.

It has been recommended that when the creatinine clearance is less than 10 ml/min the daily dosage be reduced by a half, as the apparent elimination half-life increases, with major interindividual variability (mean half-life of 5.28 hours compared to 2 hours).

In peritoneal fluid, about 3% of the dose of clindamycin phosphate is hydrolyzed to clindamycin in 2–4 hours. The peritoneal dialysis fluid must contain a concentration of 167 µg of clindamycin phosphate per ml to be certain of exerting therapeutic activity.

Elderly patients

After a single dose of 300 mg orally and intravenously the fractions absorbed were similar in young and elderly subjects (0.92 vs 0.85). The apparent elimination half-life and plasma clearance after intravenous administration did not differ significantly (2.46 vs 2.79 hours, 0.71 vs 0.75 l/kg, 3.36 vs 3.11 ml/min/kg, respectively. The dosage need not be modified in elderly subjects.

Patients with hepatic impairment

In patients with hepatic insufficiency, the apparent elimination half-life is prolonged from 5 to 15 hours compared to 2.5–3.6 hours in healthy volunteers. A reduction in the plasma clearance in cirrhotic patients has been demonstrated, with a reduction in protein binding to 79% (versus 93%).

Pregnancy

Clindamycin rapidly crosses the fetoplacental barrier. High concentrations of clindamycin have been detected in the liver, kidney and lungs, and low levels in cerebral tissue, bone or muscles of fetuses from spontaneous abortions occurring between the 10th and 22nd weeks of gestation.

Intraphagocytic concentration

The intracellular:extracellular concentration ratio in neutrophils is between 11 and 15, while in macrophages and monocytes it is 23. Inside the cell clindamycin is localized in the cytoplasm and granules. The ratio of intracellular:extracellular concentrations is of the order of 1.7:4.

Safety

The main side-effect of clindamycin is pseudomembranous colitis due to *Clostridium difficile*. Hypotension and even cardiorespiratory arrest have been reported at high intravenous doses of lincomycin, particularly after rapid administration. Skin rashes and drug fever have also been observed with clindamycin.

Streptogramins

INTRODUCTION

The streptogramins form a complex group of unique antibacterial agents. Pristinamycin (oral natural form) for historic reasons has limited clinical introduction, mainly in French-speaking countries. For the injectable form (dalfopristin–quinupristin) the clinical introduction is wide, including the USA. A new oral streptogramin – linopristin–flopristin (NXL 103, XRP 2868) – is currently in phase II clinical trials.

PROPERTIES OF NATURAL STREPTOGRAMINS

The streptogramins are composed of a mixture of two components: group A streptogramins and group B streptogramins These two components act synergistically at the microbiologic level. The two components are macrocyclic lactones and peptides, respectively.

Only two derivatives have been introduced in clinical practice: pristinamycin (Pyostacine) and virginiamycin (Staphylomycine). One injectable semisynthetic derivative has also been introduced in clinical practice, dalfopristin–quinupristin (Synercid), and one oral semisynthetic derivative is underdevelopment, linopristin–flopristin.

The ratios between the two groups of components are 30–40% for component I (group B) and 60–70% for component II (group A).

Antibacterial activities

These molecules exhibit different antibacterial activities, but their antibacterial spectra include Gram-positive cocci and bacilli, Gram-negative cocci and fastidious Gram-negative bacilli such as *Haemophilus*, *Moraxella*, *Bordetella* and certain intracellular bacteria (*Chlamydia* and *Rickettsia*).

Pristinamycin is active against Gram-positive cocci. It has good activity against methicillin-resistant or -susceptible strains and constitutive or inducible erythromycin A-resistant strains of *Staph. aureus* and against methicillin-resistant or -susceptible strains of *Staph. epidermidis*. Activities against common pathogens are given in Table 135.10 and those against other micro-organisms are given in Table 135.11. However, it is inactive against *Treponema pallidum* and *Brucella* spp.

Synergy between components A and B

The combination of the two components is synergistic in terms of activity against erythromycin A-susceptible or -resistant strains of *Staph. aureus*. This synergy is independent of the respective percentages of the two components within a large range of (20–80%) for each factor. However, *in vivo*, a higher quantity of pristinamycin II than pristinamycin I must be administered for this synergy to manifest itself in experimental *Staph. aureus* infections in the mouse because of the poor absorption of pristinamycin II_A.

Table 135.11 Antibacterial activity of streptogramins

		MIC (mg/l)					
		LINOPRISTIN–FLOPRISTIN		PRISTINAMYCIN		DALFOPRISTIN–QUINUPRISTIN	
Organism(s)	**No.**	**50**	**90**	**50**	**90**	**50**	**90**
Methicillin-sensitive *Staph. aureus*	16	0.12	0.12	0.25	0.50	0.25	0.50
Methicillin-resistant *Staph. aureus*	25	0.12	0.25	0.25	1.0	0.25	0.50
Glycopeptide intermediate-resistant *Staph. aureus*	3	0.12	–	0.25	–	0.12	–
Staph. aureus Lin^r	1	0.25	–	0.5	–	0.25	–
Strep. pyogenes	27	≤0.06	≤0.06	0.12	0.12	0.25	0.25
Strep. agalactiae	15	0.06	0.06	0.25	0.25	1.0	1.0
Group C and G streptococci	15	0.12	0.25	0.25	0.25	1.0	1.0
Viridans group streptococci	20	0.12	0.25	0.5	0.5	1.0	1.0
Strep. pneumoniae Ery^s	141	0.12	0.25	0.25	0.25	0.5	0.5
Strep. pneumoniae Ery^r	120	0.25	0.5	0.25	0.5	0.5	1.0
Staph. epidermidis	14	0.06	0.5	0.12	1.0	0.12	0.25
Enterococcus faecalis	21	1.0	2.0	2.0	8.0	8.0	16
Enterococcus faecalis Van^r	16	1.0	2.0	4.0	8.0	8.0	32
Enterococcus faecium	11	≤0.06	0.5	0.25	0.5	0.5	2.0
Enterococcus faecium VanA	12	0.12	0.25	0.25	0.5	0.5	1.0
Enterococcus faecium Lin^r	12	0.12	0.25	0.25	0.5	1.0	–
Enterococcus faecium Q/D^r	3	1.0	2.0	4.0	–	–	–
Enterococcus casseliflavus	15	0.5	0.5	1.0	1.0	2.0	4.0
Enterococcus gallinarum	15	0.25	0.5	0.5	0.5	2.0	2.0
Enterococcus avium	10	0.5	1.0	0.5	2.0	2.0	4.0
Enterococcus raffinosus	3	0.5	1.0	2.0	–	–	–
Corynebacterium jeikeium	10	≤0.03	0.25	0.12	0.5	0.25	0.5
Haemophilus influenzae β$^-$	50	0.25	0.5	1.0	2.0	2.0	4.0
Haemophilus influenzae β$^+$	79	0.25	1.0	1.0	2.0	4.0	4.0
Haemophilus influenzae^{BLNAR}	21	0.25	0.5	1.0	1.0	2.0	4.0

β$^-$, non-β-lactamase producing; β$^+$, β-lactamase producing; BLNAR, non-β-lactamase-producing strains; Ery^r, erythromycin resistant; Ery^s, erythromycin sensitive; Lin^r, lincomycin resistant; Q/D^r, quinupristin–dalfopristin resistant; VanA, vancomycin A phenotype; Van^r, vancomycin resistant.

Breakpoints

The French Antibiotic Sensitivity Test Committee has adopted a breakpoint of 2 mg/l for pristinamycin and virginiamycin. This corresponds to a diameter of ≥19 mm for a 15 µg disk.

Antibiotic combinations

The combinations of streptogramins plus aminoglycosides, rifampin or β-lactams are synergistic or additive. For staphylococci, pristinamycin combined with rifampin was bactericidal against 69% of the 16 tested strains responsible for septicemia; combination with an aminoglycoside induced bactericidal activity against 50% of the same strains.

Combination with fusidic acid or co-trimoxazole is synergistic, while the combination of pristinamycin and chloramphenicol is antagonistic.

PRISTINAMYCIN

Mechanism of action

Streptogramins inhibit bacterial protein synthesis by binding sequentially to the 50S ribosomal subunit. Pristinamycin II binds irreversibly, thus releasing a receptor site for pristinamycin I. Both streptogramins cross the bacterial cell wall by passive diffusion.

Pristinamycin II acts on the early stage of protein synthesis, especially on the elongation phase of translation. Pristinamycin I exerts inhibitory activity at the end of the process during extension of the peptide chain, causing premature detachment of the nascent peptide.

Resistance

Epidemiology

Until 1975 resistance to streptogramins was infrequent, of the order of 1–3%. In 1975 the first strain of *Staph. aureus* with plasmid-mediated pristinamycin I_A and II_A resistance was isolated in France. Outbreaks associated with this plasmid have been reported in many hospitals, with a prevalence of 4–5% of strains isolated. However, the level of resistance has remained low and affects about 1% of methicillin-susceptible strains of *Staph. aureus* (MSSA) and 10% of methicillin-resistant strains (MRSA).

In the Protek study conducted from 2000 to 2001, it was shown that less than 0.02% of *Strep. pneumoniae* strains are resistant to dalfopristin–quinupristin. For *Staph. aureus* resistant to linezolid (MIC >128 mg/l), the MIC of dalfopristin–quinupristin is ≤0.25 mg/l. For *Enterococcus faecium* resistant to linezolid, the MIC at which 50% and 90% of isolates are inhibited ($MIC_{50/90}$) for dalfopristin–quinupristin is ≤0.5/1 mg/l.

Mechanism

The antibacterial spectrum of the MLS_B group does not include Gram-negative bacilli such as Enterobacteriaceae, *Pseudomonas* spp. and other nonfermentative Gram-negative bacilli. Their intrinsic resistance is due to their outer membrane impermeability.

Acquired resistance of the MLS_B type is due to four mechanisms: modification of the antibiotic target, inactivation of the antibiotics, reduction of outer membrane permeability and efflux.

Many genes encode streptogramin resistance, a complex phenomenon which could be specific for each component.

MLS_B resistance in streptococci may be constitutive or inducible. Enterococci, *Strep. pneumoniae* and most viridans group streptococci have constitutive MLS_B resistance. Inducible expression occurs above all in group A, C, G and B streptococci and in certain viridans group streptococcal strains.

Enzymatic inactivation of pristinamycin or virginiamycin was described in 1975. Through a plasmid-mediated *O*-acetyltransferase, some strains of *Staph. aureus* are capable of acetylating the hydroxyl group at position 13, leading to a loss of ribosomal affinity and inactivating pristinamycin II. The macrolactone ring of pristinamycin I could be opened by a hydrolase which is carried by the same plasmid of *Staph. aureus*. The majority of these *Staph. aureus* strains have reduced susceptibility to lincosamides.

Pharmacokinetics

There are no published pharmacokinetic data for children, elderly subjects or patients with renal or hepatic insufficiency. In adults, pristinamycin and virginiamycin are not inactivated by gastric fluid. About 15–18% of pristinamycin II_A and virginiamycin M is absorbed in the ileojejunal section. There is also weak absorption of the I_A fraction.

After administration of 500 mg of pristinamycin or virginiamycin, the concentration in plasma is about 1 mg/l at 2 hours. Concentrations in plasma are minimal or nonexistent from 4 to 6 hours, depending on the subject.

The apparent elimination half-lives are 4–5 hours for pristinamycin I and virginiamycin M and 2.8–8 hours for pristinamycin II and virginiamycin S. After administration of 2 g orally, the peak plasma concentrations of drug for pristinamycins I and II are, respectively, 0.8 and 0.6 mg/l and are reached in about 3 hours. The apparent elimination half-lives are, respectively, 4 and 2.8 hours. The lag time is about 20 minutes. The AUCs are 2.2 and 1.2 mg.h/l. The global peak concentration in serum (pristinamycins I and II) is 1.34 ± 0.7 mg/l (mean ± standard deviation) and is reached in 3 hours. Plasma protein binding is 40–50% for pristinamycin I and 80–90% for pristinamycin II. Streptogramins are metabolized in the liver but this process has not been studied. Elimination occurs mainly in the bile and urinary elimination is weak (10% for pristinamycin I and 2% for pristinamycin II).

Drug interference

Pristinamycin does not interfere with cytochrome P450. There is the possibility of a metabolic interaction between pristinamycin and ciclosporin, which may be responsible for acute nephrotoxicity. It interferes with the metabolism of methotrexate.

DALFOPRISTIN–QUINUPRISTIN

Pristinamycin remains an antibacterial complex with good antistaphylococcal activity, particularly against methicillin- and erythromycin A-resistant strains. Its use in the hospital environment has been restricted by the absence of a parenteral formulation due to the poor water solubility of pristinamycin. The objective has been to prepare derivatives with the same antibacterial activity as pristinamycin but with greater hydrosolubility. One compound has been introduced in clinical practice, dalfopristin–quinupristin.

Semisynthesis from the isolated I_A and II_A components has yielded hydrosoluble derivatives. The semisynthetic derivative of pristinamycin I_A was obtained by introduction of an amino function at the 5δ position (5δ-thiomethylquinuclidine); the semisynthetic derivatives of pristinamycin II_A with an amino substituent at position 26 (diethylaminoethylsulfonyl) are soluble in water.

Antibacterial activity

Dalfopristin–quinupristin possesses the same antibacterial spectrum as pristinamycin and similar *in-vitro* activity.

The approved National Committee on Clinical Laboratory Standards (NCCLS) breakpoint is ≤1 µg/ml (diameter of zone of inhibition ≥18 mm) for a disk load at 7.5 µg (ratio 30:70).

Dalfopristin–quinupristin MICs ranged between 0.5 and 1.0 mg/l for glycopeptide-intermediate *Staph. aureus* (GISA) strains.

Pharmacokinetics

After repeated administrations to 10 young male volunteers of a dose of 7.5 mg of dalfopristin–quinupristin per kg for 5 days (q12h) and 4 days (q8h), there was no significant accumulation of either component. The accumulation ratio ranged between 1.16 and 1.43.

After an infusion over 1 hour, peak concentrations of dalfopristin–quinupristin were between 0.95 ± 0.22 mg/l (dose 1.4 mg/kg) and 24.20 ± 8.82 mg/l (dose 29.4 mg/kg). Quinupristin was rapidly converted to its active metabolite RP-12536. The activity of dalfopristin–quinupristin was detectable 6 hours after the end of the infusion.

Six volunteers received an infusion of a single dose of 12 mg of dalfopristin–quinupristin per kg (ratio 30:70) for 60 minutes. The peak concentrations in serum and suction blisters were, respectively, 8.65 ± 0.92 and 2.41 ± 0.75 mg/l. The AUCs were, respectively, 11.2 ± 1.45 mg.h/ml and 9.19 ± 2.02 mg.h/l. The apparent elimination half-life of dalfopristin–quinupristin was 1.48 ± 0.64 hours. The interindividual variabilities are 20–29% and 25–32% for quinupristin and dalfopristin, respectively.

Two other metabolites occur through a nonenzymatic process, similar to pristinamycin II: glutathione–quinupristin and cysteine–quinupristin.

The hepatic clearance and the fecal elimination are 74.7% and 77.5% for quinupristin and dalfopristin, respectively. Protein binding rates are 55–78% and 11–26% for quinupristin and dalfopristin, respectively.

FURTHER READING

Further reading for this chapter can be found online at http://www.expertconsult.com

Chapter 136

James CM Brust
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Oxazolidinones

The oxazolidinones are a family of bacteriostatic antimicrobials that are protein synthesis inhibitors. Because this new family works at the early stage of protein synthesis involving formation of the 70S initiation complex, there does not appear to be cross-resistance with other protein synthesis inhibitors. Originally developed for the treatment of bacterial and fungal infections of plants, these agents were subsequently found to have activity against Gram-positive bacteria. Upjohn originally developed two oxazolidinones, eperezolid and linezolid. Based on its more advantageous pharmacologic profile, linezolid was selected for further investigation. At present linezolid is the sole oxazolidinone available. The discussion below is therefore limited to this product. A number of comprehensive reviews of oxazolidinones and linezolid have recently been published.[1–5]

PHARMACOKINETICS AND DISTRIBUTION

Linezolid has similar pharmacokinetics whether administered parenterally or orally. It is completely absorbed following oral administration, achieving a bioavailability of 100%. Food causes a slight decrease in the rate of absorption but not in the overall amount absorbed. In normal volunteer studies peak plasma concentrations were achieved in 1–2 hours. Steady state concentrations were approximately 12 μg/ml and 18 μg/ml following oral doses of 375 mg and 625 mg twice daily, respectively. Following intravenous administration of 625 mg twice daily to volunteers for 7.5 days, the steady state level was 3.8 μg/ml.[6,7] The half-lives of oral and intravenously administered linezolid are 5.4 and 4.8 hours, respectively. The drug is excreted by both renal and nonrenal routes; 90% of circulating drug is not metabolized, but there are two inactive metabolites that are the result of oxidation of the morpholine ring. The P450 system does not appear to be involved in the metabolism of linezolid, thus limiting the number of possible drug–drug interactions.

Linezolid is 31% bound to plasma proteins. It has a relatively large volume of distribution of 40–50 l. Information on tissue penetration is still incomplete. However, linezolid appears to penetrate well into tissue blister fluid, pulmonary alveolar macrophages and sweat.[2,8] Preliminary studies suggest reasonable penetration of bone, fat and muscle. Mean ratios of linezolid in tissue fluid/plasma were 0.55, 1.2 and 0.71 for sweat, saliva and cerebrospinal fluid, respectively. There is little additional information at present on the tissue distribution of linezolid in humans.

ROUTE AND DOSAGE

As noted, linezolid can be administered orally or intravenously. Adjustment of dosage is not necessary when switching from one route to the other. The recommended dosage for serious infections such as nosocomial pneumonia or complicated skin and soft tissue infections is 600 mg q12h. For uncomplicated infections, including community-acquired pneumonias or uncomplicated cutaneous infections, 400 mg q12h is adequate.

In patients with mild-to-moderate renal impairment (creatinine clearance 10–79 ml/min), dosage adjustment is not necessary. However, with more severe forms of renal disease dosage adjustment may be necessary.

The pharmacokinetics of linezolid appears unaffected by age. Dosage adjustment for the elderly is unnecessary. The clearance of linezolid is more rapid in children than in adults. As a result, the dose recommended for infants and children is 10 mg/kg q8–12h. The only dosage schedule investigated to date in children has been q12h.

INDICATIONS

The oxazolidinones are primarily indicated for the treatment of bacterial infections caused by Gram-positive bacteria including staphylococci, streptococci and pneumococci, although their spectrum of antibacterial activity extends beyond these species. Linezolid has excellent *in-vitro* activity against staphylococcal species including methicillin-susceptible and resistant strains. There is little difference in the average minimal inhibitory concentrations (MICs) for methicillin-resistant and -susceptible staphylococcal isolates. Linezolid is also active against the recently described *Staphylococcus aureus* isolates that are intermediate in susceptibility to glycopeptides – *Staph. aureus* with intermediate susceptibility to vancomycin (VISA) or *Staph. aureus* with intermediate susceptibility to glycopeptides (GISA) isolates.

In contrast with the protein synthesis inhibitor combination dalfopristin–quinupristin, linezolid is active against all enterococci, including *Enterococcus faecalis* and *E. faecium*, as well as those enterococcal strains that are vancomycin resistant. It is also active against penicillin-susceptible and -resistant *Streptococcus pneumoniae*, again with comparable MIC values (Table 136.1). In addition to these common Gram-positive pathogens, linezolid also has activity (MIC = 4 μg/ml) against some of the less frequently encountered Gram-positive organisms such as *Corynebacterium* spp., *Bacillus* spp., *Listeria monocytogenes*, *Erysipelothrix rhusiopathiae* and *Rhodococcus equi*.

Linezolid is moderately active against some anaerobes including *Clostridium* spp., *Peptococcus* spp., *Bacteroides fragilis*, *Fusobacterium nucleatum* and *F. meningosepticum*. It has limited activity against some Gram-negative bacteria such as *Moraxella*, *Bordetella* and *Haemophilus* spp. and has no activity against Enterobacteriaceae or *Pseudomonas* spp. Linezolid also has activity against *Mycobacterium tuberculosis* and *M. avium* complex.

In animal studies linezolid has shown activity against methicillin-resistant *Staph. aureus* (MRSA) and vancomycin-resistant *E. faecium* in the endocarditis model.[9,10] In a rabbit model of penicillin-resistant

Table 136.1 *In-vitro* antimicrobial susceptibility for linezolid against common Gram-positive pathogens

Organism	MIC (μg/ml) 50%*	MIC (μg/ml) 90%	Overall (μg/ml)
Staph. aureus (n = 52 256)			
Oxacillin susceptible	1–4	1–4	0.5–8
Oxacillin resistant	1–4	1–4	0.5–8
Coagulase-negative staphylococci (n = 548)			
Oxacillin susceptible	0.5–2	1–4	0.25–4
Oxacillin resistant	0.5–2	1–2	0.5–4
β-Hemolytic streptococci (n = 547)	1–2	2–4	1–4
Strep. pneumoniae (n = 5454)			
Penicillin susceptible	0.5	1	<0.016–1
Penicillin resistant	0.5–1	1	0.06–4
***Enterococcus* spp**. (n = 5980)			
Vancomycin susceptible	1–4	1–4	0.5–4
Vancomycin resistant	2–4	2–4	1–4

*Minimum concentration at which 50% of strains are inhibited.
Source: Adapted from ref 1 with original data in refs 26 and 31–36 from Diekema *et al*.

and -susceptible pneumococcal meningitis, meningeal penetration of linezolid was good (38% of serum levels); however, overall it was less effective than ceftriaxone.[11] In an experimental animal model of *Staph. aureus* osteomyelitis comparing cefazolin and linezolid, treatment outcome with linezolid was inferior to cefazolin and no better than in the untreated controls.[12]

At present linezolid is approved for the treatment of infections caused by vancomycin-resistant *E. faecium* with associated bacteremia; nosocomial infections caused by both MRSA, methicillin-susceptible *Staph. aureus* (MSSA) and multidrug-resistant strains of *Strep. pneumoniae*; complicated skin and soft tissue infections caused by MRSA, MSSA, *Strep. pyogenes* and *Strep. agalactiae*; and uncomplicated skin/soft tissue infections caused by MSSA and *Strep. pyogenes*.[13,14] It is also approved by the US Food and Drug Administration (FDA) for the treatment of community-acquired pneumonia due to *Strep. pneumoniae* (including multidrug-resistant strains) and MSSA (Table 136.2). Stevens *et al*.[15] recently reported that linezolid was comparable to vancomycin in the treatment of MRSA infections in a randomized open-label trial, primarily involving skin and soft tissue infections. A recent meta-analysis of randomized clinical trials compared linezolid with glycopeptides and β-lactams in the treatment of Gram-positive infections.[5] The authors found that, overall, linezolid was more effective in clearing skin and soft tissue infections and bacteremia than the comparator antibiotics, but there was no overall difference in mortality.

There is additional clinical and experimental experience with linezolid suggesting that it may have a broader therapeutic role although these other indications have not been FDA approved. These reports, while limited, include the successful treatment of enterococcal endocarditis and meningitis; MRSA prosthetic hip infections, osteomyelitis, endocarditis and meningitis; and central nervous system infections caused by *Nocardia* spp., *Capnocytophaga* spp. and *C. jeikeium*.[16–18] There is also increasing evidence that linezolid may have utility in mycobacterial infections, most notably in the treatment of multidrug-resistant tuberculosis and *M. avium* complex.[19] It is still not entirely clear how effective linezolid is in the treatment of infections such as endocarditis that require bactericidal activity, but it appears to be a useful alternative in subjects who cannot tolerate vancomycin.[17]

Resistance to linezolid is rare but has been reported in enterococci and staphylococci. To date it has occurred more commonly in enterococci, resulting from a specific mutation in the domain V region of the 23S rRNA coding region. Enterococci carry four copies of this gene and the degree of resistance appears to correlate with the number of mutant copies. There is a fitness cost to single mutants without a significant change in MIC, which may explain the rarity of spontaneous resistance or colonization with single-mutant variants. *In-vitro* studies have shown that the appearance of resistance is strongly dependent upon the exposure dose and duration.

Table 136.2 Clinical indications for the use of linezolid

FDA-approved indications Nature of infection	Potential pathogens
Skin and soft tissue (complicated)	MSSA, MRSA, *Streptococcus pyogenes, Strep. agalactiae*
Skin and soft tissue (uncomplicated)	MSSA, *Strep. pyogenes*
Infection with bacteremia	Vancomycin-resistant *Enterococcus faecium*
Nosocomial pneumonia	MRSA, MSSA, *Strep. pneumoniae* (including multidrug-resistant strains)
Community-acquired pneumonia	*Strep. pneumoniae* (including multidrug-resistant strains), MSSA
Non-FDA-approved (off-label) indications	
Serious MRSA, VISA and VRSA infections or infections that are poorly responsive to vancomycin therapy	
Infection with bacteremia caused by *Enterococcus faecalis* (including vancomycin-resistant strains)	
Complicated infections requiring long-term oral therapy with an antistaphylococcal or enterococcal agent where β-lactams cannot be used (e.g. chronic osteomyelitis)*	
Treatment of infections (e.g. nosocomial pneumonia, endocarditis, meningitis) caused by highly resistant (but linezolid susceptible) Gram-positive bacteria where alternative agents are not available or are contraindicated	
Combination therapy for antimycobacterial infections where first- and second-line agents cannot be used	

*Close monitoring for toxicity is required with prolonged linezolid therapy.
MRSA, methicillin-resistant *Staphylococcus aureus;* MSSA, methicillin-susceptible *Staph. aureus*; VISA, *Staph. aureus* with intermediate susceptibility to vancomycin; VRSA, vancomycin-resistant *Staph. aureus*.

Linezolid is an important addition to the Gram-positive armamentarium. It is an alternative agent for the treatment of resistant Gram-positive infections (see Table 136.2). It has an antibacterial spectrum that is similar to vancomycin with advantageous pharmacokinetics. The availability of an oral preparation allows completion of therapy with the same therapeutic agent and therefore may help reduce the duration of hospital stays.[20]

Dosage in special circumstances

As noted above, adjustment of dosage for moderate degrees of renal failure is not necessary. Because linezolid, as well as the linezolid metabolites, are cleared during hemodialysis, it is recommended that patients receive a supplemental dose following dialysis. For moderate degrees of hepatic disease dosage adjustment is not necessary.

ADVERSE REACTIONS AND INTERACTIONS

Linezolid has, in general, been well tolerated. The most common adverse events in the comparator-controlled trials were diarrhea (2.8–11%), nausea (3.4–9.6%) and headaches (0.5–11.3%).

Potentially the most serious adverse event, thrombocytopenia, was seen in 2.4% of patients. It was seen most often during prolonged therapy (longer than 2 weeks) and resolved upon completion of therapy. Others have reported a higher incidence of thrombocytopenia,[21,22] and there are reports of both anemia and leukopenia associated with linezolid therapy. As a result, hematologic monitoring of these parameters is recommended during prolonged therapy, especially for subjects who are already immunocompromised.

Now that linezolid has become much more widely used, several rare but important toxicities have emerged, including an irreversible peripheral neuropathy, a partially reversible optic neuropathy and severe lactic acidosis. Both the peripheral and optic neuropathies appear to occur after prolonged therapy (median 4 months and 10 months, respectively), whereas lactic acidosis has been reported after as little as 1 week of therapy.[23]

The oxazolidinones are monoamine oxidase (MAO) inhibitors. Linezolid appears to be a relatively weak MAO inhibitor and there are now more than a dozen reports of serotonin syndrome in patients treated with linezolid while also taking selective serotonin reuptake inhibitors (SSRIs). Recommendations on the co-administration of linezolid with SSRIs vary, with some authors advocating a full washout of the SSRI before starting linezolid and other authors simply advising close clinical follow-up.[23] Data on combination antibiotic therapy with linezolid are limited.

REFERENCES


References for this chapter can be found online at http://www.expertconsult.com

Chapter 137

Richard H Drew

Aminoglycosides

INTRODUCTION

Introduced into clinical practice in the 1940s, aminoglycosides were often considered the mainstay in the treatment of many infections, most notably for serious Gram-negative infections. Currently, nine aminoglycosides (gentamicin, tobramycin, amikacin, streptomycin, neomycin, kanamycin, paromomycin, netilmicin and spectinomycin) are approved by the US Food and Drug Administration (FDA) for clinical use. A number of other aminoglycosides are in use throughout the world. Of these, gentamicin, tobramycin and amikacin are the most frequently prescribed for the treatment of severe Gram-negative infections and will be the focus of this chapter. Gentamicin and (less frequently) streptomycin are utilized as part of combination therapy for specific Gram-positive infections, while amikacin and streptomycin have also been used in the treatment of mycobacterial diseases. Use of paromomycin (for cryptosporidiosis), spectinomycin (for gonorrhea) and oral neomycin (for surgical prophylaxis) have largely been replaced by newer agents for these indications.

Popularity of the aminoglycosides declined considerably in the 1980s, due largely to concerns regarding their ototoxic and nephrotoxic potential, need for individualized dosing, serum concentration monitoring and the availability of safer therapeutic alternatives for many of their clinical indications. However, increasing Gram-negative drug resistance to β-lactams (including cephalosporins), fluoroquinolones and carbapenems, combined with a lack of development of new treatment options for serious Gram-negative infections, has resulted in a re-evaluation of the aminoglycoside class of antibiotics (most notably gentamicin, tobramycin and amikacin).

BACKGROUND

The aminoglycosides were discovered following systematic screening of soil actinomycetes for the production of substances with antimicrobial activity. These compounds exhibited particular activity *in vitro* against aerobic Gram-negative bacilli and Gram-positive cocci. Streptomycin, the first clinically useful aminoglycoside, was isolated from *Streptomyces griseus* in 1944.[1] Neomycin, kanamycin, tobramycin and paromomycin are also natural compounds that were subsequently isolated from various *Streptomyces* spp. and identified by the spelling 'mycin'. Gentamicin and sisomicin are natural products produced by *Micromonospora* spp. and are so designated by ending in 'micin'. Amikacin and netilmicin are semisynthetic aminoglycosides derived from kanamycin and sisomicin, respectively.[1]

CHEMISTRY

The chemical structures of the aminoglycosides are shown in Figure 137.1. All aminoglycosides include a central six-membered ring containing amino groups termed an aminocyclitol, which is linked to two or more amino- or non-amino-containing sugars by glycosidic bonds.[1] Spectinomycin is a pure aminocyclitol and is often considered with the aminoglycosides, but is not strictly speaking an aminoglycoside because it contains neither aminosugars nor glycosidic bonds.[1] For this reason, the complete group of compounds is more accurately referred to as aminoglycoside–aminocyclitol antibiotics.

Microbiologic activity

Mechanism of activity

Aminoglycosides act by irreversibly binding to the bacterial 30S ribosomal subunit, resulting in disruption of peptide synthesis. Bactericidal activity against susceptible organisms is rapid, most notably in aerobic Gram-negative bacilli. Numerous conditions (either *in vitro* or *in vivo*) may influence this activity.[1–3] The rate of killing is generally enhanced at higher concentrations.[2] In contrast, the presence of divalent cations, increased osmolality, an acidic pH and an anaerobic environment can significantly reduce the *in vitro* activity of aminoglycosides.[1] This may be clinically relevant in tissues and fluids with low pH (such as lung and bronchial fluids) or in anaerobic environments (such as abscesses) where activity may be significantly reduced. The microbiologic effect of aminoglycosides is not significantly altered by low or high inocula of organisms.

Aminoglycosides exhibit a postantibiotic effect (PAE), which is the continued suppression of bacterial growth after antimicrobial exposure, against both Gram-positive and Gram-negative bacteria.[4,5] The duration of such an effect is highly variable (0.5–7.5 hours) and depends greatly on the test conditions, such as the organism, exposure duration and antimicrobial concentration.[4,5] In general, the PAE observed for Gram-positive organisms (<2 hours) is shorter than that observed for Gram-negatives (up to 7 hours).[4] Similar to the rate of bactericidal activity, the duration of PAE has been noted to increase with increasing aminoglycoside concentrations (such as those seen with consolidated dosing techniques discussed later in this chapter).[4,5]

While results vary with individual organisms and other test conditions, aminoglycosides generally exhibit indifferent, additive or synergistic effects *in vitro* against numerous aerobic Gram-negative bacilli when combined with several β-lactam antibiotics.[1] A similar effect is seen *in vitro* against Gram-positive cocci when combined with vancomycin, teicoplanin and daptomycin.[1,6] Such activity has contributed to the debate over the use of aminoglycosides as part of combination therapy for the treatment of various infections (see Clinical applications, below).

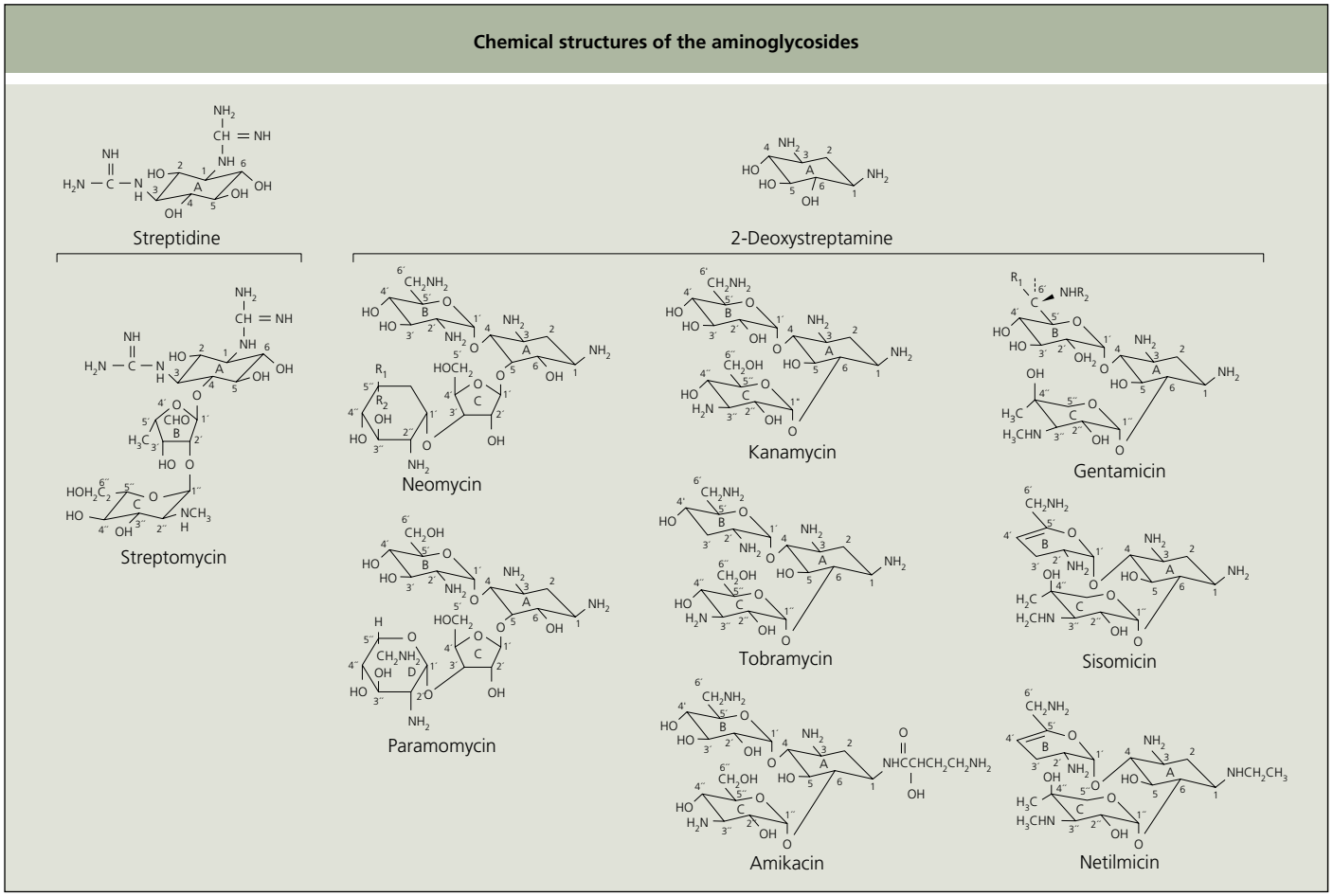


Fig. 137.1 Chemical structures of the aminoglycosides. All aminoglycosides include an aminocyclitol (a central six-membered ring containing amino groups), which is linked to two or more amino- or non-amino-containing sugars by glycosidic bonds. For streptomycin, the aminocyclitol ring is a streptidine, whereas for the remainder of the clinically available aminoglycosides it is 2-deoxystreptamine. Neomycin contains approximately equal amounts of neomycin B (R_1=H; R_2=CH_2NH_2) and neomycin C (R_1=CH_2NH_2; R_2=H). Kanamycin is principally kanamycin A, as shown. Gentamicin is gentamicin C complex with roughly equal amounts of C_1 (R_1=R_2=CH_3), C_{1a} (R_1=R_2=H) and C_2 (R_1=CH_3; R_2=H). Courtesy of Richard Quintiliani Jr, Richard Quintiliani and Charles H Nightingale.

Spectrum of activity

In general, aminoglycosides demonstrate potent activity against a wide variety of aerobic Gram-positive and Gram-negative organisms. However, anaerobic bacteria are intrinsically resistant to aminoglycosides.

Gram-positive bacteria

Aminoglycosides generally demonstrate favorable activity *in vitro* against many species of staphylococci. Gentamicin, tobramycin and amikacin demonstrated activity *in vitro* against over 95% of methicillin-susceptible *Staphylococcus aureus* (MSSA).[7–9] Activity of coagulase-negative staphylococci exceeded 84% in one report.[8] Aminoglycosides also demonstrate a high degree of activity *in vitro* against methicillin-resistant *Staph. aureus* (MRSA), including community-associated strains (CA-MRSA).[10,11] However, significant geographic variability exists, and new clonal strains of CA-MRSA resistant to aminoglycosides have been reported throughout the world.[12] Despite their generally favorable activity *in vitro*, aminoglycosides are rarely used as monotherapy in the treatment of staphylococcal infections.

Aminoglycosides generally lack sufficient activity against pneumococci. While aminoglycosides alone are not active against enterococci and viridans group streptococci, they (most commonly gentamicin) are often used in the therapy of serious infections caused by these organisms (in the absence of high-level aminoglycoside resistance) because of their frequent additive or synergistic effect when combined with other antibiotics (see Resistance, and Clinical applications, below). However, recent reports have highlighted growing concern regarding the increased incidence of high-level gentamicin resistance in enterococci.[13–15]

Gram-negative bacteria

Current clinical use of aminoglycosides is mainly due to their activity against a variety of Gram-negative pathogens. Gentamicin, tobramycin, netilmicin and amikacin often demonstrate favorable activity *in vitro* against *Acinetobacter* spp., *Citrobacter* spp., *Enterobacter* spp., *Escherichia coli*, *Klebsiella* spp., *Serratia* spp., *Proteus* spp., *Morganella* spp. and *Pseudomonas aeruginosa*.[8,16–19] However, there are differences in aminoglycoside *in-vitro* potencies against these pathogens. Among the Enterobacteriaceae, amikacin usually demonstrates the highest degree of activity *in vitro* (>90%), followed closely by gentamicin.[8,17] Relative to the Enterobacteriaceae, reduced activity is exhibited in the nonfermentative Gram-negative organisms *Acinetobacter* spp. and *P. aeruginosa* (including some multidrug-resistant strains).[18] Relative to gentamicin, tobramycin usually demonstrates superior activity *in vitro* against *P. aeruginosa* (ranging between 65% and 85% in most reports).[8,16] In contrast, gentamicin usually has superior activity *in vitro* against *Serratia* spp.[8]

Limited treatment options for specific multidrug-resistant organisms (most frequently *Klebsiella pneumoniae* and *E. coli*) producing

extended-spectrum β-lactamases (ESBL) have renewed the interest in the potential use of aminoglycosides against such pathogens.[20–23] However, *in-vitro* activity of aminoglycosides against these pathogens is not predictable and is consistently inferior to that demonstrated by other agents (such as carbapenems and tigecycline).[24]

Despite possessing activity *in vitro* against *Salmonella* spp. and *Shigella* spp., and modest activity against *Neisseria gonorrhoeae* and *Haemophilus influenzae*, these agents are rarely used for such infections due to the availability of safer alternatives. In contrast, *in-vitro* activity against *Burkholderia cepacia* and *Stenotrophomonas maltophilia*[25] is poor.

Mycobacterium

Of the aminoglycosides, streptomycin and amikacin display favorable activity *in vitro* against a variety of mycobacteria.[26] Streptomycin is particularly active against *Mycobacterium tuberculosis*.[27] Against atypical strains, amikacin is generally the most active aminoglycoside against *M. fortuitum*, *M. abscessus* and *M. chelonae*.[26] While tobramycin has demonstrated activity *in vitro* against *M. chelonae*, it lacks activity against *M. abscessus*. Amikacin has also demonstrated activity *in vitro* against *M. avium intracellulare*.[26]

Resistance

Several mechanisms of resistance to aminoglycosides have been described in the literature (both intrinsic and acquired), including impaired drug uptake, efflux, ribosomal target modification and enzymatic drug modification with subsequent inactivation. Despite these mechanisms and years of clinical application, aminoglycosides have generally maintained favorable susceptibilities against a variety of pathogens.

Intrinsic resistance is often the consequence of impaired uptake, which generally has been associated with lower-level resistance (such as that exhibited by enterococci).[28] In such settings, gentamicin minimum inhibitory concentrations (MICs) of 8–64 μg/ml and streptomycin MICs of 64–512 μg/ml are not uncommon. In such circumstances, *in-vitro* synergy may be demonstrated when enterococci with low-level aminoglycoside resistance are exposed to an aminoglycoside in combination with cell wall-active agents, notably β-lactams.[28] Such potential for synergy, however, is generally lost in the setting of high-level gentamicin resistance (MIC >500 μg/ml).[28] In such cases, only streptomycin (in strains without high-level streptomycin resistance, defined as an MIC of 2000 μg/ml or more) can be used against enterococci and staphylococci highly resistant to gentamicin.[28] In contrast, *Enterococcus faecium* often demonstrates moderate resistance to tobramycin (MIC 64–1000 μg/ml) and therefore cannot be used for synergy.

Acquisition of transposon- and/or plasmid-encoded modifying enzymes is frequently the cause of acquired resistance. In Gram-negative pathogens, these most commonly include inactivating pathways (such as phosphorylation, adenylylation or acetylation) encoded by plasmids or associated with transposable elements, or decreased drug accumulation. In addition to these more common mechanisms, efflux of the drug has been reported in resistant forms of *P. aeruginosa* and would potentially confer resistance across the class.[29]

Treatment-emergent resistance (i.e. resistance developed during treatment of an initially susceptible Gram-negative organism) is infrequent.[1] In addition, cross-resistance among the members of the aminoglycoside class is generally incomplete (except as previously noted). For example, amikacin has generally maintained high levels of activity *in vitro* against specific pathogens exhibiting high rates of resistance to gentamicin and tobramycin.

PHARMACOKINETICS

The aminoglycosides exhibit characteristics of a three-compartmental pharmacokinetic model. An initial distribution (alpha) phase is followed by a rapid elimination (beta) phase and a slow elimination (gamma) phase. However, a one-compartment model is most often used clinically for calculating dosage regimens. Because they demonstrate linear pharmacokinetics, a direct relationship is seen between dose and area under the plasma concentration–time curve (AUC).

Absorption

Aminoglycosides undergo minimal absorption when given orally, topically or by rectal instillation. Nevertheless, in patients with hepatic encephalopathy and renal impairment, large and frequent doses of neomycin have been associated with sufficient absorption into the systemic circulation to produce deafness.[30] Detectable amounts of tobramycin following oral administration as part of a selective gut decontamination regimen have been reported in patients with significant renal impairment.[31] Similarly, detectable blood levels of aminoglycosides have been reported following topical use in burn patients with large areas of denuded skin.[32] Aminoglycosides penetrate extremely well into body spaces with large serosal surfaces (e.g. pleural space, peritoneal cavity, pericardial space, synovial fluid). Intraperitoneal doses may also result in significant systemic exposure.[33]

In addition to the parenteral route of administration, aminoglycosides (most notably tobramycin) have been administered via aerosols (see Clinical applications, below). Following endotracheal administration or aerosolization of aminoglycosides, systemic absorption is usually modest. For example, mean serum tobramycin concentrations 1 hour after inhalation in 258 cystic fibrosis patients receiving 300 mg twice daily were 0.95 ± 0.50 μg/ml.[34] The estimated systemic bioavailability in this study was 11.7%

Distribution

After an intravenous or intramuscular dose, peak aminoglycoside serum concentrations occur in 30–60 minutes and 45–90 minutes, respectively. In patients with poor vascular perfusion into soft tissue structures (such as those with diabetes mellitus), there may be a delay in the time to peak concentration. The low serum protein binding of aminoglycosides (approximately 10%) facilitates wide distribution into the extravascular space. Because aminoglycosides are highly water-soluble, their volume of distribution (Vd) is similar to that of the extravascular compartment. Studies performed in normal adult volunteers have reported volumes of distribution in the range of 0.2–0.3 l/kg. In patients with increased extravascular fluid (such as those with ascites, burns or pregnancy), the Vd is increased, whereas dehydrated patients may exhibit decreases in Vd.

Penetration of aminoglycosides has been reported in numerous body fluids and tissues. For example, in the unobstructed biliary tract, aminoglycoside concentrations approximate 30% of the simultaneous serum concentration.[35] Similar penetration has been reported in lung tissues and fluids.[36] Aminoglycosides penetrate poorly into the cornea and the aqueous and vitreous humors of the eye.[37] In patients with serious eye infections such as bacterial endophthalmitis, direct intravitreal injections are usually needed. In contrast, high concentrations are observed in specialized cells which have an active transport mechanism for aminoglycosides, such as the tubular cells of the renal cortex and the hair cells of the ear (see Ototoxicity and Nephrotoxicity, below). As a consequence, concentrations in these cells can exceed those of plasma or interstitial fluid. Aminoglycoside concentrations in the urine may exceed those of simultaneous serum concentrations by 25- to 100-fold. In addition, aminoglycoside concentrations can remain above therapeutic concentrations for extended periods of time (48–200 hours) as a result of renal tubular cell absorption and the prolonged release of aminoglycosides into urine.

The penetration of aminoglycosides into cerebrospinal fluid (CSF) is poor, both in the presence and absence of meningeal inflammation. Penetration of gentamicin into CSF was studied in 26 patients aged 6–20 years with mumps meningoencephalitis after administration of a single intramuscular dose of 1.2–4.0 mg/kg. Activity was found

in only five of the 26 patients, with the highest level being 0.19 µg/ml. While use of intraventricular administration has been reported in the treatment of Gram-negative meningitis, such treatment is rarely employed.

Metabolism and elimination

After a rapid distributive phase of 15–30 minutes, the elimination phase begins. During eliminated by filtration, there is active reabsorption of aminoglycoside into the proximal renal tubular cells. A direct relationship exists between aminoglycoside clearance and glomerular filtration rate. Less than 1% of aminoglycoside is eliminated into feces, bile and saliva. Approximately 99% of the drug is excreted unchanged in the urine with a half-life of 1.5–3.5 hours in adult patients with normal renal function. In patients with increased extravascular fluid, the half-life can be prolonged. Impairment of renal function can result in considerable prolongation of the serum aminoglycoside half-life. In neonates less than 1 week of age or in small premature infants, the half-life is typically prolonged[38,39] (see Special populations, below).

Following the rapid elimination phase, there is a terminal elimination phase of 30–700 hours, secondary to prolonged release from the proximal renal tubules back into the urine. Because the amount of aminoglycoside eliminated is so low, it has no relevant effect on dosing.

PHARMACODYNAMICS

Numerous *in vitro* and animal model studies have been performed to characterize the exposure that best predicts outcome. For the aminoglycosides, the rate of bacterial eradication has been linked to both AUC/MIC and peak concentration/MIC ratios.[40] Increased rates of bactericidal activity have been noted with increasing concentrations (up to approximately 10–12 times above their MICs).

The concentration-dependent pharmacodynamic profile of aminoglycosides has stimulated *in vitro*, animal model and clinical investigations regarding the potential for consolidated dosing. In neutropenic and non-neutropenic animal models of infection, significantly more animals survive a potentially lethal challenge of bacteria if the animals are treated with a single large dose of an aminoglycoside than with the same dose divided on an 8-hour schedule.[2,41] However, the optimal exposure might be organism specific. For example, although divided doses were superior in one *in-vitro* model against *Staph. aureus*, this was not observed against *P. aeruginosa* in the same model.[42]

DOSAGE AND ADMINISTRATION

Individualized dosing of aminoglycosides is necessary to optimize the potential for favorable outcomes while minimizing the risks of treatment-related adverse effects.[40,43] Aminoglycosides are most commonly administered intravenously (over 0.5–2 hours depending upon the dose), but in specific cases may also be administered by intramuscular injection. Initial dosing should be based on indication, weight and estimates of renal function. Subsequent adjustments to the dose and/or frequency of administration should be based on results of serum concentration monitoring. Actual body weight is used in most patients to determine the initial dose. In obese patients, i.e. greater than 25% over ideal body weight (IBW), a dosing weight can be calculated using the following:

$$\text{IBW} + 0.4\ (\text{actual} - \text{ideal})$$

While estimates of creatinine clearance are most commonly performed using the Cockcroft–Gault equation,[44] disease states that limit the predictability of this equation (such as rapid changes in renal function, dialysis-dependent renal failure, liver disease and malnutrition) should be considered. Creatinine clearance estimates can be used to determine the frequency of administration.

In general, two methods of dosing and administration are employed: divided daily dosing and consolidated dosing (used most frequently for gentamicin and tobramycin).

Divided daily dosing ('traditional dosing') of gentamicin and tobramycin in adults

In the divided dosing method, a loading dose is given and followed by maintenance doses. The loading dose is dependent on target serum concentrations (based on indication and/or infection site) but independent of renal function. Peak concentrations of 2–4 µg/ml are targeted for gentamicin and tobramycin in patients with uncomplicated lower urinary tract infection and synergy with β-lactams for serious Gram-positive infections. For these indications, loading doses of 0.6–1.2 mg/kg may be administered, although loading doses are not generally required when aminoglycosides are administered for synergy against Gram-positive pathogens. In patients with Gram-negative sepsis or other serious Gram-negative infections, gentamicin and tobramycin loading doses of 2.5 mg/kg are used to target peak serum concentrations of 6–8 µg/ml. Finally, in patients with Gram-negative pneumonia or acute life-threatening Gram-negative infection in a critically ill patient, loading doses of 3 mg/kg are used to target peak serum concentrations of 7–9 µg/ml.

Following administration of a loading dose, maintenance doses should be administered based on indication and renal function. In adult patients with normal renal function, this is usually 1.7–2 mg/kg q8h.

One method to determine initial maintenance doses of gentamicin and tobramycin is outlined in Table 137.1.

All patients should undergo steady-state serum concentration monitoring (generally between the second and third dose). Peak serum concentrations should be measured 30 minutes after the intravenous infusion or 60 minutes after the intramuscular dose. Trough concentrations are obtained within 30 minutes of drug administration. Sample times should be documented in all cases. Trough concentrations for gentamicin, tobramycin and netilmicin exceeding 2 µg/ml or excessive peak concentrations indicate the need for dosing adjustment. Serum creatinine concentration should be measured at baseline and daily thereafter for the first 3 days of therapy, then every 3–5 days thereafter. Weekly renal function monitoring can be used for prolonged therapy if renal function is stable. Significant increases in serum creatinine concentration would indicate the necessity to recalculate a new dosage regimen, repeat serum monitoring or consider an alternative therapy.

Table 137.1 Initial maintenance doses of gentamicin and tobramycin in adults

Creatinine clearance (ml/min)*	Percentage of loading dose†	Dose interval (hr)
≥90	84	8
80–89	80	8
70–79	76	8
60–69	84	12
50–59	79	12
40–49	72	12
30–39	86	24
20–29	75	24–36

*Estimated using the Cockcroft–Gault equation.
†Rounded to nearest 10 mg increment.

Consolidated dosing of gentamicin and tobramycin in adults

Considerable attention has been given to optimizing the concentration-dependent pharmacodynamic properties of aminoglycosides (notably gentamicin and tobramycin) by giving the entire daily dose consolidated into one dose. This strategy is also known as 'once-daily' or 'total daily dose'. The goal of such a strategy is to increase the probability that aminoglycoside concentrations will exceed the MIC approximately 10-fold and potentially extend the PAE[45] (Fig. 137.2).

Numerous reports have described the application of consolidated dosing of aminoglycosides in a variety of clinical settings. In the largest published outcome study in adults (n=2184) treated with consolidated gentamicin or tobramycin, nephrotoxicity (defined as an increase in serum creatinine concentration of 0.5 mg/dl or more over baseline during aminoglycoside therapy) was detected in only 1.2% (27 patients) and ototoxicity in 0.1% (three patients). The incidence of nephrotoxicity was significantly lower than the 3–5% observed from the same hospital when aminoglycosides were given by the conventional dosing technique. Other experience reported in immunocompetent adults (as summarized in a meta-analysis) has demonstrated that consolidated dosing was equivalent with regard to bacteriologic cure, but showed a trend towards reduced mortality rates and reduced toxicity.[46] In addition, pharmacoeconomic analyses of consolidated dosing report the potential for significant reductions in preparation, administration, monitoring and nephrotoxicity costs, while others have documented how consolidated dosing might help to facilitate transition to outpatient care.[47,48]

While different approaches have been used to administer consolidated aminoglycoside dosing, perhaps the most popular used by US hospitals has been modeled after the one developed at Hartford Hospital. For the treatment of serious Gram-negative infections in adults, a 7 mg/kg dose of gentamicin or tobramycin is administered to target a peak serum concentration of 20 µg/ml (Fig. 137.3). Based on simulations using a one-compartment intravenous model and a Vd of 0.3 l/kg in patients with normal renal function, serum concentrations typically fall below 0.5 µg/ml within 12 hours and then remain essentially undetectable for the remainder of the day (Fig. 137.3). Kinetic studies in humans have confirmed 7 mg/kg as a reasonable target dose in adults.[49] The drug-free interval is crucial for reduced toxicity as it facilitates increased aminoglycoside egress from the renal tubular and inner ear cells relative to that seen with the conventional intermittent dosing method.

Monitoring of patients on consolidated aminoglycoside therapy most frequently involves measuring a single random serum concentration 6–14 hours following the initial dose (Fig. 137.4). The resulting concentration is then plotted to determine the subsequent dosing interval (24, 36 or 48 hours) of the 7 mg/kg dose. If the concentration falls on the line of the nomogram that separates the dosing intervals, then the longer interval is chosen. Concentrations unable to be plotted using the nomogram due to excessive concentrations should result in suspension of therapy and serial serum concentration determinations. Patients suspected of having altered aminoglycoside pharmacokinetics (such as those with rapidly changing renal function) should be considered for more intensive monitoring.

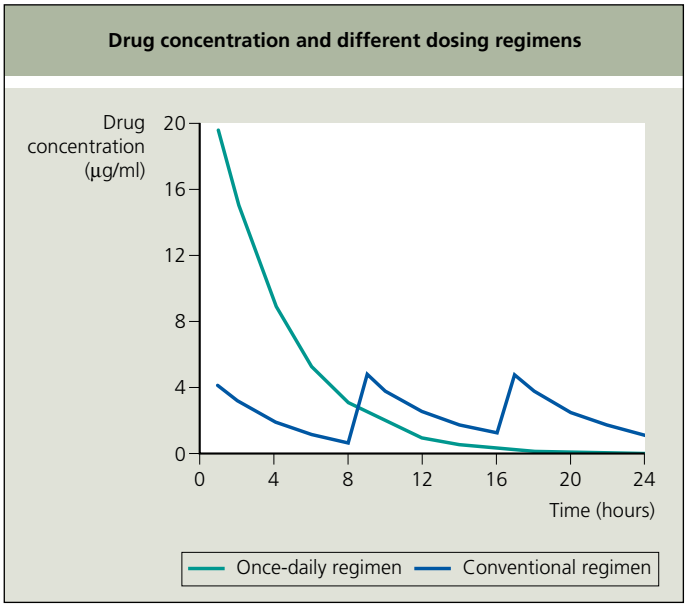


Fig. 137.2 Simulated concentration versus time profiles for once-daily 7 mg/kg and 5 mg/kg gentamicin regimens in patients with varying degrees of creatinine clearance (Clcr). Courtesy of Richard Quintiliani Jr, Richard Quintiliani and Charles H Nightingale.

Fig. 137.3 Simulated concentration versus time profiles for once-daily (7 mg/kg q24h) and conventional (1.5 mg/kg q8h) gentamicin regimens in patients with normal renal function. Courtesy of Richard Quintiliani Jr, Richard Quintiliani and Charles H Nightingale.

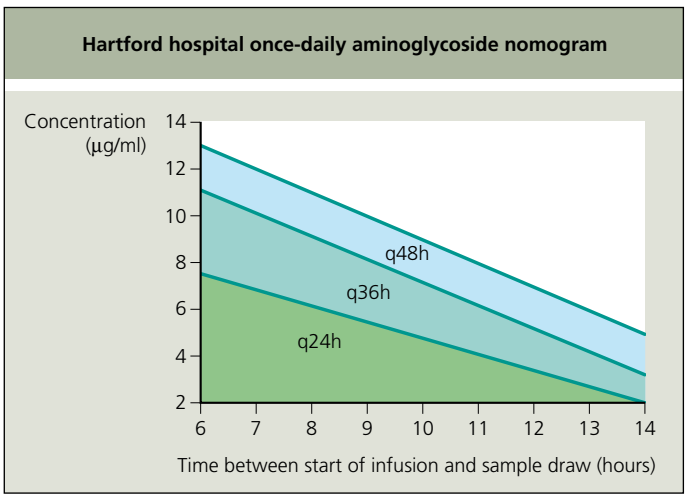


Fig. 137.4 Hartford Hospital once-daily aminoglycoside nomogram for gentamicin and tobramycin using the 7 mg/kg dose. Courtesy of Richard Quintiliani Jr, Richard Quintiliani and Charles H Nightingale.

Published experience with consolidated aminoglycoside dosing has focused on the use of either gentamicin or tobramycin. Relative to these agents, published experience with consolidated dosing of amikacin for the treatment of Gram-negative infections is limited. However, amikacin 15 mg/kg/day has been used with success.[50,51]

Similarly, netilmicin given as either 5 mg/kg q24h or 2 mg/kg q8h for 10 days was compared for the treatment of Gram-negative pyelonephritis in children in a prospective, randomized trial.[52] No difference in clinical efficacy, ototoxicity or nephrotoxicity was observed.

Published experience with consolidated aminoglycosides is expanding, including recent reports in patients with postpartum endometritis, mycobacterial infections, endocarditis,[53] osteomyelitis[54] and febrile neutropenia.[47,55] However, consolidated aminoglycoside dosing is not generally recommended for patients with severe renal insufficiency (including end-stage renal disease), pregnant women or those with major burns (>20%) or ascites due to the relative lack of data in these populations and the potential for altered pharmacokinetics. Alternative strategies for consolidated dosing are also required in the treatment of mycobacterial infections and when used for synergy (as part of combination therapy) (see Clinical applications, below).

Despite extensive published clinical experience with consolidated aminoglycoside dosing and wide acceptance in US hospitals, considerable debate persists regarding the appropriateness and optimal implementation of such therapy.[56] Alternative dosing regimens (ranging from 3 mg/kg to 7 mg/kg q24h of gentamicin or tobramycin) and dosing adjustment strategies have been advocated by some investigators. Dosing based on targeted AUC has also been proposed. For example, pharmacokinetic targets might be reached with 5 mg/kg if MIC ≤1, but higher (i.e. 7 mg/kg) if MIC ≥2.[40] However, it is uncertain which of these methods is superior.

Other aminoglycosides in adults

For amikacin and streptomycin, an initial dose of 7.5 mg/kg is administered, targeting peak concentrations of 15–30 μg/ml. Maintenance doses of amikacin are 15 mg/kg/day in divided doses q12h (for patients with normal renal function). Trough concentrations should be 5–10 μg/ml.

For netilmicin, a loading dose of 2 mg/kg is followed by 6 mg/kg/day (usually in divided doses q8h for patients with normal renal function). Similar to that previously described for gentamicin and tobramycin, maintenance doses and dosing intervals should be adjusted based on estimates of renal function. In all cases, serum concentration monitoring should be used to individualize the dose.

Special populations

The dosing of aminoglycosides should be modified in specific populations. The initial maintenance dosing to neonates and children should be based upon age, with neonates 0–7 days receiving 4–5 mg/kg/day and infants and children 5–7.5 mg/kg/day of gentamicin or tobramycin divided into two or three doses. Published experience with consolidated dosing in pediatric patients is growing, including use in neonates.[57,58] While similar pharmacodynamic principles should apply to the treatment of infections in the pediatric patient population, differences in the pharmacokinetic profile should be taken into consideration. For example, in children 6–12 months of age and those older than 1 year given a single intravenous dose of 20 mg/kg amikacin, the Vds were 0.5 l/kg and 0.33 l/kg, respectively.[59] Therefore, the Vd in children under 1 year of age is slightly higher than that of an adult, whereas that in children over 1 year of age is similar to that in adults. In addition, in this same study, the half-life in the younger children was longer than that in the older children and in adults, yet the peak and trough levels were similar. These observations suggest that children under 1 year of age may require a higher single dose (20 mg/kg) of amikacin than the dose (15 mg/kg) in older children and in adults. Other studies using a 20 mg/kg once-daily dose of amikacin in children undergoing bone marrow transplantation or with serious Gram-negative bacterial sepsis have shown no differences in efficacy or toxicity compared with standard intermittent dosing.[59]

Patients undergoing dialysis require dosing modification based on the type and frequency of dialysis. Supplemental doses of 1–2 mg/kg gentamicin or tobramycin, 2 mg/kg netilmicin and 5–7 mg/kg amikacin may be required after each hemodialysis. Those undergoing continuous arteriovenous (AV) hemofiltration may be administered initial daily doses of 2.5 mg/kg gentamicin or tobramycin followed by serum concentration monitoring to ensure adequate peak and trough concentrations. Intraperitoneal administration of aminoglycosides (gentamicin, tobramycin or netilmicin 10 mg in 2 l dialysis fluid to four bags each day), with or without a traditional loading dose given intravenously, has been used in patients undergoing continuous ambulatory peritoneal dialysis (CAPD)-related peritonitis.[33] Alternatively, high concentrations (e.g. 40 mg gentamicin, tobramycin or netilmicin in 2 l dialysate) added to one exchange of dialysis fluid per day appear to have comparable efficacy.[33] Finally, patients undergoing continuous arteriovenous hemodialysis (CAVHD) and continuous venovenous hemodialysis (CVVHD) exhibit significant interpatient variability. Multiple serial aminoglycoside concentrations are generally necessary to determine optimal dosing in such patients.

In addition to these patient populations, other groups have been reported to demonstrate alterations in aminoglycoside pharmacokinetics requiring modification of the initial empiric recommendations. For example, initial empiric dosing of gentamicin and tobramycin in patients with cystic fibrosis should be 3.0–3.3 mg/kg q8h due to the increased volume of distribution and drug elimination demonstrated in this patient population. Higher doses of gentamicin and tobramycin (i.e. 7–9 mg/kg/day) may also be necessary in cystic fibrosis patients receiving consolidated dosing.[60] Similarly, larger volumes of distribution have been reported in patients with significant burns. Therefore, maintenance doses of gentamicin or tobramycin of up to 7–8 mg/kg/day (in divided doses) may be required to achieve target serum aminoglycoside concentrations. Increased dosing requirements have also been reported in burn patients receiving consolidated dosing of amikacin.[50]

Prophylaxis

Dosing of aminoglycosides for prophylaxis of infection secondary to surgery has been described in recent guidelines.[61,62] Patients receiving gentamicin (in combination with vancomycin) for cardiac surgery may be administered a single 4 mg/kg dose within 1 hour of the surgery.[61] For such an indication, re-dosing due to cardiopulmonary bypass is not necessary.[61] Patients receiving prophylaxis for colorectal or gynecologic surgery may receive gentamicin 1.5 mg/kg (as part of combination prophylaxis) within 1 hour of the incision.[62]

Coadministration with β-lactams

Although the aminoglycosides are often used in combination with β-lactams, the combination may lead to inactivation of both drugs. The reaction, which is time- and concentration-dependent, occurs by nucleophilic opening of the β-lactam ring and acylation of an amino group of the aminoglycoside, resulting in a biologically inactive amide.[63] Gentamicin and tobramycin appear to be more susceptible to inactivation than amikacin or netilmicin. This phenomenon is probably clinically meaningful only in patients with significant renal failure in whom β-lactams accumulate to very high concentrations.[63] As such, aminoglycosides should not be mixed with β-lactams before infusion or intraperitoneal instillation, and serum samples used for aminoglycoside assay should be run immediately or frozen until used.

Aerosol administration

As previously addressed, tobramycin has been administered via aerosol to patients with cystic fibrosis.[64] In this setting, 300 mg is administered twice daily.

Local administration

Aminoglycosides have been administered in a variety of novel (i.e. nonparenteral) forms, including bone cement, irrigation solutions, impregnated beads, antibiotic locks, intraventricular injections, etc. Such use is beyond the scope of this chapter.

CLINICAL APPLICATIONS

Aminoglycosides are used most frequently as part of combination therapy (usually with cell wall-active agents such as β-lactams) for the treatment of serious Gram-negative infections. Use as part of combination therapy for the treatment of Gram-positive infections (i.e. enterococci and staphylococci) is less common. Specific aminoglycosides have been employed for the treatment of *N. gonorrhoeae* (spectinomycin), mycobacterial (streptomycin, amikacin) and protozoal (paramomycin) infections.

Severe Gram-negative infections

With the increasing use of carbapenems (e.g. imipenem/cilastatin and meropenem) and fluoroquinolones (e.g. ciprofloxacin and levofloxacin), there has been a significant increase in the emergence of multidrug-resistant bacteria. Due predominantly to their broad spectrum of bactericidal activity against Enterobacteriaceae, *Acinetobacter* spp. and *P. aeruginosa*, gentamicin, tobramycin and amikacin have played a major role in the treatment of severe to life-threatening infections suspected or documented to be caused by Gram-negative bacteria.

Recent interest has focused on the potential application of aminoglycosides for the treatment of multidrug-resistant Gram-negative infections caused by organisms producing ESBLs.[65] Among the aminoglycosides, amikacin appears to be the most active *in vitro*. However, a marked increase in the incidence of aminoglycoside resistance is seen in ESBL producers (relative to nonproducers).

Infections treated with aminoglycosides as part of a combination therapy include (but are not limited to) empiric treatment of the febrile neutropenic patient,[66] sepsis,[67] nosocomial pneumonia[68,69] and intra-abdominal infections[70] (see Chapter 37). However, despite their favorable activity *in vitro*, there are concerns regarding treatment-related toxicity, need for serum concentration monitoring, availability of alternative therapies and the general lack of controlled clinical data to support their use as part of combination therapy. Such skepticism has been reflected in the general lack of support in recently published guidelines for their empiric use in clinically stable patients for many of these infections. In contrast, many of these recommend consideration of aminoglycoside-containing regimens (usually in combination with a β-lactam) in severe refractory infections or in patients with an increased risk of a multidrug-resistant pathogen (most notably *P. aeruginosa* and *Acinetobacter* spp.).

Gram-positive infections

Enterococci

Aminoglycosides (either gentamicin or streptomycin) may be combined with either a penicillin or vancomycin in an attempt to achieve a bactericidal effect for the treatment of specific, serious enterococcal infection (e.g. enterococcal endocarditis).[71] As previously discussed, all enterococci are resistant to aminoglycosides, based on achievable serum concentrations; however, in the absence of high-level resistance, the potential exists for synergistic activity with penicillins (usually ampicillin) or vancomycin.[28]

Enterococci with high-level resistance to gentamicin contain the bifunctional aminoglycoside-modifying enzyme acetyltransferase (AAC) (6′)-phosphotransferase (APH) (2′), which abolishes synergy with all aminoglycosides except streptomycin.[28] It is important to note that, although the bifunctional enzyme modifies amikacin and abolishes synergy with β-lactams and vancomycin, it does not lead to a resistant phenotype.[28] Therefore, amikacin should not be used against strains with high-level resistance to gentamicin, even in the absence of high-level resistance to amikacin. In the absence of high-level streptomycin resistance, this aminoglycoside remains the only alternative for strains with high-level resistance to gentamicin.[28]

In general, the use of aminoglycosides for the treatment of enterococcal infections is generally reserved for severe infections. For example, target peak and trough serum gentamicin concentrations are 3–4 μg/ml and <1 μg/ml, respectively, when 3 mg/kg/day is administered in three divided doses for the treatment of enterococcal endocarditis (as part of combination therapy) in the absence of evidence for high-level aminoglycoside resistance.[71] Since conflicting animal data exist, use of consolidated dosing for this indication is generally discouraged.[1]

Staphylococci

Because of alternative treatment options, aminoglycosides are rarely used as monotherapy for the treatment of staphylococcal infections.[6] However, the role for aminoglycosides (as part of combination therapy) for most staphylococcal infections is uncertain. In one study comparing nafcillin either alone or in combination with gentamicin for the treatment of *Staph. aureus* endocarditis (predominantly left-sided), the addition of the aminoglycoside reduced the duration of bacteremia by approximately 1 day, but otherwise did not improve outcome.[72] In contrast, an increase in aminoglycoside-associated nephrotoxicity was observed. The optimal dose of gentamicin for the treatment of staphylococcal infections is also unknown. In the setting of severe infections with MSSA (most notably endocarditis), gentamicin 3 mg/kg/day in divided doses (in combination with a β-lactam for MRSA) may be considered for a period of 3–5 days or up to 2 weeks in the setting of a prosthetic valve after susceptibilities have been confirmed.[71] Based on data from *in vitro* models, higher single doses may be more effective as part of combination therapy. In the absence of endocarditis, there is little support for the routine use of gentamicin in the treatment of staphylococcal infections.[73]

Streptococci

Similar to its roles in the treatment of enterococci and staphylococci, gentamicin (in combination with ceftriaxone) is included among the recommended options for the treatment of endocarditis due to viridans streptococci and *Streptococcus bovis*,[71] recently renamed *Strep. gallolyticus.* However, in contrast to these infections, a single daily dose of 3 mg/kg may be considered for native valve endocarditis with penicillin-sensitive viridans streptococci.[53,71]

Mycobacterial infections

The clinical role of streptomycin and amikacin as well as the other aminoglycosides in the treatment of mycobacterial infections is discussed in detail in Chapter 30.

Streptomycin has the greatest *in-vitro* activity among the aminoglycosides against *M. tuberculosis*. However, increasing streptomycin resistance (noted particularly in high-prevalence areas), combined with its toxicities, have made it a second-line agent in the treatment of tuberculosis.[27] Amikacin may be considered in specific cases of multidrug-resistant tuberculosis, since some streptomycin-resistant isolates may be susceptible *in vitro* to amikacin.[27,74] Streptomycin and amikacin are usually dosed in adults with normal renal function as a single daily injection of 15 mg/kg per day (maximum 1 g/day) five to seven times per week for 2–4 months, then two to three times weekly (depending upon response).[27] Children should receive streptomycin 20–40 mg/kg/day (maximum 1 g/day) or amikacin 15–30 mg/kg/day (maximum 1 g/day) as a single daily dose. Reduced doses (i.e. 10 mg/kg/day, maximum 750 mg/day) are administered to persons >60 years of age.[27]

The role of aminoglycosides in the treatment of atypical mycobacterial infections has been summarized elsewhere.[26] For *M. abscessus* and *M. fortuitum*, amikacin 15 mg/kg/day (once daily or in divided

doses) or 25 mg/kg three times weekly can be considered. For *M. chelonae*, tobramycin is generally more active *in vitro* than amikacin. Streptomycin and amikacin may also have activity against specific strains of *M. kansasii*.[26] Three times weekly doses of amikacin or streptomycin 25 mg/kg have been employed in the treatment (as part of combination therapy) of select patients with *M. avium* complex, most often those with multilobar, cavitary pulmonary disease or those refractory to prior therapy.[26]

Other infections and indications

The principal uses of streptomycin as monotherapy have been for plague, tularemia and brucellosis (see Chapters 120, 121 and 123). Gentamicin therapy has also been used in such infections. However, streptomycin monotherapy has been associated with a high failure rate in patients with brucellosis, and is now used more commonly as part of a combination regimen.[75]

Because paromomycin is too toxic following intravenous administration and is not absorbed following oral administration, its use has been restricted to the treatment of intestinal infections, particularly cryptosporidiosis in patients who have AIDS.[76] However, the efficacy of this agent has been questioned in recent clinical trials, and the introduction of FDA-approved treatment options for this infection (i.e. nitazoxanide) will likely limit its use in the future.[76] For the treatment of intestinal amebiasis, the usual dose in adults and children is 25–35 mg/kg/day divided q8h with meals for 5–10 days. Historically, paromomycin has been used for treatment or prevention of travelers' diarrhea because it is also active against *E. coli* and *Salmonella* spp. However, such an indication has largely been replaced by newer, more widely available and better-tolerated agents.

Unlabeled uses of paramomycin include treatment of other parasitic infections such as *Dientamoeba fragilis* (25–30 mg/kg q8h for 7 days), *Diphyllobothrium latum*, *Taenia saginata*, *Taenia solium*, *Dipylidium caninum* (adult dose: 1 g every 15 minutes for four doses; pediatric dose: 11 mg/kg every 15 minutes for four doses) and *Hymenolepis nana* (45 mg/kg/day for 5–7 days).

Like paromomycin, neomycin is too toxic for systemic use. It has mainly been used by mouth as a prophylactic agent (along with erythromycin) in colonic surgery and in hepatic encephalopathy to reduce the number of aerobic enteric organisms. However, the availability of alternative prophylactic strategies has largely replaced neomycin for this indication.

In the treatment of hepatic encephalopathy, the usual dose in adults is 4–12 g/day in divided doses. Treatment is usually continued for 5–6 days. In patients with chronic hepatic insufficiency, neomycin may be required (in adults up to 4 g/day) indefinitely.

An infrequent use of gentamicin is to combine it with ampicillin in the therapy of infections due to *Listeria monocytogenes*.[77] In patients allergic to penicillin, trimethoprim–sulfamethoxazole (co-trimoxazole) can be used either alone or in combination with gentamicin.[77]

Although all aminoglycosides exhibit activity against *Neisseria* spp., only the aminocyclitol spectinomycin (not available in the USA) is used for gonococcal infection.[78] Use of aminoglycosides for such treatment, however, is very uncommon because most gonococci are susceptible to alternative therapies (such as third-generation cephalosporins). However, gentamicin (in combination with clindamycin) is considered a treatment option for pelvic inflammatory disease.[78]

Role in prophylaxis

The use of gentamicin (as part of a combination regimen) in the prophylaxis of infection has generally been restricted to colorectal or gynecologic procedures in patients at risk of Gram-negative infections unable to tolerate other options.[62] Aminoglycosides are no longer recommended for the prevention of infective endocarditis.[79] Their role as part of combination therapy (in addition to vancomycin in β-lactam allergic patients) for cardiac surgery is unclear.[61]

Aerosol therapy

Tobramycin has been administered by aerosol to patients with cystic fibrosis with the goal of providing high lung concentrations of antibiotic while minimizing systemic exposure. Data in this population have demonstrated the potential to decrease the density of *P. aeruginosa* in the sputum, thereby increasing pulmonary function and decreasing hospitalization.[64,80,81] However, its role in therapy is uncertain.

ADVERSE REACTIONS

The major adverse effects associated with aminoglycosides include neuromuscular blockade, nephrotoxicity and ototoxicity (auditory and vestibular). Aminoglycosides seldom produce hypersensitivity reactions, phlebitis, hematologic dyscrasias, hepatotoxicity, electrolyte disturbances (except those associated with renal dysfunction) or drug fevers.

Neuromuscular blockade

The neuromuscular blocking activity of aminoglycosides may manifest as weakness of the respiratory musculature, flaccid paralysis and dilated pupils. The true incidence of this reaction is unknown, but is thought to be infrequent. Risk factors include concomitant administration of drugs with neuromuscular blocking activity, rapid intravenous infusions and instillation of large concentrations into the peritoneal cavity. Infant botulism, myasthenia gravis, hypocalcemia and hypomagnesemia have also been associated with an increased risk for this adverse reaction. Consolidated dosing does not appear to increase the incidence of this reaction.[82]

Neuromuscular blockade was shown to be rapidly reversed by the administration of calcium gluconate in an animal model. With supportive care alone, in time, the blockade will resolve.

Nephrotoxicity

Except for spectinomycin, all aminoglycosides are capable of producing nephrotoxicity by interfering directly with renal tubular function and indirectly with glomerular filtration.[51] Clinical manifestations include a decreased urine concentrating ability, elevations in serum creatinine and polyuria. Because of excessive magnesium losses in the urine, hypomagnesemia may occur, which in turn can lead to secondary hypocalcemia and hypokalemia. Progression to anuric renal failure as a result of aminoglycoside nephrotoxicity is rare.

After discontinuing aminoglycoside therapy, evidence of nephrotoxicity usually disappears within several days in the absence of other causes of nephrotoxicity.[51]

Challenges exist in determining the incidence of nephrotoxicity secondary to aminoglycoside administration, including variations in study definitions and control of potential confounders. Using a definition of nephrotoxicity as a rise in serum creatinine concentration of 0.5 mg/dl above the baseline, approximately 5–20% of patients develop this adverse reaction using conventional dosing regimens. While previous clinical studies have compared the nephrotoxic potential of aminoglycosides, elements of their study design make definitive comparisons problematic. Based on animal model data, neomycin is generally considered the most nephrotoxic aminoglycoside: therefore, parenteral administration is avoided. Among the aminoglycosides most commonly prescribed (i.e. gentamicin, tobramycin, netilmicin and amikacin) there appear to be no clinically relevant differences in toxicity.

Risk factors for the development of aminoglycoside-induced nephrotoxicity include method of administration, underlying renal dysfunction, concomitant use of nephrotoxic agents, hypotension and duration of therapy.[1] Sustained elevated trough concentrations reflect impaired drug elimination and the need for dosing adjustment. Loop diuretics (such as ethacrynic acid and furosemide) may increase

the risk of aminoglycoside renal toxicity through volume depletion. Concomitant use with vancomycin, *cis*-platinum, foscarnet, amphotericin B, methoxyflurane and intravenous radiocontrast agents has also been shown to increase the incidence of aminoglycoside-induced nephrotoxicity. Although elderly patients are traditionally considered to be at higher risk for aminoglycoside nephrotoxicity, they may actually not be if one adjusts for an age-related decrease in glomerular filtration rate.

The aminoglycoside enzyme transfer system has a finite (i.e. saturable) capacity for internalizing aminoglycosides within the proximal renal tubular cell. Therefore, the amount of aminoglycoside that enters the cell is the same over 24 hours whether the dose is given in divided fashion or as a single dose.[83] Because of low or undetectable levels of aminoglycosides for 10–12 hours during the 24-hour period with once-daily dosing, most of the previously internalized drug gets transported out of the cell, resulting in less accumulation. This would explain the observation (based on animal model data) that nephrotoxicity has been shown to be greatest when the daily dose is divided into multiple small doses rather than as the same dose given once. It may also help to explain why sustained trough concentrations above the therapeutic range also predispose the patient to an increased risk of nephrotoxicity. While few adequately controlled studies are available to validate this in humans, existing clinical data generally support the potential for reductions in nephrotoxicity when consolidated dosing is utilized.

Ototoxicity

The ototoxicity of aminoglycosides includes both auditory and vestibular components. Both auditory and vestibular toxicity can occur in the same patient either during or following therapy.

Auditory toxicity occurs as a result of the accumulation of aminoglycosides in the perilymph of the inner ear with subsequent damage of the sensory cells of the organ of Corti, while the target for vestibular toxicity is the sensory hair cells of the vestibular epithelia located at the summit of the ampullary cristae.[84,85] Serious damage to these cells typically results in a permanent deficit. Risk factors for ototoxicity include age (≥60 years), pre-existing ear disease, prolonged therapy, repeated treatment with aminoglycosides, and concomitant use of loop diuretics (e.g. ethacrynic acid) and other ototoxic drugs.[86] Since ototoxicity is rarely seen when given for less than 10 days, duration of therapy is likely to be the major determinant.

Symptoms of auditory toxicity include hearing loss, tinnitus and a sensation of fullness in the ear (most frequently bilateral).[85] The onset of symptoms may occur during or after cessation of treatment.

Cochlear toxicity is usually assessed by pure-tone audiometric testing, with earliest signs usually detected at frequencies above 8 kHz. Because perception of human speech occurs in the 0.3–3 kHz range, significant cochlear damage can occur before hearing loss is apparent.

Clinical studies show that the incidence of cochlear toxicity due to aminoglycoside use varies from approximately 5% to 15% with conventional dosing of aminoglycosides.[85] The frequency varies and depends upon the method of establishing toxicity. Data in humans are insufficient to address the comparative ototoxicity of aminoglycosides given by consolidated dosing. Similar to nephrotoxicity, there is controversy regarding the comparative potential for cochlear damage among the aminoglycosides. However, it appears that there is little, if any, clinically relevant difference between gentamicin, tobramycin, amikacin and netilmicin. Compared with these agents, neomycin has more often been associated with cochlear damage. The potential for ototoxicity in humans with topical preparations remains controversial. In a study of 44 children with chronic suppurative otitis media given topical preparations containing five different aminoglycosides (four neomycin; one gentamicin), there was no evidence of ototoxicity.[87]

Symptoms of vestibular toxicity include nausea, vomiting, vertigo, nystagmus, difficulty with gait and difficulty fixating on objects. Difficulty with gait is especially prominent in the dark because of the loss of sight, which compensates for vestibular dysfunction.

The frequency of vestibular toxicity is difficult to establish because vestibular function testing is poorly standardized. Of the aminoglycosides, streptomycin is more often associated with vestibular toxicity.

Acknowledgment

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REFERENCES


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Chapter 138

Timothy Hills
Vivienne Weston
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Folate inhibitors

INTRODUCTION

Sulfonamides

Sulfonamides are competitive inhibitors of *para*-aminobenzoic acid (PABA), which is essential for folic acid synthesis in most bacteria, some protozoa and *Pneumocystis jirovecii* (formerly *P. carinii*) (Fig. 138.1).[1] A consequence of the mode of action is that sulfonamides lack activity against organisms for which PABA is not an essential metabolite (e.g. *Enterococcus* spp.). The eukaryotic cell does not use PABA and sulfonamides do not interfere with human folic acid synthesis.

Dihydrofolate reductase inhibitors

Dihydrofolate reductase is an enzyme common to mammals, bacteria and protozoa alike as part of the synthesis of tetrahydrofolate from folic acid. Trimethoprim is a diaminopyrimidine that competitively inhibits dihydrofolate reductase.[1,2] Compared with many other diaminopyrimidines with antimicrobial activity (e.g. pyrimethamine), trimethoprim has a higher affinity for bacterial dihydrofolate reductase than for the human enzyme, thus reducing the risk of folic acid deficiency in the treated patient.[3] Pyrimethamine is also a competitive dihydrofolate reductase inhibitor. It has a high affinity for protozoal enzyme.

In addition to the above modes of action, it has been proposed that trimethoprim may inhibit the adhesion of bacteria to human mucosal cells.[4]

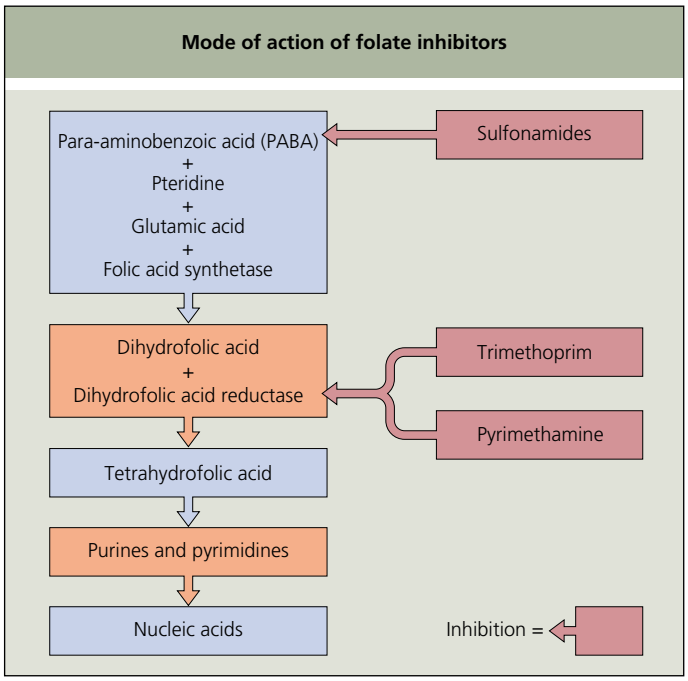


Fig. 138.1 Mode of action of folate inhibitors.

Combinations of sulfonamides and trimethoprim or pyrimethamine

Combinations of sulfonamides and trimethoprim or pyrimethamine interfere with two consecutive steps in the same metabolic chain in the micro-organism. This may lead to synergistic antimicrobial activity. The rationale for a fixed combination of trimethoprim and sulfamethoxazole is that, although both antibiotics alone are bacteriostatic, the combination may be bactericidal. The optimal trimethoprim–sulfamethoxazole (TMP–SMX) ratio for synergism inside a bacterium is 1:20. Based on the pharmacokinetics of the two agents, this ratio is obtained systemically with a 1:5 dosage combination.[5]

The clinical relevance of the synergism is difficult to prove in experimental infections and even more so in clinical trials. This has led to questioning of the clinical usefulness of the combination in comparison with trimethoprim alone for the treatment of bacterial infections.[6] An argument in favor of the combination is the possibility of the reduced risk of resistance; organisms initially susceptible to both sulfonamides and trimethoprim are less likely to develop resistance to combinations than to single drugs.

Folate inhibitor combinations are used for treating bacterial as well as fungal and protozoal infections. Because of the emergence of bacterial resistance and risks for adverse reactions, the sulfonamides have lost most of their usefulness as single agents. Emphasis will be put on combinations of sulfonamides and trimethoprim, especially the most widely used, namely TMP–SMX and pyrimethamine–sulfadoxine.

PHARMACOKINETICS

The sulfonamides are classically subdivided on the basis of their elimination time into short-acting (plasma half-life, $t_{½}$ <8 h), medium-acting ($t_{½}$=8–16 h), long-acting ($t_{½}$=17–48 h) and ultra-long-acting ($t_{½}$>48 h). Sulfonamides used as single agents today are short or medium acting.

Plasma kinetics

Currently used sulfonamides, as well as trimethoprim and pyrimethamine, are well absorbed after oral administration and have

high bioavailability. Following an oral dose of 160 mg of trimethoprim and 800 mg of sulfamethoxazole, maximal plasma concentrations of 1.6–1.9 mg/l and 26–41 mg/l, respectively, are achieved.[7] After intravenous administration of 240 mg trimethoprim and 1200 mg sulfamethoxazole q12h, peak plasma concentrations in the steady state are about 6 mg/l for trimethoprim and 180 mg/l for sulfamethoxazole.[8] The protein binding of the sulfonamides varies from less than 50% for sulfadiazine to more than 90% for sulfadoxine. Importantly, sulfonamides bind firmly to albumin and may displace other compounds (e.g. bilirubin). In newborns this may lead to toxic levels of unbound bilirubin with a subsequent risk of 'kernicterus' (see Central nervous system reactions, below).

Distribution

Trimethoprim is lipid soluble at physiologic pH and has a large volume of distribution (100–120 l), whereas sulfamethoxazole is a weak acid with poor lipid solubility at pH values above 7, leading to a volume of distribution corresponding to that of the extracellular space (i.e. 12–18 l). In tissues concentrations similar to or higher than those in plasma are achieved with trimethoprim, whereas considerably lower levels of sulfamethoxazole reach peripheral compartments. Concentrations above the minimum inhibitory concentrations (MICs) of trimethoprim-susceptible strains are achieved in most tissues and tissue fluids. With sulfamethoxazole, the peripheral concentrations are sometimes so low that it should be questioned whether therapeutic levels are reached. All of the sulfonamides as well as trimethoprim achieve high urinary concentrations.

Elimination

The main routes of elimination of sulfonamides, trimethoprim and pyrimethamine are via liver metabolism and renal excretion.[9] In patients who have normal renal function, half-life varies from less than 6 hours for sulfisoxazole and sulfamethizole to 11–17 hours for sulfamethoxazole and sulfadiazine and more than 200 hours for sulfadoxine. Trimethoprim has a half-life of about 15 hours and pyrimethamine is eliminated slowly, with a half-life of about 100 hours.

Kinetics in children

The kinetics of both sulfamethoxazole and trimethoprim differ between children and adults (Table 138.1). Elimination is faster in children, who must be given relatively higher doses than adults.

Table 138.1 Comparative kinetics of trimethoprim (T) and sulfamethoxazole (S) in children and adults

	AGE (YEARS)			
Parameter	**<1**	**1–9**	**10–19**	**20–63**
T volume of distribution (l/kg)	2.0	1.6	1.5	1.4
S volume of distribution (l/kg)	0.5	0.5	0.4	0.4
T plasma half-life (h)	11	5.6	10	16
S plasma half-life (h)	7.5	9.8	10	15

ROUTE OF ADMINISTRATION AND DOSAGE

Most sulfonamides, trimethoprim and pyrimethamine are available for oral use. Trimethoprim, sulfamethoxazole and sulfadiazine are also used intravenously; intravenous use of TMP–SMX is recommended when patients are unable to take the drug orally. Dosages for some of the sulfonamides and for combinations of sulfonamides and dihydrofolic acid inhibitors are given in Table 138.2.

Table 138.2 Adult dosages of some folate inhibitors*

Drug	Indication and recommended dose for adults
Pyrimethamine	Malaria prophylaxis (with sulfadoxine) 25 mg once weekly; malaria therapy (with sulfadoxine; Fansidar) 50–75 mg as single dose; toxoplasmosis encephalitis therapy (with sulfadiazine and calcium folinate) 75–200 mg loading dose, followed by 25–100 mg q24h for 3–6 weeks, followed by 25–50 mg q24h (maintenance therapy in AIDS); ocular toxoplasmosis (with sulfadiazine and calcium folinate) 100 mg loading dose q24h for 1–2 days followed by maintenance doses of 25–50 mg q24h
Sulfadiazine	Toxoplasmosis encephalitis therapy (with pyrimethamine and calcium folinate) 0.5–1.5 g q6h; ocular toxoplasmosis (with pyrimethamine and calcium folinate) 0.5–1 g q6h
Sulfadoxine	Malaria prophylaxis 500 mg once weekly (with pyrimethamine; Fansidar); malaria therapy 1–1.5 g (with pyrimethamine; Fansidar) as single dose
Co-trimoxazole (trimethoprim with sulfamethoxazole ratio 1 part to 5 parts)	UTI 1.92 g single dose or 480–960 mg q12h; systemic bacterial infections 960 mg q12h; pneumocystis pneumonia treatment 60 mg/kg q12h; pneumocystis pneumonia prophylaxis 960 mg thrice weekly or daily
Trimethoprim	UTI 100–200 mg q12h

*For pediatric doses, see the manufacturers' recommendations.

INDICATIONS

The activity of TMP–SMX against enterococci is controversial. There are reports in the literature of enterococcal bacteremia during treatment with TMP–SMX despite full *in-vitro* sensitivity pre-therapy, and of high failure rates and rapid emergence of resistance in enterococcal urinary tract infections (UTIs).[10]

Reduced susceptibility or resistance to penicillin G by *Streptococcus pneumoniae* seems to be coupled to resistance to TMP–SMX in a very high percentage of strains studied.[11,12] Overall, resistance to TMP–SMX in pneumococci is a rapidly increasing problem.[13]

Streptococcus pyogenes is normally sensitive to TMP–SMX, but resistance has been reported in macrolide-resistant isolates.[14] With regard to other Gram-positive organisms, *Listeria monocytogenes* is susceptible to the combination.[15]

Escherichia coli show marked variation in susceptibility to TMP–SMX, not only between but also within countries. However, there is a clear trend towards increasing frequencies of resistance and in most countries 12% or more of *E. coli* isolates are resistant to trimethoprim and TMP–SMX.[16,17]

Shigella and *Salmonella* spp. also show varying sensitivity to TMP–SMX, with frequencies of resistance ranging from less than 5% to over 50%.

Several studies have indicated a relatively high frequency of selection of resistance to TMP–SMX in Enterobacteriaceae when the antibiotic is used therapeutically or, in particular, prophylactically.[18]

Reduced rates of susceptibility of *Haemophilus influenzae* to TMP–SMX have also been reported.[13]

The spectrum of the folate inhibitors also includes micro-organisms other than bacteria. The treatment of choice for *Toxoplasma gondii* remains a combination of pyrimethamine and sulfadiazine. The combination of sulfadoxine and pyrimethamine is used for treatment but less often for prevention of falciparum malaria in areas with chloroquine resistance. However, unlike *T. gondii*, for which no resistance against pyrimethamine–sulfadiazine has yet been reported, resistance against pyrimethamine–sulfadoxine is not uncommon in *Plasmodium falciparum* and might become a problem with *Pneumocystis jirovecii*.[19,20]

Use of sulfonamides alone

Systemic sulfonamides have been used for the treatment of uncomplicated UTIs; however, this is no longer recommended.

Use of trimethoprim alone

In many European countries, trimethoprim is available as a single agent. Due to its better adverse reaction profile and avoidance of hypersensitivity reactions to sulfonamides, it is favored over TMP–SMX for some infections.

Urogenital infection

Whilst resistance can sometimes be a problem, trimethoprim is the first-line drug for the treatment of uncomplicated UTI in many European countries[21] at a dose of 200 mg orally twice daily for 3 days.

Respiratory tract infections

Trimethoprim has been shown comparable to TMP–SMX and doxycycline for the treatment of lower respiratory tract infections, although its first-line use is often hampered by emerging *Strep. pneumoniae* resistance.[22]

Use of trimethoprim–sulfamethoxazole

Trimethoprim–sulfamethoxazole has lost some of its usefulness through the emergence of resistance and increased awareness of the risk of adverse reactions, which may be serious or life-threatening.

Urogenital infections

Urogenital infections are the most common indications for TMP–SMX (Table 138.3); however, in some countries this has been replaced by the use of trimethoprim alone (see above). High frequencies of clinical and bacteriologic cure have been documented in women who have uncomplicated cystitis as well as in patients who have pyelonephritis, complicated UTI or prostatitis.

Importantly, the use of TMP–SMX is well documented for single-dose or short-term treatment of uncomplicated cystitis in women.[23] It is equally effective if given for 3 days compared with treatment for 5–10 days, and a single dose is only slightly less effective than 3-day treatment. For other types of UTI, longer treatment times are required. In countries where resistant uropathogens are uncommon, single-dose or short-term TMP–SMX is an inexpensive and effective treatment of cystitis in women. For other types of infection, where longer treatment times are required, the relatively high frequency of potentially serious adverse reactions must be taken into account, and in most adult patients alternative agents are now preferred.

Prostatitis is commonly treated with TMP–SMX and the few studies evaluating its use for this infection indicate a high degree of efficacy.[24]

Table 138.3 Clinical use of trimethoprim–sulfamethoxazole

Type of infection	Limitations
Uncomplicated UTI	None for short-term therapy (single dose or 3 days)
Other types of UTI	Resistance, safety
Shigellosis	Resistance
Salmonellosis	Resistance
Enteric fever	Resistance
Travelers' diarrhea	Resistance, safety
Otitis media	Resistance
Community-acquired pneumonia	Resistance, safety
Melioidosis	Resistance, efficacy
Prophylaxis in immunocompromised patients	Resistance, safety, efficacy
Pneumocystis jirovecii pneumonia	None

For *Haemophilus ducreyi* infections 160 mg of trimethoprim plus 800 mg sulfamethoxazole q12h for 3 days has resulted in high cure rates, whereas shorter treatment times seem ineffective.[25]

Infections caused by *Chlamydia trachomatis* respond poorly to TMP–SMX.[25]

Enteric infections

These have been extensively treated with TMP–SMX because of its activity against *Salmonella* spp. (including *Salmonella typhi*), *Shigella* spp., *Vibrio cholerae* and enterotoxigenic *E. coli*. In a well-controlled trial in patients who had enteritis of verified etiology, the causative pathogens were eliminated on treatment day 2 in 41% of patients treated with TMP–SMX compared with 23% of those receiving placebo and 91% of patients on norfloxacin.[26]

A review of patients who had shigellosis showed that 97% of 149 patients treated with TMP–SMX responded clinically and 90% bacteriologically.[27] With the comparators (ampicillin, furazolidone or sulfadimidine) the clinical success rate was 78%. However, TMP–SMX may have lost some of its usefulness in shigellosis through the emergence of resistance.

Trimethoprim–sulfamethoxazole has been shown to be as effective as chloramphenicol for the treatment of enteric fever caused by susceptible strains of *S. typhi* and *S. paratyphi*.[28] However, the emergence of multidrug-resistant strains has limited their usefulness and other agents are now preferred.[29]

In salmonellosis TMP–SMX is effective for treatment of invasive infections caused by sensitive strains, but, like other antibiotics, it seems less effective in eliminating the carrier state of *Salmonella* spp.[30]

Treatment of cholera should be aimed mainly at rehydration of the patients. However, antibiotics may reduce symptoms and shorten the duration of the carrier state of *V. cholerae*, thereby reducing the risk of transmission. Trimethoprim–sulfamethoxazole has proved effective in patients who have cholera and one study showed that it was better than tetracycline or sulfamethoxazole alone.[31]

In several studies TMP–SMX has been shown to be effective in preventing travelers' diarrhea.[32] However, it has also been found to select for resistance which, along with serious adverse reactions, makes this type of prophylaxis of doubtful value.[33]

Respiratory tract infections

Due to adverse drug reactions and the emergence of resistance in *Strep. pneumoniae*, TMP–SMX is no longer considered first line for the treatment of lower and upper respiratory tract infections.

In pneumonia caused by *Burkholderia pseudomallei*, melioidosis, TMP–SMX remains a choice for oral long-term treatment after standard treatment with ceftazidime, although some doubt exists about its effectiveness.[34] Trimethoprim–sulfamethoxazole is the drug of choice for the treatment and prevention of *P. jirovecii* pneumonia in the immunosuppressed.[35]

Other infections

Both trimethoprim and sulfamethoxazole penetrate the blood–cerebrospinal fluid barrier and the combination has been found to be effective in experimental bacterial meningitis.[36] Trimethoprim–sulfamethoxazole has therefore been used as an alternative drug for the treatment of bacterial meningitis. Favorable clinical results have been reported in the treatment of meningitis caused by *L. monocytogenes* as well as other types of meningitis.[37]

Trimethoprim–sulfamethoxazole is considered a first-line drug in the treatment of health-care associated infections due to *Stenotrophomonas maltophila*. This relatively avirulent Gram-negative bacillus can cause infection in immunocompromised patients and is often a multidrug-resistant pathogen.

In patients who have brucellosis TMP–SMX can be considered a second-line drug.

Several studies have shown excellent clinical results with TMP–SMX, alone or in combination with aminoglycosides, in the treatment of actinomycosis or nocardiosis.[38,39]

Trimethoprim–sulfamethoxazole 160/800 mg twice daily for 6 weeks may be considered an alternative in the treatment of ocular toxoplasmosis.[40]

A controversial field for the use of TMP–SMX is prophylaxis in neutropenic patients. Early studies showed significant protection against bacterial infections but its role has generally been superseded by other classes (e.g. fluoroquinolones).

Pyrimethamine–sulfadiazine and pyrimethamine–sulfadoxine

Pyrimethamine–sulfadiazine remains the first-line drug combination for the treatment of toxoplasmosis.[41]

Pyrimethamine–sulfadoxine is an alternative for the treatment of *P. falciparum* malaria in areas with chloroquine resistance.[42] However, resistance to pyrimethamine–sulfadoxine is not uncommon and safety concerns reduce its usefulness for prophylaxis.

DOSAGE IN SPECIAL CIRCUMSTANCES

Renal impairment results in prolonged elimination times. Table 138.4 gives dosages of sulfamethoxazole and trimethoprim in patients who had decreased renal function. This includes patients of advanced age. As pointed out, the above doses used in children should be higher than those in adults because of different kinetic profiles (see Table 138.1). All sulfonamides should be avoided in patients aged less than 6 weeks because of the risk of cerebral accumulation of free bilirubin (kernicterus). There are no recommendations for reduced dosage of folate inhibitors in patients who have hepatic disease. Most of the folate inhibitors pass to breast milk but at concentrations that make any toxic effects on the child unlikely. During pregnancy, particularly in the first trimester, trimethoprim and pyrimethamine should be avoided because of the possible risk of altered folate metabolism in the fetus. Sulfonamides should not be given during the last trimester of the pregnancy because of the risk of kernicterus.

ADVERSE REACTIONS AND INTERACTIONS

A summary of the potential adverse effects of folate inhibitors is given in Table 138.5.

Table 138.4 Effect of renal function on dosage of TMP–SMX and trimethoprim[53]

Creatinine clearance	Co-trimoxazole (TMP–SMX)	Trimethoprim
>30–50 ml/min	Dose as in normal renal function	Dose as in normal renal function
15–30 ml/min	Normal dose for 3 days then use 50% of the normal dose as the normal frequency	Normal dose for 3 days then use 50% of the normal dose q18h
<15 ml/min	Normal dose for 3 days then use 50% of the normal dose as the normal frequency *Note*: High-dose treatment (60 mg/kg q12h) should only be given if hemodialysis facilities are available	Give 50% of the normal dose q24h

Table 138.5 Adverse actions of folate inhibitors in humans

Body system	Sulfonamides	Trimethoprim–pyrimethamine
Central nervous system	'Kernicterus' in newborns	Aseptic meningitis, especially in patients who have collagen diseases
Liver	Toxic hepatitis	Probably none
Lung	None	None
Kidney	Crystalluria	Increased serum creatinine (inhibition of creatinine excretion); increased potassium
Prostate/ genitourinary	None	None

General safety profile

Since several studies have shown a correlation between the treatment time and the risk of adverse reactions to TMP–SMX when used for uncomplicated UTIs, short-course therapy (3 days) is encouraged.[23]

Hematologic reactions

The mode of action of trimethoprim has caused concerns over possible bone marrow toxicity. Studies in patients treated for 1 month or more with TMP–SMX have shown moderate folate deficiency.[43] The possibility of immune reactions causing hematologic adverse effects has been proposed.[44]

Serious and even fatal hematologic adverse reactions (usually cytopenias) to TMP–SMX have been reported. In a Swedish study of about 50 million daily doses an approximate frequency of fatal reactions to TMP–SMX was calculated to be 3.7/million treatments

(data from SWEDIS, Medical Products Agency, Uppsala, Sweden). It was noteworthy that the mean age of the patients who died was 78 years (range 41–96 years) and that only three of 18 patients were below the age of 70. Taking into consideration the effect of aging on renal function, the doses of TMP–SMX were high. In addition, the treatment time was long (range 3–73 days, mean 17 days, median 12 days).

Pyrimethamine hematologic toxicity is less well described. Many use folinic acid to avoid folic acid deficiency. Support is lacking and hematologic reactions may very well be due to other mechanisms.

Skin, mucocutaneous and allergic reactions

These reactions may in some cases be serious, for example Stevens–Johnson syndrome or toxic epidermal necrolysis.[45] Such reactions seem to be related to the sulfonamide component rather than to trimethoprim.

It is worth noting that, in most reports on the safety of TMP–SMX or sulfonamides, skin reactions are only rarely reported in children. Possible explanations for this are the reduced risk for overdosing in children due to efficient elimination and less risk of sensitization to trimethoprim or sulfamethoxazole from previous exposures.

High numbers of serious cutaneous reactions have been reported following treatment with pyrimethamine–sulfadoxine.[46] Between 1974 and 1989, 126 cases of mucocutaneous syndromes were reported, giving an estimated risk of about 1.1/million treatments. This risk, which is most probably related to the sulfadoxine component, is considered to be high enough to discourage routine use of the combination for malaria prophylaxis.

Patients who have AIDS and *P. jirovecii* pneumonia and are treated with high doses of TMP–SMX have high frequencies of cutaneous reactions as well as other adverse reactions.[47,48] These reactions seem to be related to dose and treatment time, and many patients who have AIDS who have developed skin reactions later tolerate low-dose TMP–SMX prophylaxis against *P. jirovecii*.

Hepatic side-effects

Cases of severe hepatic reactions to TMP–SMX have been reported and are most likely to be caused by the sulfonamide component.[49]

Gastrointestinal adverse reactions

Like many other orally administered antibiotics, TMP–SMX causes upper gastrointestinal adverse effects in some patients. Because of its low activity on the intestinal anaerobic flora it causes diarrhea only infrequently.

Renal safety

Sulfonamides with poor solubility can cause crystalluria. With sulfamethoxazole this does not seem to be a problem but, with sulfadiazine in high doses, crystalluria has been reported in AIDS patients who had toxoplasmal encephalitis.[50]

Hyperkalemia and increased serum creatinine have been reported in patients treated with TMP–SMX; elevated serum levels of creatinine seems to be related to competitive inhibition of the renal excretion of creatinine by trimethoprim.[51]

Central nervous system reactions

Aseptic meningitis is related to trimethoprim therapy. Several cases have been reported in the literature with some over-representation of patients who have collagen vascular diseases (e.g. Sjögren's syndrome).[52] The pathogenesis remains obscure but seems to be of an allergic nature, with rapid onset and relapses after provocation.

Sulfonamides can cause central nervous system toxicity in newborns (kernicterus) because of displacement of bilirubin from albumin, resulting in toxic bilirubin concentrations in the brain.

Drug–drug interactions

The sulfonamides cause variable inhibition of the cytochrome P450 enzyme CYP2C9. Trimethoprim is a possible inhibitor of CYP2C8 and CYP2C9. Many of the resultant interactions (Table 138.6) are minor, with concurrent usage often only requiring increased monitoring. The major exception is with methotrexate where concurrent usage, especially of high-dosage regimens, has led to major morbidity.

Table 138.6 Interactions between trimethoprim (TMP), TMP–SMX and sulfonamides with other drugs

Drug	Folate inhibitor	Interaction	Suggested management
Angiotensin-converting enzyme (ACE) inhibitors	TMP (and TMP–SMX)	Rarely causes serious hyperkalemia in patients with concurrent renal impairment	Monitor potassium levels
Azathioprine	TMP (and TMP–SMX)	Increased risk of hematologic toxicity	If combination necessary, monitor for leucopenia
Ciclosporin A	TMP, TMP–SMX, many other sulfonamides	Not fully defined. Reversible decrease of renal function (TMP, TMP–SMX); risk of accumulation if function deteriorates. Possible reduction in ciclosporin levels with some sulphonamides.	Monitor levels if sulfonamides are added to ciclosporin
Dapsone	TMP (and TMP–SMX)	Reduced clearance of both dapsone and trimethoprim	Monitor patient; increased dapsone toxicity has occurred
Digoxin	TMP (and TMP–SMX)	Reduced tubular secretion of digoxin	Monitor for toxicity
Folate inhibitors	TMP–SMX, sulfonamides, pyrimethamine	Concurrent use of pyrimethamine with sulfonamides has led to folate deficiency, megaloblastic anemia and pancytopenia	Supplementation with calcium folinate reduces the risk of folate deficiency
Lithium	TMP (and TMP–SMX)	Possible increased lithium levels	If combination necessary, monitor levels

Table 138.6 Interactions between trimethoprim (TMP), TMP–SMX and sulfonamides with other drugs—cont'd

Drug	Folate inhibitor	Interaction	Suggested management
Methotrexate	TMP, TMP–SMX	Potential severe bone marrow depression and pancytopenia	Combination of low-dose methotrexate with low-dose prophylactic TMP–SMX is commonly used in well monitored patients without problems. Higher doses of either drug are likely to be more problematic and have led to major morbidity. Use only if absolutely necessary with close monitoring
Nucleoside reverse transcriptase inhibitors	TMP–SMX, TMP	Reduced urinary clearance of lamivudine, zalcitabine and zidovudine. Possible interaction between TMP and stavudine	Close monitoring advised if combinations used
Phenytoin	TMP (and TMP–SMX), sulfadiazine, sulfamethizole	Reduced metabolism of phenytoin	If combination necessary, monitor phenytoin levels
Procainamide	TMP (and TMP–SMX)	Reduced clearance of procainamide	Increased risk of toxicity; lower procainamide dose may be required
Repaglinide	TMP (and TMP–SMX)	Increased repaglinide levels likely to result in hypoglycemia	Avoid if possible; monitor closely if not
Sulphonylureas	TMP, some sulfonamides, TMP–SMX	Possible increased risk of hypoglycemia	Advise patients of risk
Warfarin	TMP–SMX, some other sulfonamides	Reduced metabolism of warfarin	Monitor international normalized ratio (INR) closely when initiating and stopping TMP–SMX

REFERENCES

References for this chapter can be found online at http://www.expertconsult.com

Chapter 139

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Quinolones

INTRODUCTION

The quinolones are a heterogeneous group of synthetic antimicrobial agents. Originally deriving from 1,8-naphthyridine compounds (e.g. nalidixic acid), modern quinolones have evolved as shown in Figure 139.1

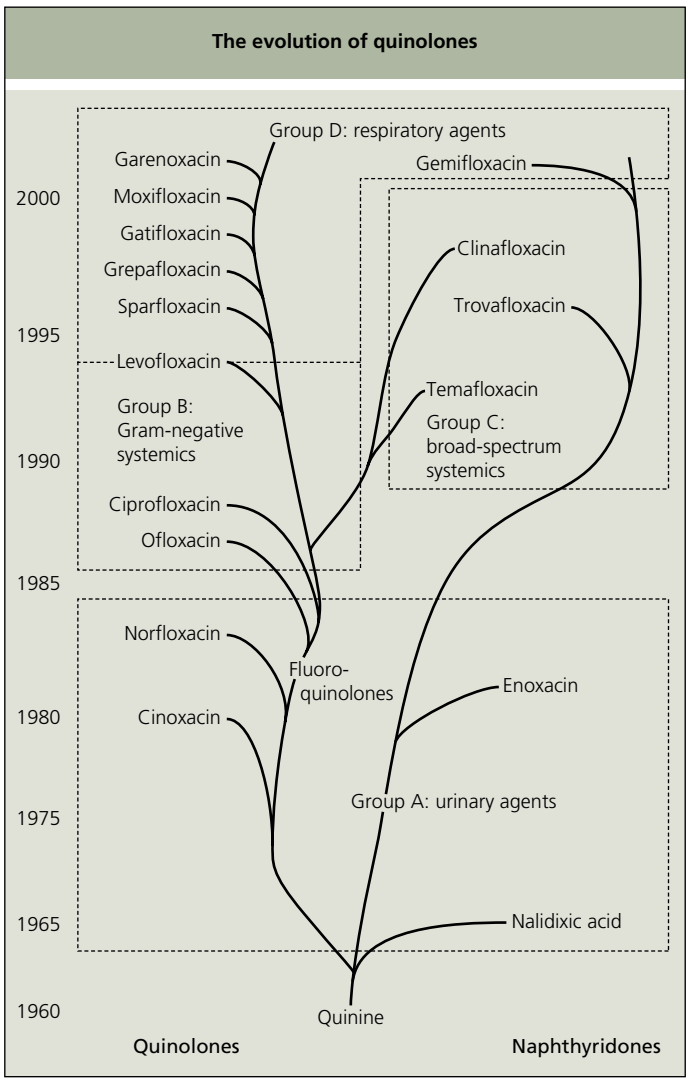


Fig. 139.1 The evolution of quinolones.

to give compounds initially with improved activity against Gram-negative bacteria (e.g. ciprofloxacin, ofloxacin) and more recently with greater activity against Gram-positives (e.g. gatifloxacin, moxifloxacin). A number of broader spectrum agents have been developed (e.g. clinafloxacin, trovafloxacin) but have had to be withdrawn due to problems with toxicity.

As quinolones have excellent tissue and tissue fluid penetration, they are suitable for infections in a wide range of organ systems. Adverse reactions are uncommon in marketed agents and relate mainly to the skin, the gastrointestinal system and central nervous system (CNS) and rarely warrant cessation of therapy. However, there are a number of potentially more serious adverse effects such as arthropathy, cardiotoxicity and phototoxicity. These occur as a class effect (although to different extents in different compounds) and have been a problem in drug development.

Modern fluoroquinolones are available in both intravenous and oral formulations. One of their major advantages has proved to be the ability to treat many serious infections with oral or intravenous–oral switch regimens, for example in the management of enteric fever, Gram-negative bacterial pyelonephritis, osteomyelitis, nosocomial pneumonia and severe exacerbations of both chronic bronchitis and cystic fibrosis. Many of the above previously demanded lengthy therapy with intravenous β-lactams, aminoglycosides or their combinations.

The activity of fluoroquinolones such as ciprofloxacin and ofloxacin in Gram-positive infections, notably those caused by pneumococci, has been disputed. Newer compounds, such as moxifloxacin, have markedly improved activity against Gram-positive pathogens and have found a place in the management of infections caused, for example, by penicillin-resistant pneumococci.

ANTIBACTERIAL SPECTRUM AND POTENCY

The antibacterial spectrum of quinolones is shown in Table 139.1. Quinolones are notable for the considerable knowledge that has been gained regarding structure–activity relationships.[1] The activity of the original naphthyridine and quinolone compounds (e.g. nalidixic acid) was limited to Gram-negative pathogens, primarily the Enterobacteriaceae, including Shigellae and Salmonellae. A major step forward in the development of the class was the addition of a fluorine at position 6, giving rise to the fluoroquinolones (Fig. 139.2). These agents are 10–100 times more active than their precursors against Gram-negative pathogens, including *Pseudomonas aeruginosa*, and have gained activity against the organisms causing atypical pneumonia. Potency, spectrum of activity and adverse effects/drug interactions are largely determined by substitutions at positions 1, 5, 6, 7 and 8:

- substitutions at position 1 (e.g. trovafloxacin) can alter potency (particularly against anaerobes) but may also affect interactions with theophyllines;
- substitutions at position 5 (e.g. grepafloxacin) can increase potency but may cause increased cardiotoxicity;

Table 139.1 Activity of quinolones against common pathogenic bacteria: MIC_{90} (mg/l)

Pathogen	Nalidixic acid	Norfloxacin	Ciprofloxacin	Ofloxacin	Levofloxacin	Grepafloxacin	Gemifloxacin	Gatifloxacin	Moxifloxacin	Garenoxacin
Streptococcus pneumoniae	>64	2–16	1–4	2–4	2	0.25	0.06	0.25	0.12	0.12
Staphylococcus aureus	>64	2	0.5–2	0.5–2	0.25	0.12	0.03	0.12	0.06	0.03
Enterococcus spp.	>64	8–16	1–8	2–8	2–8	1–16	0.25–>16	1–>16	0.5–4	0.5–8
β-Hemolytic streptococci	>64	4–8	2	4	1	0.25	0.03	0.5	0.25	0.12
Listeria spp.	NA	NA	1	2	1	NA	0.12	0.5	0.5	0.5
Haemophilus influenzae	1	0.25	0.03	0.03	0.03	0.016	0.015	0.03	0.06	0.03
Moraxella catarrhalis	4	0.25	0.06–0.25	0.12	0.06	0.015	0.03	0.12	0.12	0.03
Neisseria spp.	0.5	0.03	0.03	0.06	0.015	0.008	0.008	0.03	0.03	0.008
Escherichia coli	4–8	0.12–2	0.06–0.25	0.12–0.25	0.06–0.25	0.03	0.015	0.06–0.25	0.06	0.06–0.5
Klebsiella spp.	8–16	0.25–1	0.12–0.25	0.25–1	0.06–0.5	0.25–0.5	0.25	0.06–0.5	0.12–0.5	0.25–1
Enterobacter spp.	8–16	0.12–0.5	0.12–0.5	0.25–1	0.12–2	0.5–2	0.25–1	0.12–1	0.25	0.25–4
Salmonella spp.	2–4	0.25	0.12	0.25	0.25	0.015	0.06	0.06	0.25	0.12
Shigella spp.	8	0.25	0.12	0.25	0.03	0.008	0.008	0.03	0.06	0.03
Campylobacter spp.	8	0.25	0.12	0.25	0.12	NA	NA	0.12	0.06	0.12
Pseudomonas aeruginosa	>64	0.5–2	0.5–2	0.5–4	4	16	8	>4	8	16
Acinetobacter spp.	>64	>16	1–2	1–2	0.25–8	0.5–>16	0.5–>16	0.5–>16	0.25–16	0.12–8
Stenotrophomonas maltophilia	>64	NA	8	8	2–8	4	4	4	1	4
Bacteroides fragilis group	>64	16–32	4–16	8	2	8	1	1	0.5	0.5
Mycoplasma spp.	NA	4–16	1–2	1–2	0.5	0.12	0.12	0.12	0.12	0.06
Chlamydia spp.	NA	4–16	1–4	0.25–1	0.5	0.06	0.25	0.12	0.06	0.015
Legionella pneumophila	1	2	0.06	0.1	0.03	0.015	0.015	0.03	0.06	0.06
Mycobacterium tuberculosis	NA	2–8	1–4	0.5–2	1	NA	>4	0.25	0.12	2

NA, not available.

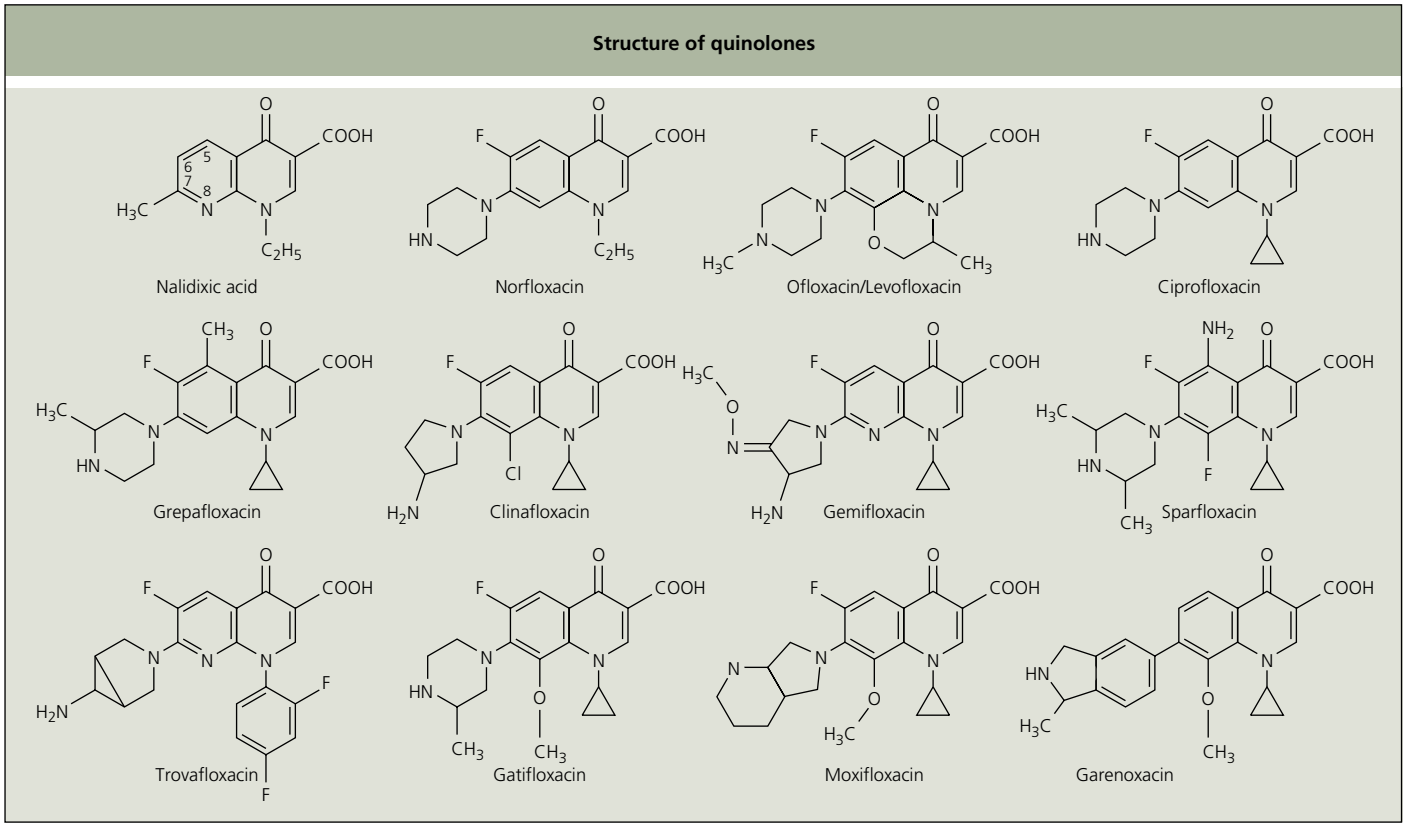


Fig. 139.2 Structure of quinolones.

- substitutions at position 7 (e.g. moxifloxacin, gemifloxacin, garenoxacin) can increase activity against Gram-positive organisms and increase the plasma half-life; and
- substitutions at position 8 (moxifloxacin, garenoxacin) can increase potency and reduce the rate of selection of resistant mutants but can be associated with increased phototoxicity (sparfloxacin).

Early representatives such as ciprofloxacin only have borderline activity against Gram-positive pathogens. However, developments such as the addition at position 7 of a five-membered ring (gemifloxacin) or an azabicyclo group (moxifloxacin, garenoxacin) have brought increased Gram-positive activity. Unfortunately, this has been partly at the expense of some activity against *P. aeruginosa*. Agents with good activity against both Gram-positive and Gram-negative bacteria have been developed (e.g. clinafloxacin) but have been withdrawn due to toxicity problems.

Fluoroquinolones have good activity *in vitro* against many intracellular pathogens such as *Legionella* spp., *Mycoplasma* spp., *Ureaplasma urealyticum*, *Chlamydia* spp., *Brucella* spp., *Salmonella typhi* and *Coxiella burnetii*. This may be enhanced by the concentration of fluoroquinolones within cells (see below). As shown in Table 139.1, *Mycobacterium tuberculosis* is susceptible to most of the fluoroquinolones with greater activity displayed by most of the newer agents (e.g. gatifloxacin and moxifloxacin). Fluoroquinolones should be used with caution in the treatment of pneumonia if there is a suspicion of tuberculosis, in order to avoid inadvertent monotherapy and the attendant risks of reduced diagnostic yield and selection of quinolone resistance. Of the other mycobacteria, *M. kansasii*, *M. marinum* and *M. fortuitum* tend to be fluoroquinolone susceptible, whereas *M. avium* complex, *M. chelonae* and *M. scrofulaceum* are more resistant.[2]

The quinolones are rapidly bactericidal against most susceptible species in a concentration-dependent manner and have a postantibiotic effect (PAE) of 2–4 hours. The pharmacodynamic determinants of efficacy are C_{max}/MIC (ratio of the maximum plasma concentration to minimum inhibitory concentration) and AUC_{0-24}/MIC (ratio of the area under the 24-hour drug concentration curve to MIC). Various groups have attempted to define the AUC_{0-24}/MIC ratio that would predict a successful outcome. It appears that the optimal ratio varies for different organisms so that a ratio of >125 has been proposed for infection caused by Gram-negative enteric pathogens and *P. aeruginosa*, but a much lower ratio of >34 is proposed for pneumococcal lower respiratory tract infections.[3,4] In addition to clinical efficacy, recent studies have focused on therapeutic strategies which may reduce the risk of fluoroquinolone resistance. The concept of the 'mutant selection window' has emerged, in which the survival of resistant organisms may be selectively encouraged by agents that only narrowly exceed the MIC of the organism. In accordance with this hypothesis, the use of the most potent agents may help to limit the risk of development of resistance by reducing the mutant selection window.[5]

MODE OF ACTION

Quinolones act by the rapid inhibition of bacterial DNA synthesis, leading to cell death. The primary targets are DNA gyrase and topoisomerase IV which are involved in the maintenance of the superhelical structure of DNA. Both enzymes are composed of two subunits that are homologous: DNA gyrase subunits encoded by *gyrA* and *gyrB*; topoisomerase IV encoded by *parC* (*grlA* in *Staphylococcus aureus*) and *parE* (*grlB* in *Staph. aureus*).

Although inhibition of these enzymes is the most important determinant of antibacterial activity, it appears that secondary activities may affect bactericidal activity. The addition of RNA and protein synthesis inhibitors or the use of high quinolone concentrations (which also inhibit RNA synthesis) can lead to a diminution in the cidality of some quinolones. Additional protein activity may be required to release the DNA ends from the DNA–gyrase–quinolone complex, with the release of free DNA ends resulting in bacterial apoptosis.[6]

BACTERIAL RESISTANCE

The major mechanism for acquired resistance to quinolones is by mutational modification of the antimicrobial target site. Mutations around the active site of *gyrA* have been identified in many strains of *Escherichia coli* and many other Gram-negative bacilli, giving rise to greater resistance to nalidixic acid than the fluoroquinolones. Alterations in *gyrB* are less common and cause lower levels of resistance.[7] The main site for resistance mutations in Gram-positive bacteria such as *Staph. aureus* and *Streptococcus pneumoniae* is the *parC* gene although mutations in *parE* have been described. In both Gram-negative and Gram-positive pathogens resistance develops in a stepwise fashion as mutations arise in one and then both targets. Following an initial mutation, the susceptibility to a quinolone will depend on the specificity of the agent for the alternative target. For example, in clinical practice it has been shown that an isolated *gyrA* mutation in *E. coli* will confer high-level resistance to nalidixic acid but only reduced susceptibility to ciprofloxacin. The acquisition of an additional *parC* mutation confers high-level resistance to ciprofloxacin.[8] For bacteria such as *P. aeruginosa* that inherently have less susceptibility to fluoroquinolones, a single mutation can give rise to clinically significant resistance.

Resistance to quinolones can also be achieved by active efflux of the drug from the bacterial cell. This has been best described in *P. aeruginosa* in which quinolone resistance has been associated with increased expression of the MexAB-OprM, MexCD-oprJ or MexEF-oprN efflux pumps.[9] In *E. coli* the pump is the acrAB-tolC system. Among Gram-positive pathogens, the *norA* pump has been described in *Staph. aureus* and the PmrA pump in *Strep. pneumoniae*.[10,11] On their own, efflux pumps will generally only cause low-level resistance and therefore may not be clinically important in inherently highly susceptible pathogens such as *E. coli*. However, the overexpression of efflux pumps becomes more significant in less susceptible organisms such as *P. aeruginosa*. The presence of efflux pumps may explain the reduced susceptibility of *Mycobacterium avium* when compared to other nontuberculous mycobacteria with similar susceptibility to gyrase inhibition.[12]

More recently, the spread of reduced susceptibility in Enterobacteriaceae by plasmid-mediated mechanisms such as Qnr proteins has been described. First discovered in the late 1990s, Qnr proteins protect gyrase and topoisomerase IV from quinolone inhibition. Despite a global distribution, Qnr proteins appear to be of low prevalence in most populations of Gram-negative bacteria. Qnr proteins confer low-level resistance which enhances the selection of resistant mutants *in vitro*, and may contribute to clinically significant levels of resistance by acting additively with other resistance mechanisms. The effect of *qnr* gene carriage on clinical outcome is as yet unknown. The aminoglycoside acetyltransferase, AAC(6′)-Ib-cr is another recently discovered mechanism for transferring low-level resistance on mobile elements. AAC(6′)-Ib-cr appears to act on piperazinyl-substituted quinolones such as ciprofloxacin.[13]

Resistance rates to quinolones continue to rise. In the UK, 2006 data from the Health Protection Agency reported that ciprofloxacin resistance among *E. coli* bacteremia isolates was 26%.[14] The increase in fluoroquinolone resistance across Europe is illustrated in Figure 139.3. Although methicillin-sensitive *Staph. aureus* is usually sensitive to fluoroquinolones, some clones of methicillin-resistant *Staph. aureus* (MRSA) – for example, epidemic MRSA (EMRSA)-16 seen in the UK – are resistant. Resistance among pneumococci remains uncommon, with most countries including the USA, Western Europe and Latin America reporting rates of resistance to levofloxacin below 1%,[15] although in some areas there is evidence that resistance is more common among penicillin-resistant pneumococci.[16]

Cross-resistance between the older fluoroquinolones is almost complete and minor differences in activity are not usually clinically exploitable. The mechanisms of resistance to antimicrobial agents are discussed in detail in Chapter 131.

PHARMACOKINETICS AND DISTRIBUTION

The quinolones are generally well absorbed and are widely distributed in body tissues and fluids, including the intracellular environment. They are excreted either by glomerular filtration or hepatic biotransformation, or a combination of these routes, and by biliary

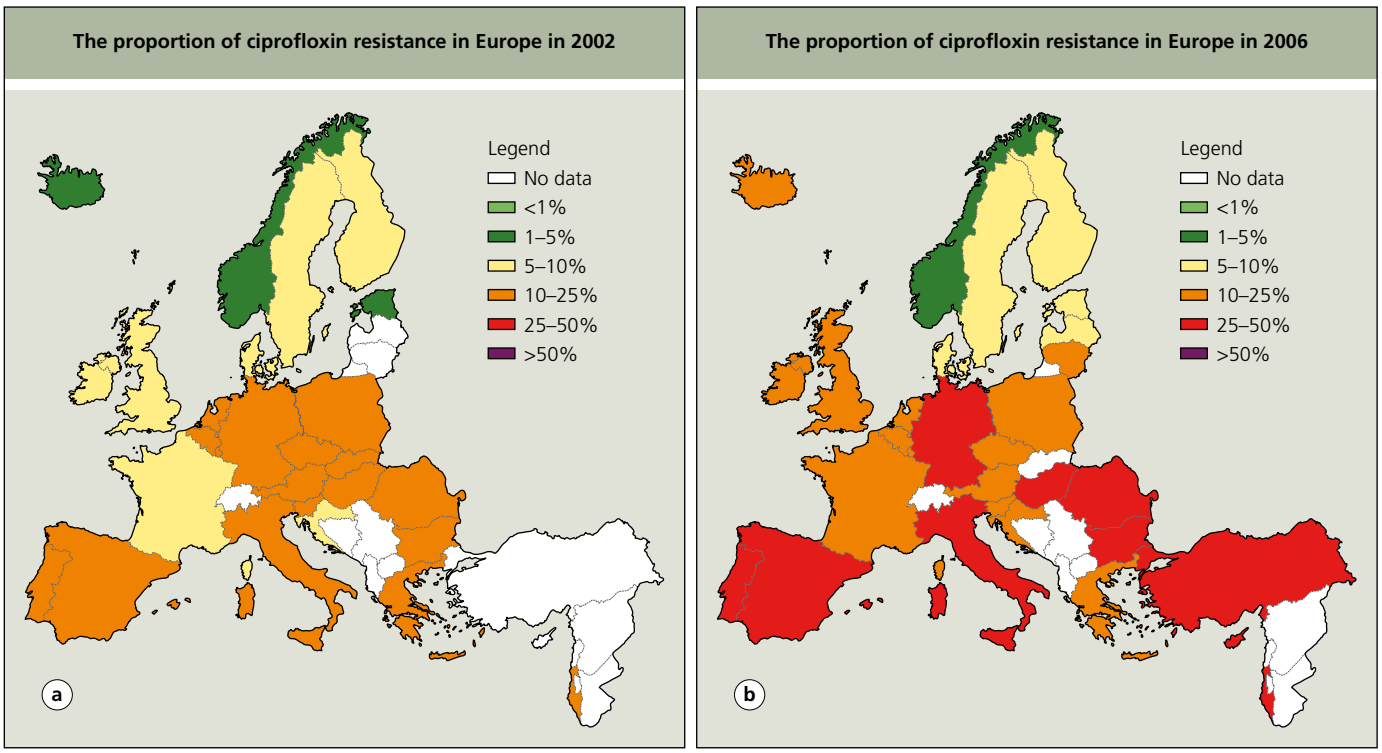


Fig. 139.3 The proportion of ciprofloxacin resistance in Europe.

Table 139.2 Basic pharmacokinetic parameters of quinolones

Agent	Dose (g)		C_{max} (mg/l)	AUC_{0-24} (mg/lh)	$t_{½}$ (h)	Protein binding %	% dose excreted unchanged in urine	Route
Nalidixic acid	1	QDS	V	V	1.5	90	<1	po
Norfloxacin	0.4	BD	2	12.5	3	15	25–40	po
Ciprofloxacin	0.75	BD	3	30	4	40	30–50	po/iv
Ofloxacin	0.4	BD	6	80	7	30	70–85	po/iv
Levofloxacin	0.5	OD	6.4	54	7	40	85–90	po/iv
Grepafloxacin	0.4	OD	2.2	14	14	50	<10	po
Gatifloxacin	0.4	OD	4.6	37	9	20	70–80	po/iv
Gemifloxacin	0.32	OD	1.8	9	7	65	30	po
Moxifloxacin	0.4	OD	4.5	44	13	50	20	po/iv
Garenoxacin	0.4	OD	6.5	84	12.5	80	40	po/iv

V, variable.

or transintestinal elimination. Bioavailability is high and protein binding usually low to intermediate. Fluoroquinolone kinetics are summarized in Table 139.2.

Absorption

Fluoroquinolones are well and rapidly absorbed after oral administration and exhibit linear absorption kinetics so that doubling the dose produces twice the plasma level.[12] Peak plasma concentrations are usually present 1–2 hours after an oral dose. Absorption may be delayed by food and is impaired by co-administration of antacids and ferrous iron, and possibly by zinc in multivitamin preparations.

Distribution

The fluoroquinolones are extensively distributed to the tissues as can be seen in Table 139.3. Apparent volumes of distribution are usually 2–3 l/kg, although values for precursor compounds are lower (e.g. 0.5 l/kg). Protein binding varies from 15% to 40% with norfloxacin, ofloxacin and ciprofloxacin[17] to 65% for gemifloxacin and higher still for garenoxacin and trovafloxacin (>80%).

Fluoroquinolones are concentrated approximately 10 times in polymorphoneutrophils (PMNs). Although it has been suggested that this may increase their *in vivo* efficacy against intracellular pathogens, there is evidence that the intracellular activity of different fluoroquinolones is variable, possibly related to where they are concentrated within the cell.[18] An additional result of the intracellular concentration of fluoroquinolones is that they may be transported by PMNs to a site of infection and then released.[19]

Elimination

Elimination half-lives vary from 1–2 hours for nalidixic acid to 3–5 hours for ciprofloxacin and 7–14 hours for newer agents.

Excretion of fluoroquinolones is primarily by renal glomerular filtration, hepatic metabolism and transintestinal elimination. The relative importance of glomerular filtration varies between agents and some compounds such as ofloxacin, levofloxacin and gatifloxacin exhibit minimal metabolism and are excreted largely unchanged in the urine. For these agents, renal clearance almost equals total clearance and dose modification is required in renal impairment.[17,20] Others, such as ciprofloxacin and moxifloxacin, have moderately extensive hepatic biotransformation (to oxo-, desethyl- and sulfo- derivatives, subsequently partly eliminated as inactive glucuronides in the bile). For these compounds, renal clearance is half of the total clearance and dosage modification may not be required in renal impairment as long as other routes of elimination are intact.[21]

Table 139.3 Tissue distribution of fluoroquinolones

Tissue	Tissue: plasma ratio
Lung	
Bronchial mucosa	1.6
Epithelial lining fluid	2.1–8.7
Alveolar macrophages	11.8–21
CSF	
Uninflamed meninges	0.1
Inflamed meninges	0.3–0.5
Brain tissue	0.9
Skin/soft tissue	
Skin	1.8
Muscle	3.3
Subcutaneous tissue	~1
Blister fluid	~1
Sweat	2.5
Prostate	
Prostatic tissue	2.1–5.7
Prostatic fluid	0.25
Seminal fluid	6–8
Eye	
Aqueous humor	0.5
Kidney	
Kidney tissue	~6
Urine	~100
Liver	
Hepatic tissue	4
Bile	6
Heart	
Myocardium	2–4
Heart valves	~1

In hepatic impairment, the dosage of agents primarily cleared by the kidney (ciprofloxacin, ofloxacin and levofloxacin) rarely requires modification. However, for extensively metabolized drugs such as grepafloxacin, dose modification is necessary in patients with cirrhosis.

Fluoroquinolones that are not primarily eliminated by the kidney are present in significant quantities in the stool, partly by biliary excretion and, notably with ciprofloxacin, by transintestinal elimination. The majority is bound to ligands in the stool.

ROUTE OF ADMINISTRATION AND DOSAGE

Most agents are available in both oral and intravenous formulations. The high oral bioavailability of fluoroquinolones means that oral administration is adequate in most situations unless this route is unavailable. The manufacturers' dosage recommendations for quinolones are given in Table 139.4.

INDICATIONS

Early quinolones such as nalidixic acid were largely used for Gram-negative urinary tract infection (UTI) and shigellosis. The development and evolution of fluoroquinolones have led to a number of agents with differences in spectrum of activity and therefore indications. Some, such as norfloxacin, are used almost exclusively for UTI. Agents such as ciprofloxacin and ofloxacin have been used for a broad range of infective syndromes. Newer compounds, such as levofloxacin, moxifloxacin and gemifloxacin, have improved activity against Gram-positive pathogens and are more appropriate for respiratory tract infections.

Genitourinary tract infections

Uncomplicated lower urinary tract infection

Oral fluoroquinolone therapy is highly effective but to limit selection pressure for resistance should be used only when bacterial resistance precludes the use of other agents. Fluoroquinolones eradicate bowel reservoirs of uropathogenic *E. coli* and may reduce the incidence of early recurrence. Long-term suppression with low-dose norfloxacin or ciprofloxacin has been shown to be effective in preventing recurrent UTI in selected patients.[22,23]

Complicated ascending urinary tract infection

Fluoroquinolones given for 1–2 weeks are the recommended agents for the treatment of ascending or complicated UTI.[24] Oral ciprofloxacin has proved as efficacious as an intravenous regimen for initial empiric therapy.[25]

Prostatitis

Fluoroquinolones are concentrated in prostatic tissue and are recommended therapy for both acute and chronic bacterial prostatitis.[26] Ciprofloxacin for 28 days can give a clinical response of 98% in chronic bacterial prostatitis although relapse may occur in up to 40% of patients.[27]

Gonorrhea

The global prevalence of fluoroquinolone resistance continues to rise. In England and Wales the prevalence of ciprofloxacin resistance was 26.5% in 2006.[28] In the USA, the Centers for Disease Control and Prevention (CDC) treatment guidelines were updated in 2007 to recommend that fluoroquinolones should no longer be used to treat gonococcal infections in any patient group.[29]

Nongonococcal urethritis/cervicitis

The antichlamydial activity of fluoroquinolones varies and ofloxacin is the most potent of the established agents. A 7-day course of ofloxacin is as effective as doxycycline therapy.[30] Newer compounds such as moxifloxacin have excellent *in vitro* activity and may have a role in therapy.

Chancroid

A single dose or 3-day course of ciprofloxacin is a recommended treatment for chancroid. Despite reports of isolates with reduced susceptibility to quinolones, a study in Nairobi showed a 92% cure rate for single-dose ciprofloxacin, comparable to a 7-day course of erythromycin.[31]

Pelvic inflammatory disease

The ideal antimicrobial treatment for acute pelvic inflammatory disease has not been established by randomized clinical trials. In view of the high rates of resistance of *Neisseria gonorrhoeae*, fluoroquinolones are no longer included in recommended treatment regimens.[29]

Respiratory tract infections

The fluoroquinolones ciprofloxacin and ofloxacin have been used extensively for upper and lower respiratory tract infections. However, there have been concerns regarding their activity against *Strep. pneumoniae*. Newer agents, such as gemifloxacin, moxifloxacin and garenoxacin, have improved activity against pneumococci, including macrolide- and penicillin-resistant strains, and are often termed 'respiratory quinolones'.

Sinusitis

Oral fluoroquinolones have comparable efficacy to macrolides or cephalosporins and give cure rates of >85% in acute sinusitis.[32–34]

Ear infections

Topical preparations of ofloxacin or ciprofloxacin are effective for the treatment of acute otitis media in children with tympanostomy tubes and for chronic suppurative otitis media.[35] Clinical cure rates of >85% for otitis externa can be obtained with the topical preparations. Malignant otitis externa, which is usually caused by *P. aeruginosa*, can be treated with oral ciprofloxacin. A prolonged course is required (3 months) and gives cure rates in excess of 90%.[36]

Acute exacerbations of chronic bronchitis

Fluoroquinolones are among the agents of choice for the management of moderate to severe exacerbations of chronic bronchitis. They have equivalent efficacy to macrolides or β-lactam/β-lactamase inhibitor combinations and achieve cure rates of >90%.[37,38]

Community-acquired pneumonia

Older quinolones are not indicated for pneumococcal pneumonia when alternative antibiotics are available. However, results with ciprofloxacin and ofloxacin suggest clinical response and bacterial eradication rates of 90% or greater and, with levofloxacin, equivalence or superiority to ceftriaxone.[39] However, concerns have been raised regarding the efficacy of ciprofloxacin in severe pneumococcal pneumonia following reports of clinical failures.[40] Failures with levofloxacin have also been reported and in Europe it is suggested that it should be given at an increased dose of 500 mg twice daily or in combination with benzyl penicillin in cases of severe pneumonia.[41,42]

Table 139.4 Dosing recommendations for quinolones (from manufacturers' data sheets)

	Nalidixic acid	Norfloxacin	Ofloxacin	Ciprofloxacin		Levofloxacin	Gatifloxacin	Moxifloxacin
	po	po	po/iv	po	iv	po/iv	po/iv	po/iv
Urinary tract infection	500–1000 mg q6h	400 mg q12h (3–21 days)	200 mg q12h (3–10 days)	100–500 mg q12h (3–14 days)	200–400 mg q12h (7–14 days)	250 mg q24h (3–10 days)	200–400 mg q24h (3–10 days)	
Chronic bacterial prostatitis		400 mg q12h (28 days)	300 mg q12h (6 weeks)	500 mg q12h (28 days)	400 mg q12h (28 days)			
Acute sinusitis				500 mg q12h (10 days)	400 mg q12h (10 days)	500 mg q24h (10–14 days)	400 mg q24h (10 days)	400 mg q24h (10 days)
Acute bacterial exacerbation of chronic bronchitis			400 mg q12h (10 days)	500–750 mg q12h (7–14 days)	400 mg q8–12h (7–14 days)	500 mg q24h (7 days)	400 mg q24h (5 days)	400 mg q24h (5 days)
Community-acquired pneumonia			400 mg q12h (10 days)			500 mg q24h (7–14 days)	400 mg q24h (7–14 days)	400 mg q24h (7–14 days)
Skin and skin structure infection			400 mg q12h (10 days)	500–750 mg q12h (7–14 days)	400 mg q8–12h (7–14 days)	500–750 mg q24h (7–14 days)		400 mg q24h (7 days)
Bone and joint infection				500–750 mg q12h (≥4–6 weeks)	400 mg q8–12h (≥4–6 weeks)			
Intra-abdominal infection				500 mg q12h (7–14 days)	400 mg q12h (7–14 days)			
Infectious diarrhea				500 mg q12h (5–10 days)				
Uncomplicated urethral and cervical gonorrhea		800 mg single dose	400 mg single dose	250 mg single dose			400 mg single dose	
Nongonococcal cervicitis/urethritis			300 mg q12h (7 days)					
Pelvic inflammatory disease			400 mg q12h (10–14 days)					
Inhalational anthrax (postexposure)				500 mg q12h (60 days)	400 mg q12h (60 days)			

Newer agents such as gemifloxacin and moxifloxacin, which have improved activity against pneumococci and atypical pathogens, show promising results in clinical trials, with clinical cure rates in excess of 90%.[43] Additionally, *in vitro* tests suggest that the use of the most potent agents, or agents of intermediate potency with altered dose administration regimens, may reduce the emergence of resistance.

While high-level penicillin resistance was found to be associated with resistance to older quinolones such as ciprofloxacin, the newer agents have comparable MIC values for both penicillin-sensitive and penicillin-resistant pneumococci.[44]

Legionellosis can be successfully treated with quinolones such as ciprofloxacin, ofloxacin or levofloxacin. There are few clinical data to show whether or not they are superior to macrolides and often they are given in combination with a macrolide or rifampin (rifampicin)[45] (see also Chapter 27).

Nosocomial pneumonia

A large-scale study of ciprofloxacin showed equivalence with imipenem in moderately to severely ill patients, most of whom required ventilation and treatment in an intensive care unit.[46] In the 20–25% with infection caused by *P. aeruginosa*, the results with both regimens were less satisfactory, underlining the need for combination therapy. Newer fluoroquinolones such as gemifloxacin and moxifloxacin have reduced *in-vitro* potency against *P. aeruginosa* and will probably not have a role in the management of hospital-acquired pneumonia where *Pseudomonas* is a likely etiologic agent. A meta-analysis of efficacy in nosocomial pneumonia concluded that quinolones are an acceptable therapy and perform comparably to other standard regimes; the authors noted the importance of considering national and local antibiotic resistance trends when using fluoroquinolones (see Chapter 28).[47]

Cystic fibrosis

Oral ciprofloxacin is effective for exacerbations caused by *P. aeruginosa*, producing results equivalent to those of standard β-lactam and aminoglycoside therapy. In the UK a 3-week course of ciprofloxacin combined with colistin is recommended for the treatment of early pseudomonal infection.[48]

Mycobacterial infections

Older fluoroquinolones have only moderate activity against *Mycobacterium tuberculosis* and current evidence does not support the use of these agents in the treatment of drug-sensitive or resistant tuberculosis. Their role in therapy is currently limited to use in combination regimens for the treatment of multiply drug-resistant *M. tuberculosis* infection.[49] Newer agents such as moxifloxacin have enhanced antimycobacterial activity and animal studies suggest they may have a future role in antituberculous chemotherapy.[50]

As noted above, the susceptibility of nontuberculous mycobacteria to fluoroquinolones is variable. *Mycobacterium avium* complex is relatively resistant to quinolones. Nevertheless, the addition of ciprofloxacin to standard therapeutic combinations has been shown to be of benefit in HIV patients with disseminated disease.[51]

Skin and soft tissue infections

The fluoroquinolones give excellent results when compared with cephalosporins for the treatment of both uncomplicated and complicated skin and soft tissue infections.[52,53] However, more effective agents are routinely available for Gram-positive infections and usefulness in MRSA infections is limited by high rates of quinolone resistance.

Skeletal infections

Oral fluoroquinolones are highly effective for Gram-negative mixed acute (or chronic) contiguous osteomyelitis, giving cure rates of 80–90% after 3- to 6-month courses. They are also effective for post-surgical cases, *Salmonella* osteitis and in some cases of chronic *P. aeruginosa* osteomyelitis (ciprofloxacin), although resistance may emerge causing a failure of treatment or relapse.[54] In patients with orthopedic prostheses infected with staphylococci, ciprofloxacin or ofloxacin in combination with rifampin have been successfully used for conservative management (i.e. preserving the prosthesis).[55,56]

Gastrointestinal infections

Typhoid and paratyphoid fevers

Although fluoroquinolones are widely regarded as the agents of choice for typhoid and paratyphoid fevers,[57,58] a Cochrane systematic review in 2005 failed to find sufficient trial evidence regarding superiority of fluoroquinolones over first-line antibiotics in children and adults.[59] However, convalescent excretion states and long-term fecal carriage are rare after fluoroquinolone therapy, thereby reducing the human reservoir and possibly leading to a fall in incidence. Carriage states persisting after other antibiotic therapy may also respond to fluoroquinolones.

Decreased quinolone susceptibility has emerged in Asia over the last 10 years. Strains are typically resistant to nalidixic acid and have raised MICs of ciprofloxacin of 0.5–1 mg/l. These strains are ciprofloxacin susceptible by Clinical and Laboratory Standards Institute (CLSI) or British Society for Antimicrobial Chemotherapy (BSAC) criteria but the clinical response to fluoroquinolones in those infected by these stains is significantly worse than with nalidixic-acid sensitive strains, and longer courses or alternative agents are recommended.[58]

Salmonellosis

A 5- to 7-day course of oral fluoroquinolone is effective in reducing the duration and severity of severe salmonellosis.

Cholera

Three-day courses of oral fluoroquinolones are equal to standard trimethoprim–sulfamethoxazole or tetracycline regimens. A cure rate of >90% can be achieved with a single 1 g dose of ciprofloxacin.[60] Reports have emerged of strains of *Vibrio cholerae* 01 with reduced susceptibility to ciprofloxacin, resulting in clinical and bacteriologic treatment failure.[61]

Shigellosis

Fluoroquinolones are the drugs of choice for invasive shigellosis. A single oral dose (ciprofloxacin 1 g) is effective in adults. Rising levels of resistance to nalidixic acid have led to the abandonment of this agent as a first-line treatment for acute shigellosis in some countries.[62]

Campylobacter

Fluoroquinolones have been used for gastrointestinal *Campylobacter* infections. However, resistance levels are increasing and may be as high as 50% in some areas of the world.[63]

Travelers' diarrhea

Ciprofloxacin or norfloxacin in full oral dosage for 5 days is effective for 80% of unprotected subjects who develop profuse diarrhea (>3–5 watery stools/day).

Other treatment indications

Ocular infections

Topical fluoroquinolones are effective for the treatment of bacterial conjunctivitis and keratitis. Penetration of systemic quinolones into

the vitreous is relatively good but may not exceed the MICs of all likely pathogens. Intravitreal ciprofloxacin has been used in the treatment of endophthalmitis.[64]

Infections associated with chronic ambulatory peritoneal dialysis

Ciprofloxacin and ofloxacin have been used with success both orally and intraperitoneally. However, the emergence of resistant staphylococcal infection has limited their usefulness as monotherapy.

Q fever

Fluoroquinolones are active against *Coxiella burnetii in vitro* and a combination of a fluoroquinolone (ofloxacin) with doxycycline has been suggested for Q fever endocarditis.[65]

Anthrax

A 60-day course of ciprofloxacin is recommended for postexposure prophylaxis against anthrax.[66] In patients with inhalational anthrax a combination of ciprofloxacin plus another active agent (e.g. doxycycline) is recommended.[67]

Meningitis

Fluoroquinolones have been successfully used for Gram-negative meningitis.[68] Newer agents such as moxifloxacin show promising results in animal models of pneumococcal meningitis.[69] Trovafloxacin had comparable efficacy to ceftriaxone in a trial of pediatric meningitis.[70]

Chemoprophylaxis

Meningococcal infection

Single-dose (500 mg) ciprofloxacin is effective in eradicating nasopharyngeal carriage in over 95% of subjects.[71]

Neutropenic patients

Norfloxacin, ofloxacin and ciprofloxacin have been widely used in the prophylaxis of opportunistic infection among neutropenic patients. Although prophylaxis has been shown to prevent febrile episodes of an infectious nature, current recommendations do not suggest their use due to concerns regarding the emergence and spread of antimicrobial resistance.[72]

Travelers' diarrhea

Once-daily prophylactic use of a fluoroquinolone (e.g. norfloxacin 400 mg or ciprofloxacin 500 mg) for the duration of potential exposure gives 75–90% protection from travelers' diarrhea caused by enterotoxigenic *E. coli* and other bacterial enteropathogens.

Surgical infections

Fluoroquinolones have been used effectively for the prevention of infection following transurethral prostatectomy and biliary surgery.

Pediatric use of fluoroquinolones

Pediatric use of fluoroquinolones has been limited by concerns regarding arthropathy observed in weight-bearing diarthrodial joints in juvenile dogs after prolonged high-dose administration. In the USA the only current licensed indications for fluoroquinolone use in patients under 18 years of age are complicated UTI, pyelonephritis and postexposure treatment for inhalational anthrax. Nevertheless, accumulated experience has established other situations in which the benefits of fluoroquinolones outweigh potential risks. These include typhoid fever, cholera and shigellosis, complicated UTI due to multiresistant pathogens, chronic suppurative otitis media caused by *P. aeruginosa*, multiresistant Gram-negative sepsis (including osteomyelitis), prophylaxis of meningococcemia (single dose) and infection in neutropenia.

Treatment of pseudomonal infections in patients with cystic fibrosis is one of the commonest indications for the use of fluoroquinolones in children. Prolonged courses are often given but there has been little evidence of related arthropathy and fluoroquinolones continue to be widely used.

DOSAGE IN SPECIAL CIRCUMSTANCES

Renal impairment

The extent to which the dosage requires modification is dependent on the degree of renal elimination. Table 139.5 shows the manufacturer's recommendations for selected quinolones. Essentially, agents such as ofloxacin and levofloxacin that are extensively renally excreted have the dose reduced to one-quarter of the normal daily dose in severe renal impairment and most other agents have the dose halved. However, as noted above, there is evidence that ciprofloxacin may not require dose modification as long as alternative routes of elimination are intact.[16] Moxifloxacin, which has only 20% renal excretion, does not require dose modification.

Hepatic impairment

Apart for extensively metabolized quinolones, such as pefloxacin, dose modification is not necessary in patients with hepatic impairment. However, experience with newer agents such as moxifloxacin in patients with severe liver failure (Child–Pugh Class C) is limited.

Elderly patients

No specific changes in dosage are required for the elderly provided appropriate changes are made for reduced renal clearance.

Pediatrics

Optimal pediatric doses have not been established. Suggested doses of ciprofloxacin are 7.5–40 mg/kg/day (oral) or 5–10 mg/kg/day (intravenous), administered on an 8–12-hourly basis.

Pregnancy and lactation

Quinolones are not approved for use in pregnancy or during lactation.

ADVERSE REACTIONS AND INTERACTIONS

Adverse drug reactions

Fluoroquinolones are generally well tolerated although there are a number of potentially serious adverse effects that have been seen in some agents.[73] When adverse effects are reported, they are usually gastrointestinal (2–20%), dermatologic (0.5–3%) and CNS (0.5–2%) reactions which rarely necessitate withdrawal of therapy (1–3%). In most cases there are no specific age or racially related effects, but adverse drug reactions are more common in neutropenic patients and possibly in people who have AIDS.

Most fluoroquinolone adverse drug reactions are class effects, but incidence varies between compounds and can often be related to the specific structure of different agents. Certain group members have specific effects or more serious class effects that have led to restrictions on

Table 139.5 Manufacturers' dosage recommendations for patients with renal impairment

	RENAL IMPAIRMENT		
	Mild	**Moderate/severe**	**Hemodialysis/CAPD**
Ciprofloxacin (iv)		200–400 mg 18–24 hourly (CC = 5–29 ml/min)	
Ciprofloxacin (po)	250–500 mg q12h (CC = 30–50 ml/min)	250–500 mg 18 hourly (CC = 5–29 ml/min)	250–500 mg q24h after dialysis
Ofloxacin	400 mg q24h (CC = 20–50 ml/min)	200 mg q24h (CC <20 ml/min)	
Levofloxacin	250 mg q24h* (CC = 20–50 ml/min)	250 mg 48 hourly* (CC = 10–19 ml/min)	250 mg 48 hourly* (CC = 10–19 ml/min)
Norfloxacin		400 mg q24h (CC <30 ml/min)	
Gatifloxacin		200 mg q24h† (CC <40 ml/min)	200 mg q24h† (CC <40 ml/min)
Moxifloxacin	No adjustment required		

CC, creatinine clearance.
*Initial loading dose of 500 mg.
†Short courses up to 3 days do not require dosage alteration.

use or withdrawal. For example, the phototoxicity of sparfloxacin has restricted licensing by some registration authorities, and temafloxacin, which caused hemolytic–uremic syndrome and hypoglycemia, was withdrawn in 1992.

Gastrointestinal reactions

The usual reported symptoms are nausea, anorexia and dyspepsia. While diarrhea, abdominal pain and vomiting are less frequent, they are more likely to result in discontinuation of treatment. Antibiotic-associated diarrhea caused by *Clostridium difficile* is associated with fluoroquinolone use, and all of the available fluoroquinolones have been implicated in *C. difficile* outbreaks.[74] Liver enzyme abnormalities occur in 2–3% of patients receiving fluoroquinolones and are usually mild and reversible. However, more severe liver abnormalities have been seen with some agents which, in the case of trovafloxacin, led to its withdrawal

Dermatologic reactions

Although non-specific skin rashes, pruritus and urticaria have been reported, it is phototoxicity that has received most attention. This is a class effect and is thought to be related to the photodegradation of the fluoroquinolone and its ability to induce free radicals. The incidence and severity of phototoxicity differ between agents. Structurally, a fluorine moeity at position 8 causes more phototoxicity; sparfloxacin, which has this moiety, has caused a higher rate of phototoxicity. Phototoxic reactions are rare with ciprofloxacin, ofloxacin and levofloxacin. Gemifloxacin can cause a nonphototoxic rash which is seen particularly in female patients between the ages of 20 and 40 years, and postmenopausal women taking hormone replacement therapy; increased risk of rash was also associated with more than 7 days of therapy.[75]

Central nervous system reactions

These occur in less than 2% of patients with most fluoroquinolones and usually manifest as headache, dizziness, mild tremor or drowsiness. Convulsions occur rarely both as a primary effect and as a result of interactions with theophylline or nonsteroidal anti-inflammatory drugs (NSAIDs). Although the mechanism of quinolone toxicity has not been fully elucidated, it is believed to be due to inhibition of $GABA_A$ receptors.

Musculoskeletal effects

Fluoroquinolones as a class produce destructive arthropathy in weight-bearing, diarthrodial joints of juvenile animals, notably dogs, by production of cartilage erosions after prolonged high dosage. Some agents, notably precursors such as nalidixic acid, are considerably more likely to induce arthropathy. This effect has never been observed in human children. MRI follow-up and autopsy studies in children receiving both nalidixic acid and modern fluoroquinolones have revealed no evidence of joint damage.[76] Experience with ciprofloxacin in 1500 children noted reversible arthralgia in 3.2% of patients treated for pulmonary exacerbations of cystic fibrosis.[77] In a retrospecive study of more 6000 fluoroquinolone-treated children, the incidence of tendon or joint disorders was <1% and comparable to the reference group of azithromycin-treated children.[78]

Tendinitis occurs rarely as a class effect although it may be more common with concomitant corticosteroid therapy. The Achilles tendon is most commonly affected and patients are usually >50 years of age. MRI can be useful for early detection of damage and discontinuation is recommended at the first sign of tendon pain or inflammation.

Cardiovascular effects

Cardiotoxicity is manifest as prolongation of the QT interval with the potential to cause ventricular arrhythmias. The significance of this effect varies between agents and appears to be affected by substitutions at position 5 (see Fig. 139.2).[79] Of the currently available fluoroquinolones, the risk of QT interval prolongation appears to be greatest with moxifloxacin and lowest with ciprofloxacin; grepafloxacin was withdrawn voluntarily following reports of seven cardiac-related fatalities. Although adverse effects due to cardiotoxicity are rare, the risk can be minimized by avoiding the use of multiple drugs that prolong the QT interval in high-risk patients, for example those with pre-existing cardiac disease or electrolyte disturbances.[80]

Dysglycemia

Two recent studies found gatifloxacin to be associated with an increased risk of both hypo- and hyperglycemia, probably due to the competing effects of the drug on both the beta cells and islet cells of the pancreas.[81] Gatifloxacin was removed from the market in 2006. In these studies levofloxacin therapy was found to be associated with a slightly increased risk of hypoglycemia, but no dysglycemic risk was noted with moxifloxacin or ciprofloxacin.

Other (rare) effects

Hypersensitivity occurs at a frequency of ~1%. Crystalluria and secondary interstitial nephritis are rare and relate to pH-associated solubility of fluoroquinolones in urine.

Interactions with other drugs

Interactions largely occur as a result of interference with fluoroquinolone absorption or by inhibition of biotransformation of unrelated drugs by the hepatic cytochrome P450 isoenzyme system. Central nervous system interactions due to GABA receptor inhibition occur with NSAIDs, notably fenbufen, and convulsions may follow, as reported with enoxacin.

Interactions affecting absorption of fluoroquinolones

The absorption of fluoroquinolones is reduced by up to 80% by co-administration of aluminum- and magnesium-containing antacids, probably by the formation of insoluble complexes and, to a lesser extent, by calcium antacids, sucralfate and ferrous iron preparations. H_2 antagonists have no effect.

Interactions affecting drug metabolism

Fluoroquinolones reduce the hepatic clearance of xanthines via the P450 cytochrome system. The effect is most marked with enoxacin and grepafloxacin, but ciprofloxacin and pefloxacin also reduce clearance of theophylline by 30% and co-administration may result in theophylline toxicity, usually nausea but possibly convulsions. Dosage of theophylline should be interrupted or reduced and serum levels monitored if enoxacin, pefloxacin or ciprofloxacin is to be administered.

A similar effect, induced by the same fluoroquinolones, is responsible for inhibition of caffeine metabolism and resultant insomnia. Metabolism of warfarin, cimetidine and ciclosporin is affected much less by P450 cytochrome inhibition and interaction may not be clinically significant.

REFERENCES


References for this chapter can be found online at http://www.expertconsult.com

Chapter 140

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Glycopeptides

Vancomycin

Vancomycin, a glycopeptide antibiotic that has been available clinically for half a century, has served as the primary therapy for Gram-positive infections due to methicillin-resistant *Staphylococcus aureus* (MRSA), other methicillin-resistant staphylococci, and ampicillin-resistant *Enterococcus* spp. Its use today is greater than any other point in its history, with the surge in the past two decades of infections caused by β-lactam-resistant staphylococci and enterococci. Vancomycin demonstrates activity against Gram-positive anaerobes, diphtheroids and *Clostridium* spp., including *C. difficile*.

Vancomycin is generally considered bactericidal antibiotic – minimum bactericidal concentration:minimum inhibitory concentration (MBC:MIC) <4 – except with enterococci, but vancomycin tolerance (MBC:MIC >32) has been increasingly documented among staphylococci as a precursor to reduced glycopeptide susceptibility. When tolerance has been demonstrated, many clinicians add a second antibiotic to the regimen in treating bacteremic disease with vancomycin. For example, vancomycin is frequently combined with gentamicin to treat bacteremia or endocarditis caused by vancomycin-susceptible strains of *Enterococcus* spp.

CLINICAL PHARMACOKINETICS

Several investigators have reported the pharmacokinetics of vancomycin in human pediatric, adult and geriatric patients (Table 140.1), and in various disease states.

Absorption

Vancomycin is administered intravenously or orally. The intramuscular route is not used because of severe discomfort and the possibility of tissue necrosis. It may be also given intraperitoneally and, based on limited data, via the intraventricular, intrathecal and intravitreal route. To treat systemic infections, vancomycin should be given intravenously, and is usually infused over 1 hour. Some recommend infusing at no more than 7.5–15 mg per minute.[6]

Oral administration of vancomycin is primarily used for the treatment of *C. difficile*-associated diarrhea (CDAD). When given orally, vancomycin is not appreciably absorbed in most patients (oral bioavailability <10%). Low concentrations of vancomycin may be found in the urine of patients with normal renal function, suggesting minimal absorption from the gastrointestinal tract. The oral administration of vancomycin does not usually result in measurable concentrations of the drug in serum, even in the presence of severely impaired renal function. However, therapeutic or potentially toxic concentrations of vancomycin have been documented in patients with impaired renal function who were administered oral vancomycin to treat CDAD.[7] Therefore, when using oral vancomycin to treat CDAD in patients with gut inflammation and renal dysfunction it may be prudent to monitor vancomycin serum concentrations.

Intraperitoneal vancomycin administration is used to treat peritonitis in patients receiving peritoneal dialysis (PD). Systemic absorption of vancomycin following intraperitoneal administration is 54–65% of a given dose in 6 hours and results in adequate serum concentrations. Peritonitis causes inflammation of the peritoneal membrane, which facilitates absorption of vancomycin from the peritoneal to plasma side of the peritoneum; however, it is poorly transferred in the opposite direction.[8] One dosing regimen[9] is to administer a loading intraperitoneal dose of 30 mg/kg followed by 1.5 mg/kg in each peritoneal exchange to yield vancomycin plasma concentrations of at least 10 mg/l at 180 hours in patients with chronic renal failure. Another dosing schedule is to administer 35 mg/kg intraperitoneally on day 1, followed by 15 mg/kg intraperitoneally once daily in automated peritoneal dialysis patients, in order to provide adequate concentrations for susceptible organisms over a 24-hour period.[8]

Intrathecal and intraventricular administration of vancomycin has been used for the treatment of central nervous system infections,[10] although systemic absorption of vancomycin following intrathecal or intraventricular administration has not been investigated. It is unlikely that intrathecal or intraventricular administration of vancomycin would achieve therapeutic or toxic serum concentrations, even in patients with impaired renal function, because the amounts of vancomycin administered by these routes are generally small (approximately 10–20 mg).

Intravitreal dosing of vancomycin has been administered for postoperative bacterial endophthalmitis.[5] As with intrathecal and intraventricular administration of vancomycin, the amounts of intravitreal vancomycin administered are small (~0.2–2 mg) so accumulation to appreciable serum concentrations is highly unlikely.

Distribution

Compared with aminoglycosides, the variability in the volume of distribution (Vd) of vancomycin is large, with a Vd ranging from 0.5 to 1.0 l/kg in non-obese adults with normal renal function (creatinine clearance >80 ml/min). In clinical practice, an average Vd of 0.7 l/kg is usually used. The Vd of vancomycin can be affected by factors such as age, gender and body weight. However, the Vd of vancomycin is far less dependent on patient fluid balance (underhydration or overhydration) than the Vd of aminoglycosides. Also, the Vd of vancomycin is not significantly correlated with creatinine clearance.

Peak serum concentrations of vancomycin are approximately proportional to the dose of drug infused. Administration of 500 mg, 1000 mg or 2000 mg of vancomycin to healthy volunteers resulted

Table 140.1 Vancomycin pharmacokinetics

Age	n	CL_{cr} (ml/min)	$t_{1/2\alpha}$ (h)	$t_{1/2\beta}$ (h)	V_c (l/kg)	V_{ss} (l/kg)	Cl_V (ml/min)	CL_R (ml/min)
Premature neonates (30–34 weeks,* <1.2 kg)[1]	5	–	–	7.8 ± 3.0	–	0.47 ± 0.21	0.72 ± 0.23 ml/min/kg	–
Premature neonates (30–42 weeks,* ≥1.2 kg)[1]	6	–	–	3.8 ± 1.4	–	0.48 ± 0.13	1.58 ± 0.65 ml/min/kg	–
Premature neonates (>42 weeks,* >2.0 kg)[1]	2	–	–	2.1 ± 0.8	–	0.47 ± 0.06	2.82 ± 0.85 ml/min/kg	–
Premature neonates (26–45 weeks*)[2]	59	–	–	–	–	0.67	3.56 l/hr/kg	–
Neonates (26–42 weeks*)[3]	108	–	–	6.0	–	0.43 ± 0.01	0.95 ± 0.03 ml/min/kg	–
Children (0.01–18 years)[4]	78	138.6 ± 49.3	–	3.9	0.27 ± 0.07	0.43	1.72 ml/min/kg	–
Adults (46.3 ± 11.6 years)[5]	10	93.4 ± 28.3	0.40 ± 0.20	5.2 ± 2.6	0.21 ± 0.11	0.50 ± 0.20	98.4 ± 24.3	88.0 ± 33.6
Adults (49.5 ± 14.3 years)[5]	14	51.0 ± 8.3	0.49 ± 0.32	10.5 ± 3.6	0.21 ± 0.14	0.59 ± 0.27	52.6 ± 17.7	48.2 ± 10.8
Adults (61.6 ± 18.4 years)[5]	13	23.9 ± 8.2	1.51 ± 0.21	19.9 ± 10.2	0.24 ± 0.12	0.64 ± 0.18	31.3 ± 14.9	19.8 ± 7.9

CL_{cr}, creatinine clearance in ml/min/1.73 m^2 unless otherwise noted; $t_{1/2\alpha}$, distribution half-life; $t_{1/2\beta}$, elimination half-life; V_c, apparent distribution volume of the central compartment; V_{ss}, distribution volume at steady-state; CL_V, total body vancomycin clearance in ml/min/1.73 m^2 unless otherwise noted; CL_R, renal vancomycin clearance in ml/min/1.73 m^2 unless otherwise noted.
*Postconceptional age, i.e. sum of gestational age at birth and chronologic age.

in vancomycin serum concentrations of approximately 2–10 mg/l, 25 mg/l and 45 mg/l, respectively, 2 hours after the dose.[11]

The disposition of vancomycin after intravenous administration has been described with one-, two-, and three-compartment models. The half-life of the first distributive phase is short (approximately 0.4 hours), whereas the half-life of the terminal phase is dependent on the patient's renal function. The one-compartment model is most widely used by clinicians but its application requires postdistribution serum samples, which are often difficult to obtain accurately. The two-compartment model results in a significant improvement in both bias and precision in predicting vancomycin concentrations.

Investigations in health volunteers and patients with normal renal function have suggested that approximately 30–55% of vancomycin is bound to plasma proteins. Data concerning the penetration of vancomycin into various body fluids and body compartments are summarized in Table 140.2.

Metabolism

Approximately 80–90% of the intravenously administered vancomycin dose can be recovered unchanged in the urine in 24 hours, with very small amounts appearing in bile. By most authorities, the metabolism of the drug is not sufficiently altered in liver failure to warrant changes in vancomycin dosing. However, some suggest dose adjustments in patients with severe liver dysfunction.[19]

Excretion

The majority (80–90%) of an intravenous vancomycin dose can be recovered unchanged in the urine of adults with normal to moderately impaired renal function. Dosage adjustment is necessary in patients with impaired renal function because elimination is primarily by glomerular filtration. The clearance of vancomycin approximates 65% of creatinine clearance. Differences between vancomycin and creatinine clearance suggests either renal tubular reabsorption or significant protein binding. Some investigators suggest that tubular secretion may be a significant component of vancomycin's net renal excretion.[20] Vancomycin clearance appears to correlate better with actual total body weight in obese patients.[21]

Creatinine clearance is often expressed as ml/kg/min and this value is multiplied by the patient's actual body weight (kg). However, it is important to exclude excessive third space fluid from the total body weight, since this weight would not be expected to be associated with an increase in the renal clearance of vancomycin. Creatinine clearance in ml/kg/min can be calculated by dividing the creatinine clearance by the patient's weight initially used to calculate the creatinine clearance.

Effects of disease states and conditions

Disease states and conditions that affect a patient's renal function will influence vancomycin elimination. In patients with normal renal function, the usual serum half-life of vancomycin is 6–10 hours, and in patients with end-stage renal disease, the half-life may approach 7 days.

The clearance rate for vancomycin increases in proportion to creatinine clearance. Patients with acute renal failure appear to eliminate vancomycin differently from patients with chronic renal failure. In patients with acute oliguric renal failure, substantial nonrenal clearance (approximately 16 ml/min; range 3.8–23.3 ml/min) of vancomycin appears to occur initially.[22] Then, as the duration of renal failure increases, the nonrenal clearance decreases, and eventually approaches the total clearance observed in patients with chronic renal failure (4–6 ml/min).

Table 140.2 Vancomycin penetration into various human body fluids and tissues following intravenous administration

Body fluid or tissue	Patient description	n	Tissue or fluid concentration range (mean), mg/l	Concomitant serum concentration range (mean), mg/l	% Tissue or fluid penetration
Cerebrospinal fluid[12]	Adults receiving ventriculoperitoneal shunts for hydrocephalus	25	0.1–1.5 (0.9)	9.1–38.7 (22.3)	4.6
Cerebrospinal fluid [13]	Hemodialysis adults with proved or suspected CNS infection	3*	<0.5–1.54 (0.92)	8.8–24.0 (15.8)	5.9
Cerebrospinal fluid [14]	Premature infants	3	2.2–5.6	–	26–68
Heart valve[15]	Adults undergoing open heart surgery	33	0–2h post-dose: 4.2 5–6h post-dose: 2.3	28.9 4.4	14.5 52.3
Pleural fluid[4]	Critically ill, ventilated patients	14	0.4–8.1 (4.5)	9–37.4 (24)	~18.8
Lung tissue[16]	Adults with normal kidney and liver function undergoing thoracotomy	26	6.9–40.6	2.4–9.6	24–41
Mammary tissue[17] [a]Capsular tissue [b]Pericapsular tissue	Adult women undergoing reconstructive surgery	24	[a]2.0–7.7 (4.6) [b]2.3–18.1 (6.4)	3.1–38.8 (14.0)	[a]58 [b]74
Peritoneal dialysis fluid[18]	Adults with peritonitis and on chronic intermittent peritoneal dialysis	6	Undetectable to 22.5	–	0–96 (mean, 27)

% Penetration = fluid or tissue vancomycin concentration/serum vancomycin concentration × 100.
*Two CSF and two serum samples obtained during each episode of meningitis.

Major burn injuries (>30% body surface area) can cause significant increases in vancomycin clearance due to increases in the basal metabolic rate, with consequential increase in glomerular filtration rate and vancomycin clearance, that occurs within 72 hours of injury. The average half-life of vancomycin in burn patients is only 4 hours. Therefore, burn patients may require more frequent dosing of vancomycin (every 6–8 hours) to maintain therapeutic trough concentrations. Due to the variability in predicting creatinine clearance from serum creatinine data in burn patients, it may be prudent to measure creatinine clearance for all predictions of vancomycin pharmacokinetics.[21]

Intravenous drug abusers, critically ill patients and obese patients with normal serum creatinine concentrations (the last due to hyperfiltration) have considerably increased vancomycin clearance.[21]

Cardiac bypass appears to have no significant effect on the pharmacokinetics of vancomycin. A recent investigation found an abrupt decrease in vancomycin serum concentrations at the onset of cardiopulmonary bypass, followed by a moderate increase possibly secondary to redistribution from tissues.[23] This rebound in vancomycin serum concentration may occur at the time of reperfusion and warming. A significant relationship was found between the rebound in vancomycin concentration in serum and the length of time between unclamping the aorta and coming off cardiopulmonary bypass (r=0.94), as well as with the increase in temperature upon rewarming (r=0.92).[24] However, it appears that a 15 mg/kg intravenous dose of vancomycin administered 1 hour prior to bypass provides therapeutic serum concentrations for up to 6 hours.

The elimination half-life of vancomycin is significantly longer in elderly persons aged >65 years (12.1 hours) compared to younger subjects (7.2 hours).[25,26] Vancomycin dosing adjustments may be necessary in the elderly, even those with normal serum creatinine concentrations, due to the longer half-live of vancomycin in this patient population.

Effects of dialysis

An important element that needs to be taken in consideration in patients undergoing hemodialysis is the elimination of vancomycin in different types of membrane and with different dialysis techniques (intermittent and continuous). Although vancomycin is not significantly dialyzable when hemodialysis is performed using a low flux membrane, significant amounts of vancomycin are cleared in the dialysate with high-flux or high-efficiency hemodialysis using membranes such as polysulfone, polyacrylonitrile and polymethylmethacrylate (Table 140.3).[27–35] Early studies estimated that up to 30% of vancomycin is removed during high-flux hemodialysis. However, due to the unrecognized redistribution of vancomycin after completion of dialysis, a more recent report indicated that only approximately 17% of vancomycin is removed during high-flux hemodialysis.[32] It is important to note that high-flux membranes have largely replaced traditional low-flux membranes in hemodialysis and dosing of vancomycin needs to reflect this transition in order to maintain adequate vancomycin concentrations in these patients.

In patients undergoing continuous ambulatory peritoneal dialysis (CAPD), the small but continuous drug loss is significant. Vancomycin is often administered intravenously approximately every 3–5 days or administered directly into the peritoneal space to maintain desired plasma concentrations.[36]

PHARMACODYNAMICS

Clinical response

Data correlating pharmacodynamic indices with clinical response to vancomycin are summarized in Table 140.4. Studies suggest that vancomycin has concentration-independent cidal activity over Gram-positive

Table 140.3 Elimination of vancomycin in hemodialysis and hemofiltration

Investigator	n	Dialysis	Membrane	CL_{HD} (ml/min)
Alwakeel *et al*.[27]	8	HD	Cuprophan	15
Lanese *et al*.[28]	6	HD	Cuprophan	9.6
Torras *et al*.[29]	8	HD	Cuprophan	9.7
Alwakeel *et al*.[27]	15	HD	Polyacrylonitrile	55
Torras *et al*.[29]	8	HD	Polyacrylonitrile	58.4
Zoer *et al*.[30]	7	HD	Polyacrylonitrile	45.7
Alwakeel *et al*.[27]	12	HD	Polysulfone	76
Foote *et al*.[31]	5	HD	Polysulfone	130.7
Lanese *et al*.[28]	6	HD	Polysulfone	44.7–85.2
Pollard *et al*.[32]	12	HD	Polysulfone	120
Touchette *et al*.[33]	8	HD	Polysulfone	108.5
Bellomo *et al*.[34]	16	CAVHDF	Polyacrylonitrile	6.9–15.4
Joy *et al*.[35]	5	CVVH	Polysulfone	5.2–22.1
Joy *et al*.[35]	5	CVVH	Polymethylmethacrylate	7.5–27
Joy *et al*.[35]	5	CVVH	Acrylonitrile	5.8–13.4

CAVHDF, continuous arteriovenous hemodiafiltration; CL_{HD}, hemodialysis clearance; CVVH, continuous venovenous hemofiltration; HD, hemodialysis.

Table 140.4 Vancomycin pharmacokinetic (PK) and pharmacodynamic (PD) data from select *in vitro*, animal and human studies predictive of success

Investigators	Organism	Model/patients	PK parameter or PD index and value
Larsson *et al*.[37]	*Staphylococcus aureus*	*In-vitro* PD model	T >MIC ≈100%
Duffull *et al*.[38]	*Staphylococcus aureus*	*In-vitro* PD model	T >MBC ≈100%
Ackerman *et al*.[39]	*Staphylococcus aureus* CONS	*In-vitro* model	No relationship between VAN concentration and killing curves
Knudsen *et al*.[40]	*Staphylococcus aureus* *Streptococcus pneumoniae*	Murine peritonitis	T >MIC, C_{max}:MIC, AUC_{24h}:MIC
Ahmed *et al*.[41]	*Streptococcus pneumoniae*	Rabbit meningitis model	CSF C_{max}:MBC ≥4
Lisby-Sutch & Nahata[1]	*Staphylococcus aureus* CONS *Enterococcus,* group D	Infants with a variety of infections	C_{max} 25–35 mg/l (SBT ≥1:8) C_{min} 5–10 mg/l (SBT 1:2 to 1:8)
Schaad *et al*.[25]	*Staphylococcus aureus* *Staphylococcus epidermidis*	Children with a variety of infections	C_{max} >25 mg/l (SBT ~1:16) C_{min} <12 mg/l (SBT ~1:4)
Louria *et al*.[42]	*Staphylococcus aureus*	Adults with a variety of infections	SBT ≥1:8
Sorrell *et al*.[43]	*Staphylococcus aureus*	Patients with bacteremia	MBC:MIC <32
Iwamoto *et al*.[44]	MRSA	Patients with pneumonia or bacteremia	C_{max} >25 mg/l C_{min} 10–15 mg/l
Moise *et al*.[45]	MRSA	Adults with lower respiratory tract infections	Clinical success: AUC_{24h}:MIC >345 Bacterial eradication: AUC_{24h}:MIC >428

CONS, coagulase-negative staphylococci; CSF, cerebrospinal fluid; MBC, minimum bactericidal concentration; MIC, minimum inhibitory concentration; MRSA, methicillin-resistant *Staphylococcus aureus*; SBT, serum bactericidal titers; UAC, area under the curve; VAN, vancomycin.

organisms, although some investigators propose the need to achieve certain peak and trough concentrations for optimal activity.[37,39,46,47] Once vancomycin concentrations exceed the MBC or four times MIC, vancomycin displays concentration-independent killing above which further increases in concentration do not increase the cidal activity according to *in-vitro* models and animal data.[41]

In-vitro pharmacodynamic models found vancomycin to display concentration-independent activity.[37,38] In one study, bolus doses achieving C_{max} values of 5, 10, 20 and 40 mg/l yielded similar time-kill curves against *S. aureus*. However, bactericidal activity was more efficient under aerobic conditions. The time to achieve a 3-log kill of bacteria was approximately twice as long in an anaerobic environment compared to an aerobic one.[48]

Ahmed and colleagues[41] reported the relationship between the bactericidal ratio of vancomycin in the cerebrospinal fluid (C_{max}:MBC ratio) and the rate of bacterial clearance of *Streptococcus pneumoniae* (vancomycin MBC = 0.5 mg/l) in a rabbit model of experimental meningitis. Animals were administered 80 mg/kg of vancomycin in two or four divided doses, with and without dexamethasone, over 36 hours. The concomitant administration of dexamethasone significantly reduced the penetration of vancomycin into the CSF from 20.1% to 14.3% ($p = 0.035$). Maximal cidal activity was achieved at concentrations fourfold greater than the MBC, above which no additional killing was observed.

A clinical investigation of *S. aureus* bacteremia[43] found an MBC:MIC ratio <32 to be predictive of successful response to vancomycin ($p = 0.04$). All 10 patients with an MBC:MIC ratio <32 experienced a vancomycin treatment success. However, only one of four infections with an MBC:MIC ratio >32 was considered a treatment success. All eight patients infected with *S. aureus* isolates having an MBC <32 mg/l were cured and three of six patients with an MBC >32 mg/l were cured of their infection. This difference was not statistically significant ($p = 0.11$). The investigators concluded that there is not a simple relationship between *in-vitro* tests and clinical response to vancomycin therapy, but tolerance was associated with poor therapeutic response. Several clinical investigators have demonstrated a relationship between successful response to vancomycin therapy and bactericidal serum titers of at least 1:8.[1,25,42]

A recent investigation[44] showed that vancomycin trough concentrations of 10–15 mg/l and peak concentrations >25 mg/l are optimal for MRSA-related pneumonia and bacteremia. Therapeutic failures have been reported in patients with endocarditis with vancomycin plasma trough concentrations <10 mg/l.[49] Small studies suggest that serum bactericidal titers of >1:8 are associated with vancomycin treatment successes.[1,25] These clinical investigations suggest that individualization of vancomycin dosage may be required for optimum therapy.

Adverse effects

Ototoxicity (<2%) and nephrotoxicity (5%) are well-recognized but relatively infrequent adverse events associated with vancomycin therapy and may be concentration related.[44,50] Attempts to relate serum concentrations of vancomycin to the development of nephrotoxicity have been difficult, although many studies suggest that the current preparations of vancomycin may have less potential for nephrotoxicity than earlier preparations. Farber and Moellering[50] reported a 5% incidence of nephrotoxicity in patients receiving vancomycin alone. In virtually all of the patients investigated, the renal function returned to baseline following discontinuation of vancomycin therapy. In contrast, when vancomycin was administered concomitantly with an aminoglycoside to adults, the incidence of nephrotoxicity was higher in some of the studies (range 22–35%).[50–53] Patients with neutropenia, peritonitis, increased age, liver disease, concurrent amphotericin B therapy and those of male gender may also have an increased risk of developing nephrotoxicity.[53]

While concerns regarding nephrotoxicity associated with vancomycin therapy have been mostly historical, their recent revival has been based on more aggressive dosing to treat *S. aureus* strains with increasing vancomycin MICs. Since it is accepted that the area under the concentration–time curve:minimum inhibitory concentration (AUC:MIC) is predictive of vancomycin treatment success in pneumonia, an increase in MIC of 0.5 mg/l to 2 mg/l would require a fourfold increase in patient exposure to the drug AUC to maintain the same ratio. If a 24-hour AUC:MIC of 400 is the target, then a 24-hour AUC of 800 would be required to treat an infection by an organism with a vancomycin MIC of 2 mg/l. This corresponds to mean serum concentrations of vancomycin of approximately 33 mg/l (peak serum concentration ~45 mg/l and trough ~25 mg/l). The doses required for such serum concentrations are frequently done with great hesitation, especially in light of some recent data suggesting potential nephrotoxicity associated with higher vancomycin doses.[54–56] While these studies are certainly not definitive in showing dose-related vancomycin nephrotoxicity, a more recent study was more definitive in associating nephrotoxicity with >4 g/day of vancomycin.[57] While this dose is higher than most clinicians are using, it is clear that these studies suggest an increasing awareness of this risk. A definitive study showing nephrotoxicity at <4 g/day may be difficult given the multitude of other causes of nephrotoxicity in the hospital environment.

Investigations on vancomycin-induced ototoxicity (hearing loss or tinnitus) have raised the possibility that ototoxicity may be related to excessively high serum concentrations (>80 mg/l), with recommendations to avoid serum vancomycin concentrations >50 mg/l.[50,58] A comparison of once- versus twice-daily vancomycin found no statistically different rates in ototoxicity rates.[59] Hearing loss developed in 1 of 31 (3.2%) and 5 of 32 (15.6%) in the once- and twice-daily groups, respectively. A recent case report has described a patient who developed ototoxicity after intrathecal administration of vancomycin,[60] although the relationship between intrathecal administration of vancomycin and ototoxicity requires further study.

A contemporary evaluation of vancomycin ototoxicity comparing baseline audiograms to audiograms after approximately 4 weeks of vancomycin therapy demonstrated 0% worsening in 32 patients aged <53 years but a 19% worsening in patients aged ≥53 years.[60A] The clinical significance and reversibility of these findings warrant a prospective clinical evaluation but they draw considerable attention to potential risks of vancomycin in older patients.

Nonconcentration-related toxicities have also been reported with vancomycin. Rapid intravenous infusion of vancomycin, >500 mg per 30 minutes in normal adults, may result in a non-IgE-mediated histamine reaction characterized by flushing, local pruritus, erythema of the neck and upper torso, tachycardia and/or hypotension.[61,62] This reaction, often referred to as 'red man syndrome', may occur at any point in the infusion and may occur for the first time after several doses or with slow infusion.[63] The effects of this reaction can be relieved by antihistamines.[64–66] The incidence of red man syndrome varies between 3.7% and 47% in infected patients,[67] and is between 30% and 90% in healthy volunteers.[62] It has been suggested that a relationship may exist between the area under the histamine concentration time curve and severity of the reaction.[68] It has been the authors' experience that some patients who experience flushing near the end of their infusion may have their symptoms relieved without discontinuation of the drug by administering the medication as a continuous infusion. It is important to realize that concomitant opiates for analgesia can potentiate the mast cell destabilization of vancomycin and patients receiving this class of medication may be at increased risk for red man syndrome.

Eosinophilia, neutropenia, rashes (including exfoliative dermatitis), Stevens–Johnson syndrome (infrequent), immune-mediated thrombocytopenia and drug fever also have been reported with vancomycin.

VANCOMYCIN RESISTANCE CONSIDERATIONS

The breakpoints of resistance to vancomycin in *S. aureus* are shown in Table 140.5. Unlike β-lactam antibiotics which bind to and interrupt the activity of penicillin-binding proteins, enzymes involved in cell

Table 140.5 Vancomycin susceptibility for *Staphylococcus aureus*

Vancomycin susceptibility	Abbreviation	MIC (μ/ml)
Susceptible	VSSA	≤2
Heteroresistant	hVISA	1–2*
Intermediate	VISA	4–8
Resistant	VRSA	≥16†

hVISA, heteroresistant vancomycin-intermediate *S. aureus*; VISA, vancomycin-intermediate *S. aureus*; VRSA, vancomycin-resistant *S. aureus*; VSSA, vancomycin-susceptible *S. aureus*.
*Consist of subpopulations (≤10^{-6}) that may grow in media containing >2 μg/ml vancomycin.
†Requires back-up primary testing with 6 μg/ml vancomycin overnight plate.
Data from Clinical Laboratory Standards Institute. Performance standards for antimicrobial disc susceptibility tests; 16th informational supplement. Report M100-S16. Wayne, PA: National Committee for Clinical Laboratory Standards; 2006.

wall synthesis, vancomycin binds with high affinity to the D-alanyl-D-alanine (D-Ala-D-Ala) *C*-terminus of late peptidoglycan precursors and prevents reactions of cell wall synthesis utilizing these precursors in transglycosylase, transpeptidase and carboxypeptidase.[69] Resistance in vancomycin-resistant enterococci (VRE) and vancomycin-resistant *S. aureus* (VRSA) is due to the presence of operons that encode enzymes which produce the low affinity precursors D-Ala-D-lactate or D-Ala-D-serine and enzymes that eliminate competitive high-affinity peptidoglycan precursors that are normally produced.

In vancomycin-intermediate *S. aureus* (VISA) strains, altered cellular physiology results in cumulative effects of mutations and/or modulation of regulatory systems. This altered physiology appears to alter cell wall metabolism in such a way as to result in increased numbers of D-Ala-D-Ala target sites for vancomycin binding. This altered cell wall results in a reduced diffusion coefficient of vancomycin, sequestration of vancomycin within the cell wall by these false targets, and prevention of vancomycin reaching its site of action.[69] In addition, studies of *S. aureus* with reduced vancomycin susceptibility and isogenic vancomycin-susceptible progenitors showed cell walls with reduced peptidoglycan cross linking, reduced cell wall turnover and reduced autolysis that investigators suggested may be due to alterations in teichoic acid structure and metabolism.[70] These metabolic changes result in considerable morphologic cell wall thickening.[71]

The phenotype of many VISA strains is unstable, suggesting that the regulatory foci can be turned on or off depending on whether glycopeptide is present. Some of the genes whose expression has been found to be altered in glycopeptide-intermediate *S. aureus* (GISA) include *agr, pbp2, pbp4, pbpD, sigB, ddh, tcaA* and *vraSR*. However, what is now clear is that the end result of altered cell wall metabolism, rather than the specific genetic mechanisms achieving this altered metabolism, is of primary importance.[72]

While VISA and VRSA remain extremely rare, cases of glycopeptide treatment failure are common. Recent studies demonstrate increased clinical failure of vancomycin therapy in MRSA infections where the isolates have increased MICs that are still within the susceptible range. For example, comparing 51 patients with infections by MRSA with vancomycin MICs of 2 mg/l with 44 patients with MICs of <2 mg/l, response was significantly lower in the high MIC group (62% vs 85%, $p=0.02$), infection-related mortality was higher (24% vs 10%) and high MIC was an independent predictor of poor response in multivariate analysis.[54] The most recent study evaluating outcome and vancomycin MIC for susceptible MRSA showed a higher mortality when vancomycin was used empirically and the organism was found to have a vancomycin MIC of 2 mg/l by E-test with an odds ratio of 6.39 ($p<0.001$).[73] Clearly these results point to a need to re-evaluate the microbiologic breakpoint for vancomycin in *S. aureus* for bacteremia and pneumonia.

Why this discrepancy in clinical and microbiologic definitions for vancomycin? The answer is complex, but may relate to the heterogeneous resistance of staphylococci to glycopeptides, whereby small subpopulations, perhaps <1 in 10^6, exist that can grow in higher concentrations of vancomycin than the MIC predicts. We refer to these strains as hetero-VISA (see Table 140.5). Such small populations may not be detected by low inocula testing used by standard microbiology laboratory methods, although these subpopulations may certainly exist *in vivo* in a pneumonia or an endocardial vegetation. The higher the vancomycin MIC, the more likely that these resistant subpopulations (hVISA) will be present[74] (see also Chapter 131).

CLINICAL APPLICATION OF PHARMACOKINETIC DATA AND DOSING CONSIDERATIONS

An understanding of the desired therapeutic range and pharmacokinetic parameters of vancomycin enables the clinician to select dosing strategies specific for the individual patient. Dosing nomograms for vancomycin are available, but due to the pharmacokinetic variability of vancomycin arising from nonrenal factors, it seems impractical to rely exclusively on nomograms that are based on renal function alone. In addition, studies have shown that the published nomograms may significantly underpredict vancomycin steady-state peak and/or trough concentrations. At this time, there appears to be no benefit in monitoring peak vancomycin serum concentrations. Trough concentrations are monitored before the fourth dose in patients whose creatinine clearance is in steady state, and then weekly for patients on vancomycin therapy for more than 2 weeks. Ideally, a baseline serum creatinine concentration and complete blood count are obtained before vancomycin therapy is initiated and then weekly thereafter. If the creatinine clearance is not in steady state or if the patient is receiving other nephrotoxic agents such as aminoglycosides or amphotericin B, serum creatinine and vancomycin serum troughs may need more frequent (two or three times per week) monitoring. Serum vancomycin trough concentrations are maintained at 10–20 mg/l, not necessarily for therapeutic benefit, but to prevent further development of glycopeptide resistance.[48,75] If serum creatinine concentrations increase by more than 0.5 mg/dl over the baseline value (or more than 25–30% over baseline for serum creatinine values >2 mg/dl), and other causes of decrease in renal function are ruled out, alternatives for vancomycin may be warranted.

Ototoxicity is generally monitored at the same time intervals as serum creatinine determination. Instead of using audiometry, clinical signs and symptoms of auditory or vestibular ototoxicity are usually monitored in the clinical setting. Symptoms of auditory ototoxicity include decreased hearing acuity in conversational range, feeling of fullness or pressure in the ears and tinnitus. Symptoms of vestibular ototoxicity include loss of equilibrium, headache, nausea, vomiting, vertigo, nystagmus and/or ataxia.

As with all antibiotics, the development of resistance to the glycopeptides is inevitable and currently there is a growing understanding that what has been traditionally rendered a susceptible definition microbiologically may not necessarily correspond to successful treatment outcome, particularly with serious MRSA infections such as bacteremia, endocarditis and pneumonia. Therefore, emerging data appear to place vancomycin in a role analogous to that of penicillin for *Streptococcus pneumoniae* infections. This means that for the serious infections mentioned above, optimal empiric therapy may consist of the newer Gram-positive antimicrobials (e.g. linezolid for pneumonia, daptomycin for bacteremia), with 'de-escalation' to vancomycin once the vancomycin is confirmed at ≤1 mg/l. While it may seem intuitive to seek alternative therapies to glycopeptides for serious MRSA infections (i.e. pneumonia and bacteremia) when the organism has a vancomycin MIC of 2 mg/l, no study has been

performed comparing vancomycin to daptomycin or linezolid under these microbiological circumstances. In addition, the role of combination therapy, for example with aminoglycosides or rifampin (rifampicin), also warrants further study. Readers should also be informed of the recent consensus statement on vancomycin administration published by leading authorities in this field during the production stages of this text.[75A]

Teicoplanin

Teicoplanin (formerly called teichomycin A) is a complex of five closely related glycopeptides that have the same heptapeptide base and an aglycone that contains aromatic amino acids, with D-mannose and *N*-acetyl-D-glucosamine as sugars, with a molecular weight of 1562–1891 Da. Teicoplanin is structurally similar to vancomycin. It is produced from the actinomycete *Actinoplanes teichomyceticus*.

Teicoplanin is similar but not identical to vancomycin in its spectrum of activity. The MICs for most Gram-positive bacteria and anaerobes are comparable, but teicoplanin is less active against some strains of *Staphylococcus haemolyticus* (MIC 16–64 mg/l compared to ≤4 mg/l for vancomycin). Its ease of administration and its low toxicity potential make it a suitable alternative to vancomycin for the treatment of Gram-positive aerobic and anaerobic bacteria in both the immunocompetent and the immunocompromised host. *In-vitro* activity against most Gram-positive organisms is equal to or greater than that of vancomycin.

In both open and comparative clinical trials, teicoplanin has been well tolerated, and adverse reactions have rarely prompted discontinuation of treatment. Nephrotoxicity caused by teicoplanin is uncommon, even when it is used concomitantly with aminoglycosides. Favorable pharmacokinetics allows for intramuscular administration as well as intravenous bolus dosing and, after appropriate loading doses, maintenance therapy may be given on a once-daily basis. The combination of all of these factors makes teicoplanin an effective, safe alternative to vancomycin in the treatment of Gram-positive infections. Although it is widely used in Europe, it is not approved by the Food and Drug Administration in the USA.

PHARMACOKINETICS AND DISTRIBUTION

Teicoplanin binds to the terminal D-Ala-D-Ala sequence of peptides that form the bacterial cell wall and, by sterically hindering the transpeptidase and transglycosylation reaction, inhibits the formation of peptidoglycan. Teicoplanin is a large polar molecule and, as it cannot penetrate the lipid membrane of Gram-negative bacteria, they are resistant to it (see Chapter 130). Enterococci expressing VanA (high-level) vancomycin resistance are also resistant to teicoplanin. Teicoplanin is not significantly absorbed from the gastrointestinal tract but it can be administered intravenously or intramuscularly, in a once-daily dosing schedule. It has a long half-life of approximately 47 hours, allowing for once-daily dosing once therapeutic serum levels are attained. In a study in which healthy volunteers were given intravenous injections of teicoplanin, doses of 3 mg/kg gave average peak plasma concentrations of 53.5 µg/ml and doses of 6 mg/kg gave average peak plasma concentrations of 111.8 µg/ml.[77] Bioavailability after intramuscular injection of teicoplanin is 90%, with peak plasma concentration occurring 2 hours after injection.[76]

Teicoplanin is approximately 90% protein-bound. It is more lipophilic than vancomycin and has excellent penetration into tissues and tissue fluids with a large volume of distribution after intravenous administration. High concentrations are achieved in peritoneal and blister fluid, bile, liver, pancreas, mucosa and bone.[77] Penetration into cerebrospinal fluid across uninflamed meninges is poor.

Teicoplanin does not undergo extensive metabolism and is excreted almost entirely by the kidneys.[78] As with vancomycin, its half-life is prolonged by renal failure.[79] Neither hemodialysis nor peritoneal dialysis significantly affects the clearance of teicoplanin.

ADMINISTRATION AND DOSAGE

Dosing recommendations for adults who have normal renal function have been based on both pharmacokinetic properties and results of open and comparative clinical trials. A single loading dose of 400 mg on the first day followed by maintenance doses of 200 mg/day (3 mg/kg/day for pediatric dose) appears adequate for the treatment of urinary tract infections, skin and soft tissue infections and lower respiratory tract infections. For serious infections, including endocarditis, osteomyelitis and sepsis, it is necessary to maintain serum concentrations of teicoplanin at 10 µg/ml or more.[80]

Treatment of endocarditis caused by *S. aureus* has been difficult with teicoplanin, especially when used as monotherapy.[80,81] In these cases, it is recommended that an aminoglycoside should be added and that teicoplanin trough levels should be maintained in the range of 20–60 µg/ml.

INDICATIONS

Indications for the use of teicoplanin are similar to those for vancomycin (Table 140.6). They include Gram-positive infections caused by strains that are resistant to penicillin, cephalosporin or methicillin, or Gram-positive infections in patients who are allergic to penicillin. In addition, teicoplanin may be used for subacute bacterial endocarditis or surgical prophylaxis, in patients who are allergic to β-lactam drugs and as an alternative to vancomycin or metronidazole in the treatment of pseudomembranous colitis caused by *C. difficile* (approved in Europe).

Teicoplanin, alone or in combination with other antibiotics, has proved effective in the treatment of various Gram-positive infections, including sepsis, endocarditis, skin and soft tissue infections, osteomyelitis and lower respiratory infections. It has also been found to be effective in both prophylaxis and treatment of Hickman catheter infections in immunocompromised patients.[82,83] Specially prepared catheters loaded with teicoplanin have been developed and tested *in vitro* and have been shown to prevent bacterial colonization for at least 48 hours, thus showing promise for inhibiting early-onset catheter infections.[84] Teicoplanin has also been used along with other antibiotics for empiric treatment of febrile neutropenic patients and in the treatment of documented Gram-positive infections in neutropenic patients.[85,86]

Table 140.6 Indications for the use of teicoplanin

Infections with:	*Staphylococcus* spp., including methicillin-resistant strains *Streptococcus* spp., *Streptococcus agalactiae, Streptococcus bovis* (recently renamed *Streptococcus gallolyticus*), *Streptococcus pneumoniae, Streptococcus pyogenes* and viridans streptococci Enterococci *Clostridium* spp., including *Clostridium difficile* *Corynebacterium* spp., including *Corynebacterium jeikeium*
The following types of infection with the above organisms	Sepsis Endocarditis Skin and soft tissue infections Osteomyelitis Lower respiratory tract infections Diarrhea associated with *Clostridium difficile* Nosocomial intravascular catheter infections (prophylaxis and treatment)

Courtesy of Jihad Slim & Leon Smith.

Oral teicoplanin is as effective as vancomycin in the treatment of diarrhea associated with *C. difficile*. A 10-day course or regimen of 100 mg oral teicoplanin q12h was found to be as effective as 500 mg oral vancomycin q6h.[87] Again, the risk of glycopeptide resistance development argues for the use of metronidazole for routine therapy for *C. difficile*-associated diarrhea.

DOSAGE IN SPECIAL CIRCUMSTANCES

A dosage nomogram for teicoplanin has been designed,[88] based on the relationship between teicoplanin clearance and creatinine clearance and an average desired steady-state concentration of 20 μg/ml. Although intravenous teicoplanin penetrates well into peritoneal fluid in normal patients, it does not penetrate well into the effluent of CAPD patients and is not recommended for the treatment of peritonitis in these patients. Intraperitoneal teicoplanin has been effective in CAPD patients who have Gram-positive peritonitis, however.

Another regimen for renal-impaired patients includes a normal loading dose followed by doubling the dosage interval for patients who have a creatinine clearance of 30–80 ml/minute and tripling the dosage interval for patients who have severe renal impairment (creatinine clearance <10 ml/minute).

ADVERSE REACTIONS AND INTERACTIONS

Teicoplanin is generally well tolerated at therapeutic dosages, with side-effects occurring in approximately 6–13% of the patients (Table 140.7).

Side-effects (including nephrotoxicity) with teicoplanin are consistently less common than with vancomycin, even when teicoplanin is used concomitantly with aminoglycosides.[83,85] The incidence of 'red man syndrome' or anaphylactoid reactions caused by teicoplanin administration is exceptionally low.

The commonest side-effects were injection site intolerance, skin rash, bronchospasm and eosinophilia. Nephrotoxicity and ototoxicity are uncommon. Like vancomycin, teicoplanin is bound and inactivated by bile-binding resins such as colestyramine.[89]

Table 140.7 Adverse reactions to teicoplanin

Hypersensitivity	Skin rash Bronchospasm Anaphylaxis
Biochemical abnormalities	Increased liver function tests
Hematologic abnormalities	Eosinophilia
Local intolerance	Redness, pain (after intramuscular administration), phlebitis (after intravenous administration)
Nonspecific reactions	Nausea Diarrhea Dizziness Tremor

Courtesy of Jihad Slim & Leon Smith.

Future Therapies in the Glycopeptide/Lipoglycopeptide Class

Oritavancin, telavancin and dalbavancin have each completed phase III trials for the management of Gram-positive infections. Dalbavancin is a semisynthetic lipoglycopeptide that inhibits cell wall synthesis. This investigational agent has a half-life of 6–10 days, which allows for once-weekly dosing. Dalbavancin 1 g given intravenously, followed by 500 mg given intravenously 8 days later, has been compared to linezolid in a randomized, double-blind trial for the management of skin and skin structure infections. At the time of writing, Pfizer globally withdrew all dalbavancin marketing applications for the treatment of skin and skin structure infections in the United States and Europe, and announced that an additional phase III study with dalbavancin is planned.

Telavancin is also a semisynthetic lipoglycopeptide with a dual mechanism; it inhibits cell wall synthesis and disrupts membrane barrier function. Telavancin has a 7–9-hour half-life and is dosed once a day (7.5–10 mg/kg/day). At the time of writing, the manufacturer of telavancin has completed phase III trials in patients with complicated skin and soft tissue infections, pneumonia and a phase II bacteremia study.

Oritavancin is a bactericidal intravenous semisynthetic glycopeptide that has a longer half-life (>10 days) than vancomycin, dalbavancin and televancin and, thus, can potentially offer a shorter duration of treatment. However, the long half-life has also raised concerns with respect to drug accumulation and potential toxicity. Oritavancin also has a dual mechanism, inhibiting cell wall synthesis and disrupting cell wall membrane function. Currently, oritavancin has completed two phase III trials for the management of complicated skin and skin structure infections using oritavancin dosed at 200 mg daily, two phase II trials for the management of bacteremia, and one phase II study investigating a single dose and an infrequent dosing regimen (first dose on day 1 with an option for a second dose on day 5). The advent of investigational agents for clinical use could provide clinicians with a variety of options for the treatment of Gram-positive infections if approved.

REFERENCES


References for this chapter can be found online at http://www.expertconsult.com

Chapter 141

Ian Chopra

Tetracyclines and chloramphenicol

Tetracyclines

INTRODUCTION

In 1948, Benjamin Duggar at the Lederle Laboratories isolated the first tetracycline, chlortetracycline, from a fungus producing a golden pigment. The fungus was therefore called *Streptomyces aureofaciens* and the antibiotic aureomycin. Interestingly, although 1948 is the acknowledged date for the discovery of the tetracyclines, it is likely that ancient civilizations were exploiting the therapeutic benefits of this class of antibiotic through consumption of beer contaminated with *S. aureofaciens*.

In 1950 oxytetracycline was isolated from a strain of *Streptomyces rimosus* by workers at Charles Pfizer & Co. and in 1953 tetracycline was produced semisynthetically from chlortetracycline. Demeclocycline, derived from a mutant strain of *S. aureofaciens*, was introduced in 1957 and in the following years the semisynthetic derivatives rolitetracycline and methacycline were also introduced. A so-called second generation of long-acting tetracyclines, doxycycline and minocycline, were synthesized in 1966 and 1972, respectively.

In response to the increasing incidence of bacterial resistance to first- and second-generation tetracyclines, a research program was initiated at Lederle Laboratories in the 1990s to seek new third-generation tetracycline analogues that would circumvent existing mechanisms of resistance. This led to the discovery of the glycylcyclines and the development of 9-(t-butylglycylamido)-minocycline (tigecycline) as a new therapeutic agent.

Chemically, the tetracyclines have the structure of a hydronaphthacene nucleus containing four fused rings. The specific analogues are obtained by substitutions on the fifth, sixth, seventh or ninth positions of the basic structure (Fig. 141.1).

Tetracyclines are mainly bacteriostatic and they act by binding to the 30S subunits of the ribosomes in susceptible micro-organisms, thereby inhibiting protein synthesis.

Tigecycline evades the major mechanisms responsible for resistance to earlier tetracyclines.

There are few reasons to use any of the older tetracyclines; however, amongst the older drugs doxycycline and to some extent minocycline should be preferred because they are better absorbed and distributed than other tetracyclines. However, one exception may be that dermatologists often seem to prefer tetracycline or another first-generation drug for the treatment of acne.

Tigecycline holds promise as a novel expanded-spectrum tetracycline and is approved for the treatment of complicated skin and soft tissue infections and complicated intra-abdominal infections. It is only available as an intravenous product. An oral formulation of the drug would expand the potential application of tigecycline in clinical practice.

ANTIMICROBIAL SPECTRUM

When they were introduced the tetracyclines were effective against a variety of Gram-positive bacteria and Gram-negative organisms within the Enterobacteriaceae group. Development of resistance has, however, led to restricted use of first- and second-generation tetracyclines for infections caused by streptococci, staphylococci, *Escherichia coli* or *Proteus* spp. However, first- and second-generation tetracyclines have retained good activity against *Mycoplasma*, *Chlamydia* and *Rickettsia* spp., *Borrelia* spp. (especially *Borrelia burgdorferi*) and *Propionibacterium acnes* (Table 141.1).[1] Tigecycline has a broad spectrum of *in-vitro* activity, including susceptible and multidrug-resistant Gram-positive and Gram-negative organisms, as well as anaerobes and atypical organisms (Table 141.1).[2,3] It is active against organisms displaying resistance to earlier tetracyclines mediated by ribosomal protection mechanisms or efflux systems belonging to the major facilitator superfamily (MFS).[4] Deficiencies in its spectrum include *Proteus* spp. and *Pseudomonas* spp. which are intrinsically resistant through expression of chromosomally encoded multidrug efflux pumps from the resistance–nodulation–division (RND) family.[5,6]

Doxycycline and minocycline often exhibit better activity *in vitro* than the other tetracyclines[7,8] but the differences are probably of little practical importance for clinical treatment.

Tigecycline exhibits antibacterial activity typical of earlier tetracyclines, but with potent activity against organisms resistant to first- and second-generation tetracyclines [2,3]

RESISTANCE

Oral therapy with earlier tetracyclines has a marked influence on the bowel flora and resistant strains may be quickly selected. This risk is somewhat reduced with doxycycline,[9] which is nearly completely absorbed.[10] Nevertheless, extensive use of first- and second-generation tetracyclines has led to widespread selection and dissemination amongst bacteria of genetic determinants encoding ribosomal protection and MFS-mediated resistance mechanisms (see also Chapter 131).[4] Most of these acquired tetracycline resistance genes reside on transposons, conjugative transposons and/or integrons which permit horizontal resistance transfer from one species to another and between unrelated genera.[4] Currently resistance to tigecycline in the clinical setting is rare.[11] However, in a recent report of treatment of two patients with tigecycline for other nosocomial infections, it appears that tigecycline-insusceptible *Acinetobacter baumannii* was selected, resulting in the development of bloodstream infections in both patients, one of whom died from the infection.[11]

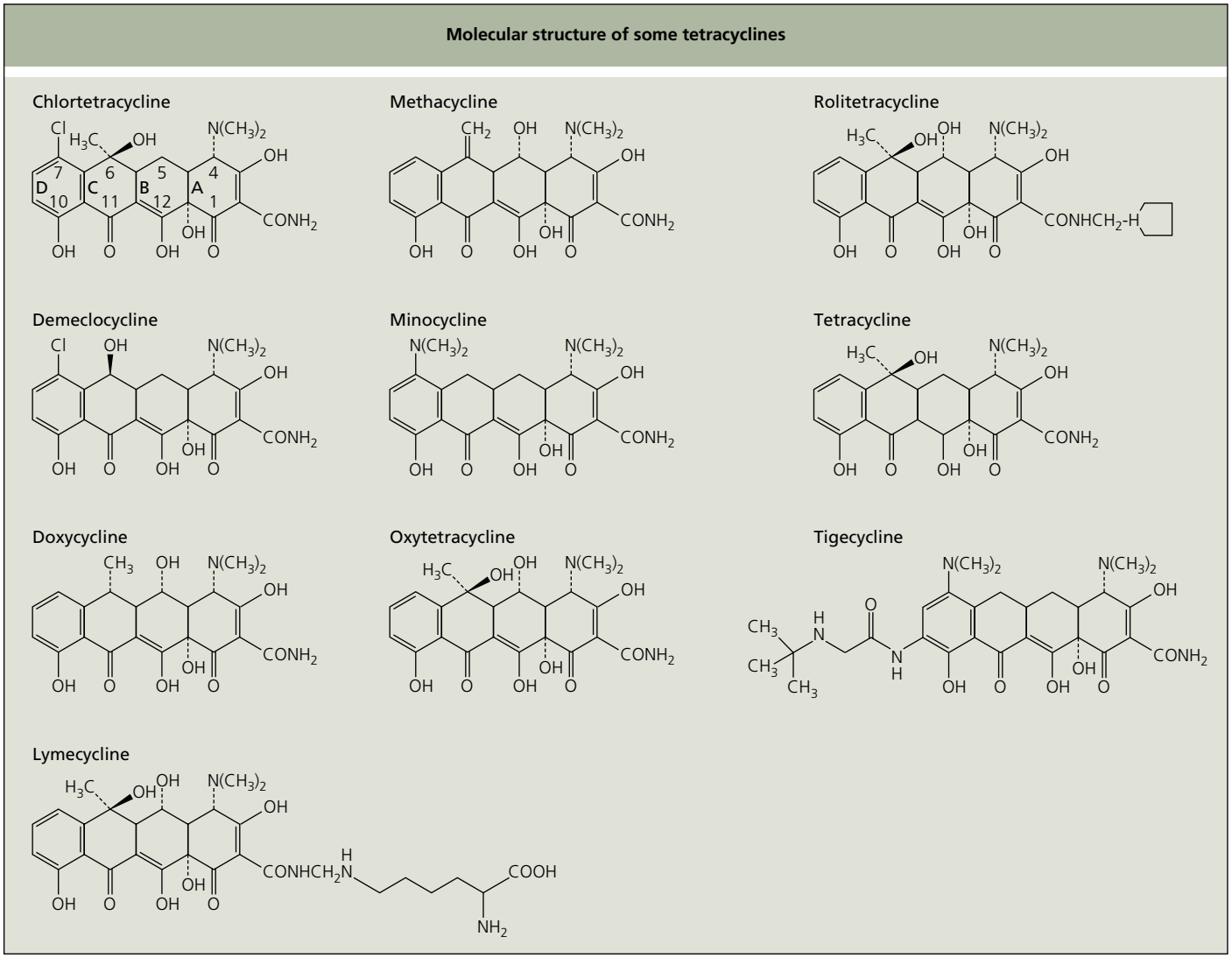


Fig. 141.1 Molecular structure of some tetracyclines.

Tetracyclines have been widely used in the veterinary field, not only to cure infections but also as a food additive to promote the growth of newborn animals. Tetracyclines are not now permitted as growth stimulators within the European Union but are still used in the USA.[4] In pigs, antibiotics promote an increase in weight of 8% and pigs are ready for slaughter 3 weeks earlier than animals that have not received antibiotics – an important economic advantage. This misuse of antibiotics is probably one important reason for the worldwide spread of tetracycline resistance within the Enterobacteriaceae group.[12–14]

Resistance to first- and second-generation tetracyclines is now common among bacteria causing respiratory tract infections such as pneumococci, *Haemophilus influenzae* and *Moraxella catarrhalis*.[15]

Obligatory intracellular pathogens such as *Chlamydia* and *Rickettsia* spp. have not yet acquired tetracycline resistance. Since these bacteria grow only inside mammalian cells, it would require that these cells be co-infected with two genera to allow gene exchange into the obligate intracellular pathogen.

PHARMACOKINETICS AND DISTRIBUTION

Clinical pharmacokinetics

After oral administration tetracyclines are absorbed from the stomach and the small intestine. Absorption is usually highest in the fasting state, but doxycycline and minocycline are also well absorbed with food. The degree of absorption and other pharmacokinetic parameters for some of the tetracyclines are shown in Table 141.2. The nearly complete absorption of doxycycline salts is reduced if the gastric pH is increased, as can occur in people who have atrophic gastritis or be caused by acid-reducing drugs. In some countries doxycycline is available in tablets bound to a polysaccharide, carragenate, which has been shown to increase absorption at higher pH.[16] There are also gelatin capsules available containing coated pellets of doxycycline hydrochloride that are resistant to gastric acid so that absorption will occur in the duodenum.[17]

Distribution

The tissue distribution of older tetracyclines is clearly related to their different lipid solubility, which is highest for doxycycline. Provided that tetracycline susceptible organisms are responsible for infection, doxycycline concentrations will be sufficient for treatment in the respiratory tract and lung tissue, the bile and the genital tract of both sexes.[18,19] Levels achieved in the central nervous system (CNS) are increased in chronic meningeal inflammation,[20] enabling treatment of neuroborreliosis.[21,22] Tigecycline has a large and variable volume of distribution.[2,23] It is concentrated in the gallbladder, lung and colon.[24] Tetracyclines, including tigecycline, cross the placenta and bind to metal ions in fetal bone and teeth. First- and second-generation tetracyclines are also known to be excreted in human breast milk. It is not known whether tigecycline is excreted in human breast milk; however,

Table 141.1 Susceptibility to first-, second- and third-generation (tigecycline) tetracyclines

	1st and 2nd generation	Tigecycline
Generally susceptible species	*Mycoplasma pneumoniae* *Ureaplasma urealyticum* *Chlamydia* spp. *Rickettsia* spp. *Brucella* spp. *Francisella tularensis* *Propionibacterium acnes* *Borrelia burgdorferi* *Yersinia* spp.	*Mycoplasma pneumoniae* *Ureaplasma urealyticum* *Chlamydia* spp. *Rickettsia* spp. *Brucella* spp. *Francisella tularensis* *Propionibacterium acnes* *Borrelia burgdorferi* *Yersinia* spp. Streptococci Enterococci Staphylococci Enterobacteriaceae *Haemophilus influenzae* Meningococci Gonococci *Serratia* spp.
Resistance common	Streptococci Staphylococci Enterobacteriaceae *Haemophilus influenzae* Meningococci Gonococci *Legionella pneumophila*	
Resistance usually found	Enterococci *Proteus* spp. *Pseudomonas* spp. *Serratia* spp. *Bacteroides* spp.	*Proteus* spp. *Pseudomonas* spp.

studies of radiolabeled drugs in rats indicate that tetracycline is readily excreted via the milk of lactating rats.[25]

Minocycline is even more lipid soluble than doxycycline. This may not be an advantage because side-effects such as vertigo and other CNS symptoms may be caused by increased drug concentrations in the cerebrosides of the brain.[26]

Elimination

Tetracyclines are metabolized in the liver in small amounts only, chlortetracycline being an exception with rapid metabolism. However, inducers of liver enzymes such as diphenylhydantoin may cause some metabolism of doxycycline. There is biliary excretion of older tetracyclines to a varying degree and possibly enterohepatic circulation. Biliary excretion is the primary route of elimination for tigecycline, with renal secretion as a secondary route.[27] Tetracyclines are partly excreted by glomerular filtration. For minocycline this excretion is less than 10% but it is more than 50% for tetracycline.

Incomplete absorption will contribute to high concentrations in feces for the older tetracyclines. Only a small fraction of doxycycline is found in active form in feces, the larger part being bound as chelate.

Doxycycline may be given in normal doses to patients who have renal insufficiency and to those undergoing hemodialysis as the reduced renal excretion is compensated for by intestinal excretion of bound substance.[28] For minocycline, caution is recommended because of a greater risk of side-effects.

No dosage adjustment of tigecycline is required in patients with renal impairment, or in patients undergoing hemodialysis.[27] Dosage adjustments are not required in patients with mild to moderate hepatic impairment, but patients with severe hepatic impairment should be treated cautiously and monitored for treatment response.

ROUTE OF ADMINISTRATION AND DOSAGE

Peak serum levels after an oral dose of 500 mg tetracycline or 200 mg of doxycycline or minocycline are usually 3–5 mg/l after 2 hours. Half-life in serum is longest for doxycycline (16–18 hours), followed by minocycline (11–13 hours) and tetracycline (8 hours). Doxycycline and minocycline can therefore be given orally once daily. For these older oral tetracyclines a higher starting dose on day 1 is recommended in order to achieve a steady-state level of the drug as soon as possible. Tigecycline is administered by intravenous infusion over 30–60 minutes. The initial dose is 100 mg followed by 50 mg every 12 hours. The peak serum level of tigecycline after a 30-minute infusion of 100 mg is approximately 1.5 mg/l. Dosages of several tetracyclines (including tigecycline) for adults are given in Table 141.3.

Doxycycline is also available for intravenous infusion but there is little difference in serum levels compared with oral administration. Tigecycline is only available for intravenous infusion.

DOSAGE IN SPECIAL CIRCUMSTANCES

In patients who have renal insufficiency or disease, doxycycline and tigecycline are the only tetracyclines that can be used safely without risk of accumulation and toxicity.

In patients who have hepatic disease, tetracyclines should generally be avoided. If treatment is important, liver tests should be performed repeatedly during treatment. However, toxicity has mostly been observed when older tetracyclines have been used at high doses or given during pregnancy.

Table 141.2 Pharmacokinetics of some tetracyclines in humans

	Tetracycline	Doxycycline	Minocycline	Tigecycline
Oral absorption in fasting state (%)	80	90–93	100	na
Serum half-life (h)	6–12	18–22	13	36
Serum protein binding (%)	24–65	80–90	55–75	68
Lipid solubility in comparison with tetracycline	1	5	10	nd
Excretion in urine (%) after: oral administration intravenous administration	 20 na	 35–40 nd	 4–9 nd	 na 15

na, not applicable; nd, not determined.

Table 141.3 Usual adult dosages for some tetracyclines*

General name	First dose	Common dosage
Oral preparations		
Tetracycline	500 mg	500 mg q6h
Oxytetracycline	500 mg	500 mg q6h
Doxycycline	200 mg	100 mg q24h
Minocycline	200 mg	100 mg q12h
Intravenous preparation		
Tigecycline	100 mg	50 mg q12h

*Doxycycline and minocycline may be given intravenously in the same doses. Higher doses of doxycycline are often used for sexually transmitted diseases and Lyme disease. Tigecycline is only available as an intravenous formulation.

During pregnancy tetracyclines should be avoided because of the depressive effect on the skeleton of the child. In lactating patients small amounts of older tetracyclines are excreted in breast milk but are harmless to the baby. Use of tigecycline in nursing women has not been adequately studied. It is not known whether tigecycline is excreted in human breast milk (but see section on 'Distribution' above).

In elderly patients kidney function is reduced according to age and the daily dose of a tetracycline should be reduced unless doxycycline or tigecycline is used.

In patients with severe hepatic impairment the initial dose of tigecycline should be 100 mg followed by a reduced dose of 25 mg q12 hours.[25]

INDICATIONS

Respiratory infections

Tetracyclines were first used for many types of respiratory tract infection. Because of increasing resistance in pneumococci and *H. influenzae*, they have been replaced in many areas by other antibiotics, mainly β-lactams.

Exacerbation of chronic bronchitis has been a classic indication for tetracyclines. Doxycycline has been found to be as effective as ampicillin. The advantage of one single dose per day may increase compliance in patients who have chronic respiratory diseases and many other medications (see Chapter 26).

Doxycycline is distributed to maxillary sinuses and can be used as a second-line drug in patients who have sinusitis and are allergic to β-lactams or in whom treatment with such drugs has failed.

Tetracyclines have good activity in pneumonias caused by *Mycoplasma pneumoniae*, *C. pneumoniae*, *C. psittaci* and *Coxiella burnetii*. Macrolides probably have an equal effect in infections caused by *M. pneumoniae* but clinical experience with the other infections is far greater with tetracyclines.

Tigecycline holds promise as a novel expanded-spectrum tetracycline, but is currently only approved for the treatment of complicated skin and soft tissue infections and complicated intra-abdominal infections.

Sexually transmitted diseases

Older tetracyclines are effective therapy for nongonococcal urethritis caused by *Chlamydia trachomatis* or *Ureaplasma urealyticum*. In an open evaluation of doxycycline in the treatment of urethritis and cervicitis caused by *C. trachomatis* the symptoms disappeared in 76% of cases.[28] Treatment should usually be given for 10 days and concurrent treatment of sexual partners is recommended (see Chapter 49).

First- and second-generation tetracyclines are usually effective for the treatment of lymphogranuloma venereum and granuloma inguinale. Nonpenicillinase-producing strains of gonococci are usually sensitive, which is an advantage in mixed infections with *C. trachomatis*.

Older tetracyclines can be used as alternative therapy for syphilis in penicillin-allergic patients. Treatment time is 15 days for early disease and 30 days for later stages of the disease.

Lyme disease and ehrlichiosis

Doxycycline can be used in penicillin-allergic patients to treat erythema migrans and is as effective as penicillin in a dose of 200 mg daily for 10 days. In erythema migrans with signs of dissemination such as multiple erythema and fever, doxycycline is often recommended as the primary drug. Treatment of neuroborreliosis with doxycycline 200 mg daily for 2 weeks has given similar results to those achieved with penicillin G (see Chapter 43).[29]

Doxycycline is also the preferred drug for infections due to *Ehrlichia* spp. (see Chapters 122 and 176).[30]

Other indications

First- and second-generation tetracyclines are very effective drugs for rickettsial infections (see Chapter 176). Doxycycline has been used for single-dose treatment of louse-borne typhus, but for other infections 7–10 days of treatment is usually needed. These infections can only be treated with tetracyclines or chloramphenicol. In children tetracyclines can often be given with minimal risk of staining the teeth if repeated treatments are avoided and the dose is kept as low as possible.

Doxycycline is also effective in a single dose for infections with *Borrelia recurrentis*. Tetracyclines are usually used in combination with other antibiotics such as streptomycin or rifampin (rifampicin) for brucellosis and tularemia.

For cholera in adults, tetracycline 500 mg q6h for 5 days or a single dose of 300 mg doxycycline have been recommended.[31]

Most of the older tetracyclines have been used for oral treatment of chronic acne. The drugs have an antibacterial effect on *P. acnes* as well as a general anti-inflammatory effect, which is probably of importance.[32] However, resistance of *P. acnes* to tetracyclines, including doxycycline, has been reported from England and the USA. Cross-resistance does not include minocycline, which may be used clinically.[33]

Doxycycline may also be used for malaria prophylaxis in areas where *Plasmodium falciparum* is resistant to other antimalarial drugs.[34]

ADVERSE REACTIONS AND INTERACTIONS

Adverse drug reactions

All oral preparations of tetracyclines can cause nausea and epigastric discomfort. It is usually an advantage if the drugs can be taken with food without decreasing absorption, as with doxycycline and minocycline. Diarrhea is less common but may occur, especially when tetracyclines with low absorption are used. Tigecycline is structurally related to other tetracyclines and therefore may share similar adverse effects to older tetracyclines.

Phototoxic reactions can occur with all tetracyclines.

Tetracyclines should not be used in children younger than 8 years because of the risk of enamel hypoplasia and tooth discoloration. They should also not be used during pregnancy. Tetracyclines, except doxycycline and tigecycline, are contraindicated in patients who have renal impairment.

Vertigo and dizziness are CNS symptoms that occur with minocycline.

Nausea, vomiting and headache are the most frequently reported adverse events associated with tigecycline therapy.

Hepatotoxicity and other severe organ reactions have occurred with older tetracyclines, mainly after parenteral therapy – often with high doses – and also when the drugs have had to be used during pregnancy.

Tigecycline is not recommended in patients under 18 years of age.

Drug–drug interactions

Tetracyclines form chelate complexes with many drugs containing metal ions (Table 141.4). When combined with diuretics the risk of accumulation of urea increases. Some drugs seem to stimulate liver enzymes, so increasing doxycycline metabolism and shortening its half-life. Tigecycline may decrease elimination of the anticoagulant warfarin, thereby increasing warfarin levels in the blood. The potential increased risk of bleeding should therefore be monitored.

In-vitro studies with human liver microsomes indicate that tigecycline does not inhibit metabolism mediated by the cytochrome P450 isoforms 1A2, 2C8, 2C9, 2C19, 2D6 or 3A4. Therefore tigecycline is not expected to alter the metabolism of drugs processed by these enzymes.[27]

Since tigecycline is not extensively metabolized, clearance of the antibiotic is not expected to be affected by drugs that induce or inhibit the activity of cytochrome P450 isoforms.[27]

Chloramphenicol

INTRODUCTION

Chloramphenicol was first isolated in 1947 from a sample of soil from Venezuela and the actinomycete was called *Streptomyces venezuelae*.

MODE OF ACTION AND SPECTRUM

Chloramphenicol inhibits protein synthesis by binding to the 50S subunit of the bacterial 70S ribosome. It is mainly a bacteriostatic agent. However, bactericidal activity on some bacteria, such as *H. influenzae*, *Streptococcus pneumoniae* and *Neisseria meningitidis*, has been reported.

Chloramphenicol has a very broad spectrum, similar to that of tetracyclines, and includes aerobic and anaerobic bacteria, spirochetes, *Rickettsia*, *Chlamydia* and *Mycoplasma* spp. It is very active against most anaerobic bacteria of clinical interest, including *Bacteroides fragilis*.

Chloramphenicol resistance can occur and is mediated by a bacterial enzyme, chloramphenicol acetyltransferase (CAT), which inactivates the drug. The gene that codes for CAT is carried on transmissible plasmids, and epidemics of chloramphenicol-resistant typhoid fever and *Shigella* infections have occurred.

Unrestricted use of chloramphenicol seems to have resulted in a resistance problem very similar to that observed with tetracyclines.

Table 141.4 Drug interactions with tetracyclines*

Interacting drug	Effect
Antacids with metal ions, calcium and iron supplements, magnesium salicylates, magnesium-containing laxatives, multivitamins	Chelate formation and impaired absorption
Diuretics	Risk of increased serum urea concentration – not with doxycycline
Rifampin (rifampicin), phenobarbital, phenytoin, carbamazepine	Half-life of doxycycline shortened

*There is a paucity of drug interaction data for tigecycline. However, at least some of the drug interactions that occur with earlier tetracyclines are also likely to occur with tigecycline.

ROUTE OF ADMINISTRATION AND DOSAGE

Chloramphenicol is conjugated with glucuronic acid and is then excreted in active form by the kidneys. The metabolites are not toxic and dose reduction is not needed in renal insufficiency.

Chloramphenicol is a small lipophilic molecule and is well distributed in the body. The serum protein binding is about 44%. It reaches the CNS better than most other antibiotics and its concentration in the cerebrospinal fluid is often 30–50% of the serum concentration. Chloramphenicol also crosses the placenta and is found in breast milk.

Chloramphenicol is well absorbed (over 90%) after oral administration. Chloramphenicol 1 g gives a serum concentration of 10 mg/l and the half-life is 3–4 hours. It may also be given intravenously as a succinate ester but intramuscular injections should be avoided as absorption is unreliable.

INDICATIONS

Chloramphenicol is toxic and should therefore be used carefully in systemic infections, when other alternatives are lacking. It can be used instead of tetracyclines for the treatment of rickettsial infections and for bacterial meningitis in the few patients who have an allergy to β-lactam drugs, including third-generation cephalosporins and meropenem. It may also have a place as an oral alternative for CNS infections, especially brain abscesses.

Topical administration of chloramphenicol in drops or ointments is widely used for superficial bacterial infections of the eyes. Such treatment is still effective in comparison with the newer drugs such as quinolones or fusidic acid, which are also used locally for eye infections.[35,36]

ADVERSE REACTIONS AND INTERACTIONS

Adverse drug reactions

Neonates have a diminished ability to conjugate chloramphenicol and to excrete the active form in the urine. A dose of 25 mg/kg/day should not be exceeded[37] otherwise the 'gray baby syndrome' may develop, with severe cyanosis and circulatory collapse. Cranial and peripheral neuropathies and optic atrophy have also been reported rarely with chloramphenicol.

Dose-related reversible bone marrow depression can occur in adults given high doses of more than 4 g/day. The daily dose should not exceed 3 g, and when the accumulated dose exceeds 25 g reticulocytes should be checked regularly (e.g. twice weekly) until treatment is stopped.

A very severe reaction is aplastic anemia, which occurs with a frequency of 1/25 000–40 000 treatment courses.[38] No clear correlation to dose or duration of treatment has been observed and no route of administration is exempt from causing this catastrophic complication. There are also reports to indicate that the use of chloramphenicol may increase the risk of leukemia in children.[39]

Drug–drug interactions

As chloramphenicol is almost completely metabolized in the liver by cytochrome P450 enzymes, there is a possible risk of interactions with other drugs if they are metabolized by the same enzyme system. Chloramphenicol will decrease the rate of metabolism of tolbutamide, phenytoin, cyclophosphamide and warfarin. Rifampin may lower chloramphenicol concentrations by induced metabolism.

REFERENCES



References for this chapter can be found online at http://www.expertconsult.com

Chapter 142

Peter J Jenks

Nitroimidazoles: metronidazole, ornidazole and tinidazole

INTRODUCTION AND MODE OF ACTION

The nitroimidazoles, which include metronidazole, tinidazole and ornidazole, were initially introduced for the treatment of trichomonal vaginitis. They were subsequently recognized to be active against other protozoa as well as facultative anaerobes (*Helicobacter pylori* and *Gardnerella vaginalis*) and anaerobic bacteria, and are used on a worldwide basis for the treatment of infections caused by these organisms.

Once they have entered the cell by diffusion, the antimicrobial toxicity of the nitroimidazoles is dependent on reduction of the nitro moiety to the nitro anion radical and other highly active compounds, including nitroso and hydroxylamine derivatives.[1,2] These reduction products are damaging to macromolecules, and have been shown to cause DNA degradation and strand breakage.[1] The nitroimidazoles are selectively toxic for micro-organisms in which the redox potential of components of the electron transport chain are sufficiently negative to reduce the nitro group of metronidazole. Aerobic bacteria are therefore intrinsically resistant to these antibiotics since they are unable to attain the low intracellular redox environment required for drug activation. In susceptible organisms, the ongoing reduction of metronidazole maintains a favorable transmembrane metronidazole concentration gradient, facilitating further diffusion into the cell. In general, micro-organisms develop resistance to the nitroimidazoles by reducing or abolishing activity of elements of the electron transport reactions, with appropriate compensatory modifications of the normal fermentative pathway.[2–5] Resistance to metronidazole in *Bacteroides* spp. has been shown to be associated with specific nitroimidazole (*nim*) resistance genes that encode a 5-nitroimidazole reductase, which prevents the formation of the free radicals responsible for bactericidal activity.[6]

ROUTE OF ADMINISTRATION AND DOSAGE

The two most frequently used nitroimidazoles, metronidazole and tinidazole, have parenteral and oral formulations, and are also available as suppositories, as a gel for periodontitis and for vaginal administration. Dosing regimens of metronidazole are given in Table 142.1. The doses of tinidazole and ornidazole are similar but, because their half-lives are longer, these drugs can be administered at longer intervals. Recommendations in the USA for certain infections are for shorter dose intervals, although it is recognized that the half-life of the nitroimidazoles means that dose intervals shorter than 8 hours should not be needed and in most cases 12-hour regimens should be adequate.

PHARMACOKINETICS

Following oral administration, all nitroimidazoles are almost completely absorbed.[7] After rectal administration of metronidazole the bioavailability is approximately 60%, with considerable variability between individuals. When given vaginally the bioavailability of metronidazole is 20% or less. Following a 400 mg oral dose of metronidazole or tinidazole, peak plasma concentrations of about 10 mg/l are achieved after 3–5 hours. Dose proportional kinetics have been seen for doses up to 2 g. The concentrations after normal oral doses are well above the minimum inhibitory concentrations (MICs) for anaerobes but are borderline for *G. vaginalis*. The nitroimidazoles are well distributed to peripheral compartments, including brain tissue and cerebrospinal fluid,[7] but concentrations in subcutaneous fat are low (15% or less of concurrent serum levels).[8] Nitroimidazoles are metabolized mainly by the liver and excreted in the urine. The plasma half-life is about 8 hours for metronidazole and 12–13 hours for tinidazole. Metronidazole is partly metabolized to hydroxymetronidazole, which has a half-life of 10–13 hours.

Metronidazole elimination is prolonged in premature newborns and in patients with impaired liver function, and dose reduction is necessary in these groups. Although dose adjustment is not normally required in patients with renal impairment, the hydroxymetronidazole metabolite may accumulate in patients with end-stage disease and dose reduction may be necessary. Hemodialysis increases the clearance of metronidazole, shortening the half-life to 2–3 hours.

INDICATIONS

Bacterial infections

Nitroimidazoles are the most active antibiotics for the treatment and prevention of infections caused by anaerobic bacteria (Table 142.2) and resistance in these organisms is rare.[11] Nitroimidazoles are therefore important in the treatment of intra-abdominal and gynecologic sepsis, abscesses and tetanus. They are also an important component of prophylactic regimens for surgical procedures where contamination with anaerobic flora is likely. Nitroimidazoles are used to treat bacterial vaginosis (frequently associated with *G. vaginalis*) and dental infections, including acute necrotizing ulcerative gingivitis (Vincent's angina). Metronidazole continues to be first-line treatment for mild/moderate antibiotic-associated diarrhea caused by *Clostridium difficile* but is inferior to vancomycin in patients with severe, recurrent, complicated or fulminant disease (see also Chapter 35).[12] Nitroimidazoles are also a component of modern triple eradication regimens for *H. pylori*, although resistance may affect 10–50% of strains isolated in developed countries and virtually all strains from developing countries.[13]

Table 142.1 Dosages of metronidazole

Type of infection	Adult dose	Child dose*	Duration of treatment	Notes
Trichomoniasis	2 g	Not applicable	Single dose	Or 400 mg q12h for 5–7 days (250–500 mg q8h)†
Giardiasis	400 mg q8h (250 mg q12h or q8h)	15 mg/kg q12h	5–7 days	Or 2 g q24h for 3 days
Amebic dysentery	800 mg q8h (500–750 mg q8h)	20 mg/kg q12h	5–10 days	
Amebic abscess	800 mg q8h (500–750 mg q8h)	20 mg/kg q12h	10 days	
Bacterial vaginosis	400 mg q12h (500 mg q12h)	Not applicable	5–7 days	Or 2 g as a single dose
Helicobacter pylori	400 mg q8h (250 mg q8h)	Not applicable	7–14 days	
Clostridium difficile	800 mg then 400 mg q8h (250 mg q6h or 500 mg q8h)	7.5 mg/kg q12h	10–14 days	
Anaerobic infection (treatment)	800 mg then 400 mg q8h (500 mg q12h–q6h)	7.5 mg/kg q8h	7–10 days	
Anaerobic infection (prophylaxis)	400 mg (15 mg/kg then 7.5 mg/kg 6 and 12 h after initial dose)	7.5 mg/kg	Single dose	Repeat every 3 h for prolonged procedures

*Child dose is for children aged 8 weeks or more.
†US dosages in parentheses.

Table 142.2 Activity of metronidazole against anaerobic bacteria

Organism	Metronidazole MIC (mg/l) for 90% isolates	Percent sensitive (NCCLS)
Bacteroides fragilis	1–4	100
Prevotella spp.	4	100
Fusobacterium nucleatum	0.25–2	100
Fusobacterium spp.	0.25–4	100
Peptostreptococcus spp.	0.5–4	100
Propionibacterium acnes	>1.0	0
Clostridium difficile	0.5–4	100
Clostridium spp.	2–16	95

NCCLS, National Committee for Clinical Laboratory Standards.
Data from Wexler et al.[9] and Spangler et al.[10]

Protozoal infections

The nitroimidazoles are highly active against protozoa and provide the first-line treatment for giardiasis, amebiasis and trichomonal vaginitis. Although resistant strains of *Entamoeba histolytica* and *Trichomonas vaginalis* are rarely encountered, up to 20% of *Giardia lamblia* isolates may be resistant in general clinical practice.

Other

Topical metronidazole is used to reduce the odor produced by anaerobic bacteria in fungating cutaneous lesions and has also been used in the management of acne rosacea. Metronidazole may be beneficial for the treatment of diarrhea and perianal involvement in patients with active Crohn's disease.

ADVERSE REACTIONS AND INTERACTIONS

The most common side-effects of the nitroimidazoles are gastrointestinal (including nausea and diarrhea) and a metallic taste, especially when high doses are used. A reversible peripheral neuropathy may occur in patients receiving high doses for prolonged periods. Severe central nervous system effects have also been reported and the drug should be discontinued if any abnormal neurologic symptoms are reported. If combined with alcohol, metronidazole may cause a disulfiram-like reaction, with nausea, vomiting, flushing of the skin, tachycardia, hypotension and palpitations.

Although nitroimidazoles have been found to be mutagenic and carcinogenic in animal studies, there is a lack of evidence that they are carcinogenic to humans.[14] Although theoretical concerns of teratogenicity have not been confirmed,[15,16] it seems prudent that metronidazole should only be used in pregnancy when the benefits outweigh the risks, and should be avoided altogether in the first trimester. Because the concentrations of metronidazole in breast milk are similar to those in serum, a risk assessment should be performed before using this drug in lactating mothers.

REFERENCES


References for this chapter can be found online at http://www.expertconsult.com

Chapter 143

John M Grange
Alimuddin Zumla

Antituberculosis agents

INTRODUCTION

The discovery of the tubercle bacillus by Robert Koch in 1882 raised hopes that an effective remedy for tuberculosis would soon be found and many workers attempted to develop immunotherapeutic agents. The best known of these attempts was that of Koch himself who advocated injections of old tuberculin. Old tuberculin was later shown to be useless as a therapeutic, but priceless as a diagnostic test. The British bacteriologist Sir Almroth Wright also conducted extensive studies, which are immortalized in George Bernard Shaw's play *The Doctor's Dilemma*.

It was, however, the discovery of streptomycin in 1944 by Albert Schatz and Selman Waksman in the USA that opened the door to effective therapy and led to the belief that the disease would soon be conquered. Early jubilation turned to disappointment when it was found that patients treated with streptomycin often made an initial improvement but soon relapsed because their tubercle bacilli became resistant to this agent. Fortunately, other active antituberculosis agents were soon discovered and, as a result of extensive trials initiated by Sir John Crofton in the UK, multidrug regimens that cured patients and prevented the emergence of drug resistance were developed.[1]

Therapy of tuberculosis with these early drug regimens, usually consisting of streptomycin, isoniazid and *para*-aminosalicylic acid, was beset with problems. Streptomycin had to be given by injection and *para*-aminosalicylic acid caused such severe gastrointestinal effects that patients often failed to comply with therapy. In addition, it was necessary to treat patients for 18–24 months in order to achieve a cure.

The second therapeutic revolution came in the early 1970s when regimens containing rifampin (rifampicin) were developed. The introduction of this drug had three major effects on the treatment of tuberculosis. First, the duration of therapy could be reduced to only 6 months so that the era of 'short course' therapy had arrived. Secondly, regimens could be entirely by the oral route and thirdly, as a consequence, hospitalization was often unnecessary.

Modern short-course therapy, properly used, can achieve a cure in around 98% of patients and is among the most effective and cost-effective of all therapeutic interventions for a chronic disease.[2] Far from being conquered, however, tuberculosis remains one of the most prevalent causes of mortality and morbidity worldwide. It is responsible for one in seven deaths among young adults and it was declared a global emergency by the World Health Organization (WHO) in 1993. The problem is currently exacerbated by the HIV pandemic, the increasing prevalence of multidrug-resistant tuberculosis (MDRTB), defined as resistance to at least rifampin and isoniazid among the first-line drugs, and the more recent emergence of extremely drug-resistant tuberculosis (XDRTB), as defined below. There is therefore an urgent need to develop new therapies and to use the available therapies in a much more responsible manner.

CLASSIFICATION

Antituberculous agents can be classified in several ways (Table 143.1). They can be divided into:

- those that are synthetic molecules and those that are antibiotics or semisynthetic antibiotic derivatives;
- agents with a broad spectrum of activity and those only active against mycobacteria or, specifically, members of the *Mycobacterium tuberculosis* complex;
- first-line drugs that form the basis of the modern short-course regimens advocated by the WHO and second-line drugs that are used in cases of drug resistance and when toxic reactions prevent the use of one or more first-line drugs; and
- bacteriostatic and bactericidal agents, with a clinically important distinction of the latter between those that are bactericidal *in vitro* and those that are able to sterilize lesions of tubercle bacilli *in vivo* (Table 143.2).

MODE OF ACTION AND PHARMACOKINETICS

The sequencing of the genome of *M. tuberculosis*[3] has led to considerable advances in our understanding of the genetic basis of action of the drugs used for treating mycobacterial disease as well as the delineation of metabolic pathways that could serve as targets for novel drugs.

The targets of streptomycin and other aminoglycosides, rifamycins and fluoroquinolones are the same in mycobacteria as in other bacterial genera, and resistance is due to single amino acid substitutions in the target proteins. The targets of many important antituberculosis agents, notably isoniazid, pyrazinamide, ethambutol, ethionamide and prothionamide, are components of the complex and lipid-rich mycobacterial cell wall. The mode of action and target genes are discussed under the individual drug headings below and are summarized in Table 143.3; the targets are shown in Figure 143.1. Most of the antituberculosis agents are readily absorbed from the gastrointestinal tract. Exceptions are streptomycin and other aminoglycosides, capreomycin and viomycin, which must therefore be given parenterally. Binding to serum proteins varies from agent to agent, as does entry into the cerebrospinal fluid (CSF). Agents that enter into the CSF poorly in health often pass the inflamed meninges so that therapeutically useful levels are achieved in cases of tuberculous meningitis. The pharmacokinetics of the conventional antimycobacterial drugs, their principal metabolites and routes of excretion are summarized in Tables 143.4 and 143.5.

Table 143.1 Spectrum of activity, class of compound and cross-resistances of the antituberculosis agents*

Agent	Class of compound	Spectrum of activity	Cross-resistance to other antituberculosis agents
First-line agents			
Rifampin (rifampicin)	Antibiotic	Broad	Other rifamycins
Isoniazid	Synthetic	Tubercle bacilli	None
Pyrazinamide	Synthetic	Tubercle bacilli	None
Ethambutol	Synthetic	Tubercle bacilli	None
Streptomycin	Antibiotic	Broad	Other aminoglycosides, viomycin, capreomycin
Second-line agents			
Thiacetazone	Synthetic	Tubercle bacilli	Ethionamide and prothionamide
Para-aminosalicylic acid	Synthetic	Tubercle bacilli	None
Ethionamide and prothionamide	Synthetic	Tubercle bacilli	Thiacetazone
Capreomycin	Antibiotic	Tubercle bacilli	Aminoglycosides, viomycin
Viomycin	Antibiotic	Tubercle bacilli	Aminoglycosides, capreomycin
Cycloserine	Synthetic	Broad	None
Ofloxacin	Antibiotic	Broad	None

*Agents that are active against tubercle bacilli (*Mycobacterium tuberculosis* complex) may also show activity against some other species of mycobacteria. Strains of *Mycobacterium bovis* are naturally resistant to pyrazinamide. There are only limited data on other activities of capreomycin and viomycin.

Table 143.2 Efficacy of antituberculosis agents*

Agent	Early bactericidal activity	Sterilizing activity	Prevention of emergence of drug resistance
Rifampin (rifampicin)	Fair	Good	Good
Pyrazinamide	Poor	Good	Poor
Isoniazid	Good	Fair	Good
Ethambutol	Fair	Poor	Fair
Streptomycin	Poor	Poor	Fair
Thiacetazone	Poor	Poor	Poor

*In sterilizing lesions, reducing viable bacterial population rapidly and preventing the emergence of drug resistance.

Table 143.3 Antituberculosis agents: targets and genes for resistance

Agent	Target	Gene(s) encoding target(s) or those in which mutations conferring resistance occur
Isoniazid	Mycolic acid synthesis	*inhA, katG, KasA, oxyR-ahpC*
Rifampin (rifampicin)	DNA-dependent RNA polymerase	*rpoB*
Pyrazinamide	Fatty acid synthetase-1	*pncA*
Ethambutol	Arabinosyl transferase, involved in cell wall arabinogalactan synthesis	*embA, embB and embC*
Streptomycin	30S ribosomal subunit	*rspL* (encodes for ribosomal protein S12)
Other aminoglycosides	30S ribosomal subunit	Genes encoding 16S-rRNA (and possibly *aac(2')* encoding aminoglycoside acetyltransferase)

Table 143.3 Antituberculosis agents: targets and genes for resistance—cont'd

Agent	Target	Gene(s) encoding target(s) or those in which mutations conferring resistance occur
Thiacetazone	Mycolic acid synthesis	Unknown
Para-aminosalicylic acid	Mycobactin synthesis (?)	Unknown
Ethionamide and prothionamide	Mycolic acid synthesis	*inhA*
Fluoroquinolones	Inhibition of bacterial DNA gyrase topoisomerase IV	*gyrA* and *gyrB* (gyrase) *parC* and *parD* (topoisomerase IV)
Capreomycin and viomycin	50S or 30S ribosomal subunit	*vicA* (50S) or *vicB* (30S)
Clofazimine	Unknown; possibly RNA polymerase	–
Cycloserine	Peptidoglycan	*alrA*

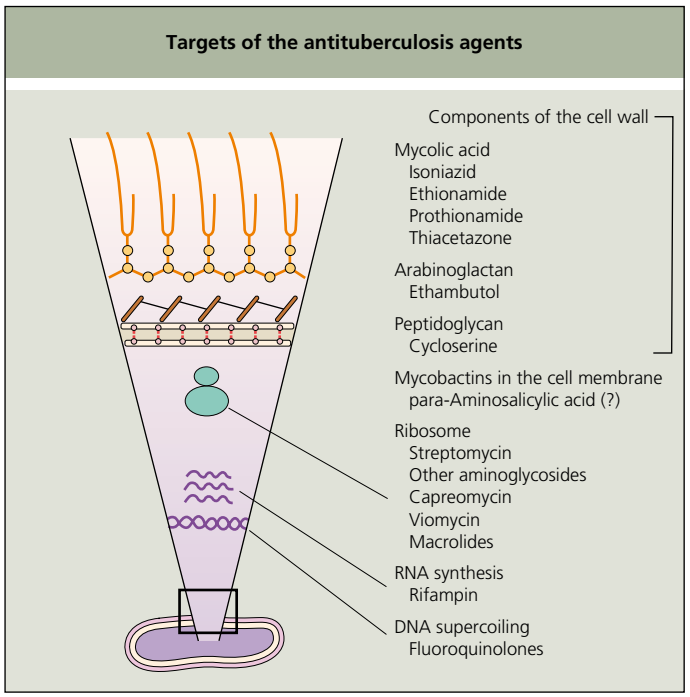


Fig. 143.1 Targets of the antituberculosis agents.

DRUG TOXICITY

Although all antituberculosis drugs have some untoward side-effects, drug toxicity is, in general, not a serious problem in modern short-course chemotherapy based on the WHO-recommended first-line agents and is a small price to pay for the very real curative benefits. The major side-effects are hepatotoxicity, peripheral neuropathy, mental disturbances, skin reactions (Figs 143.2–143.4) and fevers. Side-effects are particularly likely to occur in HIV-positive patients and, of these, skin reactions due to thiacetazone are particularly serious and may be fatal.

The principal drugs used in modern short-course regimens – isoniazid, rifampin, pyrazinamide and ethambutol – are all potentially hepatotoxic, but this is seldom a problem in clinical practice. Some physicians, however, advocate regular liver function tests during therapy.[4] The adverse effects of the various drugs are discussed under the individual headings below and are summarized in Table 143.6.

FIRST-LINE DRUGS

Isoniazid (isonicotinic acid hydrazide)

This is included in all modern regimens and is also used as preventive monotherapy for infected (tuberculin-positive, without clinical disease; also known as latent tuberculosis) persons, particularly in the USA.

It has a powerful bactericidal action against actively replicating tubercle bacilli and thus rapidly reduces infectiousness by reducing the number of viable bacilli in cavities. It has little or no activity against slowly replicating bacilli but is included in the continuation phase of modern short-course therapy to kill any rifampin-resistant mutants that commence replication. It inhibits the synthesis of mycolic acids – long-chain fatty acids that form an important part of the mycobacterial cell wall. Although mycolic acids are common to all mycobacteria, and similar molecules occur in the genera *Nocardia* and *Corynebacterium*, susceptibility to isoniazid is essentially restricted to the *M. tuberculosis* complex and some strains of *M. xenopi* and *M. kansasii*. Isoniazid is a prodrug requiring oxidative activation by the mycobacterial catalase-peroxidase enzyme KatG and the commonest causes of isoniazid resistance are point mutations in, or deletion of, the *katG* gene that encodes this enzyme. Less frequent resistance-determining mutations are in the *inhA* locus (or in its promoter region) which codes for an NADH-dependent enoyl-acyl carrier protein reductase involved in mycolic acid synthesis and the *oxyR–ahpC* locus which, like the *katG* locus, is involved in protection against oxidative stress.[5] The predominant mutations conferring resistance to isoniazid differ between the various lineages of *M. tuberculosis* and therefore show geographic variations in their distribution.[6]

Isoniazid is readily absorbed from the gastrointestinal tract and is converted to inactive metabolites, principally by acetylation, the rate of which is genetically determined. Thus, patients can be divided into rapid acetylators and slow acetylators, in whom the elimination half-lives of the drug are 0.5–1.5 hours and 2–4 hours, respectively. About half of Caucasian and black patients but over 80% of Chinese and Japanese patients are rapid acetylators. If administered regularly, response to therapy is unaffected by acetylator status.

Owing to its widespread use since the 1950s, resistance to isoniazid is common and many strains that are resistant to other antituberculosis drugs, particularly to rifampin, are also resistant to isoniazid.

Adverse events are usually mild and are more likely to occur in slow acetylators. They include several neurologic effects, including insomnia, restlessness, peripheral neuropathy, optic neuritis and mild psychiatric disturbances. More serious, but less common, neurologic effects include severe psychiatric disturbance and encephalopathy. The latter is particularly likely to occur in renal dialysis patients.[7]

Table 143.4 Pharmacokinetics of the antituberculosis agents

Agent	Binding to serum proteins	Absorption from gastrointestinal tract (time to reach peak serum level)	Entry into CSF (with healthy meninges)
Isoniazid	Very low	Very rapid (30–60 minutes)	Good
Rifampin (rifampicin)	High (up to 95%)	Rapid (2 hours)	Poor
Pyrazinamide	Very low	Rapid (1–2 hours)	Good
Ethambutol	Binds to erythrocytes	Rapid (2 hours); 80% of dose absorbed	Poor
Streptomycin	Moderate (30–35%)	Not absorbed	Poor
Thiacetazone	Not bound	Rapid (2 hours)	Limited data
Para-aminosalicylic acid	High (60–65%)	Very rapid	Poor
Ethionamide and prothionamide	Limited data	Very rapid (30 minutes)	Good
Capreomycin	Limited data	Not absorbed	Poor
Viomycin	Limited data	Not absorbed	Poor
Clofazimine	Limited data	Slow (8–12 hours)	Limited data
Cycloserine	Not bound	Rapid (3 hours)	Good
Ofloxacin	Low	Rapid (1–1.5 hours)	Moderate

Table 143.5 Principal metabolic products and excretion of the antituberculosis agents

Agent	Principal metabolic products	Excretion
Isoniazid	Acetyl derivatives: rate of acetylation is genetically controlled	Unchanged and as acetyl derivatives in urine (ratio depends on rate of acetylation)
Rifampin (rifampicin)	Desacetyl derivative	As desacetylrifampin in bile
Pyrazinamide	Pyrazinoic acid	Mostly as pyrazinoic acid in urine
Ethambutol	Oxidation products and aldehydes	Mostly unchanged in urine; about 15% as metabolites
Streptomycin	None	Unchanged in urine
Other aminoglycosides	None	Unchanged in urine
Thiacetazone	Unknown	20% eliminated in urine, fate of remainder unknown
Para-aminosalicylic acid	Acetylation products and glycine conjugates	About 80% in the urine, mostly in the acetylated form
Ethionamide and prothionamide	Sulfoxide (biologically active) and methyl derivatives	Less than 1% unchanged in urine
Capreomycin	None	Unchanged in urine
Viomycin	None	Unchanged in urine
Clofazimine	Very small amounts of unidentified metabolites	Unchanged in urine and feces
Cycloserine	Up to 35% converted to unidentified metabolites	Varying amounts unchanged in urine
Ofloxacin	5% metabolized to oxides and dimethyl derivatives	70–95% unchanged in urine, small amounts in bile

Other adverse effects include hepatitis, particularly in patients aged over 35 years, arthralgia, fever and skin rashes. Very rare complications include hyperglycemia and agranulocytosis.

Adverse effects, particularly neurologic ones, are usually preventable by administration of pyridoxine (vitamin B6) 10 mg daily. In particular, pyridoxine should be given to patients who have liver disease, pregnant women, alcoholics, renal dialysis patients, HIV-positive patients, the malnourished and the elderly. Encephalopathy in renal dialysis patients may not respond to pyridoxine but usually resolves when isoniazid is withdrawn.[7]

Rifampin (rifampicin)

This is one of the rifamycins, semisynthetic derivatives of rifamycin S, a metabolite of *Amycolatopsis* (*Streptomyces*) *mediterranei*. Rifampin inhibits protein synthesis by a very specific inhibition of bacterial DNA-dependent RNA polymerase, thereby blocking the synthesis of mRNA. The corresponding mammalian enzyme is inhibited only by very high concentrations of rifampin. Resistance is due to single amino acid mutational changes in the *rpoB* gene, which encodes for the β subunit of the polymerase. Rifampin is a particularly effective drug because



Fig. 143.2 Severe dermal reaction to isoniazid. Courtesy of Dr P Mwaba, Zambia.



Fig. 143.3 Erythema multiforme reaction to rifampin. Courtesy of Dr P Mwaba, Zambia.

it kills both rapidly dividing bacilli and those that exhibit only occasional short bursts of metabolism. Ideally, therefore, it is given throughout the course of therapy. It is also used in the treatment of leprosy and for some other mycobacterial diseases.

Rifampin is rapidly absorbed from the gastrointestinal tract, although absorption is delayed if it is given with food, and it is widely distributed in the internal organs. Only small amounts enter the CSF in health but much more enters when the meninges are inflamed. Rifampin enters cells and is therefore active against intracellular mycobacteria. It is metabolized by hepatic microsomal enzymes to the desacetyl derivative, which is excreted in the bile. As this enzymatic activity is inducible, the rate of plasma clearance of rifampin increases as treatment proceeds. Although principally excreted in the bile, some rifampin and the desacetyl derivative enter the urine and impart an orange-red color to it. It also enters saliva and lachrymal secretions and may cause pink staining of soft contact lenses. The induction of microsomal enzymes may have clinically significant effects on the metabolism of several other drugs (see below).



Fig. 143.4 Stevens–Johnson syndrome induced by thiacetazone. Courtesy of Dr P Mwaba, Zambia.

Rifampin may cause an influenza-like syndrome, which, paradoxically, occurs less often if the drug is given daily rather than intermittently. It causes transient abnormalities in liver function and, occasionally, clinically evident cholestatic hepatitis, although this is usually mild. Other adverse effects include gastrointestinal disturbances, skin rashes and antibody-mediated thrombocytopenia.

Acute renal failure is a rare complication, although in some regions it is more frequent; in one center in India it accounted for 11 of 607 (1.8%) admissions for acute renal failure.[8] The renal prognosis is usually favorable. It typically occurs after re-introduction of rifampin and intermittent therapy is a risk factor.

Although the evidence that rifampin is teratogenic is very limited, it is best avoided if possible during the first 3 months of pregnancy. For the same reason, women receiving rifampin should avoid becoming pregnant. In this respect it is important to note that this drug interferes with the action of oral contraceptives, and women of childbearing age should be advised to use another form of birth control while receiving rifampin.

Pyrazinamide (pyrazinoic acid amide)

This is regularly included in the initial intensive phase of short-course chemotherapy because it has the important property of killing intracellular tubercle bacilli and, possibly, extracellular bacilli in anoxic, acidic inflamed lesions. It is inactive in neutral or alkaline microenvironments. The mode of action of pyrazinoic acid is poorly understood but the available evidence suggests that it disrupts bacterial membrane function. There is also evidence that it inhibits the mycobacterial fatty acid synthase (FAS)-1 enzyme.[9] Pyrazinamide requires conversion to pyrazinoic acid by mycobacterial pyrazinamidase enzymes encoded for by the 600 base-pair *pncA* gene. Resistance is usually associated with a wide range of point mutations in this gene, which are detectable by various techniques including microarrays.[10] Pyrazinamidase activity is not detectable in most pyrazinamide-resistant mutants of *M. tuberculosis* or in strains of *M. bovis*, which are naturally resistant to this agent. A few pyrazinamide-resistant strains, however, lack mutations in the *pncA* gene, suggesting alternative mechanisms for resistance to this agent, including defects in transportation of the agent into the bacterial cell.[11]

Pyrazinamide is readily absorbed from the gastrointestinal tract and freely enters the CSF, in which levels similar to those in plasma are found. It is metabolized in the liver; the metabolites, mostly pyrazinoic acid, are excreted in the urine.

Table 143.6 Adverse reactions to the antituberculosis agents

Agent	Adverse reactions
Isoniazid	
Uncommon reactions	Hepatitis, cutaneous hypersensitivity reactions including erythema multiforme, peripheral neuropathy
Rare reactions	Vertigo; convulsions; optic neuritis and atrophy; psychiatric disturbance; hemolytic anemia; aplastic anemia; dermal reactions including pellagra, purpura and lupoid syndrome; gynecomastia, hyperglycemia, arthralgia
Rifampin (rifampicin)	
Uncommon reactions	Hepatitis, flushing, itching with or without a rash, gastrointestinal upsets, 'flu-like syndrome', headache
Rare reactions (usually associated with intermittent therapy)	Dyspnea, hypotension with or without shock, Addisonian crisis, hemolytic anemia, acute renal failure, thrombocytopenia with or without purpura, transient leukopenia or eosinophilia, menstrual disturbances, muscular weakness, pseudomembranous colitis
Pyrazinamide	
Common reactions	Anorexia
Uncommon reactions	Hepatitis, nausea and vomiting, urticaria, nausea, arthralgia
Rare reactions	Sideroblastic anemia, photosensitization, gout, dysuria, aggravation of peptic ulcer
Ethambutol	
Uncommon reactions	Optic neuritis, arthralgia
Rare reactions	Hepatitis, cutaneous hypersensitivity including pruritus and urticaria, photosensitive lichenoid eruptions, paresthesia of the extremities, interstitial nephritis
Streptomycin	
Uncommon reactions	Vertigo, ataxia, deafness, tinnitus, cutaneous hypersensitivity
Rare reactions	Renal damage, aplastic anemia, agranulocytosis, peripheral neuropathy, optic neuritis with scotoma, severe bleeding due to antagonism of factor V, neuromuscular blockade in patients receiving muscle relaxants and in those with myasthenia gravis
Other aminoglycosides	
Uncommon reactions	Cutaneous hypersensitivity, vertigo, deafness
Rare reactions	Renal damage, hypoglycemia, hypokalemia
Thiacetazone	
Common reactions	Gastrointestinal upsets, cutaneous hypersensitivity, vertigo, conjunctivitis
Uncommon reactions	Hepatitis, erythema multiforme, exfoliative dermatitis, hemolytic anemia
Rare reactions	Agranulocytosis
***Para*-aminosalicylic acid**	
Common reactions	Gastrointestinal upsets
Uncommon reactions	Cutaneous hypersensitivity, hepatitis, hypokalemia
Rare reactions	Acute renal failure, hemolytic anemia, thrombocytopenia, hypothyroidism
Ethionamide and prothionamide	
Common reactions	Gastrointestinal upsets, salivation, metallic taste
Uncommon reactions	Cutaneous hypersensitivity, hepatitis
Rare reactions	Alopecia, convulsions, deafness, diplopia, gynecomastia, hypotension, impotence, psychiatric disturbance, menstrual irregularity, hypoglycemia, peripheral neuropathy
Capreomycin and viomycin	
Common reactions	Eosinophilia (with capreomycin), pain and induration at injection site
Uncommon reactions	Loss of hearing, vertigo, tinnitus, electrolyte disturbances including hypokalemia, leukopenia or leukocytosis
Rare reactions	Renal impairment, hepatitis, thrombocytopenia

Table 143.6 Adverse reactions to the antituberculosis agents—cont'd

Agent	Adverse reactions
Clofazimine	
Common reactions	Discoloration of skin and body fluids, nausea, vomiting, abdominal pain, diarrhea
Uncommon reactions	Dryness of skin, ichthyosis, photosensitivity
Rare reactions	Intestinal obstruction
Cycloserine	
Common reactions (especially with daily doses exceeding 500 mg)	Convulsions, drowsiness, sleep disturbance, headache, tremor, vertigo, confusion, irritability, aggression and other personality changes, psychosis (sometimes with suicidal tendencies)
Uncommon reactions	Cutaneous hypersensitivity, hepatitis, megaloblastic anemia
Rare reactions	Congestive heart failure
Fluoroquinolones	
Uncommon reactions	Gastrointestinal upsets, headache, dizziness, insomnia, cutaneous hypersensitivity reactions
Rare reactions	Restlessness; convulsions; psychiatric disturbances including psychotic reactions and hallucinations; edema of face, tongue and epiglottis; disturbance of taste and smell; anaphylactoid reactions

Adverse effects are uncommon. It causes raised serum transaminase levels but overt hepatotoxicity, contrary to earlier reports, is uncommon. It should be used with caution in alcoholics and in patients who have pre-existing hepatic disease, who should have regular liver function tests. Other adverse effects include anorexia, nausea, photosensitization of the skin,[12] arthralgia and gout caused by the inhibition of the excretion of uric acid by pyrazinoic acid.

Ethambutol

Ethambutol (*S,S'*-2,2'-(ethylenediimino)di-1-butanol) is included in the initial 2-month intensive phase of the WHO-recommended short-course antituberculosis regimens and also in some regimens for disease caused by other slowly growing mycobacteria, particularly members of the *M. avium* complex (MAC), *M. kansasii, M. xenopi* and *M. malmoense*. By inhibiting the enzyme arabinosyl transferase, ethambutol blocks the synthesis of the polysaccharide arabinogalactan, a macromolecule essential for the structural integrity of the mycobacterial cell wall. Resistance is associated with mutations in the *embA*, *embB* and *embC* cluster of genes (principally *embB*), which code for this enzyme.[13]

There is conflicting evidence on its effect on resistance to other antituberculosis agents. Thus there is evidence that it may enhance the activity of some of the other drugs by affecting cell-wall permeability, particularly in the MAC but possibly also in multidrug-resistant strains of *M. tuberculosis*.[14] There is also evidence that, in addition to determining resistance to ethambutol, a mutation in codon 306 of the *embB* gene predisposes *M. tuberculosis* to the development of resistance to a range of antituberculosis agents and transmission of such mutants has resulted in clusters of cases of drug-resistant tuberculosis.[15]

About 80% of the dose of ethambutol is absorbed from the gastrointestinal tract. Absorption is inhibited by antacids containing aluminum hydroxide. It does not cross the healthy meninges but up to 40% of the plasma level is found in the CSF in cases of tuberculous meningitis. It is mostly excreted unchanged in the urine but up to 15% is excreted as metabolites.

The principal side-effect is optic neuritis which may have an irreversible effect on vision, but this is rare if the drug is given for no longer than 2 months at a daily dose of 25 mg/kg, or for longer at a dose not exceeding 15 mg/kg. Nevertheless, care should be observed in the use of this drug, its recommended dose and duration of therapy should never be exceeded, and the patient should be informed of the risk of visual impairment and advised to discontinue the drug if such impairment occurs. Loss of color discrimination is the first sign of visual toxicity. Where facilities are available, visual acuity should be assessed before therapy and at intervals during it.

Most guidelines recommend that the drug should not be given to children under the age of 5 years because their visual acuity cannot be readily assessed, even though ocular complications in such young children are extremely rare.

Other side-effects of ethambutol include skin rashes, arthralgia, peripheral neuritis, hyperuricemia and, rarely, jaundice and thrombocytopenia.

Streptomycin

This was the first of the antituberculosis drugs to be discovered and it still has an important role in the treatment of tuberculosis. It inhibits protein synthesis by binding to the 30S subunit of the bacterial ribosome. It is active in neutral or alkaline environments such as the cavity wall but not in the more acidic environment of the closed, inflammatory foci and is therefore not a good sterilizing drug. It is very poorly absorbed from the gastrointestinal tract and must be given parenterally.

Streptomycin is toxic for the eighth cranial nerve (vestibular > cochlear), including that of the fetus, and its use should therefore be avoided in pregnancy. Other adverse reactions include impairment of renal function (uncommon) and hypersensitivity reactions – usually mild skin rashes or fever but occasionally anaphylactic reactions or exfoliative dermatitis.

SECOND-LINE DRUGS

There are, in addition to some agents used on anecdotal evidence of efficacy, six classes of second-line antituberculosis agents; namely, aminoglycosides, polypeptides, thioamides, fluoroquinolones, cycloserine and *para*-aminosalicylic acid.

Aminoglycosides

In addition to streptomycin, the aminoglycosides kanamycin, amikacin and aminosidine (paromomycin) have activity against *M. tuberculosis*.

Cross-resistance with streptomycin is usual. Kanamycin or amikacin is included in some regimens for the treatment of MDRTB and amikacin in some regimens for the treatment of disease due to the MAC, particularly in HIV-positive patients. In common with streptomycin, these aminoglycosides are not absorbed from the gastrointestinal tract and must therefore be given parenterally. Nephrotoxicity and ototoxicity are the major toxicities.

Polypeptides – capreomycin and viomycin

In common with the aminoglycosides, these structurally closely related, cyclic polypeptides inhibit protein synthesis by blocking ribosomal function and must be given by intramuscular injection. They are completely cross-resistant and highly resistant mutants are cross-resistant to the aminoglycosides. They do not readily enter cells or the CSF and are mostly excreted unchanged in the urine. Adverse effects include ototoxicity, nephrotoxicity and pain, bleeding and induration at the injection site. There is some evidence that capreomycin is active against dormant or nonreplicating cells of *M. tuberculosis*.[16] Viomycin is rarely used and is seldom available.

Thioamides – ethionamide and prothionamide

Ethionamide (ethylthioisonicotinamide) and prothionamide (propylthioisonicotinamide) are closely related drugs that are structurally similar to isoniazid; in common with isoniazid they inhibit the synthesis of mycobacterial mycolic acids by targeting the same molecule – enoyl-acyl carrier protein reductase encoded by the *inhA* gene.[17] Thus, in common with isoniazid, resistance is often associated with mutations in the *inhA* gene but, surprisingly, complete cross-resistance to isoniazid does not develop and partial cross-resistance is uncommon. Also in common with isoniazid, these agents are prodrugs that require activation by an enzyme encoded by the *ethA* locus but less than half the mutations conferring resistance to this agent occur in this locus.[18]

These two agents are degraded into several metabolites in the liver and less than 1% is excreted unchanged in the urine. The common occurrence of gastrointestinal irritation with these agents, even when they are given as enteric-coated tablets, limits their use in the treatment of tuberculosis. Prothionamide is slightly better tolerated and is used in some regimens for leprosy. Other adverse effects include skin reactions, hepatitis, impotence and gynecomastia in male patients, menstrual irregularities and various neurologic complications such as convulsions, mental disturbance and peripheral neuropathy.

Fluoroquinolones

These agents inhibit the enzyme DNA gyrase encoded by the *gyrA* and *gyrB* genes, and DNA topoisomerase IV encoded by the *parC* and *parD* genes involved in the regulation of DNA supercoiling. Several fluoroquinolones have early bactericidal activities approaching those of isoniazid, and although results of clinical trials have been rather variable,[19] there is evidence that the addition of ofloxacin to the standard regimen of isoniazid, rifampin and pyrazinamide enables the duration of therapy to be reduced by 1–2 months.[20]

Fluoroquinolones are readily absorbed from the gastrointestinal tract and enter tissues and fluids, including the CSF. Although metabolized to some extent by the liver, they are largely excreted unchanged in the urine. Doses therefore require modification in patients who have renal failure. Adverse effects include nausea and abdominal pain and various neurologic abnormalities including headache, vertigo, insomnia, restlessness, epileptiform attacks and psychiatric disturbances. They should be used with care in epileptic patients.

Cycloserine

This D-alanine analogue, a cyclic derivative of serine hydroxamic acid, inhibits synthesis of peptidoglycan. A related agent, terizidone, is a condensation product containing two cycloserine molecules. Cycloserine is bacteriostatic and thus of limited efficacy, although it is used in some cases of MDRTB. Psychiatric symptoms, including acute psychotic episodes, occur commonly and further limit the usefulness of this drug, although the risk may to some extent be reduced by giving pyridoxine. Allergic skin rashes are rare.

Para-aminosalicylic acid

The mode of action of this bacteriostatic drug is poorly understood although there is some evidence that it inhibits the salicylate-dependent synthesis of the mycobactins – a class of iron-chelating lipids unique to the mycobacteria. It is readily absorbed from the intestine and rapidly acetylated in the liver. About 80% is excreted in the urine, mostly in the acetylated form.

It is rarely used as adverse effects are common. Gastrointestinal effects, including nausea, abdominal pain and diarrhea, occur in up to 30% of patients. Other adverse effects include thyroid dysfunction, crystalluria, blood dyscrasias and, rarely, Löffler syndrome and encephalitis.

Other antituberculosis agents

In common with isoniazid, thiacetazone (acetylaminobenzaldehyde thiosemicarbazone) inhibits the synthesis of mycolic acid, but by a poorly understood mechanism. It is readily absorbed from the gastrointestinal tract. About 20% is eliminated in the urine but the fate of the remainder is unknown. Resistance develops readily and adverse effects, particularly skin reactions, are common. Less frequent adverse effects include gastrointestinal upsets, hepatitis, hemolytic anemia and, rarely, agranulocytosis. A very high incidence of severe, sometimes fatal, skin reactions – exfoliative dermatitis and Stevens–Johnson syndrome – occurs in HIV-positive tuberculosis patients. For this reason, and in view of its limited bacteriostatic activity, the WHO has advised that thiacetazone should no longer be used.

Rifabutin and rifapentine are closely related to rifampin, being semisynthetic derivatives of rifamycin S. Although rifabutin (ansamycin) is considerably more active than rifampin *in vitro*, its *in-vivo* action against *M. tuberculosis* is similar to that of rifampin. Cross-resistance between the rifamycins is usual, so the place for rifabutin in the treatment of MDRTB is limited except in patients receiving antiretroviral therapy (see Chapter 91).

Macrolides are broad-spectrum antibiotics that inhibit protein synthesis by binding to the ribosomal 50S subunit. The newer macrolides – clarithromycin, azithromycin and roxithromycin – are included in regimens used to treat disease due to MAC and other slowly growing mycobacteria including *M. kansasii* and *M. xenopi*. They have only partial *in-vitro* activity against *M. tuberculosis* and evidence for their clinical usefulness is limited. They are well absorbed from the gastrointestinal tract and are excreted in urine. Adverse effects include gastrointestinal upsets with occasional cases of pseudomembranous colitis and various psychiatric disorders, including rare episodes of acute mania.

Certain agents usually used for other infections may have useful activity against *M. tuberculosis*. For example, a combination of amoxicillin and clavulanic acid has *in-vitro* activity against *M. tuberculosis* and there is anecdotal evidence of a beneficial effect against MDRTB.[21]

EXPERIMENTAL ANTITUBERCULOSIS AGENTS

Very little research into new antituberculosis agents was undertaken for around three decades after the discovery of rifampin as it was considered that sales of novel agents would not cover the financial outlay required for their development. In October 2000, however, the Global

Table 143.7 Principal novel antituberculosis agents under investigation in the year 2007

Agent	Mode of action	Investigational status
Moxifloxacin	DNA gyrase and topoisomerase inhibition	Phase III clinical trials
Nitroimidazoles	Dual action: inhibition of protein synthesis and cell wall lipid synthesis	Phase I clinical trials
Pleuromutilins	Interaction with bacterial 50S ribosome subunit to block protein synthesis	*In-vitro* studies
InhA inhibitors	Direct inhibition of InhA, the target of isoniazid but avoiding metabolism by KatG and therefore not being cross-resistant with isoniazid	Identification of agents
Bacterial topoisomerase inhibitors	Similar to fluoroquinolones but binding outside the gyrase region responsible for resistance to fluoroquinolones	Lead identification
Electron transport inhibitors	Disrupt bacterial cell membrane energy functions	Lead identification
Peptide deformylase inhibitors	Inhibit protein maturation	Lead identification

Alliance for TB Drug Development was founded and includes various governmental and nongovernmental organizations, funding agencies and pharmaceutical companies.[22,23] This led to a resurgence of activity and several agents are now in various stages of evaluation. The stages of development of these agents are reported in the Global Alliance annual reports and updated on the alliance website.[23] The principal agents under investigation in 2007 were new quinolones, notably moxifloxacin, and quinolizinones, riminophenazines (related to clofazimine), rifamycins and ethambutol analogues. Compounds with novel modes of activity and those undergoing clinical trials are listed in Table 143.7.

Attempts to treat tuberculosis by enhancing or modifying immune defense mechanisms have been made with reported success.[24] In view of the increasing prevalence of MDRTB, and especially XDRTB, further extensive clinical studies are urgently required.

THE BASIS AND DESIGN OF THERAPEUTIC REGIMENS

The aims of modern chemotherapeutic regimens are:

- to cure the patient;
- to reduce infectivity as rapidly as possible; and
- to prevent the emergence of drug resistance.

In order to cure patients, it is necessary to destroy all the tubercle bacilli in the tissues; if even a few survive there is a high chance of relapse. In this respect, drugs that are bactericidal *in vitro* may not effectively sterilize the tissues *in vivo*.[25] This difference occurs because tubercle bacilli *in vivo* are in a number of different physiologic states or 'compartments':

- freely dividing extracellular bacilli, found mainly in the cavity walls;
- slowly dividing bacilli, found within macrophages and in acidic, inflammatory lesions; and
- dormant and near-dormant bacilli, within cells and in firm caseous material.

The antituberculosis drugs vary in their ability to destroy bacilli in these compartments and in preventing the emergence of resistance to a second drug.

During chemotherapy with modern short-course regimens, freely replicating bacilli in the walls of the cavities are rapidly killed; this is termed the early bactericidal effect. Subsequently, the slowly replicating and near-dormant bacilli are destroyed, but at a much slower rate.

Isoniazid plays a key role in achieving the early bactericidal effect because it is particularly effective in destroying the freely multiplying extracellular bacilli, particularly those in the walls of cavities. It has little or no effect on near-dormant bacilli and is therefore not a good sterilizing drug. Rifampin also contributes to the early bactericidal effect.

Ethambutol has bactericidal activity in the early stage of therapy but is not a sterilizing drug. Streptomycin is bactericidal in the slightly alkaline cavity walls but is likewise not a sterilizing agent because it is ineffective in the acidic environment within cells and caseous lesions. By contrast, pyrazinamide is effective within macrophages and acidic, anoxic inflammatory lesions, but not in the neutral or alkaline environment.

Thus, modern regimens commence with an intensive phase of therapy, usually lasting for 2 months, to optimize the early bactericidal effect, thereby eliminating most of the bacilli and rendering the patient noninfectious.[26] The principal drugs used are:

- isoniazid (active against bacilli in the cavity walls);
- pyrazinamide (active against bacilli in acidic closed lesions);
- rifampin (active against both); and
- ethambutol (active in the early stage of therapy and included as a fourth drug as drug resistance is increasingly prevalent).

The daily drug doses are listed in Table 143.8 and the intermittent doses in Table 143.9.

The intensive phase is followed by a continuation phase, usually lasting 4 months, in which any remaining dormant or near-dormant bacilli are destroyed. For this purpose, rifampin is the most powerful sterilizing drug. Although isoniazid is not a sterilizing drug, it is, by its potent activity against replicating bacilli, very good at preventing the emergence of rifampin-resistant mutants. It is thus given together with rifampin throughout the regimen.

Accordingly, the most effective and widely used modern short-course regimens are based on a 2-month phase of rifampin, isoniazid, pyrazinamide and ethambutol, followed by a 4-month phase of rifampin and isoniazid. The WHO has issued clear recommendations on drug regimens for the four categories of tuberculosis seen in clinical practice (Table 143.10).[26]

In most regimens drugs are given daily but, provided that therapy is closely supervised so that all doses are taken, they may be given three times weekly during the continuation phase or, in some regimens, throughout. Intermittent therapy renders the direct administration of the drugs less of a burden for both patients and supervisors.

There is general agreement, supported by clinical trials, that the modern chemotherapeutic regimens discussed above are suitable for the treatment of all types of tuberculosis. Some physicians, however, continue therapy for up to 12 months as a precautionary measure for some extrapulmonary forms of tuberculosis; particularly tuberculous meningitis in which a relapse would be particularly devastating.

Table 143.8 Daily doses of the antituberculosis agents

Agent	DAILY DOSE	
	Adults	Children
Rifampin (rifampicin)	450 mg if body weight <50 kg 600 mg if body weight ≥50 kg	10 mg/kg to maximum of 600 mg
Isoniazid	200–300 mg	5 mg/kg
Pyrazinamide	1.5 g if body weight <50 kg 2.0 g if body weight ≥50 kg	25 mg/kg
Ethambutol	15 mg/kg	
Streptomycin	750 mg if body weight <50 kg 1 g if body weight ≥50 kg 750 mg if age ≥40 years 500 mg if age ≥60 years	15 mg/kg to maximum of 0.75 g
Thiacetazone	150 mg	50 mg
Para-aminosalicylic acid	10–12 g	300 mg/kg
Ethionamide and prothionamide	500 mg if body weight <50 kg 750 mg if body weight ≥50 kg	15–20 mg/kg
Capreomycin	1 g	Avoid
Viomycin	1 g	Avoid
Cycloserine	500 mg if body weight <50 kg 750 mg if body weight ≥50 kg	Avoid
Ofloxacin	800 mg	Avoid

Table 143.9 Doses of the first-line antituberculosis agents in three times weekly intermittent therapy

Agent	Dose (adults and children) (mg/kg)	Maximum dose
Isoniazid	15	750 mg
Rifampin (rifampicin)	15	600 mg
Pyrazinamide	50	2.0 g if body weight <50 kg 2.5 g if body weight ≥50 kg
Ethambutol	30	1.8 g
Streptomycin	15–30	750 mg if body weight <50 kg 1 g if body weight ≥50 kg

DRUG-RESISTANT TUBERCULOSIS

Resistance to any given anti-infective agent occurs by mutation at a low but constant rate, so that treatment with a single drug, however powerful, will inevitably lead to selection of resistant mutants. This is largely, but not entirely, avoided by the use of multidrug regimens. Under ideal conditions and in the absence of drug resistance, relapses after completion of a modern short-course chemotherapeutic regimen are uncommon and are mostly due to drug-susceptible bacilli. Unfortunately, ideal treatment conditions have long been the exception rather than the rule and many deficiencies in the use of multidrug regimens has led to the increasing emergence of strains resistant to one or more drugs.[27]

Although resistance to isoniazid is common, patients whose disease is caused by such resistant strains usually respond to short-course chemotherapy. Resistance to rifampin is much more serious in view of the unique ability of this drug to eliminate near-dormant persisting bacilli. Many strains resistant to rifampin are also resistant to isoniazid and, because these drugs are the principal components of modern regimens, this combination renders such regimens ineffective. Thus, the term multidrug resistance has been adopted by the WHO to refer to strains that are resistant to these two drugs, with or without resistance to additional drugs.[28]

A combined initiative by the WHO and the International Union Against Tuberculosis and Lung Disease was launched in 1994 to perform a global survey of resistance to first-line antituberculosis drugs. The first report, published in 1997 and covering 35 countries, showed that resistance to these drugs was more widespread than was previously recognized and revealed certain 'hotspot' areas with a very high incidence, including Estonia, Latvia, Argentina, the Dominican Republic, Côte d'Ivoire and parts of China and Russia.[29] The second report was published in 2000, the third in 2004 and the fourth, covering 138 geographic settings in 114 countries and two special administrative regions (SARs) of China, in 2008, and confirmed the widespread occurrence of MDRTB.[30] The incidence of resistance to any drug and multidrug resistance in new and previously treated patients is summarized in Table 143.11. The range of all types of drug resistance varied enormously from setting to setting, being low in most Western European countries but high in some countries of the former Soviet Union and some SARs of China. According to WHO estimates, almost 490 000 cases of MDRTB emerged worldwide in 2006, 4.8% of the estimated total number of new cases of tuberculosis.[30]

Table 143.10 Principal antituberculosis regimens recommended by the World Health Organization

Diagnostic category	Characteristics of patients	Initial phase	Continuation phase
I	New smear-positive pulmonary TB; new smear- negative pulmonary TB with extensive parenchymal involvement; concomitant HIV disease or severe forms of extrapulmonary TB	*Preferred* 2 HRZE *Optional* 2 $(HRZE)_3$ *or* 2 HRZE	4 HR *or* 4 $(HR)_3$ 4 $(HR)_3$ *or* 6 HE
II	Previously treated sputum smear-positive pulmonary TB: relapse or treatment after default	*Preferred* 2 HRZES/1 HRZE *Optional* 2 $(HRZES)_3$/1 $(HRZE)_3$	5 HRE 5 $(H\ RE)_3$
	Treatment failure of category I in settings with adequate program performance; representative DRS data showing high rates of MDRTB and/or capacity for DST if cases and availability of category IV regimens	Specially designed standardized or individualized regimens	
	In settings where representative DRS data show low rates of MDRTB or individualized DST shows drug-susceptible disease, or in settings of poor programme performance; absence of representative DRS data insufficient resources to implement category IV treatment	*Preferred* 2 HRZES/1 HRZE *Optional* 2 $(HRZES)_3$/1 $(HRZE)_3$	5 HRE 5 $(HRE)_3$
III	New smear-negative pulmonary TB (other than category I); less severe forms of extrapulmonary TB	*Preferred* 2 HRZE *Optional* 2 $(HRZE)_3$ *or* 2 HRZE	4 HR, 4 $(HR)_3$ 4 $(HR)_3$ *or* 6 HE
IV	Chronic (still sputum-positive after supervised re-treatment); proven or likely MDRTB cases	Specially designed standardized or individualized regimens	

E, ethambutol; H, isoniazid; R, rifampin (rifampicin); S, streptomycin; Z, pyrazinamide. DRS, drug-resistance surveillance; MDRTB, multidrug-resistant tuberculosis. Prefixed numbers show duration of treatment in months. The subscripted figure 3 indicates thrice weekly intermittent dosing.

Extremely drug-resistant tuberculosis (XDRTB)

Tuberculosis resistant to many of the second-line, as well as first-line drugs, has been described in many countries and has been termed extremely drug-resistant tuberculosis (XDRTB). Although there was disagreement over the definition of XDRTB, at the WHO Emergency Global Task Force on XDRTB in October 2006 it was defined as resistance to at least rifampin and isoniazid among the first-line agents, to any fluoroquinolone and to at least one of the injectable second line agents (amikacin, kanamycin and capreomycin).[31]

There are few data on the global incidence and prevalence of XDRTB as a result of limited facilities for testing for susceptibility to a wide range of antituberculosis agents and technical difficulties in accurately determining resistance to the second-line agents. A total of 17 690 strains of *M. tuberculosis* from many countries, 11 939 of them from South Korea, isolated between 2001 and 2004, were subjected to extensive drug-susceptibility testing; 3520 were found to be multidrug resistant (MDR).[32] Of these, 347 (10%) were extremely resistant (XDR). The proportion of MDR strains being XDR was highest in South Korea (15%) and Eastern Europe/Western Asia (14%). Cases of XDRTB were found throughout the world, including industrialized countries, and the proportion of MDR strains being XDR rose over the study period in most regions, from an average of 5% to 7%.

The serious threat posed by XDRTB is illustrated by an outbreak in the Kwazulu-Natal province of South Africa where, between January 2005 and March 2006, 221 of 1539 patients had MDRTB and 53 of these had XDRTB. These included 44 patients who were tested for HIV infection and all were positive. Despite antituberculosis and antiretroviral therapy, all except one with XDRTB died, and among 42 whose date of death was recorded, the median survival was only 16 days after diagnosis.[33]

Causes of antituberculosis drug resistance

Two forms of drug resistance are encountered – acquired and primary (or initial):

- acquired resistance is the result of suboptimal therapy that encourages selective growth of mutants resistant to one or more drugs; and
- primary resistance is due to infection from a source case who has drug-resistant disease.

In practice, it is difficult to be sure that a patient with apparent primary resistant tuberculosis has, in fact, not received any antituberculosis therapy, and some workers therefore prefer the term initial resistance. The division of resistance into acquired and primary forms is of epidemiologic value because an increasing incidence of the former indicates that drug regimens or the supervision of therapy are suboptimal, whereas the continuing occurrence of primary resistance indicates that the transmission of the disease in the community is not being adequately controlled.

The development of drug resistance is due to many avoidable failures in the management of the disease (Table 143.12).

The traditional explanation for treatment failure is noncompliance or nonadherence to therapy and, though there will always be a small number of patients who will default on treatment in any situation, there is ample evidence that it is more often the health services

Table 143.11 Global prevalence of resistance to antituberculosis agents

Any drug resistance – new cases	
Weighted mean*	17.0% (95% CI 13.6–20.4)
Range	0–85.9%
Settings with over 30% resistance	13
Settings with very high prevalence of resistance	4
Multidrug resistance – new cases	
Weighted mean	2.9% (95% CI 2.2–3.6)
Range	0–22.3%
Settings with over 5% resistance	14
Any drug resistance – previously treated cases	
Weighted mean	35.0% (95% CI 24.1–45.8)
Range	0–85.9%
Settings with over 50% resistance	16
Multidrug resistance – previously treated cases	
Weighted mean	15.3% (95% CI 9.6–21)
Range	0–60%

*Means weighted for size of populations surveyed.

Table 143.12 Factors leading to suboptimal therapy and the emergence of drug and multidrug resistance

- Intermittent drug supplies
- Use of time-expired drugs
- Unavailability of combination preparations
- Use of poorly formulated combination preparations
- Prescription of inappropriate drug regimens
- Unregulated over-the-counter sale of drugs, including cough mixtures containing isoniazid
- Addition of single drugs to failing regimens in the absence of bacteriologic control
- Poor supervision of therapy
- Unacceptably high cost to patient in respect of the drugs, travel to the clinic and time off work

than the patient that are at fault. In order to enhance effective tuberculosis control, the WHO Global Plan to Stop Tuberculosis program has widely advocated the DOTS strategy.[34] Originally DOTS was an acronym for directly observed therapy short-course but it is now the 'brand name' for a control strategy with five components:

- government commitment to tuberculosis control;
- provision of a regular supply of good-quality drugs free at the point of delivery;
- passive case finding by sputum microscopy;
- directly observed therapy; and
- regular evaluation of the efficacy of the control program.

Combination drug preparations have been used to prevent patients from receiving monotherapy, but irregular and intermittent use of such preparations has led to drug and multidrug resistance.[30] In addition, the use of poorly formulated combination preparations has led to reduced bioavailability of the constituent agents and the risk of development of drug resistance.

There is no doubt that the blind addition of drugs to a failing regimen is likely to generate multidrug resistance. Unfortunately, the determination of drug resistance is far from easy and most parts of the world lack the facilities for conducting drug susceptibility tests under good quality control. Errors are not uncommon even in the most sophisticated centers in the developed world and may be noticed only when laboratory reports are considered in the light of clinical data. The presence of HIV infection does not by itself lead to an upsurge of MDRTB. Despite the increase in HIV-associated tuberculosis seen in most African countries, such an upsurge has not been the usual finding. On the other hand, several well-documented epidemics of MDRTB have occurred in institutions such as hospitals, prisons or common lodging facilities where immunocompromised HIV-infected persons are crowded together and exposed to an infectious source case.[35,36]

Ethnic minority communities, originating in countries where drug resistance is common, often have higher levels of drug resistance than the majority populations.[37]

The incidence of single-drug-resistant tuberculosis in a community can be reduced by establishment of good disease control programs.[34] This raises the question of whether the implementation of the WHO DOTS strategy would also reduce the incidence of MDRTB in a region. According to one mathematical model,[38] it would have such an effect, but the model assumes that multidrug-resistant strains of *M. tuberculosis* are less virulent than their drug-susceptible counterparts. Since the publication of this model, it has been established that strains of *M. tuberculosis* are divisible into several lineages that differ in their virulence, immunogenicity and ability to retain virulence on mutation to drug or multidrug resistance.[39] In a recently evolved sublineage of the Beijing or W-Beijing lineage, multidrug-resistant strains appear able to retain high virulence and to cause spreading epidemics of multidrug-resistant tuberculosis in certain geographic regions.[40] Further studies are therefore required in order to determine and predict the likely epidemiologic patterns of MDRTB and XDRTB and to take the necessary control measures.

Therapy of multidrug-resistant tuberculosis

Tuberculosis due to bacilli resistant to isoniazid alone usually responds to short-course drug regimens based on four drugs during the intensive phase but, by contrast, resistance to both isoniazid and rifampin (i.e. multidrug resistance) requires prolonged treatment with drugs that are much more costly, less effective and more toxic. The cost of such therapy is high; in the USA it can exceed $US250 000, compared with the cost of $US2000 for treating a patient who has drug-susceptible disease. The prognosis for patients who have MDRTB has improved considerably and, provided that the patient is diagnosed before severe lung damage has occurred and that the best supervised therapy and laboratory support is available, the outlook is good in the majority of cases. Under these optimal conditions, cure rates of 96% have been achieved.

The successful management of MDRTB requires laboratory support and a team of dedicated supervisors of therapy, the so-called 'DOTS-plus' strategy.[41] Ideally regimens should be designed for each patient on the basis of *in-vitro* susceptibility. Various regimens have been used and there have been few comparisons between them. Currently used regimens are usually based on a fluoroquinolone with at least two other drugs to which the strain is susceptible, such as kanamycin and ethionamide (or prothionamide).[42,43] Other agents include rifabutin, amikacin, capreomycin, clofazimine, cycloserine and *para*-aminosalicylic acid. Great care and dedication are required for the successful management of MDRTB (Table 143.13).

Treatment outcomes of patients with MDRTB and XDRTB were assessed in Latvia and the USA and were significantly less favorable in the latter.[32] Alternative forms of treatment, including immunotherapy, are therefore urgently required.

Table 143.13 Principles for management of multidrug-resistant tuberculosis

- Single agents should never be blindly added to failing regimens
- Before drug susceptibility tests become available, patients should be started on three agents that they have never received before
- All therapy should be fully supervised (use of an injectable agent enhances adherence to therapy)
- Therapy should last at least 24 months and should be continued for at least 18 months after bacteriologic conversion
- Drug susceptibility tests should be repeated if cultures remain positive after 3 months of therapy

TREATMENT OF PATIENTS IN SPECIAL CIRCUMSTANCES

Patients who have renal or hepatic disease

Modification of drug regimens and dosages may be required when there is substantial renal impairment or liver disease. The first-line drugs (rifampin, isoniazid, pyrazinamide) and also ethionamide and prothionamide are either completely metabolized or eliminated in the bile. They may therefore be used safely at normal doses in patients who have renal impairment. Isoniazid occasionally causes encephalopathy in patients who have renal failure and in those on dialysis but the risk is reduced, although not eliminated, by administering pyridoxine.[7] Although ethambutol is mainly eliminated by the kidney it can be used in reduced doses in patients who have impaired renal function. Streptomycin and other aminoglycosides are eliminated entirely by the kidney; as they are potentially nephrotoxic, special care must be taken.

In severe renal failure the dose of isoniazid should be reduced to 200 mg once daily (ensuring that pyridoxine is given to prevent peripheral neuropathy). Streptomycin and ethambutol are excreted by the kidney and adjustment to doses is necessary in renal failure. Streptomycin levels must be monitored and doses and spacing adjusted to achieve a peak level of 40 μg/ml and a trough level of <5 μg/ml to avoid toxicity. For patients on dialysis, a supplemental streptomycin dose should be given at approximately 5 mg/kg after hemodialysis.

Ethambutol dosages are dependent on creatinine clearances. For patients who have creatinine clearances between 50 ml/min and 100 ml/min, the dose is 25 mg/kg three times weekly; at 30–50 ml/min, the dose is 25 mg/kg twice a week; and at 10–25 ml/min the dose is 15 mg/kg at 2-day intervals. Patients on hemodialysis may be given 25 mg/kg ethambutol 6 hours before the procedure.

The catabolism of immunosuppressive agents administered to renal transplant patients, including azathioprine, corticosteroids, ciclosporin, sirolimus and tacrolimus, is increased by rifampin and dose adjustments may be required.[44] Streptomycin should be avoided in transplant patients as it increases the available level of ciclosporin, resulting in nephrotoxicity.

There is no clear evidence that the potentially hepatotoxic drugs rifampin and pyrazinamide are any more toxic in patients who have impaired hepatic function. Nevertheless, if they are used, hepatic function should be carefully and regularly monitored during therapy. Some physicians avoid them and treat such patients with isoniazid and ethambutol for 1 year, with the addition of streptomycin for the first 2–3 months. An alternative is to use a fluoroquinolone such as ofloxacin instead of rifampin.[45]

If rifampin is used, it should be used with caution; doses should be reduced in patients who have bilirubin concentrations exceeding 50 mmol/l. Liver function should be regularly monitored, where possible, in alcoholics, the elderly, malnourished children and children under 2 years of age.

If jaundice develops during antituberculosis therapy, treatment should be stopped until the jaundice resolves. In many cases resumption of treatment does not cause a recurrence of the jaundice. If the patient is seriously ill with tuberculosis, he or she may be treated with streptomycin and ethambutol even in the presence of jaundice.

HIV-positive patients who have tuberculosis

The treatment of tuberculosis in HIV-positive patients (see also Chapter 93) follows the same well-established principles used in the treatment of non-HIV-infected patients.[46] Despite a good bacteriologic response to treatment, patients who have HIV-related tuberculosis in Africa who are not receiving antiretroviral therapy have an extremely poor prognosis. They are almost four times as likely to die within 13 months of diagnosis as HIV-negative patients, with most deaths occurring during the first month of treatment.[47] This is largely due to other opportunistic infections but may also, in part, be due to an apparent synergistic immunosuppressive action of HIV infection and active tuberculosis. For this reason, prevention of tuberculosis is preferable to attempts to treat tuberculosis in HIV-positive persons. Drug reactions tend to be more severe in HIV-positive patients than in HIV-negative patients. In particular, thiacetazone often causes severe dermal reactions, with some patients developing fulminant and potentially fatal exfoliative dermatitis.

Pregnancy and the postpartum period

There is general agreement that the management of tuberculosis in pregnancy and in the postpartum period should be similar to that in other patients, although some advocate avoiding pyrazinamide. Short-course regimens seem to have a minimal risk of causing fetal abnormalities, and side-effects in the pregnant woman are no higher than in those who are not pregnant.[48] Opinions concerning the safety of pyrazinamide differ because there are limited experimental data on its effect on the fetus. Nonetheless, it is often used, particularly in regions where drug resistance is common. Streptomycin is avoided owing to its ototoxic properties. The treatment of drug-resistant tuberculosis, especially MDRTB, during pregnancy and the management of the neonate requires careful consideration, and experience is very limited.[49] Expert clinical and laboratory guidance, if available, is required.

An increased incidence of isoniazid-related epileptiform attacks and other neurologic symptoms has been reported in pregnant women but these are preventable by the prescription of pyridoxine 10 mg daily. Mothers taking antituberculosis drugs at the time of birth can care for their infants with little risk, unless the mother's disease is drug resistant or not responding to therapy. Likewise, although some of the drugs enter the milk in small concentrations, breast-feeding has no adverse effects on the infant.[50]

Adjunct corticosteroid therapy in tuberculosis

It has been postulated that corticosteroids, by reducing the host's immune response, would allow dormant bacilli to replicate freely and thereby facilitate killing by the drugs. There is little evidence to support this, and their use has not been shown to affect the outcome of modern short-course chemotherapy. On the other hand, in some forms of tuberculosis corticosteroids aid recovery and reduce long-term sequelae by suppressing inflammatory reactions and limiting subsequent scar formation (see also Chapter 30).

Chemoprophylaxis and preventive therapy of tuberculosis

Chemoprophylaxis is defined as the prescription of antituberculosis drugs for uninfected persons who are exposed to a risk of infection; preventive

therapy refers to the treatment of persons who have already been infected with tubercle bacilli (as indicated by tuberculin testing) but who show no clinical or radiologic evidence of active disease. Chemoprophylaxis is principally used to protect children who are at risk of infections, particularly those under the age of 3 years, who are prone to develop serious extrapulmonary forms of tuberculosis, including meningitis.

Preventive drug therapy for those infected by *M. tuberculosis* (i.e. tuberculin reactors) is used in some countries, notably the USA, where tuberculosis is uncommon and where bacille Calmette–Guérin (BCG) is not used. Although highly efficacious, it is not without its problems. Isoniazid monotherapy, for up to 1 year, is the most widely used form of preventive therapy, although 6-month regimens are becoming more common. Although there is a theoretic risk of generating isoniazid resistance, this does not seem to happen in practice, probably because there are so few bacilli present. Hepatic toxicity has given cause for concern, particularly in older adults, and so such preventive therapy is generally not recommended for those aged over 35 years.[51]

Policies for the use of preventive therapy vary from country to country. National guidelines should be consulted for indications for such therapy and for the recommended drug regimens. In general, preventive therapy in tuberculosis control in regions with a high incidence of tuberculosis has, in view of problems of compliance and organization, not played a major role in tuberculosis control programs.

Transplant recipients receiving corticosteroids and other immunosuppressive drugs are at risk of developing tuberculosis. It has been suggested that such patients should be given isoniazid 300 mg and pyridoxine 25–50 mg daily if they have one or more of the following:[52]

- history of inadequately treated tuberculosis;
- an abnormal chest radiograph compatible with antecedent infection with tuberculosis;
- positive tuberculin test of more than 10 mm in diameter; and
- recent contact with a case of active tuberculosis.

Prophylaxis of tuberculosis in HIV-infected patients

In view of the very high risk of an HIV-infected person developing active tuberculosis, and the adverse effect of this disease on the immune status and survival of the patient, there is a very good theoretical case for provision of preventive therapy for those at risk. Several placebo-controlled studies of isoniazid monotherapy in patients co-infected with *M. tuberculosis* and HIV have shown that such preventive therapy is effective.[53] In practice, serious problems have been encountered in diagnosing dual infection, ruling out active tuberculosis and ensuring compliance with therapy without breach of confidence or enhancement of stigma. Studies of varying design from Haiti, Zambia and Uganda have shown that preventive therapy in HIV-infected adults significantly reduced the incidence of tuberculosis. The questions of how long the protection lasts, whether prophylactic treatment is safe and whether it can lead to the emergence of drug-resistant strains of tuberculosis require attention.

Although preventive therapy leads to a reduction of the risk of tuberculosis by around 60% in tuberculin-skin-test-positive adults who have HIV infection, identifying HIV-infected individuals is difficult in resource-poor settings. A major problem is ensuring compliance with therapy. It is important to ensure that HIV-positive persons receiving preventive therapy do not have active tuberculosis or there is a strong risk of masking the disease and encouraging the emergence of drug resistance. It is also necessary to supervise the therapy, and this adds another burden to stretched tuberculosis control services.

Isoniazid monotherapy for 9 months is suitable for preventive therapy in HIV-positive persons; continuation of therapy to 12 months adds very little. Shorter prophylactic regimens, such as rifampin with pyrazinamide for 2 months, have also been evaluated. These include a 3-month course of a rifamycin (rifampin or rifabutin) plus isoniazid, and a 2-month course of a rifamycin plus pyrazinamide. Although completion of treatment is significantly higher with the shorter regimens, the incidence of hepatic toxicity is much higher,[54] and as there have been some deaths due to liver failure such short courses of preventive therapy should only be used where there are facilities for regular assessment of hepatic function. The relatively short-term benefit of preventive therapy is a further problem. By 18 months, the incidence of tuberculosis in those who receive such therapy is similar to that in those not receiving such therapy, indicating the need to consider repeated courses or, perhaps, lifelong preventive therapy.[55] As a general rule, prevention is more effective in those who have relatively limited immunosuppression (positive tuberculin tests, high lymphocyte counts and high hemoglobin levels). This, together with the difficulty in detecting co-infection, has led to the current recommendation to restrict preventive therapy to tuberculin-positive, HIV-positive persons (see Chapter 93 for further discussion).

Another problem with preventive therapy is the high incidence of multidrug-resistant tuberculosis in some regions, against which isoniazid monotherapy would afford no protection. It is understandable that health care staff are anxious about the risk to their health if reliance is placed on chemoprophylaxis rather than BCG vaccination. In general, in view of problems of compliance and organization, preventive therapy has not played a major role in tuberculosis control.[56]

DRUG INTERACTIONS

Clinically significant interactions between the first-line antituberculosis drugs themselves have been described but are uncommon;[57] however, such reactions could well occur when more complex regimens are used to treat MDRTB and XDRTB. Antituberculosis drugs may interact with drugs used to treat unrelated conditions (Table 143.14). Rifampin is the most important in this respect because it is a potent inducer of cytochrome isoenzymes involved in the metabolism of many drugs. The increased metabolism and clearance of these drugs may lead to therapeutic failure unless levels are adjusted and then readjusted when rifampin therapy ceases. Patients on oral contraceptives should be advised to use alternative forms of birth control.

Rifampin reduces the plasma concentrations and half-lives of the imidazole and triazole antifungals and these agents reduce plasma levels of rifampin. Because some patients, notably those who are HIV positive, may also require antifungal therapy, these interactions, which may lead to treatment failure, are of increasing importance. Patients who are HIV positive may also be receiving trimethoprim–sulfamethoxazole for prevention or treatment of *Pneumocystis jirovecii* (formerly *P. carinii*) infection. This agent significantly increases the serum levels and half-life of rifampin, leading to an increased incidence of adverse effects, including hepatotoxicity.[58]

Drug interactions with isoniazid are more pronounced in slow acetylators. The effects of isoniazid are potentiated by insulin and opposed by prednisone (prednisolone); its absorption from the intestine, and that of ethambutol and the quinolones, is inhibited by antacids containing aluminum hydroxide. The effects of carbamazepine and phenytoin are potentiated by isoniazid and those of enflurane are opposed.

Drug interactions with antiretroviral agents

The available antiretroviral agents all interact with rifampin, but this is most marked with protease inhibitors such as saquinavir, ritonavir, indinavir and nelfinavir.[59] Rifampin accelerates the metabolism of protease inhibitors (through induction of hepatic P450 cytochrome), resulting in subtherapeutic levels of these agents and thereby increasing the risk of the development of viral resistance. In addition, protease inhibitors retard the metabolism of rifampin, resulting in increased serum levels and the likelihood of increased drug toxicity (see Chapter 93).

When prescribing antiretrovirals with antituberculosis drugs, it is important to refer to the latest guidelines on the subject since these are updated frequently. As new antiretroviral agents are being discovered and used, new interactions with antituberculosis drugs are being discovered. Updates on these periodically appear on the US Centers for Disease Control and Prevention website – http://www.cdc.gov/mmwr.

Table 143.14 Clinically significant drug interactions with antituberculosis agents

Effects opposed by rifampin (rifampicin)	
Antiretroviral agents	Opioids
Azathioprine	Oral contraceptives
Corticosteroids	Phenytoin
Ciclosporin	Propranolol
Diazepam	Quinidine
Digoxin	Theophylline
Haloperidol	Tolbutamide
Imidazoles	Warfarin
Potentiates the effects of rifampin (rifampicin)	
Trimethoprim–sulfamethoxazole	
Effects potentiated by isoniazid	
Phenytoin Carbamazepine	
Potentiates the effects of isoniazid	
Insulin	
Effects opposed by isoniazid	
Enflurane	
Opposes the effects of isoniazid	
Prednisone Antacids (inhibit absorption)	
Effects potentiated by streptomycin	
Neuromuscular blocking agents	
Effects potentiated by quinolones	
Aminophylline and theophylline	
Potentiates the effects of quinolones	
Cimetidine	
Opposes the effects of quinolones	
Antacids, iron preparations, sucralfate, didanosine (all inhibit absorption)	

The timing of antiretroviral therapy (ART) in HIV-infected patients receiving antituberculosis therapy has been the subject of some controversy. The priority is to treat tuberculosis and while it is desirable to treat the HIV also without delay, complicating factors including the possible development of the immune reconstitution inflammatory syndrome (IRIS)[60] make it safer to delay ART. In 2006, the WHO recommended that ART should be delayed for 2–8 weeks in those with $CD4^+$ counts of <200 cells/mm^3 and in those whose $CD4^+$ counts are unknown, and for 8 weeks in those with $CD4^+$ counts of 200–350 cells/mm^3. Those with counts of >350 cells/mm^3 should be evaluated after 8 weeks and, unless their clinical condition is deteriorating, ART can be withheld until the course of treatment for tuberculosis has been completed.[61]

Regimens not containing a rifamycin are much less effective than those that are rifamycin based for treatment of HIV-infected patients. Thus, except in cases of resistance or intolerance to these agents, regimens containing a rifamycin throughout should be used and any interactions between these and the antiretroviral agents should be managed appropriately. Whenever possible, rifampin should be replaced by rifabutin, a much less powerful inducer of cytochrome enzymes, and to commence or continue with the antiretroviral drugs.[62] The clinical experience of antiretroviral regimens based on a non-nucleoside reverse-transcriptase inhibitor such as efavirenz plus two nucleoside analogues with rifampin-based antituberculosis treatment regimens in terms of tolerance and impact on both tuberculosis and HIV disease has been very favorable.[63,64]

DRUG SUSCEPTIBILITY TESTING

The purpose of drug susceptibility testing is not to detect small numbers of drug-resistant mutants, which will inevitably be present in every patient who has tuberculosis and in every culture, but rather to determine whether the great majority of the bacilli are susceptible to levels of the drugs that are achieved clinically. In the developed nations with the requisite facilities, and particularly where MDRTB is common, susceptibility testing of all clinical isolates is definitely indicated. In most developing countries, facilities for conducting drug susceptibility tests are very limited.

Drug susceptibility testing is of epidemiologic importance in monitoring the global incidence and distribution of acquired and primary drug resistance. Unfortunately, susceptibility testing is expensive and time-consuming and requires good laboratory facilities, a high level of technical expertise and rigid quality-control procedures. There is no point in doing such testing unless high standards of accuracy can be maintained because much harm may be done by modifying regimens to include less effective and more toxic drugs on the basis of false reports of resistance.

Global surveys on drug resistance have been compromised by the variety of methods used for surveillance and for drug susceptibility testing and the lack of standardization of the methods. The WHO has therefore prepared guidelines for standardized surveillance techniques and has established a network of supranational reference laboratories to co-ordinate surveillance and to provide technical guidance and assistance.[65]

Methods of drug susceptibility testing

Methods for drug susceptibility testing can be divided into:

- those that are based on inhibition of bacterial growth on drug-containing standard media;
- those that detect growth inhibition by automated systems;
- those that use biologic indicators of bacterial viability, such as enzyme activity and bacteriophage replication; and
- those that use nucleic-acid-based technology to detect mutations in genes determining susceptibility to drugs.

Methods for drug susceptibility testing based on growth on conventional media are well established but have the great disadvantage that there is a long delay before results are available. Four methods are currently in use (Table 143.15).

In an (unpublished) investigation carried out by members of the European Society of Mycobacteriologists, there were only minor discrepancies between results obtained by different workers using the first three of the methods listed in Table 143.15. All these methods may be used either for direct susceptibility tests on smear-positive sputum or for indirect susceptibility tests on cultures. The relative merits and usefulness of these methods in differing circumstances have been reviewed.[66]

Conventional tests for susceptibility to pyrazinamide pose particular problems because the drug acts only in acidic environments in which bacterial growth is poor. Thus, the tests require careful standardization and interpretation.

Automated techniques are widely used in developed countries. They are more costly than the conventional methods but the rapidity of the results justifies the extra cost, especially where multidrug resistance is common. Susceptibility to all antituberculosis drugs, including pyrazinamide, can be determined by these methods. Although radiometric systems were used originally,[67] these have been largely replaced by nonradiometric systems, principally based on the unquenching of a fluorescent dye when oxygen is consumed by mycobacterial metabolism or on color changes in dyes when carbon dioxide is liberated from nutrients in the medium.[68]

Several rapid methods for the detection of mutations in the *rpoB* gene conferring resistance to rifampin have been described; one of these, based on a number of DNA probes for wild-type and mutated regions of the gene (line hybridization assay), is obtainable in a commercially available kit form. The sites of mutations responsible for resistance to some other antituberculosis drugs, including streptomycin, pyrazinamide, isoniazid and ethionamide, are also known, and so

Table 143.15 Techniques used to determine susceptibility to antituberculosis agents *in vitro*

Technique	Where technique is used	Description of technique
Proportion method	USA and some European countries	Drug-free and drug-containing media are inoculated with test strains and the colony counts are compared; strains are reported as resistant if the colony count on the drug-containing medium is over 1% of that on the drug-free medium
Absolute concentration method	Some parts of Europe	Based on growth on media containing doubling dilutions of a known concentration of drug, so that the minimal bactericidal concentrations of drugs may be determined
Resistance ratio method	UK and those countries influenced by British bacteriologists	Similar to the absolute concentration method except that results are expressed as the ratio of the drug concentration inhibiting the test and drug susceptible control strains, rather than as the actual inhibiting concentration
Disk diffusion method	Rarely used	Similar to absolute concentration method and resistance ratio method but technically simpler, as disks containing the drugs are placed on the solid media, thereby avoiding the need to prepare batches of media containing the various drugs

commercially available kits for rapid detection of resistance to these and other drugs may soon be obtainable.[69]

Bacteriophages have been used to detect bacterial viability in the presence of antituberculosis agents.[70]

CONCLUSIONS

Chemotherapy is the mainstay of tuberculosis control and modern short-course regimens are among the most effective and cost-effective of all therapeutic interventions for any human disease. Sadly, this potent intervention has been so badly used that tuberculosis remains the leading infectious cause of death worldwide and control of the disease is now seriously threatened by the emergence of multidrug resistance. Although recent advances in immunology and molecular biology may eventually yield novel preventive and therapeutic agents, the overwhelming need at the present time is ensure that the available disease control tools are universally deployed and used in the most effective ways possible.

REFERENCES

References for this chapter can be found online at http://www.expertconsult.com

Chapter 144

Timothy Hills
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Miscellaneous agents: fusidic acid, nitrofurantoin and spectinomycin

This chapter deals with three antibiotics that are chemically different and have different antibacterial spectra and clinical uses.

Fusidic Acid

Fusidic acid has a steroidal chemical structure.

MECHANISM OF ACTION

It inhibits protein synthesis by interfering with elongation factor G preventing the release of energy required for the translocation of peptidyl tRNA from the A site to the P site.

SPECTRUM OF ACTIVITY

Fusidic acid has a narrow spectrum of activity. It exhibits good activity against the staphylococci including both methicillin-sensitive and methicillin-resistant strains of *Staphylococcus aureus* (MSSA and MRSA), as well as coagulase-negative staphylococci, but activity against other Gram-positive cocci is poor. In addition, fusidic acid has anaerobic activity, with the exception of *Fusobacterium* spp., has been found to be active against some mycobacteria and has limited activity against the Gram-negative bacteria *Neisseria* spp. and *Legionella pneumophila*.[1]

MECHANISMS OF RESISTANCE

The most clearly described resistance in *Staph. aureus* is conferred through single point mutations at various points of the *fusA* gene, leading to amino acid changes in the elongation factor G protein structure.[2] Other described mechanisms are altered cell permeability, binding by chloramphenicol acetyltransferase type 1 and enhanced drug efflux.

PHARMACOKINETICS, ROUTE OF ADMINISTRATION AND DOSAGE

Fusidic acid is available for intravenous, oral or topical administration. The bioavailability after oral administration is 75–90% with tablets but only about 23% in children given a suspension.[3] After an oral dose of 500 mg given as tablets, the initial plasma concentration is approximately 30 µg/ml and in steady state the concentration is about 100 µg/ml after a dosage of 500 mg q8h. The protein binding is about 97%.

Fusidic acid is lipid soluble and is efficiently distributed to peripheral compartments, including brain tissue, but excluding cerebrospinal fluid.[4] The elimination half-life is about 9 hours. Fusidic acid is eliminated mainly by conjugation to glucuronide in the liver and subsequent biliary excretion.

The normal dosage is:

- adults 500 mg (as sodium fusidate tablets) q8h orally or intravenously;
- neonates 50 mg/kg (as fusidic acid suspension) in three divided doses;
- child 1 month–1 year, 15 mg/kg q8h;
- child 1–5 years, 250 mg q8h;
- child 5–12 years, 500 mg q8h;
- child 12–18 years, 750 mg q8h.

Doses should be reduced in patients who have hepatic disease, particularly biliary obstruction. Full doses can be given to patients who have renal insufficiency and to the elderly. Because of the high protein binding, administration of fusidic acid should be avoided during the last trimester of pregnancy and to newborn children (risk for accumulation of bilirubin in the central nervous system – 'kernicterus').

INDICATIONS

Fusidic acid is mainly used as an antistaphylococcal agent but has to be used in combination with other antibiotics to prevent the emergence of resistance during treatment. In experimental staphylococcal endocarditis it was shown that, when fusidic acid was used as a single agent, resistance emerged in five of 12 animals, whereas no resistance was seen in animals treated with vancomycin plus fusidic acid.[5] Resistance has also emerged as a problem clinically when it has been used as a single agent topically for skin infections; a number of clonal outbreaks of childhood impetigo due to fusidic acid-resistant MSSA in Scandinavian and European countries have been associated with the use of topical preparations of fusidic acid.[6,7]

Both *in-vivo* and *in-vitro* studies have shown that the antibacterial activity of the combination of fusidic acid with other antibiotics is unpredictable so that synergistic, antagonist, additive or indifferent effects have all been described.[8,9] For example, in a clinical study of 104 consecutive cases of *Staph. aureus* meningitis, a better outcome was demonstrated in those treated with fusidic acid in combination with another antistaphylococcal agent (mainly a penicillinase-stable penicillin, e.g. methicillin) than those treated with other regimens including methicillin alone.[10] However, in an experimental model of *Staph. aureus* meningitis, antagonism between methicillin and fusidic acid was observed.[11]

Fusidic acid is widely used for the treatment of bone and joint infections due to *Staph. aureus* as high bactericidal levels are achieved in infected joints and bone, including sequestra. It is usually combined with a penicillinase-stable penicillin (e.g. flucloxacillin) to prevent the emergence of resistance, although there are no formal studies showing that the combination is better than a penicillinase-stable penicillin alone.

The spectrum of fusidic acid makes it one of the few antibiotics that can be used for the oral treatment of methicillin-resistant staphylococci. It should then preferably be combined with another antibiotic to avoid the emergence of resistance. The combination of oral rifampin (rifampicin) with fusidic acid has been used successfully for the decolonization of respiratory tract carriage of MRSA and follow-on oral treatment of vancomycin-intermediate *Staph. aureus* (VISA) infections, but monitoring of liver function is advisable as hepatic failure has been described on this combination.[12,13]

ADVERSE REACTIONS AND INTERACTIONS

Table 144.1 lists the most important adverse reactions to fusidic acid. Most common adverse reactions are minor (diarrhea, abdominal discomfort, contact dermatitis with topical therapy). Intravenous fusidic acid causes local irritation and thrombophlebitis in about 15% of patients treated, and hemolysis may occur following rapid infusion (normal infusion time is 2 hours or more). Additionally, intravenous use appears to increase the risk of jaundice and liver impairment. In a cohort study including 131 patients with staphylococcal bacteremia treated with fusidic acid, reversible jaundice occurred in 48% of those treated intravenously compared to 13% treated orally (and 2% with other therapies).[14] These, along with sodium fusidate's high oral bioavailability, favor the oral route. In newborns fusidic acid may cause kernicterus. Fusidic acid may interact with coumarin derivatives and oral contraceptives, reducing the bioavailability of these drugs through interference with the fecal flora.

Nitrofurantoin

Nitrofurantoin has the structure 1-{[(5-nitro-2-furanyl)methylene] amino}-2,4-imidazolidinedione and is the only compound of the nitrofuran class that has been used widely; the active component is the 5-nitro structure.

MECHANISM OF ACTION

Although this is not fully understood, it is known that the active form is produced intracellularly by reduction of the nitro group by cellular nitroreductases, which produce highly active intermediate metabolites that have been shown to bind to bacterial ribosomes, halting protein synthesis, and are thought to result in damage of bacterial DNA.

Table 144.1 Serious adverse reactions to fusidic acid

Adverse reaction	Risk factor
Thrombophlebitis	Use of intravenous preparation for more than 24 h
Jaundice, liver impairment	Intravenous preparation, abnormal liver function

SPECTRUM OF ACTIVITY

Nitrofurantoin has broad-spectrum activity against Gram-negative and Gram-positive bacteria, including enterococci but excluding *Pseudomonas aeruginosa* and the Proteae (e.g. *Proteus, Morganella* and *Providencia* spp.) and some limited activity against *Bacteroides* spp.[15] Resistance is still rare in *Escherichia coli* and other extended-spectrum β-lactamase (ESBL)-producing Enterobacteriaceae.[16,17] As nitrofurantoin loses most of its antibacterial activity in alkaline pH, it is not active against the Proteae, even if susceptibility testing shows sensitivity.

MECHANISMS OF RESISTANCE

Acquisition of resistance is rare and the mechanism has not been fully elucidated. It is thought to be due to loss of nitroreductase activity or prevention of adequate intracellular concentrations of the drug being reached.

PHARMACOKINETICS, ROUTE OF ADMINISTRATION AND DOSAGE

Nitrofurantoin is administered orally as a microcrystalline or macrocrystalline formulation, of which the latter has a slower absorption rate. Absorption is almost complete, with 2–4% of the dose being recovered from the feces.[18] Serum concentrations are not measurable, except in patients who have severe renal failure. This is because of destruction of nitrofurantoin in the tissues and, in particular, a very rapid renal elimination by glomerular filtration (20%) and tubular secretion, resulting in a serum half-life of only 20 minutes in patients who have normal renal function.[18] Excretion is complete within 6 hours after intake and urine concentrations of 200–400 mg/l are achieved after a dose of 100 mg q8h. In patients who have renal failure – who should not be given nitrofurantoin – there are measurable but still very low serum and urine concentrations.[19]

Therapeutic doses of nitrofurantoin are 50–100 mg q8h or q6h for adults and 3 mg/kg/day q12h or q8h for children. Prophylactically, the adult dose is 50–100 mg and the pediatric dose 1–2 mg/kg at bedtime. The duration of treatment when nitrofurantoin is used therapeutically should be 5–7 days. Dosages are not affected by liver function.

INDICATIONS

Nitrofurantoin should only be used for the treatment and prevention of bacterial cystitis.

In a study comparing 3-day treatment regimens of trimethoprim–sulfamethoxazole 160–800 mg q12h, cefadroxil 500 mg q12h, amoxicillin 500 mg q8h and nitrofurantoin 100 mg q6h for the treatment of uncomplicated cystitis in women, significantly better results were obtained with trimethoprim–sulfamethoxazole than with the other three regimens, which did not differ from each other.[20]

However, similar efficacy results have been reported for trimethoprim–sulfamethoxazole and nitrofurantoin when they are used for 7 days. Despite these findings, nitrofurantoin has a place in the therapy of cystitis, and use is currently increasing with the emergent problem of multiresistant ESBL-positive *E. coli* and other enteric Gram-negative bacilli isolates which are resistant to the usual first-line oral therapies.

The use of a single dose of nitrofurantoin at night to prevent cystitis has been shown to be well tolerated with no emergence of nitrofurantoin-resistant organisms in the fecal flora.[21] Use in most stages

of pregnancy appears to be safe. However, some authorities advise avoidance in pregnant women at term (38–42 weeks gestation) when labor is imminent due to the possible risk of hemolytic anemia in the newborn.[22]

ADVERSE REACTIONS

Table 144.2 lists the most important adverse reactions to nitrofurantoin. They occur at low frequencies (<0.5%).[23] The risk for these reactions can be markedly reduced by:

- avoiding long-term (>7 days) treatment, especially in the elderly;
- avoiding daily doses higher than 300 mg in adults; and
- reducing the dosage for elderly patients and patients who have renal impairment.

Nitrofurantoin should not be used for patients who have renal failure because the urine levels are too low and there is an increased risk of adverse reactions. Upper gastrointestinal adverse reactions (nausea, vomiting, anorexia) may occur and seem to be more common with the historical microcrystalline formulation than with the macrocrystalline one now in use.

Spectinomycin

Spectinomycin is an aminocyclitol antibiotic with a chemical structure similar to that of the aminoglycosides.

MECHANISM OF ACTION

Spectinomycin acts by inhibiting bacterial protein synthesis at the 30S ribosomal level.[24]

Table 144.2 Serious adverse reactions to nitrofurantoin

Adverse reaction	Risk factor
Eosinophilic lung infiltrates, fever	Prolonged treatment time, high doses
Pulmonary fibrosis	Elderly female patients, high doses
Polyneuropathy	High dose relative to renal function
Hepatitis	Long treatment time
Hemolytic anemia	Hereditary glucose-6-phosphate dehydrogenase deficiency

SPECTRUM OF ACTIVITY

Spectinomycin is most active against *Neisseria gonorrhoeae* where resistance is uncommon.[25,26] It is also active against various Gram-negative Enterobacteriaceae including *Escherichia coli, Klebsiella, Enterobacter* and some *Serratia* spp.[27] Activity against Gram-positive bacteria is more limited, with some strains of *Streptococcus pyogenes, Strep. pneumoniae* and *Staphylococcus* sensitive.

MECHANISMS OF RESISTANCE

Resistance is conferred through enzymic adenylylation via aminoglycoside adenylyltransferase.

PHARMACOKINETICS, ROUTE OF ADMINISTRATION AND DOSAGE

Spectinomycin is always given intramuscularly. It is rapidly and completely absorbed and a concentration of about 80 mg/l is achieved after a dose of 2 g.[28] Its protein binding is low (<5%) and it has an apparent volume of distribution of 0.3 l/kg. Elimination is renal, with a half-life of about 1 hour. It has no reported effects per se on any organ system.

Spectinomycin is given as a single intramuscular dose of 2 g for gonococcal urethritis and at a dose of 2 g q12h for 3 days for disseminated gonococcal infection. Dose reductions are not necessary in any patient category.

INDICATIONS AND ADVERSE REACTIONS

Spectinomycin is indicated in the treatment of gonococcal infection of the cervix, urethra or rectum. Although not normally used as first-line therapy, it should be considered as an alternative when the patient is allergic to cephalosporins.[29]

REFERENCES

References for this chapter can be found online at http://www.expertconsult.com

Chapter 145

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Antiretroviral agents

INTRODUCTION

The field of antiretroviral therapy is well into its third decade. It began shortly after the discovery of HIV-1 in 1983, following which *in-vitro* cultivation of the virus permitted the screening of agents for antiviral activity. The first antiretroviral agent approved was zidovudine, a nucleoside analogue reverse transcriptase inhibitor, in 1987. The identification of viral-specific targets and a selected host target (Fig. 145.1) has facilitated high throughput screening and rational drug design efforts, which resulted in the availability of 25 US Food and Drug Administration (FDA)-approved agents by early 2009 (Table 145.1). Potent combination therapy, commonly referred to as highly active antiretroviral therapy (HAART), has led to dramatic reductions in morbidity and mortality in the developed world and has begun to demonstrate similar benefits in the developing world as a result of large-scale rollout efforts.[1] The availability of simpler, less toxic initial regimens and the recognition of the importance of 'non-AIDS' events linked to uncontrolled viremia have moved the pendulum to initiating therapy earlier. In parallel, the approval of a number of new agents in the past few years with activity against drug-resistant strains of HIV-1 has changed the perspective on the management of highly treatment experienced patients with multidrug resistance in resource-rich settings, permitting the goal of therapy to be complete viral suppression in all patients.[2,3]

Despite these advances, the challenges of adherence, toxicities and drug resistance have placed recognizable limits on the currently available agents. Thus, continued drug development is a necessity if further progress is to be made. The clinical use of antiretroviral agents involves a pathogenesis-based, combination treatment approach. The principles and approach to antiretroviral therapy and clinical monitoring tools are addressed in Chapters 99 and 100. The reader is also referred to published antiretroviral therapy guidelines from the Department of Health and Human Services,[2] the International AIDS Society–USA,[3] and the European AIDS Clinical Society.[4] This chapter will review approved antiretroviral agents. Selected investigational agents in advanced phase clinical testing are discussed in the web version.

NUCLEOSIDE ANALOGUE REVERSE TRANSCRIPTASE INHIBITORS

Nucleoside reverse transcriptase inhibitors (NRTIs) were the first class of antiretroviral agents developed (Fig. 145.2). These drugs share a common mechanism of action. As purine or pyrimidine analogues, they require intracellular anabolic phosphorylation to triphosphate forms to be active inhibitors of the HIV reverse transcriptase. The NRTI triphosphates act as competitive inhibitors of the normal nucleotides and act as chain terminators during proviral DNA synthesis.

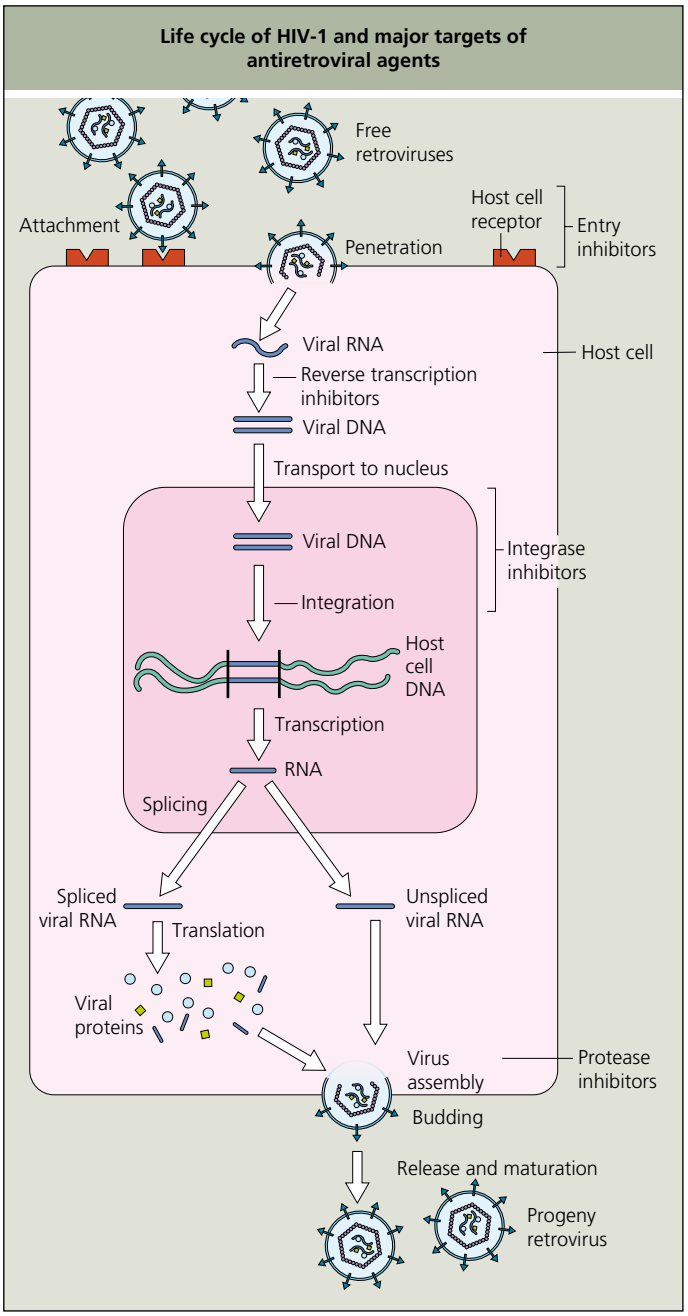


Fig. 145.1 Life cycle of HIV-1 and major targets of antiretroviral agents.

Table 145.1 List of FDA-approved agents*†

Nucleoside/nucleotide reverse transcriptase inhibitors	Non-nucleoside reverse transcriptase inhibitors	Protease inhibitors	Entry inhibitors	Integrase inhibitors
Zidovudine (Retrovir) Didanosine (Videx, Videx EC) Stavudine (Zerit) Lamivudine (Epivir) Abacavir (Ziagen) Emtricitabine (Emtriva) Tenofovir disoproxil fumarate (Viread)	Nevirapine (Virammune) Delavirdine (Rescriptor) Efavirenz (Sustiva, Stocrin) Etravirine (Intelence)	Saquinavir (Invirase) Indinavir (Crixivan) Ritonavir (Norvir) Nelfinavir (Viracept) Lopinavir/ritonavir (Kaletra, Aluvia) Atazanavir (Reyataz) Fosamprenavir (Lexiva, Telzir) Tipranavir (Aptivus) Darunavir (Prezista)	Enfuvirtide (Fuzeon) Maraviroc (Selzentry, Celsentri)	Raltegravir (Isentress)

*Includes US and non-US brand names.
†Combination products are not listed; please refer to text.

Zidovudine

Description

Zidovudine – 3′-azido-,3′-deoxythymidine (AZT, ZDV; see Fig. 145.2) – is converted to ZDV triphosphate sequentially by cellular thymidine kinase, thymidylate kinase and nucleoside diphosphate kinase. Zidovudine triphosphate possesses a 100-fold greater selectivity for the HIV-1 reverse transcriptase than for the cellular DNA polymerase alpha, thus accounting for its viral specificity. The drug is active against HIV-1, HIV-2 and human T-cell lymphotropic virus type 1 (HTLV-1).

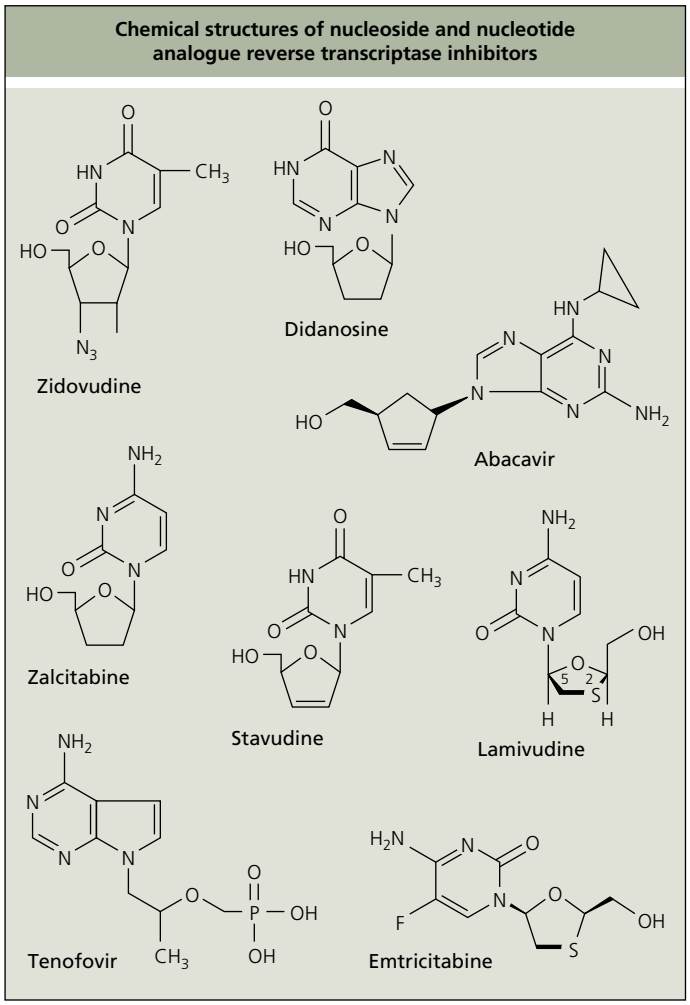


Fig. 145.2 Chemical structures of nucleoside and nucleotide analogue reverse transcriptase inhibitors.

Pharmacokinetics and distribution

Zidovudine is rapidly and well absorbed following oral administration, but exhibits a mean systemic bioavailability of about 64% due to significant first-pass metabolism. Peak plasma concentrations of intravenous and orally administered zidovudine range from 1.5 to 18 µmol/l with single and multiple doses of 1–10 mg/kg. Zidovudine is highly lipophilic and widely distributed throughout the body. Concentrations in the cerebrospinal fluid (CSF) in adults have ranged from 15% to 135% of plasma concentrations. Zidovudine is approximately 25% protein-bound and is primarily metabolized by hepatic 5′-glucuronidation followed by renal excretion.[5] The serum half-life of ZDV is approximately 1 hour, but the intracellular half-life of zidovudine 5′-triphosphate is approximately 3 hours.

Use

The usual adult therapeutic dosage of zidovudine is 300 mg q12h. Typically, it is paired with another NRTI to form a dual nucleoside component of a three- or four-drug regimen. The second NRTI is most commonly lamivudine or emtricitabine but should never be stavudine because of demonstrated antagonism.[6] In antiretroviral-naive patients, these dual nucleoside components need to be prescribed with a ritonavir-boosted protease inhibitor (PI) or a non-nucleoside reverse transcriptase inhibitor (NNRTI) to form a combination regimen capable of suppressing plasma HIV-1 RNA to less than 50 copies/ml. Zidovudine is less frequently used than tenofovir or abacavir as part of the dual nucleoside or nucleotide–nucleoside component of initial regimens because of its toxicity profile. The use of zidovudine in treatment-experienced patients should be guided by the previous treatment history and the results of drug resistance testing. The presence of NRTI class cross-resistance often limits the efficacy of zidovudine in second- and third-line regimens.

Resistance

The mechanism of resistance[7] is thought to be mediated by pyrophosphorolysis, which facilitates the removal of zidovudine after its incorporation into the proviral DNA chain. Resistance to zidovudine is conferred primarily by six mutations, which include M41L, D67N, K70R, L210W, T215F/Y and K219Q/E. High-level zidovudine resistance requires the accumulation of three to four mutations. These zidovudine-associated mutations are referred to as thymidine or nucleoside–nucleotide analogue-associated mutations (TAMs, NAMs) because of the increasing recognition of their role in cross-resistance to other members of the NRTI class (Fig. 145.3). Resistance to zidovudine



Fig. 145.3 HIV drug resistance mutations. For each amino acid residue the letter above the bar indicates the amino acid associated with wild-type virus and the letter(s) below indicate the substitution(s) that confer viral resistance. The number shows the position of the mutation in the protein. HR1, first heptad repeat. Courtesy of International AIDS Society–USA (for full details and footnotes see http://www.iasusa.org).

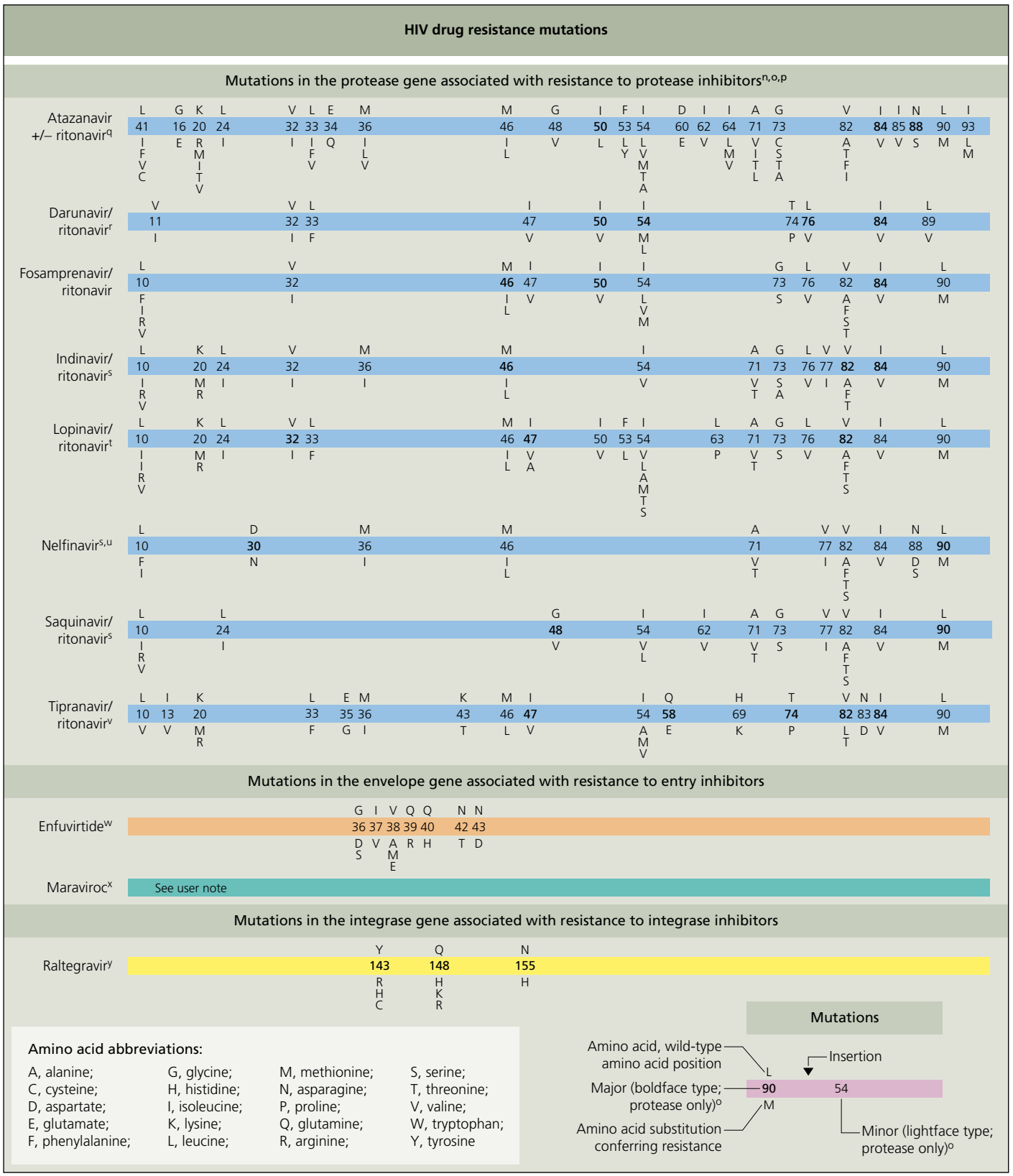


Fig. 145.3—cont'd.

can also be conferred by two multi-nucleoside resistance complexes: the Q151M complex (A62V, V75I, F116Y, Q151M) and a T69S 6 bp insertion in the presence of NAMs (see Fig. 145.3). Mutations in the RNase H (Q509L) and connection (N348I, A371V) domains of the reverse transcriptase increase the degree of zidovudine resistance in the presence of TAMs, probably by facilitating zidovudine excision.[8]

Dosage in special circumstances

Dosage adjustment is necessary in patients with severe renal disease (Table 145.2). In patients with hepatic dysfunction, zidovudine clearance is reduced and dosage modification is recommended. Limited data are available regarding specific dosage recommendations for patients with liver disease. Based on limited pharmacokinetic data in 14 patients with liver cirrhosis, a zidovudine dose reduction by 50% or a doubling of the interval has been recommended.[5]

Zidovudine crosses the placenta with concentrations in the fetal circulation approximately 85% of maternal plasma concentrations. Teratogenic effects with zidovudine have only been reported in rodents when exposed to lethal maternal doses (pregnancy category C).[9]

Adverse reactions and drug interactions

The major toxicities of zidovudine include nausea, headache, anemia, neutropenia and myopathy (Table 145.3). The myopathy is manifested by myalgias, muscle tenderness and proximal muscle weakness, mainly in the lower extremities. NRTI class toxicities are listed in Table 145.4. Clinically significant drug interactions are minimal because zidovudine is predominantly renally excreted; however, the use of zidovudine with stavudine is not recommended due to antagonism, and the concomitant use of drugs with myelosuppressive effects (e.g. ganciclovir, flucytosine) may increase the potential for hematologic adverse effects.

Didanosine

Description

Didanosine – 2′,3′-dideoxyinosine (ddI; see Fig. 145.2) – is sequentially converted intracellularly to 2′,3′-dideoxyinosine monophosphate by 5′-nucleotidase, and to 2′,3′-dideoxyadenosine monophosphate by adenylosuccinate synthetase and adenylosuccinate lyase. It is then converted to the ddA-triphosphate, which is the active form of the drug, possessing activity against HIV-1 and HIV-2.

Pharmacokinetics and distribution

Didanosine bioavailability varies from 21% to 54% following oral administration in adults. It is recommended that didanosine be administered on an empty stomach to increase absorption. Administration of delayed release capsules (Videx EC) with food decreases the didanosine area-under-the-curve (AUC) by approximately 19%. Didanosine exhibits linear pharmacokinetics with peak plasma concentrations ranging from 0.52 to 2.79 mg/l after oral doses of 125–375 mg q12h.[10] Results of pharmacokinetic studies suggest similar AUC values comparing the standard twice-daily regimen to the same total daily dose administered once daily.[11] Didanosine is less than 5% protein-bound. Concentrations in the CSF have been reported to be approximately 21% of those in plasma.[10] The plasma half-life of didanosine is short (<2 hours), but the *in-vitro* intracellular half-life of the triphosphate appears prolonged (>25 hours).[12] Didanosine is partially metabolized to ddATP or uric acid, or enters the purine metabolic pool.

Use

In adults, the usual dosage of didanosine is 400 mg q24h for those weighing over 60 kg and 250 mg q24h for those under 60 kg. It is typically prescribed as part of the dual nucleoside component of three- or four-drug combination regimens. It is most commonly paired with zidovudine or abacavir. It should not be prescribed with stavudine because of the risk of metabolic toxicities including lipodystrophy and lactic acidosis. Didanosine-containing dual NRTI components must be used with an agent or agents from another major antiretroviral drug class or classes to provide a potent combination regimen. The drug can be used in both treatment-naive and experienced patients but is generally reserved as a second-line agent. Its use should be driven by the treatment history and the drug resistance profile.

Resistance

Low-fold changes in susceptibility to didanosine are sufficient to compromise the response to the drug and this must be recognized to properly interpret phenotypic resistance results. Genotypically, the signature mutation conferring didanosine resistance is L74V but K65R and M184V have also been associated with low-fold changes in susceptibility. The Q151M complex, the T69S insertions and multiple NAMs also confer diminished susceptibility to didanosine (see Fig. 145.3).

Dosage in special circumstances

Didanosine clearance is significantly reduced in patients with renal disease and dosage modification is necessary (see Table 145.2). No dosage adjustment is recommended in patients with liver disease. Didanosine crosses the placenta. Studies evaluating long-term carcinogenicity and teratogenicity have produced negative results (pregnancy category B).[9]

Adverse reactions and drug interactions

The major toxicities of didanosine include pancreatitis, peripheral neuropathy and diarrhea (see Table 145.3). The drug can also cause hepatotoxicity and has been associated with hepatotoxicity and lactic acidosis when used in combination with stavudine in pregnant women (for NRTI class toxicities, see Table 145.4).

The didanosine oral solution has the potential for drug interactions with agents affected by concomitant antacid administration and with any agents that require gastric acidity for absorption. Clinically significant drug interactions have been reported between didanosine formulations and tenofovir disoproxil fumarate (tenofovir DF), delavirdine and indinavir. Administration of once-daily didanosine enteric-coated capsules (Videx EC) 2 hours before tenofovir DF administered with a light meal resulted in an approximate 48% increase in didanosine exposure. The administration of both of these agents simultaneously with a light meal resulted in an approximate 60% increase in didanosine exposure. There appears to be no effect of didanosine on the levels of tenofovir DF. If didanosine and tenofovir are used concomitantly, the dosage of didanosine should be reduced to 250 mg q24h (≥ 60 kg) or 200 mg q24h (<60 kg). Debate surrounds the use of the combination of didanosine and tenofovir DF because of reports of blunted CD4 cell responses.[13,14]

Stavudine

Description

Stavudine – 2′,3′-didehydro-3′-deoxythymidine (d4T; see Fig. 145.2) – is a thymidine analogue that is converted to the active form, stavudine triphosphate, by a series of cellular kinases; the initial phosphorylation is the rate limiting step. The drug has activity against both HIV-1 and HIV-2.

Pharmacokinetics and distribution

Stavudine is rapidly absorbed and exhibits linear pharmacokinetics. Peak concentrations of about 0.9 μg/ml are achieved within 2 hours. The oral bioavailability is 82–86%. The mean serum half-life is short

Table 145.2 Nucleoside and nucleotide dosage modifications in patients with renal dysfunction and with dialysis

Drug		Usual adult dose CrCL >50 ml/min	CrCL 30–50 ml/min	CrCL 10–30 ml/min	CrCL <10 ml/min	Hemodialysis*	Peritoneal dialysis	Continuous renal replacement therapy
Abacavir (po)		300 mg q12h or 600 mg q24h	300 mg q12h or 600 mg q24h	300 mg q12h or 600 mg q24h	300 mg q12h or 600 mg q24h	300 mg q12h or 600 mg q24h	300 mg q12h or 600 mg q24h	300 mg q12h or 600 mg q24h
Didanosine (po)	≥60 kg <60 kg	400 mg q24h 250 mg q24h	200 mg q24h 125 mg q24h	125 mg q24h 125 mg q24h	125 mg q24h 75 mg q24h	125 mg q24h 75 mg q24h	125 mg q24h 75 mg q24h	125 mg q24h 125 mg q24h
Emtricitabine (po)	Capsule Oral solution	200 mg q24h 240 mg q24h	200 mg q48h 120 mg q24h	200 mg q72h 80 mg q24h	200 mg q96h 60 mg q24h	200 mg q96h 60 mg q24h	200 mg q96h 60 mg q24h	200 mg q48–72h 80–120 mg q24h
Lamivudine (po)		150 mg q12h or 300 mg q24h	150 mg q24h	150 mg x1, then 100 mg q24h	150 mg x1, then 50 mg q24h	150 mg x1, then 25–50 mg q24h	150 mg x1, then 25–50 mg q24h	150 mg q24h
Stavudine (po)	≥60 kg <60 kg	40 mg q12h 30 mg q12h	20 mg q12h 15 mg q12h	20 mg q24h 15 mg q24h	20 mg q24h 15 mg q24h	20 mg q24h 15 mg q24h	20 mg q24h 15 mg q24h	N/A N/A
Tenofovir DF (po)		300 mg q24h	300 mg q48h	300 mg q72–96h	300 mg every 7 days	300 mg every 7 days	300 mg every 7 days	N/A
Zidovudine (po)		300 mg q12h	300 mg q12h	300 mg q12h	100 mg q8h	100 mg q8h	100 mg q8h	100 mg q8h

CrCL, creatinine clearance; N/A, specific dosing recommendations not available.
*Drug should be administered after the hemodialysis session.

Table 145.3 List of nucleoside and nucleotide reverse transcriptase inhibitor-specific toxicities

Zidovudine	Didanosine	Stavudine	Lamivudine	Abacavir	Emtricitabine	Tenofovir DF
Anemia or neutropenia Anorexia Bone marrow suppression Headache Insomnia Malaise Nausea Vomiting	Diarrhea Nausea Pancreatitis Peripheral neuropathy	Pancreatitis Peripheral neuropathy	Diarrhea Headache Insomnia Nausea Neuropathy Pancreatitis	Headache Hypersensitivity reaction* Malaise/fatigue Nausea Vomiting	Abdominal pain Asthenia Depression Diarrhea Dizziness Fatigue Headache Insomnia Nausea Skin hyperpigmentation	↓ Bone mineral density Asthenia Diarrhea Headache Nausea New onset/worsening renal impairment

*Hypersensitivity reaction can be fatal: symptoms may include fever, rash, nausea, vomiting, malaise, fatigue, loss of appetite, respiratory symptoms such as pharyngitis, dyspnea or cough.

Table 145.4 List of nucleoside reverse transcriptase inhibitor and protease inhibitor class toxicities

Nucleoside reverse transcriptase inhibitors	Protease inhibitors
Hepatomegaly with steatosis Lactic acidosis Lipoatrophy Mitochondrial toxicity	Cardiovascular effects (myocardial infarction, cerebrovascular accident) Fat redistribution Gastrointestinal intolerance Hepatitis Hyperglycemia (insulin resistance/diabetes mellitus) Hyperlipidemia (except atazanavir) Possible increased bleeding episodes in patients with hemophilia

(1–1.67 hours), with an intracellular half-life of about 3–4 hours. Protein binding is minimal. Stavudine penetrates into the CSF, achieving levels approximately 40% of plasma levels.[15] Stavudine is excreted by renal and nonrenal routes with approximately 50% of a dose excreted unchanged in the urine.[16]

Use

For adults weighing over 60 kg, the dose is 40 mg q12h; for those under 60 kg, the dose is 30 mg q12h. Like zidovudine, stavudine is used as part of a dual NRTI component, most commonly with lamivudine. These are then typically combined with a ritonavir-boosted PI or an NNRTI to form potent combination regimens. Stavudine can be used in treatment-naive patients or as an alternative to zidovudine in those patients who exhibit intolerance to zidovudine in the first few weeks after initiation of therapy. The association of stavudine with peripheral neuropathy, lipoatrophy, mitochondrial toxicity and lactic acidosis has led to a much diminished role for stavudine. The drug's use in treatment-experienced persons should be dictated by the treatment history and the results of drug resistance testing.

Resistance

Low-fold changes in susceptibility can impair the response to stavudine *in vivo* and this is important to keep in mind in the interpretation of phenotypic resistance testing. Genotypically, the V75T has been thought to be a signature mutation for stavudine resistance but this mutation is only rarely seen in clinical isolates. Recent data suggest that stavudine and stavudine–didanosine can select for zidovudine-associated mutations (TAMs, NAMs) and the Q151M complex. Zidovudine and stavudine share cross-resistance at both the virion and enzyme levels. Zidovudine-resistant isolates should be considered resistant to stavudine (see Fig. 145.3).

Dosage in special circumstances

Stavudine requires dose adjustment in patients with renal disease (see Table 145.2). No dosage modification is recommended in patients with liver disease.

Stavudine crosses the placenta and teratogenicity studies in rodents were negative with a decrease in sternal calcium noted at high doses (pregnancy category C).[9]

Adverse reactions and drug interactions

The major toxicity of stavudine is peripheral neuropathy and the drug has been strongly implicated in lipoatrophic and mitochondrial dysfunction syndromes as noted above (see Table 145.3). Clinically significant drug interactions are minimal. The combination of stavudine and didanosine should not be used due to the increased risk for peripheral neuropathy and lactic acidosis.

Lamivudine

Description

Lamivudine – (–)2′,3′-dideoxy-3′-thiacytidine (3TC; see Fig. 145.2) – is the (–) enantiomer of a sulfur-containing cytidine analogue. This enantiomer was chosen on the basis of its potency and cytotoxicity profile. It is phosphorylated to its active form, lamivudine triphosphate, by cellular kinases. The drug is active against HIV-1, HIV-2 and hepatitis B virus (HBV).

Pharmacokinetics and distribution

Lamivudine is well absorbed following oral administration with a mean bioavailability of over 80% in adults. Systemic drug exposure is not influenced by administration with food. Peak and trough concentrations of approximately 2 µg/ml and 0.33 µg/ml, respectively, have been achieved following oral administration of 150 mg q12h.[17] The mean serum half-life is approximately 4–6 hours, with the intracellular half-life ranging from 10.5 to 15.5 hours.[18] Lamivudine is less than 36% protein-bound. It penetrates the CSF, but the CSF to serum ratio is lower than that of other nucleoside analogues. Lamivudine is not significantly metabolized and is eliminated primarily unchanged via the kidney.

Use

The usual adult dosage is 150 mg q12h or 300 mg q24h. Lamivudine is one of the cornerstones of current antiretroviral therapeutics given its potency and excellent tolerability. It is commonly prescribed as part of the nucleoside component of initial regimens, typically paired with zidovudine, tenofovir or stavudine. When combined with a ritonavir-boosted PI or an NNRTI, this NRTI combination creates a potent regimen for treatment-naive patients. In treatment-experienced patients, the M184V mutation is commonly present. This mutation confers high-level resistance to lamivudine (see below) but resensitizes virus to zidovudine and tenofovir. Thus, this well-tolerated drug is often continued in treatment-experienced patients with multidrug-resistant virus to try to maximize the activity of the nucleoside/nucleotide component of the regimen. Lamivudine is widely used as part of maternal therapies and as part of maternal–fetal transmission interruption regimens.

Resistance

High-level phenotypic resistance (>500-fold change in susceptibility) quickly and nearly uniformly develops in patients treated with partially suppressive regimens containing lamivudine (e.g. dual nucleoside regimens). This is mediated through the lamivudine signature mutation, M184V. The latter has also been reported to increase the fidelity of the HIV reverse transcriptase and to decrease replicative fitness. The M184V mutation can delay the emergence of zidovudine resistance and reverse zidovudine resistance when the T215F/Y mutation is present. However, high-level zidovudine–lamivudine co-resistance can develop when multiple zidovudine-associated mutations (TAMs) and the M184V are present. This mutation can also partially reverse the resistance to tenofovir conferred by the K65R mutation.[19]

The Q151M complex and the T69S insertions also confer lamivudine resistance. Multiple TAMs alone, however, do not reduce susceptibility to lamivudine. This distinguishes lamivudine from the other approved NRTIs (see Fig. 145.3).

Dosage in special circumstances

Dosage adjustment is required in patients with renal disease (see Table 145.2). No dosage adjustment is necessary in patients with liver disease. Lamivudine crosses the placenta. No carcinogenicity or teratogenicity has been observed in long-term animal studies (pregnancy category C).

Adverse reactions and drug interactions

Lamivudine is generally very well tolerated. Insomnia, headache, pancreatitis and peripheral neuropathy can occur (see Table 145.3). Clinically significant drug interactions are minimal. Hepatitis and pancreatitis have been described in children.

Abacavir

Description

Abacavir sulfate – (1S, 4R)-4-[2-amino-6-(cyclopropylamino)-9H-purin-9-yl]-2-cyclo pentene-1-methanol (ABC; see Fig. 145.2) – is converted to carbovir intracellularly. Adenosine phosphotransferase catalyzes the first phosphorylation step. A cytosolic 5′-nucleotidase then converts abacavir monophosphate to carbovir monophosphate. Cellular kinases then complete the di- and triphosphorylation steps. Carbovir triphosphate is active against HIV-1 and HIV-2.

Pharmacokinetics and distribution

Abacavir is rapidly and well absorbed with a reported absolute oral bioavailability of approximately 83%. Administration with food does not significantly affect the oral bioavailability. Peak concentrations achieved following multiple dose administration of 300 mg q12h are approximately 2.2 μg/ml.[20] The mean plasma half-life of abacavir is less than 2 hours, but the intracellular half-life of the active carbovir triphosphate is approximately 20 hours. Abacavir is 50% protein-bound. It is highly lipophilic and penetrates into the CSF, with concentrations approximately 30% of plasma levels.[21] Abacavir undergoes extensive hepatic metabolism by alcohol dehydrogenase and glucuronosyl transferase.

Use

The usual adult dosage is 600 mg q24h or 300 mg q12h. In treatment-naive individuals it is usually prescribed as part of a dual nucleoside combination with lamivudine along with a ritonavir-boosted PI or an NNRTI. Abacavir–lamivudine has been reported to be inferior to tenofovir–emtricitabine when combined with efavirenz or atazanavir–ritonavir in patients with baseline plasma HIV-1 RNA levels >100 000 copies/ml although debate concerning this point continues. The triple nucleoside combination of abacavir–zidovudine–lamivudine has been shown to be virologically inferior to efavirenz-containing regimens and is best avoided.[22] In treatment-experienced patients, abacavir's usefulness depends on the degree of cross-resistance that may have been conferred by previous NRTI therapy (see below).

Resistance

Changes in susceptibility of eightfold or greater compromise the clinical efficacy of abacavir. A number of mutations confer resistance to abacavir. The M184V mutation alone confers a twofold change in abacavir susceptibility and the drug should still be useful in this situation. However, the M184V mutation in the presence of multiple TAMs and/or L74V and K65R will confer higher level abacavir resistance and compromise the drug's efficacy. The Q151M complex and the T69S insertion also confer abacavir resistance (see Fig. 145.3).

Dosage in special circumstances

Dosage adjustment is not necessary in patients with renal disease. No specific dosage modification is recommended in patients with liver disease. Abacavir crosses the placenta. Developmental toxicity secondary to abacavir has been observed in rats (pregnancy category C).[21]

Adverse reactions and drug interactions

The major abacavir toxicity of concern is the idiosyncratic hypersensitivity reaction, which has at least a 5% incidence and can be fatal (see Table 145.3). Abacavir hypersensitivity has been linked to the HLA B*5701 genotype and screening for this HLA haplotype is recommended prior to initiating abacavir.[23,24] Abacavir may increase the risk for myocardial infarction but conflicting data on this question exist.[25,26]

Emtricitabine

Description

Emtricitabine – 5-fluoro-1-[(2R,5S)-2-(hydroxymethyl)-1,3-oxathiolan-5-yl]cytosine (FTC; see Fig. 145.2) – is a cytidine analogue with activity against HIV-1, HIV-2 and HBV.

Pharmacokinetics and distribution

Emtricitabine is rapidly and almost completely absorbed with bioavailabilities of 93% and 75% for the oral capsules and oral solution, respectively. Following multiple dose oral administration, a mean peak concentration of approximately 1.8 μg/ml is achieved. Protein binding of emtricitabine is negligible (<4%). The plasma half-life is approximately 10 hours, but the intracellular half-life of the

triphosphate form is longer, 29 hours, allowing once-daily administration.[27] Emtricitabine is not significantly metabolized (13%) and is eliminated primarily unchanged via the kidney.

Use

The usual adult dose is 200 mg once daily for the capsules and 240 mg once daily for the oral solution. The clinical applications of emtricitabine are essentially the same as lamivudine. Most commonly, it is used in combination with tenofovir as a dual nucleoside/nucleotide component of ritonavir-boosted PI- or NNRTI-based regimens in treatment-naive individuals. The availability of the fixed-dose combination of emtricitabine, tenofovir and efavirenz permits a single pill, once-daily potent regimen that is the benchmark for simplicity and potency.

Resistance

The M184V mutation confers high-level resistance to emtricitabine and is the most commonly selected mutation in patients failing emtricitabine-containing regimens. Clinical trial data suggest that the rate of emergence of the M184V mutation with emtricitabine–tenofovir is lower than that seen with lamivudine–zidovudine in potent combination regimens. K65R, the Q151M complex and the T69S insertions also confer resistance to emtricitabine.

Dosage in special circumstances

Dosage adjustment is required in patients with renal disease (see Table 145.2). No dosage adjustment is necessary in patients with liver disease. In animal studies emtricitabine was not associated with fetal variations or malformations in mice and rabbits at drug levels 60- and 120-fold higher than used in humans. Emtricitabine is classified as pregnancy category B.

Adverse reactions and drug interactions

Emtricitabine is generally well tolerated. The most frequently reported adverse effects are mild to moderate headache, nausea, diarrhea, skin discoloration (hyperpigmentation on palms and soles) and skin rash (see Table 145.3).

NUCLEOTIDE ANALOGUE REVERSE TRANSCRIPTASE INHIBITOR

Tenofovir disoproxil fumarate (DF)

Description

Tenofovir DF – (R)-9-(2-phosphonomethoxypropyl) adenine (TDF; see Fig. 145.2) – is a prodrug of the nucleoside phosphonate 9-R-(2-phosphonomethoxypropyl)adenine (PMPA). Tenofovir DF is the first nucleotide reverse transcriptase inhibitor (NtRTI) approved for the treatment of HIV infection. These drugs differ from the nucleoside RTIs by having a phosphate group in the parent molecule. They thus require only diphosphorylation to be converted to their active compounds. Tenofovir DF is converted to tenofovir by serum esterases. Tenofovir is then activated by phosphorylation by cellular kinases. The drug is active against HIV-1, HIV-2 and HBV.

Pharmacokinetics and distribution

Tenofovir is administered orally as a prodrug, tenofovir DF. The oral bioavailability of tenofovir is approximately 25% and 40% in the fasting and fed state, respectively, as compared with 1 mg/kg intravenous dosing. Oral dosing of tenofovir DF at 300 mg q24h results in steady state peak concentrations of 303 ng/ml with an estimated half-life of approximately 14 hours.[28] The intracellular half-life of tenofovir diphosphate ranges from 12 to 50 hours.[29] Tenofovir is primarily eliminated renally (70–80%) via a combination of glomerular filtration and active tubular secretion.

Use

The usual adult dosage is 300 mg q24h. The major use of tenofovir DF is as part of initial regimens in treatment-naive persons because of its potency, safety profile and convenience.[30–34] The combination of tenofovir with abacavir–lamivudine or didanosine–lamivudine results in an unacceptably high rate of early virologic failure associated with the emergence of K65R and M184I/V mutations, and should be avoided.

In HIV–HBV co-infected patients, the combination of tenofovir–emtricitabine or tenofovir–lamivudine (when used in combination with a PI or NNRTI) provides three active drugs versus HIV and two active drugs versus HBV. This approach is often used to try to treat both infections effectively while avoiding the emergence of drug resistance in either pathogen (see Practice Point 44).

Resistance

A phenotypic change in susceptibility of over four-fold compromises the virologic response to tenofovir DF. The K65R is a signature mutation for tenofovir DF but its appearance in patients treated with tenofovir DF is relatively infrequent. Four or more TAMs (especially M41L and L210W) and the T69S insertion also confer resistance to tenofovir DF. Tenofovir retains activity against viruses harboring the Q151M mutational complex. Interestingly, the M184V mutation enhances susceptibility to tenofovir DF.[19]

Dosage in special circumstances

Dosage adjustment and monitoring for drug toxicity are necessary in patients with renal disease (see Table 145.2). Dose adjustment is necessary in patients with creatinine clearance <50 ml/min; such recommendations are based not on safety and effectiveness, but rather on single-dose pharmacokinetic data in this population. The presence of hepatic insufficiency is likely to have a limited effect on tenofovir pharmacokinetics and no specific dosage modifications are recommended in this population. Tenofovir is classified as pregnancy category B. In rats and rabbits, studies have found no evidence of impaired fertility or teratogenicity.

Adverse reactions and drug interactions

Neutropenia, headache, fatigue, pancreatitis, elevated creatinine and hypophosphatemia have been reported (see Table 145.3). Tenofovir has been associated with reductions in bone mineral density and osteomalacia.[35] Concomitant use of tenofovir DF with didanosine increases exposure to didanosine and increases the potential for drug toxicity. The combination may also result in early virologic failure and a blunted CD4 cell response.[13,14]

NON-NUCLEOSIDE REVERSE TRANSCRIPTASE INHIBITORS

The NNRTI class of antiretroviral agents is a chemically heterogeneous group of compounds that share a common mechanism of action. These agents differ from the NRTIs in that the parent compound is active and no intracellular metabolism is necessary. The drugs in this class allosterically bind in a noncompetitive fashion to a hydrophobic pocket near the active site of the reverse transcriptase and 'lock' the enzyme into an inactive state. The agents in this class also differ from NRTIs in that they are active against HIV-1 except for subtype O and are inactive against HIV-2 strains.

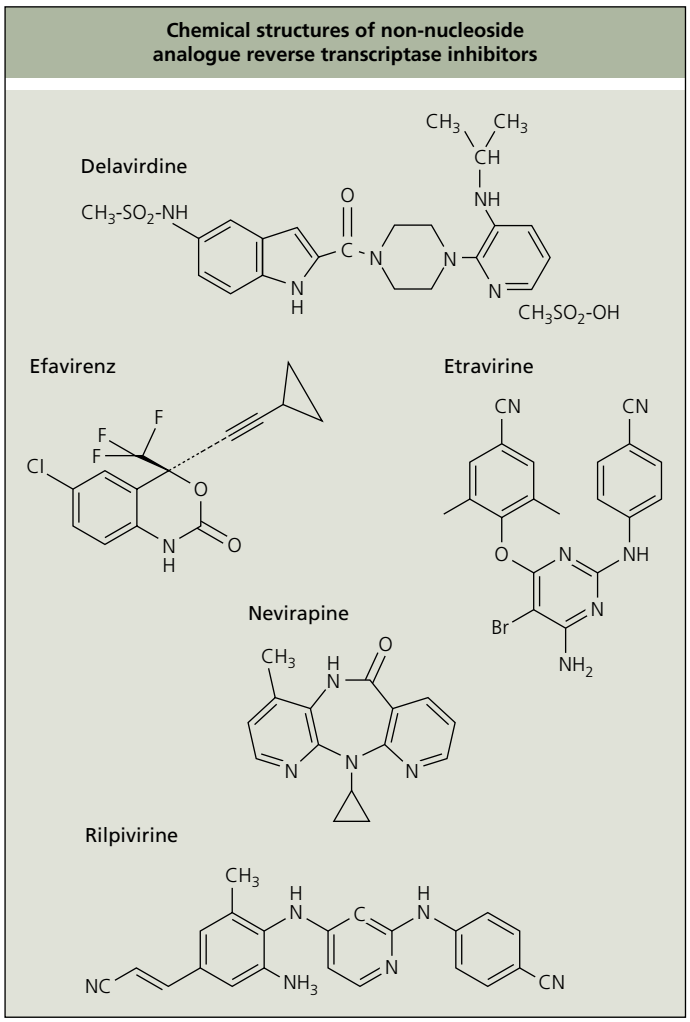


Fig. 145.4 Chemical structures of non-nucleoside reverse transcriptase inhibitors.

Nevirapine

Description

Nevirapine – 11-cyclopropyl-5,11-dihydro-4-methyl-6H-dipyrido [3,2-b:2′,3′-][1,4]diazepin-6-one (NVP; Fig. 145.4) – is the lead compound in this class and was the first NNRTI approved in the USA.

Pharmacokinetics and distribution

Nevirapine is well absorbed with an oral bioavailability of 90%. Absorption does not appear to be affected by co-administration with food or antacids. Maximum concentrations are achieved approximately 2 hours after an oral dose with a second peak occurring approximately 14 hours after a dose, presumably due to enterohepatic recycling.[36] Nevirapine exhibits linear pharmacokinetics. Following administration of a single 200 mg dose, a peak concentration of 7.5 µmol/l is achieved. Following 200 mg daily, average steady state plasma concentrations of peak and trough concentrations are 27.1 µmol/l and 15–17 µmol/l respectively. Nevirapine is very lipophilic and widely distributed throughout the body.[37] It is approximately 60% protein-bound. Concentrations in the CSF are approximately 45% of those achieved in plasma.[37] Nevirapine is primarily metabolized by the CYP3A4 and CYP2B6 isoenzymes to hydroxy-nevirapine metabolites and induces both of these enzyme systems.[38] Autoinduction of its own metabolism has been demonstrated. The half-life of nevirapine is approximately 25–30 hours.

Use

The usual adult dosage is 200 mg q24h for 14 days followed by 200 mg q12h. This dose escalation regimen is recommended to reduce the incidence and severity of rash during treatment initiation.

Due to the vulnerability of nevirapine to single-step, high-level resistance (i.e. a low genetic barrier to resistance), it needs to be used in potent combination regimens designed to suppress plasma HIV-1 RNA levels to less than 50 copies/ml. Partially suppressive regimens or poor drug adherence carry a high risk of engendering nevirapine resistance.

In individuals intolerant to the central nervous system (CNS) side-effects of efavirenz or in women of child-bearing age for whom access to effective contraception is problematic, nevirapine is an appropriate alternative to efavirenz. In previously naive persons who are virologically suppressed on a PI-containing regimen, a switch of the PI to nevirapine can maintain virologic suppression and improve serum lipid abnormalities.

Nevirapine has assumed an important role in the prevention of maternal–fetal HIV transmission in the developing world[39] (see Chapter 98).

In the prophylaxis of accidental needlestick exposure in health-care workers, the use of nevirapine should be avoided given the reports of severe hepatotoxicity when used in this setting.

Resistance

Low-level changes in susceptibility to nevirapine (and other NNRTIs), of the order of 2.5- to 10-fold, are the result of natural polymorphisms in wild-type strains and do not affect the response to these agents. Higher level resistance compromises or eliminates the virologic response to nevirapine. The signature mutation for nevirapine is Y181C, but other nevirapine-associated mutations include L100I, K103N, V106A/M, V108I, Y188C/L/H and G190A (see Fig. 145.3). Drug class cross-resistance among the first generation of approved NNRTIs (nevirapine, delavirdine, efavirenz) is nearly complete.

Dosage in special circumstances

Dosage modification is not required for patients with renal dysfunction. Limited data are available in patients with hepatic impairment and no specific dosage modification is currently recommended. Nevirapine is not associated with teratogenicity in rabbits or rats (pregnancy category C).[9] It rapidly crosses the placenta.

Adverse reactions and drug interactions

The major toxicities associated with nevirapine are rash and hepatotoxicity (Table 145.5). The risk of hepatotoxicity, which can be fatal, appears higher in women and in those with higher CD4 cell counts, namely >250 cells/µl in women and >400 cells/µl in men. Nevirapine is a moderate inducer of CYP3A4. Further discussion of the importance of drug interactions in HIV-infected patients can be found in Practice Point 45.

Table 145.5 List of non-nucleoside reverse transcriptase inhibitor specific toxicities

Nevirapine	Delavirdine	Efavirenz	Etravirine
Hepatotoxicity Hyperlipidemia Rash	Diarrhea Fatigue Nausea Rash	CNS symptoms* Hepatotoxicity Hyperlipidemia Rash	Nausea Rash

*CNS symptoms may include dizziness, insomnia, impaired concentration, somnolence, abnormal dreams, euphoria, confusion, agitation, hallucinations.

Delavirdine

Description

Delavirdine – 1-(5-methanesulfonoamido-1H-indol-2-ylcarbonyl)-4-[3-(1-methylethylamino)pyridinyl]piperazine (DLV; see Fig. 145.4) – was the second NNRTI approved in the USA.

Its use in clinical practice has been very restricted because of the high pill burden, the reluctance to use this agent if a severe reaction to nevirapine or efavirenz has occurred and the cross-resistance within this class of agents.

Efavirenz

Description

Efavirenz – (S)-6-chloro-4-(cyclopropylethynyl)-1,4-dihydro-4-(trifluoromethyl)-2H-3,1-benzoxazin-2-one (EFV; see Fig. 145.4) – is one of the most widely prescribed NNRTIs because of its potency, once-daily administration and lower incidence of rash compared to nevirapine.

Pharmacokinetics and distribution

Efavirenz is well absorbed with peak concentrations achieved 5 hours after oral administration. Absorption appears unaffected by administration with meals containing a moderate fat content. When administered with high-fat meals, a mean increase in AUC of 50% has been shown, and concomitant administration with high-fat meals is not recommended. Efavirenz exhibits linear pharmacokinetics. Average steady state plasma C_{min} and C_{max} following oral administration of 600 mg daily are approximately 6 µmol/l and 13 µmol/l, respectively.[37] Efavirenz is over 99% bound to plasma proteins, predominantly albumin, which limits crossing the blood–brain barrier, with CSF concentrations on average 0.69% of total plasma concentrations.[40] Efavirenz is metabolized in the liver, predominantly to inactive metabolites by CYP3A4 and CYP2B6. After multiple-dose oral administration, the half-life of efavirenz is approximately 40–55 hours. Efavirenz induces CYP3A4 *in vivo*, but has also been shown to inhibit CYP3A4, CYP2C9 and CYP2C19 *in vitro*.[41]

Use

Efavirenz is administered orally. The usual adult dosage is 600 mg q24h, which is available in a single tablet formulation and in a fixed-dose combination with tenofovir–emtricitabine.

Efavirenz plays a major role in the initial treatment of antiretroviral-naive patients and, in combination with tenofovir–emtricitabine, is a standard-of-care regimen for treatment-naive patients. The drug should be avoided in patients with a history of significant psychiatric illness because of its CNS side-effect profile. It is also contraindicated in the first trimester of pregnancy because of demonstrated teratogenicity.

Resistance

The most common mutation encountered clinically is K103N, which confers cross-resistance to efavirenz and delavirdine. Other clinically relevant mutations are L100I, V106M, V108I, Y181C, Y188L, G190S/A and P225H. High-level resistance is seen with the double mutations K103N+V108I and L100I+K103N (see Fig. 145.3).

Dosage in special circumstances

No dosage adjustment is required in patients with renal disease. Following a single-dose study in patients with chronic liver disease, efavirenz C_{max} was reduced and the half-life increased with no significant change in AUC compared to healthy volunteers.[37] Administration of the standard dose with close monitoring for toxicity is recommended in patients with liver disease.

Efavirenz crosses the placenta. Teratogenicity has been noted in primates and humans and administration to pregnant women should be avoided (pregnancy category D).[9]

Adverse reactions and drug interactions

The major toxicities associated with efavirenz are CNS related (e.g. impaired concentration, abnormal dreams, euphoria, anxiety and depression) and rash (see Table 145.5). As above, the drug is teratogenic. Efavirenz acts as an inducer or inhibitor of CYP3A4 depending on the concomitantly administered drug. Further discussion of the importance of drug interactions in HIV-infected patients can be found in Practice Point 45.

Etravirine

Description

Etravirine – 4-[[6-amino-5-bromo-2-[(4-cyanophenyl)amino]-4-pyrimidinyl]oxy]-3,5-dimethylbenzonitrile (ETR; see Fig. 145.4) – is a diarylpyrimidine analogue, 'second-generation' NNRTI. This designation arises from etravirine's antiviral activity against strains of HIV-1 harboring the K103N and a limited number of other NNRTI-associated resistance mutations.[42,43] This activity may be a result of the conformational and positional flexibility of etravirine. Etravirine is active against HIV-1 only.

Pharmacokinetics and distribution

Etravirine reaches peak concentrations following oral administration in 2.5–4 hours. The oral bioavailability of etravirine is unknown. After 1 and 8 days of etravirine 200 mg twice daily, the mean peak concentration of etravirine is 125.8 ng/ml and 451.3 ng/ml, respectively. Etravirine should be administered following a meal as the mean C_{max} is reduced 44% and the mean AUC is reduced 51% when administered in the fasted state compared to after a meal.[44] Etravirine is highly protein-bound (99.9%) and is primarily metabolized by CYP3A4, CYP2C9 and CYP2C19 enzymes in the liver. The half-life of etravirine is approximately 41 hours.

Use

Etravirine is administered orally as a tablet formulation. The usual adult dosage is 200 mg twice daily following a meal. Etravirine is indicated in individuals harboring multidrug-resistant virus who have experienced virologic failure on a first-generation NNRTI (e.g. efavirenz, nevirapine). Etravirine's clinical efficacy was demonstrated in trials which enrolled patients with virologic failure and multidrug-resistant virus.[45] The greatest experience with etravirine is in combination with darunavir–ritonavir so caution needs to be exercised when combining the drug with other PIs.

Resistance

Etravirine's activity is unaffected by the K103N mutation and good virologic responses are seen when there are fewer than three other NNRTI-associated mutations at baseline. Virologic response is diminished when three or more of the following mutations are present: V90I, A98G, L100I, K101E/P, V106I, V179D/F/T, Y181C/I/V and G190S/A. In patients who have experienced virologic failure on an NNRTI-based initial regimen and exhibit only the K103N mutation on resistance testing, etravirine should be combined with at least two other fully active agents as other NNRTI mutations may exist at low frequency and be undetected by routine resistance testing.

Dosage in special circumstances

Etravirine does not require dosage adjustment in patients with renal dysfunction. The pharmacokinetics of etravirine are not affected by

mild-to-moderate liver disease (Child-Pugh Class A or B). Due to the lack of data, it is not recommended to use etravirine in patients with severe liver disease (Child-Pugh Class C).

Etravirine appears safe in pregnant animal models, but no adequate studies in pregnant women have been conducted. Etravirine is classified as pregnancy category B.

Adverse reactions and drug interactions

The most common adverse reactions associated with etravirine are rash, including Stevens–Johnson syndrome, and nausea (see Table 145.5). Etravirine is an inducer of CYP3A4 and inhibitor of CYP2C9 and CYP2C19, and has the potential for serious drug interactions and toxicity. Etravirine should not be co-administered with other NNRTIs, all unboosted PIs, or ritonavir-boosted tipranavir or fosamprenavir. It can be co-administered with darunavir–ritonavir, lopinavir–ritonavir (limited data), raltegravir and maraviroc (with dose adjustment of maraviroc). Further discussion of the importance of drug interactions in HIV-infected patients can be found in Practice Point 45.

PROTEASE INHIBITORS

Mature HIV virions are produced as the virus buds off the cell surface and gag and gag–pol polyprotein precursors are cleaved by a virally encoded aspartyl protease. Successful inhibition of this enzyme marked a revolution in antiretroviral therapy starting in 1996. The nine currently approved PIs all bind to the active site of the protease enzyme and inhibit both HIV-1 and HIV-2.

Saquinavir

Description

Saquinavir – N-tert-butyldecahydro-2-[2(R)-hydroxy-4-phenyl-3-(S)-[[N-(2-quinolylcarbonyl)-l-asparaginyl]-amino]butyl](4aS,8aS)-isoquinoline-3(S)-carboxamide (SQV; see Fig. 145.5) – was the first PI approved in the USA.

Pharmacokinetics and distribution

Saquinavir-hard gel capsule (hgc) is poorly absorbed, with the mean absolute bioavailability of a 600 mg oral dose administered with food averaging only 4%. This is presumed to be due to limited absorption and extensive first-pass metabolism.[46] Absorption is improved upon administration with food or up to 2 hours after a meal. Saquinavir is approximately 98% bound to plasma proteins and is extensively hepatically metabolized to mono- and di-hydroxylated inactive compounds, primarily by CYP3A4 (>90%).[46] It is also a substrate of P-glycoprotein. The half-life following intravenous administration is approximately 7 hours.

Use

Saquinavir is administered orally. Given its poor oral bioavailability, the hgc formulation should only be prescribed with low-dose ritonavir enhancement. The dose of saquinavir-hgc is 1000 mg twice daily, always with ritonavir 100 mg twice daily. Saquinavir is used with low-dose ritonavir enhancement and for initial therapy is typically combined with two NRTIs. In patients with treatment failure on an NNRTI-based regimen, saquinavir–ritonavir can be a useful component of the second-line regimen. In the case of treatment failure in the presence of PI-associated resistance mutations, lopinavir–ritonavir, tipranavir–ritonavir or darunavir–ritonavir are, in general, better options for the alternative PI component of the new regimen, taking into account the specific resistance testing results.

Resistance

Resistance to saquinavir–ritonavir is mediated principally by the L90M mutation and to a lesser extent the G48V mutation. Other codon alterations that can contribute to saquinavir resistance include L10I/R/V, L24I, I54V/L, I62V, A71V/T, G73S, V77I, V82A/F/T/S and I84V (see Fig. 145.3). L90M is one of the major PI mutations associated with drug class cross-resistance.

Dosage in special circumstances

Saquinavir does not require dosage adjustment in patients with renal disease. Saquinavir pharmacokinetics has not been studied in patients with liver disease. No specific dosage recommendations are available in this patient population.

Saquinavir only minimally crosses the placenta. Animal studies have shown no mutagenicity or teratogenicity at 40–50% of AUC values achieved in humans (pregnancy category B).[9]

Adverse reactions and drug interactions

The major toxicity associated with saquinavir is gastrointestinal side-effects (Table 145.6), including diarrhea, abdominal discomfort and nausea. For PI class toxicities, see Table 145.4. Saquinavir (in the absence of ritonavir) is a weak inhibitor of CYP3A4. Further discussion of the importance of drug interactions in HIV-infected patients can be found in Practice Point 45.

Ritonavir

Description

Ritonavir: 10-hydroxy-2-methyl-5-(1-methylethyl)-1-[2-(1-methylethyl)-4-thiazolyl]-3,6-dioxo-8,11-bis(phenylmethyl)-2,4,7,12-tetraazatridecan-13-oic-acid, 5-thiazolyl-methyl ester [5S-(5R*, 8R*, 10R*, 11R*)] (RTV; see Fig. 145.5). Its intolerability at full therapeutic doses and its potent CYP3A4 inhibitory activity have combined to position this drug exclusively as a pharmacoenhancer of other PIs.

Pharmacokinetics and distribution

Ritonavir's oral bioavailability is estimated to range from 60% to 80%.[47] Relative to the fasting state, the AUC of ritonavir from the capsule formulation is approximately 15% higher when administered with food. For the oral solution, the AUC is decreased 7% when administered with food.[47] Following oral administration of ritonavir 600 mg q12h, the C_{max} and C_{min} are 11 mg/l and 4 mg/l, respectively. Ritonavir is greater than 98% protein-bound, both to albumin and α_1-acid glycoprotein. CSF concentrations are low and reported to be less than 0.05 mg/l.[47] Ritonavir is extensively metabolized, primarily by CYP3A4 isoenzymes, with the CYP2D6 isoenzyme also contributing to the production of the isopropylthiazolyl oxidation metabolite.[47] The half-life of ritonavir ranges from 3 to 5 hours.

Use

When administered as the sole PI, the adult dose of ritonavir is 600 mg q12h. However, ritonavir's major role is now as a pharmacoenhancer of other PIs. Pharmacoenhancement doses depend upon the co-administered PI(s) and whether an inducer of CYP3A4, such as efavirenz or nevirapine, is also included in the regimen. Most typically, ritonavir doses of 100–200 mg once or twice daily are used in pharmacoenhanced regimens.

Resistance

The major mutations conferring resistance to ritonavir are V82A/F/T/S and I84V. When used in low dose as a pharmacoenhancer of a second

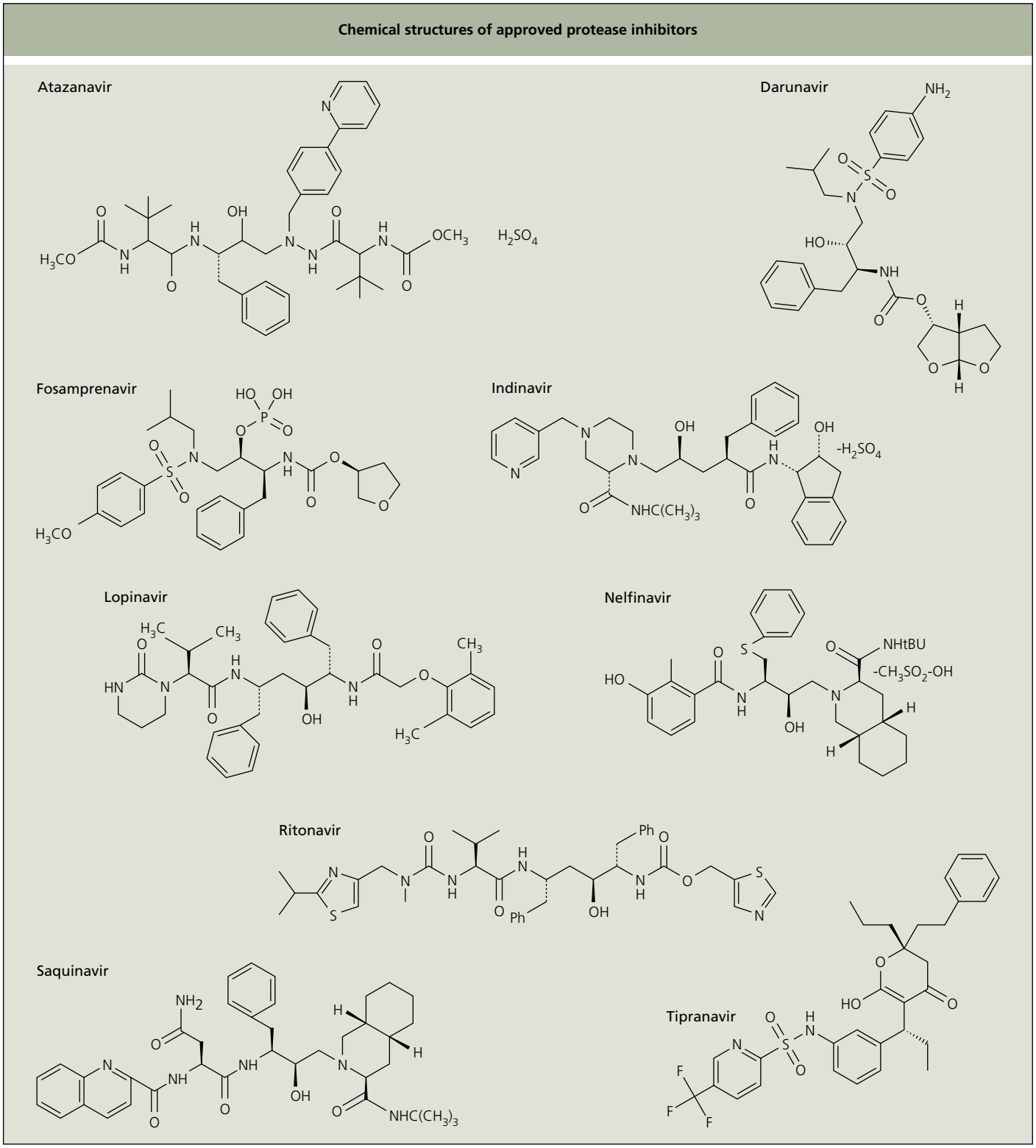


Fig. 145.5 Chemical structures of approved protease inhibitors.

PI, the pattern of mutations that emerges with virologic failure may be influenced by the presence of ritonavir.

Dosage in special circumstances

Renal disease is expected to have little effect on ritonavir pharmacokinetics and no dosage modification is necessary. In patients with mild-to-moderate hepatic insufficiency, the ritonavir pharmacokinetics vary little compared to patients with normal hepatic function when the dosage is reduced by 20%.[47] In addition, the elimination half-life increases from 4.6 hours in patients with normal hepatic function to 6.3 hours in patients with moderate hepatic disease. No specific dosage recommendations are available for patients with liver disease.

Table 145.6 List of protease inhibitor-specific toxicities

Saquinavir	Indinavir	Ritonavir	Nelfinavir	Lopinavir/r	Atazanavir	Fosamprenavir	Tipranavir	Darunavir
Nausea Diarrhea Headache	Nephrolithiasis Nausea Indirect hyperbilirubinemia Headache Asthenia Dizziness Rash Alopecia Hemolytic anemia	Nausea Vomiting Diarrhea Paresthesias (circumoral and extremities) Asthenia Taste perversion	Diarrhea	Nausea Vomiting Diarrhea Asthenia	Indirect hyperbilirubinemia Prolonged PR interval Nephrolithiasis	Rash Diarrhea Nausea Vomiting Headache	Rash Intracranial hemorrhage	Rash Diarrhea Nausea

Less than 10% of ritonavir appears to cross the placenta. Ritonavir is not mutagenic in bacteria or mammalian cells and teratogenicity has only been seen in rats at maternally toxic doses (pregnancy category B).[9]

Adverse reactions and drug interactions

The major toxicities associated with ritonavir are headache, diarrhea, altered taste, circumoral and peripheral paresthesias and hyperlipidemia (see Table 145.6). For PI class toxicities, see Table 145.4. Ritonavir is the most potent inhibitor of the cytochrome P450 system of all the PIs. Ritonavir inhibits CYP3A4 and CYP2D6 and also increases glucuronosyl transferase activity. Ritonavir also induces CYP3A4 activity and has been shown to induce its own metabolism. Further discussion of the importance of drug interactions in HIV-infected patients can be found in Practice Point 45.

Indinavir

Description

Indinavir – N-(2(R)-hydroxy-1(S)-indanyl)-2(R)-(phenylmethyl)-4(S)-hydroxy-5-[1-[4-(3-pyridylmethyl)-2(S)-(N-tert-butylcarbamoyl)piperazinyl]]pentanamide (IDV; see Fig. 145.5) – was the third PI approved in the USA but is infrequently used in current antiretroviral therapy because of its complex pharmacology and side-effects.

Nelfinavir

Description

Nelfinavir – [3S-(3R*, 4aR*, 8aR*, 29S*, 39S*)]-2-[2′-hydroxy-3′-phenylthiomethyl-4′-aza-5′-oxo-5′-(2″-methyl-3″-hydroxyphenyl) pentyl]decahydroisoquinoline-3-N-t-butyl-carboxamide (NFV; see Fig. 145.5) – was the mainstay of antiretroviral regimens for several years but has now been largely replaced by more potent, ritonavir-boosted, protease inhibitors.[48]

Lopinavir (co-formulated with ritonavir)

Description

Lopinavir – [1S-[1R*, (R*), 3R*, 4R*]]-N-[4-[[2,6-dimethylphenoxy) acetyl]amino]-3-hydroxy-5-phenyl-1-(phenylmethyl)pentyl] tetrahydro-alpha-(1-methylethyl)-2-oxo-1(2H)-pyrimidineacetamide (LPV; see Fig. 145.5) – is co-formulated with ritonavir (lopinavir–ritonavir).

Pharmacokinetics and distribution

Lopinavir is poorly bioavailable because it is rapidly metabolized by NADPH and cytochrome P450 3A4/5-dependent enzyme systems; thus, the rationale for co-formulation with ritonavir. Ritonavir's inhibition of the metabolism of lopinavir results in an over 100-fold increase in lopinavir AUC.[49] Concentrations achieved following administration of lopinavir–ritonavir tablets are not affected by food, but administration with food increases the AUC by 80% for liquid formulations. Therefore, the tablets can be administered with or without food, while the oral solution should be administered with food. Lopinavir is 98–99% protein-bound, both to albumin and alpha-1-acid glycoprotein. At steady state, lopinavir peak and trough concentrations are 9.6 μg/ml and 5.5 μg/ml, respectively, following twice-daily lopinavir–ritonavir 400/100 mg.[49] Lopinavir undergoes extensive oxidative metabolism via CYP3A. The half-life of lopinavir–ritonavir is approximately 6 hours.

Use

Lopinavir–ritonavir tablets contain 200 mg of lopinavir and 50 mg of ritonavir or 100 mg of lopinavir and 25 mg of ritonavir. The usual adult dosage is lopinavir–ritonavir 400 mg/100 mg (two tablets) q12h. This total daily dose may be administered once daily in treatment-naive patients, but not in patients also receiving efavirenz, nevirapine, fosamprenavir or nelfinavir. In patients taking these concomitant antiretrovirals, the lopinavir–ritonavir dosage should be increased to 500 mg/125 mg q12h.

In antiretroviral-naive patients, lopinavir–ritonavir in combination with two NRTIs is an effective regimen.[48] Lopinavir–ritonavir is also effective in constructing regimens for treatment-experienced patients, particularly those who have experienced virologic failure on their initial, NNRTI-based regimen, or those with a limited number of protease inhibitor-associated resistance mutations. Treatment history and the results of drug-resistance assays should guide whether lopinavir–ritonavir should be used for treatment-experienced patients with virologic failure.

Resistance

The major mutations conferring lopinavir–ritonavir resistance are V32I, I47V/A, V82A/F/T/S, L10F/I/R/V, K20M/R, L24I, L33F, M46I/L, I50V, F53L, I54V/L/A/M/T/S, L63P, A71V/T, G73S, I84V and L90M (see Fig. 145.3). The pharmacoenhancement of lopinavir by ritonavir permits the drug to be active against viruses with up to 10- and possibly 40-fold changes in susceptibility to lopinavir.

Dosage in special circumstances

Dosage adjustment is not necessary in patients with renal disease. In patients with mild-to-moderate hepatic impairment, the AUC of lopinavir is increased 30% compared to patients with normal hepatic function. Close monitoring is advised in patients with liver disease. No specific dosage recommendations are available for this patient population.

Lopinavir has been shown to cross the placenta in rats with resulting developmental toxicities (decreased fetal viability, decreased

fetal body weight, skeletal variations) at maternally toxic doses. No embryonic or fetal developmental toxicities have been seen in rabbits. Lopinavir–ritonavir is classified as pregnancy category C.[9]

Adverse reactions and drug interactions

The principal toxicities associated with lopinavir–ritonavir include gastrointestinal side-effects, hyperlipidemia and liver enzyme abnormalities (see Table 145.6). Lopinavir is a moderate inhibitor of CYP3A4 and the combination of lopinavir with ritonavir (a potent CYP3A4 inhibitor) has a number of significant drug interactions. Further discussion of the importance of drug interactions in HIV-infected patients can be found in Practice Point 45.

Atazanavir

Description

Atazanavir sulfate – dimethyl (3S, 8S, 9S, 12S)-9-benzyl-3,12,di-tert-butyl-8-hydroxy-4,11-dioxo-6-(p-2pyridylbenzyl)-2,5,6,10,13-pentaazatetradecanedioate sulfate (ATV; see Fig. 145.5) – is an azapeptide PI whose advantage is once-daily administration and the relative lack of induction of hyperlipidemia.

Pharmacokinetics and distribution

Atazanavir is rapidly absorbed and requires an acidic environment for adequate absorption. The mean C_{max} at steady state is 5.35 µg/ml at a median time of 2.5 hours following the administration of 400 mg once daily. The co-administration of ritonavir increases the atazanavir AUC 2.4-fold and the C_{min} 10.9-fold.[50] Atazanavir administration with a light meal or a high-fat meal increased the AUC by 70% and 35%, respectively, compared to the fasted state. Co-administration with ritonavir in similar situations decreases the pharmacokinetic variability. Atazanavir is 86% protein-bound, both to alpha-1-acid glycoprotein and albumin, and CSF to plasma ratios are reported to be between 0.0021 and 0.0226. Atazanavir is extensively metabolized in the liver, primarily by the CYP3A4 isoenzyme. The mean half-life of atazanavir is approximately 7 hours.

Use

Atazanavir is administered orally with or without ritonavir boosting. The usual dose of atazanavir in adults is 400 mg once daily or atazanavir 300 mg with ritonavir 100 mg once daily.

Atazanavir's once-daily administration, efficacy and lesser tendency to induce hyperlipidemia have made atazanavir–ritonavir one of the preferred choices for initial therapy. In patients with treatment experience, unboosted atazanavir should not be used. Atazanavir–ritonavir is useful in patients in whom an NNRTI-based initial regimen has failed but in patients who have PI-associated mutations, atazanavir–ritonavir is generally not as efficacious as tipranavir–ritonavir or darunavir–ritonavir.[51,52]

Resistance

The major mutations conferring resistance to atazanavir or atazanavir–ritonavir are I50L, I84V and N88S. Twenty-one minor mutations have been reported to contribute to atazanavir or atazanavir–ritonavir resistance (see Fig. 145.3).

Dosage in special circumstances

No dosage adjustment is necessary in patients with renal dysfunction. A dosage reduction to 300 mg once daily when prescribed without ritonavir boosting is recommended in patients with moderate hepatic impairment (Child-Pugh Class B) and the drug is not recommended in patients with severe hepatic impairment (Child-Pugh Class C).

Atazanavir is classified as pregnancy category B. Cases of lactic acidosis have been reported in pregnant women taking atazanavir with nucleoside analogues. Hyperbilirubinemia has also been reported. No adverse fetal effects or teratogenicity have been reported in animal reproductive and developmental studies.

Adverse reactions and drug interactions

In general, atazanavir has been well tolerated with the relative absence of hyperlipidemia a notable feature. When the drug is combined with low-dose ritonavir, some lipidogenic effect of ritonavir can be seen. The most common adverse effects are gastrointestinal complaints. Unconjugated, benign, reversible hyperbilirubinemia is the most frequent laboratory abnormality noted and may be associated with scleral icterus or clinical jaundice, but rarely requires treatment discontinuation. Atazanavir competitively inhibits UGT1A1 in the liver which is responsible for bilirubin glucuronidation and the proportion of patients with hyperbilirubinemia varies with distinct UGT1A1 genotypes.[53] Atazanavir can prolong the PR interval and must be used with caution in patients with pre-existing conduction system disease and with other drugs that may prolong the PR interval. Nephrolithiasis has also been reported with atazanavir.

Atazanavir is an inhibitor of CYP3A and UGT1A1, and a weak inhibitor of CYP2C8. Further discussion of the importance of drug interactions in HIV-infected patients can be found in Practice Point 45. Atazanavir plasma concentrations are expected to be reduced when co-administered with proton-pump inhibitors, antacids, buffered medications or H_2-receptor antagonists.

Amprenavir–fosamprenavir

Description

Amprenavir–(3S)-tetrahydro-3-furyl-N-[(1S, 2R)-3-(4-amino-N-isobutylbenzenesulfonamido)-1-benzyl-2-hydroxypropyl] carbamate (APV; see Fig. 145.5). Fosamprenavir (FPV) is the prodrug of amprenavir and is now the marketed drug as it has improved the bioavailability and lowered the pill burden of amprenavir. It is rapidly hydrolyzed to amprenavir and inorganic phosphate by cellular phosphatases in the gut epithelium.

Pharmacokinetics and distribution

Fosamprenavir is rapidly absorbed following oral administration. The peak concentration of amprenavir is approximately 4.82 µg/ml and is achieved within 1–2 hours following administration of 1400 mg q12h. When fosamprenavir 1400 mg is co-administered with ritonavir 100 mg, both once daily, the peak concentration of amprenavir is approximately 7.93 µg/ml. The relative bioavailability of amprenavir oral solution is 14% less than amprenavir oral capsules. Administration of a single 1400 mg dose of fosamprenavir as the oral solution in the fed state was associated with a 46% reduction in C_{max}, a delayed T_{max} and a 28% reduction in amprenavir AUC. Amprenavir is approximately 90% protein-bound, predominantly to alpha-1-acid glycoprotein. Amprenavir is hepatically metabolized by CYP3A4. The plasma half-life of amprenavir is about 7.7 hours.

Use

The dosage in therapy-naive adults without ritonavir enhancement is 1400 mg q12h. With ritonavir, the dosage is fosamprenavir 1400 mg with ritonavir 100 mg or 200 mg once daily, or fosamprenavir 700 mg with ritonavir 100 mg twice daily. In treatment-experienced patients, the twice daily regimen with ritonavir is recommended.

Fosamprenavir–ritonavir is primarily used as an alternative PI for initial treatment. For treatment-experienced persons, fosamprenavir–ritonavir can be used as part of a new regimen following failure on an NNRTI-based regimen and in those patients with limited PI experience.

In patients with greater treatment experience and multiple PI-associated mutations, tipranavir–ritonavir or darunavir–ritonavir generally has greater utility if a PI is chosen to be part of the next regimen.

Resistance

The major mutations conferring resistance to fosamprenavir–ritonavir are I50V and I84V. Viral isolates resistant to the other, earlier approved PIs may remain susceptible to amprenavir. The major mutations conferring amprenavir resistance are I50V (a signature mutation) and I84V. Other important mutations include L10F/I/R/V, V32I, M46I/L, I47V, I54L/V/M, G73S, L76S, V82A/F/S/T and L90M (see Fig. 145.3).

Dosage in special circumstances

Data on the effects of renal disease on amprenavir pharmacokinetics are limited and no dosage modification is necessary. The AUC of amprenavir in patients with moderate and severe cirrhosis is significantly greater than in patients with normal hepatic function.

Fosamprenavir is classified as pregnancy category C.

Adverse reactions and drug interactions

The major toxicities associated with fosamprenavir include gastrointestinal symptomatology (diarrhea, nausea, vomiting) and rash (see Table 145.6). Amprenavir is a moderate inhibitor of CYP3A4. Further discussion of the importance of drug interactions in HIV-infected patients can be found in Practice Point 45.

Tipranavir

Description

Tipranavir disodium – [R-(R*, R*)]-N-[3-[1-[5,6-dihydro-4-hydroxy-2-oxo-6-(2-phenylethyl)-6-propyl-2H-pyran-3-yl]propyl]phenyl]5-(trifluoromethyl)-2-pyridinesulfonamide disodium salt (TPV; see Fig. 145.5) – is a dihydropyrone, nonpeptidic PI whose molecular advantage is the flexibility of binding to the active site of the HIV protease, thus retaining activity against viral strains with diminished susceptibility to other approved PIs.[54]

Pharmacokinetics and distribution

Tipranavir's bioavailability has not been quantified but a high-fat meal enhances its absorption. Co-administration with aluminum- or magnesium-based liquid antacids decreases the tipranavir AUC by 25%. Tipranavir is always co-administered with ritonavir as ritonavir increases tipranavir exposure by 24- to 70-fold; tipranavir is a substrate and inducer of P-glycoprotein and may initially induce its own metabolism. Tipranavir 500 mg with ritonavir 200 mg administered twice daily results in a tipranavir mean C_{max} of 94.8 µM and 77.6 µM in females and males, respectively. Tipranavir is highly protein-bound (>99.9%) to both albumin and alpha-1-acid glycoprotein. Tipranavir is hepatically metabolized predominantly by CYP3A4. The half-life of tipranavir is approximately 5–6 hours.

Use

Tipranavir is always administered with ritonavir. The usual adult dosage is 500 mg tipranavir with ritonavir 200 mg administered twice daily with food. Tipranavir contains a sulfonamide moiety and the potential for cross-sensitivity in patients with a sulfa allergy is unknown.

Tipranavir–ritonavir is useful in the setting of substantial PI resistance and its use is generally reserved for this circumstance.[55]

Resistance

The major mutations conferring tipranavir–ritonavir resistance are L33F, I47V, Q58E, Y74P, V82L/T and I84V. At least 11 minor mutations, which can contribute to tipranavir resistance, have been described (see Fig. 145.3).

Dosage in special circumstances

No dosage adjustment of tipranavir–ritonavir is necessary in patients with renal dysfunction. Effects of moderate or severe hepatic impairment have not been evaluated and usage is not recommended. No dosage adjustment is necessary in patients with mild hepatic impairment.

Tipranavir is classified as pregnancy category C.

Adverse reactions and drug interactions

The most frequent adverse effects associated with tipranavir–ritonavir are diarrhea, nausea and vomiting (see Table 145.6). Elevations in hepatic transaminases and deaths due to hepatitis and liver decompensation have been reported, especially in patients with concomitant hepatitis B or C. Rash may occur in patients with or without history of sulfa allergy. Tipranavir–ritonavir has been associated with reports of both fatal and nonfatal intracranial hemorrhage. Many of these cases occurred in patients with contributing factors for intracranial hemorrhage. Tipranavir inhibits platelet aggregation and must be used cautiously in patients who may be at increased risk of bleeding. The drug interaction profile of tipranavir–ritonavir is complex. Tipranavir is both an inducer and inhibitor of the hepatic CYP3A4 isoenzyme. When co-administered with ritonavir, the net effect is inhibition. Tipranavir is also a potent inducer of P-glycoprotein and a weak P-glycoprotein inhibitor, while ritonavir is a P-glycoprotein inhibitor. When co-administered, the net effect on P-glycoprotein is induction. Further discussion of the importance of drug interactions in HIV-infected patients can be found in Practice Point 45.

Darunavir

Description

Darunavir – [(1S,2*R*)-3-[[(4-aminophenyl)sulfonyl](2-methylpropyl)amino]-2-hydroxy-1-(phenylmethyl)propyl]-carbamicacid(3*R*,3aS,6a*R*)-hexahydrofuro[2,3-*b*]furan-3-yl ester monoethanolate (DRV; see Fig. 145.5) – is another protease inhibitor with excellent activity against HIV-1 strains resistant to other PIs. It is a synthetic nonpeptide analogue of amprenavir, with a critical change at the terminal tetrahydrofuran group (THF), namely two THF groups forming a bis-THF moiety. This structure increases interactions with the protease enzyme and increases the binding energy.[56]

Pharmacokinetics and distribution

Darunavir is administered orally. Its absolute oral bioavailability following a single 600 mg dose increases from 37% to 82% after co-administration with ritonavir 100 mg twice daily. Peak darunavir concentrations of approximately 11.2–14.9 µg/ml (co-administered with ritonavir) are achieved within 2.5–4 hours. When administered with food, the C_{max} and AUC of darunavir co-administered with ritonavir are increased 30% compared to the fasting state. Darunavir is highly protein-bound (95%) primarily to alpha-1-acid glycoprotein. Darunavir is extensively liver metabolized to oxidative metabolites, primarily by the CYP3A isoenzymes. The half-life is approximately 15 hours when co-administered with ritonavir.

Use

Darunavir is always co-administered with ritonavir. The usual adult dose and frequency of administration are dependent on the treatment history of the patient, namely 800 mg with ritonavir 200 mg once daily with food in treatment-naive patients and 600 mg with ritonavir 100 mg twice daily with food in treatment-experienced patients.

Darunavir/ritonavir has proven most useful in highly treatment-experienced persons with multidrug-resistant virus.

Resistance

The major mutations conferring resistance to darunavir–ritonavir are I50V, I54M/L, L76V and I84V. Other mutations which contribute to darunavir–ritonavir resistance are V11I, V32I, L33F, I47V, L74P and L89V (see Fig. 145.3).

Dosage in special circumstances

No dosage adjustment is necessary in patients with renal dysfunction as darunavir is extensively metabolized in the liver. No dosage adjustment is necessary in patients with mild-to-moderate hepatic dysfunction (Child-Pugh Class A and B), but no pharmacokinetic or safety data are available for patients with severe liver disease.

Darunavir is classified as pregnancy category C.

Adverse reactions and drug interactions

The most common adverse effects associated with darunavir–ritonavir are gastrointestinal symptoms, nausea and headache. Drug-induced hepatitis and rash, including Stevens–Johnson syndrome, have also been reported. Given the sulfa moiety in darunavir, it is unclear if the rash is associated with the presence of a sulfa allergy. Darunavir–ritonavir is an inhibitor of CYP3A and CYP2D6 isoenzymes. Further discussion of the importance of drug interactions in HIV-infected patients can be found in Practice Point 45.

ENTRY INHIBITORS

Remarkable advances have been made in the past several years in our understanding of the HIV entry process. Specifically, the identification of HIV co-receptors (e.g. CCR5 and CXCR4) and the understanding of the events involved in fusion of the viral envelope with the cell membrane have created new therapeutic targets. Entry inhibitors can be divided into three subcategories: attachment inhibitors, chemokine receptor antagonists and fusion inhibitors.[57,58]

Enfuvirtide

Description

Enfuvirtide – $C_{204}H_{301}N_{51}O_{64}$, T-20 – is a 36-amino acid peptide derived from the HR2 region of HIV-1_{LAI}, which binds to the HR1 region of the HIV gp41 fusion peptide and prevents the coil–coil zipping reaction, which leads to six-helix bundle formation and eventual viral–host membrane fusion (Fig. 145.6). Enfuvirtide is active against both CCR5-using (R5) and CXCR4-using (X4) viral strains with susceptibility influenced by the time that the gp41 HR1 target is exposed to the drug as the viral entry process proceeds. Co-receptor density and affinity may influence the susceptibility of HIV strains to enfuvirtide.

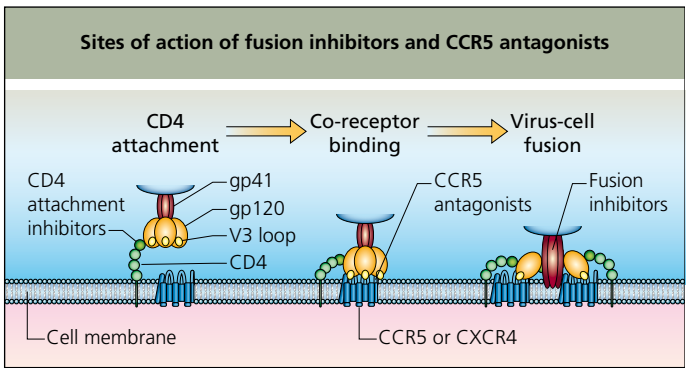


Fig. 145.6 Sites of action of fusion inhibitors and CCR5 antagonists. (Adapted from Moore JP, et al. Proc Natl Acad Sci USA 2003; 100; 10598–602.)

Pharmacokinetics and distribution

Enfuvirtide is rapidly digested by peptidases in the gastrointestinal tract and consequently is not orally bioavailable. Following subcutaneous dosing of enfuvirtide, the mean C_{max} and C_{min} at steady state were reported as 2626 ng/ml and 972 ng/ml, respectively, at 50 mg q12h, and 4725 ng/ml and 1774 ng/ml, respectively, at 100 mg q12h.[59] The time to maximal concentrations was approximately 4 hours. The serum half-life of enfuvirtide after intravenous administration has been reported as approximately 2 hours, but more sustained concentrations throughout the 12-hour dosing interval have been reported following subcutaneous administration.

Use

Enfuvirtide is administered by subcutaneous injection. The adult dosage is 90 mg q12h.

Given the parenteral nature of the drug and its activity against drug-resistant virus, its role lies in the management of patients with treatment failure in whom other options are constrained. It is important to try to have at least two (and preferably more) other active drugs to administer with enfuvirtide so that enfuvirtide-resistant virus does not quickly emerge.

Resistance

Resistance to enfuvirtide has been documented to occur *in vivo* with most mutations mapping to positions 36–45 of the amino terminal (HR1 region) of gp41. The most commonly described mutations are G36D/S, I37V, V38A/M/E, Q39R, N42T and N43D (see Fig. 145.3). Interestingly, enfuvirtide-resistant viruses may be less fit than wild-type isolates. Thus an immunologic (and presumably clinical) benefit may persist beyond the point of virologic failure, similar to what has been described for PIs.

Dosage in special circumstances

The dose of the drug should not be influenced by renal or hepatic dysfunction given its peptide nature.

Adverse reactions and drug interactions

The major toxicity of enfuvirtide is injection site reaction, which occurs in a large proportion of patients to varying degrees. Painful, erythematous, indurated nodules have been reported in up to 98% of patients, but uncommonly require drug discontinuation. Rare cases of hypersensitivity have been reported.

Maraviroc

Description

Maraviroc – 4,4-difluoro-N-[(1R)-3-[(1S,5R)-3-(3-methyl-5-propan-2-yl-1,2,4-triazol-4-yl)-8-azabicyclo[3.2.1]octan-8-yl]-1-phenylpropyl]cyclohexane-1-carboxamide (MVC; see Fig. 145.7) – is the first in a new class of agents called CC chemokine receptor 5 (CCR5) antagonists. Chemokine receptors, namely CCR5 and the CXCR4, play a critical role in the entry of HIV into the $CD4^+$ cell as they serve as co-receptors following the binding of the virus to the primary receptor, the CD4 molecule (see Fig. 145.6). HIV-1 strains that exclusively use CCR5 for cell entry are termed R5-tropic, and strains that exclusively use CXCR4 are termed X4-tropic. Strains that are able to use both receptors for cell entry are termed R5/X4-dual tropic. Mixed populations of

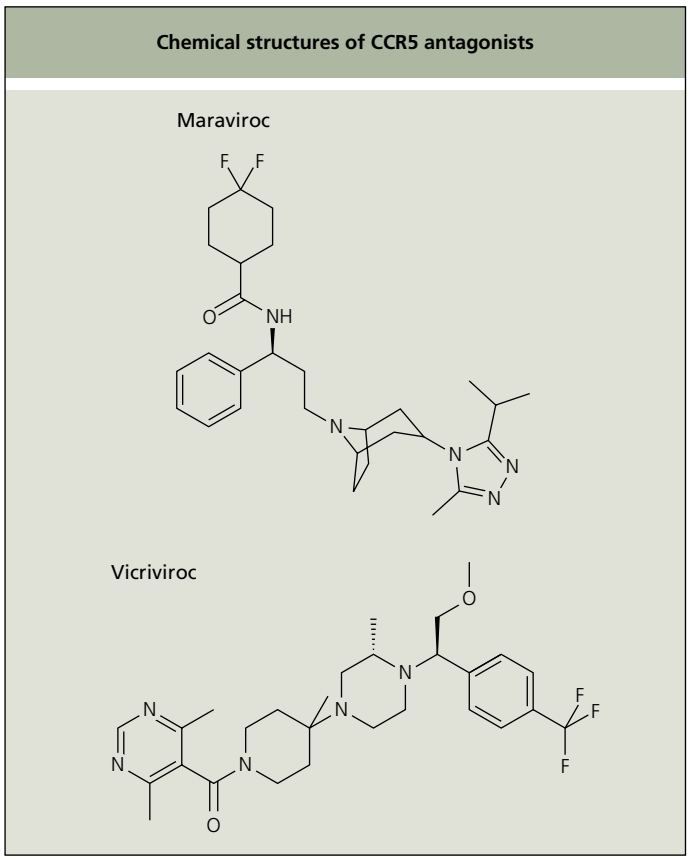


Fig. 145.7 Chemical structures of CCR5 antagonists.

R5 and X4 virus are termed R5/X4-mixed tropic. Maraviroc is a selective, reversible (noncompetitive) antagonist of the binding between human CCR5 and HIV-1 gp120 required for cell entry, thereby resulting in blocking of CCR5-tropic virus entry into cells. It represents a watershed in antiretroviral drug development because it is the first approved antiretroviral agent which targets a host cell molecule.

Pharmacokinetics and distribution

Maraviroc is administered orally with an absolute bioavailability of 23% and 33% (predicted) after a 100 mg and a 300 mg dose, respectively. Peak concentrations of 266–888 ng/ml have been reported in patients administered 300 mg twice daily, with peak concentrations achieved within 0.5–4 hours following a dose. Maraviroc is 76% protein-bound to both albumin and alpha-1-acid glycoprotein. It is primarily metabolized in the liver by CYP3A isoenzymes with a half-life of 14–18 hours.

Use

Maraviroc is available as an oral tablet formulation. The dosage regimen depends on the co-administration of other drugs that may inhibit or induce CYP3A. The usual adult dose with NRTIs, tipranavir–ritonavir, nevirapine, and drugs devoid of inhibition or induction of CYP3A4 is 300 mg twice daily. In the presence of potent CYP3A inhibitors, the dosage must be reduced to 150 mg twice daily. When co-administered with potent CYP3A4 inducers, the dosage must be increased to 600 mg twice daily.

Maraviroc has proven efficacy in the setting of multidrug-resistant virus when combined with an optimized background regimen.[60] In general, patients with more advanced or longer-standing HIV disease have increasingly detectable proportions of X4 or dual–mixed tropic virus on tropism testing. Thus, the key to using maraviroc efficiently is to determine at what level of treatment failure to introduce the drug.

Resistance

Resistance to maraviroc appears to occur primarily via two mechanisms. The first, and most frequent, is the outgrowth of X4 virus subpopulations which existed but were undetected at baseline. The selective pressure of maraviroc on the R5 majority population allows the X4 virus to proliferate. The second is the result of mutations in the HIV-1 gp120 region which binds the CCR5 receptor which permit the virus to bind to the co-receptor and enter the cell despite the presence of bound maraviroc.[61–63] Early concerns that maraviroc would induce co-receptor switching on individual $CD4^+$ cells have not been borne out.

Dosage in special circumstances

No dosage adjustment of maraviroc is necessary in patients with renal dysfunction or mild to moderate liver disease. No pharmacokinetic data are available in patients with severe hepatic impairment (Child-Pugh Class C).

Animal studies have not demonstrated increased pregnancy-related concerns with maraviroc. No adequate or well-controlled studies have been performed in pregnant women (pregnancy category B).

Adverse reactions and drug interactions

Maraviroc is generally very well tolerated. The most common adverse effects reported are upper respiratory tract infections, cough, pyrexia, rash and dizziness.

INTEGRASE INHIBITORS

HIV integrase is essential for viral replication and has been an attractive target since the early 1990s. However, it took the recognition of the key function of the HIV-1 integrase enzyme, the proviral DNA strand-transfer reaction, to develop true integrase inhibitors with clinical effectiveness.[64–66] Figure 145.8 illustrates the function of the HIV-1 integrase enzyme and the point at which integrase strand transfer inhibitors block virus replication.

Raltegravir

Description

Raltegravir – *N*-[(4-Fluorophenyl)methyl]-1,6-dihydro-5-hydroxy-1-methyl-2-{1-methyl-1-[[(5-methyl-1,3,4-oxadiazol-2-yl)carbonyl]amino]ethyl}-6-oxo-4-pyrimidinecarboxamide monopotassium salt (RAL; see Fig. 145.9) – is the lead drug in a new and important class of antiretrovirals called integrase inhibitors. Raltegravir binds to divalent cations in the catalytic core of integrase, preventing the formation of covalent bonds between integrase and host DNA. Three classes of integrase inhibitors have been identified: diketoacids, hydroxyquinolones and polyphenols. Raltegravir is a member of the diketoacid class.[66,67]

Pharmacokinetics and distribution

Raltegravir is administered orally with a mean peak concentration of 4.5 µmol/l achieved approximately 3 hours after dosing. It may be administered without regard to food, but a high-fat meal has been shown to increase the AUC by about 20% and slow the rate of absorption. Approximately 83% of raltegravir is protein-bound.

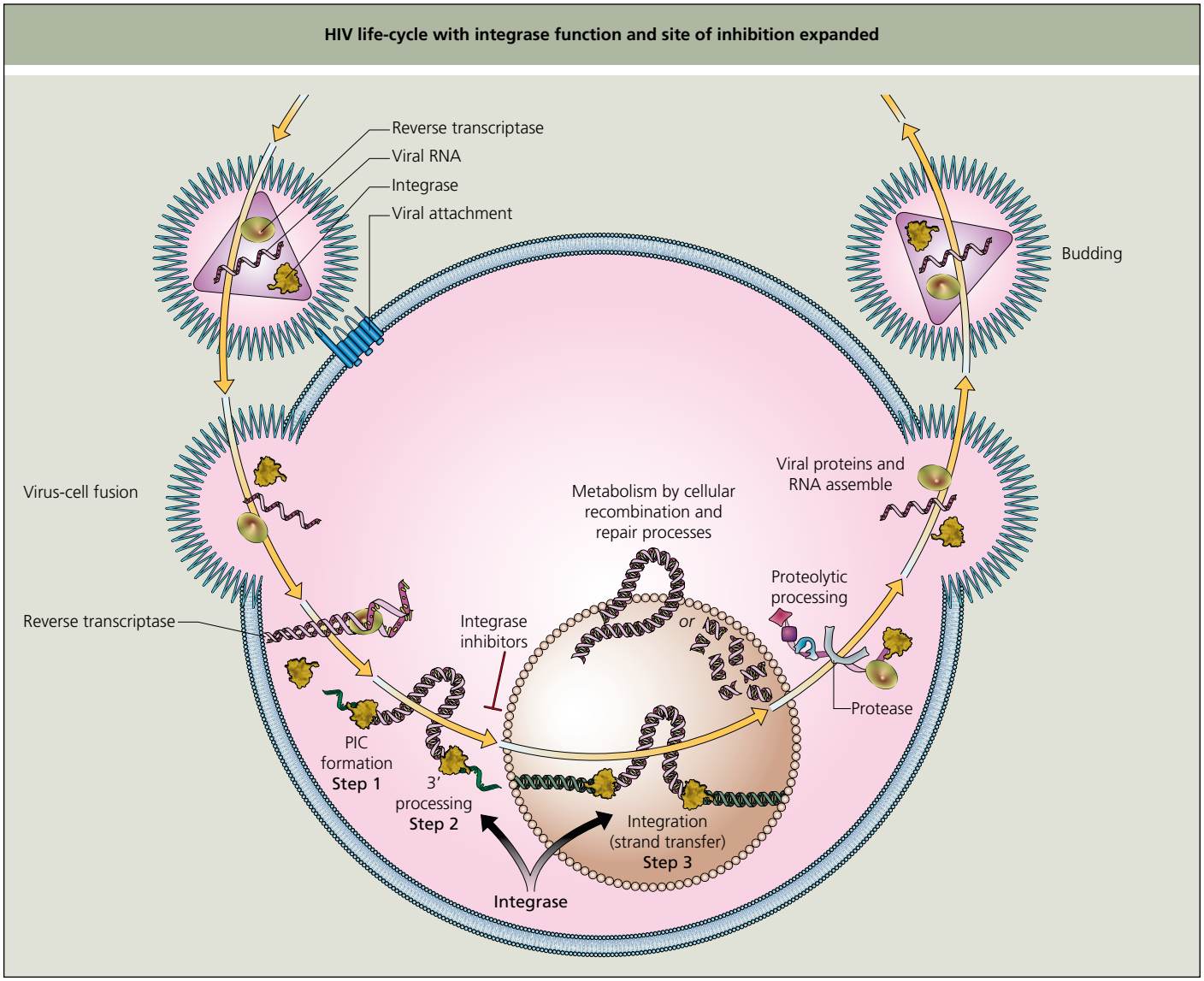


Fig. 145.8 HIV life-cycle with integrase function and site of inhibition expanded. (Courtesy, Dara Hazuda, Merck Research Laboratories.)

Raltegravir is metabolized by uridine diphosphate-glucuronosyltransferase (UGT) to a glucuronide metabolite, mainly by UGT1A1. The dose is eliminated in both the urine (32%) and feces (51%), with metabolite accounting for 23% and 0% in the urine and feces, respectively. The half-life of raltegravir is approximately 9 hours.

Use

The usual adult dosage is 400 mg twice daily.

Raltegravir is primarily used in combination with other antiretroviral agents in persons with treatment-experience and multidrug-resistant virus. Raltegravir's approval was based on the results of two phase III studies which demonstrated clear superiority when raltegravir was added to an optimized background antiretroviral regimen compared to optimized background alone.[68,69]

Raltegravir has also demonstrated powerful, rapid, and durable antiretroviral activity in treatment-naive patients and studies are ongoing to determine what this agent's role might be in first-line therapy.[3,70,71]

Resistance

Raltegravir resistance is associated with mutations in HIV-1 integrase and evolves along one of three mutational pathways. The signature mutations for these pathways are Y143R/H/C, Q148H/K/R and N155H (see Fig. 145.3). Q148H+G140S is the most common pathway described thus far. Additional mutations seen are L74M+E138A and E138K. Additional mutations in the N155H pathway include L74M, E92Q, T97A, Y143H, G163K/R, V151I and D232N. The Y143R/H/C mutational pathway appears to be uncommon.[68]

Dosage in special circumstances

No dosage adjustment is necessary in patients with renal dysfunction. Raltegravir pharmacokinetics have not been studied in patients with severe hepatic dysfunction, but no dosage adjustment is necessary in patients with mild-to-moderate hepatic impairment.

Developmental and reproductive toxicity animal studies suggest possible fetal toxicity and therefore raltegravir is classified as pregnancy category C.

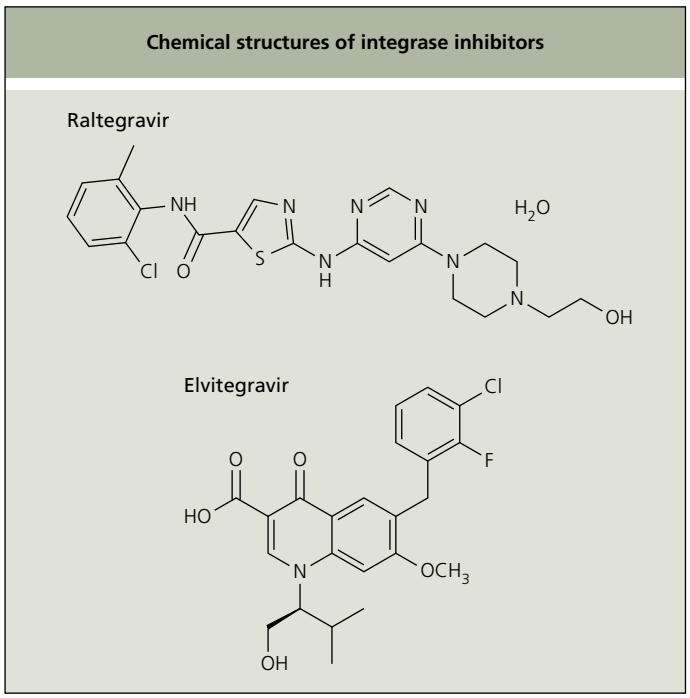


Fig. 145.9 Chemical structures of integrase inhibitors.

Adverse reactions and drug interactions

Raltegravir is generally well tolerated. The most commonly associated adverse effects reported with raltegravir are nausea, headache and diarrhea. Raltegravir is not a CYP450 substrate, inducer or inhibitor and therefore is not expected to interact with drugs metabolized by CYP450 enzymes. As raltegravir is metabolized by UGT1A1, strong inducers of UGT1A1 such as rifampin (rifampicin) are expected to significantly decrease raltegravir concentrations and must be used with caution. Less strong inducers and inhibitors such as atazanavir have not been shown to significantly decrease raltegravir concentrations or increase toxicity, respectively.

CONCLUSION

The field of antiretroviral therapy has shown dramatic growth over the past 22 years with 25 agents in seven drug classes/subclasses approved over this time period. Clinicians and patients now have numerous choices for initial and subsequent therapy such that long-term viral suppression, immunologic preservation and clinical health should be achievable in most patients. The challenges of long-term adherence, toxicities and emerging drug resistance remain, however, such that the drug development pipeline needs to remain robust. In addition, and most critically, the advances in the developed world must continue to be translated into public health approaches for resource-limited settings where 90% of individuals with HIV infection live (see also Chapter 96).

REFERENCES

References for this chapter can be found online at http://www.expertconsult.com

Chapter 146

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Drugs for herpesvirus infections

DRUGS FOR TREATMENT OF HSV AND VZV INFECTIONS

Aciclovir and valaciclovir

Mechanism of action and *in-vitro* activity

Aciclovir, an acyclic analogue of guanosine, is a selective inhibitor of the replication of herpes simplex virus (HSV) types 1 and 2 and varicella-zoster virus (VZV). Valaciclovir is an orally administered prodrug of aciclovir with improved pharmacokinetic properties. Aciclovir is converted to its monophosphate derivative by virus-encoded thymidine kinase (TK), a reaction that does not occur to any significant extent in uninfected cells (Fig. 146.1). Subsequent diphosphorylation and triphosphorylation steps are catalyzed by cellular kinases, producing high concentrations of aciclovir triphosphate within HSV- or VZV-infected cells. Aciclovir triphosphate inhibits viral DNA synthesis by competing with deoxyguanosine triphosphate as a substrate for viral DNA polymerase. Since aciclovir triphosphate lacks the 3´-hydroxyl group required for further DNA chain elongation, incorporation into viral DNA results in obligate chain termination. Viral DNA polymerase has much higher affinity for aciclovir triphosphate than does cellular DNA polymerase, resulting in little incorporation of aciclovir into cellular DNA. Aciclovir exhibits good *in-vitro* activity against HSV-1, HSV-2 and VZV, with median inhibitory concentrations (IC_{50}) of 0.04, 0.10 and 0.50 µg/ml, respectively. Human cytomegalovirus (CMV) is not inhibited by aciclovir at clinically achievable concentrations.

Pharmacokinetics and distribution

Following oral administration, aciclovir is slowly and incompletely absorbed, with bioavailability of about 15–30%. After oral doses of 200 mg or 800 mg of aciclovir, mean plasma peak concentrations (C_{max}) at steady state are about 0.66 µg/ml and 1.6 µg/ml, respectively. Steady-state peak plasma aciclovir concentrations after intravenous doses of 5 mg/kg or 10 mg/kg of body weight every 8 hours are about 10 µg/ml and 20 µg/ml, respectively. Plasma protein binding is less than 20%. Aciclovir penetrates well into most tissues, including the central nervous system (Table 146.1). The CSF aciclovir area under the concentration–time curve (AUC) is about 20% of the serum AUC. In non-inflamed eyes, the mean vitreous-to-serum concentration ratio for aciclovir is about 24%. Significant concentrations of aciclovir (up to 300% of the serum concentration) can be found in breast milk. Aciclovir is minimally metabolized and about 85% of an administered dose is excreted unchanged in the urine via glomerular filtration and renal tubular secretion. The terminal plasma half-life of acyclovir is 2–3 hours in adults and 3–4 hours in neonates with normal renal function, but is extended to about 20 hours in anuric subjects.

Valaciclovir is an orally administered prodrug of aciclovir designed to overcome the problem of poor oral bioavailability. Valaciclovir, the L-valine ester of aciclovir, is well absorbed from the gastrointestinal tract via a stereospecific transporter and undergoes essentially complete first pass conversion in the gut and liver to yield aciclovir and L-valine. This prodrug improves bioavailability to about 54%, yielding peak plasma aciclovir levels that are three- to fivefold higher than those achieved with oral aciclovir. Oral doses of 500 mg or 1000 mg of valaciclovir produce peak plasma aciclovir concentrations of 3.3 µg/ml and 5–6 µg/ml, respectively. After administration of valaciclovir at a dose of 2 g orally four times daily, plasma aciclovir AUC values approximate those produced by aciclovir given intravenously at a dose of 10 mg/kg every 8 hours. Following enzymatic conversion of valaciclovir to aciclovir, the antiviral spectrum of activity, pharmacokinetic properties and excretion are the same as those described above.

Route of administration and dosage

Aciclovir is available in topical, oral and intravenous formulations. Outside of the USA, aciclovir is also available as a 3% preparation for ophthalmologic use. The dermatologic preparations consist of 5% aciclovir in a polyethylene glycol ointment or propylene glycol cream base. Oral aciclovir products include a 200 mg capsule, 400 mg and 800 mg

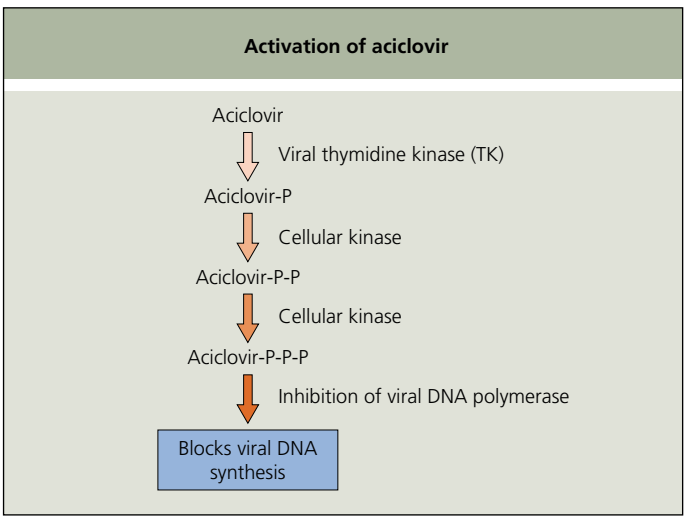


Fig. 146.1 Activation of aciclovir is dependent on monophosphorylation via viral thymidine kinase (TK). Aciclovir triphosphate inhibits the activity of viral DNA polymerase, thus blocking viral replication. Penciclovir and ganciclovir are activated by similar mechanisms.

Table 146.1 Tissue distribution of aciclovir*

	CSF/CNS	Lung	Liver	Kidney	Breast milk	Amniotic fluid	Heart
Aciclovir concentration (μg/ml)	10	26.7	26.7	206	66.7	<0.1–2.6	26.7

*Based on a steady-state plasma concentration (20.6 μg/ml) after intravenous aciclovir dosing of 15 mg/kg q8h.

tablets, and a liquid suspension (200 mg/5 ml). Aciclovir sodium for intravenous infusion is supplied as a sterile water-soluble powder that must be reconstituted and diluted to a concentration of 50 mg/ml.

The recommended dose of aciclovir will vary with the specific indication (Table 146.2). Because of the greater intrinsic resistance of VZV to aciclovir, the doses required for treating VZV infections are higher than those used for HSV infections. In adults with normal renal function, oral aciclovir is given at a dose of 200 mg (for HSV) to 800 mg (for VZV) five times daily. The recommended dose of intravenous aciclovir is 5 mg/kg every 8 hours for HSV infections and 10 mg/kg every 8 hours for VZV infections, although higher doses (12–15 mg/kg every 8 hours) are sometimes used for life-threatening infections, especially in immunocompromised patients.

Valaciclovir is available as 500 mg and 1000 mg tablets. The recommended doses are 500 mg every 12 hours for episodic treatment of recurrent genital HSV infections and 1000 mg every 8 hours for treatment of herpes zoster. A suspension preparation is not available.

Indications

HSV infections

Aciclovir and valaciclovir are effective for treatment of initial and recurrent episodes of genital herpes as well as for suppressive therapy (see Table 146.3).[1,2] There is no evidence of cumulative toxicity or emergence of drug-resistant HSV in immunocompetent patients even after years of suppressive therapy.[3] Suppression with aciclovir or valaciclovir will also significantly reduce (but not eliminate) the frequency of asymptomatic viral shedding and reduce the risk of transmission of genital herpes to an uninfected partner.[4,5]

Antiviral prophylaxis with aciclovir or valaciclovir can significantly reduce the necessity for Caesarian section due to active genital HSV lesions present at the onset of labor.[6] For babies that develop neonatal herpes, intravenous aciclovir therapy can significantly reduce both morbidity and mortality.

Valaciclovir (2 g every 12 hours for 1 day) has been shown to reduce the duration of an episode of recurrent herpes labialis by 1.1 days.[7] Suppressive therapy with valaciclovir (1 g daily) can reduce the frequency of clinical recurrences by about 50%.[8] Combination therapy with an antiviral drug (such as aciclovir or valaciclovir) plus corticosteroids is widely used for treatment of Bell's palsy, but the demonstrated benefit from the antiviral therapy has varied among clinical trials.[9] Oral aciclovir and valaciclovir have both been used successfully for treatment and prophylaxis of herpetic keratitis.

Aciclovir or valaciclovir prophylaxis of HSV infections is highly effective in severely immunocompromised patients, particularly those undergoing induction chemotherapy or organ transplantation. Clinical experience suggests that intravenous aciclovir (10 mg/kg every 8 hours) is the treatment of choice for disseminated or visceral HSV infection (e.g. pneumonitis, hepatitis, esophagitis, etc.) in immunocompromised patients, although data from controlled clinical trials are lacking. Aciclovir is the drug of choice for HSV encephalitis and should be given intravenously at a dose of 10–15 mg/kg every 8 hours for 14–21 days.

VZV infections

Oral aciclovir therapy (20 mg/kg, up to a maximum of 800 mg, every 6 hours) is effective for immunocompetent children with chickenpox, but the benefits are modest and many pediatricians consider antiviral treatment of chickenpox to be optional. Adolescents and adults are at greater risk for varicella complications; aciclovir therapy (800 mg orally five times daily for 7 days) is recommended for those who present within 24–48 hours of disease onset. Valaciclovir is also likely to be effective in this setting, but data from controlled clinical trials are lacking. Immunocompromised patients with chickenpox should receive intravenous aciclovir (10 mg/kg or 500 mg/m^2 every 8 hours for 7–10 days). Clinical experience suggests that intravenous aciclovir is the treatment of choice for patients with VZV infections complicated by visceral involvement (e.g. pneumonitis, encephalitis, etc.).

Both aciclovir and valaciclovir are effective for treatment of localized herpes zoster in immunocompetent patients if therapy is initiated within 48–72 hours of rash onset. Valaciclovir has the advantage of a simpler dosing regimen. Severely immunocompromised patients (e.g. bone marrow transplant, cancer chemotherapy, etc.) with herpes zoster are at high risk for disseminated VZV infection and should be treated with intravenous aciclovir (10 mg/kg every 8 hours).

Other viral infections

While aciclovir is ineffective for established CMV infections, high-dose oral aciclovir or valaciclovir may have value for CMV prophylaxis in high-risk populations such as organ transplant recipients.[10] Aciclovir or valaciclovir can induce regression of Epstein–Barr virus (EBV)-associated oral hairy leukoplakia in HIV-infected patients. Aciclovir is considered the drug of choice for therapy of rare human infections

Table 146.2 Indications for aciclovir therapy

Infection	Route and dosage*
Genital HSV	
Initial episode	200 mg po 5 times/d (or 400 mg q8h) × 10 days
Initial episode with complications	5 mg/kg iv q8h × 5–7 days
Recurrent episodes	200 mg po 5 times/d (or 400 mg q8h) × 5 days
Suppression	400 mg po q12h daily
Mucocutaneous HSV in immunocompromised patient	400 mg po 5 times/d × 10–14 days, or 5 mg/kg iv q8h × 10 days
Disseminated or visceral HSV (including encephalitis)	10–15 mg/kg iv q8h × 14–21 days
Neonatal HSV	10–15 mg/kg iv q8h × 14–21 days
Varicella (chickenpox)	
Normal host	20 mg/kg (max. 800 mg) po 4–5 times/d × 5 days
Immunocompromised patient	10–15 mg/kg iv q8h × 7–10 days
Herpes zoster (shingles)	
Normal host	800 mg po 5 times/d × 7–10 days
Immunocompromised patient (disseminated or visceral VZV)	10–15 mg/kg iv q8h × 7–10 days

*Given doses are indicated for patients with normal renal function.

Table 146.3 Oral antiviral therapy for genital herpes*

Drugs	Initial episode	Episodic therapy	Suppressive therapy
Aciclovir	200 mg q5h × 7–10 days *Alternative dose*: 400 mg q8h × 7–10 days	200 mg q5h × 5 days *Alternative doses*: 400 mg q8h × 5 days 800 mg q12h × 5 days 800 mg q8h × 2 days	400 mg q12h daily
Famciclovir	250 mg q8h × 7–10 days	125 mg q12h × 5 days *Alternative dose*: 1000 mg q12h × 1 day	250 mg q12h daily
Valaciclovir	1000 mg q12h × 7–10 days	500 mg q8h × 3 days *Alternative dose*: 1000 mg q24h × 5 days	500 mg once daily *Alternative doses*: 1000 mg q24h daily 500 mg q12h daily

*Recommended doses for immunocompetent adults with normal renal function.[1,50]

caused by cercopithecine herpesvirus-1 (B virus). Aciclovir is not directly active against HIV; however, studies of HIV/HSV-2 co-infected men have shown significant reductions in HIV plasma levels in those patients receiving valaciclovir.[11]

Dosage in special circumstances

Aciclovir is cleared primarily by renal mechanisms and dosage modification of aciclovir and valaciclovir is required for patients with significant renal dysfunction (Tables 146.3, 146.4). The mean elimination half-life of aciclovir after a single 1 g dose of valaciclovir is about 14 hours in patients with end-stage renal disease. No specific dosage modification is required for patients with hepatic impairment. Aciclovir and valaciclovir are not approved for use in pregnancy, but have been widely used to treat HSV and VZV infections in pregnant women without evidence of maternal or fetal toxicity.

In a study of pediatric cancer patients, the bioavailability of aciclovir after valaciclovir dosing was 64%. Aciclovir AUC values after oral valaciclovir dosing are slightly higher in elderly individuals when compared with younger control groups, due to age-related decline in renal function. Because no liquid valaciclovir preparation is available, experience with this drug in very young children is limited.

Adverse reactions

Aciclovir and valaciclovir are extremely well-tolerated drugs with few significant adverse effects. Allergic reactions to aciclovir or valaciclovir have been reported, but are very uncommon. With intravenous aciclovir therapy, inflammation and phlebitis may occur following localized drug extravasation. Renal dysfunction (including cases of acute renal failure) resulting from accumulation of aciclovir crystals in the kidneys has been observed following administration of large doses of aciclovir by rapid intravenous infusion, but is uncommon and usually reversible.

The risk of nephrotoxicity can be minimized by administering aciclovir by slow infusion (over 1 hour) and ensuring adequate hydration. Rarely, nephrotoxicity has been observed following oral dosing of aciclovir or valaciclovir. Risk factors for aciclovir-induced

Table 146.4 Aciclovir dosage modification for renal impairment

	ADJUSTED DOSAGE REGIMEN		
Normal dosage regimen	**CrCl (ml/min/1.73 m²)**	**Dose**	**Dosing interval (hr)**
Aciclovir 200 mg po q4h	>10 0–10	200 mg 200 mg	4 (5 times/day) 12
Aciclovir 400 mg po q12h	>10 0–10	400 mg 200 mg	12 12
Aciclovir 800 mg po q4h	>25 10–25 0–10	800 mg 800 mg 800 mg	4 (5 times/day) 8 12
Aciclovir 5 mg/kg iv q8h	>50 25–50 10–25 0–10	5 mg/kg 5 mg/kg 5 mg/kg 2.5 mg/kg	8 12 24 24
Aciclovir 10 mg/kg iv q8h	>50 25–50 10–25 0–10	10 mg/kg 10 mg/kg 10 mg/kg 5 mg/kg	8 12 24 24

CrCl, creatinine clearance.

nephropathy include pre-existing renal dysfunction and concomitant use of other nephrotoxic drugs.

Reports have linked administration of aciclovir with CNS disturbances, including agitation, hallucination, disorientation, tremors, clonus and seizures. Neurotoxicity has most often been recognized in elderly patients with underlying CNS abnormalities and renal insufficiency. Elevated concentrations of the main aciclovir metabolite, 9-carboxymethoxymethylguanine (CMMG), have been detected in serum and CSF of patients who developed neuropsychiatric symptoms while receiving aciclovir or valaciclovir and could play an etiologic role.[12] Aciclovir-induced neurologic toxicity has been successfully treated by emergent hemodialysis.

Patients receiving oral aciclovir or valaciclovir therapy occasionally complain of nausea, diarrhea, rash or headache, but at rates that do not differ from the placebo recipients. The safety of oral aciclovir and valaciclovir for long-term administration has been confirmed in patients receiving the drug for over 5 years for suppression of recurrent genital herpes.[3]

A syndrome of thrombotic microangiopathy (TMA) was described in HIV-infected patients receiving investigational high-dose valaciclovir (8 g/day) for prevention of CMV disease. A causal relationship between high-dose valaciclovir and TMA has not been proven. The TMA-like syndrome, which is characterized by fever, microangiopathic hemolytic anemia, thrombocytopenia and renal dysfunction, has not been observed in immunocompetent patients receiving valaciclovir at approved doses (up to 3 g/day). There is no contraindication to using valaciclovir at standard doses in HIV-infected patients and it has proven safe in wide clinical experience.[13]

Significant interactions between aciclovir and other drugs are extremely uncommon. Probenecid decreases the renal clearance of aciclovir and can prolong the plasma excretion half-life. Additive aciclovir-induced nephrotoxicity in patients receiving concomitant ciclosporin A therapy has been suggested. Lethargy has been reported in a few patients receiving both aciclovir and zidovudine, but a causative role for aciclovir has not been established. Concomitant administration of cimetidine and probenecid reduces the rate of valaciclovir conversion to aciclovir, but the effect is not clinically significant.

Resistance

Herpes simplex virus resistance to aciclovir can develop through mutation of the viral genes encoding thymidine kinase or DNA polymerase.[14] Most aciclovir-resistant clinical HSV isolates are TK deficient and are, therefore, unable to phosphorylate aciclovir. Consequently, these isolates will also be resistant to valaciclovir, penciclovir, famciclovir and ganciclovir, all of which have the same mechanism of action and require viral TK for activation.

Disease caused by aciclovir-resistant HSV isolates occurs almost exclusively in immunocompromised patients. The most common clinical presentation of infection caused by aciclovir-resistant HSV is chronic, progressive mucocutaneous ulcerations. Approximately 5–6% of HSV isolates recovered from HIV-seropositive patients are aciclovir-resistant (IC_{50} > 2.0 µg/ml).[15] Aciclovir-resistant VZV isolates (which are less frequently encountered than resistant HSV isolates) are occasionally recovered from AIDS patients. Clinical disease caused by aciclovir-resistant VZV has usually been limited to cutaneous involvement, often characterized by atypical lesions. The options for treatment of aciclovir-resistant HSV or VZV disease are foscarnet and cidofovir.

Penciclovir and famciclovir

Mechanism of action and *in-vitro* activity

Penciclovir is an acyclic guanine derivative that is similar to aciclovir in structure, mechanism of action and spectrum of antiviral activity. In HSV- or VZV-infected cells, penciclovir is first monophosphorylated by virally encoded TK and then further phosphorylated to the triphosphate moiety by cellular enzymes. Penciclovir triphosphate blocks viral DNA synthesis through competitive inhibition of viral DNA polymerase. Unlike aciclovir triphosphate, penciclovir triphosphate is not an obligate chain terminator and can be incorporated into the extending DNA chain. Compared with aciclovir triphosphate, intracellular concentrations of penciclovir triphosphate are much higher. For example, the half-life values for penciclovir triphosphate and aciclovir triphosphate in HSV-1-infected cells are 10 hours and 0.7 hour, respectively. However, this potential advantage is offset by a much lower affinity of penciclovir triphosphate for viral DNA polymerase. The *in-vitro* activities of penciclovir against HSV-1, HSV-2 and VZV are similar to those of aciclovir, with median IC_{50} values of 0.4, 1.5 and 4.0 µg/ml, respectively, in MRC-5 cells.

Just as valaciclovir is a prodrug of aciclovir, famciclovir is a prodrug of penciclovir. Because penciclovir is very poorly absorbed, famciclovir (the diacetyl ester of 6-deoxy-penciclovir) was developed as the oral formulation. The first acetyl side chain of famciclovir is cleaved by esterases found in the intestinal wall. On first pass through the liver, the second acetyl group is removed and oxidation catalyzed by aldehyde oxidase occurs at the 6 position, yielding penciclovir, the active antiviral compound.

Pharmacokinetics and distribution

Intravenous infusion of penciclovir at 10 mg/kg over 1 hour yields a peak plasma concentration of 12.1 µg/ml. Plasma protein binding of penciclovir is <20%. The drug is cleared by renal tubular secretion and passive filtration. The plasma elimination half-life of penciclovir is about 2 hours and approximately 70% is recovered unchanged in the urine.

When administered as the famciclovir prodrug, the bioavailability of penciclovir is about 77%. Following a single oral dose of 250 mg or 500 mg of famciclovir, peak plasma penciclovir concentrations of 1.9 µg/ml and 3.5 µg/ml, respectively, are achieved at 1 hour. The pharmacokinetics of penciclovir are linear and dose independent over a famciclovir dosing range of 125–750 mg. Food slows famciclovir absorption and lowers the peak plasma penciclovir concentration, but does not alter the AUC value.

Route of administration and dosage

Famciclovir is available as 125 mg, 250 mg and 500 mg tablets. Recommended dosages will vary with the indication. The usual dose of famciclovir is 125 mg every 12 hours for episodic therapy of recurrent genital herpes and 500 mg every 8 hours for herpes zoster. The intravenous preparation of penciclovir has not been commercially released. A topical preparation of penciclovir is available as a 1% cream for treatment of HSV labialis.

Indications

Genital HSV infections

Famciclovir is effective for initial and recurrent genital herpes and also provides effective suppressive therapy[1,16,17] (see Table 146.3). Famciclovir suppressive therapy reduces mucosal viral shedding from HSV-2 infected persons,[18] but its effect on virus transmission has not been studied. Famciclovir is also effective for suppression and treatment of recurrent mucocutaneous (orolabial and anogenital) infections in HIV-infected patients.

Three drugs (aciclovir, valaciclovir and famciclovir) with proven efficacy for long-term suppression of recurrent genital herpes are currently available. Direct comparative trials are not available to select the most effective treatment.[19] All three compounds are safe and well tolerated. Considerations in selecting the appropriate drug may include cost and dosing convenience. For any patient, the goal is to establish a suppressive regimen that is effective, economical and convenient. For these drugs, a dose–response relationship exists, meaning that higher total daily doses generally produce more complete suppression. Dose titration of the selected drug will be needed to identify optimal treatment for an individual patient (see Table 146.3).

Herpes labialis

Topical 1% penciclovir cream (applied every 2 hours while awake) and oral famciclovir (1500 mg) have demonstrated efficacy for treatment of herpes labialis.[20]

Herpes zoster

Famciclovir accelerated cutaneous healing and reduced the duration of viral shedding and postherpetic neuralgia in immunocompetent patients with dermatomal herpes zoster.[21] Famciclovir was proven effective for herpes zoster therapy in bone marrow transplant, cancer and HIV-seropositive patients. In the USA, the recommended dose of famciclovir for uncomplicated herpes zoster is 500 mg three times daily. Doses of 250 mg three times daily and 750 mg once daily are approved in Europe and the UK.

Three drugs (aciclovir, valaciclovir and famciclovir) are currently available in the USA for treatment of uncomplicated herpes zoster in immunocompetent patients (a fourth drug, brivudin, is available in some countries and is discussed below) (Table 146.5). All three drugs are well tolerated and appear to be comparable in clinical efficacy. In a large randomized clinical trial, valaciclovir and famciclovir were shown to be therapeutically equivalent for treatment of herpes zoster.[22] Because of their improved pharmacokinetic profiles and simpler dosing regimens, valaciclovir and famciclovir are preferred over aciclovir for this indication.

Other viral infections

Famciclovir suppresses hepatitis B virus (HBV) replication by targeting the viral polymerase, but treatment of hepatitis B using famciclovir monotherapy resulted in rapid emergence of HBV resistance. Case reports have suggested that famciclovir is effective for treatment of EBV-induced oral hairy leukoplakia in HIV-infected patients.

Dosage in special circumstances

Penciclovir is cleared predominantly by renal mechanisms, so adjustments of famciclovir dosing are required in patients with advanced renal insufficiency. In patients with hepatic insufficiency, the rate of conversion of famciclovir to penciclovir is decreased, but the plasma AUC value for penciclovir is not significantly changed; no famciclovir dosage modification is necessary. Plasma penciclovir concentrations are slightly higher in elderly patients treated with famciclovir due to age-related reduction in glomerular filtration rates, but dosage modifications are not required. Absorption after topical application is minimal and no dosage modifications are required for use of penciclovir 1% cream. No liquid preparation of famciclovir is currently available and few data regarding use in small children are available. Famciclovir has not been approved for use during pregnancy.

Adverse reactions

Safety data collected from over 3000 patients given famciclovir have shown the drug to be very safe and well tolerated. The most frequently reported adverse experiences have included occasional headache, nausea and diarrhea.

No clinically significant drug interactions have been noted with famciclovir. Co-administration of famciclovir with cimetidine or theophylline will increase the penciclovir AUC by about 20%. Co-administration of famciclovir and digoxin results in a 19% increase in the peak digoxin concentration, but no change in the AUC.

Table 146.5 Oral antiviral therapy for herpes zoster*

Treatment options
- Aciclovir 800 mg q4h for 7–10 days
- Famciclovir 500 mg q8h for 7 days
- Valaciclovir 1000 mg q8 for 7 days
- Brivudin 125 mg q24h for 7 days

*Recommended doses for immunocompetent adults with normal renal function.

Resistance

The majority of clinically encountered aciclovir-resistant HSV and VZV isolates are TK deficient and thus will generally be resistant to penciclovir and famciclovir, which also require viral TK for activation. However, some HSV strains that are aciclovir resistant by virtue of altered TK or DNA polymerase mutations may retain susceptibility to penciclovir.

Brivudin

Brivudin is a highly potent antiviral agent selectively active against VZV and HSV-1 with therapeutic equivalence to aciclovir. Because of concerns about potential toxicity, commercial development of brivudin has halted in many countries (including the USA), but the drug is widely available in Europe.

Mechanism of action and *in-vitro* activity

Brivudin, a 5′-halogenated thymidine nucleoside analogue, is sequentially phosphorylated by viral TK and cellular kinases to form BVDU triphosphate, a competitive inhibitor of viral DNA polymerase and an alternative substrate for incorporation in viral DNA. The intracellular half-life in virus-infected cells is about 10 hours. The drug is highly active against VZV, with IC_{50} values 28–1100-fold lower than those of aciclovir. Brivudin is active against HSV-1, but not against HSV-2. Thus, the clinical development of the drug has focused on its role for herpes zoster therapy.

Pharmacokinetics and distribution

Brivudin is well absorbed after oral administration and absorption is not affected by food. There is high first-pass metabolism in the liver to bromovinyluracil, which lacks antiviral activity. At steady state (brivudin 125 mg daily for 5 days), the C_{max} and C_{min} are 1.7 μg/ml (at 1 hour) and 0.06 μg/ml, respectively. The plasma terminal elimination half-life is approximately 16 hours; metabolites are excreted in urine (65%) and plasma (21%). Brivudin is highly protein-bound (>95%).[23]

Route of administration and dosage

Brivudin is available as a 125 mg tablet and as a 0.1% ointment for ophthalmologic use. The standard dose for herpes zoster is 125 mg orally once daily for 7 days.

Indications

In randomized clinical trials, brivudin has been compared with aciclovir and famciclovir in immunocompetent patients with herpes zoster and was equivalent to the comparator drugs for end points of zoster lesion healing and pain resolution.[24] With its once-daily dosing, brivudin offers a potential advantage of convenience and improved patient adherence.

Dosage in special circumstances

The pharmacokinetic properties of brivudin in elderly patients or in patients with renal or hepatic failure are not significantly changed from those seen in healthy volunteers.

Adverse reactions

In clinical trials with brivudin, the most commonly observed adverse effects were nausea (2.1%), abdominal pain (0.8%), vomiting (0.5%) and headache (1%) and did not differ significantly from adverse

effects reported with aciclovir or famciclovir. Hypersensitivity reactions to brivudin are rare.

Brivudin has a critically important drug interaction that has been an obstacle to its regulatory approval in some countries. Bromovinyluracil (the primary metabolite of brivudin) irreversibly inhibits dihydropyrimidine dehydrogenase (DPD), an enzyme that regulates nucleoside metabolism.[25] Co-administration of brivudin with 5-fluorouracil (5-FU, a cancer chemotherapeutic agent) results in a 15-fold increase in systemic exposure to 5-FU, causing potentially lethal bone marrow suppression and gastrointestinal toxicity. Full recovery of DPD activity requires at least 18 days after brivudin dosing. Potential interactions with brivudin may also occur with other fluoropyrimidines such as flucytosine (5-FC), tegafur, floxuridine and capecitabine. Brivudin should be used with extreme caution in cancer patients to avoid concomitant dosing with 5-FU.

Other drugs

Vidarabine

Vidarabine was the first intravenous antiviral drug accepted for widespread clinical use, but has now been replaced by more potent antiviral drugs. Vidarabine 3% ophthalmic ointment is used for treatment of HSV keratoconjunctivitis.

Trifluridine

Trifluridine, a fluorinated pyrimidine nucleoside with good *in-vitro* activity against HSV, is a competitive inhibitor of HSV DNA polymerase. Trifluridine is widely used as a 1% ophthalmic solution for topical therapy of HSV keratitis. Topical trifluridine has also been used with moderate success for treatment of aciclovir-resistant mucocutaneous infections in AIDS patients.

Idoxuridine

Idoxuridine is an iodinated thymidine derivative with activity against HSV. Use of idoxuridine has been limited to topical application, since systemic administration is associated with significant myelosuppression. Idoxuridine (in topical 1% solution and 0.5% ointment formulations) has been used successfully for treatment of HSV keratitis, but has largely been replaced by topical trifluridine and aciclovir for this indication. Topical application of 15% idoxuridine in dimethyl sulfoxide was shown to shorten the course of herpes labialis in a placebo-controlled trial.

n-Docosanol

n-Docosanol is a 22-carbon fatty alcohol with *in-vitro* activity against several enveloped viruses, including HSV-1 and HSV-2. The drug acts by interfering with viral entry into target cells. n-Docosanol is available over the counter in a 2 g tube of 10% cream and is indicated for recurrences of herpes labialis.

DRUGS FOR TREATMENT OF CYTOMEGALOVIRUS INFECTIONS

Ganciclovir and valganciclovir

Mechanism of action and *in-vitro* activity

Ganciclovir is a nucleoside analogue that is structurally similar to aciclovir, but has a hydroxymethyl group at the 3´ position of the acyclic side chain. This relatively minor structural modification accounts for enhanced activity of ganciclovir against human CMV and also for the drug's greater toxicity. Ganciclovir triphosphate is a potent inhibitor of herpesvirus DNA replication, acting as both an inhibitor of and a substrate for viral DNA polymerase.[26]

In HSV- or VZV-infected cells, monophosphorylation of ganciclovir is induced by viral TK, as also occurs with aciclovir. In CMV-infected cells, ganciclovir monophosphorylation is carried out by a protein kinase encoded by the UL97 gene. The di- and tri-phosphorylation steps are mediated by cellular kinases. On a molar basis, aciclovir triphosphate is actually a more potent inhibitor of CMV than is ganciclovir triphosphate. However, aciclovir is a poor substrate for phosphorylation by the UL97 gene product; consequently, the concentration of ganciclovir triphosphate in CMV-infected cells is 10-fold higher than that of aciclovir triphosphate. Furthermore, the half-life of ganciclovir triphosphate in CMV-infected cells is 16.5 hours, compared with 2.5 hours for aciclovir triphosphate. Ganciclovir triphosphate does not function as a chain terminator, and can be incorporated into elongating viral DNA (and, to a much lesser extent, human DNA) where it functions to slow DNA chain extension.

Ganciclovir and aciclovir have similar *in-vitro* activity against HSV-1, HSV-2 and VZV. However, ganciclovir is much more active against CMV, with IC_{50} values of 0.1–1.8 μg/ml against clinical isolates.

Pharmacokinetics and distribution

Intravenous infusion of ganciclovir at a dose of 5 mg/kg yields peak and trough plasma levels of approximately 8 μg/ml and 1 μg/ml, respectively. Plasma protein binding is 1–2%. Reported plasma-to-CSF ratios for ganciclovir have ranged from 24% to 70%. Ganciclovir is not metabolized and is cleared by renal mechanisms, with an elimination half-life of about 3 hours. Ganciclovir is poorly absorbed after oral administration, with bioavailability of only 5–9%. Following oral dosing of ganciclovir at 1000 mg every 8 hours, steady-state plasma peak and trough concentrations of 1.2 μg/ml and 0.2 μg/ml, respectively, are achieved.

To overcome the limited oral bioavailability of ganciclovir, a prodrug called valganciclovir has been developed.[27] Valganciclovir, the L-valyl ester of ganciclovir, is rapidly and almost completely hydrolyzed to ganciclovir in the liver and intestinal wall. Bioavailability of ganciclovir is about 60% from the prodrug formulation and is significantly increased with food administration. Maximum plasma ganciclovir concentrations are four- to fivefold higher than those achieved after oral dosing with the parent drug. Oral valganciclovir doses of 450 mg and 875 mg once daily for 3 days produced peak plasma ganciclovir concentrations of 3.3 μg/ml and 6.1 μg/ml, respectively. The AUC of ganciclovir after administration of 900 mg valganciclovir is about 26 μg/ml/h, which is comparable to the AUC following administration of ganciclovir dosed at 5 mg/kg intravenously.

Route of administration and dosage

Ganciclovir is available as an oral capsule, an intravenous formulation and a delayed-release intraocular implant device. Recommended doses will vary with the indication. For treatment of acute CMV disease, the usual dose of intravenous ganciclovir is 5 mg/kg every 12 hours. Oral ganciclovir, supplied as 250 mg capsules, can be used for maintenance therapy of CMV disease at a dosage of 1000–2000 mg every 8 hours, but has largely been replaced by oral valganciclovir.[28,29]

Valganciclovir is available as a 450 mg tablet. The recommended dose for induction therapy of acute CMV retinitis is 900 mg orally every 12 hours for a total of 21 days, followed by maintenance therapy of 900 mg orally once daily. The dosage recommended for prophylaxis of CMV disease in kidney, heart and kidney–pancreas transplant recipients is 900 mg orally once daily. All dosages should be administered with food.

Indications

Treatment of CMV disease

Intravenous ganciclovir is used for therapy of serious CMV infections in immunocompromised patients, including CMV retinitis, pneumonitis, encephalitis and gastrointestinal disease. The usual dose of

Table 146.6 Systemic antiviral therapy for CMV retinitis*

Drugs	Induction therapy*	Maintenance therapy†
Ganciclovir	5 mg/kg iv q12h × 4–21 days	5 mg/kg iv daily, or 1000–2000 mg po q8h daily
Valganciclovir	900 mg po q12h × 14–21 days	900 mg po daily
Foscarnet	90 mg/kg iv q12h (or 60 mg/kg iv q8h) × 14–21 days	90–120 mg/kg iv daily
Cidofovir	5 mg/kg iv weekly × 2–3 weeks	5 mg/kg iv every other week

*Recommended doses for adults with normal renal function.
†Other therapeutic options include intraocular drug implants or intravitreal drug injections.

ganciclovir for induction therapy is 5 mg/kg given intravenously every 12 hours for 14–21 days, followed by a maintenance regimen (5 mg/kg once daily).

Intravitreal injections of ganciclovir have been used effectively for treatment of CMV retinitis, although ganciclovir intraocular implants are a better option for patients who cannot tolerate systemic ganciclovir therapy.

Ganciclovir, valganciclovir, foscarnet and cidofovir (see below) are all effective for initial and maintenance therapy of CMV retinitis in AIDS patients (Table 146.6). All of these drugs are associated with significant toxicity; drug selection in an individual patient hinges, to some extent, on which adverse effects would be most tolerable. Ganciclovir is primarily myelosuppressive, while foscarnet and cidofovir are nephrotoxic. Ganciclovir would be preferred in a patient who has baseline renal dysfunction or who requires therapy with other nephrotoxic drugs. Conversely, foscarnet might be a better choice in a patient with significant baseline neutropenia. Despite the survival benefits shown for foscarnet therapy in some studies, most clinicians use ganciclovir or valganciclovir for initial therapy on the basis of more predictable adverse effects. Combination therapy (e.g. ganciclovir plus foscarnet) may be more effective for CMV disease in selected patients.

Prophylaxis of CMV disease

Benefits of intravenous and oral prophylaxis of CMV infection in solid-organ transplant recipients have varied with the transplant type, immunosuppressive regimen and CMV serologic status of the donor and recipient.[30] Prophylaxis with intravenous ganciclovir or oral valganciclovir significantly reduces the incidence of CMV disease in high-risk immunocompromised patients, but is often complicated by drug-induced neutropenia. An alternative scheme is to withhold ganciclovir until there is early laboratory evidence (e.g. by polymerase chain reaction or antigenemia assay) of CMV activation. This pre-emptive therapy approach permits initiation of antiviral treatment before CMV disease becomes symptomatic, while avoiding the risk of neutropenia associated with long-term ganciclovir administration.[31–38]

Dosage in special circumstances

Because ganciclovir is cleared by renal mechanisms, dosage reduction is necessary in patients with creatinine clearance of <70 ml/min (Table 146.7). About 50% of an administered dose is removed during 4 hours of hemodialysis and dosing after dialysis is recommended. Valganciclovir dosage adjustment is required for patients with creatinine clearance <60 ml/min; the drug is not recommended for patients on hemodialysis. No dosage adjustment for hepatic impairment is necessary.

Ganciclovir is mutagenic, carcinogenic and causes reproductive toxicity in animal models. Use of ganciclovir or valganciclovir in pregnant or nursing women is not recommended without careful consideration of the risk–benefit ratio. Data on ganciclovir or valganciclovir use in children are currently limited. High inter- and intrapatient variability in ganciclovir levels was observed in kidney, bone marrow and pediatric liver and kidney transplant recipients.[39] Oral solution valganciclovir given to neonates with symptomatic congenital CMV disease resulted in plasma levels comparable to administration of intravenous ganciclovir;[40] however, valganciclovir is not commercially available in this formulation.

Adverse reactions

The most important adverse effects of ganciclovir noted in AIDS patients being treated for CMV retinitis were neutropenia and thrombocytopenia. About 40% developed granulocytopenia (absolute neutrophil count <1000/mm^3) and 15% had thrombocytopenia (platelet count <50 000/mm^3). Hematologic toxicity is also seen, although less commonly, in organ transplant recipients. Neutropenia and thrombocytopenia are usually reversible when ganciclovir therapy is discontinued. In many patients requiring ganciclovir therapy, neutropenia can be prevented or treated by co-administration of granulocyte colony-stimulating factor. Renal dysfunction has been reported in up to 20% of transplant recipients receiving ganciclovir prophylaxis, although this may be related to co-administration of other nephrotoxic drugs. In animal models, ganciclovir produces significant reproductive toxicity, especially azoospermia.

Valganciclovir appears to have hematologic toxicity similar to intravenous ganciclovir. Pooled data from two different studies indicated that 50% of patients developed granulocytopenia (absolute neutrophil count <1000/mm^3), 35% experienced anemia (hemoglobin <9.5 g/dl) and 23% developed thrombocytopenia (platelets <100 000/µl). Gastrointestinal complaints were also common; 41% reported diarrhea, 30% nausea, 21% vomiting and 15% abdominal pain. In a study of valganciclovir 450 mg twice daily in kidney transplant recipients to prevent CMV disease, 5.7% (four patients) developed agranulocytosis (neutrophil count <500 cells/mm^3) an average of 74 days after transplantation. Onset was abrupt and asymptomatic, with all patients recovering after discontinuation of valganciclovir.

In vitro, ganciclovir and zidovudine have mutually antagonistic antiviral activity, but this observation has not been shown to be clinically significant. Ganciclovir should be used with caution in combination with other myelosuppressive drugs such as zidovudine because of the risk of additive hematologic toxicity. When co-administered with didanosine, ganciclovir increases the AUC of didanosine from 50% to 114%; thus patients should be monitored for didanosine toxicity when these two drugs are administered together. Probenecid can reduce renal clearance of ganciclovir, resulting in clinically significant increases in ganciclovir AUC. Seizures have been reported in patients receiving concomitant therapy with ganciclovir and imipenem.

Resistance

HSV and VZV isolates that are TK deficient and aciclovir resistant will also be cross-resistant to ganciclovir. Ganciclovir resistance *in vitro* is defined as an IC_{50} >6 µM (1.5 µg/ml). In a study of 72 AIDS patients treated with ganciclovir, 5 of 13 culture-positive patients treated for >3 months excreted resistant virus. Ganciclovir-resistant CMV has been identified as a cause of retinitis, encephalitis and polyradiculopathy in AIDS patients and enteritis and viremia among solid-organ transplant patients. Among solid-organ transplant recipients, resistance rates of

Table 146.7 Ganciclovir and valganciclovir dosage modification for renal impairment

ADJUSTED DOSAGE REGIMEN			
Normal dosage regimen	**CrCl (ml/min)**	**Dose**	**Dosing interval (hr)**
Ganciclovir 5 mg/kg iv q12h	≥70 50–69 25–49 10–24	5 mg/kg 2.5 mg/kg 2.5 mg/kg 1.25 mg/kg	12 12 24 24
Ganciclovir 5 mg/kg iv q24h	≥70 50–69 25–49 10–24 HD	5 mg/kg 2.5 mg/kg 1.25 mg/kg 0.625 mg/kg 0.625 mg/kg	24 24 24 24 Post-HD (TIW)
Ganciclovir 1000 mg po q8h	≥70 50–69 25–49 HD	1000 mg 500 mg 500 mg 500 mg	8 8 24 Post-HD (TIW)
Valganciclovir 900 mg po q12h	>60 40–59 25–39 10–24 HD	900 mg 450 mg 450 mg 450 mg NR	12 12 24 48 –
Valganciclovir 900 mg po q24h	>60 40–59 25–39 10–24 HD	900 mg 450 mg 450 mg 450 mg NR	24 24 48 Twice weekly –

CrCl, creatinine clearance; HD, hemodialysis; NR, not recommended; post-HD, after each dialysis; TIW, three times weekly.

1.5–14% have been reported.[41,42] CMV resistance to ganciclovir is usually secondary to mutations in the UL97 gene, although alterations in the DNA polymerase gene have also been described. UL97 mutants remain susceptible to foscarnet, although polymerase mutants cross-resistant to both ganciclovir and foscarnet have been identified. Foscarnet and cidofovir are therapeutic alternatives for treatment of disease caused by ganciclovir-resistant CMV.

Foscarnet

Mechanism of action and *in-vitro* activity

Foscarnet is an analogue of inorganic pyrophosphate that functions as a noncompetitive inhibitor of herpesvirus DNA polymerase.[43] Foscarnet blocks the pyrophosphate binding site, preventing cleavage of pyrophosphate from deoxynucleotide triphosphates. Viral DNA polymerase is inhibited at foscarnet concentrations 100-fold lower than those required to inhibit cellular DNA polymerase. Unlike the aciclovir-like drugs discussed above, foscarnet is not a nucleoside analogue, does not require intracellular activation by viral kinase, and is not incorporated into the viral DNA chain. Therefore, TK-deficient HSV and VZV isolates that are resistant to aciclovir will remain susceptible to foscarnet. Foscarnet has *in-vitro* activity against HSV, VZV, CMV, EBV and human herpesvirus 6 (HHV-6). The IC_{50} for most clinical isolates of CMV is in the range of 100–300 µM, but varies considerably with the experimental conditions. Foscarnet can also inhibit viral reverse transcriptase and has *in-vitro* activity against hepatitis B virus and HIV.

Pharmacokinetics and distribution

Foscarnet has low oral bioavailability (approximately 17%) and is administered only by the intravenous route. Peak plasma concentrations after steady-state dosing at 60 mg/kg every 8 hours or 90 mg/kg every 12 hours are about 500 µM and 700 µM, respectively. Plasma protein binding is about 15%. CSF foscarnet levels demonstrate wide interpatient variability, but average about 66% of plasma levels at steady state. Foscarnet is not metabolized and about 80% of an administered dose is excreted unchanged in the urine by glomerular filtration and tubular secretion within 36 hours. About 20% of the foscarnet dose is retained in bone, presumably due to the drug's structural similarity to inorganic phosphate. This results in a complex pattern of drug disposition, in which the initial elimination half-life is about 4.5 hours, followed by a prolonged terminal half-life of about 88 hours as drug is released from bone. Plasma foscarnet levels are reduced about 50% following hemodialysis; dosing after dialysis is recommended.

Route of administration and dosage

Foscarnet is available only as an intravenous formulation. The usual dose for induction therapy of CMV retinitis is 90 mg/kg every 12 hours, with a maintenance dose of 90–120 mg/kg every 24 hours. For aciclovir-resistant HSV, the usual dose of foscarnet is 40 mg/kg every 8–12 hours. When given via a central venous catheter, the drug can be diluted to 24 mg/ml; for infusion through peripheral vein catheters, foscarnet must be diluted to 12 mg/ml to avoid local phlebitis. The foscarnet dose must be administered over at least 1 hour using an intravenous infusion pump; bolus infusion can result in severe toxicity.

Indications

Intravenous foscarnet is used primarily to treat diseases caused by drug-resistant strains of HSV, VZV or CMV, or to treat patients who are intolerant of first-line antiviral therapy. Use of foscarnet for prophylaxis is limited by toxicity.[44,45]

Dosage in special circumstances

Foscarnet is excreted by renal mechanisms and dosage adjustment is required even for minor degrees of renal insufficiency. Serum creatinine should be monitored at least every other day during foscarnet therapy to assess the need for further dose adjustment. Dosage modification in hepatic impairment is not required. The safety of foscarnet during pregnancy has not been adequately evaluated and use is not recommended unless no other alternative therapy is available. Little information has been published regarding foscarnet safety and tolerance in neonates and children.

Adverse reactions

The most important adverse effect caused by foscarnet is nephrotoxicity. Dose-limiting renal toxicity occurs in at least 15–20% of patients treated with foscarnet for CMV retinitis. The primary mechanism of renal toxicity appears to be acute tubular necrosis, although interstitial nephritis and crystalline nephropathy have also been described. Loading the patient with intravenous saline prior to foscarnet infusion can help reduce the risk of nephrotoxicity. In most cases, the renal dysfunction is reversible and serum creatinine will return to normal within 2–4 weeks after foscarnet therapy is discontinued. However, irreversible renal failure may occur in patients who are volume depleted or who receive concomitant therapy with other nephrotoxic medications.

Foscarnet can induce a variety of electrolyte and metabolic abnormalities, most notably hypocalcemia. Hypercalcemia, hypomagnesemia, hypokalemia and hypo- and hyperphosphatemia have also been reported. The acute decline in ionized serum calcium that can occur with foscarnet infusion may be due to formation of a complex between foscarnet and free calcium. Further depletion of total serum calcium seen with long-term drug administration may be caused by renal calcium wasting, abnormal bone metabolism, concurrent hypomagnesemia, or some combination of these factors. Foscarnet-induced electrolyte disturbances can predispose the patient to cardiac arrhythmias, tetany, altered mental status or seizures. It is mandatory that serum creatinine and electrolyte levels be closely monitored during foscarnet therapy.

Foscarnet is much less myelosuppressive than ganciclovir, but anemia was reported in 10–50% of AIDS patients receiving foscarnet. Patients, especially uncircumcised males, may develop genital ulcerations due to local toxicity of high foscarnet concentrations in urine. Nausea and vomiting has been reported by 20–30% of patients receiving foscarnet. Other infrequent adverse effects include headache, diarrhea and abnormal liver function tests. When possible, foscarnet should be administered through a central venous line to avoid peripheral thrombophlebitis.

Specific drug interactions with foscarnet have not been described, although there is significant potential for additive toxicity. Concurrent therapy with foscarnet and intravenous pentamidine can result in severe and potentially fatal hypocalcemia. Concomitant administration of foscarnet with other potentially nephrotoxic drugs (such as amphotericin B or aminoglycosides) can compound the risk of serious nephrotoxicity. Foscarnet can be safely administered to patients receiving zidovudine, although there may be an increased risk of anemia. Due to foscarnet's chelating properties, a number of drugs may precipitate when administered through the same intravenous catheter. Thus, review of the package insert is recommended for dosing recommendations and drug incompatibilities prior to administration.

Resistance

Although uncommon, foscarnet-resistant isolates of CMV, VZV and HSV have been encountered in AIDS patients receiving foscarnet therapy. Resistance is due to a mutation in the DNA polymerase gene, which means that, in some circumstances, the foscarnet-resistant isolate may remain susceptible to aciclovir or ganciclovir. However, CMV isolates cross-resistant to both ganciclovir and foscarnet (containing both polymerase and UL97 mutations) have been recovered. Cidofovir may be an effective alternative drug in this setting, but *in-vitro* antiviral susceptibility testing is necessary to guide drug selection.

Cidofovir

Mechanism of action and *in-vitro* activity

Cidofovir is a nucleotide analogue of cytosine monophosphate with potent broad-spectrum antiviral activity.[46] Unlike aciclovir and other nucleoside analogues which require monophosphorylation by viral kinases for activation, cidofovir already carries a phosphonate group and does not require viral enzymes for conversion to cidofovir diphosphate, the active antiviral compound. Cidofovir diphosphate competitively inhibits the DNA polymerases of herpesviruses, thereby blocking DNA synthesis and viral replication. Cidofovir diphosphate inhibits viral DNA polymerases at concentrations much lower than those required to inhibit cellular DNA polymerases, accounting for its selectivity of action.

Cidofovir has potent *in-vitro* activity against human CMV, with IC_{50} values in the range of 0.1–0.9 µg/ml. Cidofovir retains activity against most CMV clinical isolates that are resistant to ganciclovir. Cidofovir also demonstrates *in-vitro* activity against HSV and VZV (including TK-deficient, aciclovir-resistant isolates), adenovirus, poxvirus (including variola or smallpox virus) and human papillomavirus.

Pharmacokinetics and distribution

Serum cidofovir concentrations are dose proportional over a dosing range of 1.0–10.0 mg/kg. Intravenous infusion of cidofovir at a dosage of 5 mg/kg produces peak plasma concentrations of about 11 µg/ml. The terminal half-life is 2.6 hours. Approximately 90% of the intravenous cidofovir dose is excreted by the kidneys within 24 hours, with clearance involving both glomerular filtration and tubular secretion. At cidofovir doses higher than 3 mg/kg, concomitant administration of probenecid can block tubular secretion of cidofovir and reduce its renal clearance. Cidofovir diphosphate and its metabolites have prolonged intracellular half-lives, which permit cidofovir to be effectively administered at extended dosing intervals.

Route of administration and dosage

Cidofovir for intravenous administration is supplied as 375 mg of an aqueous solution (75 mg/ml). The selected dose is diluted in 100 ml of normal saline prior to administration. For induction therapy, the usual dose of cidofovir is 5 mg/kg infused over 1 hour once weekly for 2 weeks. The dose for maintenance therapy for CMV disease is 5 mg/kg administered once every 2 weeks. To minimize nephrotoxicity, patients should receive 1 liter of normal saline intravenously over 1–2 hours immediately prior to cidofovir dose and an additional 1 liter of normal saline immediately following the cidofovir dose. Probenecid is given at a dose of 2 g orally 3 hours before the cidofovir dose, then 1 g doses at 2 hours and 8 hours after completion of the cidofovir infusion, for a total probenecid dose of 4 g.

Indications

Cidofovir is used primarily to treat diseases caused by drug-resistant strains of HSV, VZV or CMV, or to treat patients who are intolerant of first-line antiviral therapy.[47,48] Use of cidofovir for prophylaxis or preemptive therapy is limited by toxicity.

Dosage in special circumstances

Because intravenous cidofovir can cause significant nephrotoxicity, initiation of therapy in patients with pre-existing renal dysfunction – serum creatinine >1.5 mg/dl, calculated creatinine clearance <55 ml/min or proteinuria >100 mg/dl (>2+) – is not recommended. Declining

renal function during cidofovir therapy mandates dosage adjustment. If the serum creatinine increases by 0.3–0.4 mg/dl above baseline, the cidofovir dose should be reduced from 5 mg/kg to 3 mg/kg. If the serum creatinine increases >0.5 mg/dl above baseline or if proteinuria >3+ develops, cidofovir therapy should be discontinued. Dosage adjustment in patients with hepatic impairment is not required. Cidofovir is embryotoxic in animals and the drug should not be used during pregnancy unless there are no other therapeutic options. In small studies of pediatric patients, cidofovir toxicity was similar to that seen in adults,[47] although large randomized trials are lacking.

Adverse reactions

The most important safety concern with cidofovir therapy is nephrotoxicity. Pretreatment with intravenous hydration and probenecid (which blocks cidofovir tubular secretion) reduces the incidence of nephrotoxicity. In a clinical trial using cidofovir 5 mg/kg plus probenecid, proteinuria occurred in 5 of 41 patients (12%) and elevated serum creatinine levels in 2 of 41 patients (5%). Neutropenia (ANC <750 WBC/mm^3) was observed in 15% of cidofovir recipients. Anemia, thrombocytopenia and hepatotoxicity have not been observed with cidofovir therapy. Ocular complications (including iritis, anterior uveitis and hypotony) have been described following intravenous or intravitreal cidofovir administration.

Probenecid, a benzoic acid derivative with a sulfa moiety, can cause allergic symptoms (e.g. fever, chills, rash) in patients allergic to sulfonamides. Patients with a history of severe sulfa hypersensitivity should not be treated with probenecid and, consequently, should not receive systemic cidofovir. Other probenecid-related adverse effects include headache, nausea and vomiting, which can be minimized by administering the drug on a full stomach. In a CMV retinitis trial, probenecid-related adverse effects occurred in 23 of 41 patients (56%) and were dose limiting in three patients (7%).

Cidofovir injections in rats were associated with mammary adenocarcinomas, but surveillance studies in treated patients have not demonstrated any excess frequency of tumors. Cidofovir administration causes embryotoxicity and impaired spermatogenesis in animals; male and female patients are advised to use adequate birth control during and for 3 months after completion of cidofovir therapy.

No specific drug interactions with cidofovir have been described, although concomitant therapy with other nephrotoxic drugs may result in additive toxicity. Probenecid, however, is known to alter the renal excretion of a wide variety of drugs. The dose of zidovudine should be reduced to 50% on days when probenecid administration is planned.

Resistance

Instances of clinical failure of cidofovir therapy due to drug resistance have been reported. CMV resistance to cidofovir results from a mutation in the viral polymerase gene and resistant isolates may exhibit cross-resistance to ganciclovir and/or foscarnet. *In-vitro* susceptibility testing is necessary in this circumstance to guide appropriate drug selection.

Fomivirsen

Mechanism of action and *in-vitro* activity

Fomivirsen is a 21-nucleotide phosphorothioate oligonucleotide designed as an antisense molecule with activity against CMV.[49] The oligonucleotide is complementary to mRNA from the immediate-early region 2 of CMV. Antisense inhibition of target gene expression, while necessary for optimal antiviral activity, only partially explains the activity of fomivirsen against CMV. Nonspecific interactions between the oligonucleotide and virus particles may prevent adsorption or lead to inhibition of enzymes required for viral DNA synthesis. Fomivirsen has activity against clinical CMV isolates, including isolates resistant to conventional antiviral drugs, with a median IC_{50} of 0.37 µM in tissue culture.

Phamacokinetics and distribution

When cynomolgus monkeys were given 11, 57 or 115 µg of fomivirsen intravitreally, maximum vitreal concentrations (ranging from 0.11 to 1.28 µM) were achieved 2 days after injection of all doses. For the same dosages, retinal concentrations increased in a logarithmic pattern, with maximal concentration obtained 2 days after injection for all doses, ranging from 0.12 to 0.88 µM. For the 115 µg dose, the vitreal and retinal half-lives were 22 hours and 78 hours, respectively. Electrophoretic analyses of retina and vitreous specimens indicate that the oligonucleotide is metabolized by exonucleolytic cleavage. Both intact drug and its metabolic products diffuse from the vitreous humor to the retina and it is hypothesized that active metabolism occurs in both compartments.

Route of administration and dosage

Fomivirsen is supplied in single use vials containing 0.25 ml of 6.6 mg/ml solution. Dosage for induction therapy of CMV retinitis is 330 µg given intravitreally every other week for two doses. Maintenance therapy is 330 µg given intravitreally every 4 weeks.

Indications

Fomivirsen is indicated for treatment of CMV retinitis in AIDS patients who have failed or are intolerant of other CMV therapies, including those with CMV that is resistant to ganciclovir and foscarnet. It is not recommended for use in patients who have received intravitreal or intravenous cidofovir. Fomivirsen is not indicated for systemic CMV therapy and will not protect the contralateral eye from involvement.

Dosage in special circumstances

As there is little systemic exposure to fomivirsen after intravitreal injection, no dose adjustments are required. This drug has not been studied in pregnant or lactating women, or in pediatric or geriatric populations.

Adverse reactions

The most common adverse reactions reported were increased intraocular pressure and mild to moderate intraocular inflammation of the anterior and posterior chambers. The combined incidence of ocular reactions was 10–12% of patients treated every other week and 20% in patients treated weekly. Other adverse reactions reported in 5–20% patients included abnormal or blurred vision, conjunctival hemorrhage, retinal detachment and retinal edema. Topical steroids have been used with success to ameliorate some of these effects.

Resistance

A fomivirsen-resistant CMV isolate has been developed by exposing a laboratory strain to increasing concentrations of the drug. This resistant virus did not prove to have mutations which would have altered specificity for the fomivirsen target sequence, supporting the hypothesis that both antisense-specific and nonspecific mechanisms are involved in the drug's activity against CMV. There have been no reports of fomivirsen-resistant CMV isolates recovered from patients.

REFERENCES

References for this chapter can be found online at http://www.expertconsult.com

Chapter 147

Michael G Ison

Antiviral agents against respiratory viruses

M2 Inhibitors

Introduction and mechanism of action

Amantadine (Symmetrel) and rimantadine (Flumadine) are symmetric tricyclic amines that specifically inhibit the replication of influenza A viruses at low concentrations (<1.0 μg/ml) by blocking the action of the M2 protein. M2, an acid-activated ion channel found only in influenza A viruses, is a membrane protein required for efficient nucleocapsid release after viral fusion with the endosomal membrane (Fig. 147.1). Unfortunately, widespread resistance to all M2 inhibitors has been documented in circulating H3N2 strains. As a result, this class of agents is not currently recommended for the prevention or treatment of influenza unless the strain is known to be H1N1.

Amantadine and rimantadine share two concentration-dependent mechanisms of antiviral action.[1,2] Low concentrations of the drugs inhibit the ion channel function of the M2 protein, which inhibits viral uncoating or disassembly of the virion during endocytosis and, in H7 subtypes, alters hemagglutinin (HA) maturation during viral assembly. Amantadine and rimantadine also increase the lysosomal pH, which in turn may inhibit virus-induced membrane fusion events for several enveloped viruses. However, such effects are generally not seen at drug concentrations observed in humans and clinically relevant antiviral activity is confined to influenza A viruses, although studies in chronic hepatitis C are in progress.

Pharmacokinetics

Amantadine is rapidly absorbed, with a 53–100% bioavailability, and reaches peak plasma levels of 475 μg/l within 4.5 hours after 100 mg doses in healthy adults.[3] The drug is predominately excreted unchanged in the urine by glomerular filtration and tubular secretion. The plasma elimination half-life is about 11–15 hours in persons with normal renal function. Elimination is markedly prolonged in patients with renal impairment and decreases about twofold in the elderly so that dose adjustments are required (Table 147.1). Amantadine is widely distributed, with salivary levels equivalent to those of blood and nasal mucus comparable to plasma at 8 hours after dosing.[5,6] Amantadine crosses the placenta and blood–brain barrier, with cerebrospinal fluid (CSF) levels equal to 56–96% of serum levels, and distributes in breast milk (Table 147.2).

In experimental human influenza infection, a trough concentration at steady state of 300 μg/l, which corresponds to that observed after 200 mg/day dosing, was associated with lower infection rate than placebo.[6] Adverse events are dose related, and daily doses >200 mg in young adults and 100 mg in the elderly are associated with excess CNS adverse effects.[3]

Rimantadine has nearly complete oral bioavailability and achieves maximal plasma concentration 3–5 hours after ingestion. Peak concentrations average 416 μg/l after a 200 mg oral dose. Rimantadine levels in nasal secretions average 1.5 times those of plasma levels. The plasma half-life is long and ranges from 24 to 36 hours. Rimantadine undergoes extensive metabolism, including hydroxylation, conjugation and glucuronidation in the liver before being excreted in the urine. Only 25% of the parent drug is excreted unchanged in the urine. Dose adjustments are recommended for advanced renal or hepatic failure and older age (see Table 147.1).

Resistance

Resistance to the two agents occurs as the result of amino acid substitutions in the transmembrane portion of the M2 protein. Although resistant wild-type virus is uncommonly found (<1%),[9] it may rapidly emerge within 2–4 days after the start of therapy in up to 30% of patients.[10] Emergence of resistant virus does not appear to cause a rebound in illness in immunocompetent adults but may be associated with protracted illness and shedding in immunocompromised hosts.[11] Importantly, resistant virus can be spread to others and has caused failures of antiviral prophylaxis under close contact conditions, as in nursing homes and households.[10] The resistant virus appears to retain wild-type pathogenicity and causes an influenza illness indistinguishable from susceptible strains. Cross-resistance occurs to all M2 inhibitors without affecting susceptibility to the neuraminidase inhibitors and ribavirin. Recently, most circulating H3N2 viruses have been documented to be resistant to the M2 inhibitors; resistance among H1N1 viruses is increasing as well.[12] Among H5N1 viruses, resistance is commonly seen with clade 1 and clade 2 subclade 1 viruses; current susceptibility data should be obtained from reference viral isolation laboratories if M2 inhibitor therapy is being considered. The novel swine-origin 2009 H1N1 viruses are all currently resistant to the M2 inhibitors as well.

Indications

Amantadine and rimantadine are indicated for the prevention and treatment of influenza A virus illness; given significant resistance, these agents are not currently recommended for the prevention or treatment of influenza unless the susceptibility of circulating strains of H1N1 are known. In the majority placebo-controlled studies of these drugs in the management of influenza, most have been conducted in previously healthy persons.[6]

Prophylaxis

Prophylaxis with amantadine or rimantadine is approximately 70–90% effective in preventing symptomatic influenza A infections.[13] Administration is advised for postexposure prophylaxis in

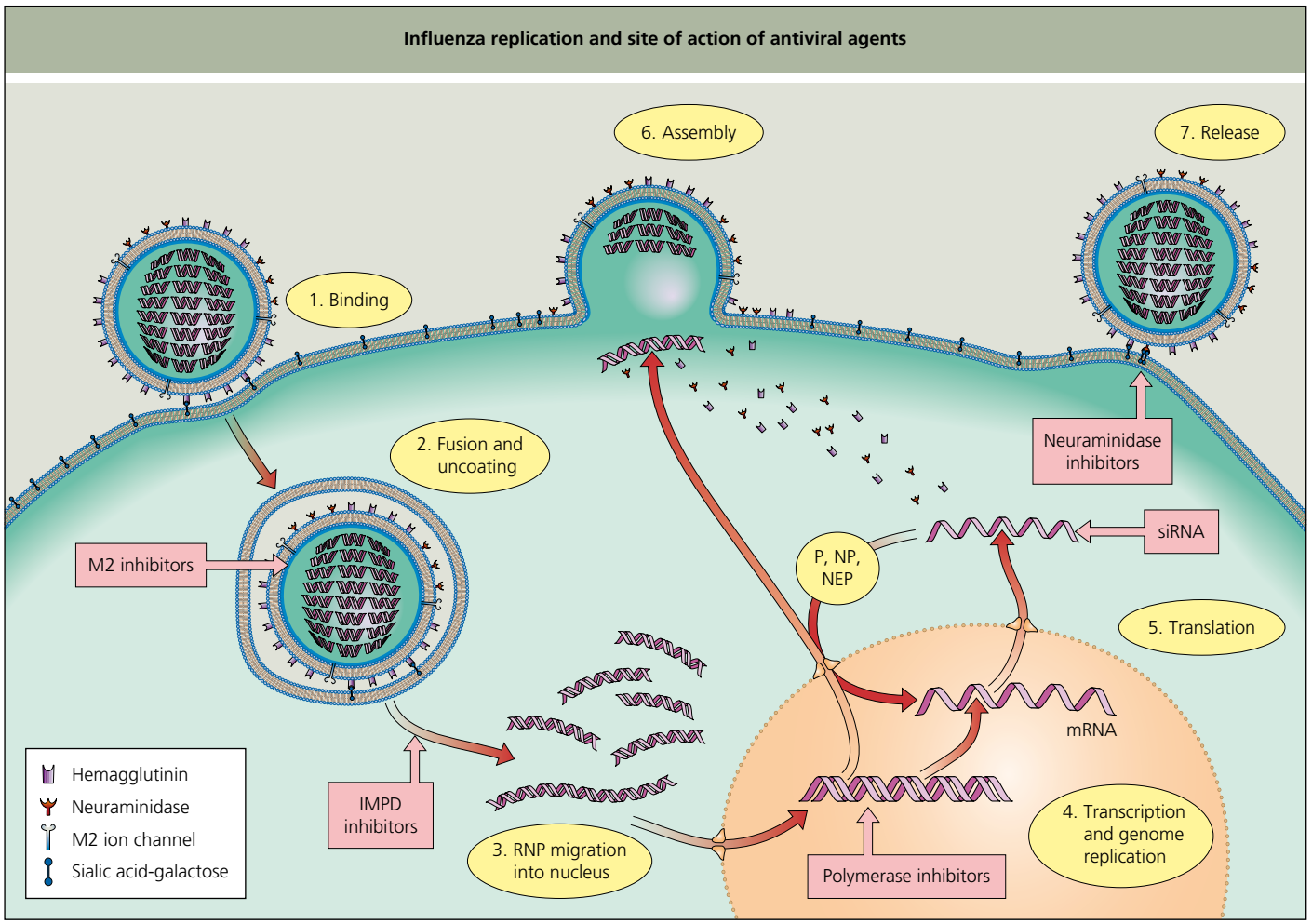


Fig. 147.1 Influenza replication and site of action of antiviral agents. IMPD, inosine monophosphate dehydrogenase; NEP, nuclear export protein; NP, nucleoprotein; P, polymerase; RNP, ribonuclear protein; siRNA-small inhibitory ribonucleic acid. From Beigel J, Bray M. Current and future antiviral therapy of severe seasonal and avian influenza. Antiviral Res 2008;78:91–102.

nursing home populations at 100 mg/day for 14 days or for at least 7 days after the last culture-confirmed illness in the ward or building; this regimen should be given with concomitant influenza vaccination for those not previously provided.[4] Rimantadine is better tolerated in this population.[14] Seasonal prophylaxis, during the 4–8 weeks of peak influenza virus circulation within the community, can be used for protection of high-risk patients who cannot tolerate immunization, who do not develop an adequate immune response to vaccine or when the strain circulating in the community does not match the vaccine strain. Postexposure prophylaxis in households appears protective when not used in conjunction with treatment of an ill index case.

Treatment

Amantadine or rimantadine therapy reduces duration of fever and symptoms in patients with documented influenza by about 1 day compared to placebo, when the medication is initiated within 48 hours of symptom onset.[15] Treatment is also associated with more rapid functional recovery[15] and resolution of small airways functional abnormalities. Studies comparing the therapeutic activity of amantadine and rimantadine are few but generally show comparability.[15] Amantadine appears safe and efficacious in reducing length of fever and illness in children older than 2 years of age.[15] Pediatric studies have found variable clinical benefits relative to acetaminophen controls and document the frequent emergence of drug-resistant variants.[15]

Controlled data to support the use of M2 inhibitors in treating severe influenza or in preventing complications is lacking; one retrospective study found no important differences in duration of illness or hospitalization between the amantadine-treated and untreated patients hospitalized with influenza.[16] In hematopoietic stem cell transplant and acute leukemia patients who received therapy with one of the M2 inhibitors, a reduced risk of progression to pneumonia (35% vs 76%) was found compared to no treatment.[17] However, resistance emergence is common in such patients.[11] One retrospective study of nursing home residents suggested that early treatment might reduce lower respiratory complications.[18] Amantadine is ineffective for treating influenza B or C infections.

Dosage and special consideration

Amantadine and rimantadine come as 100 mg tablets and a syrup formulation (50 mg/5 ml). In adults, the usual dose for treatment or prevention of influenza A infection is 100 mg q12h for both drugs (see Table 147.1).

Dosing of amantadine and rimantadine should be adjusted in the setting of renal failure (see Table 147.1). Neither M2 inhibitor is cleared by hemodialysis. Patients who are over 65 years of age should have the dose of both medications reduced to 100 mg once daily to avoid side-effects. Rimantadine needs dose adjustment to 100 mg/day for serious hepatic insufficiency. Amantadine and rimantadine are embryotoxic and teratogenic in preclinical tests and amantadine may be associated with birth defects. As a result, neither drug should be used in pregnant

Table 147.1 Agents used to prevent and treat influenza[4]

	USUAL ADULT DOSAGE[#]			
Drug	**Prophylaxis**	**Treatment**	**Dose adjustment state**	**Suggested dosage**
Amantadine	100 mg q12h	100 mg q12h	Age 1–9 years	5 mg/kg to max of 150 mg in two divided doses
			CrCl 30–50 ml/min	100 mg q24h
			CrCl 15–30 ml/min	100 mg q24h
			CrCl 10–15 ml/min	100 mg q week
			CrCl 10 ml/min	100 mg q week
			Age ≥65 years	100 mg q24h
Rimantadine	100 mg q12h	100 mg q12h	Age 1–9 years*	5 mg/kg to max of 150 mg in two divided doses
			CrCl <10 ml/min	100 mg q24h
			Severe hepatic dysfunction	100 mg q24h
			Age ≥65 years	100 mg q24h
Zanamivir[†]	2 puffs	2 puffs	No dose adjustment needed	
Oseltamivir[‡]	75 mg q24h	75 mg q12h	CrCl <30 ml/min[§]	Treatment: 75 mg q24h Prophylaxis: 75 mg every other day
			≤15 kg	30 mg q12h (2.5 ml[¶])
			15–23 kg	45 mg q12h (3.8 ml[¶])
			23–40 kg	60 mg q12h (5 ml[¶])
			>40 kg	75 mg q12h (6.2 ml[¶])

Recommendations based on those provided by the Advisory Committee on Immunization Practices.[4]
*Investigational: not approved for treatment of children by the US Food and Drug Administration.
[†]Zanamivir is indicated for prophylaxis in children ≥5 years old and for treatment in children ≥7 years old.
[‡]Oseltamivir is indicated for prophylaxis and treatment in children ≥1 year old.
[§]No treatment or prophylaxis dosing recommendations are available for patients undergoing renal dialysis.
[¶]Volume of suspension.
[#]Duration of treatment is usually 5 days. Duration of prophylaxis depends on clinical setting.

Table 147.2 Pharmacokinetic properties of antivirals with activity against influenza[5–8]

Drug	**Dose**	**Route**	**C_{max} (μg/l)**	**T_{max} (h)**	**$AUC_{0–12\ h}$ (μg/ml•h)**	**$T_{½}$ (h)**	**Bioavailability (oral, %)**	**Protein binding (%)**
Amantadine[5,6]*	200 mg	Oral (young)	510 (140)	2.1 (1)	10.2 (3.4)	14.4 (6)	62–93	67
	200 mg	Oral (elderly)	800 (200)	2.2 (2.1)	17.6 (6.5)	19 (9.1)	54–100	
Rimantadine[5,6]*	200 mg	Oral (young)	240 (70)	4.6 (2.1)	9.8 (4.5)	36.5 (17.3)	75–93	40
	200 mg	Oral (elderly)	250 (50)	4.0 (2.4)	11.5 (3.0)	36.5 (14.5)	NA	
Zanamivir[7†]	16 mg	Inhaled	29 (23–69)	0.75 (0.08–2)	0.03 (0.02–0.06)	3.6 (2.2–9.4)	4–17	10
	16 mg	Inhaled	54 (34–96)	0.75 (0.25–1)	0.16 (0.02–0.32)	—	4–17	
Oseltamivir[8]*	100 mg q12h	Oral (18–55 years)	439 (40.8)	3.5 (1)	3.85 (0.6)	6–10	79	42
	100 mg q12h	Oral (≥65 years)	575 (83.8)	3.5 (1.4)	4.94 (1.0)	—	—	

C_{max}, maximum serum drug concentration; T_{max}, time to C_{max}; $T_{½}$, serum elimination half-life; AUC, area under the serum drug concentration versus time for the dose interval; bioavailability, percentage of intravenous C_{max}.
*Values are mean (SD); [†]Values are median (range).

women unless the benefits of therapy clearly outweigh the potential risks (pregnancy category C). The recommended pediatric dosage of both amantadine and rimantadine is 5 mg/kg/day to a maximum of 150 mg/day in two divided doses in children younger than 10.[4]

Safety issues

The most common side-effects of the M2 inhibitors are minor central nervous system (CNS) complaints (anxiety, difficulty concentrating, insomnia, dizziness, headache and jitteriness) and gastrointestinal upset. Patients who receive amantadine may develop antimuscarinic effects, orthostatic hypotension and congestive heart failure at low frequencies. Particularly in the elderly or those with renal failure, serious CNS side-effects due to amantadine and less often rimantadine, include confusion, disorientation, mood alterations, memory disturbances, delusions, nightmares, ataxia, tremors, seizures, coma, acute psychosis, slurred speech, visual disturbances, delirium, oculogyric episodes and hallucinations.[6] Amantadine causes CNS side-effects in about 15–30% of persons, as well as dose-related abnormalities in psychomotor testing.[6] The incidence and severity of CNS adverse effects are less common with rimantadine.[14]

Concomitant ingestion of antihistamines or anticholinergic drugs increases the CNS effects of amantadine. Trimethoprim–sulfamethoxazole and triamterene–hydrochlorothiazide decrease the renal clearance of amantadine, which enhances the risk of CNS toxicity. Quinine and quinidine likewise reduce the clearance of amantadine. Co-administration with monoamine oxidase inhibitors may precipitate life-threatening hypertension. The drug does not appear to interact with the cytochrome P450 system. Cimetidine is associated with 15–20% increases and aspirin or acetaminophen with 10% decreases in plasma rimantadine concentrations; however, such changes are unlikely to be of clinical significance.[19] Patients receiving either amantadine or rimantadine along with drugs affecting CNS function, such as antihistamines, antidepressants and minor tranquilizers, should be monitored closely.

Conclusions

Given the emergence of resistance among influenza A/H3N2 virus, the M2 inhibitors, as a class, are not recommended for the prevention or treatment of influenza when significant resistance is present in circulating strains. They may have a role in the management of patients with susceptible H1N1 and H5N1 viruses; current recommendations of the Centers for Disease Control and Prevention (CDC) and the World Health Organization (WHO) should be consulted before using this class clinically.

Neuraminidase Inhibitors

Introduction and mechanism of action

Influenza A and B viruses possess a surface glycoprotein with neuraminidase activity whereas influenza C viruses do not (see Fig. 147.1). This enzyme cleaves terminal sialic acid residues and destroys the receptors recognized by viral hemagglutinin. This activity is essential for release of virus from infected cells, for prevention of viral aggregates, and for viral spread within the respiratory tract.[20] Zanamivir (Relenza) and oseltamivir (Tamiflu, a prodrug of the active carboxylate) are sialic acid analogues that potently and specifically inhibit influenza A and B neuraminidases by competitively and reversibly interacting with the active enzyme site.[21] These drugs are active against all nine neuraminidase subtypes in nature including most of the avian strains of influenza A H5N1 and H9N2 that infect humans.

Resistance

Zanamivir and oseltamivir carboxylate resistance *in-vitro* results from mutations in the viral hemagglutinin and/or neuraminidase.[22,23] In the hemagglutinin variants, mutations in or near the receptor binding site make the virus less dependent on neuraminidase action, whereas neuraminidase mutations directly affect interaction with the inhibitors. The altered neuraminidases typically show reduced activity or stability, and the mutated viruses usually have decreased infectivity in animals.[24] The particular neuraminidase mutation determines the degree of resistance and cross-resistance (i.e. R229K and H274Y cause high-level resistance to oseltamivir but not zanamivir).[22] One combined hemagglutinin and neuraminidase mutant has been recovered from an immunocompromised child with prolonged virus excretion despite receiving nebulized zanamivir.[25] Oseltamivir-resistant variants have been recovered from <1% of treated adults and about 4% of treated children.[21] The possible clinical and epidemiologic significance of such variants requires study and a global Neuraminidase Inhibitor Susceptibility Network has been established to address these concerns.[23] Unfortunately, resistance to oseltamivir among H1N1 strains (with H274Y change in the NA gene) has been described globally.[26] The impact and persistence of these resistant variants are still under investigation. Although resistance has been rarely documented, most novel swine-orgin 2009 H1N1 viruses are susceptible to oseltamivir.

ZANAMIVIR

Pharmacokinetics

The oral bioavailability of zanamivir is low (<5%) and most clinical trials have used intranasal or dry powder inhalation delivery. The current dry powder formulation is mixed with lactose (5 mg zanamivir per 20 mg lactose). Following inhalation of the dry powder, approximately 7–21% is deposited in the lower respiratory tract and the remainder in the oropharynx.[7,27] Median zanamivir concentrations are above 1000 ng/ml in induced sputum 6 hours after inhalation and remain detectable up to 24 hours. The peak plasma concentration averages 46 µg/l after a single 16 mg inhalation of zanamivir. The proprietary inhaler device for delivering zanamivir is breath-actuated and requires a cooperative patient.[28]

In both experimental and natural influenza, once-daily dosing appears protective.[29] Twice-daily administration is therapeutically active but increasing the dose frequency to four times per day does not appear to increase efficacy in treating natural influenza.[29] Intranasal dosing is protective against experimental infection but not natural infection and does not substantially increase the overall therapeutic response to inhaled zanamivir.

Indications

Prophylaxis

Zanamivir is indicated as once-daily inhalations for the prevention of influenza in patients more than 5 years old. Once-daily inhaled zanamivir for 4 weeks was 84% efficacious in preventing laboratory-confirmed illness with fever and 31% effective in preventing influenza infections, irrespective of symptoms.[29] When used for postexposure prophylaxis, inhaled zanamivir for 10 days reduced the risk of secondary influenza illness by 79% in households.[29] In nursing homes experiencing influenza outbreaks, inhaled zanamivir was more effective for prevention of influenza A illness than oral rimantadine, in part because of frequent resistance emergence to the M2 inhibitor.[29]

Treatment

In the USA, zanamivir is indicated for the treatment of uncomplicated acute illness due to influenza A and B virus in adults and pediatric patients 7 years and older who have been symptomatic for no more than 2 days. Inhaled zanamivir in adults has consistently shown at least one less day of disabling influenza symptoms, and most studies have found a reduction in the number of nights of disturbed sleep, in time to resumption of normal activities, and in the use of

symptom relief medications.[29,30] Similar therapeutic benefits have also been shown in children aged 5–12 years old.[31] Greatest benefit was noted in patients who were febrile at the time of enrollment, those started on therapy within 30 hours after the onset of symptoms, and in adults aged 50 years and older.[29] Zanamivir has also been associated with a 40% reduction in lower respiratory tract complications of influenza leading to antibiotics, particularly bronchitis and pneumonia.[32] Zanamivir appears generally well tolerated and effective in treating influenza in patients with mild to moderate asthma or, less often, chronic obstructive pulmonary disease.[30,32]

An uncontrolled study found zanamivir to be safe and possibly effective in allogeneic stem cell transplant recipients, although viral shedding persisted an average of 2 weeks on therapy.[24] Further studies are needed in immunocompromised populations. Likewise, there may be benefit to using neuraminidase inhibitors beyond the initial 48 hours of symptoms in patients with severe illness, particularly if the patients are ill enough to warrant hospital admission (see oseltamivir treatment section for more details).

Dosage and special consideration

Zanamivir is delivered by inhalation with a proprietary breath-activated device (Diskhaler). The usual adult treatment dose is two inhalations (10 mg) twice a day for 5 days. Although the plasma elimination half-life increases with creatinine clearance ≤70 ml/min, drug accumulation is negligible after inhalation and dose adjustment is not necessary for renal or hepatic dysfunction. Certain populations, particularly very young, frail or cognitively impaired patients, may have difficulty using the drug delivery system.[28]

Safety issues

Topically applied zanamivir is generally well tolerated in controlled studies, including those involving patients with asthma and chronic obstructive pulmonary disease.[30] No difference in adverse events between zanamivir and placebo (lactose) recipients have been found.[30] Less than 5% zanamivir recipients have reported diarrhea, nausea, sinusitis, nasal signs and symptoms, bronchitis, cough, headache, dizziness, and ear, nose and throat infections. Postmarketing reports indicate that bronchospasm may be an uncommon but potentially severe problem, particularly in patients with acute influenza and underlying reactive airway disease.[33] Anecdotal reports of hospitalization and fatality indicate that inhaled zanamivir should be used cautiously in such patients. Current guidelines advise against the use of zanamivir in patients with underlying airway disease, unless the patient is closely monitored and has a fast-acting inhaled bronchodilator available when inhaling zanamivir.[4]

Low bioavailability is associated with low exposure to circulating zanamivir, and no clinically significant drug interactions have been recognized. *In-vitro* studies suggest that zanamivir does not inhibit or induce cytochrome P450 enzymes. The drug does not affect the immunogenicity of concomitant immunization with inactivated virus vaccines. It is uncertain whether inhaled zanamivir might reduce the immunogenicity of intranasal, live-attenuated vaccine if administered concurrently. Although not associated with teratogenic effects in preclinical studies, zanamivir should only be used in pregnancy when the potential benefit justifies the potential risk to the fetus (category C).

OSELTAMIVIR

Pharmacokinetics and distribution

Oral oseltamivir ethyl ester is well absorbed and rapidly cleaved by esterases in the gastrointestinal tract, liver or blood. The bioavailability of the active metabolite, oseltamivir carboxylate, is estimated to be ~80% in previously healthy persons.[8] Mean peak oseltamivir carboxylate concentrations of 456 µg/l are reached at 5 hours after oral administration of 150 mg doses in healthy adults, and the plasma elimination half-life is 6–10 hours. Although drug concentrations over time are 25–35% higher in the elderly at steady state, no dose adjustments are deemed necessary. Administration with food appears to decrease the risk of gastrointestinal upset without decreasing bioavailability. Both the prodrug and parent are eliminated primarily unchanged through the kidney by glomerular filtration and anionic tubular secretion. The dose should be reduced by half for patients with a creatinine clearance less than 30 ml/min.[34] Distribution is not well characterized in humans, but peak bronchoalveolar lavage levels are similar to plasma levels in animals.[8] Drug levels in middle ear fluid and sinus aspirates are similar to those in blood.[8]

There is no clear association between the area under the serum concentration versus time curve (AUC) for the oseltamivir carboxylate drug and viral titer after experimental infection; early therapy of experimental infection is associated with reduced median nasal lavage concentrations of interleukin 6 (IL-6), tumor necrosis factor and interferon gamma (IFN-γ) as compared to placebo.[8] In natural influenza, once-daily dosing appears as effective as twice daily dosing for prevention of influenza illness.[8] Doses of 75 mg and 150 mg twice daily provide comparable antiviral and clinical effects in the treatment of acute influenza A illness[8] and of experimental influenza B in adults.

Indications

Prophylaxis

Oseltamivir is indicated for the prevention of influenza infection in patients ≥1 year, with dosing once a day. The efficacy of once-daily oseltamivir 75 mg for 6 weeks in preventing influenza illness in healthy, nonimmunized adults was 84% and in preventing influenza infection irrespective of symptoms was 50%.[8] In immunized nursing home residents, the efficacy of prophylaxis was 92% against illness compared to placebo.[8] Similar efficacy was seen in a household-contact prophylaxis study,[8] and protection against influenza has been shown in children.[8] A small observational study among stem cell transplant recipients also documented that oseltamivir, when taken as directed, was highly effective in preventing infection. A prospective study among immunocompromised patients is currently underway to determine the safety and efficacy of long-term prophylaxis in these populations.

Treatment

Oseltamivir 75 mg twice daily for 5 days when started within the first 2 days of symptoms was associated with a shorter time to alleviation of illness (29–35 hours shorter) and with reductions in severity of illness, duration of fever, time to return to normal activity, quantity of viral shedding, duration of impaired activity, and complications leading to antibiotic use, particularly bronchitis, compared to placebo in previously healthy adults.[8] Preliminary analyses indicate that early treatment can reduce hospitalizations. In a pediatric study enrolling children between the ages of 1 and 12 years, oseltamivir 2 mg/kg every 12 hours for 5 days significantly reduced illness duration and severity, time to resumption of full activities, and the occurrence of complications leading to antibiotic use, particularly acute otitis media.[35] Little published information is available about therapeutic efficacy in elderly or high-risk persons, including those with underlying cardiopulmonary conditions or immunodeficiency. A recent observational study among hospitalized patients with influenza who were treated, irrespective of the duration of clinical symptoms, found that antiviral therapy (typically with oseltamivir) was associated with a significant reduction in mortality (OR 0.21, 95% confidence interval 0.06–0.80; p <0.03). Based on these data, antiviral therapy should be considered, irrespective of time since symptom onset, in patients with severe disease or who warrant hospital admission, if viral replication is documented.[36]

Dosage and special consideration

Oseltamivir comes as 75 mg tablets and as a white tutti-frutti-flavored suspension. The suspension comes in bottles containing 25 ml of suspension after constitution equivalent to 300 mg oseltamivir base. The typical adult dose for treatment is 75 mg twice daily for 5 days and for prophylaxis is 75 mg once daily. Pediatric dosing is based on weight and is outlined in Table 147.1. Oseltamivir dose should be reduced to 75 mg once a day for treatment and 75 mg every other day or 30 mg of suspension daily for prophylaxis when a patient has a creatinine clearance of <30 ml/min. Doses of oseltamivir should be given after hemodialysis. The safety and pharmacokinetics in patients with hepatic impairment have not been evaluated. No clinical studies have been conducted to assess the safety of neuraminidase inhibitors in pregnancy. Despite this, current CDC guidelines state that 'pregnancy should not be considered a contraindication to oseltamivir or zanamivir use. Because of its systemic activity, oseltamivir is preferred for treatment of pregnant women.' The recommended pediatric dosage is listed in Table 147.1.

Safety issues

Oral oseltamivir is generally well tolerated and no serious end-organ toxicity has been found in controlled clinical trials. Oseltamivir is associated with nausea, discomfort and, less often, emesis in a minority of treated patients. Nausea and vomiting occur at approximately 10–15% excess in oseltamivir recipients. Gastrointestinal complaints are usually mild-to-moderate in intensity, usually resolve despite continued dosing, and are ameliorated by administration with food.[37] Clinical studies comparing 75 mg and 150 mg twice daily found similar frequencies of adverse events with the two doses. Other infrequent possible adverse events include insomnia, vertigo and fever. Postmarketing reports suggest that oseltamivir may be associated rarely with skin rash, hepatic dysfunction or thrombocytopenia. Additionally, there have been reports of abnormal neurologic and behavioral symptoms which have rarely resulted in deaths among mostly children; most of these reports have come from Japan. It is unclear if this unusual behavior is secondary to oseltamivir or the result of their influenza illness. It is currently recommended that patients be monitored closely for behavioral abnormalities.[37]

No clinically significant drug interactions have been recognized. However, probenecid blocks tubular secretion and doubles the half-life of oseltamivir. Studies with amoxicillin, aspirin and acetaminophen have found no clinically important interactions. No interactions with the cytochrome P450 enzymes occur *in vitro*. Protein binding is below 10%. Oseltamivir should not affect the immunogenicity of concomitant vaccination with inactivated virus but might impair the immunogenicity of concurrent live-attenuated intranasal influenza vaccine.

Conclusions

The neuraminidase inhibitors are currently the preferred agents for the prevention and treatment of influenza infection. Recently, low level, clonal resistance to oseltamivir in influenza A/H1N1 strains globally has been described. It is unclear if these viruses will be persistent and what their impact will be globally; the CDC and WHO influenza guidance programs should be consulted regularly in order to stay up to date with the current prevention and treatment recommendations.

Ribavirin

Introduction and mechanism of action

Ribavirin (Virazole, Rebetol) is a guanosine analogue with a wide range of antiviral activity including influenza viruses, respiratory syncytial virus (RSV) and parainfluenza viruses. Ribavirin is rapidly phosphorylated by intracellular enzymes and the triphosphate inhibits influenza virus RNA polymerase activity and competitively inhibits the guanosine triphosphate-dependent 5′-capping of influenza viral messenger RNA. In addition, ribavirin depletes cellular guanine pools.[38,39] Ribavirin also shows antiproliferative and immunomodulatory effects.

Pharmacokinetics

Oral ribavirin has a bioavailability of 33–45% in adults and children and achieves peak plasma concentration of 0.6 μg/ml 1–2 hours after ingestion of a 400 mg dose in adults. Ribavirin has a short initial (0.3–0.7 hour) and a long terminal (18–36 hours) phase half-life and is eliminated by hepatic metabolism and renal clearance.[40–43] After aerosol administration, plasma levels increase with exposure and range from 0.2 to 1 μg/ml. Respiratory secretions have levels up to 1000 μg/ml, which decline with a half-life of 1.4–2.5 hours.

Indications

Ribavirin aerosol is currently indicated for the treatment of severe RSV in children. Oral ribavirin is indicated in combination with interferon-α or pegylated interferon for the treatment of chronic hepatitis C (see Chapter 148). Trials of aerosolized ribavirin for the treatment of severe RSV infection in infants have shown no consistent effect on duration of hospitalization time or mortality.[44] Earlier studies were confounded by the use of water aerosol as placebo which may induce bronchospasm. Long-term follow-up of ribavirin recipients has likewise found no consistent benefits on pulmonary function.[44] Current guidelines recommend that aerosolized ribavirin be considered in the treatment of high-risk infants and young children, as defined by congenital heart disease, chronic lung disease, immunodeficiency states, prematurity and age <6 weeks, as well as for those hospitalized with severe illness.[44,45] Administration of a more concentrated aerosol solution (60 mg/ml) over 2 hours q8h appears well tolerated and easier to administer.[46] Aerosolized ribavirin has shown minimal efficacy in treating influenza in hospitalized children.[47]

Ribavirin has also been studied for the treatment of RSV and parainfluenza virus infections in immunocompromised patients. Intravenous ribavirin appears to be ineffective in reducing RSV-associated mortality in hematopoietic stem cell transplant (HSCT) patients with RSV pneumonia; there may be benefit among lung transplant recipients.[48] Aerosolized ribavirin may provide benefit in selected patient groups with less severe RSV disease. Survival was improved when treatment was started before respiratory failure or when infection was limited to the upper respiratory tract.[48] Although there have not been any reported prospective studies of aerosolized ribavirin versus combined ribavirin–antibody therapy (either intravenous immunoglobulin, RespiGam or palivizumab), combination therapy appears more effective, particularly when started before severe respiratory distress.[48] Oral ribavirin has been tried in the management of RSV with variable success. Systemic therapy is associated with a higher risk of adverse effects, particularly hemolytic anemia.[48] In the management of parainfluenza virus in bone marrow transplant recipients, two case series found that aerosolized ribavirin failed to improve 30-day mortality or reduce the duration of viral replication relative to no treatment.[48, 49]

Dosage and special consideration

Ribavirin comes in three formulations: oral, intravenous (investigational in USA) and aerosol. Ribavirin for aerosolization is available as a 6 g/100 ml solution which is diluted to a final concentration of 20 mg/ml and delivered by small particle aerosol for 12–18 hours with a proprietary device (SPAG-2 nebulizer). A higher concentration of aerosol solution (60 mg/ml) has been given over 2 hours three times daily in some studies and appears well tolerated. Ribavirin also comes in 200 mg tablets and sterile solution for injection.

Systemic ribavirin is contraindicated in patients with creatinine clearance <50 ml/min and the dose should be reduced by one-third

for patients under 10 years of age. Dose adjustment is needed if there is a substantial decline in hematocrit and the drug should be discontinued if the hemoglobin drops below 8.5 g/dl.

Safety issues

Systemic ribavirin can cause a dose-related extravascular hemolytic anemia and, at higher doses, suppression of bone marrow release of erythroid elements. Severe anemia may require dose adjustment or cessation or use of erythropoietin. Aerosolized ribavirin can cause bronchospasm, mild conjunctival irritation, rash, psychological distress if administered in an oxygen tent, and rarely acute water intoxication. Bolus intravenous administration may cause rigors. Antagonism of both drugs may occur when ribavirin is combined with zidovudine. Ribavirin is contraindicated in pregnant women and in male partners of women who are pregnant because of teratogenicity of the drug. Pregnancy should be avoided during therapy and for 6 months after completion of therapy in both female patients and in female partners of male patients taking ribavirin (pregnancy category X).

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References for this chapter can be found online at http://www.expertconsult.com

Chapter 148

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Drugs to treat viral hepatitis

INTRODUCTION

Tremendous progress in the treatment of viral hepatitis has been made over the past two decades, and with many new drug classes in development, the next 20 years hold similar promise. This chapter describes the mechanism of action, pharmacokinetics, indications and safety of the drugs used for the treatment of chronic hepatitis B and C infections and highlights some of the agents in development. The diagnosis and management of hepatitis B and C are covered in Chapter 38. Vaccination and prevention strategies are discussed in Chapters 38 and 154. Chronic hepatitis C infection is treated with pegylated interferon and ribavirin, while oral nucleoside or nucleotide analogue therapy represents standard initial treatment for chronic hepatitis B infection. The response to the drugs described in this chapter is affected by both host (e.g. age, gender, race, co-morbid conditions such as HIV, stage of illness, immune response) and viral factors (e.g. genotype, RNA/DNA level, resistance mutations). Therefore, the choice of the drug, dose and duration must take these factors into consideration. As no targeted treatments exist for hepatitis A or E infections, they will not be discussed.

INTERFERONS

Interferons (IFNs) are a family of naturally occurring proteins produced by eukaryotic cells that function as cytokines in an early response to viral infection. Interferons do not have direct antiviral activity, but instead induce an antiviral state in exposed cells and activate other immune functions.

Interferons are broadly characterized as type I (IFN-α and -β) and type II (IFN-γ). Interferon-α and -β have primary antiviral activity while IFN-γ has more potent immunoregulatory functions.

Interferons act through cell surface receptors to induce a complex series of intracellular events, with inhibition of viral protein production appearing to be the primary target.[1] A variety of purified and recombinant IFN-α preparations is available for clinical use. The pharmaceuticals are termed 'IFN-alfa' to distinguish them from naturally occurring IFN-α.

Recently, pegylated ('peg') varieties of IFN have become available, and their usage in hepatitis C virus (HCV) treatment has for the most part replaced standard interferon. Pegylation involves the addition of a polyethylene glycol side chain to the protein molecule, which extends the half-life significantly. Two formulations are currently available, pegIFN alfa-2a and pegIFN alfa-2b. PegIFN alfa-2a has a larger branched peg molecule of about 40 kDa attached at several sites. PegIFN alfa-2b has a smaller, linear peg molecule of about 12 kDa attached at several sites. Their proposed mechanism of action is similar to that of standard IFNs.[2]

Pharmacokinetics and distribution

Standard pharmacokinetic measurements may not be relevant for IFN therapy. In healthy male volunteers, maximum serum levels of IFN alfa-2b occur 3–12 hours after subcutaneous administration, and serum concentrations are undetectable after 16 hours. IFN alfa is filtered through the glomeruli and then undergoes rapid proteolytic degradation during tubular resorption. Negligible amounts of IFN are excreted in the urine. A small percentage of the administered dose undergoes hepatic metabolism and biliary excretion. PegIFN alfa-2b reaches maximum concentrations by 20 hours and has a half-life of 40 hours. PegIFN alfa-2a reaches maximum concentrations by 72–96 hours and has a longer $t_{½}$ of 80 hours compared to pegIFN alfa-2b.

Indications

Chronic hepatitis B virus infection

IFN alfa-2b and pegIFN alfa-2a are approved for the treatment of chronic hepatitis B virus (HBV) infection. A response to IFN therapy is judged by a loss of HBV DNA and hepatitis B e antigen (HBeAg) from the plasma (in eAg-positive patients), along with biochemical and histologic improvements. A meta-analysis of 16 randomized trials examining standard IFN versus control in HBeAg-positive patients found that loss of HBeAg occurred in 33% of patients while clearance of HBV DNA occurred in 37% of patients (although the DNA assays used in these studies were less sensitive than current methods).[3] Control patients experienced these outcomes in only 12% and 17% of cases, respectively. Clinical predictors of response to IFN in patients infected with HBV include low level of HBV DNA, elevated serum transaminases and evidence of active hepatic inflammation.[4]

PegIFN is thought to result in similar or slightly superior outcomes than standard IFN, although limited data exist from head-to-head comparisons. In HBeAg-positive patients, 48 weeks of pegIFN alfa-2a therapy was associated with loss of eAg in 30% and seroconversion in 27%, with persistence of these finding 24 weeks after discontinuation of therapy.[5] However, only 25% of patients achieved undetectable levels of HBV DNA. Studies of pegIFN alfa-2b yielded similar results.[6] In contrast, IFN and pegIFN treatment in HBeAg-negative patients results in high rates of HBV viral suppression; however, the response is generally not durable after treatment discontinuation.[7]

Recent years have seen a variety of new and effective oral medications for the treatment of HBV infection with improved rates of HBV DNA suppression. Oral agents have more favorable side-effect profiles compared to IFN, but HBeAg seroconversion rates are not as high. As a result of these therapies, IFN is no longer considered first-line treatment for HBV infection in most circumstances (see also Chapter 38).

Hepatitis C virus infection

Interferon treatment of acute HCV infection is currently recommended, yet proper timing of treatment, regimen and duration remain uncertain. A landmark study reported a 98% sustained virologic response rate with 24 weeks of IFN monotherapy; however, the study had no control group and some patients experience spontaneous resolution if left untreated.[8] As additional data have accumulated, the use of pegIFN has been advocated in the treatment of acute HCV, although the need for combination therapy with ribavirin remains controversial.

Treatment of chronic HCV infection is generally recommended in patients experiencing detectable HCV RNA levels and evidence of fibrosis on liver biopsy. A sustained virologic response (SVR) of HCV to therapy is defined as an HCV RNA assay below the limit of assay detection (using an assay with a cutoff of 50 IU/ml or lower) at 24 weeks after completion of therapy.[9] Over the past 10 years, it has been established that pegIFN plus ribavirin combination therapy is superior to combination therapy with standard interferon or pegIFN monotherapy and is now considered the standard of care.

Hepatitis C virus has a half-life of about 3 hours and infection results in the production of about 12 billion virions daily. The frequent dosing and pharmacokinetics of standard IFN result in wide variations in serum IFN levels and fluctuating antiviral activity. Standard IFN monotherapy results in SVR rates of less than 10% after 24 weeks of treatment and SVR rates of less than 20% after 48 weeks of therapy.[10] Pegylation of the IFN molecule maintains rapid absorption and rapid time to peak drug level, while providing a dramatically lengthened half-life. Studies show that the pegIFNs provide significantly improved response rates over standard IFNs. Monotherapy with pegIFNs yields SVR rates of 25–39% in treatment-naive patients with HCV infection.[11] These improved results over standard IFN apply to both pegIFN alfa-2a as well as pegIFN alfa-2b.

Route of administration and dosing

Interferons are administered subcutaneously for the treatment of chronic viral hepatitis. Dosages vary considerably according to the specific preparation and indication (Table 148.1). Guidelines for adjusting IFN dosage in patients who have renal insufficiency are poorly defined. Only limited data are available regarding administration to patients who have decompensated liver disease, but IFN may be poorly tolerated in this population and its use is generally not advised. In pregnant monkeys, IFN has abortifacient effects and should not be used in pregnant women unless the potential benefits clearly outweigh the potential risks to the fetus.

Table 148.1 Medication dosage regimens for adults. Preferred initial regimens are shown in bold

Disease	Therapy	Route	Dose	Duration
Chronic hepatitis B	IFN alfa-2b*	sc	5×10^6 IU/day or 10×10^6 IU three times/week	HBeAg-positive: 16–24 weeks† HBeAg-negative: 12+ months†
	PegIFN alfa-2a	sc	180 µg/week	48 weeks†
	Lamivudine	po	100 mg q24h	Optimal duration unknown
	Adefovir dipivoxil	po	10 mg q24h	Optimal duration unknown
	Entecavir	**po**	**0.5 mg q24h (lamivudine-inexperienced)** or 1.0 mg (lamivudine-experienced)	**Optimal duration unknown**
	Telbivudine	po	600 mg q24h	Optimal duration unknown
	Tenofovir‡	**po**	**300 mg q24h**	**Optimal duration unknown**
	Emtricitabine§	po	200 mg q24h	Optimal duration unknown
Chronic hepatitis C	IFN alfa-2a*	sc	3×10^6 IU three times/week	24–48 weeks¶
	IFN alfa-2b*	sc	3×10^6 IU three times/week	24–48 weeks#
	IFN alfacon*	sc	9 µg three times/week**	24-48 weeks¶
	PegIFN alfa-2a	**sc**	**180 µg/week**	**24 weeks genotype 2,3††** **48 weeks genotype 1,4**
	PegIFN alfa-2b	**sc**	**1.5 µg/kg/week††** (or 1.0 µg/kg/week)‡‡	**24 weeks genotype 2,3**** **48 weeks genotype 1,4**
	Ribavirin	**po**	**800–1400 mg/day divided in two doses§§**	**Co-administer with IFN or pegIFN for entire duration**

*The use of standard interferon has been replaced for the most part by the use of pegylated interferon for reasons of efficacy and convenience. Standard interferon may be used in certain situations (e.g. prior to liver transplant) when a shorter half-life is preferred.
†Optimal dosing and duration of interferons is unclear for HBV. Whether lower dose or shorter treatment of pegIFN may suffice for HBeAg-positive and whether longer treatment will improve response for HBeAg-negative patients is not known.
‡First-line therapy for HIV-infected patients who also require HIV treatment, usually in combination with lamivudine or emtricitabine as part of a combination antiretroviral regimen.
§ FDA-approved for treatment of HIV infection but not HBV monoinfection.
¶Optimal duration of IFN therapy with these agents is based on genotype and what has become standard of care may vary from the package inserts.
#Dosing of 15 µg sc three times/week is recommended for **nonresponders** to prior treatment or **relapsers**. Daily dosing has also been studied and may be appropriate in certain circumstances.
**For HIV-infected patients, duration of treatment is extended to 48 weeks.
††Dual therapy with ribavirin.
‡‡Monotherapy.
§§Optimal dose of ribavirin varies with the co-administration of IFN products as well as patient weight and HCV genotype. Readers should refer to specific package inserts and practice guidelines.

Adverse reactions

Interferon therapy is associated with an extensive list of toxicities (see Fig. 148.1). Most patients receiving standard IFN doses of over one million units experience an 'influenza-like' syndrome characterized by fever, chills, headache, myalgias and arthralgias. These symptoms appear a few hours after the IFN injection, and usually resolve within 12 hours. The symptoms can often be prevented by premedication with antipyretics. Tolerance develops in many patients with continued therapy. The most frequent dose-limiting adverse effects are leukopenia and thrombocytopenia. Before beginning IFN therapy, a baseline complete blood count, liver function profile, urinalysis, antinuclear antibody screen and thyroid function tests should be obtained.

The adverse events seen with pegIFN are similar to those seen with standard IFN. The most common hematologic toxicity associated with pegIFNs is neutropenia with as many as 18% of patients requiring dose adjustments as a result.[12] Dose reduction or use of hematopoietic growth factors is often helpful in patients experiencing significant generalized or hematologic toxicity, respectively. The pegylated IFNs are also associated with psychiatric disturbances including depression, irritability, insomnia and suicidal ideation. Caution is warranted when using IFNs and pegIFNs in patients with a history of a psychiatric disorder.

RIBAVIRIN (SEE ALSO CHAPTER 147)

Ribavirin is a guanosine analogue that has broad-spectrum antiviral activity. Its mechanism of action is not clearly defined, but it is believed to act against HCV by one or more of the following mechanisms:

- enhancing T-cell mediated immune responses to HCV by shifting the balance towards a T-helper-1 response;
- inhibiting cellular inosine monophosphate dehydrogenase, thereby decreasing the intracellular guanosine triphosphate pool needed for viral RNA replication;
- directly inhibiting HCV polymerase; and
- acting as an RNA virus mutagen, thereby reducing viral fitness.[13]

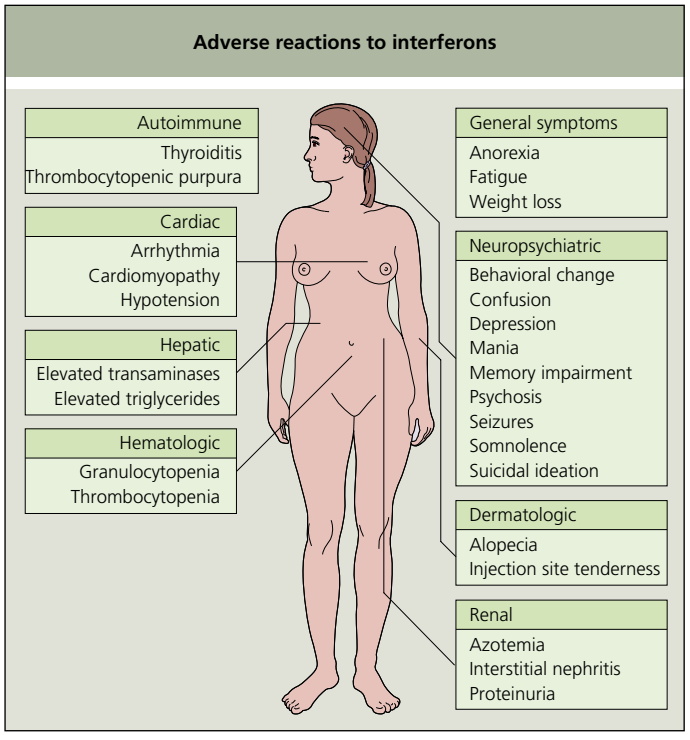


Fig. 148.1 Adverse reactions to interferons.

Pharmacokinetics

Ribavirin is well absorbed after oral administration, with bioavailability ranging from 33% to 69% of the dose and about 40% of the drug being eliminated via the kidneys.

Indications

Hepatitis C virus infection

Ribavirin is used in combination with IFN or pegIFN for the treatment of chronic HCV infection. As the benefits of ribavirin monotherapy in HCV infection disappear rapidly when the treatment is discontinued, monotherapy is not recommended.

Route of administration and dosing

Ribavirin is administered orally for the treatment of hepatitis C. The recommended dose of ribavirin varies by weight and HCV genotype (see Table 148.1). Retrospective analysis of phase III studies of pegIFN plus ribavirin revealed that response is inversely correlated with the patient's body weight. In the study by Manns *et al.*, patients receiving at least 10.6 mg/kg of ribavirin had significantly better response rates for the same doses of pegIFN.[12] A randomized clinical trial confirmed that weight-based dosing of ribavirin improves sustained virologic response rates in patients with genotype 1 infection.[14]

Adverse reactions and interactions

The major side-effect of ribavirin is hemolytic anemia, which may necessitate discontinuation of therapy. Ribavirin is highly teratogenic and birth control is required if the drug is used by women of childbearing age or their partners. In HIV-infected individuals, ribavirin should not be co-administered with didanosine because of excess toxicity, and co-administration with zidovudine should be avoided due to potentiation of anemia (see also Practice Point 46).

COMBINATION THERAPY FOR HEPATITIS C VIRUS INFECTION

Ribavirin given in combination with IFN has been shown to be more effective than the use of IFN alone for the treatment of HCV.[15] An SVR may occur in up to 40% of patients treated with combination therapy compared with approximately 20% of patients treated with standard IFN alone. Studies combining pegIFN alfa-2b with ribavirin had a 54% SVR compared to a 47% SVR with standard IFN alfa-2b plus ribavirin.[12] The SVR in patients infected with HCV genotype 1, which is associated with a poorer response to therapy, was also improved (42% vs. 33%). Trials of pegIFN alfa-2a plus ribavirin also demonstrate improved responses overall and for genotype 1 when compared to pegIFN alone (46% vs. 21% for genotype 1) and standard IFN plus RBV (46% vs. 36% for genotype 1).[11] A randomized trial of treatment-naive, HCV genotype 1 patients comparing the two available preparations of pegIFN (pegIFN alfa-2a and pegIFN alfa-2b) given in combination with weight-based ribavirin dosing demonstrated no significant difference in sustained virologic response for the two combinations. In patients with HIV/HCV co-infection, combination therapy with pegIFN and ribavirin for HCV has also been shown to be superior to pegIFN monotherapy or combination therapy using standard IFN[16–18] (see Practice Point 46).

As data on the use of these drugs have accumulated, refinements in the recommended ribavirin dose and total treatment duration have been made based on HCV genotype and HIV co-infection status (see Table 148.1). In addition, validated criteria for stopping treatment have been developed based on lack of response at 12 and 24 weeks. The rapidity of treatment response has also been explored as a rationale for foreshortening or extending the duration of

therapy, but the usefulness of this approach has not been clearly demonstrated. In practice, many physicians extend the treatment duration in genotype 1 patients who take 24 weeks to achieve undetectable HCV RNA.

THERAPIES IN DEVELOPMENT FOR HEPATITIS C

Hepatitis C virus is a single-stranded (ss) RNA virus of about 9.4 kb in length. It is translated into a single polypeptide precursor, which is cleaved into several functional gene products, including structural proteins, RNA polymerase, helicase and serine protease.[19] Many drugs are in preclinical and clinical development to target key components of the viral life cycle, including inhibitors of viral entry, RNA polymerase activity (e.g. nucleoside and non-nucleoside inhibitors), protein translation, polyprotein processing (e.g. serine protease inhibitors) and viral assembly and release (Fig. 148.2).

Telaprevir, a protease inhibitor, is the agent furthest along in clinical development. Telaprevir given in combination with pegIFN and ribavirin was recently shown in phase II studies to achieve superior SVR rates over pegIFN plus ribavirin.[20,21] Similarly, the combination of pegIFN and ribavirin with boceprevir, another HCV protease inhibitor in clinical development, resulted in significantly higher SVR rates than pegIFN and ribavirin in phase II studies. Since monotherapy with telaprevir, boceprevir, and similar agents results in unacceptable rates of resistance, strategies for the use of newer agents typically involve co-administration with pegIFN and ribavirin. Nitazoxanide, an approved antiparasitic agent, has also been shown to possess antiviral properties and its use in combination with pegIFN and ribavirin is being studied.

In addition to novel strategies, longer acting and oral forms of interferon as well as analogues of ribavirin are also being investigated. Therapies targeted at preventing fibrosis are also in development.

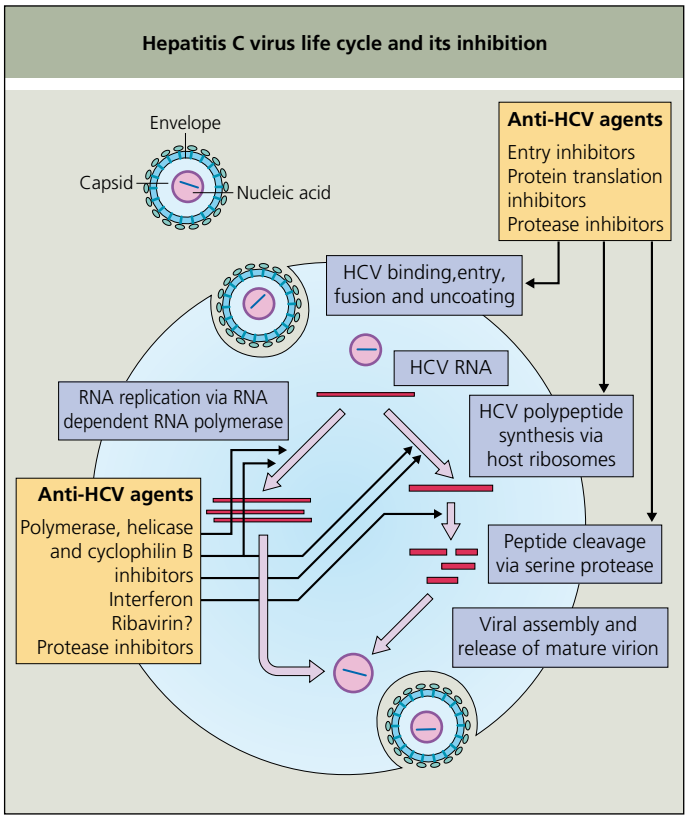


Fig. 148.2 Hepatitis C virus life cycle and its inhibition.

Disappointingly, a recent randomized clinical trial designed to investigate the long-term use of pegIFN maintenance therapy as an antifibrotic over a 4-year period did not show benefit in terms of fibrosis.[22]

NUCLEOSIDE AND NUCLEOTIDE ANALOGUES

Nucleoside and nucleotide analogues have been developed for use in the treatment of several viral pathogens and function either as nucleic acid chain terminators or as inhibitors of polymerase enzymes. A nucleotide differs from a nucleoside by the presence of one or more phosphate groups covalently bound to the molecule's sugar group. Intracellular phosphorylation is required before incorporation into the elongating nucleic acid chain. Nucleoside analogues include lamivudine, emtricitabine, entecavir and telbivudine, while nucleotide analogues are represented by adefovir and tenofovir. These drugs inhibit a critical step in the HBV life cycle where the reverse transcriptase activity of its DNA polymerase catalyzes the conversion of RNA to DNA. Resistance to nucleoside(tide) analogues can be caused by mutations in the active site of the DNA polymerase enzyme, including the highly conserved amino acid motif YMDD which confers resistance to lamivudine and emtricitabine.[23]

Nucleoside(tide) analogue therapy is generally indicated for patients with hepatitis B who have evidence of active viral replication and either persistent elevations in transaminases or histologically active disease (see Chapter 38). Based on the need for long-term treatment and their higher barrier to resistance, entecavir and tenofovir represent the currently preferred first-line monotherapy choices. The other available agents are no longer felt to be suitable for monotherapy in most circumstances due to unacceptably high rates of resistance. Viral breakthrough due to the development of resistance or discontinuation of this drug class may be associated with severe exacerbations of hepatitis.

Lamivudine (3TC)

Lamivudine was the first nucleoside analogue approved for HBV infection. It is also used for the treatment of HIV infection. Detailed pharmacokinetic and distribution data are provided in Chapter 145. The dose used for HBV (100 mg q24h) is lower than that used for HIV. Lamivudine therapy is associated with a significant improvement in hepatic histology, normalization of hepatic enzymes and suppression of plasma HBV DNA.[24] In most patients, however, values return to baseline levels when lamivudine is discontinued. Furthermore, the emergence of drug-resistant HBV limits the value of lamivudine for long-term therapy and may be associated with hepatitis flares.[25] Regimens combining lamivudine with other active compounds to enhance efficacy and limit the development of resistance are under investigation. Lamivudine (300 mg dose) is also available as a co-formulation with other drugs used in the treatment of HIV.

Adefovir

Adefovir is an approved nucleotide analogue for the treatment of HBV infection. Originally developed as an antiretroviral agent, it is associated with significant renal toxicity at the higher doses needed to inhibit HIV replication and is not approved for that indication.

Pharmacokinetics and distribution

Adefovir is administered as the prodrug adefovir dipivoxil. Unaffected by food, it achieves 60% oral bioavailability. Its half-life is about 12–30 hours and it undergoes renal excretion without significant observed metabolites. It does not substantially affect the cytochrome P450 system.

Indications

Chronic hepatitis B virus infection

Adefovir is highly active against HBV, producing reductions of HBV DNA of >3.5 logs. A phase III study in HBeAg-positive, treatment-naive patients randomized patients to adefovir 10 mg, 30 mg or placebo; HBV viral load reduction, histologic improvement and HBeAg seroconversion rates were superior in the adefovir arms.[26] The drug does not exhibit cross-resistance with lamivudine and is effective in the treatment of lamivudine-resistant HBV. As mutations associated with adefovir resistance emerge in 15% of patients by 96 weeks,[27] its use as first line-therapy is largely being supplanted by entecavir and tenofovir. Its role in combination therapy is also being explored.

Route of administration and dosing

Adefovir is approved for oral use only. The drug displays anti-HBV activity at doses above 5 mg/day. It is approved for use at 10 mg/day for HBV infection.

Adverse reactions and interactions

Adefovir appears well tolerated at the 10 mg dose. Renal impairment becomes significant, however, at the 30 mg dosage which is not used for the treatment of HBV.[25] Dose adjustment is recommended for patients with creatinine clearances (CrCl) <50 ml/min. The most common adverse reactions are headache, gastrointestinal upset and elevated transaminases. No significant drug interactions have been documented.

Tenofovir disoproxil fumarate (TDF)

Tenofovir is an orally bioavailable, nucleotide analogue approved for treatment of HIV and recently approved for treatment of chronic hepatitis B. Detailed pharmacokinetic and safety data are given in Chapter 145. Tenofovir exhibits potent viral suppression, with 93% and 76% of HBeAg-negative and HBeAg-positive patients having undetectable HBV DNA, respectively, at 1 year.[28] No patients in these studies exhibited genotypic resistance, and only case reports of drug resistance have been described to date.

Tenofovir has activity against lamivudine- and adefovir-resistant HBV isolates, and its efficacy has been shown in clinical trials of lamivudine- and adefovir-experienced patients. Tenofovir (usually in combination with lamivudine or emtricitabine) is the HBV treatment of choice for patients who require concomitant treatment of HIV infection (see Practice Point 44). It is available as a co-formulation with emtricitabine or with emtricitabine and efavirenz.

Emtricitabine (FTC)

Emtricitabine is an orally bioavailable, cytosine nucleoside analogue approved for treatment of HIV infection with efficacy against HBV. Because of its structural similarity to lamivudine, it shares a common resistance profile and its use in the treatment of HIV is felt to be interchangeable with lamivudine. Detailed pharmacokinetic and safety data are given in Chapter 145. In a study of both HBeAg-positive and HBeAg-negative patients, the 200 mg dose of emtricitabine was associated with viral suppression in 54% compared to 2% of placebo-treated patients at 48 weeks.[29] As with lamivudine, resistance with monotherapy is unacceptably high, with emtricitabine resistance mutations detected in 13% of patients in this study. It is available as a co-formulation with tenofovir or with tenofovir and efavirenz.

Entecavir

Entecavir is an orally bioavailable guanosine nucleoside analogue that functionally inhibits all three activities of the HBV DNA polymerase: base priming, reverse transcription of the negative strand and synthesis of the positive strand.

Pharmacokinetics and distribution

Entecavir's active phosphorylated form has a half-life of 15 hours. Entecavir is cleared by the kidneys primarily, and dose adjustment is recommended for patients with CrCl <50 ml/min.

Indications

Chronic hepatitis B virus infection

A dose-ranging study of entecavir at 0.01 mg, 0.1 mg and 0.5 mg versus lamivudine demonstrated reductions in HBV DNA of 2.4, 4.3 and 4.7 logs at each entecavir dose, respectively, compared with a 3.4 log reduction with lamivudine.[30] In studies of lamivudine-naive, HBeAg-positive subjects comparing entecavir and lamivudine, entecavir was associated with superior rates of virologic suppression at 48 weeks compared to lamivudine (67% vs. 36% <300 copies/ml); however, rates of eAg loss were similar.[31] In lamivudine-naive HBeAg-negative patients, entecavir was also shown to achieve superior rates of virologic suppression at 48 weeks.[32] Longer term treatment shows continued high rates of suppression through 4–5 years.

The presence of resistance mutations associated with lamivudine and telbivudine predisposes to the development of entecavir resistance, and its efficacy is reduced in the setting of prior lamivudine failure (e.g. cumulative development of resistance was 43% in lamivudine-refractory, compared to 1.2% in lamivudine-naive, subjects at 4 years).[33] An increased dose is recommended for patients with probable or known lamivudine resistance; however, use of alternative drugs without cross-resistance may be preferable. Because it is capable of inducing mutations in HIV reverse transcriptase associated with lamivudine resistance,[34,35] it should not be used in HBV/HIV co-infected patients who are not receiving antiretroviral therapy (see Practice Point 44).

Route of administration and dosing

Entecavir is approved for oral use only. The dose is 0.5 mg once daily except for patients with probable or known lamivudine resistance for whom the recommended dose is 1.0 mg daily. As its absorption is decreased by food, it is recommended that entecavir be administered on an empty stomach (at least 2 hours before or after a meal).

Adverse reactions and interactions

In studies, safety was similar between entecavir and lamivudine or entecavir and placebo. The most commonly observed adverse events that were possibly related to entecavir included headache, fatigue, dizziness and nausea.

Telbivudine (LdT)

Telbivudine is an oral thymidine nucleoside analogue with activity against HBV on both first and second strand synthesis.

Pharmacokinetics and distribution

Telbivudine distributes widely into the tissues and is excreted primarily by the kidneys. Dose adjustments are recommended for CrCl <50 ml/min. There are no significant known drug interactions.

Route of administration and dosing

Telbivudine is administered orally as 600 mg once daily with or without food as its absorption is not affected by food.

Indications

Chronic hepatitis B virus infection

Telbivudine showed superior rates of HBV DNA viral suppression when compared to lamivudine for both HBeAg-positive and

HBeAg-negative patients.[36] However, at 2 years, the development of genotypic resistance occurred in 21.6% and 8.6%, respectively.[37] The most commonly observed resistance mutation during treatment with telbivudine (M204I) confers cross-resistance to lamivudine. For these reasons, as with lamivudine, telbivudine monotherapy for HBV should generally be avoided. Telbivudine's use has not been investigated in patients with HIV co-infection.

Adverse reactions and interactions

Telbivudine was tolerated similar to lamivudine in studies comparing the two drugs except that grade 3–4 creatine kinase elevations were observed more frequently with telbivudine (9% vs. 3%). Myopathy with associated muscle weakness was rare (<1%).[37]

THERAPIES IN DEVELOPMENT FOR HEPATITIS B

Clevudine is an oral, once-daily nucleoside analogue which is approved for use in Korea. In phase III studies comparing clevudine with placebo, 24 weeks of treatment with clevudine resulted in HBV DNA suppression in 59% of HBeAg-positive and 92% of HBeAg-negative patients during treatment, and a higher than expected median DNA suppression 24 weeks after discontinuation of treatment.[38,39] Phase III studies are ongoing in the USA and Europe.

COMBINATION THERAPY FOR HEPATITIS B VIRUS INFECTION

Studies of combination therapy involving nucleoside analogues plus IFN demonstrated that while combination therapy could enhance viral suppression, post-treatment end points such as HBeAg seroconversion were not affected.[5,7] Combination studies involving use of two nucleoside(tide) analogues to date have provided signals that combination therapy may enhance viral suppression and increase the barrier to resistance but have yet to establish superiority of combination therapy compared to potent monotherapy. Thus, ongoing studies involving more potent combinations compared to potent monotherapy must be completed to determine whether particular combinations can be recommended definitively. For HIV/HBV co-infected patients who require combination therapy for the treatment of HIV, the use of the combination of tenofovir plus emtricitabine (available as a co-formulation) or tenofovir plus lamivudine is recommended as part of the regimen.

REFERENCES


References for this chapter can be found online at http://www.expertconsult.com

Chapter **149**

Shmuel Shoham
Andreas H Groll
Thomas J Walsh

Antifungal agents

Invasive fungal infections have emerged as important causes of morbidity and mortality in patients with severe underlying diseases. For more than three decades, treatment had been limited to amphotericin B deoxycholate with or without flucytosine. Therapeutic options only emerged with the clinical development of fluconazole and itraconazole in the late 1980s. The past 15 years, however, have witnessed a major expansion in our antifungal armamentarium through the introduction of less toxic formulations of amphotericin B, the development of improved antifungal triazoles and the advent of the echinocandin lipopeptides, a new class of antifungal agents that target the fungal cell wall. This chapter reviews the clinical pharmacology of approved and investigational antifungal agents for treatment of invasive fungal infections.

POLYENE ANTIFUNGAL AGENTS

Amphotericin B deoxycholate

Amphotericin B (AmB) is a natural polyene macrolide antibiotic and consists of seven conjugated double bonds, an internal ester, a free carboxyl group and a glycoside side chain with a primary amino group (Fig. 149.1). It is amphoteric, virtually insoluble in water, and not orally or intramuscularly absorbed. For parenteral use, AmB has been solubilized with deoxycholate as micellar suspension, and this formulation has now been available for more than 40 years.

Mechanism of action

Amphotericin B primarily acts by binding to ergosterol, the principal sterol in the cell membrane of most fungi, leading to the formation of ion channels and concentration-dependent cell death (Fig. 149.2). These pores, composed of AmB multimers, form most readily when ergosterol is present in the cell membrane.[1,2] With less avidity, the compound also binds to cholesterol, the main sterol of mammalian cell membranes, which is believed to account for most of its adverse effects. Amphotericin B may also induce cell damage through a cascade of oxidative reactions with formation of free radicals or an increase in membrane permeability.[3–5]

Antifungal activity

Amphotericin B has broad-spectrum antifungal activity that includes most pathogenic fungi. Primary resistance has been associated with qualitative or quantitative variations in membrane sterols, but may also be related to increased catalase activity with decreased susceptibility to oxidative damage. Resistance remains uncommon in *Cryptococcus neoformans* and *Candida* spp. although AmB appears

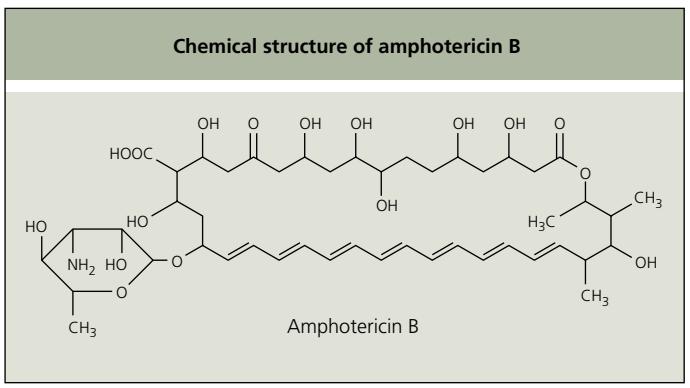


Fig. 149.1 Chemical structure of the polyene antifungal agents.

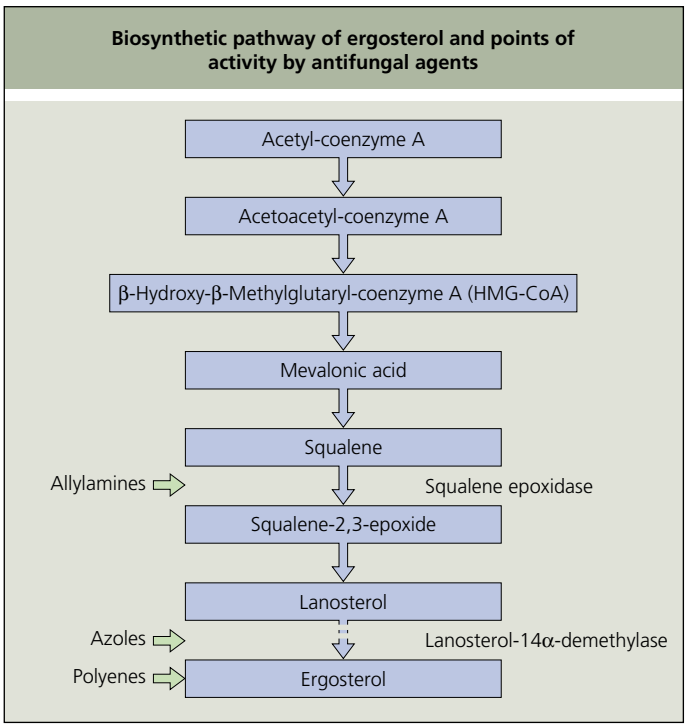


Fig. 149.2 Biosynthetic pathway of ergosterol and points of activity by antifungal agents.

somewhat less active against *C. guilliermondii*, *C. parapsilosis* and *C. tropicalis*. *Candida lusitaniae* isolates are often resistant to AmB, and this resistance can develop during treatment.[6] *Aspergillus* spp. and other opportunistic molds, but not the dimorphic molds, tend to have more variable susceptibility. *Aspergillus terreus*, *Fusarium* spp., *Pseudallescheria boydii*, *Scedosporium prolificans*, certain other dematiaceous fungi and *Trichosporon* spp. can be resistant to AmB at concentrations safely achievable in patients.[6–8] Acquisition of secondary resistance has been anecdotally reported but its clinical role has not been fully defined.

Pharmacodynamics

AmB displays concentration-dependent fungicidal activity against susceptible *Candida albicans*, *Cr. neoformans* and *Aspergillus fumigatus* in time-kill assays and exhibits a postantifungal effect of up to 12 hours' duration against *C. albicans* and *Cr. neoformans in vitro*.[9–11] Studies in laboratory animals with disseminated candidiasis suggest that peak serum level/minimum inhibitory concentration (MIC) correlates best with efficacy *in vivo*. AmB may have immune stimulatory properties that enhance phagocyte responses to *Aspergillus* and *Fusarium* spp.[12,13]

Antifungal activity in biofilms has gained increasing attention. Biofilm-associated candidiasis typically occurs with infection of biomedical devices. Compared to planktonic yeast cells, biofilm-associated sessile cells show decreased susceptibility to AmB deoxycholate. By contrast, lipid formulations of AmB may remain active against sessile yeast.[14] Animal models suggest a role for AmB lipid formulations as antibiotic lock therapy in catheter-associated candidiasis.[15]

Pharmacokinetics

After intravenous administration, AmB dissociates from its vehicle and becomes highly protein-bound before distributing predominantly into liver, spleen, bone marrow, kidney and lung. A unique property of AmB is that protein binding in plasma is enhanced with increasing drug concentration.[16] Clearance from plasma is slow with a terminal half-life of 5 days and longer.[17] Concentrations in body fluids other than plasma may vary greatly between patients and are substantially lower than serum drug levels.[18,19] The clinical relevance of this is unclear and despite mostly undetectable concentrations in the cerebrospinal fluid (CSF), AmB is effective in central nervous system (CNS) fungal infections. AmB is mostly excreted in urine and feces as unchanged drug and no metabolites have been identified.[17] Dose adjustment of AmB is not necessary in patients with unrelated renal or hepatic dysfunction. Because of its high protein binding, hemodialysis usually does not affect plasma concentrations. Plasma drug clearance is inversely correlated with age.

Adverse effects

Infusion-related reactions and nephrotoxicity are major problems and often limit therapy. Infusion-related reactions (fever, rigors, chills, myalgias, arthralgias, nausea, vomiting and headaches) are mediated by cytokine release from monocytes. Whether this immune activation contributes to the effectiveness of AmB as an antifungal agent is not known. Infusion-related events can be noted in up to 73% of patients during the first dose, but often improve during continued therapy.[20] Slowing the infusion rate or premedication with acetaminophen (10–15 mg/kg), hydrocortisone (0.5–1.0 mg/kg) or meperidine (0.2–0.5 mg/kg) can blunt these reactions. Continuous 24-hour infusion of AmB further reduces symptoms, but data on the antifungal efficacy of this dosing strategy are limited.[21] Cardiac arrhythmias and cardiac arrest due to acute potassium release may occur with rapid infusion (<60 min), especially if there is pre-existing hyperkalemia and/or renal impairment. True allergic reactions are rare.[22]

AmB-associated nephrotoxicity occurs due to alterations of membrane permeability in renal tubular and vascular smooth muscle cells. Tubular transport defects and decreased glomerular filtration rate (GFR) due to vasoconstriction are responsible for potassium and magnesium wasting, tubular acidosis, impaired urinary concentrating ability and azotemia. Decreased renal blood flow and recurrent ischemia may lead to permanent structural nephrotoxic effects.[3] Hypokalemia can be refractory to replacement until hypomagnesemia is corrected. Tubular acidosis and impaired urinary concentrating ability are rarely of clinical significance. Azotemia is common. In a large prospective clinical trial, baseline serum creatinine rose by more than double in over one-third of patients receiving AmB.[20] Renal toxicity is related to dose and duration of therapy, concomitant use of nephrotoxic agents and pre-existing renal dysfunction. AmB has the potential to lead to renal failure and dialysis. Often, however, azotemia stabilizes on therapy and is usually reversible after discontinuation of the drug. Avoiding concomitant nephrotoxic agents, and normal saline loading (10–15 ml 0.9% NaCl/kg/day) may lessen azotemia.

Amphotericin B may also cause hypotension, hypertension, flushing, vestibular disturbances and a normocytic, normochromic anemia after chronic administration.[15] As AmB deoxycholate is topically irritating, a central line should be used for infusion. Local instillation, including intrathecal administration, can cause focal areas of necrosis and requires expert consultation.[23]

Drug interactions

In rats, AmB decreases the concentration of hepatic microsomal cytochrome P450 and inhibits propafenone metabolism.[24] Drug interactions due to shared metabolic pathways have not been described in humans. Hypokalemia may be aggravated by corticosteroids, and hypomagnesemia may become especially profound in cancer patients with platinum-associated nephropathy. Amphotericin B may enhance plasma levels and toxicity of renally cleared drugs, including aminoglycosides, vancomycin, flucytosine and ciclosporin. Simultaneous infusion of granulocytes has been associated with acute pulmonary reactions and should be used with extreme caution.

Indications and dosing

Despite its toxicity profile, AmB deoxycholate remains an important option for initial treatment of many life-threatening fungal infections. Depending on the type of infection and the host, recommended daily dosages range from 0.5 mg/kg/day to 1.5 mg/kg/day administered over 2–4 hours as tolerated; the standard dosage for empiric therapy in persistently febrile neutropenic patients is 0.5–0.6 mg/kg/day.[22] Continuous infusion of AmB deoxycholate is counterintuitive to the concentration-dependent pharmacodynamics of AmB and is not recommended. Whether the enhanced plasma clearance in infants and young children has implications for dosing remains unknown. Currently, dosage recommendations for all pediatric age groups do not differ from those in adults. Treatment should be started at the full target dosage with careful bedside monitoring during the first infusion to allow for prompt intervention for infusion-related reactions.

Therapeutic monitoring

Historically, 1-hour postinfusion plasma concentrations of twice the MIC of the fungal isolate have been proposed as a target for the treatment of yeast infections.[25] However, monitoring of AmB concentrations in plasma or CSF appears of little value, since relationships between plasma and tissue concentrations and clinical efficacy or toxicity have not been adequately characterized.[10]

Amphotericin B lipid formulations

Three lipid formulations of AmB have been approved in the USA and most of Europe:

- AmB colloidal dispersion (ABCD, Amphocil or Amphotec);
- AmB lipid complex (ABLC or Abelcet); and
- a small unilamellar vesicle (SUV) liposomal formulation (L-AmB, AmBisome).

Because of their reduced nephrotoxicity in comparison to AmB deoxycholate, these compounds allow for the delivery of higher dosages of AmB. However, data from animal models also suggest that higher dosages are required for equivalent antifungal efficacy.

Physicochemical properties and pharmacokinetics

Each of the lipid formulations confers distinct plasma pharmacokinetic properties to AmB. All three formulations preferentially distribute to organs of the mononuclear phagocytic system (MPS); sequester lipid-associated drug within deep tissues and functionally spare the kidney. L-AmB, the only truly liposomal AmB, is composed of small spherical unilamellar lipid vesicles averaging 60–70 nm in size. This preparation has a prolonged circulation time in plasma, achieves strikingly high peak plasma concentrations and AUC values and is only slowly taken up by the MPS. However, most of the AmB in plasma remains liposome associated and free unbound drug concentrations are low.[16,17] ABLC is composed of ribbon-like lipid aggregates. It is efficiently opsonized by plasma proteins and rapidly taken up by the MPS to achieve high concentrations in cellular components of blood, liver, lung and spleen. ABLC exists as a depot in these tissues and free AmB is slowly released. Fungal and inflammatory cell lipases at the site of infection act to release AmB from the lipid carrier.[26] ABCD, a colloidal dispersion of microscopic disc-shaped particles, also preferentially collects in tissues of the MPS and free drug is slowly released over time. Drug levels in the plasma and kidney are low and terminal elimination half-life is long (Table 149.1). Whether these distinct pharmacokinetic features translate into different pharmacodynamic properties is largely unknown. Comparisons of all four formulations of AmB against defined infections suggest potentially important differences in efficacy depending on agent, dose, type and site of infection.[7]

Safety and antifungal efficacy

Safety and efficacy of the lipid formulations have been demonstrated in phase I/II studies in immunocompromised patients with a wide spectrum of underlying disorders.[27–29] The overall response rates in these trials ranged from 53% to 84% for invasive candidiasis and 34% to 5%, respectively, in probable or documented invasive aspergillosis. Clinical and microbiologic responses were observed even when lipid formulations were used after failure of conventional AmB deoxycholate. Patients with neutropenia responded to therapy with lipid formulations; however, as with other antifungal agents, outcomes were dependent on host factors. Several randomized controlled trials have compared lipid formulations with AmB deoxycholate. These studies have consistently shown at least equivalent efficacy and reduced nephrotoxicity in comparison to AmB deoxycholate. A meta-analysis of 1149 neutropenic patients from three trials found that prophylactic or empiric use of L-AmB tended to be more effective than AmB deoxycholate in preventing breakthrough fungal infections, was associated with less nephrotoxicity but did not alter survival.[30] Infusion-related side-effects are less frequent with L-AmB but may be increased with ABCD.[20,31,32] Substernal chest discomfort, respiratory distress and sharp flank pain have been noted during infusion of L-AmB. Hypoxic episodes associated with fever and chills are more frequent with ABCD than AmB deoxycholate.[22] Mild increases in serum bilirubin and alkaline phosphatases have been observed with lipid formulations. Generally mild increases in serum transaminases and pancreatic enzymes have been observed with L-AmB.

Indications and dosing

The lipid formulations are indicated for the treatment of patients with AmB-susceptible invasive mycoses who are refractory to, or intolerant of, AmB deoxycholate and are limited to L-AmB for empiric therapy of persistently neutropenic patients. Preliminary pediatric pharmacokinetic and safety data indicate no fundamental differences in comparison to adults. The optimal dosages of each formulation for the various types and sites of invasive fungal infection remain to be defined. Based on animal data and the few randomized studies that have used AmB deoxycholate as comparator,[33] the authors and most other experts in the field consider a dose of 5 mg/kg/day of ABCD, ABLC and

Table 149.1 Amphotericin B formulations: physicochemical properties and pharmacokinetic parameters

	DAMB	ABCD	ABLC	LAMB
Lipids (molar ratio)	Deoxycholate	Cholesterylsulfate	DMPC/DMPG (7:3)	HPC/CHOL/DSPG (2:1:0.8)
Mol% drug	34	50	50	10
Lipid configuration	Micelles	Micelles	Membrane-like	SUVs
Particle diameter (μm)	0.05	0.12–0.14	1.6–11	0.08
Dosage (mg/kg)	1	5	5	5
C_{max} (μg/ml)	2.9	3.1	1.7	58
$AUC_{0-\infty}$ (μg/ml.h)	36	43	14	713
VD_{ss} (l/kg)	1.1	4.3	131	0.22
Cl (l/h/kg)	0.028	0.117	0.476	0.017
Half-life (h)	39	28	6–18	7–10

DAMB, amphotericin B deoxycholate; ABCD, amphotericin B colloidal dispersion; ABLC, amphotericin B lipid complex; LAMB, liposomal amphotericin B; DMPC, dimiristoyl phosphatidylcholine; DMPG, dimiristoyl phosphatidylglycerol; HPC, hydrogenated phosphatidylcholine; CHOL, cholesterol; DSPG, disteaoryl phosphatidylglycerol; SUVs, small unilamellar vesicles (liposomes); C_{max}, peak plasma concentration; $AUC_{0-\infty}$, area under the concentration vs time curve from time zero to infinity; VD_{ss}, apparent volume of distribution at steady state; Cl, plasma clearance; Half-life, apparent plasma half-life during the dosing interval of 24 hours.
Data represent mean values, stem from adult patients and were obtained after different rates of infusion. Modified from Groll *et al.*[52]

L-AmB equivalent to a dosage of 1 mg/kg/day of AmB deoxycholate. Accordingly, an initial dosage of 5 mg/kg/d of ABCD, ABLC or L-AmB is recommended for suspected or documented life-threatening infections, and 3 mg/kg/day when L-AmB is selected for empiric antifungal therapy in persistently febrile neutropenic patients; 3 mg/kg/day may be effective in histoplasmosis and selected patients with candidiasis.[34] Some clinicians have successfully used L-AmB at 10 mg/kg/day and 15 mg/kg/day in patients with recalcitrant invasive fungal infections. However, such doses may provide no additional efficacy and more nephrotoxicity.[35]

Use of the lipid formulations has been limited by their higher costs. Comprehensive analyses that include hospital and societal costs due to excess nephrotoxicity relating to the use of conventional AmB deoxycholate are indicating that the higher cost of lipid formulations of AmB may be supplanted by the even greater cost of AmB deoxycholate-induced nephrotoxicity. Among patients receiving AmB, even slight decreases in renal function are associated with longer hospitalizations and severe nephrotoxicity is a significant predictor of death.[36] At an increasing number of centers the current practice is to limit use of AmB deoxycholate to patients having the lowest risk for nephrotoxicity and to switch to a lipid formulation with decreasing renal function.

Aerosolized amphotericin B

Nebulized AmB is increasingly used as antimold prophylaxis in lung transplant and neutropenic patients. All four AmB formulations can be aerosolized. This approach appears promising in animal studies and early clinical trials.[37,38] Aerosol size and uniformity vary with AmB formulation and nebulizer technique.[39] These variables may impact delivered drug dose to the lung. Furthermore, within individual patients, delivered dose may be suboptimal in some lung segments.[33]

Current protocols employ varying doses, regimens and delivery systems. When tolerated, as in lung transplant recipients, aerosolized AmB is effective at preventing aspergillosis. Common side-effects are cough, adverse taste and nausea.[40,41] Tolerability remains a major drawback in very fragile and weak patients. Cough and inability to use an aerosol delivery system are common limitations.[37]

FLUCYTOSINE

Flucytosine (5-fluorocytosine; 5-FC) is a low molecular weight water-soluble synthetic fluorinated analogue of cytosine (Fig. 149.3). It has no antifungal activity of its own. It is taken up by the fungus-specific enzyme cytosine permease and converted in the cytoplasm by cytosine deaminase to 5-fluorouracil which causes RNA miscoding and inhibits DNA synthesis.[42,43] Although it was originally synthesized as a potential anti-tumor agent, 5-FC is relatively nontoxic to mammalian cells.

Comparison of the chemical structures of cytosine, flucytosine and fluorouracil

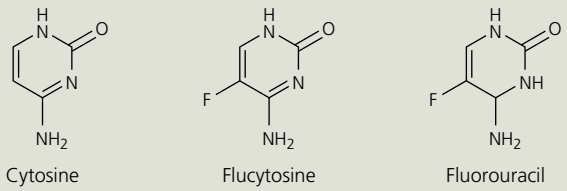


Fig. 149.3 Comparison of the chemical structures of cytosine, flucytosine and fluorouracil.

Antifungal activity

The antifungal spectrum of 5-FC *in vitro* encompasses *Candida* spp., *Cr. neoformans*, *Saccharomyces cerevisiae* and selected dematiaceous molds; 5-FC has little to no activity against *Aspergillus* spp. and other hyaline molds.[43] Synergistic or additive effects in combination with AmB have been observed against *Candida* spp. and in combination with AmB, fluconazole or posaconazole against *Cr. neoformans*.[44,45] With the exception of *Candida krusei*, primary resistance occurs very rarely in *Candida* spp.[46] Resistance (MIC ≥32 μg/ml) has been observed in 1.6–2.2% of *Cr. neoformans* isolates.[47] In contrast to primary resistance, secondary resistance, which occurs predominantly by selection of resistant clones, can evolve rapidly and appears to result from a single point mutation. In *Cr. neoformans* resistance has been observed in 30–40% of isolates from patients with meningitis who relapsed following monotherapy. Resistance in susceptible fungi may involve either mutations in the enzymes necessary for cellular uptake, transport or metabolism, or competitive upregulation of pyrimidine synthesis.[42] As a consequence, 5-FC is rarely given alone but in combination with AmB or, more recently, fluconazole.

Pharmacodynamics

Flucytosine has demonstrated predominantly concentration-independent fungistatic (99% reduction in CFU) activity against *Candida* spp. and *Cr. neoformans* in time-kill assays and prolonged concentration- and exposure-dependent postantifungal effects of up to 10 hours.[48,49] Pharmacodynamic studies in mice with disseminated candidiasis indicate that time above the MIC and AUC/MIC are the most important parameters in predicting efficacy. The C_{max}/MIC ratio was the least important parameter and maximum efficacy was observed when levels exceeded the MIC for only 20–25% of the 24-hour dosing interval.[50] Thus, lower dosages or less frequent dosing may yield identical efficacy while reducing the mostly dose-dependent toxicities.

Pharmacokinetics

Oral 5-FC is readily absorbed from the gastrointestinal tract, has negligible protein binding and distributes evenly into tissues and body fluids, including the CSF, peritoneal fluid, inflamed joints and the eye. Intravenous 5-FC results in significantly higher serum drug levels, but the clinical or microbiologic implications of this are unknown.[51] At usual dosages, the drug undergoes little hepatic metabolism and is eliminated predominantly in active form by glomerular filtration into the urine with a half-life in plasma of 3–6 hours. Individual dosage adjustment is necessary in patients with impaired renal function and those undergoing hemofiltration. In patients undergoing hemodialysis, a dose of 37.5 mg/kg is recommended following dialysis; in peritoneal dialysis, 5-FC can be administered systemically or intraperitoneally.[42] Impaired liver function does not appear to alter 5-FC disposition.[52] As the pharmacokinetics of 5-FC in children have not been formally characterized, uniform dosing recommendations cannot be made.

Adverse effects

Common adverse effects of 5-FC include gastrointestinal intolerance and reversible elevations of hepatic transaminases and alkaline phosphatase. More rare are rashes, ulcerative colitis and bowel perforation, eosinophilia and crystalluria.[52] Hematologic adverse effects may include neutropenia, thrombocytopenia or pancytopenia. Among 202 AIDS patients receiving combination therapy with AmB and 5-FC for cryptococcal meningitis, the drug toxicity withdrawal rate of 3% was similar to those receiving AmB monotherapy.[53] Although adverse affects are usually reversible following discontinuation of the drug or dosage reduction, fatal outcomes have been reported. Some adverse effects may be due to conversion of 5-FC to 5-fluorouracil by gastrointestinal bacteria and toxic effects of endogenous metabolites.[42]

In patients treated with intravenous 5-FC, in whom the drug presumably did not undergo metabolism by intestinal microflora, 5-fluorouracil levels were not detected, but some toxicity was still observed. Hematologic adverse effects are less frequent if plasma levels do not exceed 100 µg/ml.[52]

Drug interactions

Orally administered, nonresorbable antibiotics and aluminum/magnesium hydroxide-based antacids may delay oral 5-FC absorption.[52] Flucytosine is not known to interfere with the CYP450 enzyme system. However, drugs causing reduction in the GFR may increase 5-FC serum levels. Cytosine arabinoside competitively inhibits 5-FC and both drugs should not be given concomitantly.

Indications and dosing

Due to the propensity for secondary resistance, 5-FC is generally not administered alone. An established indication for its use in combination with AmB deoxycholate is for induction therapy of cryptococcal meningitis.[53] In cryptococcosis, sterilization of CSF is significantly faster when AmB is used in combination with 5-FC.[55] This combination can also be recommended for deep tissue candidiasis, particularly in critically ill patients and when non-*albicans Candida* spp. are involved. This includes meningitis, endophthalmitis, endocarditis, vasculitis and peritonitis, as well as osteoarticular, renal and chronic disseminated candidiasis.[52] Flucytosine may delay hematopoietic recovery after cytotoxic chemotherapy and is not recommended as part of empiric therapy of persistently febrile neutropenic patients. Flucytosine with fluconazole may be used for cryptococcal meningitis, when treatment with AmB is not feasible. This combination may also be useful as second-line therapy for individual patients with invasive *Candida* infections involving aqueous body compartments. We recommend a starting dosage for both adults and children of 100 mg/kg/day divided into three or four doses. However, when used in combination with AmB for disseminated candidiasis, 5-FC dosage of 25 mg/kg/day in four divided doses may suffice.[56]

Therapeutic monitoring

Monitoring of plasma concentrations is essential to adjust dosage to changing renal function and to avoid toxicity. Most patients have levels outside the expected therapeutic range and high levels are particularly common in neonates. In one report, nearly 10% of patients had potentially toxic levels (>100 µg/ml).[57] Following oral administration, near peak levels 2 hours post dosing overlap with trough levels as patients reach steady state and are thus sufficient for therapeutic monitoring. Peak plasma levels between 40 µg/ml and 60 µg/ml correlate with antifungal efficacy and are seldom associated with hematologic adverse effects.[54] The need for monitoring plasma concentrations has limited the use of 5-FC as this test is often restricted to referral laboratories.

ANTIFUNGAL TRIAZOLES

The antifungal azoles are a class of synthetic compounds that have one or more azole rings and – attached to one of the nitrogen atoms – a more or less complex side chain. The imidazoles miconazole and ketoconazole were the first azoles developed for systemic treatment of human mycoses. Severe toxicities associated with the drug carrier (miconazole), erratic absorption and significant interference with the human cytochrome P450 system (ketoconazole) have limited their clinical usefulness.[52] The subsequently developed triazoles (Fig. 149.4), however, have become extremely useful agents. They possess an expanded spectrum of activity and greater target specificity, and are overall well tolerated.

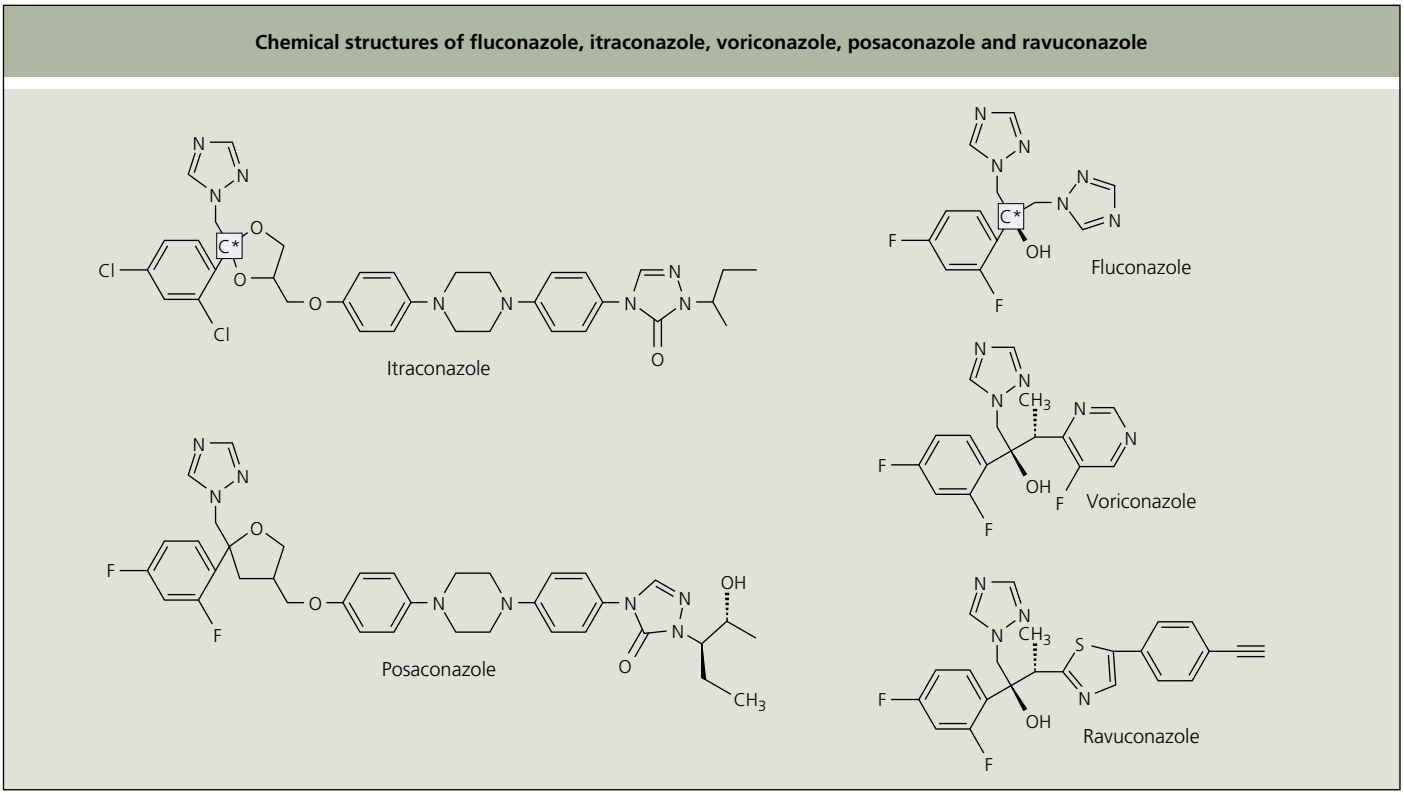


Fig. 149.4 Chemical structures of fluconazole, itraconazole, voriconazole, posaconazole and ravuconazole.

Mechanism of action

The antifungal azoles target ergosterol biosynthesis by inhibiting the fungal cytochrome P450-dependent enzyme lanosterol 14-α-demethylase. This inhibition interrupts the conversion of lanosterol to ergosterol, which leads to accumulation of aberrant 14-α-methylsterols and depletion of ergosterol in the fungal cell membrane (see Fig. 149.2). This alters cell membrane physiology and, depending on organism and compound, may lead to cell death or inhibition of cell growth and replication. The azoles also inhibit cytochrome P450-dependent enzymes of the fungal respiration chain.[52]

Antifungal activity

Fluconazole and itraconazole are principally active against dermatophytes, *Candida* spp., *Cr. neoformans*, *Trichosporon* spp. and some other uncommon yeast-like organisms, and against dimorphic fungi such as *Histoplasma capsulatum*, *Coccidioides immitis*, *Blastomyces dermatitidis*, *Paracoccidioides brasiliensis* and *Sporothrix schenkii*.[52] These azoles have less activity against *Candida glabrata* and almost none against *C. krusei*.[58] Itraconazole is active against *Aspergillus* spp. and dematiaceous molds. Neither is active against *Fusarium* spp. and the zygomycetes.[52] The second-generation triazoles, voriconazole and posaconazole, are active against *C. albicans* and *C. krusei*, *Aspergillus* spp. and some *Fusarium* spp. Posaconazole is also active against zygomycetes.[59]

Resistance

Selection and nosocomial spread of azole-resistant *Candida* spp. is a matter of increasing concern. Resistance in *Candida* is encountered most commonly in the form of a primarily resistant species or through selection of resistant subclones during exposure to azoles. Several mechanisms have been identified, including alterations at the target binding site, increased target expression and induction of cellular efflux pumps. *Candida* in biofilm is significantly less susceptible to fluconazole, voriconazole and posaconazole.[28] This is at least partly mediated by expression of efflux pumps and alteration in sterol composition.[60]

Azole-resistant oropharyngeal and esophageal candidiasis remains a challenge in some AIDS patients.[61] Emergence of *C. glabrata* and *C. krusei* infections in association with fluconazole prophylaxis has been sporadically observed in several bone marrow transplant centers.[62] While cross-resistance of *Candida* spp. to antifungal azoles is common,[63] it is not obligate: patients with microbiologic and clinical fluconazole-resistant mucosal candidiasis may respond to other triazoles. Acquired azole resistance has been documented in patients with *Cr. neoformans* meningitis receiving maintenance therapy. Azole-resistant *A. fumigatus* has emerged in the past decade and up to 6% of clinical isolates may have decreased susceptibility to itraconazole, voriconazole and posaconazole.[64]

Drug interactions

Caution is advised when antifungal azoles are co-administered with CYP3A4 substrates that have the potential to prolong the QTc interval and when co-administered with HMG-CoA reductase inhibitors that have the potential to cause rhabdomyolysis. The concurrent use of terfenadine, astemizole, cisapride, pimozide, halofantrine, dofetilide, quinidine and vincristine is contraindicated.

Fluconazole

Pharmacodynamics

Conventional time-kill assays performed over incubation periods of 24–48 hours in susceptible *Candida* spp. and *Cr. neoformans* show fungistatic activity of fluconazole with variable concentration-related growth effects.[65,66] However, with extended incubation of up to 14 days and under nonproliferating growth conditions, fungicidal activity has been observed against *C. albicans*.[67] In serum-free growth media, fluconazole displays no measurable postantifungal effect (PAFE) against *C. albicans* and *Cr. neoformans*, but concentration-dependent PAFEs of 1–3.6 hours were observed in the presence of fresh serum.[10] Clinical data and pharmacodynamic studies in murine models of disseminated *C. albicans* infection collectively suggest that the AUC/MIC ratio is the most predictive pharmacodynamic parameter of fluconazole.[68,69] Overall, the dose-independent pharmacokinetics and the available experimental and clinical data are in support of once-daily dosing regimens.

Pharmacokinetics

Fluconazole is available for oral and parenteral use, and exhibits linear plasma pharmacokinetics that are independent of route and formulation. Steady state is generally reached within 4–7 days following once-daily dosing, but can be rapidly achieved by doubling the dose on the first day. Bioavailability may vary greatly among critically ill surgical patients.[70] Protein binding is low, and the drug distributes evenly into virtually all tissue sites and body fluids, including the CSF, brain tissue and the eye. More than 90% of a dose is renally excreted, with approximately 80% recovered as unchanged active drug and 11% recovered as inactive metabolites. In patients with a creatinine clearance of ≤50 ml/min, a 50% reduction in dosage is required, and below 21 ml/min, a 75% reduction is needed. The initial loading dose need not be adjusted. Fluconazole is dialyzable; in patients undergoing hemodialysis, 100% of the target dose is given after each dialysis session. In continuous hemofiltration, clearance may be faster, requiring therapy at maximum approved dosages; in patients undergoing peritoneal dialysis, the compound can be administered either systemically or intraperitoneally. Hepatic insufficiency does not require dose adjustments, but careful monitoring of additional hepatic toxicity is warranted.[52]

The pharmacokinetics in children reflect developmental changes characteristic for a water-soluble drug with minor metabolism and predominantly renal elimination. Except for premature neonates, where clearance is initially decreased, children tend to have an increased weight-normalized clearance rate from plasma that leads to a shorter half-life in comparison to adults. As serum levels in neonates depend upon both gestational age at birth and postnatal age,[71] dosages at the high end of the recommended dosage range are necessary for the treatment of invasive mycoses in children.

Adverse effects

In adults, fluconazole has been safely administered over prolonged periods of time at dosages of up to 1600 mg/day.[72] Compiled data from adults who received dosages of 100–400 mg/day indicate an incidence of significant adverse effects or laboratory abnormalities leading to drug discontinuation of 2.8%. Gastrointestinal symptoms are seen in <5%, rashes and headaches in <2% and are usually reversible, and asymptomatic transaminase elevations in up to 7% of adult patients. QT segment prolongation and ventricular tachycardia have been described with fluconazole.[73] In children of all age groups, at dosages of up to 12 mg/kg/day, fluconazole is generally well tolerated with no differences in frequency and profile of adverse events.[29] Severe side-effects, including liver failure and exfoliative dermatitis, have been reported anecdotally.[52] Alopecia can be seen with higher doses (400 mg/day) given for 2 months or longer and is reversible with reduced dosage or drug discontinuation.[74]

Drug interactions

Fluconazole undergoes minimal CYP-mediated metabolism, but inhibits intestinal and hepatic CYP3A4 and several other CYP isoforms *in vitro*. Fluconazole interacts with enzymes involved in glucuronidation and is a substrate of the drug transporter P-glycoprotein, leading to a number of significant drug interactions (Table 149.2). Interactions

Table 149.2 Drug–drug interactions of fluconazole and itraconazole

Mechanism and drug involved	Triazole involved	Comment
Decreased plasma concentration of triazole		
Decreased absorption of triazole • Antacids, H_2 antagonists • Omeprazole, sucralfate • Didanosine, grapefruit juice	Itra**	Take antacids and antifungal agent at least 2 hours apart
Increased metabolism of triazole • Isoniazid, rifampin (rifampicin) • Rifabutin, phenytoin • Phenobarbital, carbamazepine	Itra**, flu	Potential for therapy failure; increased potential for hepatotoxicity
Increased concentration of co-administered drug through inhibition of its metabolism by triazole		
Terfenadine, astemizole, cisapride	Flu‡, Itra‡	Concom. use prohibited
Lovastatin, simvastatin, atorvastatin	Itra‡, Flu‡	Concom. use prohibited
Phenytoin	Flu*, Itra*	Monitor levels
Benzodiazepines	Flu†, Itra†	Monitor closely
Carbamazepine	Flu†	Monitor closely
Haloperidol	Itra†	Monitor closely
Rifampin (rifampicin), rifabutin	Flu†, Itra†	Monitor closely
Clarithromycin	Itra†	Monitor closely
Indinavir, ritonavir	Itra†	Monitor closely
Vincristine, vinblastine, vindesine	Itra*	Avoid concom. use
Busulfan	Itra†	Avoid concom. use
All-trans retinoic acid	Flu†	Monitor closely
Nifedipine, felodipine	Itra†, Flu†	Monitor closely
Ciclosporin A, tacrolimus	Flu†, Itra†	Monitor serum level
Sulfonylurea drugs, warfarin, prednisolone	Flu†, Itra†	Monitor closely
Digoxin, quinidine	Itra†	Monitor levels (digoxin)
Zidovudine, theophyllin	Flu†	Monitor closely

Flu, fluconazole; Itra, itraconazole.
*Major significance; †moderate significance; ‡contraindicated.
Modified from Groll *et al.*[52]

with other CYP3A4 substrates, including calcineurin inhibitors, may be more pronounced with oral fluconazole.[75] On the other hand, drugs notorious for hepatic enzyme induction – for example, rifampin (rifampicin) – may decrease fluconazole levels.[52] Altogether, the number of relevant drug interactions is lower than with ketoconazole and itraconazole.

Indications and dosing

Fluconazole is highly effective for treatment of superficial and invasive candidiasis, including infections in neutropenic patients.[76] However, in unstable patients and in recipients of azole prophylaxis, an alternative therapy should be considered. Other indications for fluconazole include consolidation therapy for chronic disseminated candidiasis, cryptococcal meningitis and infections by *Trichosporon* spp. Fluconazole is the drug of choice for treatment of coccidioidal meningitis and has effectiveness in nonmeningeal coccidioidal infections;[77,78] against paracoccidioidomycosis, blastomycosis, histoplasmosis and sporotrichosis, fluconazole is less active than itraconazole.[79–82] In the prophylactic setting, fluconazole has proven efficacy for primary prevention of invasive candidiasis in high-risk patients with acute leukemia, bone marrow and liver transplantation, for primary prevention of cryptococcosis and histoplasmosis in advanced AIDS and for secondary prevention of cryptococcosis and coccidioidomycosis in AIDS.[83,84]

In adults, the recommended dosage range for treatment of invasive infections is 400–800 mg/day and 100–400 mg/day in the preventive setting. In children, the recommended dosage range is 6–12 mg/kg/day. In view of the faster clearance rate, the larger volume of distribution and its safety profile, 12 mg/kg/day may be more appropriate for serious infections in term neonates, infants and children. Given the extreme variability in extravascular water content and renal function, particularly in very low birth weight preterm infants, predictably effective and safe treatment with fluconazole may not be possible during the first days of life.

Itraconazole

Pharmacodynamics

Itraconazole (ITC) exerts species- and strain-dependent fungistatic or fungicidal pharmacodynamics *in vitro*. Time-kill experiments have demonstrated concentration-independent, fungistatic activity of ITC against *Candida* spp. and *Cr. neoformans*.[10] Against *Aspergillus* spp., however, ITC displayed time- and concentration-dependent fungicidal activity with >87% to >97% killing within 24 hours of drug exposure.[85] Persistent effects have not been reported thus far, and it remains to be determined which pharmacodynamic parameter best predicts antifungal efficacy *in vivo*.[10]

Pharmacokinetics

Itraconazole is a high-molecular weight, highly lipophilic bis-triazole. It is available as capsules and as an oral solution in hydroxypropyl-β-cyclodextrin (HP-β-CD). Absorption from the capsule form is dependent on a low intragastric pH and is compromised in the fasting state, and thus is unpredictable in granulocytopenic cancer patients and in

patients with hypochlorhydria. Absorption is improved when the capsules are taken with food or an acidic cola beverage. Itraconazole oral solution in HP-β-CD provides better oral bioavailability that is further enhanced in the fasting state.[86]

Following oral administration, peak plasma concentrations occur within 1–4 hours; systemic absorption of the cyclodextrin carrier is negligible. With once-daily dosing, steady state is achieved after 7–14 days, but can be reached more rapidly by doubling the dose over the first 2–3 days. Itraconazole is highly protein-bound and is extensively distributed throughout the body. Although concentrations in nonproteinaceous body fluids are negligible, tissue concentrations in many organs, including the brain, exceed corresponding plasma levels by 2–10 times.[86]

Itraconazole is extensively metabolized in the liver and excreted in metabolized form into bile and urine. The major metabolite, hydroxyitraconazole (OH-ITC), possesses antifungal activity similar to ITC.[52] The elimination of ITC from plasma follows a biphasic pattern; in comparison to single dosing, the elimination half-life at steady state is about twice as long, reflecting saturable excretion mechanisms.[52]

The dosage of oral ITC does not need to be adjusted with renal insufficiency or dialysis. In patients with severe hepatic insufficiency, the elimination half-life of ITC can be prolonged and additional hepatic toxicity or possible drug interactions should be carefully monitored.[86] The pharmacokinetics of oral HP-β-CD ITC in pediatric patients beyond the neonatal period appear similar to that of adults.[87–89]

Adverse effects

Itraconazole is usually well tolerated, with a similar spectrum and frequency of adverse effects as fluconazole. Adverse events leading to the discontinuation of ITC occur in approximately 4% of patients treated for systemic fungal infections at dosages of up to 400 mg/day. Most observed reactions are transient, and include nausea and vomiting (<10%), hypertriglyceridemia (9%), hypokalemia (6%), elevated hepatic transaminases (5%), rash and/or pruritus (2%), headaches or dizziness (<2%) and pedal edema (1%).[90] Gastrointestinal intolerance is frequent with oral HP-β-CD ITC at dosages exceeding 400 mg/day. Only a few cases of more severe hepatic injury or hepatitis have been described. Itraconazole can have negative inotropic effects; because of a possible risk of cardiac toxicity, ITC should not be administered to patients with ventricular dysfunction.[91]

Oral HP-β-CD ITC was safe and well tolerated in pharmacokinetic studies in pediatric patients.[92] Vomiting (12%), abnormal liver function tests (5%) and abdominal pain (3%) were the most common adverse effects in 103 neutropenic pediatric cancer patients who received the drug at 5 mg/kg/day or 2.5 mg/kg q12h for antifungal prophylaxis; 18% of patients withdrew from the study because of adverse events.[93]

Drug interactions

In comparison to fluconazole, both propensity and extent of drug–drug interactions are greater (see Table 149.2). Itraconazole is a substrate of CYP3A4, but also interacts with the heme moiety of CYP3A, resulting in noncompetitive inhibition of oxidative metabolism of many CYP3A substrates and increased and potentially toxic concentrations of co-administered drugs. Increased metabolism of ITC resulting in decreased plasma levels can result from co-administration of inducers of hepatic enzymes. Finally, the systemic availability of ITC depends in part on the activity of intestinal CYP3A4 and P-glycoprotein, which contributes to its variable bioavailability after oral administration.

Indications and dosing

Itraconazole is useful for dermatophytic infections, pityriasis versicolor and all forms of cutaneous and mucosal candidiasis; however, its clinical efficacy in invasive candidiasis has not been evaluated. Itraconazole has been successful for consolidation and maintenance treatment of cryptococcosis in the setting of HIV infection.[94] Itraconazole is approved as a second-line agent for treatment of invasive aspergillosis; few data exist on its use for first-line treatment in neutropenic patients.[97] Itraconazole may be useful in the management of infections by certain dematiaceous molds, but is inactive against zygomycosis and fusariosis. Itraconazole is the current treatment of choice for lymphocutaneous sporotrichosis and non-life-threatening, nonmeningeal paracoccidioidomycosis, blastomycosis and histoplasmosis. In progressive nonmeningeal coccidioidomycosis, ITC appears at least as active as fluconazole.[77] However, AmB remains the treatment of choice for most immunocompromised patients and for life-threatening infection.

Itraconazole has been successful as antifungal prophylaxis in patients undergoing stem cell transplantation or intensive chemotherapy.[93] HP-β-CD ITC may reduce the incidence of proven or suspected invasive fungal infections in neutropenic patients with hematologic malignancies.[96] Itraconazole was at least as effective as conventional AmB and less toxic as empiric therapy in persistently febrile, neutropenic patients,[97] which has led to the approval of this indication by the US Food and Drug Administration (FDA).

The recommended dosage range of oral itraconazole is 100–400 mg/day (capsules) and 2.5 mg/kg q12h (HP-β-CD solution). For life-threatening infections, higher doses may be necessary. For such conditions, we recommend a loading dose of 600–800 mg/day for 3–5 days, followed by a maintenance dose of 400–600 mg/day, and monitoring of serum levels. Itraconazole is not approved for patients <18 years of age; based on the available pharmacokinetic data, a starting dosage of 2.5 mg/kg q12h of oral HP-β-CD ITC can be advocated. The recommended dosage range for the capsule formulation is 5–8 mg/kg/day with a loading dose of 4 mg/kg q8h for the first 3 days.

Therapeutic monitoring

Data from a large cohort of patients with acute leukemia receiving ITC have demonstrated an association of trough concentrations <0.5 μg/ml with occurrence of invasive fungal infections. We therefore recommend rapid achievement and maintenance of trough levels of ≥0.5 μg/ml when ITC is given for prevention or treatment of invasive fungal infections.

Second-generation antifungal triazoles

Further improvements in the structure–activity relationship of antifungal triazoles have led to a new group of synthetic compounds that are collectively known as second-generation triazoles, comprising voriconazole, posaconazole, ravuconazole and isavuconazole. Voriconazole and posaconazole are approved for clinical use. Ravuconazole (BMS-207147 and its prodrug BMS 292655; Eisai Inc., Woodcliff Lake, New Jersey) and isavuconazole (BAL8557; Basilea Pharmaceutica Ltd, Basel, Switzerland) are currently in clinical development. Ravuconazole and voriconazole are structurally related to fluconazole, and posaconazole is similar in structure to itraconazole.

Pharmacodynamics

The second-generation triazoles possess enhanced target activity and specificity. They are active against a wide spectrum of clinically important fungi, including *Candida* spp., *Trichosporon* spp., *Cr. neoformans*, *Aspergillus* spp., *Fusarium* spp. and other hyaline molds, and dematiaceous as well as dimorphic molds. They have demonstrated efficacy in animal models of invasive fungal infections. Similar to itraconazole, these novel triazoles exert fungistatic activity against susceptible yeast-like organisms and strain-dependent fungicidal activity against susceptible filamentous fungi. Fundamental differences in potency, spectrum and antifungal efficacy between posa-, isavu-, ravu- and voriconazole have not surfaced, but posaconazole appears to have the best activity against zygomycetes. In *Candida* spp. and *Cr. neoformans* voriconazole exhibits nonconcentration-dependent pharmacodynamics. Near-maximal fungistatic activity is achieved at a drug concentration

of approximately three times the MIC.[98] A postantifungal effect of up to 4 hours has been observed with *C. albicans*.[99] Voriconazole also has immunomodulatory properties that may enhance the immune response to *A. fumigatus*.[100]

Voriconazole

Pharmacokinetics

Voriconazole is available as a tablet and parenteral solution that uses sulfobutyl ether-β-cyclodextrin (SE-β-CD) as a solubilizer. In tablet form, voriconazole is rapidly absorbed from the gastrointestinal tract. Although bioavailability is near 100%, steady-state plasma concentrations are achieved more rapidly with an intravenous loading dose. Multiple enzymes of the cytochrome P450 system including CYP2C19, which exhibits genetic polymorphism in certain racial groups, metabolize voriconazole. Drug levels in poor metabolizers, which may include up to 20% of Asian populations, can be significantly elevated. Plasma levels in extensive or ultrarapid metabolizers may be much lower.[101] In adults, voriconazole exhibits nonlinear pharmacokinetics, possibly due to saturable metabolism and systemic clearance.[102] In contrast, elimination of voriconazole in children is linear and higher relative dosages may be required to achieve exposures consistent with those in adults.[103] Given the multiplicity of factors that may impact voriconazole pharmacokinetics, therapeutic drug monitoring is increasingly used and may improve outcomes.[104]

Voriconazole has been used successfully in patients with central nervous system mycoses. Cerebrospinal fluid concentrations vary greatly, but are generally approximately half the serum concentration and have a linear relationship to dose.[105] Voriconazole has been evaluated in ocular infections, and concentrations in the vitreous and aqueous are 38% and 53% of serum levels, respectively.[106] The addition of a 1% topical voriconazole solution can further increase vitreous and aqueous levels and therapeutic concentrations against many pathogenic fungi may be achieved with topical therapy alone.[107,108] Voriconazole may also be injected directly into the cornea, the anterior chamber and the vitreous in cases of invasive fungal keratitis and endophthalmitis.

Following its metabolism in the liver, the drug is mostly excreted in urine. The pharmacokinetics are unaffected by renal function. Concern has arisen regarding the potential for accumulation of the renally metabolized, and potentially nephrotoxic, intravenous carrier solution SE-β-CD in patients with renal impairment. Whether accumulation of SE-β-CD in such patients is clinically relevant is unclear.[109,110]

Drug interactions

In addition to undergoing metabolism by the P450 system, voriconazole inhibits several CYP enzymes, leading to extensive potential for drug–drug interactions. Extreme care must be taken when co-administering voriconazole with agents metabolized through the P450 system, particularly with drugs having narrow therapeutic windows such as calcineurin inhibitors and sirolimus.

Adverse affects

Mild and reversible visual disturbances occur in approximately 30% of patients. This may manifest as altered color perception, photophobia or blurred vision. The mechanism is unknown, but is thought to be retinal. Other common adverse reactions include rashes and phototoxicity, gastrointestinal disturbances and hepatic abnormalities. Visual and/or auditory hallucinations occur in up to 16.6% of patients, may be related to higher serum drug levels and are particularly associated with intravenously administered voriconazole.[111]

Clinical studies

Voriconazole is indicated for primary treatment of aspergillosis and candidemia, as well as for salvage therapy for fusariosis and scedosporiosis. In a randomized, open label trial of patients with invasive aspergillosis, initial therapy with voriconazole led to a significantly greater response rate, improved survival and less toxicity than the standard approach of initial therapy with AmB deoxycholate.[112] Voriconazole also has been used successfully in the treatment of invasive fungal infections including aspergillosis in children who were refractory to, or intolerant of, conventional antifungal therapy.[113] It has also been shown to be effective in oropharyngeal and esophageal candidiasis in immunocompromised patients and as primary therapy for candidemia in non-neutropenic patients.[114,115] A large, randomized, multicenter trial has been completed that compared 12-hourly administration of voriconazole (3 mg/kg intravenously or 200 mg orally) with liposomal AmB (3 mg/kg intravenously) for empiric antifungal therapy. This study showed comparable composite success rates, but less proven and probable breakthrough infections, infusion-related toxicity and nephrotoxicity in the voriconazole-treated cohort. However, patients receiving voriconazole had significantly more frequent episodes of transient visual disturbances and hallucinations.[116]

For treatment of invasive fungal infections in adults, an intravenous loading dose of 6 mg/kg q12h on day 1 followed by 4 mg/kg q12h thereafter is employed. For children, a dosage of 7 mg/kg intravenously q12h is used. Voriconazole by the oral route is administered at 200 mg q12h in adults. As SE-β-CD clearance is related to GFR, oral voriconazole should be used, when feasible, in patients whose creatinine clearances is <50 ml/min. In patients with mild to moderate hepatic cirrhosis, the standard loading dose is recommended, but the maintenance dose should be halved.

Posaconazole

Pharmacokinetics

At present, posaconazole is available as an oral suspension only and achieves optimal exposure when administered in two to four divided doses given with food or a nutritional supplement. The compound has dose-proportional pharmacokinetics in the 50–800 mg dose range, with saturation of absorption occurring above 800 mg. Following repeat dosing, steady state is achieved after 7–10 days with a six- to eight-fold accumulation of plasma concentrations.[117] Posaconazole has a large volume of distribution on the order of 5 l/kg and a prolonged elimination half-life of approximately 20 hours. It is not significantly metabolized through the cytochrome P450 enzyme system but primarily excreted unchanged in the feces. Approximately 10% of the compound is metabolized through glucuronidation.

Adverse effects

The safety profile of posaconazole appears to be comparable to that of fluconazole or itraconazole.[118,119] The overall safety of posaconazole has been assessed in more than 400 patients with invasive fungal infections from two open label clinical trials who received posaconazole suspension (800 mg/day in divided doses).[120,121] Treatment-related adverse events occurred in 38% of patients (164/428); the most common were nausea (8%), vomiting (6%), headache (5%), abdominal pain (4%) and diarrhea (4%). Treatment-related abnormal liver function test results were observed in up to 3% of patients. The most common severe adverse events were altered drug levels, increased hepatic enzymes, nausea, rash, and vomiting (1% each).

Drug interactions

Posaconazole is not significantly metabolized through the cytochrome P450 enzyme system, but inhibits cytochrome P3A4; it has no effect on 1A2, 2C8, 2C9, 2D6 and 2E1 isoenzymes.[122–125] As such, monitoring of ciclosporin, tacrolimus and sirolimus blood concentrations is mandatory, and dose adjustments should be made accordingly.

Indications and dosing

Posaconazole is indicated for treatment of aspergillosis, fusariosis, chromoblastomycosis and coccidioidomycosis in patients who are refractory to, or intolerant of, standard therapies. It is also indicated for prophylaxis of invasive *Candida* and *Aspergillus* infections in high-risk patients ≥13 years of age with allogeneic hematopoietic stem cell transplantation and graft-versus-host disease or those with hematologic malignancies and prolonged neutropenia. In a large phase II study including 330 patients with invasive fungal infections mostly refractory to standard therapies, the rates of successful outcome were 42% for aspergillosis (107 patients),[126] 39% for fusariosis (18 patients), 69% (16 patients) for coccidioidomycosis and 81% for chromoblastomycosis (11 patients).[127] Efficacy has also been found against other invasive fungal infections, including zygomycosis, candidiasis, cryptococcosis and refractory oropharyngeal candidiasis.[128–133] Two studies demonstrated the preventive efficacy of posaconazole, in particular against invasive *Aspergillus* infections in high-risk patients, including a statistically significant survival benefit in patients with acute myeloid leukemia/myelodysplastic syndrome undergoing remission induction chemotherapy.[118,119]

The recommended daily dosage for salvage treatment is 400 mg q12h given with food; for patients not tolerating solid food, a dosage of 200 mg q6h is recommended, preferentially together with a nutritional supplement. The dosage for primary treatment of oropharyngeal candidiasis is 100 mg/day (day 1: 100 mg q12h) and 400 mg q12h for refractory disease; for prophylaxis of invasive *Candida* and *Aspergillus* infections, the recommended dosage is 200 mg q8h. The pharmacokinetics of posaconazole in pediatric patients (<18 years of age) have not been adequately studied.[134]

ECHINOCANDIN LIPOPEPTIDES

Anidulafungin, caspofungin, micafungin

The echinocandins are a novel class of semisynthetic amphiphilic lipopeptides composed of a cyclic hexapeptide core linked to a variably configured lipid side chain. The echinocandins act by noncompetitive inhibition of the synthesis of 1,3-β-glucan, a polysaccharide in the cell wall of many pathogenic fungi (Fig. 149.5). Together with chitin, the rope-like glucan fibrils are responsible for the cell wall's strength and shape. They are important in maintaining the osmotic integrity of the fungal cell and play a key role in cell division and cell growth. The structures of caspofungin, anidulafungin and micafungin are shown in (Fig. 149.6).

Antifungal activity

The current echinocandins appear to possess very similar pharmacologic properties. All three have potent and broad-spectrum fungicidal *in-vitro* activity against *Candida* species and potent inhibitory activity against *Aspergillus* spp.; their antifungal efficacy against these organisms *in vivo* has been demonstrated in various animal models. While still generally susceptible, *C. parapsilosis* isolates generally show elevated MICs for the echinocandins.

The current echinocandins have variable activity against dematiaceous and endemic molds, and are inactive against most hyalohyphomycetes, *Cr. neoformans* and *Trichosporon* spp. In *vitro*, echinocandins have limited activity against zygomycetes,[135] but animal models of zygomycosis and clinical experience suggest a role for echinocandins when combined with AmB.[136,137] *In-vitro* resistance has been observed in fungal isolates overexpressing a gene encoding for a Golgi protein involved in the transport of cell wall components.[138] The MIC breakpoint for echinocandin susceptibility is 2 µg/ml.

Primary resistance to echinocandins in otherwise susceptible fungal yeast species is rare and resistance induction studies have demonstrated a low potential for secondary resistance in *Candida* spp.[139] The frequency of primary echinocandin resistance among clinical isolates of *Aspergillus* spp. is unknown, but induction of secondary resistance has been achieved *in vitro*.[140]

Pharmacodynamics

The echinocandins demonstrate a species-dependent mode of antifungal activity. They have fungicidal activity against most *Candida* spp. but not against *Aspergillus* spp. In *Aspergillus* spp., microscopic examination of exposed hyphae show a dose-dependent formation of microcolonies with progressively truncated, swollen hyphal elements that appear to be cell-wall deficient, but that can regain their cell walls upon subculture in the absence of drug. In a process akin to pruning, caspofungin preferentially kills cells at active centers of cell wall synthesis within *A. fumigatus* hyphae.[141] These observations indicate differences in functional target sensitivity in both species that are not fully understood. *In-vitro* pharmacodynamic studies in *Candida* spp. have shown predominantly concentration-dependent

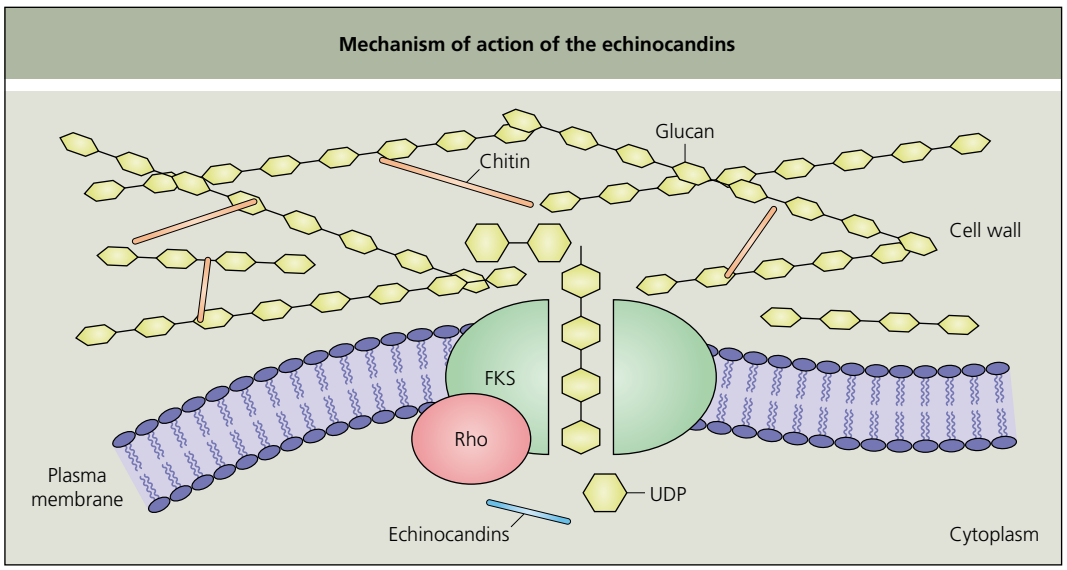


Fig. 149.5 Mechanism of action of the echinocandins.

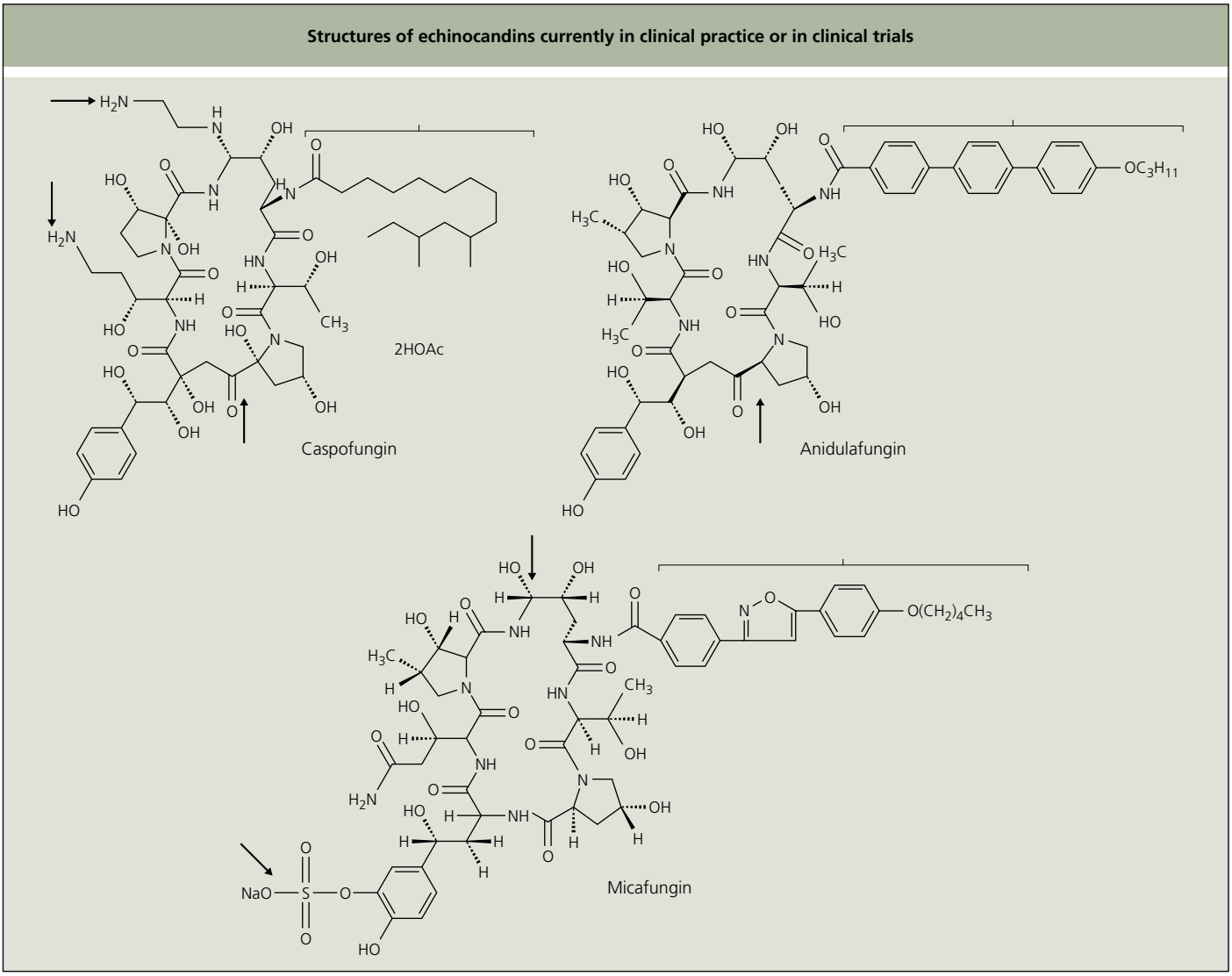


Fig. 149.6 Structures of echinocandins currently in clinical trials.

(≥99.9% reduction in CFU) fungicidal activity and rate of kill, and concentration-dependent prolonged postantifungal effects of up to 12 hours' duration.[9,142] Animal studies suggest similar concentration-dependent activity of the current echinocandins.

The echinocandins may have a role in biofilm-associated candidiasis. Therapeutic concentrations of echinocandins display potent *in-vitro* activity against *C. albicans* in biofilms.[143] When used as catheter lock solutions, echinocandins may effectively reduce or control fungal biofilms.[144] Combined systemic and catheter intraluminal lock therapy was highly effective in rabbits with venous catheter *C. albicans* infection.[145] Clinical studies are needed to define the role of the echinocandins in biofilms.

Data have emerged regarding interactions between echinocandins and phagocytic cells. Echinocandins alone do not completely inhibit *in-vitro* growth of *A. fumigatus*, but a powerful antifungal effect is seen in combination with phagocytes. Echinocandins enhance neutrophil activity against mold hyphae by unmasking β-glucans and activating host antifungal pathways via the Dectin-1 receptor.[146,147]

Pharmacokinetics

All current echinocandins are only available intravenously. They exhibit dose-proportional plasma pharmacokinetics with a β-half-life of 10–15 hours that allows for once-daily dosing. Echinocandins are >95% protein-bound and distribute into all major organ sites including the brain; however, concentrations in uninfected CSF are low. The echinocandins are hepatically metabolized and slowly excreted into urine and feces; only small fractions are excreted into urine in unchanged form.[148] The pharmacokinetic parameters are generally lower in children than in adults. Drug levels are especially low in smaller, younger children. The β-half-life in children is reduced by over one-third relative to adults.

Adverse effects and drug interactions

At the currently investigated dosages, all echinocandins are generally well tolerated. Only a small fraction of patients enrolled in clinical trials (<5%) discontinued therapy due to drug-related adverse events. The most frequently reported adverse effects include increased liver transaminases, gastrointestinal upset and headaches. Like other basic polypeptides, the echinocandins have the potential to cause histamine release; however, histamine-mediated symptoms have been observed in isolated cases only.

The current echinocandins appear to have no significant potential for drug interactions mediated by the CYP450 enzyme system. Caspofungin reduces the AUC of tacrolimus by approximately 20%

but has no effect on ciclosporin levels. However, ciclosporin increases the AUC of caspofungin by approximately 35%; because of transient elevations of hepatic transaminases in single-dose interaction studies, concomitant use of both drugs is not recommended. Finally, inducers of drug clearance and/or mixed inducers/inhibitors – namely efavirenz, nelfinavir, nevirapine, phenytoin, rifampin (rifampicin), dexamethasone and carbamazepine – may reduce caspofungin concentrations.

Clinical studies

The clinical efficacy of anidulafungin, caspofungin and micafungin against *Candida* spp. has been investigated in phase II or III studies of esophageal candidiasis.[149] Echinocandins are also effective for treatment of azole-refractory esophageal and oropharyngeal candidiasis.[150] All agents were well tolerated without serious adverse events, and achieved efficacy at least comparable with standard agents. Phase III studies of echinocandins for initial therapy of invasive candidiasis (mostly candidemia) indicate that these agents are effective, but less toxic than AmB and noninferior to fluconazole.[151–153]

Caspofungin has been studied for empiric therapy in patients with persistent fever and neutropenia and was as effective as and better tolerated than liposomal AmB.[154] The role of echinocandins in established invasive aspergillosis is less clear. In a salvage trial using caspofungin monotherapy, a complete or partial response was observed in 45% of patients. Early data suggest a possible role for echinocandins in combination with other drugs active against *Aspergillus* spp. and this approach is increasingly used.[155–157] Clinical trials are needed to further define the safety and efficacy of combination therapy in invasive aspergillosis.

The recommended daily doses in adults with systemic infections are 100 mg for micafungin and anidulafungin, and 50 mg for caspofungin. A loading does of 200 mg for anidulafungin and 70 mg for caspofungin is required on the first day. In children, doses of caspofungin at 50 mg/m^2/day and anidulafungin at 1.5 mg/kg/day provide comparable exposures to that of adults treated with standard dosing.[158,159]

TERBINAFINE

Terbinafine is a highly lipophilic and keratophilic allylamine inhibitor of ergosterol biosynthesis. It is a potent noncompetitive inhibitor of fungal squalene epoxidase and prevents squalene epoxidation, an important early step in the synthesis of ergosterol. Treated fungi accumulate squalene and become deficient in ergosterol. Cell death is associated with the development of high intracellular squalene concentrations, which interfere with fungal membrane function and cell wall synthesis. In addition to its antifungal properties, terbinafine is also anti-inflammatory and can act as a free radical scavenger.

Terbinafine is active *in vitro* against a wide range of pathogenic fungi, including dermatophytes, molds, dimorphic fungi, *Cr. neoformans* and some but not all *Candida* and *Aspergillus* spp.[160] *In-vitro* studies with *Aspergillus* have shown terbinafine and amphotericin to have additive to synergistic interactions depending on the isolate. In combination with itraconazole or voriconazole, terbinafine displays potent synergistic interactions against *Aspergillus*. Fluconazole also increases the activity of terbinafine in an additive to synergistic fashion.[161] *In-vitro* combinations of terbinafine with voriconazole can overcome azole resistance in *Candida* spp. Terbinafine in combination with AmB or voriconazole is synergistic against some zygomycete isolates. Resistance to terbinafine has been observed following a single gene mutation in *Aspergillus*.[162] In *Candida*, cross-resistance following treatment with fluconazole can occur due to upregulation of target enzymes. Multidrug efflux pumps may also reduce susceptibility.[163]

Oral terbinafine is rapidly absorbed, with peak concentration occurring at 60–90 minutes post dose. It is quickly converted to multiple metabolites, which co-exist in plasma with the parent compound. Drug is delivered to peripheral tissues via sebum and by direct diffusion through the dermal layers. It is detected in sebum and hair within the first week of administration and by week 3 in stratum corneum and nail samples. The terminal half-life is approximately 3 weeks and fungicidal concentrations persist in peripheral tissues for weeks to months after administration of the last dose. Increasing age and concomitant hypertension are associated with higher plasma concentrations and smokers have lower levels than do nonsmokers.[164] When given as a 1% cream, terbinafine concentrations in the horny layer of skin far exceed the minimum inhibitory concentrations for common dermatophytes and effective levels of drug remain in skin well beyond discontinuation of therapy.[165]

Multiple cytochrome P450 enzymes metabolize terbinafine, and agents that affect this system may alter drug concentrations.[166] Terbinafine competitively inhibits CYP2D6 and elevated levels of desipramine have been observed when the drugs are co-administered. Terbinafine may reduce the level of ciclosporin A. Most of the drug and drug metabolites are eliminated in the urine. The drug is generally well tolerated, but gastrointestinal disturbances, skin rashes and headaches occur occasionally. Rare adverse reactions include hepatobiliary dysfunction, induction and exacerbation of lupus, agranulocytosis and severe skin reactions.

Oral terbinafine at doses of 250 mg/day in adults and 62.5–250 mg/day in children is effective and generally safe for cutaneous mycoses that warrant systemic therapy. It is highly efficacious as treatment for onychomycosis. At this time, however, this agent cannot be recommended for the treatment of systemic mycoses. Terbinafine 1% cream is effective against a variety of cutaneous mycoses. The length of treatment with either topical or oral formulation depends on the specific infection.

GRISEOFULVIN

Griseofulvin, a metabolic product of *Penicillium*, was the first oral agent available for treatment of dermatomycoses. This compound inhibits fungal cell mitosis and nucleic acid synthesis and disrupts spindle and cytoplasmic microtubule function.[167] Griseofulvin is active against many, but not all, dermatophytes. High-level resistance can develop following drug exposure and a multiple-layered thick cell wall, which may limit griseofulvin entry, has been observed in resistant isolates. Griseofulvin has extremely low water solubility and moderate lipid solubility. Absorption from the gastrointestinal tract is variable and depends on the amount of dissolved drug that reaches the intestines. Absorption is enhanced by a fatty meal. The bioavailability of the ultra-microsize formulation is higher compared to the microsize formulation. Once absorbed, the drug is highly protein-bound.

Griseofulvin is detected in the outer layer of the stratum corneum soon after it is ingested and is diffused from the extracellular fluid and sweat. Deposition of drug in growing cells may account for entry into hair and nails.[167] Concentration in plasma peaks at 3–4 hours and in skin blister fluid at 6 hours. The terminal half-life in plasma and in skin blisters is approximately 9–10 hours. During chronic administration, plasma and skin blister levels equilibrate. Griseofulvin is largely metabolized in the liver and degradation metabolites are excreted in the urine. Griseofulvin is also effective in several inflammatory skin conditions, possibly due to anti-inflammatory properties mediated by modulation of the expression of cell adhesion molecules on leukocytes and vascular endothelial cells.[168]

Adverse effects include gastrointestinal disturbances, headaches, hepatitis and rashes. Liver and thyroid neoplasia, abnormal germ cell maturation, teratogenicity and embryotoxicity have been observed in animal studies. The reproductive toxicity as well as the induction of chromosome aberrations in somatic cells may result from disturbance of microtubule formation. Griseofulvin also induces accumulation of porphyrins and formation of Mallory bodies in hepatocytes. These may represent additional carcinogenic mechanisms. Less common adverse events include exacerbation of lupus, porphyrias and blood dyscrasias. Drug interactions are related to induction of hepatic enzymes and include phenobarbital, oral anticoagulants and oral contraceptives.

For onychomycosis, griseofulvin has been largely supplanted by newer antifungals. Currently, its main indication is for the treatment

of tinea capitis and other cutaneous mycoses. Doses of 500–1000 mg/day in adults and 15–20 mg/kg/day in children have been used successfully. Length of treatment depends on the site and type of infection.

AMOROLFINE

The morpholine derivative amorolfine is currently only used as a topical agent. This compound interferes with ergosterol biosynthesis and leads to the depletion of ergosterol and the subsequent accumulation of sterol precursors within the cell membrane. Amorolfine also weakly inhibits the fungal enzyme squalene epoxidase. *In-vitro* amorolfine has a broad spectrum of activity that includes dermatophytes, dimorphic, dematiaceous and filamentous fungi, and yeasts.[169] In patients, amorolfine has eradicated infections due to both *C. albicans* and a broad range of dermatophytes. In a murine model of dermatophytosis the combinations of amorolfine with griseofulvin, terbinafine, itraconazole and fluconazole were synergistic, but 5-FC appears to be antagonistic in some fungal isolates.

Amorolfine has been formulated as 0.125%, 0.25% and 0.5% creams and as a 5% lacquer. Following application, the amorolfine penetrates the nails rapidly and within 24 hours of contact exceeds the MIC of most fungi that cause onychomycosis. Following topical application, an active concentration of amorolfine is retained in the skin for several days. In experimental models of systemic mycosis amorolfine shows no significant activity, which may be due to strong protein binding and/or rapid metabolism. The bioavailability of topical amorolfine is 4–10% and drug is excreted in urine and feces.

Treatment-related adverse events are generally limited to burning, itching, erythema, skin dryness and scaling. Topical amorolfine has been used successfully in a variety of dermatomycoses and for the treatment of onychomycosis. Combination therapy with oral itraconazole or terbinafine may be a promising option for patients with severe disease.[170]

CICLOPIROX

Ciclopirox is a poorly absorbable, synthetic hydroxypyridone antifungal agent. The mechanism of action is related to its affinity for trivalent cations. By chelating essential metal co-factors ciclopirox inhibits fungal enzymes that are responsible for diverse metabolic processes and ultimately causes membrane and cytoplasmic disruption.[171] Ciclopirox at subinhibitory concentrations impairs candidal adherence to mucosal surfaces. This agent has potent antifungal activity against a broad range of dermatophytic and nondermatophytic fungi.

Ciclopirox is available in a variety of topical formulations including a 0.77% gel and 8% nail lacquer. It readily penetrates the skin via the epidermis and the hair follicles and achieves the highest concentration in the horny layer. When used as 8% nail lacquer, the drug achieves uniform distribution within the nail plate after daily use for 1 week, reaching a maximum at 3–4 weeks with uniform distribution to all nail layers.[172] Within the nail the drug achieves concentrations in excess of inhibitory and fungicidal concentrations for most pathogens. The bioavailability of topical ciclopirox is 2–5% and absorbed drug is mainly metabolized by glucuronidation. Excretion is predominantly renal with an elimination half-life of approximately 2 hours.[172]

Treatment-related adverse events are uncommon, but local reactions such as pruritus and burning sensation can occur. Ciclopirox has been used successfully for a variety of cutaneous mycotic infections, mucosal candidiasis, seborrheic dermatitis and as treatment for onychomycosis.

FUTURE DIRECTIONS

The past decade has seen a considerable expansion in antifungal drug research and the clinical development of several new compounds targeted against invasive fungal infections. Major progress has been made in defining paradigms for antifungal intervention and in designing and implementing clinical trials. Several novel and promising antifungal compounds are currently in early stages of preclinical investigation and the pursuit of novel biochemical and molecular targets will result in further candidate drugs. Cognizant of past and present epidemiologic trends, invasive fungal infections will likely remain a frequent and important complication in immunocompromised patients. An expanded drug arsenal, elucidation of resistance mechanisms, integration of pharmacokinetic and pharmacodynamic relationships, and combination therapies offer hope for further substantial progress in prevention and treatment.

REFERENCES


References for this chapter can be found online at http://www.expertconsult.com

Chapter 150

Samuel L Stanley, Jr

Antiparasitic agents

INTRODUCTION

Antiparasitic agents are used to treat infestations caused by a diverse and complex group of organisms encompassing the unicellular protozoa, which have intricate life cycles often involving more than one host, as well as the helminths, which have highly developed organ systems. Many antiparasitic agents are old drugs that have never been subjected to the rigorous testing of efficacy and safety currently required by agencies in various countries, such as the US Food and Drug Administration.

The treatment options by organism or disease entity, along with the recommended adult and pediatric dosages, are listed in Table 150.1. Agents that are not readily available in the USA are listed in Table 150.2. Few of the antiparasitic agents have been extensively studied in pregnancy. Table 150.3 divides the drugs into those that are probably safe on the basis of clinical experience, those that are possibly safe on the basis of anecdotal experience or are safe during certain trimesters, and those that are known to be hazardous or for which too little information is known to make a recommendation. In general, however, the decision to use of any of these agents in a pregnant patient must be made on an individual basis, weighing the severity of the illness and the benefit of treatment to the mother against the potential toxicity to the fetus.

Antiprotozoal Agents

AMODIAQUINE

Amodiaquine is a 4-aminoquinoline with antimalarial activity and a mechanism of action similar to that of chloroquine.[6,7] The side-effect profile is also similar to that of chloroquine but agranulocytosis and severe hepatitis have been reported with long-term use (as chemoprophylaxis). The activity of amodiaquine against some chloroquine-resistant strains of *Plasmodium falciparum* has led to a revival in its use, particularly as part of combination therapy with artesunate.[8] While resistance to amodiaquine in certain parts of Africa may limit the usefulness of this combination in those regions, the artesunate–amodiaquine combination has proved very effective in areas where responses to amodiaquine alone exceed 80%.[9,10]

AMPHOTERICIN B

Amphotericin B, a polyene antifungal agent, is the drug of choice for primary amebic meningoencephalitis caused by *Naegleria* spp.[11] (see Chapter 182), and is used for the treatment of visceral leishmaniasis (see Chapter 117).[12,13] Its pharmacokinetics and side-effects are detailed in Chapter 149. Lipid-associated formulations of amphotericin B are effective in the treatment of visceral leishmaniasis, and in one study in India single-dose therapy with liposomal amphotericin B gave cure rates of more than 92% with minimal toxicity.[12,13]

ANTIFOLATE AGENTS

Antifolate agents act at various steps in the folic acid cycle. For *Plasmodium* spp., *Toxoplasma* spp. and other sensitive parasites, reduced folic acid derivatives are essential for de-novo pyrimidine synthesis. Unlike mammalian cells, these parasites cannot use preformed pyrimidines. Antifolate agents are most commonly used in combination to block sequential steps in the folic acid metabolic pathway (see Chapter 138).

Pyrimethamine

Pyrimethamine is a diaminopyrimidine that inhibits plasmodial dihydrofolate reductase at a concentration that is 1000 times less than that required to inhibit the mammalian enzyme.[6,7] It is effective against the erythrocytic stages of all *Plasmodium* spp. that are pathogenic for humans, but resistance has significantly limited its usefulness as a single agent.[14] In combination with sulfadiazine, clindamycin or atovaquone, it is used for the treatment of *Toxoplasma gondii*.[15,16] Pyrimethamine also has activity against *Isospora belli*.[17] The drug is available in oral form; it is slowly but completely absorbed, is 85% protein-bound and is extensively metabolized by the liver (<3% is excreted unchanged). The half-life is 4–6 days.

Although pyrimethamine is available as 25 mg tablets, it is almost exclusively used in combination with a sulfonamide (sulfadiazine, sulfadoxine) or a sulfone (dapsone; see below). The dosage for toxoplasmosis is listed in Table 150.1. Some clinicians give an initial pyrimethamine loading dose of 200 mg. In patients who cannot tolerate sulfonamides, clindamycin (1.8–2.4 g/day in divided doses) or atovaquone (1.5 g q12h) may be substituted. Side-effects of pyrimethamine include blood dyscrasias, rash and, very rarely, seizures or shock. At high doses, pyrimethamine causes bone marrow suppression, which can be prevented by concurrent administration of folinic acid. Sulfadoxine–pyrimethamine as 25 mg pyrimethamine and 500 mg sulfadoxine is used in combination with artesunate for the treatment of *P. falciparum* malaria in areas where susceptibility to sulfadoxine and pyrimethamine remains high (South America, the Middle East, South Asia).[9]

Table 150.1 Antiparasitic agents and dosages (Organism or disease entity is listed alphabetically. Both adult and pediatric dosages are indicated)

Infection	Drug	Adult dosage (po unless otherwise indicated)	Pediatric dosage (po unless otherwise indicated)
Acanthamoeba (keratitis)			
	Polyhexamethylene biguanide 0.02% plus 0.1% propamidine isethionate (topical)		
Amebiasis (***Entamoeba histolytica***)			
Asymptomatic			
or following metronidazole or tinidazole therapy	Paromomycin	25–35 mg/kg/day in three doses for 7 days	25–35 mg/kg/day in three doses for 7 days
Alternatives	Diloxanide furoate	500 mg q8h for 10 days	20 mg/kg/day in three doses for 10 days
OR	Iodoquinol	650 mg q8h for 20 days	30–40 mg/kg/day (maximum 2 g) in three doses for 20 days
Mild-to-moderate intestinal disease			
	Metronidazole	500–750 mg q8h for 5–10 days	35–50 mg/kg/day in three doses for 10 days
OR	Tinidazole	2 g/day for 3 days	≧3 years: 50 mg/kg (maximum 2 g) per day for 3 days
Severe intestinal disease and extraintestinal disease			
	Metronidazole	750 mg q8h for 5–10 days	35–50 mg/kg/day in three doses for 10 days
OR	Tinidazole	2 g/day for 5 days	≧3 years: 50 mg/kg or 60 mg/kg (maximum 2 g) per day for 5 days
Amebic meningoencephalitis (primary or granulomatous amoebic encephalitis)			
***Naegleria* spp.**			
	Amphotericin B	1.5 mg/kg/day iv, uncertain duration	1 mg/kg/day iv, uncertain duration
Acanthamoeba* spp.			
	Pentamidine, ketoconazole, flucytosine, uncertain duration		
Balamuthia mandrillaris*			
	Clarithromycin	500 mg q8h, uncertain duration	
	Fluconazole	400 mg q24h, uncertain duration	
	Sulfadiazine	1.5 g q6h, uncertain duration	
	Flucytosine	1.5 g q6h, uncertain duration	
Sappinia diploides*			
	Azithromycin	250 mg q24h, uncertain duration	
	Pentamidine	300 mg iv q24h, uncertain duration	
	Itraconazole	200 mg q12h, uncertain duration	
	Flucytosine	2.75 g q6h, uncertain duration	
***Ancylostoma caninum* (eosinophilic enterocolitis)**			
	Mebendazole	100 mg q12h for 3 days	100 mg q12h for 3 days
OR	Pyrantel pamoate	11 mg/kg (maximum 1 g) for 3 days	11 mg/kg (maximum 1 g) for 3 days
OR	Albendazole	400 mg, single dose	400 mg, single dose
Angiostrongyliasis			
Angiostrongylus cantonensis			
	Mebendazole	100 mg q12h for 5 days	100 mg q12h for 5 days
Angiostrongylus costaricensis			
	Mebendazole	200–400 mg q8h for 10 days	200–400 mg q8h for 10 days
Alternative	Thiabendazole	75 mg/kg/day in three doses for 3 days (maximum 3 g/day)	75 mg/kg/day in three doses for 3 days (maximum 3 g/day)
Anisakiasis (*Anisakis* spp.)			
Treatment of choice		Surgical or endoscopic removal	
Ascariasis (***Ascaris lumbricoides*, roundworm**)			
	Mebendazole	100 mg q12h for 3 days or 500 mg, single dose	100 mg q12h for 3 days or 500 mg, single dose
OR	Pyrantel pamoate	11 mg/kg, single dose (maximum 1 g)	11 mg/kg, single dose (maximum 1 g)
OR	Albendazole	400 mg, single dose	400 mg, single dose

(Continued)

Table 150.1 Antiparasitic agents and dosages (Organism or disease entity is listed alphabetically. Both adult and pediatric dosages are indicated)—cont'd

Infection	Drug	Adult dosage (po unless otherwise indicated)	Pediatric dosage (po unless otherwise indicated)
Babesiosis (*Babesia* spp.)			
The recommendations below are for normal hosts. Immunocompromised individuals may require more prolonged (6 weeks or more) courses of therapy to achieve cure (see Krause *et al*., Clin Infect Dis 2008;46:370–6).			
	Clindamycin	1.2 g q12h iv or 600 mg q8h po for 7 days	20–40 mg/kg/day po in three doses for 7 days
PLUS	Quinine	650 mg q8h for 7 days	25 mg/kg/day in three doses for 7 days
OR	Atovaquone	750 mg q12h for 7–10 days	20 mg/kg q12h for 7–10 days
PLUS	Azithromycin	600 mg q24h for 7–10 days	12 mg/kg q24h for 7–10 days
Balantidiasis (*Balantidium coli*)			
	Tetracycline	500 mg q6h for 10 days	40 mg/kg/day (maximum 2 g) in four doses for 10 days
Alternatives	Iodoquinol	650 mg q8h fro 20 days	40 mg/kg/day in three doses for 20 days
	Metronidazole	750 mg q8h for 5 days	35–50 mg/kg/day in three doses for 5 days
Baylisascariasis (*Baylisascaris procyonis*)			
	No drug proven effective – albendazole 25 mg/kg/d for 20 days recommended for known exposure to raccoon feces		
***Blastocystis hominis* (clinical significance controversial)**			
	Metronidazole	750 mg q8h for 5–10 days	
OR	Iodoquinol	650 mg q8h for 20 days	
Capillariasis (*Capillaria philippinensis*)			
	Mebendazole	200 mg q12h for 20 days	200 mg q12h for 20 days
Alternative	Albendazole	400 mg/day for 10 days	400 mg/day for 10 days
Cryptosporidiosis (*Cryptosporidium parvum*)			
No agent has yet been conclusively proven to be effective in AIDS patients. Nitazoxanide, in the doses listed below, showed efficacy in clearing infection from immunocompetent individuals.			
	Nitazoxanide	500 mg q12h for 3 days	1–3 years: 100 mg q12h for 3 days 4–11 years: 200 mg q12h for 3 days
Cutaneous larva migrans (creeping eruption, dog and cat hookworm)			
	Thiabendazole	Topical administration	Topical administration
OR	Ivermectin	150–200 µg/kg, single dose	150–200 µg/kg, single dose
OR	Albendazole	400 mg/day for 3 days	400 mg/day for 3 days
Cyclosporiasis (*Cyclospora cayetanensis*)			
	TMP–SMX	TMP 160 mg, SMX 800 mg q12h for 7–10 days	TMP 5 mg/kg, SMX 25 mg/kg q12h for 7–10 days
Dientamoeba fragilis			
	Iodoquinol	650 mg q8h for 20 days	40 mg/kg/day (maximum 2 g) in three doses for 20 days
OR	Paromomycin	25–30 mg/kg/day in three doses for 7 days	25–30 mg/kg/day in three doses for 7 days
OR	Tetracycline	500 mg q6h for 10 days	10 mg/kg q6h (maximum 2 g) for 10 days
Dracunculiasis (*Dracunculus medinensis*, guinea worm)			
		Physical removal of worm	
Entamoeba polecki			
	Metronidazole	750 mg q8h for 10 days	35–50 mg/kg/day in three doses for 10 days
Enterobiasis (*Enterobius vermicularis*, pinworm)			
	Pyrantel pamoate	11 mg/kg, single dose (maximum 1 g); repeat in 2 weeks	11 mg/kg, single dose (maximum 1 g); repeat in 2 weeks
OR	Mebendazole	100 mg, single dose; repeat in 2 weeks	100 mg, single dose; repeat in 2 weeks
OR	Albendazole	400 mg, single dose; repeat in 2 weeks	400 mg, single dose; repeat in 2 weeks
Filariasis			
Wuchereria bancrofti, Brugia malayi			
	Diethylcarbamazine	Day 1: 50 mg after food Day 2: 50 mg q8h Day 3: 100 mg q8h Days 4–14: 6 mg/kg/day in three doses	Day 1: 1 mg/kg after food Day 2: 1 mg/kg q8h Day 3: 1–2 mg/kg q8h Days 4–14: 6 mg/kg/day in three doses

Table 150.1 Antiparasitic agents and dosages (Organism or disease entity is listed alphabetically. Both adult and pediatric dosages are indicated)—cont'd

Infection	Drug	Adult dosage (po unless otherwise indicated)	Pediatric dosage (po unless otherwise indicated)
Loa loa			
	Diethylcarbamazine	Day 1: 50 mg after food Day 2: 50 mg q8h Day 3: 100 mg q8h Days 4–21: 9 mg/kg/day in three doses	Day 1: 1 mg/kg after food Day 2: 1 mg/kg q8h Day 3: 1–2 mg/kg q8h Days 4–21: 9 mg/kg/day in three doses
Mansonella ozzardi			
	Ivermectin	200 µg/kg single dose	
Mansonella perstans			
	Mebendazole	100 mg q12h for 30 days	
OR	Albendazole	400 mg q12h for 10 days	
Mansonella streptocerca			
	Ivermectin	150 µg/kg, single dose	
OR	Diethylcarbamazine	6 mg/kg/day for 14 days	
Tropical pulmonary eosinophilia			
	Diethylcarbamazine	6 mg/kg/day in three doses for 14 days	6 mg/kg/day in three doses for 14 days
***Onchocerca volvulus* (river blindness)**			
	Ivermectin	150 µg/kg, single dose, repeated every 6–12 months	150 µg/kg, single dose, repeated every 6–12 months
Fluke (hermaphroditic) infection			
***Clonorchis sinensis* (Chinese liver fluke)**			
	Praziquantel	75 mg/kg/day in three doses for 1 day	75 mg/kg/day in three doses for 1 day
OR	Albendazole	10 mg/kg for 7 days	
***Fasciola hepatica* (sheep liver fluke)**			
	Triclabendazole	10 mg/kg, single dose	
Alternative	Bithionol	30–50 mg/kg on alternate days for 10–15 doses	30–50 mg/kg on alternate days for 10–15 doses
***Fasciolopsis buski, Heterophyes heterophyes, Metagonimus yokogawai* (intestinal flukes)**			
	Praziquantel	75 mg/kg/day in three doses for 1 day	75 mg/kg/day in three doses for 1 day
***Metorchis conjunctus* (*North American liver fluke*)**			
	Praziquantel	75 mg/kg/day in three doses for 1 day	75 mg/kg/day in three doses for 1 day
Nanophyetus salmincola			
	Praziquantel	60 mg/kg/day in three doses for 1 day	60 mg/kg/day in three doses for 1 day
***Opisthorchis viverrini* (South East Asian liver fluke)**			
	Praziquantel	75 mg/kg/day in three doses for 1 day	75 mg/kg/day in three doses for 1 day
***Paragonimus westermani* (lung fluke)**			
	Praziquantel	75 mg/kg/day in three doses for 2 days	75 mg/kg/day in three doses for 2 days
Alternative	Bithionol	30–50 mg/kg on alternate for 10–15 doses	30–50 mg/kg on alternate for 10–15 doses
Giardiasis (***Giardia lamblia***)			
	Metronidazole	250 mg q8h for 5 days	15 mg/kg/day in three doses for 5 days
OR	Tinidazole	2 g, single dose	50 mg/kg, single dose (maximum 2 g)
Alternatives	Nitazoxanide	500 mg q12h for 3 days	1–3 years: 100 mg q12h for 3 days 4–11 years: 200 mg q12h for 3 days
OR	Furazolidone	100 mg q6h for 7–10 days	6 mg/kg/day q6h for 7–10 days
OR	Paromomycin	25–35 mg/kg/day in three doses for 7 days	
OR	Quinacrine	100 mg q8h for 5 days (maximum 300 mg/day)	2 mg/kg q8h for 5 days (maximum 300 mg/day)
Gnathostomiasis (***Gnathostoma spinigerum***)			
Treatment of choice		Surgical removal	
OR	Ivermectin	200 µg/kg/day for 2 days	200 µg/kg/day for 2 days
OR	Albendazole	400 mg q12h for 21 days	400 mg q12h for 21 days
Hookworm infection (*Ancylostoma duodenale, Necator americanus*)			
	Mebendazole	100 mg q12h for 2 days or 500 mg, single dose	100 mg q12h for 2 days or 500 mg, single dose
OR	Pyrantel pamoate	11 mg/kg (maximum 1 g) for 3 days	11 mg/kg (maximum 1 g) for 3 days
OR	Albendazole	400 mg, single dose	400 mg, single dose

(Continued)

Table 150.1 Antiparasitic agents and dosages (Organism or disease entity is listed alphabetically. Both adult and pediatric dosages are indicated)—cont'd

Infection	Drug	Adult dosage (po unless otherwise indicated)	Pediatric dosage (po unless otherwise indicated)
Isosporiasis (***Isospora belli***)			
	TMP—SMX	160 mg TMP, 800 mg SMX q6h for 10 days, then q12h for 3 weeks	
Leishmaniasis (*Leishmania mexicana, Leismania tropica, Leishmania major, Leishmania braziliensis, Leishmania donovani* (kala-azar), *Leishmania infantum*)[‡]			
Visceral or mucosal disease			
	Lipid-encapsulated amphotericin B	15–20 mg/kg (total dose over 5 days or longer)	15–20 mg/kg (total dose over 5 days or longer)
OR	Sodium stibogluconate	20 mg Sb/kg/day iv or im for 20–28 days	20 mg Sb/kg/day iv or im for 20–28 days
OR	Meglumine antimonate	20 mg Sb/kg/day for 20–28 days	20 mg Sb/kg/day for 20–28 days
OR	Miltefosine	2.5 mg/kg/day (maximum 150 mg per day) for 28 days	2.5 mg/kg/day (maximum 150 mg per day) for 28 days
Alternatives	Amphotericin B	0.5–1 mg/kg by slow infusion daily or every 2 days for up to 8 weeks	0.5–1 mg/kg by slow infusion daily or every 2 days for up to 8 weeks
OR	Paromomycin	15 mg/kg/day for 21 days	15 mg/kg/day for 21 days
Cutaneous disease			
	Sodium stibogluconate	20 mg Sb/kg/day iv or im for 20–28 days	20 mg Sb/kg/day iv or im for 20–28 days
OR	Meglumine antimonate	20 mg Sb/kg/day iv or im for 20–28 days	20 mg Sb/kg/day iv or im for 20–28 days
OR	Paromomycin	Topically q12h for 10–20 days	Topically q12h for 10–20 days
OR	Pentamidine	2–3 mg/kg iv or im q24h or qod for 4–7 doses	2–3 mg/kg iv or im q24h or qod for 4–7 doses
Malaria treatment (*Plasmodium falciparum, Plasmodium ovale, Plasmodium vivax, Plasmodium malariae*)			
Chloroqine-resistant *Plasmodium falciparum* (oral regimens)			
	Atovaquone/proguanil	Two adult tablets (250 mg atovaquone/100 mg proguanil) q12h for 3 days or four adult tablets q24h for 3 days	5–8 kg: two peds tablets/day (62.5 mg atovaquone/25 mg proguanil) for 3 days 9–10 kg: three peds tablets/day for 3 days 11–20 kg: one adult tablet/day for 3 days 21–30 kg: two adult tablets/day for 3 days 30–40 kg: 3 adult tablets/day for 3 days >40 kg: adult dose
OR	Quinine sulfate	650 mg q8h for 3–7 days	25 mg/kg/day q8h for 3–7 days
PLUS	Doxycycline	100 mg q12h for 7 days	2 mg/kg/day for 7 days
OR PLUS	Clindamycin	900 mg q8h for 5 days	20–40 mg/kg/day q8h for 5 days
Alternatives	Mefloquine	750 mg followed by 500 mg 12h later	15 mg/kg, single dose (if body weight <45 kg), followed by 10 mg/kg 12h later
OR	Artesunate[†]	4 mg/kg/day for 3 days	
PLUS	Mefloquine	750 mg followed by 500 mg 12h later	15 mg/kg, single dose (if body weight <45 kg), followed by 10 mg/kg 12h later
OR	Artemether/lumefantrine	Four tablets/dose administered at 0, 8, 24, 36, 48 and 60 hours	<15 kg: one tablet/dose at same intervals as adults 15–25 kg: two tablets/dose at same intervals 24–35 kg: three tablets/dose at same intervals >35 kg: same as adult dosage
Chloroquine-resistant *Plasmodium vivax*			
	Atovaquone/proguanil	Two adult tablets (250 mg atovaquone/100 mg proguanil) q12h for 3 days or four adult tablets/day for 3 days	5–8 kg: two peds tablets/day (62.5 mg atovaquone/25 mg proguanil) for 3 days 9–10 kg: three peds tablets/day for 3 days 11–20 kg: one adult tablet/day for 3 days 21–30 kg: two adult tablets/day for 3 days 30–40 kg: three adult tablets/day for 3 days >40 kg: adult dose
OR	Mefloquine	750 mg followed by 500 mg 12h later	15 mg/kg, single dose (if body weight <45 kg), followed by 10 mg/kg 12h later
Alternative	Quinine sulfate	650 mg q8h for 3–7 days	25 mg/kg/day in three doses for 3–7 days
PLUS	Doxycycline	100 mg q12h for 7 days	2 mg/kg/day for 7 days
All regimens followed by:			
	Primaquine phosphate	26.3 mg (15 mg base)/day for 14 days or 79 mg (45 mg base)/week for 8 weeks	0.3 mg base/kg/day for 14 days

Table 150.1 Antiparasitic agents and dosages (Organism or disease entity is listed alphabetically. Both adult and pediatric dosages are indicated)—cont'd

Infection	Drug	Adult dosage (po unless otherwise indicated)	Pediatric dosage (po unless otherwise indicated)
All *Plasmodium spp*. except chloroquine-resistant *Plasmodium falciparum* and chloroquine-resistant *Plasmodium vivax*			
	Chloroquine phosphate	1 g (600 mg base), then 500 mg (300 mg base) 6h later, then 500 mg (300 mg base) at 24h and 48h	10 mg base/kg (maximum 600 mg base), then 5 mg base/kg 6h later, then 5 mg base/kg at 24h and 48h
All *Plasmodium* spp. (parenteral regimens)			
	Quinidine gluconate	10 mg/kg iv loading dose (maximum 600 mg) in normal saline slowly over 1–2h, followed by continuous infusion of 0.02 mg/kg/min until oral therapy can be started	10 mg/kg iv loading dose (maximum 600 mg) in normal saline slowly over 1–2h, followed by continuous infusion of 0.02 mg/kg/min until oral therapy can be started
OR	Quinine dihydrochloride	20 mg/kg iv loading dose in 5% dextrose over 4h, followed by 10 mg/kg over 2–4h, q8h (maximum 1800 mg/day) until oral therapy can be started	20 mg/kg iv loading dose in 5% dextrose over 4h, followed by 10 mg/kg over 2–4h, q8h (maximum 1800 mg/day) until oral therapy can be started
Alternatives	Artemether†	3.2 mg/kg im, then 1.6 mg/kg q24h	3.2 mg/kg im, then 1.6 mg/kg q24h
	Artesunate†	2.4 mg/kg/dose iv for 3 days with doses at 0, 12, 24, 48 and 72 hours	2.4 mg/kg/dose iv for 3 days with doses at 0, 12, 24, 48 and 72 hours
Prevention of relapses (*Plasmodium vivax* and *Plasmodium ovale* only)			
	Primaquine phosphate	26.3 mg (15 mg base)/day for 14 days or 79 mg (45 mg base)/week for 8 weeks	0.3 mg base/kg/day for 14 days
Malaria prevention			
Chloroquine-sensitive areas			
	Chloroquine phosphate	500 mg (300 mg base) once per week	5 mg/kg base once per week, up to adult dose of 300 mg base
Chloroquine-resistant area			
	Atovaquone/proguanil	One adult tablet/day	5–8 kg: ½ ped tablet/day 9–10 kg: ¾ ped tablet/day 11–20 kg: one ped tablet/day 21–30 kg: two ped tablets/day 31–40 kg: three ped tablets/day >40 kg: adult dose
OR	Mefloquine	250 mg once per week	Weight <5 kg, no data; weight 5–9 kg, 1/8 tablet; weight 10–19 kg, ¼ tablet; weight 20–30 kg, ½ tablet; weight 31–45 kg ¾ tablet; weight >45 kg, one tablet
OR	Doxycycline	100 mg/day	2 mg/kg/day, up to 100 mg/day
Alternatives	Primaquine	0.5 mg/kg base/day	0.5 mg/kg base/day
Microsporidiosis			
Ocular microsporidiosis (*Encephalitozoon hellem, Encephalitozoon cuniculi, Vittaforma corneae* (*Nosema corneum*)			
	Albendazole	400 mg q12h	
PLUS	Fumagillin eye drops		
Intestinal microsporidiosis (*Enterocytozoon bieneusi, Encephalitozoon* (*Septata*) *intestinalis*)			
	Albendazole	400 mg q12h	
OR	Fumagillin	60 mg q24h for 14 days	
Disseminated microsporidiosis (*Enterocytozoon hellem, Enterocytozoon cuniculi, Enterocytozoon intestinalis, Pleistophora* ssp.)			
	Albendazole	400 mg q12h	
Moniliformis moniliformis			
	Pyrantel pamoate	11 mg/kg, single dose, repeat twice 2 weeks apart	11 mg/kg, single dose, repeat twice 2 weeks apart
Oesophagostomum bifurcum			
	Albendazole or pyrantel pamoate		
Schistosomiasis (bilharziasis)			
Schistosoma haematobium			
	Praziquantel	40 mg/kg/day in two doses for 1 day	40 mg/kg/day in two doses for 1 day
Schistosoma japonicum			
	Praziquantel	60 mg/kg/day in three doses for 1 day	60 mg/kg/day in three doses for 1 day

(Continued)

Table 150.1 Antiparasitic agents and dosages (Organism or disease entity is listed alphabetically. Both adult and pediatric dosages are indicated)—cont'd

Infection	Drug	Adult dosage (po unless otherwise indicated)	Pediatric dosage (po unless otherwise indicated)
Schistosoma mansoni			
	Praziquantel	40 mg/kg/day in two doses for 1 day	40 mg/kg/day in two doses for 1 day
Alternative	Oxamniquine	15 mg/kg, single dose	20 mg/kg/day in two doses for 1 day
Schistosoma mekongi			
	Praziquantel	60 mg/kg/day in three doses for 1 day	60 mg/kg/day in three doses for 1 day
Strongyloidiasis (*Strongyloides stercoralis*, threadworm)			
	Ivermectin	20 µg/kg/day for 1–2 days	200 µg/kg/day for 1–2 days
Alternative	Thiabendazole	50 mg/kg/day in two doses (maximum 3 g/day) for 2 days	50 mg/kg/day in two doses (maximum 3 g/day) for 2 days
Tapeworm infection (adult (intestinal stage))			
***Diphyllobothrium latum* (fish), *Taenia saginata* (beef), *Taenia solium* (pork), *Dipylidium caninum* (dog)**			
	Praziquantel	5–10 mg/kg, single dose	5–10 mg/kg, single dose
Alternative	Niclosamide	2 g single dose	50 mg/kg, single dose
***Hymenolepis nana* (dwarf tapeworm)**			
	Praziquantel	20 mg/kg, single dose	25 mg/kg, single dose
Tapeworm infection (larval (tissue stage))			
***Echinococcus granulosus* (hydatid cyst)**			
	Albendazole	400 mg q12h for 28 days, repeated as necessary	15 mg/kg/day for 28 days, repeated as necessary
Echinococcus multilocularis			
Treatment of choice		Surgical excision	
***Cysticercus cellulosa* (*cysticercosis*)**			
	Albendazole	400 mg q12h for 8–30 days, repeated as necessary	15 mg/kg/day (maximum 800 mg) in two doses for 8–30 days, repeated as necessary
OR	Praziquantel	50 mg/kg/day in three doses for 15 days	50 mg/kg/day in three doses for 15 days
Alternative	Surgery		
Toxoplasmosis (*Toxoplasma gondii*)			
	Pyrimethamine	25–100 mg/day for 3–4 weeks	2 mg/kg/day for 3 days, then 1 mg/kg/day (maximum 25 mg/day) for 4 weeks
PLUS	Sulfadiazine	1–1.5 g q6h for 3–4 weeks	100–200 mg/kg/day for 3–4 weeks
Alternative	Spiramycin	3–4 g/day	50–100 mg/kg/day for 3–4 weeks
Trichinosis (*Trichinella spiralis*)			
	Corticosteroids for severe symptoms		
PLUS	Mebendazole	200–400 mg q8h for 3 days, then 400–500 mg q8h for 10 days	
Trichomoniasis (*Trichomonas vaginalis*)			
	Metronidazole	2 g, single dose or 500 mg q12h for 7 days	15 mg/kg/day in three doses for 7 days
OR	Tinidazole	2 g, single dose	50 mg/kg, single dose (maximum 2 g)
Trichostrongyliasis (*Trichostrongylus* spp.)			
	Pyrantel pamoate	11 mg/kg, single dose (maximum 1 g)	11 mg/kg, single dose (maximum 1 g)
Alternative	Mebendazole	100 mg q12h for 3 days	100 mg q12h for 3 days
OR	Albendazole	400 mg, single dose	400 mg, single dose
Trichuriasis (*Trichuris trichiura*, whipworm)			
	Mebendazole	100 mg q12h for 3 days or 500 mg, single dose	100 mg q12h for 3 days or 500 mg, single dose
Alternative	Albendazole	400 mg, single dose	400 mg, single dose
Trypanosomiasis			
***Trypanosoma cruzi* (American trypanosomiasis, Chagas disease)**			
	Nifurtimox	8–10 mg/kg/day in three or four doses for 90–120 days	1–10 years: 15–20 mg/kg/day in four doses for 90 days 11–16 years: 12.5–15 mg/kg/day in four doses for 90 days
OR	Benznidazole	5–7 mg/kg/day for 30–90 days	≤12 years: 10 mg/kg/day in two doses for 30–90 days

Table 150.1 Antiparasitic agents and dosages (Organism or disease entity is listed alphabetically. Both adult and pediatric dosages are indicated)—cont'd

Infection	Drug	Adult dosage (po unless otherwise indicated)	Pediatric dosage (po unless otherwise indicated)
***Trypanosoma brucei gambiense* (West African trypanosomiasis) – hemolymphatic stage**			
	Difluoromethylornithine (eflornithine)	400 mg/kg/day iv in four divided doses for 14 days	
OR	Pentamidine isethionate	4 mg/kg/day im for 10 days	4 mg/kg/day im for 10 days
Alternative	Suramin	100–200 mg (test dose) iv, then 1 g on days 1, 3, 7, 14 and 21	20 mg/kg in on days 1, 3, 7, 14 and 21
***Trypanosoma brucei rhodesiense* (East African trypanosomiasis) – hemolymphatic stage**			
	Suramin	100–200 mg (test dose) iv, then 1 g on days 1, 3, 7, 14 and 21	20 mg/kg iv on days 1, 3, 7, 14 and 21
Late disease with central nervous system involvement, both *T. brucei gambiense* and *T. brucei rhodesiense*			
	Melarsoprol	2–3.6 mg/kg/day iv for 3 days; after 1 week 3.6 mg/kg/day iv for 3 days; repeat again after 10–21 days	18–25 mg/kg over 1 month; initial dose of 0.36 mg/kg iv, increasing gradually to maximum 3.6 mg/kg at intervals of 1–5 days for a total of 9–10 doses
OR	Difluoromethylornithine	400 mg/kg/day iv in four divided doses for 14 days	
Visceral larva migrans (toxocariasis)			
	Albendazole	400 mg q12h for 3–5 days	400 mg q12h for 3–5 days
OR	Mebendazole	100–200 mg q12h for 5 days	100–200 mg q12h for 5 days

Sb, antimony; SMX, sulfamethoxazole; TMP, trimethoprim.
*These recommendations are based on a handful of cases for each type of infection as summarized in Visvesvara *et al*.[2]
†Artesunate, artemether and other artemenisin derivatives should be paired with another antimalarial to avoid high rates of recrudescence (see text).
‡There appears to be significant variation in the susceptibility of *Leishmania* spp. to different agents, hence the choice of therapies should reflect the known sensitivities of organisms within the region where disease was acquired.
Data modified from Med Lett Drugs Ther 2007;(5):11.[1]

Trimethoprim

Trimethoprim (TMP) is another diaminopyrimidine that inhibits microbial dihydrofolate reductase. It has activity against:

- a variety of bacteria (see Chapter 138);
- *Pneumocystis jirovecii* (see Chapter 91); and
- the parasites *Isospora belli* and *Cyclospora cayetanensis* (see Chapter 180).[17]

Trimethoprim is readily absorbed, widely distributed and 50% protein-bound. Less than 20% is hepatically metabolized to inactive metabolites and the drug is excreted both in the urine and bile. The half-life is 9–11 hours.

For parasitic infections, TMP is used in fixed combination with sulfamethoxazole (SMX; see below). Side-effects include rashes, pruritus, nausea, vomiting, glossitis, elevated liver enzymes, cytopenias, megaloblastic anemia, fever, aseptic meningitis and impaired renal function.

Sulfonamides

Sulfonamides, which are derivatives of sulfanilamide, interfere with microbial folic acid synthesis by competitively inhibiting the enzyme dihydropteroate synthase.[6,7] This enzyme is involved in the step in folic acid synthesis that precedes the step blocked by pyrimethamine and TMP. Sulfonamides are separated into four groups:

- short- and intermediate-acting agents;
- long-acting agents;
- agents that are limited to the bowel lumen; and
- topical agents.

Only agents from the first two of these categories are used to treat parasitic diseases; these are generally combined with either pyrimethamine or TMP.

Sulfamethoxazole

Sulfamethoxazole is an intermediate-acting sulfonamide. It is rapidly absorbed, widely distributed, 50–70% protein-bound, hepatically metabolized and renally excreted. The half-life is 7–12 hours. It is available in a fixed combination with TMP (see below) for numerous indications.

Sulfadiazine

Sulfadiazine, another intermediate-acting sulfonamide, is also rapidly absorbed, widely distributed (including within the cerebrospinal fluid), 45–55% protein-bound, hepatically metabolized and renally excreted. The half-life is 12 hours. It is used with pyrimethamine in the treatment of toxoplasmosis, as detailed above.

Sulfadoxine

Sulfadoxine, a long-acting sulfonamide, is rapidly absorbed but slowly eliminated and has a half-life of 7–9 days. It is available in a fixed combination with pyrimethamine for the treatment of malaria (see pyrimethamine above).

Side-effects of sulfonamides

Side-effects of sulfonamides are numerous. Nausea, vomiting and anorexia occur in 1–2% of patients. Hypersensitivity reactions include:

- drug eruptions (ranging from morbilliform rash to severe exfoliation);
- fever;
- serum sickness; and
- hepatocellular dysfunction and necrosis.

Table 150.2 Availability* of antiparasitic agents

Agent	Trade name	Manufacturer
Available from the drug service provided by the US Centers for Disease Control and Prevention (CDC)		
Artesunate		Walter Reed Army Institute of Research
Bithionol	Bitin	Tanabe (Japan)
Diethylcarbamazine		CDC
Melarsoprol	Arsobal	Rhône-Poulenc Rorer
Nifurtimox	Lampit	Bayer
Sodium stibogluconate	Pentostam	Wellcome Foundation
Suramin	Germanin	Parke–Davis
Commercially available only outside the USA		
Amodiaquine	Camoquin Flavoquine	Parke–Davis Aventis
Artemether	Artenam	Arenco (Belgium)
Benznidazole	Rochagan	Roche
Diloxanide furoate	Furamide	Boots (UK)
Fumagillin	Flisinit	Sanofi-aventis (France)
Meglumine antimonate	Glucantime	Aventis
Niclosamide	Yomesan	Bayer
Ornidazole	Tiberal	Roche
Oxamniquine	Vansil	Pfizer
Proguanil	Paludrine	AstraZeneca (UK)
Quinacrine	Atabrine	Sanofi
Quinine dihydrochloride	(Generic)	ACF Chemiefarma NV (The Netherlands)
Spiramycin	Rovamycine	Aventis
Triclabendazole	Egaten	Novartis

*It is often difficult to obtain antiparasitic agents in the USA. This is not a comprehensive list; many drugs have multiple trade names and manufacturers, only some of which are included. Agents are divided into those available from the drug service provided by the Centers for Disease Control and Prevention (call 404-639-3670 during business hours, 404-639-2888 at other times) and those that are commercially available only outside the USA. Some of the latter may be available through compounding pharmacies within the USA. These include: Panorama Compounding Pharmacy, 6744 Balboa Blvd, Lake Balboa, CA 91406. Tel: (800) 247-9767, http://www.uniquerx.com; Medical Center Pharmacy, 800 Howard Avenue, New Haven, CT 06511. Tel. 203-688-6816. The National Association of Compounding Pharmacies (800-687-7850) and the Professional Compounding Centers of America (800-331-2498) can provide the names of additional compounding pharmacies.

Table 150.3 Safety of antiparasitic agents in pregnancy*

Category 1 drugs: probably safe	Category 3 drugs: insufficient data or established as unsafe (contraindicated)
Amphotericin B Azithromycin Chloroquine Clindamycin Niclosamide Paromomycin Praziquantel Pyrantel pamoate Proguanil Spiramycin	Albendazole – highest risk in first trimester Artemisinin and derivatives Atovaquone Benznidazole – contraindicated Bithionol Diethylcarbamazine Difluoromethylornithine – contraindicated Diloxanide furoate Doxycycline – contraindicated Fumagillin Furazolidone – contraindicated at term Iodoquinol Ivermectin Melarsoprol Miltefosine – contraindicated Nifurtimox Nitazoxanide Oxamniquine – contraindicated Pentamidine isethionate Primaquine Quinacrine Quinidine-large doses contraindicated Quinine Sodium stibogluconate Suramin Tetracycline Thiabendazole Triclabendazole
Category 2 drugs: possibly safe or safe during certain trimesters	
Dapsone Mebendazole Mefloquine Metrifonate Metronidazole Piperazine Pyrimethamine – contraindicated in first trimester Sulfonamides – contraindicated at term Trimethoprim – contraindicated at term	

*There are no well-controlled studies proving the safety of any of these antiparasitic agents in pregnancy. Category 1 drugs are those for which extensive clinical experience has demonstrated safety in pregnancy. Category 2 drugs are those that have been reported as safe only anecdotally or those that have been used safely in certain trimesters only. Category 3 drugs fall into two categories: those that should not be used because inadequate information makes risk assessment difficult, or those that are contraindicated because of documented fetal harm. Drugs in this group should be used only when there is a strong clinical indication for immediate treatment, there are no safer alternatives available, and the benefit of treatment to the mother outweighs the potential or known risks to the fetus.[3–5]

Acute hemolytic anemia, agranulocytosis and aplastic anemia are rare. Reversible bone marrow suppression is not uncommon in immunocompromised patients, particularly those who have AIDS. Crystalluria can occur with sulfadiazine and can be avoided by increasing fluid intake or alkalinizing the urine.

Trimethoprim–sulfamethoxazole

Trimethoprim–sulfamethoxazole (TMP–SMX) is a combination used to treat bacterial infections (see Chapter 138), *P. jirovecii* (see Chapter 91) and the parasites *I. belli* and *C. cayetanensis*.[17] This combination also has some efficacy against *P. falciparum*, but resistance to the TMP component limits its use.[18] It is available as single-strength tablets (80 mg TMP and 400 mg SMX) and as double-strength tablets (160 mg TMP and 800 mg SMX). An oral suspension (40 mg TMP and 200 mg SMX per 5 ml) and an intravenous formulation (80 mg TMP and 400 mg SMX per 5 ml vial) are available as well.

The dose for isosporiasis is one double-strength tablet orally q6h for 10 days, followed by one double-strength tablet q12h for 3 weeks. Immunocompromised patients usually require maintenance therapy of one double-strength tablet daily or three times weekly. For cyclosporiasis, one double-strength tablet q12h for 7–10 days is generally used, but some clinicians extend treatment to 14 days. Immunocompromised patients sometimes require four tablets per day and usually need maintenance therapy as well.

Side-effects of TMP–SMX include those listed for each of the two component drugs, as detailed above. Dermatologic reactions (3–4%) and gastrointestinal disturbances (3–4%) are the most common side-effects in nonimmunocompromised patients. For unclear reasons, patients who have AIDS have a much higher rate of complications, ranging in different series from 45% to 90%.

Trimetrexate

Trimetrexate is a lipid-soluble dihydrofolate reductase inhibitor that was originally developed as a myelosuppressive agent, but was found to have antiparasitic activity against *P. jirovecii* and *T. gondii*.[15] It is available for intravenous injection only. Adverse effects include rash, leukopenia, elevated liver enzymes and a reversible peripheral neuropathy. Folinic acid is administered concurrently to diminish the incidence of bone marrow suppression.

Proguanil

Proguanil is a biguanide that inhibits plasmodial dihydrofolate reductase.[6,7] Although it is seldom used for monotherapy because of its slow action, in combination with atovaquone it is effective in the prevention and treatment of *P. falciparum* malaria (see below, under Atovaquone).[19–21]

Proguanil is slowly absorbed after oral administration, is 75% protein-bound, is metabolized to the active triazine metabolite cycloguanil, and is excreted in urine (40–60%) and feces (10%). The drug is safe and well tolerated. Pancytopenia has been rarely reported. Nausea, vomiting, abdominal pain, diarrhea and hematuria are associated with the use of high doses.

ARTEMISININ AND ITS DERIVATIVES

A major change in malaria therapy over the past 5 years has been the growing use of combination therapy for chloroquine-resistant *Plasmodium falciparum* infections. Artemisinin-based combination treatments (ACTs) have become a foundation of this effort.[9] Artemisinin, or qinghaosu, is a sesquiterpene lactone derived from the leaves of the sweet wormwood *Artemisia annua*.[7,9,22,23] It has been used for centuries in traditional Chinese medicine and is now known to be active against intraerythrocytic forms of *P. falciparum* and *P. vivax*, *Schistosoma mansoni*, *Schistosoma japonicum*, *Clonorchis sinensis* and *Naegleria fowleri*. Its main clinical use is the treatment of drug-resistant *P. falciparum* infections.

Route of administration and dosage

Artemisinin and its derivatives, the water-soluble hemisuccinate artesunate, the oil-soluble derivatives artemether and artemotil, and the active metabolite dihydroartemisinin are the most rapidly acting of known antimalarials and appear to be quite safe. These compounds can be given by several routes:

- artemisinin is available in oral and suppository forms;
- artesunate is available in oral, intravenous and intramuscular forms;
- artemether is available in oral and intramuscular forms;
- artemotil is available for intramuscular injection; and
- dihydroartemisinin is available in oral form.

They are rapidly absorbed and eliminated, with half-lives ranging from minutes (artesunate) to hours (artemether). The parent drug and derivatives are hepatically hydrolyzed to the active metabolite, dihydroartemisinin. These compounds are believed to act by disrupting parasite protein synthesis via the production of oxygen free radicals.

Artemisinin and its derivatives are best administered in conjunction with a longer acting antimalarial (e.g. mefloquine, lumefantrine, amodiaquine, sulfadoxine–pyrimethamine) to decrease the emergence of resistance and enhance efficacy. Recrudescence is a significant problem when artemisinin compounds are used as single agents. Artesunate (4 mg/kg orally) is usually given q24h for 3 days and is followed by a course of mefloquine. A fixed combination of artesunate (200 mg) and mefloquine (400 mg) base dosed at mefloquine 8 mg/kg/day for 3 days has been used with good success and, as noted above, the combination of artesunate and amodiaquine has been effective in certain regions.[9,10,24] Artemether can be given at a dose of 3.2 mg/kg intramuscularly initially, followed by 1.6 mg/kg q24h. It is available orally in combination with lumefantrine (artemether 80 mg/lumefantrine 480 mg) as a six-dose regimen. This has been used effectively and is well tolerated, but requires twice daily dosing and co-administration of fat for optimal absorption.[9,25] The fixed dose combination of dihydroartemisinin (40 mg) and piperaquine (a 4-aminoquinolone related to chloroquine) (320 mg) has been used successfully to treat *P. falciparum* infections in Asia and Africa.[9,26]

Adverse reactions

Adverse events from artemisinin and its derivates include diarrhea, abdominal pain, transient first-degree heart block and reversible mild decreases in reticulocyte and neutrophil counts. Neurotoxicity has been described in animals but not with clinical use in humans. Resistance to artemisinin has occurred in murine malaria, and the resistant parasites also developed cross-resistance to chloroquine, quinine and mefloquine. In the USA the only artemisinin derivative currently available is the intravenous form of artesunate, which must be obtained from the US Centers for Disease Control and Prevention (www.cdc.gov/malaria/features/astesunate_now_available) and is currently released only for patients that cannot receive or tolerate quinidine.[20]

ATOVAQUONE AND ATOVAQUONE–PROGUANIL

Atovaquone, a synthetic hydroxynaphthoquinone derivative, has activity against *P. jirovecii* (see Chapter 91), *P. falciparum*, *T. gondii* and *Babesia microti*.[16,20,21,27] It interferes with pyrimidine synthesis by uncoupling mitochondrial electron transport. Because of its erratic absorption it is usually administered with a fatty meal. It is hepatically metabolized and excreted in the bile and urine. Atovaquone is an alternative oral agent for the treatment of mild to moderate *P. jirovecii* pneumonia in those who are intolerant of TMP–SMX, and experimental data indicate that it is synergistic with pyrimethamine or sulfadiazine for *T. gondii* infection. Atovaquone has also been used with azithromycin in the treatment of babesiosis.

The combination of atovaquone and proguanil has become a treatment of choice for *P. falciparum* malaria contracted in areas where chloroquine resistance is present (most of the malarious world with the exception of Central America west of the Panama Canal, Mexico, Hispaniola, parts of China and the Middle East)[20] and the combination pill containing atovaquone 250 mg and proguanil 100 mg has rapidly become a leading drug for malaria prophylaxis in travelers.[20,21,28,29] A randomized controlled trial comparing mefloquine and atovaquone–proguanil for malaria prophylaxis in nonimmune travelers found equivalent efficacy for the two agents, with a similar number of adverse events, but fewer adverse effects of moderate or severe intensity were reported in the atovaquone–proguanil group.[29] The adult dosage for prophylaxis is one tablet q24h (250 mg atovaquone/100 mg proguanil), beginning 1–2 days prior to arrival in the malarious area and continuing for 1 week after return. Side-effects include rash, nausea, vomiting, diarrhea, headache, fever, anemia, elevated liver function tests, hyponatremia and hyperglycemia. *P. falciparum* strains resistant to atovaquone–proguanil resulting in treatment failures have now been identified, with most strains showing mutations in the parasite cytochrome b gene.[30] Combination therapy of artesunate with atovaquone–proguanil has been tested and found to be well tolerated and highly effective in limited trials.[31]

BENZNIDAZOLE

Benznidazole is a nitroimidazole derivative that is active against both the trypomastigote and amastigote forms of *Trypanosoma cruzi*.[6,7,32] It is available in oral form and has a half-life of 12 hours. Side-effects

include malaise, nausea, photosensitivity rash, peripheral neuropathy, bone marrow suppression and psychiatric disturbances.

CHLOROQUINE

Chloroquine, a 4-aminoquinoline that was first synthesized in 1934 but did not become popular until the end of the Second World War, has been the agent most widely used for treating the erythrocytic stage of uncomplicated malaria caused by *P. vivax, P. ovale, P. malariae* and chloroquine-sensitive *P. falciparum*.[6,7,20,21] Its precise mechanism of action has not been delineated, but chloroquine and its metabolites inhibit the ability of the parasite to polymerize the heme moiety of hemoglobin, resulting in toxic levels of free heme.

Pharmacokinetics and distribution

Absorption after oral ingestion is excellent (90%), and the volume of distribution is large owing to its extensive tissue sequestration, particularly in the liver, spleen, kidneys and erythrocytes. It is approximately 50% bound to plasma proteins and is eliminated slowly. Its half-life of 4–6 days permits weekly dosing for prophylaxis. Chloroquine is metabolized by the liver to the active metabolite desethylchloroquine, but 50% is cleared by the kidneys unchanged. Thus, dosing need not be altered for abnormal renal function, but caution must be exercised in patients who have hepatic, gastrointestinal, neurologic or hematologic disorders.

Route of administration and dosage

The drug is formulated as a phosphate, sulfate or hydrochloride salt and is dosed by base content. It can be administered orally or rectally or by intravenous, intramuscular or subcutaneous injection. In the USA, chloroquine is marketed as Aralen phosphate in 500 mg salt tablets (equal to 300 mg base). The dosage for the treatment and prophylaxis of malaria is given in Table 150.1. If chloroquine hydrochloride is given intravenously, it must be administered by slow, constant infusion to avoid respiratory depression, hypotension, heart block, cardiac arrest and seizures that may occur with transient toxic levels. A dose of 300 mg base q8–12h may be given by intramuscular injection.

Adverse reactions

Reversible side-effects include headache, gastrointestinal disturbances, blurred vision, dizziness, fatigue and pruritus. Rarer side-effects include hair depigmentation, weight loss, myalgias, leukopenia and eczematous eruptions. Very rarely, acute psychosis may occur. Permanent retinal damage has been observed with long-term (longer than 5 years) prophylactic use. The drug is contraindicated in patients who have retinal disease, psoriasis and porphyria. An oral dose of 5 g is fatal without immediate mechanical ventilation, adrenaline (epinephrine) and diazepam.

Resistance of *Plasmodium falciparum* to chloroquine

Resistance of *P. falciparum* to chloroquine is ubiquitous in regions where malarial transmission occurs with the exception of Central America west of the Canal Zone, Mexico, Haiti, the Dominican Republic and much of the Middle East (although there are reports of resistance from Yemen, Oman and Iran).[20] There is a report that withdrawal of chloroquine as preferred treatment from a region can, after an 8-year period, result in the re-emergence of chloroquine-sensitive *P. falciparum*.[33] Resistance to chloroquine among *P. vivax* isolates has been reported in Brazil, Colombia, India, Myanmar, Papua New Guinea and Indonesia.[20,34,35] A single oral dose of mefloquine (15 mg base/kg) has been used successfully in such cases.

CLINDAMYCIN

Clindamycin, a lincosamide antibiotic, is active against bacteria (see Chapter 135), *P. falciparum*, *T. gondii* and *Babesia* spp.[15,36,37] It is well absorbed after oral administration and is 90% protein-bound and widely distributed. It is hepatically metabolized and excreted in the urine and bile; its half-life is 2.5–3 hours.

Dosages for malaria and babesiosis are listed in Table 150.1. For cerebral toxoplasmosis in the case of sulfonamide hypersensitivity, 1.8–2.4 g divided into three daily doses is combined with a course of pyrimethamine. Clindamycin has been used in combination with quinine for short-course (3-day) treatment of travelers who have *P. falciparum* malaria, with excellent results.[38] Side-effects include rash, diarrhea, nausea, vomiting, abdominal pain, pseudomembranous colitis, hepatotoxicity and cytopenias.

DIFLUOROMETHYLORNITHINE

Difluoromethylornithine is an ornithine decarboxylase inhibitor that is effective in the treatment of both early and late sleeping sickness caused by *Trypanosoma brucei gambiense*.[7,39,40] It has variable efficacy against *T. brucei rhodesiense* because many strains are resistant. Difluoromethylornithine inhibits ornithine decarboxylase, an enzyme involved in the first step in polyamine synthesis. It is available as the hydrochloride salt for both oral and intravenous administration. It has a half-life of approximately 3 hours, and 80% of the drug is excreted unchanged by the kidneys.

Side-effects of difluoromethylornithine include anemia, thrombocytopenia, leukopenia, abdominal pain, nausea, vomiting, weight loss, arthralgias, seizures, hearing loss and alopecia. Overall, difluoromethylornithine is less toxic than other available antitrypanosomal agents, but it has not seen widespread use because of its high cost.

DILOXANIDE FUROATE

Diloxanide furoate is a dichloroacetamide derivative that is a luminally active agent used to eradicate cysts of *E. histolytica* in asymptomatic carriers and in those who have mild, noninvasive disease, as well as after treatment with metronidazole in those who have invasive amebiasis.[6,41] It is not useful in extraintestinal disease. After oral administration, diloxanide furoate is hydrolyzed by intestinal esterases, thus releasing diloxanide, the absorbable component, and the ester furoic acid, which is not well absorbed and thus attains higher intraluminal concentrations in the colon. Both compounds are amebicidal, but the mechanism of action is not known. The drug has a half-life of 6 hours, is hepatically conjugated to form a glucuronide and is 60–90% excreted in the urine.

Side-effects are mild; they include flatulence and, less commonly, nausea, vomiting, diarrhea, pruritus and urticaria. Because it is relatively inexpensive, diloxanide furoate is an attractive agent for use in developing countries.

FUMAGILLIN

Fumagillin, a water-insoluble antibiotic derived from *Aspergillus fumigatus*, was discovered in 1949 and originally used in humans as an amebicide. Fumagillin is an inhibitor of parasite RNA synthesis, but may also act by inhibiting a key proteinase, type 2 methionine aminopeptidase.[42] A water-soluble preparation (Fumidil B) is used to control nosematosis, a disease of honey bees that results from infection with microsporidian *Nosema apis*. Topical fumagillin has been used to treat microsporidial keratoconjunctivitis caused by *Encephalitozoon*

hellem, *Encephalitozoon cuniculi*, *Encephalitozoon* (*Septata*) *intestinalis* and, with less success, *Vittaforma corneae* (*Nosema corneum*) in AIDS patients.[43] Studies of oral fumagillin for intestinal microsporidiosis have provided promising results.[44]

FURAZOLIDONE

Furazolidone is a nitrofuran derivative that is commonly used to treat giardiasis in children because of its availability in a liquid form for oral use.[6,45] Furazolidone also has activity against *I. belli* and *Trichomonas vaginalis* as well as many enteropathogenic bacteria, and is also used for treatment of *Helicobacter pylori* infections. The mechanism of action involves damage to DNA. It is well absorbed and is excreted mainly in the urine.

Adverse reactions include diarrhea, fever, nausea and vomiting. Urticaria, serum sickness, hypoglycemia and orthostatic hypotension occur rarely. Furazolidone has disulfiram-like properties and patients should therefore be warned to avoid alcohol. Furazolidone has monoamine oxidase inhibitor activity, but hypertensive crises have not been reported in association with this agent. Furazolidone may cause hemolysis in patients who have glucose-6-phosphate dehydrogenase (G6PD) deficiency.

HALOFANTRINE

Halofantrine is an oral synthetic 9-phenanthrene methanol with activity against the intraerythrocytic stages of chloroquine-sensitive and chloroquine-resistant *P. falciparum* and *P. vivax*.[6,7,21,46] It is more active and generally better tolerated than mefloquine, but is poorly absorbed. Ingestion with fatty meals increases absorption. Halofantrine is hepatically metabolized and excreted in feces, with a half-life of 1–2 days for the parent compound and 3–5 days for the active metabolite. Its mechanism of action is poorly understood.

There is some evidence of cross-resistance with mefloquine; therefore halofantrine may not be useful for those patients in areas with mefloquine resistance. Its side-effects include prolongation of the PR and QT_c intervals on the electrocardiogram, diarrhea, abdominal pain, pruritus and rash. Because of reports of sudden death associated with halofantrine therapy, it is absolutely contraindicated in individuals with a history of congenital QT prolongation, other conduction defects or anyone taking medications known to prolong the QT interval.

IODOQUINOL

Iodoquinol, a halogenated hydroxyquinoline, is a luminal amebicide used to eradicate cysts in patients who have asymptomatic *E. histolytica* infection.[6,41] It is also given after metronidazole therapy to eradicate cysts in patients who have invasive disease. Iodoquinol is the drug of choice for *Dientamoeba fragilis* infection and is an alternative for *Balantidium coli*.[47] It has been used to treat *Blastocystis hominis*, but the pathogenicity of this protozoan and its need for treatment are controversial.[48] Iodoquinol also has activity against *Giardia lamblia* and *T. vaginalis*, but other agents are typically employed. The mechanism of action of iodoquinol is uncertain. It is available in oral form, but is poorly absorbed and should be given with meals. Side-effects include nausea, vomiting, diarrhea, abdominal pain, headache, fever, seizures and encephalopathy.

Iodochlorhydroxyquin, a related compound, is better absorbed than iodoquinol, but is rarely used because of the high incidence of subacute myelo-optic neuropathy described with its use in Japan in the early 1970s. Because iodoquinol may rarely cause this syndrome when given at high dose or for prolonged periods, treatment recommendations should not be exceeded. For this reason many clinicians prefer alternative agents such as paromomycin or diloxanide furoate for these indications.

LUMEFANTRINE

Lumefantrine is an aryl amino alcohol compound that is structurally related to mefloquine and halofantrine. It is active against all *Plasmodium* spp. that infect humans.[9] As noted above, a fixed tablet combination of lumefantrine and artemether has proven to be highly effective in the treatment of multidrug-resistant *P. falciparum* infection.[9]

MACROLIDE ANTIBIOTICS

Spiramycin

Spiramycin is used in Europe to prevent the transmission of *T. gondii* from mother to fetus.[15] The drug is concentrated in the placenta and has been shown to reduce transmission by 60%. It is given at a dose of 1 g orally q8h on an empty stomach. If fetal infection has not occurred (as assessed by amniotic fluid polymerase chain reaction testing for *T. gondii*), spiramycin is continued until delivery. Because spiramycin does not cross the placenta well, it cannot be used to treat fetal toxoplasmosis; pyrimethamine and sulfadiazine are recommended in this situation. Oral spiramycin is generally well tolerated; gastrointestinal distress is the main side-effect.

Azithromycin

Azithromycin (see Chapter 135), both alone and in combination with pyrimethamine, has been shown to be effective in cerebral toxoplasmosis in AIDS patients.[15] It is considered relatively safe in pregnancy, but has not been extensively studied in preventing the vertical transmission of *T. gondii*. Azithromycin has also been used with both quinine and atovaquone for babesiosis.[36] Azithromycin has activity against *Plasmodium* spp., and is being evaluated as a component of combination therapy for *P. falciparum* paired with pyrimethamine–sulfadoxine, artesunate or quinine.[49]

MEFLOQUINE

Mefloquine is a fluorinated 4-quinoline methanol derivative of quinine. It is an oral formulation that was developed as part of a search for new antimalarials.[6,7,20,21,23] It is a blood schizonticide effective against all *Plasmodium* spp. that infect humans, including *P. falciparum* isolates that are resistant to chloroquine and pyrimethamine–sulfadoxine. It is ineffective against exoerythrocytic forms and gametocytes. The mechanism of action is unknown, but mefloquine may interfere with the function of *Plasmodium* food vacuoles or inhibit the polymerization of heme. The drug is slowly absorbed, has a bioavailability of 85% and is almost completely protein-bound in plasma. The long elimination half-life of 2–3 weeks allows for weekly prophylaxis. Mefloquine is extensively metabolized and is excreted in bile and feces.

Common side-effects at therapeutic doses include nausea, vomiting, dizziness, weakness and dysphoria.[29,50] Neuropsychiatric reactions, including acute psychosis, sleep disturbances and seizures, have been documented in approximately 0.5% of patients taking therapeutic doses and in less than 0.5% of those taking prophylactic doses. Thus, the drug is not recommended for those who have a history of seizures or psychiatric disorders. Judicious use is suggested for those whose occupations require spatial discrimination and fine motor coordination. Cardiac rhythm and conduction abnormalities and at least one instance of nonfatal cardiac arrest have occurred in patients on β-adrenergic blockers who took mefloquine; caution should be exercised in any patient who has cardiac disease. Mefloquine should not be co-administered with quinine, quinidine or halofantrine owing to potentially fatal prolongation of the QT_c interval. Mefloquine may also decrease the response to the live *Salmonella typhi* oral vaccine,

and thus the vaccine series should be completed at least 3 days before beginning mefloquine prophylaxis.

Mefloquine resistance in *P. falciparum* isolates has been increasing along the Thailand–Myanmar and Thailand–Cambodia borders, in western Africa and in the Amazon region. In these areas, doxycycline at a dose of 100 mg/day or atovaquone–proguanil may be used for prophylaxis. Even the combination of mefloquine and artesunate may no longer be effective for treatment of acute falciparum malaria in those regions.[24,51] Treatment options include:

- quinine plus tetracycline or doxycycline for 7 days;[52]
- lumefantrine and artemether;[9]
- doxycycline plus artesunate;[53]
- atovaquone–proguanil and artesunate;[31]
- quinine plus clindamycin;[37] and
- dihydroartemisinin and piperaquine.[9]

MELARSOPROL

Melarsoprol is a trivalent arsenical compound introduced in 1949 and used for the treatment of late-stage African trypanosomiasis caused by either *T. brucei gambiense* or *T. brucei rhodesiense*.[6,7,40] It is also effective in treating the early or hemolymphatic stage of infection, but its toxicity prohibits routine use for this stage and it should be used only in patients who have failed to respond to suramin and pentamidine.

Melarsoprol acts by interacting with protein sulfhydryl groups and subsequently inactivating enzymes, a nonspecific action that is also responsible for the toxicity of the drug. Melarsoprol, formulated as a 3.6% weight per volume solution in propylene glycol, is given intravenously. A small, but adequate amount of the drug penetrates the cerebrospinal fluid, where it is taken up and concentrated by susceptible trypanosomes. Resistant organisms appear to concentrate the drug poorly. Melarsoprol is rapidly excreted in the urine.

Melarsoprol is highly toxic. It is irritating to tissues and care must be taken to prevent extravasation. Fever is commonly seen. Reactive encephalopathy occurs in up to 18% of patients and may be fatal; it usually occurs during the first 3–4 days of therapy.[54] It is manifested by headache, confusion, dizziness, mental slowing and ataxia, with seizures and a progressive decline in mental status, and it is felt to be an immunologic reaction to parasite antigens released during therapy. Corticosteroids have been used to treat the encephalopathy with some success. Very rarely, a hemorrhagic encephalopathy, which is almost always fatal, may occur. Arthralgias, rash, hypertension, proteinuria and hepatic dysfunction have been seen. Abdominal pain and vomiting may be minimized by slow administration of the drug to a patient who is supine and fasting. Erythema nodosum leprosum may be precipitated in patients who have leprosy. Hemolysis may be seen in G6PD-deficient patients.

MILTEFOSINE

Miltefosine, an alkyl phospholipids compound, was developed as an anticancer agent, but had dose-limiting gastrointestinal toxicity that outweighed its clinical benefits. However, it was discovered to have excellent antileishmanial activity and has become an important oral drug in the treatment of visceral leishmaniasis.[55,56] Its mechanism of action remains unknown, but parasite death appears to occur via apoptosis.[57] A 21-day course of miltefosine has proven safe and effective in the treatment of adults with visceral leishmaniasis in India.[58] It has also been studied in the treatment of cutaneous leishmaniasis in the Americas where it showed variable efficacy depending on the region and parasite strain.[59] Gastrointestinal side-effects including nausea, vomiting and diarrhea are frequent, occurring in roughly one-third of subjects. Elevations in transaminases may be seen in 15% of recipients, and increases in serum creatinine in 10%; both tend to normalize during treatment.

NIFURTIMOX

Nifurtimox, an oral nitrofuran, is used to treat acute Chagas disease (American trypanosomiasis), although benznidazole is gaining favor as a first-line agent in some regions with endemic Chagas disease (see Chapter 118).[6,7,32] Nifurtimox has also been used against resistant strains of *T. brucei gambiense*.[39] It acts by inhibiting nucleic acid synthesis by oxygen free radical formation. It is rapidly absorbed, has a half-life of approximately 3 hours, is extensively metabolized by the liver by a first-pass effect that results in low serum and tissue levels, and is excreted by the kidneys. There is considerable geographic variation in responsiveness to nifurtimox; better results are obtained in Argentina and Chile than in Brazil and other countries. Effectiveness in indeterminate-phase and chronic-phase infection is variable and organ damage is not reversible.

Gastrointestinal side-effects including nausea, vomiting, anorexia and abdominal pain; weight loss may occur. Neurologic side-effects include headache, restlessness, insomnia, disorientation, paresthesias, polyneuritis, weakness and seizures. Rash, decreased sperm counts and neutropenia have also been described. Adherence to a full 4 months of therapy is often poor, and better agents are needed.

NITAZOXANIDE

Nitazoxanide is a nitrothiazole benzamide derivative with *in-vitro* activity against a wide variety of bacterial, protozoal and helminthic pathogens. In randomized, double-blind, placebo-controlled clinical trials it showed efficacy comparable to metronidazole in the treatment of giardiasis and amebiasis, and was very successful in eradicating helminths from individuals in Egypt and Mexico.[60] Healthy adults treated with nitazoxanide cleared cryptosporidia from their stool more rapidly than did placebo controls, but the efficacy of nitazoxanide in AIDS patients remains to be established.[60]

NITROIMIDAZOLE DERIVATIVES

Metronidazole

Metronidazole (see Chapter 142) has activity against many anaerobic parasites. It is the drug of choice for the treatment of:

- invasive enterocolitis and liver abscess caused by *E. histolytica* and the rarely reported *Entamoeba polecki*;[6,7,41]
- vaginitis caused by *T. vaginalis*;[61] and
- enteritis caused by *G. lamblia*.[45]

It has been used to treat *Blastocystis hominis* in the stool (although its efficacy remains unproven) and is considered an alternative agent for *Balantidium coli* infection. Metronidazole is also used in the treatment of infections with the guinea worm, *Dracunculus medinensis*; it decreases inflammation and facilitates worm removal, but has no direct toxic effect on the worm itself.

Metronidazole acts as an electron sink under anaerobic or microaerophilic conditions, depriving the parasite of necessary reducing equivalents such as nicotinamide adenine dinucleotide phosphate, reduced form (NADPH). Reduced metronidazole (i.e. drug molecules that have gained electrons) causes a loss of the helical structure of DNA and strand breakage.

Metronidazole is available for oral and intravenous use. It is rapidly and almost completely absorbed orally, has limited protein binding and is widely distributed throughout the body. The half-life is 6–11 hours and metabolism is hepatic. Although excretion is mainly by the kidney, dosage adjustments are seldom needed in renal failure because the metabolites are less active compounds. However, the dosage should be modified in patients who have liver failure.

The most common side-effects are headache, metallic taste, dry mouth and nausea. Less frequent are urticaria, pruritus, urethral burning, reversible neutropenia, and vaginal and oral candidiasis. Rarely,

patients may experience central nervous system toxicity, including dizziness, vertigo, ataxia, encephalopathy and seizures, as well as peripheral neuropathy. Acute pancreatitis has been reported. Patients should be advised to avoid consuming alcohol because of the disulfiram-like effects of metronidazole, including headache, flushing, abdominal pain and vomiting. Some patients may experience a red–brown discoloration of the urine owing to the presence of metabolites of metronidazole.

Tinidazole and ornidazole

Tinidazole and ornidazole are two other nitroimidazole derivatives (see Chapter 142). Their antimicrobial spectrum is similar to that of metronidazole,[6,7,41] and tinidazole has been used successfully for single-dose therapy of amebic liver abscess.[62] Tinidazole has also been used to treat metronidazole-resistant *Trichomonas* spp. and may show greater efficacy in the treatment of bacterial and trichomonal vaginitis.[61] These compounds are well absorbed orally and are widely distributed. Tinidazole has a half-life of 14 hours and ornidazole has a half-life of 12–13 hours; both compounds are probably hepatically metabolized and are excreted primarily in the urine. Generally, these drugs are better tolerated than metronidazole; the main side-effects are headache, dizziness and anorexia.

PAROMOMYCIN

Paromomycin (also known as aminosidine) is a nonabsorbable aminoglycoside antibiotic (see Chapter 137) that is concentrated in the lumen of the colon. It is active against *E. histolytica, D. fragilis* and *G. lamblia* as well as the cestodes *Taenia saginata, Taenia solium, Diphyllobothrium latum, Dipylidium caninum* and *Hymenolepis nana*.[6,41,45,47] Paromomycin with methylbenzethonium chloride has been used topically in the treatment of cutaneous leishmaniasis and systemically for visceral leishmaniasis.[39,63] Paromomycin is available as the sulfate salt for oral administration.

The dose is 25–35 mg/kg/day in three divided doses for 7 days. Side-effects include cramps, nausea, vomiting, diarrhea, rash, headache and vertigo. Burning may occur with topical preparations.

PENTAMIDINE ISETHIONATE

Pentamidine isethionate, an aromatic diamidine derivative, is effective in the treatment of the early or hemolymphatic stages of sleeping sickness caused by *T. brucei gambiense*, some forms of leishmaniasis and *P. jirovecii* pneumonia (see Chapters 105 and 117).[6,7,39] It is less effective against *T. brucei rhodesiense*. It has also been used in the treatment of disseminated *Acanthamoeba* spp. infections and babesiosis.[64,65] The mechanism of action of pentamidine is unclear, but it may involve the binding of DNA and the interruption of DNA replication. The drug is available for parenteral and inhalational use; the latter mode is used only in the prophylaxis and treatment of *P. jirovecii* pneumonia because little of the inhaled drug is absorbed systemically. Parenterally administered pentamidine isethionate penetrates extensively and is excreted slowly from tissues such as liver, spleen, kidneys and adrenal glands. Very little crosses the blood–brain barrier, accounting for the lack of utility of pentamidine in late-stage trypanosomiasis.

Route of administration and dosage

There are different recommendations for dosing pentamidine isethionate. For early *T. brucei gambiense* infection, the CDC recommends 4 mg/kg/day for 10 days. The World Health Organization (WHO) recommends 3–4 mg/kg/day or every other day for 7–10 doses. Because of the rapidity with which *T. brucei rhodesiense* invades the central nervous system, this drug is generally not used for this organism. Pentamidine has also been used for prophylaxis against infection with *T. brucei gambiense* at a dose of 4 mg/kg (to a maximum of 300 mg) given every 3–6 months.

For leishmaniasis, the CDC recommends 2–4 mg/kg/day or every other day for 12–15 doses; a second course is sometimes given after an interval of 1–2 weeks. Alternatively, the WHO recommends 4 mg/kg three times a week for 5–25 weeks or longer. The dosage regimen varies slightly depending on the species of *Leishmania* and the region of the body affected. A study of 315 patients in Surinam found good results in the treatment of cutaneous leishmaniasis with a single dose weekly for 3 weeks.[66]

Adverse reactions

Pentamidine isethionate may cause toxicity in 50% of patients. Precipitous hypotension with dizziness, dyspnea, tachycardia, headache, vomiting and syncope can occur with rapid intravenous infusion. Intramuscular administration may result in sterile abscesses. Hypoglycemia, which may be life threatening, pancreatitis, hyperglycemia and diabetes mellitus probably result from a direct toxic effect of pentamidine on pancreatic β cells. Reversible renal failure occurs in up to 25% of patients. Other side-effects include fever, arrhythmias (particularly torsades de pointes), hypocalcemia, confusion, hallucinations, leukopenia, thrombocytopenia and elevated transaminases.

PENTAVALENT ANTIMONIAL COMPOUNDS

The pentavalent antimonial compounds remain a mainstay of therapy for leishmaniasis and are less toxic than the older trivalent compounds.[6,7,39,67] Sodium stibogluconate has been the most extensively studied and is the only pentavalent antimonial available in the USA. Meglumine antimonate is used largely in French-speaking countries and parts of Latin America. These compounds appear to inhibit bioenergetic pathways such as glycolysis and fatty acid oxidation in *Leishmania* amastigotes. *Leishmania* strains resistant to pentavalent antimony compounds are becoming more common, especially in India, and this has led to treatment failures and the need for alternative agents.[68]

These compounds are available as aqueous solutions for intravenous or intramuscular use only. Each milliliter of sodium stibogluconate contains the equivalent of 100 mg of pentavalent antimony, whereas each milliliter of meglumine antimonate contains 85 mg. They are rapidly absorbed and are eliminated in two phases. The first has a half-life of 2 hours, but the second is longer, with a half-life of between 33 hours (after intravenous administration) and 76 hours (after intramuscular administration). This slow terminal elimination may result from a conversion to trivalent antimony, which thus may be responsible for the toxicity seen with long-term therapy. Excretion is primarily renal.

Pentavalent antimonials are relatively well tolerated, but malaise, nausea, vomiting, abdominal pain, headache, arthralgias, myalgias, fever, rash, elevated transaminases, nephrotoxicity and pancreatitis may be seen. Dose-related electrocardiographic changes include T-wave flattening and inversion and QT_c-interval prolongation. Arrhythmias and sudden death have been described with high-dose therapy.

PIPERAQUINE

Piperaquine phosphate is a bisquinolone antimalarial drug that is structurally related to chloroquine and has activity against *P. vivax* and *P. falciparum*. Piperaquine is used in combination with dihydroartemisinin for the treatment of chloroquine-resistant *P. falciparum*. The drug is generally well tolerated, with relatively few adverse events noted to date.[69]

PRIMAQUINE

Primaquine, an 8-aminoquinoline active against hypnozoites of *P. vivax* and *P. ovale* in the liver, is the only agent with the potential for yielding complete resolution of malaria caused by these organisms.[6,7,20,21] Primaquine combined with clindamycin is also effective in the treatment of *P. jirovecii* pneumonia (see Chapter 91). Recently, primaquine in a dose of 30 mg/day showed efficacy as prophylaxis against *P. falciparum* (88% protection) and *P. vivax* (92% protection) malaria.[70] Primaquine acts by interfering with plasmodial mitochondrial function, possibly through its effects on the electron transport chain and pyrimidine biosynthesis. Primaquine phosphate, which is available only in oral form, is rapidly absorbed (bioavailability 96%), widely distributed and hepatically converted to three metabolites, yielding an elimination half-life of 6–7 hours. It is unclear whether the parent compound or the metabolites possess the antimalarial activity.

Primaquine phosphate is formulated in tablets containing 26.3 mg of the salt, equivalent to 15 mg of the base. Dosages are given in Table 150.1. Relapse of *P. vivax* after conventional primaquine treatment has been described in up to 30% of cases in Papua New Guinea, the Solomon Islands, Thailand and other parts of South East Asia.[71] Therefore, for cases acquired in South East Asia or Oceania, the dose should be increased to 22.5 mg base per day.

The principal toxicity of primaquine is hemolysis in patients who are G6PD-deficient, and thus G6PD levels should be measured before therapy is begun. Headache, nausea, vomiting and abdominal cramps have been reported. At higher doses, mild anemia, cyanosis (due to methemoglobinemia) and leukopenia may occur. Rarely, neurotoxicity, arrhythmias, hypertension and agranulocytosis occur.

QUINACRINE

Quinacrine is an acridine dye derivative that is effective against *G. lamblia*.[6,45] It has recently been used as combination therapy with metronidazole for individuals who failed therapy with metronidazole alone.[72] It also has activity against adult cestodes, but for this indication it has been supplanted by less toxic alternatives. In the Second World War, quinacrine was used for malaria prophylaxis and treatment. The mechanism of antiparasitic action is unclear, but the drug has been shown to intercalate with DNA and inhibit nucleic acid synthesis. Quinacrine is available in an oral formulation and is well absorbed and widely distributed. It has extensive tissue binding and has been detected in the urine 2 months after stopping therapy. Its metabolic fate is poorly understood.

The dosage in giardiasis is 100 mg q8h for 5–7 days. A second treatment course may be given 2 weeks later. The drug has a bitter taste and may induce nausea and vomiting. Dizziness and headache are also common. Reversible yellow skin discoloration (with spared sclerae) is seen in 4–5% of those treated with quinacrine for giardiasis. Under Wood's light, a bright yellow–green fluorescence distinguishes this side-effect from hyperbilirubinemia. Toxic psychosis may occur in 0.1–1.5% of patients. Other rare side-effects include blood dyscrasias, ocular toxicity and urticaria. Patients who have psoriasis may experience exfoliative dermatitis. Quinacrine has a disulfiram-like effect, and thus patients should be advised to avoid alcohol consumption while taking it. It also interferes with the metabolism of primaquine, and toxic levels of primaquine can result from co-administration of quinacrine with primaquine.

QUINIDINE

Quinidine is the dextrostereoisomer of quinine. It is a blood schizonticide and is the parenteral therapy of choice for chloroquine-resistant *P. falciparum* as a result of its wide availability as an antiarrhythmic.[6,7,20,21] It is supplied as the gluconate salt for intravenous use, with a half-life of 6–8 hours. It is 80–90% protein-bound, hepatically metabolized and renally excreted.

During treatment, continuous electrocardiographic and blood-pressure monitoring are recommended. Widening of the QRS complex and prolongation of the QT_c interval may be seen, and hypotension may ensue if the drug is infused rapidly. Other side-effects are similar to those of quinine.

QUININE

Quinine is an alkaloid derived from the bark of the South American cinchona tree. It has been used as an antimalarial for over 350 years.[6,7,20,21] It is effective against the asexual blood stages of all four *Plasmodium* spp. that cause malaria in humans, and is used for chloroquine-resistant *P. falciparum* infections. Quinine is also used with clindamycin in the treatment of *Babesia microti* infection.[36] The basis for the antimalarial activity of quinine is unclear, but three mechanisms have been proposed:

- intercalation with parasite DNA, interrupting replication and transcription;
- interaction with erythrocyte fatty acids, promoting hemolysis and preventing schizont maturation; and
- alkalinization of parasite digestive vacuoles, interfering with hemoglobin degradation.

Pharmacokinetics and distribution

Quinine is available as the sulfate, bisulfate, hydrochloride, dihydrochloride, hydrobromide and ethylcarbonate salts for oral administration and as the dihydrochloride salt for parenteral use. It is rapidly absorbed after oral administration (bioavailability 80%), extensively protein-bound (90%), hepatically metabolized (80%) and renally excreted. The therapeutic range in plasma is 8–15 mg/l, which is achieved within 1–3 hours after a single oral dose. The half-life is approximately 11 hours. In cases of severe illness, the volume of distribution decreases, clearance is reduced and the half-life is prolonged. Thus, on a given dosage schedule, plasma quinine concentrations are elevated with acute illness and decrease as the patient improves. Monitoring blood levels is recommended in those who have renal or hepatic dysfunction; dosage reduction is needed with severe renal failure.

Route of administration and dosage

The oral dose of quinine sulfate (unlike other antimalarial agents, it is dosed by weight of salt) is 650 mg salt q8h for 3–7 days. A longer course is preferred for those in areas where *P. falciparum* is less sensitive to quinine, including South East Asia and western Africa.[73]

Intravenous quinine dihydrochloride may be used for severe infections. A 20 mg salt/kg loading dose in 5% dextrose is given over 4 hours, followed by 10 mg salt/kg over 2–4 hours q8h (maximum 1800 mg salt/day) until oral therapy can be given. The loading dose should be omitted in those who have received oral quinine, quinidine or mefloquine during the previous 24 hours. Intravenous quinidine gluconate has become the parenteral therapy of choice worldwide (see above).

Adverse reactions

The term cinchonism refers to a cluster of dose-related and reversible side-effects of quinine, including tinnitus, decreased hearing, headache, nausea, vomiting, dysphoria and visual disturbances. Hypoglycemia can occur secondary to quinine stimulation of insulin release in conjunction with parasite consumption of glucose. Skin rashes (urticaria, flushing), pruritus, hepatitis, thrombocytopenia, agranulocytosis and massive hemolysis with hemoglobinuria (with resultant bilirubinuria, termed blackwater fever) occur rarely. Quinine can cause respiratory depression in patients who have myasthenia gravis and hemolysis in those who have

G6PD deficiency. Myocardial depression, vasodilatation and shock may result from rapid intravenous infusion. Overdose can result in delirium, seizures, coma, respiratory depression, cortical blindness, shock and death. An oral quinine dose of 2–8 g may be fatal for adults.

SURAMIN

Suramin is a sulfated naphthylamine introduced in 1920 and is used in the treatment of the early or hemolymphatic stage of African trypanosomiasis.[6,7,39] It is more effective against *T. brucei rhodesiense* than against *T. brucei gambiense*, for which pentamidine is often used for early disease. Suramin has also been used for prophylaxis in those who have intense exposure. Additionally, suramin is active against the adult forms of *Onchocerca volvulus*, but is rarely used for this infection because of its toxicity.

The mechanism of action of suramin is unclear; it is a polyanion that inhibits many cellular enzymes. Notably, its antitrypanosomal activity correlates with inhibition of glycerol-3-phosphate oxidase and dehydrogenase, enzymes involved in energy metabolism.

Pharmacokinetics and distribution

Suramin is 99% protein-bound and persists at low levels in plasma for 3 months, which supports its use in prophylaxis. It is not metabolized and is excreted mainly by the kidneys. The large, polar, polyanionic structure affords poor cellular penetration. Very little drug penetrates into the cerebrospinal fluid, accounting for the lack of efficacy of suramin in late-stage disease.

Route of administration and adverse reactions

Suramin is available only for intravenous use. The dosage for trypanosomiasis is given in Table 150.1 Suramin has a variety of side-effects, which are generally more severe in malnourished patients. Immediate reactions include malaise, nausea, vomiting, fatigue, fever, urticaria, shock, loss of consciousness and, rarely, death. Late reactions include fever, rash, stomatitis, exfoliative dermatitis, lacrimation, photophobia, headache and hyperesthesia. Renal dysfunction (hematuria, proteinuria, casts and elevated creatinine), hepatic dysfunction (elevated transaminases and bilirubin), diarrhea, thrombocytopenia and agranulocytosis may occur. Additional side-effects during treatment for onchocerciasis include pruritus, dermal edema, papular eruptions, palmoplantar paresthesias and iridocyclitis.

TETRACYCLINES

Tetracycline (see Chapter 141) is used in combination with quinine in the treatment of drug-resistant *P. falciparum* in South East Asia, where resistance to chloroquine, Fansidar and quinine is common.[6,7,20,21,52] Doxycycline, a longer-acting derivative, is used for malaria prophylaxis in this area, and worldwide in individuals unable to tolerate mefloquine, although atovaquone–proguanil is now preferred for this indication.[20,21,74] Tetracycline is also the drug of choice for infection with the ciliate, *Balantidium coli*.

Tetracyclines are well absorbed after oral administration and are probably active against parasite protein synthesis. Side-effects include gastrointestinal distress, photosensitivity and vaginal candidiasis.

TRYPARSAMIDE

Tryparsamide, a pentavalent arsenical first described in 1919, is used primarily for the treatment of advanced *T. brucei gambiense* infections resistant to other therapy.[6,7] It has poor efficacy against *T. brucei rhodesiense*. Side-effects of tryparsamide include fever, rash, abdominal pain, vomiting, tinnitus, optic atrophy and blindness, and encephalopathy.

Anthelmintic Agents

ALBENDAZOLE

Albendazole is a benzimidazole carbamate that has a broad spectrum of anthelmintic activity, including against *Ascaris lumbricoides, Enterobius vermicularis, Ancylostoma duodenale, Necator americanus, Strongyloides stercoralis, Echinococcus* spp. and *T. solium* cysticerci.[6,7,75–78] The drug has also been used to treat eosinophilic enterocolitis caused by *Ancylostoma caninum, Capillaria philippinensis*, cutaneous and visceral larva migrans, *C. sinensis, Gnathostoma spinigerum, Oesophagostomum bifurcum* and *Trichostrongylus* spp. It is also used in combination with diethylcarbamazine or ivermectin for mass treatment of lymphatic filariasis (*Brugia malayi* and *Wuchereria bancrofti*).[79] Additionally, it has variable efficacy in the treatment of microsporidiosis caused by *Encephalitozoon hellem, E. cuniculi, E. intestinalis, E. bieneusi* and *Vittaforma corneae*.[80] Albendazole has some activity against *G. lamblia*.[45]

The mechanism of action is similar to that of mebendazole with blockade of parasite microtubule assembly. Albendazole is poorly soluble in water and should be taken with a fatty meal to enhance absorption. It undergoes extensive first-pass metabolism in the liver, and albendazole sulfoxide is responsible for most of the systemic anthelmintic effects. This metabolite has a half-life of 9–15 hours and is mostly excreted renally.

Single doses of albendazole are generally well tolerated; abdominal discomfort, diarrhea or migration of *Ascaris* into the mouth and nose occur infrequently. Prolonged, high-dose treatment can be associated with reversible aminotransferase elevations, bone marrow suppression and alopecia.

BITHIONOL

Bithionol is a chlorinated bisphenol that is used for infections with *Fasciola hepatica*, but may have been supplanted by triclabendazole. Bithionol is also an alternative agent against *Paragonimus* spp.[6,7] It has activity against many other flukes, but has been replaced by praziquantel. Its mechanism of action is poorly understood.

Side-effects include anorexia, abdominal pain, nausea, vomiting, headache, dizziness, diarrhea, urticaria and proteinuria, some of which may be allergic responses to liberated fluke antigens.

DIETHYLCARBAMAZINE

Diethylcarbamazine is a piperazine derivative used in the treatment of filariasis. It is microfilaricidal for *W. bancrofti, B. malayi* and *B. timori*.[6,7,75,81,82] It appears to be macrofilaricidal for these species as well (i.e. it kills adult worms) and is considered to be the drug of choice for these three infections. Diethylcarbamazine is a key component of mass chemotherapy approaches for the eradication of lymphatic filariasis.[79] It has been used as a sole agent for single-dose therapy, administered long term as diethylcarbamazine-fortified dietary salt, and as a component of combination single-dose regimens with ivermectin or albendazole. Diethylcarbamazine is also the mainstay of therapy against *Loa loa* and *Mansonella streptocerca*. It has been used to treat tropical pulmonary eosinophilia, supporting the contention that the pulmonary infiltrates in this disorder are due to migrating microfilariae. It has also been used for visceral larva migrans.

Diethylcarbamazine is effective in eliminating microfilariae of *O. volvulus* in the skin and eye, but the resulting inflammation can cause permanent ocular damage, including uveitis, punctate keratitis and retinal pigment epithelium atrophy. Adult *Onchocerca* worms are not killed, however, and the infection may return once treatment has stopped. Ivermectin has largely replaced diethylcarbamazine for ocular onchocerciasis. Diethylcarbamazine also has activity against *A. lumbricoides*.

The mechanism of action of diethylcarbamazine involves two processes:

- first, filarial muscular activity decreases, probably secondary to hyperpolarization of membranes by the piperazine moiety of diethylcarbamazine; and
- second, microfilarial surface membranes are altered by making them more susceptible to host defenses.

Diethylcarbamazine is available as the citrate salt in 50 mg tablets. It is rapidly absorbed, widely distributed in the body, hepatically metabolized and renally eliminated. It has a half-life of approximately 10 hours.

Side-effects, although common, are usually mild and transient; they include headache, malaise, arthralgias, anorexia, nausea and vomiting. Toxicity can result from the destruction of organisms and release of antigens which provoke an inflammatory response. This reaction is most severe in patients who are heavily infected with *O. volvulus*. This is termed the Mazzotti reaction and consists of severe pruritus, edema, rash, arthralgias, lymphadenopathy, fever, hypotension, increased eosinophilia, proteinuria and splenomegaly. These symptoms persist for 3–7 days. Nodular swellings along lymphatics and lymphadenitis may occur with *W. bancrofti* and *B. malayi* infections. Patients heavily infected with *L. loa* may experience encephalopathy and other neurologic complications.[83] Pre-treatment with corticosteroids may lessen the severity of these inflammatory responses.

FLUBENDAZOLE

Flubendazole is a fluorine analogue of mebendazole and the two drugs have similar spectra of activity.[6,7] Flubendazole is poorly absorbed after oral administration. It has been used against many of the common intestinal helminths and, with limited success, in the treatment of neurocysticercosis.

IVERMECTIN

Ivermectin is a derivative of avermectin B1, a type of macrocyclic lactone that was discovered in the 1970s as a product of the actinomycete *Streptomyces avermitilis*.[6,7,76,84] Ivermectin is used as a broad-spectrum veterinary agent for infections with helminths and arthropods. Since the 1980s, it has become the drug of choice for onchocerciasis because it kills microfilariae in the skin and the eye while provoking much less inflammation than diethylcarbamazine. The response to ivermectin is rapid and can last for 6–12 months. Adult worms appear to be unaffected by ivermectin, but the drug seems to prevent developing larvae from leaving the uterus. The drug also has activity against *W. bancrofti, B. malayi, B. timori, L. loa, Mansonella ozzardi* and *Mansonella streptocerca*, and is being used as a component of mass chemotherapy for lymphatic filariasis.[79] Ivermectin is effective against *S. stercoralis* and is active against *E. vermicularis, A. lumbricoides* and *Trichuris trichiura*. Ivermectin causes tonic paralysis of the helminth musculature, but the mechanism of action is poorly understood, although it is known to include γ-aminobutyric acid (GABA) blockade.

Ivermectin is available as 6 mg tablets. It is highly protein-bound, has an elimination half-life of 50–60 hours, is concentrated in liver and adipose tissue, and is almost entirely excreted in the feces (only 1–2% appears in the urine). Side-effects include headache, fever, pruritus, lymphadenopathy, myalgias, arthralgias and, less commonly, orthostatic hypotension.

MEBENDAZOLE

Mebendazole is a benzimidazole carbamate with a broad range of anthelmintic activity.[6,7,75,76] It is active against the larvae and adults of *E. vermicularis, A. lumbricoides, T. trichiura, N. americanus* and *A. duodenale*. It is ovicidal for *Ascaris* and *Trichuris* spp. It is less effective than thiabendazole against *S. stercoralis*. Mebendazole has been used at high doses and for long periods in the treatment of *C. philippinensis*. It can also be used for infections caused by *Angiostrongylus cantonensis, Angiostrongylus costaricensis, Toxocara canis* and *Trichostrongylus* spp. The drug has activity against adult *Trichinella spiralis*, with some activity against larval forms, and it is currently recommended in the treatment of trichinosis.

Mebendazole is also effective in the treatment of certain types of filariasis; it is considered the drug of choice against *Mansonella perstans* (diethylcarbamazine is ineffective) and has been shown to have efficacy against *L. loa, O. volvulus* and *Dracunculus medinensis* infections. The drug has activity against *T. saginata, T. solium* and *Hymenolepis nana*, although praziquantel is more effective. Although mebendazole does not eradicate echinococcal infection, the drug prevents progression of existing cysts and the development of new cysts when administered in high dose for a prolonged period. Mebendazole has largely been replaced by albendazole for echinococcosis.

Mebendazole acts by binding parasite tubulin, thus blocking microtubule assembly and interfering with glucose absorption. Susceptible helminths become paralyzed and depleted of energy stores, but death and clearance of the worms from the gastrointestinal tract can take days. Mebendazole, formulated as 100 mg tablets, has low water solubility and is poorly absorbed. It is 95% protein-bound in plasma and undergoes rapid and extensive first-pass metabolism in the liver. Thus, systemic bioavailability is low, accounting not only for its poor tissue levels and relative lack of usefulness in extraintestinal infections, but also for its low rate of side-effects.

Abdominal pain and diarrhea may occur after mebendazole administration. The drug also has been reported to prompt the migration of adult *Ascaris* spp. into the mouth and nose. At high doses, reversible bone marrow suppression with neutropenia, alopecia, allergic skin reactions, hepatitis, vertigo and oligospermia occur rarely.

METRIFONATE

Metrifonate is an organophosphate inhibitor of acetylcholinesterase that was originally developed as an insecticide. It has activity against *Schistosoma haematobium*[6,7,85] but has largely been replaced by praziquantel as primary therapy.

The dosage is 7.5–10 mg/kg orally once every 2 weeks for three cycles. Side-effects include nausea, vomiting, vertigo and lethargy. Patients receiving metrifonate should neither be exposed to other insecticides nor receive neuromuscular blocking agents in the 2 days before or after taking metrifonate.

NICLOSAMIDE

Niclosamide is a salicylamide derivative that is active against the cestodes *Diphyllobothrium latum, D. caninum, H. nana, T. saginata* and *T. solium*, as well as the trematodes *Echinostoma* spp., *Fasciolopsis buski* and *Heterophyes heterophyes*.[6] It acts by interfering with oxidative phosphorylation and production of adenosine triphosphate. Treatment

failures of *Taenia* spp. with niclosamide have been reported, and praziquantel has been used successfully in these cases.[86]

Niclosamide is supplied as 500 mg tablets that should be chewed thoroughly because of its very poor absorption. The dosage is 2 g as a single dose (or 1 g and 1 g, given 1 hour apart), except for *H. nana* infection, which requires 2 g then 1 g q24h for 6 days. Side-effects include gastrointestinal distress, dizziness and rash.

OXAMNIQUINE

Oxamniquine is a tetrahydroquinoline that is effective in *S. mansoni* infections.[6,7,85] Its mechanism of action is unclear, but it causes adult worms to become paralyzed and dislodged from the veins they inhabit, resulting in subsequent killing by host defenses. The drug is available in 250 mg capsules that are rapidly absorbed and extensively metabolized in the liver. The half-life is approximately 2 hours, and 70% of the drug is excreted by the kidneys. Side-effects include drowsiness, dizziness, orange–red discoloration of the urine and, rarely, seizures. The drug should be given cautiously to patients who have a history of seizures.

PIPERAZINE

Piperazine has activity against *A. lumbricoides* and *E. vermicularis*.[6,7,74] In many parts of the world it has been replaced by less toxic agents such as mebendazole. However, because of its lower cost, piperazine is still frequently used. Piperazine blocks the helminth muscle response to acetylcholine by altering membrane ion permeability and causing hyperpolarization and decreased action potentials. Flaccid paralysis ensues and the worms are eliminated in the stool.

Piperazine is available as the citrate salt in 250 mg tablets. There is good oral absorption and a small amount of hepatic metabolism; 60% of the drug is excreted in the urine unmodified. Different dosage schedules exist, but a single dose of 75 mg/kg (maximum 4 g) per day for 2 days is effective for ascariasis, and a single dose of 65 mg/kg/day for 7 days is used for enterobiasis.

Side-effects include gastrointestinal disturbances, headache, dizziness and urticaria. Seizures occur rarely, and piperazine is contraindicated in patients who have a history of a seizure disorder. Piperazine and pyrantel pamoate are antagonistic and should not be co-administered.

PRAZIQUANTEL

Praziquantel, a pyrazinoisoquinoline derivative developed in the early 1970s, has broad activity against trematodes and cestodes but not nematodes.[6,7,75,85] All *Schistosoma* spp. that infect humans are susceptible. The drug also has activity against the trematodes *C. sinensis, Dicrocoelium dendriticum, Echinostoma* spp., *F. buski, H. heterophyes, Metagonimus yokogawai, Metorchis conjunctus, Nanophyetus salmincola, Opisthorchis viverrini* and *Paragonimus westermani* and other *Paragonimus* spp. *Fasciola hepatica* does not appear to be adequately treated with praziquantel; bithionol is used instead. Praziquantel is effective in treating adult cestodes, including *D. latum* and other *Diphyllobothrium* spp., *D. caninum, H. nana, Hymenolepis diminuta, T. saginata* and *T. solium*. It has been used successfully to treat neurocysticercosis (larval *T. solium*), but it is not useful in echinococcosis. The drug has several actions, including promoting calcium influx and parasite muscle contraction and causing vacuolization and bleb formation in the helminth tegument, thereby activating host defenses.

Praziquantel is available as 600 mg tablets that are nearly insoluble in water. There is good oral absorption, 80% protein binding and rapid first-pass metabolism. The half-life is 1.5 hours. About 80% of the drug is excreted in the urine. Side-effects are common but transient and include headache, dizziness, nausea, vomiting and abdominal pain. Fever and rashes are occasionally seen.

PYRANTEL PAMOATE

Pyrantel pamoate, a tetrahydropyrimidine that was originally developed as a veterinary anthelmintic, has a broad range of activity in humans.[6,7,75,76] It is considered by many to be the treatment of choice for *E. vermicularis*. It is also effective for *A. lumbricoides*, eosinophilic enterocolitis caused by *Ancylostoma caninum*, hookworm, the acanthocephalan *Moniliformis moniliformis* and *Trichostrongylus* spp. It does not have activity against *T. trichiura*. Oxantel pamoate, an *m*-oxyphenol derivative, can be given in a single dose for *Trichuris* spp. Pyrantel pamoate and its analogues act by causing depolarizing neuromuscular blockade and by blocking acetylcholinesterase, which result in spastic paralysis and muscle contracture, respectively, and allow expulsion of the worms.

Pyrantel pamoate is available as an oral suspension of 250 mg of pyrantel base per 5 ml. It is poorly absorbed. Less than 15% is excreted in the urine and most remains in the feces unmodified. Side-effects include headache, dizziness, insomnia, nausea, vomiting, anorexia and abdominal pain. Pyrantel pamoate, which causes depolarization and increased spike frequency in worm muscle cells, should not be given with piperazine, which causes hyperpolarization and a reduction in spike frequency.

THIABENDAZOLE

Thiabendazole is a substituted benzimidazole compound that has better activity against *S. stercoralis* and *Strongyloides fuelleborni* than mebendazole.[6,7,75,76,85] It is active against *A. costaricensis, C. philippinensis, D. medinensis, Trichostrongylus* spp. and *T. spiralis*. In trichinosis, however, larval stages are often resistant to thiabendazole. The drug is also used in the treatment of both cutaneous and visceral larva migrans. Thiabendazole has some activity against *A. lumbricoides*, hookworm, *E. vermicularis* and *T. trichiura*, but mebendazole is less toxic and is thus preferred. The drug acts by inhibiting parasite fumarate reductase, and it may bind tubulin as well.

Thiabendazole is available in tablet and liquid form and, in contrast to other benzimidazole derivatives, is rapidly absorbed. It is extensively metabolized by the liver, has a half-life of 1 hour and is mainly excreted by the kidneys.

Side-effects are frequent; they include nausea, vomiting, anorexia and dizziness. Pruritus, epigastric pain, headache, drowsiness, giddiness and diarrhea are less common. Rarer still are tinnitus, hallucinations, numbness, seizures, altered olfaction, altered color perception, hypotension, bradycardia, crystalluria, leukopenia, elevated liver enzymes and intrahepatic cholestasis. Allergic manifestations, including fever, angioneurotic edema, erythema multiforme and Stevens–Johnson syndrome, have been described and may result from the release of parasite antigens during treatment. Increased theophylline levels and consequent nausea and vomiting may result from the coadministration of theophylline and thiabendazole.

TRICLABENDAZOLE

Triclabendazole, a benzimidazole derivative used as a veterinary fasciolicide, has been used safely and successfully in cases of human chronic hepatic fascioliasis.[87,88] It is now considered the drug of choice for human hepatic fascioliasis, but in the USA it is available only through compounding pharmacies.

REFERENCES

References for this chapter can be found online at http://www.expertconsult.com

Chapter 151

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Acute gastroenteritis viruses

Acute gastroenteritis is a major cause of morbidity and mortality worldwide, most commonly affecting children both in developing and developed countries. Its importance was clearly defined in the 1980s and 1990s (Table 151.1).[1–4] Recent studies indicate that it continues to be a significant burden for human health.[5,6] While fewer than 5% of stool samples in patients with diarrhea show a bacterial or parasite pathogen,[7] recent technology allows for identifying an increasing number of viral etiologies.

APPROACH TO MANAGEMENT

No specific antiviral exists for any of the gastroenteritis viruses. Primary objectives of management are prevention and treatment of dehydration and malnutrition.[8] Most patients will improve with the administration of oral rehydration solutions (ORS) and continuous feeding with their regular diet. Rarely, intravenous fluids may be needed for severe dehydration, intractable vomiting or very high stool output. Although lactose malabsorption develops frequently during acute gastroenteritis, most children can be fed their usual lactose-containing formulas in small frequent feeds. Human milk has high lactose content, yet it reduces stool output and provides excellent nutrients and anti-infectious factors; therefore, breast-feeding should be continued. Moderate to severe malnourished children with diarrhea present a special problem. Malnutrition causes total body sodium excess, potassium and micronutrient deficiencies and increased energy requirements. ReSoMal is recommended for these patients. It is a specially formulated ORS that provides less sodium and more potassium and glucose. Magnesium, zinc and copper are added to ReSoMal.[9]

APPROACH TO PREVENTION

Viruses that cause gastroenteritis are transmitted mainly by the fecal–oral route, through direct person-to-person contact or contaminated food, water and fomites. Aerosol transmission from respiratory secretions or vomit occurs rarely. Hygienic measures decrease person-to-person spread. However, because these measures are difficult to enforce, particularly in young children, and because some viruses are resistant to commonly used disinfectants, transmission of the disease continues even in developed countries. Outbreaks require isolating ill personnel, thoroughly cooking food, disinfecting the environment and proper handling of food, water and sanitation. Careful hand washing cannot be overemphasized, especially among food handlers and personnel from hospitals, schools and day-care centers. Hospitalized or other institutionalized patients should have universal precautions and contact isolation until 48–72 hours after symptom resolution. The best preventive method will be effective, safe and affordable vaccines. Currently, such a vaccine is commercially available only for rotavirus.[10]

APPROACH TO DIAGNOSIS

An etiologic diagnosis is not necessary for the management of most cases of acute gastroenteritis. For viral gastroenteritis, an etiologic diagnosis may be desired in specific clinical circumstances or for epidemiologic and research purposes. Detection of viral antigens by immunoassays (IAs) is the method of choice. IAs are simple and rapid, have good sensitivity and specificity, and are practical

Table 151.1 Morbidity and mortality rates of diarrheal diseases in children*

	DEVELOPING COUNTRIES			USA–CANADA
	Snyder & Merson (1982)[1]	**Claeson & Merson (1990)[2]**	**Bern *et al.* (1992)[3]**	**Glass *et al.* (1991)[4]**
No. of studies evaluated	24	276	22	4
Diarrheal episodes/year (millions)	1000	1500	1000	21–37
Episodes/child/year (median)	2.2–3.0	3.3	2.6	1.3–2.5
No. of diarrheal deaths/year	4.6 million	4.0 million	3.3 million	325–425

*Estimates from longitudinal, prospective, community-based studies in developing and developed countries.

for testing large numbers of samples. IAs are commercially available for rotaviruses, adenoviruses and astroviruses. Reverse transcriptase polymerase chain reaction (RT-PCR) techniques have been developed for many gastroenteritis viruses. Because they are more sensitive than IAs, they allow viral detection not only in clinical specimens (stools, vomitus) but also in contaminated food, water and fomites. These techniques are most useful for caliciviruses. Electron microscopy (EM) and immune electron microscopy (IEM) identify viruses of distinct morphology and excreted in large numbers (>10^6 virions/ml of stools), such as rotavirus or astrovirus. However, these techniques are not suitable for a large number of samples or routine laboratory diagnosis. Viral culture is essential for virus characterization, the study of their pathogenesis and the production of diagnostic reagents but is slow and cumbersome. Not all gastroenteritis viruses can be cultured.

VIRAL AGENTS OF GASTROENTERITIS

The morphologic, epidemiologic and clinical characteristics of the main gastroenteritis viruses are presented in Tables 151.2–151.4. Most cases of acute gastroenteritis worldwide are caused by caliciviruses in all age groups and rotaviruses in children; all the other viruses are responsible for approximately 10% of cases.

Caliciviruses (Norovirus, Sapovirus)

In 1972, Kapikian *et al.*, investigating a gastroenteritis outbreak in Norwalk, Ohio, visualized 27 nm particles in a stool filtrate and noted that infected individuals developed a specific antibody response against the particles. This was the first confirmation of a virus as an etiologic agent of gastroenteritis.[11] The virus was named Norwalk virus (NV) and was believed to belong to the Picornaviridae family. Similar viruses were named by the location where they were first identified (Hawaii, Sapporo, etc.) and, as a group, were known as Norwalk-like viruses or small round structured viruses (SRSVs). Soon, it became evident that the SRSVs were different from the picornaviruses in structure, mode of replication and other characteristics. Cloning of the NV genome[12] led to the classification of these viruses within the Caliciviridae family.

NATURE

Caliciviruses are small (27–40 nm), nonenveloped viruses with a single-stranded, positive-sense RNA genome.[13] They have an icosahedral capsid with cup-like depressions on the viral surface (*calici* is derived from the Latin word *calyx*, which means cup) (Fig. 151.1).

Four genera have been identified in the Caliciviridae family: Lagovirus, Vesivirus, Norovirus (NoV) and Sapovirus (SaV). Lagovirus and Vesivirus only infect animals. Norovirus and Sapovirus have human and animal strains and are further classified into genogroups and genetic clusters (Table 151.5).

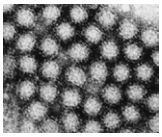
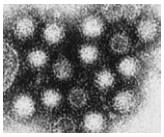
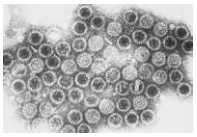
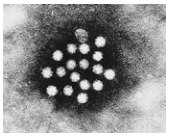
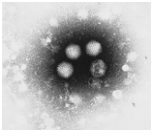
The calicivirus genomes are about 7.3–8.5 kb in length and organized in two or three open reading frames (ORFs). In the genera with three ORFs, Norovirus and Vesivirus, the first ORF (ORF1) encodes for a large protein that by proteolytic cleavage produces the nonstructural proteins. ORF2 encodes for the single major structural protein (viral protein 1, VP1) and ORF3 encodes for a minor structural protein (viral protein 2, VP2). Sapovirus and Lagovirus have only two ORFs. In these viruses, VP1 and VP2 are encoded in ORF1 and ORF2, respectively.[14]

The molecular weight of VP1 is approximately 60 kDa. Ninety VP1 dimers form the capsid with 90 arch-like protruding capsomers arranged in such a way as to leave 32 calices on the viral surface. VP1 has two major domains: the shell (S) domain and the protruding (P) domain. The S domain forms the inner part of the capsid. It has 225 amino acids (aa), including aa 10–49 corresponding to the *N*-terminal region of the protein (N subdomain), which faces the interior of the capsid. The P domain includes amino acids 226–520 and is subdivided into subdomains P1 and P2. The P1 subdomain (aa 226–278 and 406–520) forms the sides of the capsomers, whereas the P2 subdomain (aa 279–405) forms the most protruding part of the capsomer arches (see Fig. 151.1). VP1 self-assembles into virus-like particles (VLPs) without RNA or VP2 participation. The VLPs are morphologically and antigenically similar to natural virions.[15]

Each virion has only one or two copies of VP2 (12–29 kDa). The function of VP2 is unknown; however, in feline caliciviruses, intact VP2 is needed to produce infection. A third structural protein, VPg, is also present as one or two copies per virion. It is linked to the genomic or subgenomic RNA and may function as a nonstructural protein during replication.[14]

The caliciviruses have seven nonstructural proteins (NSP1–NSP7). The function of some of them has been inferred based on similar sequences in picornavirus proteins. NSP3 is a nucleoside triphosphatase,

Table 151.2 Structure and morphological characteristics of gastroenteritis viruses

Characteristics	Norovirus	Sapovirus	Rotavirus	Astrovirus	Enteric adenovirus
Family	*Caliciviridae*	*Caliciviridae*	*Reoviridae*	*Astroviridae*	*Adenoviridae*
Virion size (nm)	27–35	27–40	70–75	41	70–80
Envelop	Non-enveloped	Non-enveloped	Non-enveloped	Non-enveloped	Non-enveloped
Capsid	Icosahedral	Icosahedral	Triple shelled	Icosahedral	Icosahedral
Genome type	Positive sense ssRNA	Positive sense ssRNA	Segmented dsRNA	Positive sense ssRNA	dsDNA
Morphology on electron microscopy	Round surface, cup-shaped indentations	Round surface, cup-shaped indentations	Wheel-like capsid with radiating spokes	Round, 28–30 nm, 5–6-pointed star shape	Fiber-like projections from vertices
Electron micrograph					

ds, double-stranded; ss, single-stranded.
Norovirus and sapovirus electron micrographs courtesy of C Humphrey (CDC); rotavirus, astrovirus and enteric adenovirus electron micrographs courtesy of S Spangenberger.

Table 151.3 Epidemiological characteristics of gastroenteritis viruses

Characteristic	Norovirus	Sapovirus	Rotavirus	Astrovirus	Enteric adenovirus
Age group	All ages	Children	6–24 months	<7 years, elderly	<4 years
Seasonality	No	No	Winter	Winter	Summer
Disease pattern	Outbreaks, endemic	Endemic, outbreaks	Endemic, annual epidemics	Endemic, nosocomial outbreaks	Endemic
Transmission	Person-to-person, water, food, shellfish	Person-to-person, water, cold foods, shellfish	Person-to-person, food, water	Person-to- person, food, water	Person-to-person
Fecal excretion (days)	7–13	–	10	–	Persistent, months
Outpatient prevalence (%)	Endemic: 10–25 Outbreaks: 90	1–10	5–10	7–8	4–8
Inpatient prevalence (%)	Rare	3–5	35–40	3–5	5–20

Table 151.4 Usual clinical characteristics of gastroenteritis virus infections

Signs and symptoms	Norovirus	Sapovirus	Rotavirus	Astrovirus	Enteric adenovirus
Prodrome (days)	1–2	1–3	1–3	3–4	8–10
Diarrhea: watery	66–95% 4–8/day Adults >children	88–95% Mild	96–100% 10–20/day	72–100% 2–4/day	97% 1/3 >14 days
Vomitus	57–95% Children >adults	44–65%	80–90% Early	20–50% 1/day	79% Early
Fever	24–48% Low grade	18–34%	60–65% Moderate	20% Low grade	Occasionally Low grade
Abdominal pain	11–91% Cramps	–	Colicky	50%	–
Dehydration	~1%	Infrequent	Frequent in young children	Infrequent	Infrequent
Other symptoms	Myalgia 26%, headache 22%	Respiratory 22%, myalgia, headache	Respiratory 22–52%	Malaise, respiratory	Respiratory occasionally
Duration of illness (days)	0.5–2.5	4	3–8	2–3	5–12

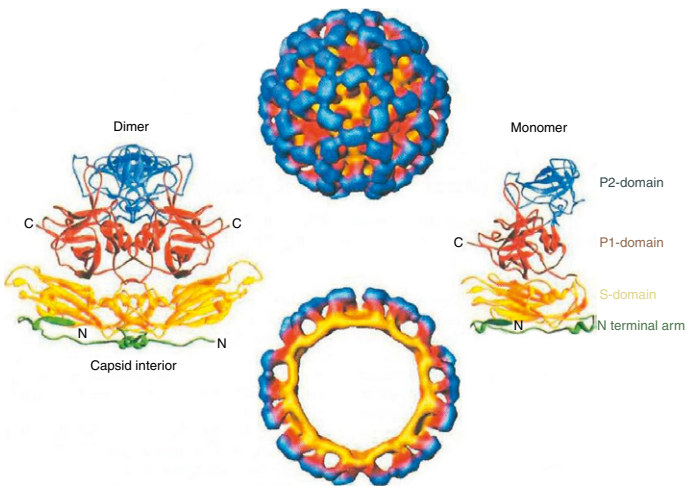


Fig. 151.1 Norwalk virus-like particle. Adapted from Hutson *et al*. Trends Microbiol 2004;12:279–87, with permission.

NSP6 is a proteinase and NSP7 is an RNA-dependent RNA polymerase. While the function of the other NSPs is unknown, they may play a role in the replication process.[14]

EPIDEMIOLOGY

Human caliciviruses (HuCV) have worldwide distribution. Noroviruses infect persons of all ages, both in developed and developing countries. They are the most common cause of gastroenteritis outbreaks and are also recognized as the most common cause of viral gastroenteritis in community-based studies.[16] Studies show an increasing prevalence of specific antibodies since infancy, reaching 80–90% in young adults.[17] The prevalence of specific antibodies rises faster in developing countries, indicating early infections.[18] Genogroups I and II produce the majority of community infections. Most infections are caused by a single circulating strain. For example, Genogroup II.4 became the most common cause of outbreaks around the world in the 1990s. Co-infections occur, giving the opportunity for the exchange of genetic material between strains and the generation of new virus strains.[19] Outbreaks occur mainly in

Table 151.5 Taxonomy of the Caliciviridae family

Genus	Genogroups	Genetic clusters	Representative species	Representative strain*
Norovirus (Nov)	I II III IV V	1–8 1–19 1–2 1 1	Norwalk virus Hawaii norovirus Bovine norovirus Alphatron virus Murine norovirus	Hu/NoV/GI.1/Norwalk/1968/US Hu/Nov/GII.1/Hawaii/1971/US Bo/NoV/GIII.1/Jena/1980/DE Hu/Nov/GIV.1/Alphatron98-2/1998/NL M/NoV/GV.1/MNV-1/2003/US
Sapovirus (SaV)	I II III IV V	1–3 1–3 1 1 1	Sapporo virus London sapovirus Swine sapovirus Houston sapovirus Argentina sapovirus	Hu/SaV/G1.1/Sapporo/1982/JP Hu/SaV/GII.1/London/1992/UK Sw/SaV/GIII.1/PEC-Cowden/1980/US Hu/SaV/GIV.1/Hou7-1181/1990/US Hu/Sav/GV.1/Argentina 39/AR
Lagovirus (LaV)			Rabbit hemorrhagic disease virus European brown hare syndrome virus	Ra/LaV/RHDV/GH/1988/DE Ha/LaV/EBHSV/GD/1989/FR
Vesivirus (VeV)			Vesicular exanthema of swine virus Feline calicivirus	SW/VeV/VESV/A48/1948/US Fe/VeV/FCV/F9/1958/US

Host species abbreviations: Hu, human; Bo, bovine; M, murine; Sw, swine; Ra, rabbit; Ha, hare; Fe, feline. **Country abbreviations**: US, United States; DE, Germany; NL, Netherlands; JP, Japan; UK, United Kingdom; AR, Argentina; FR, France.
*Host species/genus/species or genogroup/strain name/year of occurrence/country of origin.
Adapted with permission from Green *et al.*[13]

day-care centers, schools, colleges, hospitals, nursing homes, military personnel, restaurants, vacation facilities and cruise ships. Noroviruses also cause travelers' diarrhea. They were the only pathogens isolated from Hurricane Katrina evacuees with gastroenteritis in a large shelter in Houston, Texas.[20] Noroviruses can spread internationally through contaminated food or beverages.[21] A study of 8271 food-borne outbreaks of gastroenteritis reported to the US Centers for Disease Control and Prevention (CDC) shows a median of affected persons of 25 vs 10 for the bacterial outbreaks. Ten percent of the individuals required medical care and 1% were hospitalized.[22]

Caliciviruses are ubiquitous and stable in the environment, providing a persistent source of infection. Noroviruses survive freezing, heating to 140°F (60°C) for 30 minutes and are stable in water chlorinated to 6.25 mg/l; most municipal water systems contain <5 mg/l of chlorine. The virus is also acid-resistant and ether-stable.[23,24]

PATHOGENICITY

Caliciviruses are transmitted mainly by the fecal–oral route, but airborne transmission has also been reported.[25,26] The infectious dose has been estimated at less than 10 virions.[16] The incubation period ranges from 10 to 51 hours with a mean of 24 hours.[23,27] Norovirus infection mainly affects the proximal portions of the small intestine with broadening and blunting of the villi, crypt cell hyperplasia, cytoplasm vacuolization and mononuclear cell infiltration in the lamina propria. There is malabsorption of D-xylose, lactose and fat, and a decrease in brush border enzymes.[28,29] Pathologic changes are transient and resolve within 2 weeks. No histologic lesions are seen in the gastric or rectal mucosa, and the secretion of hydrochloric acid, pepsin and intrinsic factor remain normal.[30] Gastric emptying is markedly delayed, which could explain the frequent occurrence of nausea and vomiting; however, the degree of delay does not correlate with the severity of vomiting.[31] Virus excretion in the stool may begin during the prodrome stage,[32] last 7–13 days,[33] and persist for several weeks after resolution of symptoms, especially in infants,[34] and for months to years in immunocompromised patients.[35]

The cell and tissue tropism of HuCV are largely unknown. Calicivirus replication may be similar to that of other positive-strand RNA viruses. Following attachment to specific receptors, the virus enters the cell and is uncoated, and ORF1 produces the NSPs. Synthesis of negative-strand RNA begins. This serves as a template for positive-strand genomic and subgenomic RNA, which will serve as a template for the synthesis of VP1 and VP2. It is known that VP1 can self-assemble to form the capsid, but the process of packing of the viral RNA within the capsid, maturation and release of the virions is poorly understood.[14] It is assumed that replication occurs in the epithelial cells of the upper gastrointestinal tract; however, no viral particles have been observed by EM of the jejunal mucosa. While there is no animal model for the human caliciviruses, it has been shown that radiolabeled Norwalk VLPs bind and, in some cases, enter various cell lines.[36] The development of a human cell line for norovirus and a murine norovirus model will advance the study of norovirus pathogenesis.[37,38]

Susceptibility to NV infection is peculiar. Some individuals have natural resistance to the infection. They lack virus receptors; repeated challenges with the virus fail to produce illness or antibody response. The virus receptors are histo-blood group antigens (HBGAs). These are membrane carbohydrates present on the surface of red blood cells, enterocytes and other mucosal cells. The carbohydrates have an α1,2-linked fucose in common, e.g. Fucoα2Galβ3GlcNac. The protruding domain of VP1 of NV VLPs attaches to this trisaccharide or similar molecules, allowing binding and subsequent internalization of the VLPs. The receptors are host and viral-strain specific. Receptor production is genetically controlled by alleles at the ABO, FUT2 and FUT3 loci. Individuals with O blood group are more easily infected, while B blood group individuals have decreased risk. Individuals with the FUT2 –/– genotype are highly resistant to NV infections and represent about 20% of the European population.[39,40]

Acquired immunity is mostly short term and type specific. Challenges with the virus produce illness and specific antibody response. Although re-exposure to the same virus strain within 6–14 weeks does not result in illness, a re-challenge 27–42 weeks later results in disease and serum antibody response.[23,41,42] Serum or local jejunal antibodies do not correlate with resistance in volunteer studies.[33,43,44]

On the other hand, a rapid mucosal IgA response, associated with resistance to illness, has been shown in volunteers.[40] Cell-mediated immune responses have been observed in volunteers immunized with Norwalk VLPs.

PREVENTION

To date, no commercially available vaccine exists against HuCV. Vaccine development has been hampered by the following factors:

- partial understanding of HuCV immunogenicity;
- multiple virus types and infrequent cross immunity;
- inability to culture the virus; and
- lack of a small animal model for human caliciviruses.[45]

Nevertheless, rNV VLPs given orally to mice and human volunteers are safe and induce IgG_1 and IgA antibody responses.[46] While waiting for the vaccine, preventive measures should follow the procedures mentioned above. Contaminated water supplies can be treated with chlorine concentrations >10 mg/l.

DIAGNOSTIC MICROBIOLOGY

RT-PCR is the most widely used technique. It is very sensitive and allows virus detection in clinical specimens and the environment. Primer selection is important as genetically diverse strains may not be detected; RT-PCR with multiple primers identified Norwalk-like viruses as the etiologic agent of 96% of gastroenteritis outbreaks.[14,16,47] Immunoassays to detect viral antigens in stools are less sensitive; however, IAs to detect antibodies to the viruses have been very useful in sero-epidemiologic and vaccine development studies.

CLINICAL MANIFESTATIONS

The usual clinical characteristics of norovirus and sapovirus infections are presented in Table 151.4. Most of the time, infections are acute, short lived or asymptomatic; however, severe illness, including disseminated intravascular coagulation, has been described in healthy soldiers under severe environmental conditions.[48]

MANAGEMENT

No specific therapy exists for HuCV infections. Treatment is supportive with oral or intravenous rehydration. Bismuth subsalicylate reduces severity and duration of illness in experimental infections in adults,[49] but the use of symptomatic medications for the management of acute gastroenteritis in children is not recommended by the American Academy of Pediatrics.[50]

Rotaviruses

First described in 1973 by Bishop *et al.*,[51] human rotaviruses (RVs) represent the main agent of acute gastroenteritis in infants and young children.

NATURE

Rotaviruses belong to the family Reoviridae.[52] Intact 75 nm particles have a triple-layered capsid with core, inner and outer layers. Sixty spike-like structures (capsomers) radiating from the inner to the outer layer give the virus its characteristic wheel-like appearance. The core capsid encloses the viral genome, consisting of 11 segments of double-stranded RNA. Each segment encodes for one protein, except segment 11 which encodes for two. Six proteins (VP1–VP4, VP6–VP7) form the virion structure, and six nonstructural proteins (NSP1–NSP6) are expressed only in the infected cell (Fig. 151.2, Table 151.6).[53,54] The core is made of VP1, VP2 (its major contituent) and VP3. VP6 is the sole component of the inner capsid. The outer capsid is composed of VP7 (90%) and VP4, which forms the capsomers.

VP6 defines seven antigenic groups (A–G), with group A rotaviruses causing most human infections. The outer capsid proteins VP4 and VP7 determine the serotypes. Those defined by VP7 are called G (for glycoprotein) serotypes, and those defined by VP4 are called P (for protease-sensitive protein) serotypes. There is full concordance between VP7 serotypes and genotypes; there are at least 15 G serotypes designated G1–G15. The concordance between VP4 serotypes and genotypes is poor. There are 14 VP4 serotypes (P1–P14) and 26 genotypes (1–26). P1 and P5 include subtypes A and B; P2 includes subtypes A, B and C; Genotype 5 has a 5A subtype. Rotaviruses are formally designated using the G and P letters followed by the serotype number; a second number in parentheses after the P indicates the genotype. For example, the human RV strain Wa is designated G1P1A[8].

EPIDEMIOLOGY

Transmission of RVs is mainly person to person by the fecal–oral route. Fecal excretion starts immediately before the onset of symptoms and lasts for 5–7 days. Spread is favored by the large number of virions excreted in feces (1 trillion per ml) and the low infective dose (10 RV particles). Contamination of food and water has been implicated in some outbreaks. Since RVs can survive for 60 days on environmental surfaces at different temperatures (39.2–68°F/4–20°C) and humidities (50–90%), fomites may play a role in such settings as day-care centers and nurseries.[55] Respiratory transmission has been suspected in a few outbreaks and is supported by the way the annual RV epidemic spreads in North America. However, attempts to isolate RV from respiratory secretions have been mostly unsuccessful.

Rotaviruses are the most commonly identified viral enteropathogens of infants and young children. The proportion of diarrhea cases caused by RVs increases from community to clinic to hospital populations, reflecting the tendency of the virus to produce dehydration. In the USA, RVs cause 5–10% of all diarrheal episodes and 30–50% of severe diarrhea in children under 5 years of age, resulting in 3.5 million cases, 55 000 hospitalizations, 20–40 deaths and costs in excess of one billion dollars.[56] Worldwide, the estimates are 111 million episodes, 2 million hospitalizations and 600 000 deaths annually. RVs account for a larger proportion of cases in developed countries than in developing ones (38–89% vs 20–46%, respectively) and in high-income groups than in low-income ones (60% vs 4–30%, respectively).[57]

Incidence rates peak at age 6–24 months. Neonates are affected infrequently. Adults exposed to infected children become infected frequently (11–70%) but rarely develop clinical disease.[58,59] Outbreaks have been described in nursing homes for the elderly, hospital wards and military bases. RVs have been detected in as many as 20% of cases of travelers' diarrhea. Asymptomatic shedding of RVs occurs in 10–15% of individuals.

In temperate climates, RVs appear in characteristic and predictable winter epidemics. In North America, the epidemic starts in late fall in Mexico and the southwestern USA, spreads in a northeast direction and ends in the spring in the northeastern USA and the Maritime provinces of Canada.[60] The reasons for this spread pattern are not clear; climate, virus characteristics or other factors may play a role. In tropical climates, RVs are endemic throughout the year, with some clustering in the cooler, drier months. Globally, group A serotypes G1–G4, in conjunction with P[8] or P[4], constitute most human infections. However, there is much geographic and temporal variability, with G9, G8, G5 and others emerging as prevalent in some parts of the world.[53,61] Multiple serotypes can co-circulate during a specific year.

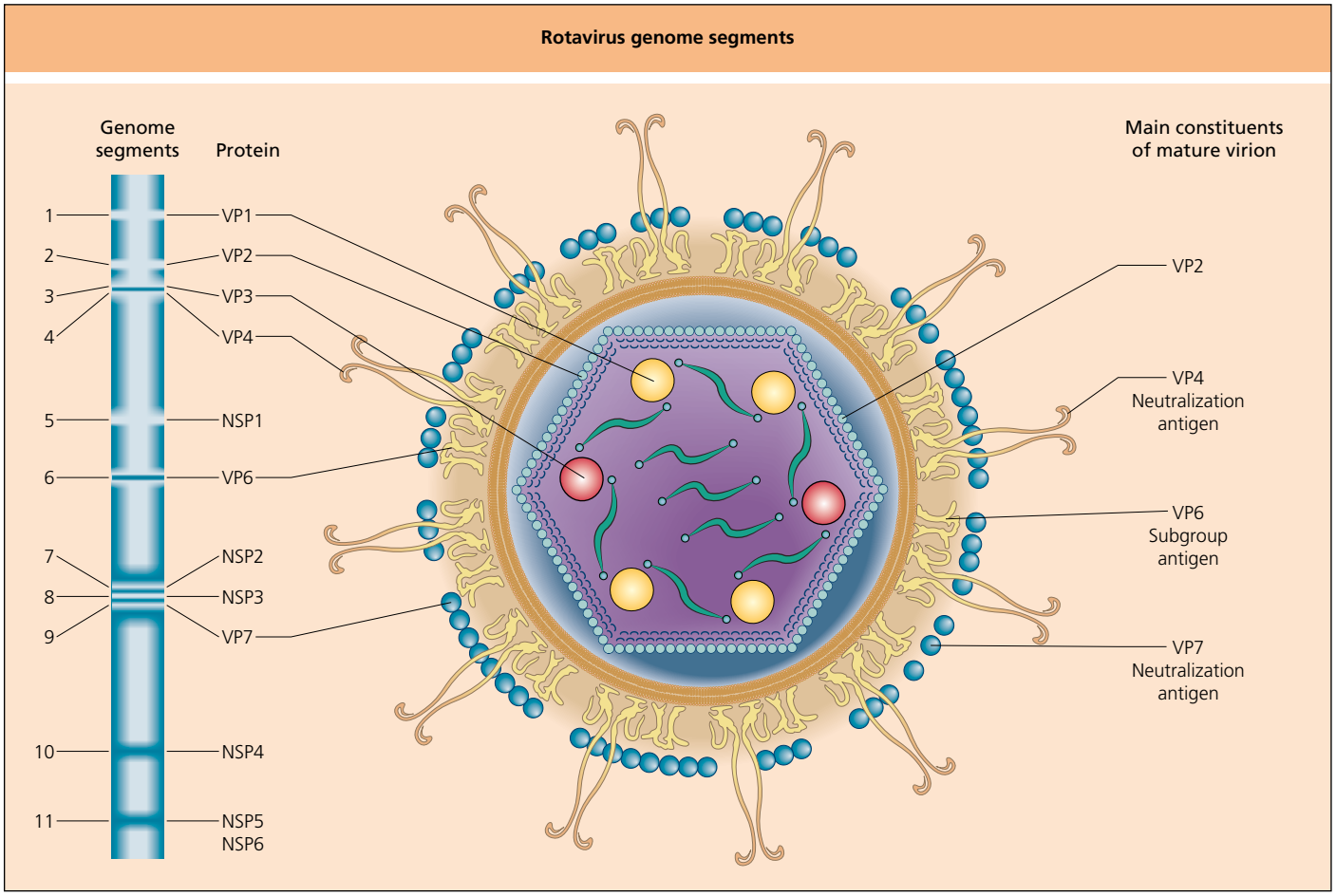


Fig. 151.2 Diagram showing rotavirus genome segments, protein products and their location in the viral particle. Adapted with permission from Gentsch *et al.*[53]

Group B RVs have been associated with epidemics of diarrhea among adults in China, and group C RVs have been identified in Central and South America, Europe, Australia and Asia.

PATHOGENICITY

Rotaviruses preferentially infect the mature enterocytes of the small intestine. Infected cells change from columnar to cuboidal, with enlarged cisternae of the endoplasmic reticulum and fewer and shorter microvilli. The cells are eventually killed and sloughed off and, with denudation of the tip cells, the villi become shortened. Mononuclear leukocyte infiltration is minimal. These changes occur within 24 hours of infection, start proximally and progress caudally.[51,62] Recently, RV RNA has been detected in blood and cerebrospinal fluid, suggesting that RV may represent a systemic infection.[63] Diarrhea results from decreased absorption of salt and water secondary to enterocyte damage and replacement of absorptive intestinal cells by secreting cells from the crypts. Loss of disaccharidases at the damaged brush border results in carbohydrate malabsorption and osmotic diarrhea. NSP4, the first described viral enterotoxin, increases plasma membrane chloride permeability, leading to chloride secretion and secretory diarrhea.[64]

The replication cycle of RV has been reviewed elsewhere.[54] Some key points are noted here. The nature of the receptors on the host cell remains elusive. On the virus side, VP4 mediates attachment, and its cleavage into VP5* and VP8* by proteases-like trypsin is essential for cell penetration. Cell penetration also requires VP7. The virus enters the cell either by direct membrane penetration or by receptor-mediated endocytosis; intracellular levels of Ca^{2+} trigger virus uncoating. RNA synthesis occurs in the core of the virion, mediated by an endogenous RNA-dependent RNA polymerase. The RNA transcripts encode the RV proteins, which are translated by the cellular translational machinery. The initial steps of virus replication occur in cytoplasmic inclusions called viroplasms. The viral subparticles bud through the membrane of the endoplasmic reticulum and become mature particles. NSP4 plays a key role in the assembly process, which is Ca^{2+} dependent. Lastly, mature virus particles are released by cell lysis in nonpolarized cells or by nonclassic vesicular transport in polarized epithelial cells.

Immunity against RV infection and illness are not completely understood.[65] Clinical protection may involve local (mucosal) and systemic (serum) antibodies as well as cellular immunity. VP7- and VP4-induced serum-neutralizing antibodies against the infecting serotype (homotypic) appear within 2 weeks of infection. Heterotypic antibodies against different serotypes also occur, but mostly among adults, and vary with the infecting strain. There is a correlation between the presence of homotypic neutralizing antibodies and protection.[58] The duration of homotypic protection is probably longer than that of heterotypic but is both incomplete and short lived, as shown by the occurrence of re-infections with the same serotype. Antibody levels are high at birth, decline by 3–6 months, rise to a peak at 2–3 years and remain elevated throughout life (this is probably because of repeated, mostly asymptomatic infections).[66] Serum antibodies do not always prevent the infection. Mucosal immunity may be more important than systemic immunity. It develops 4 weeks after the illness and persists for several months, eventually decreasing with advancing age.[67]

Passively acquired mucosal immunity by breast-feeding or orally administered immune globulins has conferred protection to high-risk individuals. Cell-mediated immunity also seems to be important.

Table 151.6 Rotavirus genome segments and their corresponding viral proteins

Genome segment (size, bp)	Protein product (MW, kDa)	Location in virus particles	Function
1 (3302)	VP1 (125)	Core capsid	RNA-dependent RNA polymerase, complex with VP3
2 (2729)	VP2 (94)	Core capsid	Main constituent of core, RNA binding
3 (2591)	VP3 (88)	Core capsid	Complex with VP1, guanylyl and methyl transferase, synthesis of capped mRNA transcripts
4 (2359)	VP4 (86.7)	Outer capsid	Hemagglutinin, cell attachment, neutralization antigen, determines P serotypes, cleaved by trypsin into VP5* (52.9) and VP8* (24.7), virulence
5 (1566)	NSP1 (58.6)	Nonstructural	Basic protein, RNA binding
6 (1356)	VP6 (44.8)	Inner capsid	Main constituent of inner capsid, determines group specificity, protection, required for transcription
7 (1074)	NSP3 (34.6)	Nonstructural	Acidic protein, RNA binding, inhibits host cell translation
8 (1059)	NSP2 (36.7)	Nonstructural	Basic protein, RNA binding, forms viroplasms with NSP5
9 (1062)	VP7 (37.4)	Outer capsid	Glycoprotein, major constituent of outer capsid, determines G serotypes
10 (750)	NSP4 (20.2)	Nonstructural	Role in morphogenesis, interacts with viroplasms, modulates intracellular Ca^{2+} and RNA replication, enterotoxin, virulence
11 (664)	NSP5 (21.7)	Nonstructural	Role in morphogenesis, forms viroplasms with NSP2, interacts with VP2 and NSP6
11 (664)	NSP6 (12)	Nonstructural	Interacts with NSP5, present in viroplasms

bp, base pairs; MW, molecular weight.
Adapted with permission from Estes & Kapikian.[54]

In mice, RV-specific cytotoxic T cells appear in the intestinal mucosa soon after infection. Mice with severe combined immmunodeficiency are able to clear RV infection when reconstituted with CD8 T cells, despite their lack of antibodies against the virus.[68] Recent experimental data suggest a role for non-neutralizing anti-VP6 antibodies in protection, though its significance in human infection is still unclear.[69]

PREVENTION

Breast-feeding reduces the overall incidence of diarrhea. For RVs, however, the effect of breast-feeding may be not so much in reducing incidence as in reducing the severity and duration of illness.[70]

Careful hand washing is important to reduce virus transmission. Fecal contamination of surfaces and objects occurs frequently, and RVs can survive in the environment for weeks.[71] Effective disinfectants are 6% hydrogen peroxide, 2500 ppm chlorine, 80% ethanol, ethanophenolic disinfectants, ultraviolet radiation and heat; drying and phenolic disinfectants are not effective;[72] hypochlorites are inactivated by fecal organic matter. Nondisposable objects are better cleaned by washing at 176°F (80°C) for at least 1 minute. Household laundry should be washed with detergent and bleach, followed by a drying cycle.

The first commercial RV vaccine, RotaShield by Wyeth-Ayerst Laboratories, was licensed in the USA in 1998. It was a rhesus–human reassortant live vaccine containing strains with specificities for G1–G4 serotypes. Three doses given orally at 2, 4 and 6 months of age were 49% effective for all RV diarrhea and 80% effective for severe RV diarrhea, thereby decreasing physician intervention by 73% and basically eliminating all cases of RV dehydration.[73] The vaccine was suspended less than 1 year later due to an association with intussusception, estimated as one additional case/10 000 infants vaccinated.[74]

More recently a new vaccine, RotaTeq, has been licensed in the USA (Table 151.7). This is also a live reassortant vaccine but of bovine–human origin and containing five strains of G1–G4 and P1[8] specificities. Three doses of the vaccine administered at 2, 4 and 6 months of age have demonstrated 74% efficacy to prevent any RV disease and 98% efficacy for severe disease with no detected association to intussusception.[75] A second RV vaccine, Rotarix (Table 151.7), also prevents any (73%) or severe (85%) RV disease without association to intussusception.[76] Rotarix is a live monovalent vaccine derived of a human attenuated strain of G1P[8]; two doses at 2 and 4 months are recommended. It is licensed in Latin America, Europe and recently in the US. Both vaccines decrease the overall burden of diarrhea hospitalization by ~50%, which may significantly improve the worldwide health of children. Both vaccines are recommended for universal immunization of children in the US.

DIAGNOSTIC MICROBIOLOGY

Various antigen detection kits based on enzyme immunoassays (EIAs) and the latex agglutination test are commercially available and represent the tests of choice for most clinical circumstances. They are relatively inexpensive and permit rapid diagnosis with high sensitivity and specificity (70–100%). Newborns and breast-feeding children might have higher false-positive results. Samples should be obtained during the symptomatic period to optimize the performance of the test. If samples are not to be processed immediately, they can be stored at 39.2°F (4 °C) or frozen. In special situations, other tests can be considered, such as EM, gel electrophoresis of viral RNA, hybridization of radiolabeled nucleic acid probes to the viral RNA, amplification by RT-PCR and viral culture. Serologic tests are rarely used. Neutralizing antibodies can be detected by plaque reduction or cytopathic effect inhibition.

Table 151.7 Comparison of two licensed rotavirus vaccines

Name	RotaTeq®	Rotarix™
Producer	Merck & Co., Inc	GlaxoSmithKline
Vaccine type	Live, bovine–human reassortant	Live-attenuated human RV strain (RIX4414)
Serotypes	Pentavalent: G1, G2, G3, G4, P1[8]	Monovalent G1P[8]
Dose	>2 × 10^6 infective doses, each	>1 × 10^6 infective doses
Administration	Oral, three doses at 2, 4 and 6 months of age	Oral, two doses at 2 and 4 months of age
Intussusception risk*	1.6 (0.4–6.4)	0.85 (0.30–2.42)
Efficacy:†		
RV GE, any severity	74 (67–80)	73 (27–91)
RV GE, severe	98 (88–100)	85 (72–92)
All diarrhea hospitalization	59 (52–65)	42 (29–53)
Virus shedding	9%	50–80%

*Odds ratio vaccine vs placebo (95% CI).
†Percent decrease vaccine vs placebo (95% CI).
GE, gastroenteritis; RV, rotavirus.

CLINICAL MANIFESTATIONS

The clinical spectrum of RV infections ranges from asymptomatic to severe disease with dehydration and death. The usual clinical picture is presented in Table 151.4. The disease is self-limiting; chronic infection has not been described in the normal host.[66] Neonatal RV infections are symptomatic in <10–20% of cases, and usually mild. Severe infections may occur among premature infants and in special care units.

MANAGEMENT

Rehydration and appropriate feeding are the main therapies for RV diarrhea. Oral immune globulin and colostrum or human milk containing RV antibodies have been used in the treatment of RV diarrhea with good results.[77] The anti-RV titer is low in human preparations but higher in colostrum from cows immunized against RV. This form of therapy might prove useful for immunocompromised patients and those with chronic or severe disease. Also, formulas supplemented with *Bifidobacterium bifidum* and *Streptococcus thermophilus* reduce the incidence of diarrhea and RV shedding in infected children.[78] Interference with the overgrowth of bacteria and promotion of the intestinal immune response to RV have been suggested as possible mechanisms. Recently, nitazoxanide (a broad-spectrum anti-infective) has been reported to decrease the duration of severe RV diarrhea.[79]

Astroviruses

Astroviruses belong to the family Astroviridae. In 1975, they were described by Madeley & Cosgrove as small (28 nm) particles with a five- or six-pointed star appearance by EM.[80] Later studies showed an icosahedral, 41 nm morphology with well-defined spikes (Fig. 151.3). If the viruses are subjected to high pH, they transform to the previously described star.[81] The virus is nonenveloped, with a single-stranded, positive-sense RNA genome that contains three ORFs. ORF1a and ORF1b encode viral protease and polymerase, respectively. ORF2 encodes a protein capsid precursor, which gives rise to VP32, VP29 and VP26 (structural capsid proteins). VP26 and VP29 appear to be responsible for antigenic variation.[82] Eight serotypes have been described (HAstV-1 to HAstV-8).

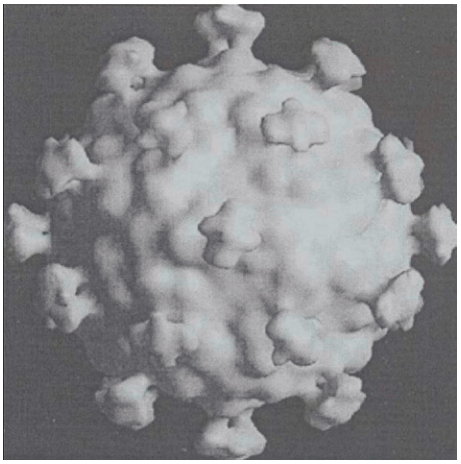


Fig. 151.3 Three-dimensional reconstruction of cryoelectron microscopy image of human astrovirus. Reproduced with permission from Mendez & Arias.[85]

Human astrovirus are responsible for 2–4% of endemic diarrhea in children worldwide; HAstV-1 is the most prevalent.[83,84] Astroviruses are the 'second or third most common cause of viral diarrhea in young children'.[85] They have been associated with outbreaks in day-care centers, schools and pediatric wards. Infection confers protective antibodies, which become more prevalent with increasing age. More than 80% of adults have antibodies against the virus. Immunocompromised subjects and the elderly are also affected.

Astrovirus pathogenesis is not completely understood. Histopathologic studies in an immunocompromised patient show infection in mature epithelial cells of the small intestine,[86] without inflammatory response. Mild crypt cell hyperplasia, also without inflammation, has been observed in turkeys.

Prevention or control of outbreaks can be accomplished by following the procedures mentioned above. The virus is inactivated by methanol 70–90% and heating at 140°F (60°C) for 10 minutes; however, it is resistant to chloroform and ethanol. As yet there is no vaccine.

Diagnosis can be made by commercial IAs with good sensitivity and specificity when compared with EM and RT-PCR.[84] The clinical characteristics of astrovirus infection are presented in Table 151.4. Symptoms are similar to those of RV infection but less severe. Management is supportive.

Enteric Adenoviruses

The Adenoviridae family has 51 human adenovirus serotypes, which are divided into six species or groups (A–F). Described in 1975,[87] enteric adenoviruses belong to species F and comprise serotypes 40 and 41. Other serotypes have also been associated with gastroenteritis, but their significance is less clear.

Adenoviruses are nonenveloped, with a dsDNA genome surrounded by an icosahedral capsid with fiber-like projections from each of the 12 vertices. Morphologic characteristics are presented in Table 151.2. Each virion contains 240 hexons (major surface protein) and 12 pentons. Each penton consists of a base and a fiber. Genus-specific antigens are located in the hexon. Type-specific antigens are located in the hexon and the fiber, which are in the virion surface and give rise to serum neutralizing antibodies.

The fiber protein of most adenoviruses binds to the coxsackie–adenovirus receptor (CAR) of epithelial cells and the penton base mediates internalization of the virus. Infected cells degenerate in typical ways. The process is related to the E3 virus proteins.[88] No specific receptors to explain species tissue tropism have been identified. The pathogenesis of gastroenteritis produced by serotypes 40 and 41 remains elusive.

Enteric adenoviruses have worldwide distribution, mainly affecting young children, and are responsible for 4% of acute gastroenteritis episodes seen in outpatient clinics and 2–22% seen in hospitalized children.[89] Outbreaks lasting 7–44 days have occurred in hospitals and day-care centers; approximately 40% of children were infected, half of them asymptomatically.[90]

Transmission is person to person by the fecal–oral route. Infected persons developed group- and type-specific antibodies. The latter are needed for long-term immunity and can be measured by neutralization or hemagglutination inhibition (HAI) tests. Stool excretion of adenoviruses lasts 10–14 days, from 2 days before and 5 days after diarrhea. Asymptomatic excretion may last months to years; thus, their isolation in diarrheic stools does not necessarily mean acute infection.

Outbreak control can be accomplished following the procedures mentioned above. Adenoviruses are less resistant than RVs and are rapidly inactivated at 133°F (56°C) and by exposure to ultraviolet light or formalin. No vaccine is under development.

Viral antigen detection by immunoassay is the diagnostic test of choice. IAs are simple, quick and inexpensive; sensitivity and specificity are 98% when compared with EM; however, real-time RT-PCR has proven superior to IAs and EM.[91] Enteric adenoviruses do not grow in routine tissue culture.

The clinical manifestations of enteric adenoviruses are presented in Table 151.4. In general, the disease is milder but more prolonged than that with RV. Treatment is supportive. Cidofovir alone or with ribavirin have been used for immunocompromised patients.

Other Viruses

Cases of human gastroenteritis have been associated with picornaviruses (Aichi virus) coronaviruses, parvoviruses, pestiviruses, toroviruses, picobirnaviruses, Breda viruses and others, but their role as agents of gastroenteritis is under study.

REFERENCES

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Chapter 152

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Peter Morgan-Capner

Measles, mumps and rubella viruses

INTRODUCTION

The epidemiology of the viruses discussed in this chapter (measles, rubella and mumps) has changed dramatically since the successful introduction of live attenuated vaccines. As infections such as measles and rubella become less frequent, clinicians are less familiar with their clinical presentations and surveillance based solely on clinical recognition is not always sufficiently accurate. Laboratory confirmation of suspected clinical cases is essential and should be complemented by genotyping of circulating viral strains for effective surveillance.[1]

Measles

NATURE

Measles (rubeola) was first identified over 2000 years ago, although its infectious nature was not recognized until the mid 19th century when epidemics in island communities were described. It is one of the infectious diseases targeted by the World Health Organization (WHO).

Measles virus is a member of the *Morbillivirus* genus of the Paramyxoviridae and was first isolated in cell culture in 1954; the first live attenuated vaccine became available in 1963. It is pleomorphic, with a diameter of 150 nm or more. Single-stranded RNA is enclosed in a capsid of helical symmetry of 18 nm. This is enclosed within an envelope, the surface of which carries the hemagglutinin and fusion proteins. Replication is mainly cytoplasmic; however, there is some nuclear involvement. Although there is only one measles virus serotype, 23 genotypes of measles virus have been described, which are organized in eight clades (A–H).[2]

EPIDEMIOLOGY

Measles is endemic worldwide except in those countries in which complete control has been achieved by immunization (Fig. 152.1). Infection in childhood before the impact of immunization is almost 100%, confirming measles as one of the most infectious of microbial pathogens. Epidemics occur every 2–3 years, but are less defined in the tropics.

The epidemiology of measles has been profoundly modified by immunization strategies, with a 74% reduction in measles death globally from 757 000 in 2000 to 197 000 in 2007. The target set by the global immunization vision and strategy program developed by the WHO and UNICEF is to reduce mortality due to measles by 90% by 2010.[3]

Despite efforts to eradicate measles, outbreaks continued to occur in Europe during 2006 and 2007 and the number of measles cases reported to the Centers for Disease Control and Prevention (CDC) in the first half of 2008 was the highest year-to-date figure since 1996.[4,5] Most measles cases were reported in unvaccinated children and suboptimum vaccine uptake due to parenteral concerns and/or religious reasons remains a major obstacle towards achieving elimination of the disease in Europe.

PATHOGENICITY

Infection is spread by droplet from person to person. The incubation period to onset of rash is about 14 days, with prodromal symptoms starting 1–3 days earlier. Maximum infectivity is during the prodrome, although patients are considered to be infective from 4 days before to 4 days after onset of the rash.

After infection, initial replication occurs in the respiratory epithelium with local spread by lymphatics and a primary viremia 2–3 days after infection. A secondary viremia occurs 3–4 days later and lasts for up to 7 days. The peak viremia coincides with the prodromal symptoms. The rash results from the immunologic reaction between the virus antigens and host antibody, with involvement of capillary walls. Intrauterine infection has not been convincingly reported.

PREVENTION

Live attenuated measles vaccine became available in 1963 based on the Edmonston strain, but those in current use such as Schwarz and Moraten strains are further attenuated. The measles vaccine is available in monovalent form, as well as combined with mumps and rubella vaccine (MMR). Two doses of the vaccine are necessary to achieve high levels of immunity (>98% efficacy). They are included in the routine childhood immunization scheme at age 12–15 months and at school entry (4–6 years). Administration before 12 months can result in lower protection because of interference by residual maternal antibody. This is especially problematic for developing countries, where many cases of measles occur in infants under 12 months of age. For most developing countries, the WHO recommends immunization at 9 months of age.[3]

In HIV-infected children the vaccine is less effective but protective antibody levels can be achieved in HIV-infected children on highly active retroviral therapy (HAART) with immune recovery.[6] Measles vaccine is contraindicated in pregnancy and in those with impaired cell-mediated immunity because measles-specific complications, as well as potential side-effects, such as localized pain and tenderness, mild fever (about 10%) and transient rashes (about 5%), may occur.

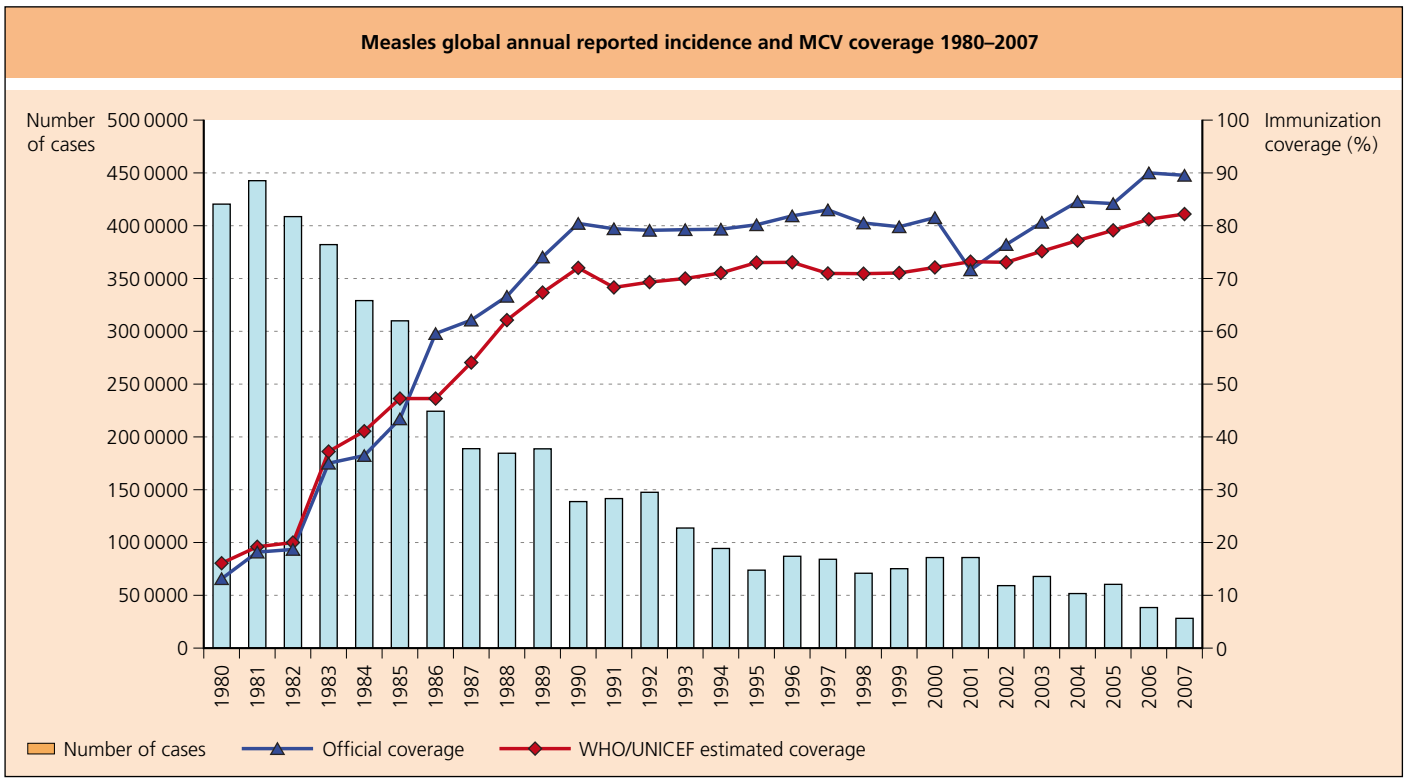


Fig. 152.1 Measles global annual reported incidence and measles-containing vaccine coverage 1980–2007.

Encephalitis has been reported in one in a million doses, but its causal relationship with the vaccine is uncertain. Anxieties had been expressed as to whether MMR immunization may lead to inflammatory bowel disease and autism,[7] but strong evidence has been published since then against an association of autism with MMR vaccine exposure.[8,9]

DIAGNOSTIC MICROBIOLOGY

Virus isolation may be attempted from throat swabs, conjunctival swabs, nasopharyngeal aspirates (NPAs) and sedimented cells from urine, but is technically demanding and unreliable. The cell line used in global reference laboratories for the isolation of measles virus is a Vero/SLAM cell line – a Vero cell line transfected with a plasmid encoding human SLAM (signaling lymphocyte-activation molecule) which is a cellular receptor for the measles virus. The cytopathic effect after inoculation is not immediately apparent as multinucleated giant cells can take up to 15 days to develop. Measles virus can also be detected by hemadsorption (monkey, human or guinea-pig red blood cells) and isolation can be confirmed by specific neutralization of the hemadsorption or by immunofluorescence.

Immunofluorescent antigen detection in NPA cells may be performed and is particularly useful for diagnosing atypical cases or infection in the immunocompromised host. The measles virus genome, amplified from cells obtained by throat swab or NPA by reverse transcriptase polymerase chain reaction (RT-PCR) can be used for genetic characterization and to determine the geographic origin of the infecting strain.[2]

As RT-PCR can detect the viral genome without viable virus being present, a diagnosis can be made up to several weeks after rash onset. Serologic diagnosis is dependent on demonstrating seroconversion, rising antibody titer or detecting specific IgM. Hemagglutination inhibition and complement fixation testing (CFT) have been the established methods in the past, but are now being replaced by immunoglobulin-specific enzyme immunoassays (EIAs). Serum-based IgM EIAs are currently recommended for the rapid laboratory detection of measles. IgM assays are available in the format of IgM capture assays or as indirect assays. False-negative IgM assays can occur if serum is collected within 3 days of rash onset.[10]

IgG assays require the collection of two serum specimens about 10–30 days apart. A fourfold rise in the IgG titer is considered to be significant. IgG assays are not routinely used for the diagnosis of acute measles virus infection. They are used for the clarification of equivocal IgM results and in seroprevalence studies.[1] Simultaneous detection of IgM and viral RNA from dried blood specimens or from oral fluid sampling are possible alternatives for diagnosis and surveillance of measles, especially in countries where specimen transport or refrigeration is challenging.

Subacute sclerosing panencephalitis may be diagnosed by detection of the genome or isolation of virus by co-cultivation from brain tissue. A more usual approach, however, is the detection of elevated concentrations of measles antibody in serum and concentrations indicating intrathecal synthesis in cerebrospinal fluid (CSF) by CFT.

CLINICAL MANIFESTATIONS

Toward the end of the incubation period, the prodromal symptoms of fever and malaise appear and persist for up to 4 days before the rash appears. Pyrexia may rise to 103–104°F (39.4–40°C) and is often accompanied by conjunctivitis, cough and coryza (the three 'C's of measles). Koplik's spots are a pathognomonic feature of measles, and can be seen for up to 2 days before rash onset as punctate white spots on an erythematous background on the buccal mucosa.

The rash usually begins on the face and neck, and evolves to the body and limbs (Fig. 152.2). Although lesions are usually discrete, they can coalesce, and desquamation may occur. The rash usually



Fig. 152.2 Measles. A disseminated erythematous rash can be seen over the trunk and arms.



Fig. 152.3 Cancrum oris. Necrosis of the upper lip.

Table 152.1 Complications of measles (% of cases)

	USA			UK
Age	**5 years**	**5–19 years**	**>20 years**	**All ages**
Otitis media	14	2	2	5
Pneumonia	9	2	7	4
Encephalitis	0.2	0.1	0.3	0.1
Death	0.3	0.2	0.3	0.25
Hospitalization	25	8	24	1

USA data are broken down into three age groups, whereas UK data are not; however, UK data primarily reflect infection in early childhood.[11,12]

persists for 5–6 days. Generalized lymphadenopathy and diarrhea are common.

Complications are most common in younger and older patients (Table 152.1) and those with underlying malnutrition, particularly vitamin A deficiency. Pneumonia, either viral or, more usually, secondary bacterial, occurs in up to 4% of patients. Neurologic complications include convulsions, in up to 1% of cases, and encephalitis, which has been reported in 1/1000 cases with a reported mortality rate of 15% and residual neurologic deficit rate of 25%. Overall, estimates of the mortality rate for measles are 1 per 1000 patients in developed countries but higher in the developing world.

Subacute sclerosing panencephalitis (SSPE) manifests from months to years after the initial natural measles or measles immunization; the mean interval is 7 years after natural measles and 3.3 years after immunization. The risk is less after measles immunization (1:1 million) than after natural infection (1:100 000), and is three times more common in boys than in girls. Specific risk factors for the development of SSPE have not been identified. SSPE is a progressively fatal degenerative disease of the central nervous system, manifests itself as a progressive intellectual impairment, which may not be noticed initially and moves to convulsions, motor abnormalities and coma; death is inevitable after a progressive deterioration over months or years. Its pathogenesis is not well understood, but persistent infection of the central nervous system with a genetic variant of measles virus may be involved. SSPE virus has unique phenotypic features that are different from those of wild-type measles virus, which are referred to as nonmutated measles. For example, some SSPE virus strains are highly neuropathogenic in hamsters, mice and other rodents. Also, SSPE virus produces few infectious virus particles, if any, and thus its spread from infected to uninfected cells is almost completely dependent on cell-to-cell infection. This defective feature is thought to be caused by a variety of mutations affecting primarily the viral matrix (M) protein and the cytoplasmic tail of the fusion (F) glycoprotein. This results in the lack of functional M protein and is thought to be responsible, at least partly, for the viral inability to produce virus particles and for chronic progressive neuropathogenicity in the host. Other viral genes, such as the F gene, also undergo unique mutations that are not found in the wild-type measles virus. These viral genetic mutations must have been accumulated during viral persistence over a long period of time, especially after the onset of SSPE symptoms, when the virus is actively proliferating in the brain.[13]

The immunocompromised patient with T-cell deficiencies, such as those who have leukemia or HIV, is at particular risk for the development of complications. A typical rash is often missing and infection persists, manifesting as a giant cell pneumonitis or rapidly progressive encephalitis; the fatality rate is high.[14]

Measles in pregnancy seems to carry a higher risk of complications but has not been associated with congenital abnormalities, although there is an increased risk of intrauterine death or premature delivery.[15]

Measles presents special problems in developing countries, where case fatality rates of up to 25% have been described. The high mortality rate is associated with malnutrition and, in particular, vitamin A deficiency.[16] Death usually results from bacterial superinfection, such as pneumonia, or diarrheal illness, although cancrum oris, a progressive oral necrosis, is also seen (Fig. 152.3).

MANAGEMENT

Postexposure prophylaxis with human normal immunoglobulin is indicated for those who are susceptible and who would be at risk from complications, in particular immunocompromised children and pregnant women. In outbreak situations and in immunocompetent persons administration of measles vaccine within 3 days of contact can provide protection.

Uncomplicated measles is managed symptomatically, with vitamin A supplementation for malnourished children. Antimicrobial therapy active against *Streptococcus pneumoniae* and *Haemophilus influenzae* is indicated for presumed bacterial superinfection. In those with major complications, such as pneumonitis in immunocompromised patients, specific antiviral treatment with ribavirin may be of benefit;[17] however, such treatment is of no value in SSPE.

Rubella

NATURE

Although rubella had been recognized as a distinct clinical illness since the 18th century, it was not until 1941 that Sir Norman Gregg, an Australian ophthalmologist, made the association between rubella in early pregnancy and congenital abnormalities.[18] In 1962 the causative virus was isolated, leading to techniques for specific diagnosis and the development of attenuated live vaccines.

Rubella is a single-stranded RNA virus, with an icosahedral nucleocapsid surrounded by an envelope. Replication occurs in the cytoplasm of infected cells. It is the sole member of the genus *Rubivirus* within the family Togaviridae. There are three major virus polypeptides: C, E_1 and E_2. Polypeptide E_1 is present in the envelope and has hemagglutinating properties. Only one serotype of the virus is recognized, but phylogenetic analysis of the coding region of E_1 revealed at least seven distinct genotypes grouped in two clades [19] and natural infection of species other than humans has not been shown.

EPIDEMIOLOGY

Rubella is a mild disease presenting with fever and rash. Existing vaccines, single or in combination with measles and/or mumps, have been proven to be highly efficacious. Rubella elimination is one of the primary targets of the WHO as infection during the early months of pregnancy can lead to fetal death or serious congenital birth defects (congenital rubella syndrome, CRS). It is estimated that more than 100 000 infants are born with CRS each year, the majority occurring in countries without an effective rubella vaccination program.[20]

PATHOGENICITY

Infection is transmitted by the airborne route, by direct contact or indirectly through fomites. Patients are potentially infective from 1 week before to 1 week after the onset of rash. After replication in the upper respiratory tract and local lymph nodes, a viremia infects target organs such as skin, joints and placenta. Similarly to measles, the rash is immunologically mediated and marks the production of specific antibodies.

If the patient is pregnant, placental infection can occur; transmission to the fetus is possible but not inevitable.[21] Confirmed rubella during the first trimester of pregnancy resulted in damage to 50–90% of fetuses (see Table 152.2).[22]

PREVENTION

Passive prophylaxis with human normal immunoglobulin has not been demonstrated to reduce the risk of rubella after contact, although it may attenuate the illness. Control of rubella has resided in using the live attenuated vaccines available since the early 1970s. The vaccine strain RA27/3 induces seroconversion in over 95% of susceptible vaccinees, and protection persists for at least 15–20 years.[23] Vaccine virus can be isolated from the throat of vaccinees, but transmission to susceptible contacts has not been demonstrated. A mild, transient rubelliform rash illness with arthralgia can occur 2–3 weeks after immunization, but chronic joint problems have not been associated with administration of the vaccine. If vaccine virus is inadvertently administered to pregnant women, fetal infection occurs in approximately 1% of cases, but extensive postvaccine surveillance did not detect any fetal damage.[24] Rubella immunization presents no significant risk to the individual who has HIV infection.

With rubella being a mild illness, control has focused on preventing infection in pregnant women. Two approaches have been used. First, as exemplified in the UK until 1988, rubella vaccine was targeted at adolescent girls and susceptible women, with rubella remaining endemic in children and men. Although cases of congenital rubella were markedly reduced, there were still 2–3% of women susceptible and at risk of rubella in pregnancy. The alternative approach, as used in the USA and in the UK from 1988, was to combine rubella vaccine with measles and mumps vaccines (MMR), and offer it to all children in the second year of life to eradicate rubella from the community. This policy was supported by identifying and immunizing susceptible women. If uptake rates of more than 90% can be obtained, control of endemic rubella is achievable (Fig. 152.4). Elimination of the congenital rubella syndrome has been accomplished in the USA and in other developed countries although sub-Saharan Africa still remains a problem.[25]

DIAGNOSTIC MICROBIOLOGY

With the clinical diagnosis of rubella being unreliable, laboratory confirmation of infection is required. In pregnancy it is imperative to

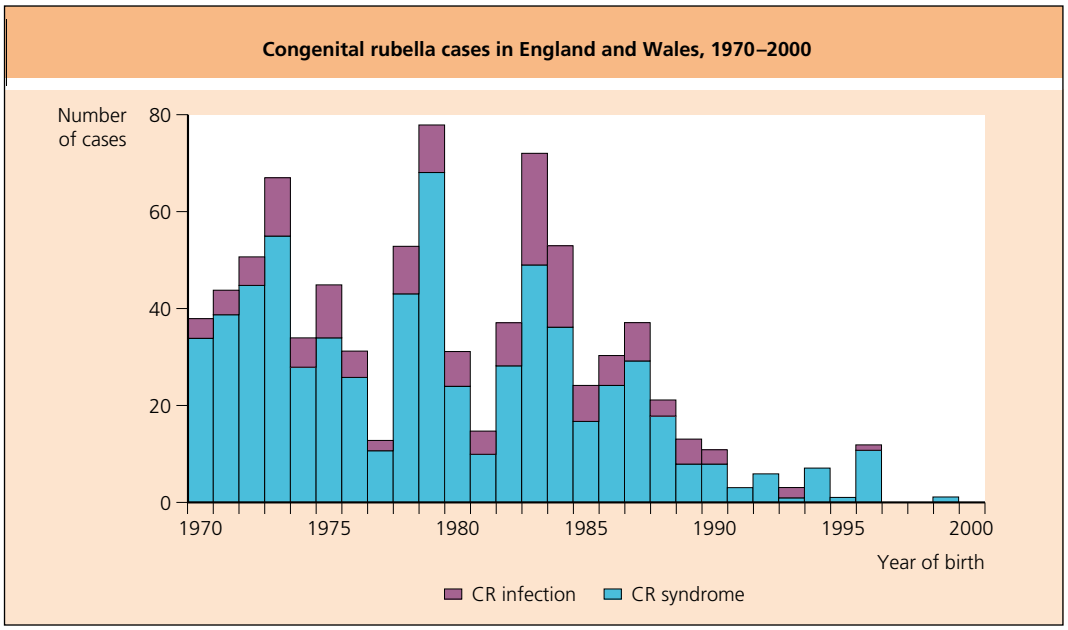


Fig. 152.4 Cases of congenital rubella (CR) infection and syndrome in the UK. The number of confirmed cases was substantially reduced by the introduction of MMR vaccine in 1988. Data from National Congenital Rubella Surveillance Programme and the PHLS Communicable Disease Surveillance Centre.

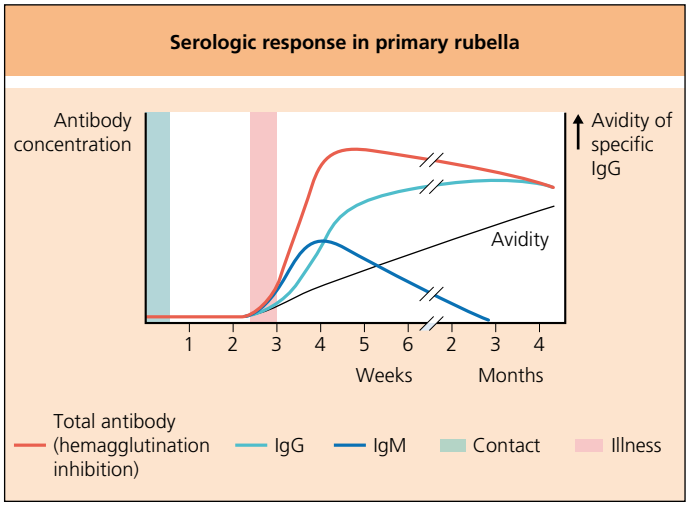


Fig. 152.5 Serologic response in primary rubella. The development of specific IgG and IgM and the increasing avidity of specific IgG are illustrated.

investigate both a rash illness and contact with a rash illness because subclinical rubella may occur, and this has been proven to be a risk to the fetus.[26] It is debatable whether such investigation of contacts should be pursued if there is a documented history of past detection of rubella antibody or vaccine. However, vaccine is not 100% effective in inducing protection, and if there has been only one previous detection of rubella antibody, there is a remote possibility that a laboratory error may have occurred.

The diagnosis of postnatal rubella infection is made by serology and the serologic response is detailed in Figure 152.5; tests for total rubella antibody (such as hemagglutination inhibition) have been progressively replaced with tests specific for IgG and IgM, particularly EIAs. Serum should be obtained as soon as possible after onset of illness or after contact. Detection of rubella-specific IgG, but failure to demonstrate specific IgM, indicates immunity to rubella and that the illness being investigated is not rubella. If neither rubella-specific IgG nor specific IgM are detected, repeat testing of a later serum is needed. The incubation period after contact may be up to 21 days, and it may take up to 10 days after onset of illness for rubella antibody to be detectable. Hence in the susceptible person it will not be until about 4 weeks after contact that a subclinical illness can be excluded.

Detection of rubella-specific IgM indicates recent primary rubella infection, but care is needed in interpretation. Rubella-specific IgM reactivity persists for about 1–3 months and false-positive IgM results occur in a range of other infections such as parvovirus B19 infection and Epstein–Barr virus infection (infectious mononucleosis). Rubella-specific IgM reactivity may also be nonspecific and may occur in rubella re-infection (see below). In the first 2 days after rash onset RT-PCR testing of oral fluid specimens can identify more cases than IgM testing.[27] Other serologic approaches, such as determining specific IgG avidity, are of value in confirming primary rubella,[28] with specific IgG early after infection being less tightly bound to antigen (low avidity) than the antibody found in the mature antibody response (high avidity; see Fig. 152.5).

For the diagnosis of congenital rubella isolation of virus in cell culture is of value because infected infants can excrete high titers of virus in the throat and urine during the first year of life; congenitally infected infants can be highly infectious to susceptible contacts until approximately 6 months of age. Rubella isolation can be performed in many cell culture lines, but a cytopathic effect is only produced in a few of them, for example RK 13. Immunofluorescence is probably the best method for detecting virus growth.

Congenital rubella is routinely diagnosed by detecting specific IgM; all congenitally infected infants are positive for the first 3 months of life and most for the first 6 months. As passively transmitted maternal IgG antibodies have declined by 6 months of age, persistence of IgG antibodies beyond this time period indicates fetal infection as well.

Occasionally there may be problems with diagnosis or management of rubella in pregnancy and intrauterine diagnosis may be considered. Approaches used, but of limited availability, have included virus isolation and genome detection from amniotic fluid or trophoblast, and detection of specific IgM in fetal blood; this latter method is unreliable before 24 weeks' gestation, however, because the infected fetus may not be capable of an IgM response until that age.

Oral fluid samples can be used both for detection of antibodies and for amplification of the viral genome by RT-PCR. Detection of IgM in saliva is especially helpful for clarifying the etiology of rashes in childhood because they are noninvasive and more acceptable for infant sampling.

The majority of developed countries have programs in place to identify susceptible women of child-bearing age and offer immunization. Tests for specific IgG are many and include EIAs and latex agglutination. An area of contention is the concentration of rubella-specific IgG taken to indicate protection (or, more correctly, as not necessitating immunization), with opinions varying from any confirmed antibody (possibly as low as 3–5 IU) up to 15 IU; 10 IU has been recommended in the USA.[29]

CLINICAL MANIFESTATIONS

In childhood, primary rubella is subclinical in approximately 50% of patients, but in adolescence or older it is usually (90% or more) clinically apparent. After an incubation period of up to 21 days (usually 15–17 days) a pinkish-red maculopapular rash develops, starting on the face and neck and rapidly spreading over body and limbs (Fig. 152.6). Individual spots may coalesce but the rash usually clears in 3–4 days. Nonspecific symptoms, such as fever, malaise and upper respiratory tract symptoms, may precede and accompany the rash. In childhood the illness is benign and may have no systemic impact. Lymphadenopathy commonly occurs, the suboccipital nodes being frequently involved.

In adolescence and adulthood, rubella is often far more severe, not only because of complications but also because of a more severe systemic illness. Arthralgia is a frequent complication in adults, with women (30% or more) suffering more often than men. Joints frequently involved are those of the hands and wrists; resolution

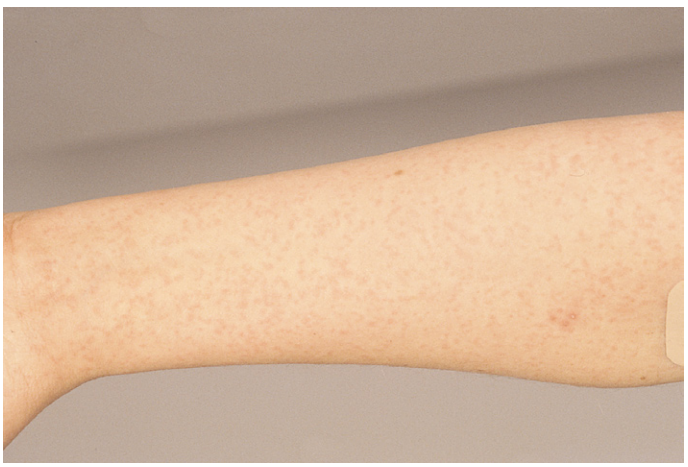


Fig. 152.6 Rubella. A pink macular rash can be seen on the forearm.

Table 152.2 Features of congenital rubella
Cardiovascular defects Persistent ductus arteriosus Pulmonary artery stenosis Myocarditis
Ocular defects Cataracts (unilateral or bilateral) Pigmentary retinopathy Micro-ophthalmus Glaucoma Iris hypoplasia
Auditory defects Sensorineural deafness (unilateral or bilateral)
Central nervous system Microcephaly Psychomotor retardation Meningoencephalitis Behavioral disorders Speech disorders
Intrauterine growth retardation
Hepatitis/hepatosplenomegaly
Thrombocytopenia, with purpura
Bone 'lesions'
Pneumonitis *
Diabetes mellitus *
Thyroid disorders *
Progressive rubella panencephalitis *
*These disorders become apparent in infancy or later.

usually occurs in 2–4 weeks but can persist for some months or even years. Thrombocytopenia and postinfectious encephalitis are rare complications, occurring in less than 1/5000–10 000 patients, with fatal outcome virtually unknown. Even in patients with HIV infection and other immunocompromised patients, rubella rarely carries any additional risk.[30]

Primary rubella infection in the first 16 weeks of pregnancy presents major risks to the fetus. Consequences for the fetus and the pregnancy include abortion, miscarriage or stillbirth or multiple birth defects (Table 152.2). The congenital rubella triad comprises cardiac, ophthalmic and auditory lesions, but purpura, intrauterine growth and neurologic problems are also frequent.

The gestation at which the mother suffers her rubella infection is critical for the outcome. Onset of rubella before conception carries little, if any, risk,[31] whereas from conception to the 12th week the risk is about 90%.[23] Between 12 and 16 weeks' gestation the risk falls to about 20%, with sensorineural deafness being the only consequence. Beyond 16 weeks' any risk is minimal, with only rare cases of sensorineural deafness.

It has been established for many years that re-infections can occur in those with a past history of natural rubella or successful immunization. Such re-infections are rarely clinically apparent and are usually identified by a serologic response after exposure, which is usually to a close contact such as the patient's own child. Fetal infection and damage can occur after maternal re-infection, but this is rare, with the fetal risk probably being less than 5%.[32] Because of the difference in risk to the fetus, it is critical to distinguish between subclinical primary rubella and re-infection in early pregnancy. This may be difficult in the absence of past rubella-specific IgG test results because a specific IgM response can occur in both; IgG avidity testing will usually resolve the problem because re-infection would be characterized by the presence of high avidity IgG.

MANAGEMENT

Rubella is a self-limiting illness that usually runs a benign course. Supportive and symptom-relieving therapy is indicated for complications such as arthralgia, and the patient can be reassured as to the unlikely occurrence of symptoms persisting beyond a few months.

Management of rubella in pregnancy is first dependent on achieving a correct diagnosis. A serum sample must be taken as soon as possible after contact or onset of illness, and the testing laboratory given full details of past testing and/or vaccination. On the basis of results and the clinical details, the laboratory will advise on diagnosis and any further testing required. If primary rubella or re-infection in the first 16 weeks of pregnancy is diagnosed, further management will have to take into account social, legal and religious perspectives because in many countries the risk to the fetus is sufficient to justify consideration of termination of pregnancy.

Mumps

NATURE

Mumps was first identified as a distinct clinical illness by Hippocrates in the 5th century BC, but has only attracted attention in the past two to three centuries, particularly because of the impact of outbreaks in the armed forces. Natural infection is limited to humans and is endemic worldwide.

Mumps virus is a member of the *Paramyxovirus* genus of the Paramyxoviridae family. It was first isolated in the chick embryo in 1945, with the first live attenuated vaccine being licensed in 1967. On electron microscopy it is pleomorphic (Fig. 152.7) and varies in size from 80 to 350 nm, although it is usually approximately 200 nm. The envelope is studded with projections containing hemagglutinin/neuraminidase or fusion proteins. Within the envelope the nucleoprotein has helical symmetry with a diameter

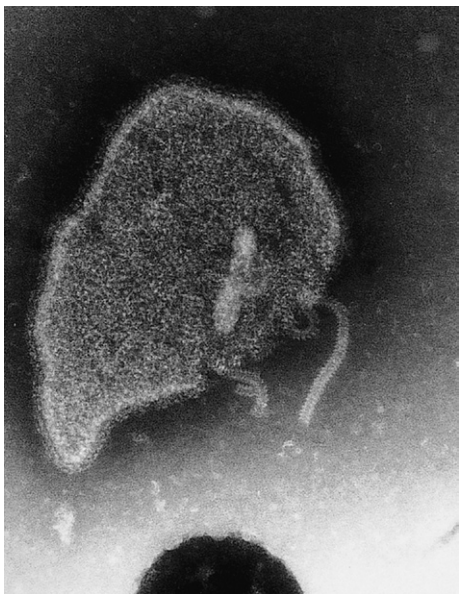


Fig. 152.7 Electron micrograph of mumps virus. Courtesy of Dr A Curry.

of 15–19 nm. The RNA is single stranded and of negative sense; the nucleocapsid contains an RNA-dependent RNA polymerase. This enzyme enables replication of the RNA in the cytoplasm once the cell has been penetrated. Mature virions are released from the cell by budding.

Twelve mumps virus genotypes (A–L) with different geographic distribution have been described. There is only one serologic type although studies suggest that cross-neutralization between genotypes might not be complete.[33]

EPIDEMIOLOGY

Mumps is endemic worldwide, with epidemics occurring every 2–3 years in populations not influenced by widespread use of mumps vaccine. In the prevaccine era 50% of children aged 4–6 years and 90% of children aged 14–15 years had positive serology for mumps.[34] With the introduction of mumps vaccines the epidemiology of the disease shifted, depending on the number of vaccine doses, age at vaccination and vaccine uptake. Mumps outbreaks are not uncommon in countries with routine mumps vaccination; in 2004/2005 the UK experienced a large scale outbreak [35] and in 2006 more than 5800 cases of mumps were reported in the USA.[36] In both countries the majority of infections occurred in young adults attending college. Although the majority of college students in the USA had been MMR vaccinated, suboptimal vaccine effectiveness and insufficient vaccine uptake allowed transmission in this population.[36] In addition, whereas natural infection seems to result in lifelong immunity, immunity induced by vaccination can decrease.[37]

PATHOGENICITY

Infection is acquired by droplet spread, direct contact or contaminated fomites. Replication in the upper respiratory epithelium is followed by spread to local lymph nodes and subsequent primary viremia, with seeding of target organs such as parotid glands, central nervous system and genital organs. A secondary viremia occurs at the onset of symptoms.

The incubation period is usually 16–18 days, with infectivity commencing 2 days before onset of symptoms and persisting for 5–7 days after onset. Both in the health-care setting and in the community patients with mumps should be isolated with droplet and contact precautions for a 5-day period after onset of parotitis.[38] Intrauterine infection has been reported only rarely.

PREVENTION

Two live attenuated vaccine strains have been mainly used since 1967: Jeryl Lynn and Urabe strain vaccines. Both are produced in chick embryo cell culture and are available in monovalent form or combined with measles and rubella vaccine (MMR). The vaccine is usually administered as MMR at 12–15 months of age, with second doses being given later in childhood. The protective efficacy is 75–85%, with clinically apparent mumps in immunized individuals being well documented. Minor complications of mumps vaccine include mild parotitis, fever and, very rarely, orchitis. Although Urabe strain vaccines were withdrawn in some countries in the 1990s due to higher rates of aseptic meningitis occurring with this vaccine strain, the WHO justifies the use of the Urabe strain in national vaccination programs as aseptic meningitis postvaccination is generally benign and the Urabe strain seems to be more immunogenic and is cheaper.[39]

Mumps vaccine is contraindicated in those with previous anaphylaxis to egg and in pregnancy, although adverse implications for the fetus have not been reported. It should also not be given to immunocompromised patients, with the exception of children with HIV infection, in whom no adverse consequences have been seen.

DIAGNOSTIC MICROBIOLOGY

Virus isolation may be achieved from throat swabs, saliva, CSF and possibly urine. Mumps virus is readily isolated in either Vero cells (African green monkey kidney) or Caco-2 (human colorectal adenocarcinoma epithelium). Although cytopathic effect may sometimes be observed (multinucleated giant cells), isolation is usually confirmed by immunofluorescence staining. RT-PCR is performed directly on the clinical specimen and sequencing of the amplified product can be used to identify individual genotypes. RT-PCR was found to be more sensitive than culture methods in detecting virus in cerebrospinal and oral fluid.[40]

Serologic diagnosis is usually achieved by detection of virus-specific IgM antibody by direct or indirect enzyme-linked immunosorbent assay (ELISA). False-negative IgM results can occur for serum collected before day 4 of clinical presentation.[41] A fourfold rise of IgG titer between acute and convalescent serum is considered diagnostic as well. Although ELISA is routinely used for the diagnosis of infection, it can give false-positive results in assessing immunity. Serologic testing can also be performed on CSF specimens in patients with mumps, meningitis or encephalitis. Saliva specimens can be used for specific IgM detection and are valuable for surveillance of mumps-like illness in the community.[34]

CLINICAL MANIFESTATIONS

Towards the end of the incubation period of 16–18 days prodromal symptoms such as pyrexia and malaise develop, which is followed by the characteristic tender swelling of the parotid glands. There is often accompanying headache and earache. Recovery is usually complete within 4–5 days. Asymptomatic mumps is common and occurs in about one-third of infections. Parotitis is present in 95% of symptomatic infections and is unilateral in about one-quarter; other salivary glands are involved in about 10% of patients. Parotitis similar to that seen with mumps may also be found in other virus infections, for example coxsackievirus and Epstein–Barr virus B infection.

Complications are common;[39] the risk is the same at all ages except for orchitis and oophoritis, which are virtually limited to post puberty. Orchitis generally arises a few days after parotitis. The risk of orchitis in the adolescent or adult male is about 35%, with bilateral involvement in one-third of these. There may be some persisting testicular atrophy, but sterility is remarkably uncommon, despite public perceptions. Oophoritis is only observed in about 5% of mumps in adult women and causes lower abdominal pain; it is uncertain whether there may be long-term consequences.

Meningeal involvement, as shown by CSF pleocytosis, occurs in about 50% of patients, with signs of meningitis or meningeal irritation in about 1–10%. Up to 50% of meningitis cases occur without concomitant parotitis.

Mumps virus may also cause encephalitis, presenting as impaired level of consciousness, seizures, aphasia, etc. Mumps meningitis is, in general, a benign disease; persistent sequelae are found in a small percentage of encephalitis and are more common in adults.

The incidence of encephalitis is about 1/6000 patients, with a mortality rate of 1.4%. Transient hearing impairment as a result of a labyrinthitis is not uncommon, with persisting deafness occurring in about 1/20 000 patients. Other complications include pancreatitis, which is usually mild, arthritis, mastitis, thyroiditis and myocarditis. Investigation of renal function will often demonstrate kidney involvement, but there are no clinical consequences.

Mumps in immunocompromised patients seems to carry no undue risk and persistent infection has not been described.

Infection in the first trimester can lead to spontaneous abortion, but infection later in pregnancy carries no increased risk, and congenital infection and damage have not been convincingly shown.

MANAGEMENT

Postexposure prophylaxis with normal immunoglobulin is of no benefit, and specific mumps immunoglobulin is no longer available, although it may have reduced the risk of orchitis in adult men. Mumps vaccine administered as postexposure prophylaxis is of no benefit.

There is no specific antiviral treatment available for mothers and management of infection is symptomatic, with the patient being reassured that recovery occurs in a few days, even when complications are present.

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References for this chapter can be found online at http://www.expertconsult.com



Chapter **153**

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Inmaculada Casas
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Human enteroviruses

The Viruses

DISCOVERING THE ENTEROVIRUSES

History

The history of enteroviruses is the history of poliovirus. The first known clinical description of poliomyelitis is attributed to Michael Underwood, a British physician, who in 1789 reported an illness which appeared to target primarily children with paralysis and fever. In 1840, von Heine published a monograph describing the clinical symptoms. Later, the Swedish physician Medin contributed to the descriptions. The syndrome was named Heine–Medin disease. However, it was not until the early 20th century that the number of paralytic poliomyelitis cases began to reach epidemic proportions. Poliovirus was discovered to be the causative agent of poliomyelitis in 1908 by Viennese investigators Landsteiner and Popper who successfully transmitted the clinical disease and its pathology to monkeys using a filtrate from paralytic patients. Prevention of infantile paralysis and control of poliovirus spread was attempted by the use of two vaccines, one inactivated (Salk; intramuscular route) and one attenuated (Sabin; oral route). The real impact of those early studies is demonstrated by the ongoing success of the global poliovirus eradication program.

In addition to polioviruses, during these initial studies on poliomyelitis, other enteric viruses (thus named enteroviruses) were discovered. In 1948, an antigenically distant virus to poliovirus was isolated from the feces of a paralytic child in Coxsackie, New York, during a poliomyelitis epidemic. It was aptly termed Coxsackie virus. Later, when a second diverse virus was isolated in the same area from cases of aseptic meningitis, the first virus become the first member of the Coxsackie A virus group, while the second formed the Coxsackie B virus group. Finally, in 1951 from the stool of asymptomatic individuals, several antigenically independent viruses were isolated. They were named echoviruses, corresponding to *e*nteric isolates, *c*ytopathogenic in tissue culture, isolated from *h*umans and *o*rphans (no association with known clinical disease). After 1969, new serotypes were numbered sequentially.

Physical and chemical properties

The pathology, transmission and general epidemiology of enteroviruses are all shaped by their biophysical properties (Table 153.1). Their structural stability and their resistance are key factors in their mechanism of transmission. They may also explain their cytolytic life cycle and their singular characteristics within the Picornaviridae family.

Table 153.1 Physical and chemical properties of enterovirus particles

Characteristic	Properties
Sedimentation	Constant buoyant density in cesium chloride (irrespective of pH or time of centrifugation)
Stability	Stable in weak acid, retain the infectivity at pH values up to 3.0 (allow replication in the alimentary tract)
Structural stability	Insensitive to lipid solvents (ether and chloroform) Stable in many detergents at ambient temperature Relatively resistant to laboratory disinfectants (70% ethanol, isopropanol, lysol, quaternary ammonium compounds)
Inactivation	Chemical: formaldehyde, glutaraldehyde, strong acid, sodium hypochlorite, free residual chlorine Environmental: temperatures higher than 42°C, ultraviolet light, drying on surfaces

Classification

Members of the family Picornaviridae are small, single-stranded RNA viruses (ssRNA). The family is subdivided into nine genera: Enterovirus, Rhinovirus, Cardiovirus, Aphthovirus, Hepatovirus, Parechovirus, Erbovirus, Kobuvirus and Teschovirus. The genus Enterovirus can be further grouped into eight species, four of them infecting humans, each with a variable number of serotypes (Table 153.2).

The original classification was based on the clinical manifestations observed in human infections as well as on the pathogenesis in intracranially and subcutaneously inoculated experimental suckling mice. This classification defines the following homogeneous antigenic groups:

- polioviruses, which cause flaccid paralysis (poliomyelitis) in humans;
- Coxsackie A viruses (CAV), which are linked to human central nervous system (CNS) disease and herpangina, as well as acute flaccid paralysis in suckling mice;
- Coxsackie B viruses (CBV), associated with ailments of the human cardiac and central nervous systems, necrosis of the fat pads between the shoulders, focal lesions in skeletal muscle, brain and spinal cord, as well as spastic paralysis in the suckling mouse experimental model; and
- echoviruses, which were not originally associated with human disease or with paralysis in mice.

Table 153.2 Classification of enterovirus species and serotypes

Species	Serotypes
Human enterovirus A	CVA2–8, CVA10, CVA12, CVA14, CVA16, HEV71, HEV76, HEV89–91 (contains most of the old Coxsackie A virus group)
Human enterovirus B	CVA9, CVB1–6, E1–7, E9, E11–21, E24–27, E29–33, EV69, EV73–75, EV77–88, EV93, EV97, EV98, EV100, EV101 (contains all Coxsackie B virus group and all echoviruses of the old nomenclature)
Human enterovirus C	CVA1, CVA11, CVA13, CVA15, CVA17–22, CVA24, PV1–3, EV95, EV96, EV99, EV102, EV104 (contains the remainder of the Coxsackie A virus group and the poliovirus)
Human enterovirus D	EV68, EV70, EV94

Since 1969, new enterovirus types have been assigned type numbers, starting with 68 and today reaching 101.

The introduction of molecular typing methods is the basis for the modern classification scheme. Under the new genetic scheme, human enteroviruses are subdivided into four species of human enterovirus A to D supported by genome organization, sequence similarity and biologic properties. Incorporation of polioviruses into the human enterovirus C species is pending approval by the International Committee on Taxonomy of Viruses (ICTV) (http://www.picornastudygroup.com).[1] Nowadays, new enteroviruses are being phylogenetically grouped into their species[2–8] instead of being antigenically characterized. The ICTV has proposed genetic criteria for inclusion of a new enterovirus in one concrete species. Similarly, criteria to define new serotypes are under discussion (Table 153.3).

Table 153.3 Criteria for species and serotype classification

Taxonomic level	Criteria
Enterovirus species (ICTV)	Members of a species of the genus Enterovirus: • Share greater than 70% aa identity in P1 • Share greater than 70% aa identity in the nonstructural proteins 2C+3CD • Share a limited range of host cell receptors • Share a limited natural host range • Have a genome base composition (G+C) which varies by no more than 2.5% • Share a significant degree of compatibility in proteolytic processing, replication, encapsidation and genetic recombination
Enterovirus serotype (proposed)	Members of a serotype of the a species of Enterovirus: • Share greater than 75% nucleotide similarity in the VP1-coding sequence • Share greater than 85% amino acid similarity in the VP1-coding sequence Members of different serotypes of species of Enterovirus: • Share less than 70% nucleotide similarity in the VP1-coding sequence • Share less than 85% amino acid similarity in the VP1-coding sequence

Structure and genome organization

The structure of poliovirus 1 was determined by X-ray crystallography in 1985.[9] Virions are spherical with a diameter of about 30 nm. The naked RNA genome is surrounded by proteins without a lipid envelope and its infectivity is not affected by organic solvents. The capsid is composed of four structural proteins – VP1, VP2, VP3 and VP4 – which are arranged with icosahedral symmetry (20 triangular faces and 12 vertices). A total of 60 structural proteins are involved. One copy of each of the four proteins builds the capsid promoter. VP1, VP2 and VP3 have no sequence homology but have the same topology. The internal VP4 is essential for the stability of the virion.

The three-dimensional structure of the poliovirus particle was solved as a star-shaped plateau (mesa) at the fivefold axis of symmetry surrounded by a deep depression (canyon) which is proposed as the receptor-binding site. The connecting loops and the *C*-terminus of the capsid proteins are located in the outer surface of the virion and determine the viral serotype since this area contains the major neutralization antigenic sites. The RNA genome stabilizes the capsid by interactions with conserved residues of VP4.

The genome of the enteroviruses is a single, positive-stranded RNA molecule. The genome varies in length from 7209 nt to 8450 nt and consists of three distinct regions: the 5′ nontranslated region (NTR), a single open reading frame (ORF) and the 3′ NTR (Fig. 153.1).

Infection by Enterovirus

PATHOGENICITY

Viral cycle

The picornavirus cycle of replication occurs, in general, in the cell cytoplasm. The cycle can be divided into:

- adsorption;
- penetration and uncoating;
- synthesis of virus-specific protein and RNA; and
- virus assembly and release from the infected host cell.

Again, the cycle of polioviruses is the most studied and best characterized.

Adsorption

Enterovirus adsorption at the cell surface is regulated by binding to cell-specific receptors. Its mechanism of pathogenesis appears to be mainly determined by cell tropism which depends on the specific recognition of the virus by receptors on the surface of susceptible cells (Table 153.4).

The poliovirus receptor (PVR, CD155) – a glycosylated, three-domain membrane protein that appears in different isoforms (molecular weight 67–80 kDa) – was identified by DNA transformation. Briefly, it was known that although mouse cells were permissive to poliovirus replication, they were not susceptible to poliovirus infection.[10] Thus, poliovirus-susceptible mouse cells were generated by introducing human genomic DNA into mouse cells. Later, these findings were confirmed *in vivo* using transgenic mice.[11] Although PVR transgenic mice are not susceptible to infection by the oral route they have proved to be a valuable model for studying the pathogenesis of poliomyelitis.[12] The poliovirus receptor presents three extracellular immunoglobulin-like domains: a membrane-distal V-type domain followed by two C2-type domains. The first domain contains the site that binds poliovirus.

Some species C enteroviruses use intercellular adhesion molecule-1 (ICAM-1, CD54) as their receptor. Their physiologic ligand is the lymphocyte function-associated molecule-1 (LFA-1), at the surface of leukocytes. The binding site is located in the first immunoglobulin-like domain. The superfamily of integrins also contains other enterovirus receptors: Decay-accelerating factor (DAF, CD55), vitronectin ($\alpha_v\beta3$) and very late-activating antigen-2 (VLA-2).

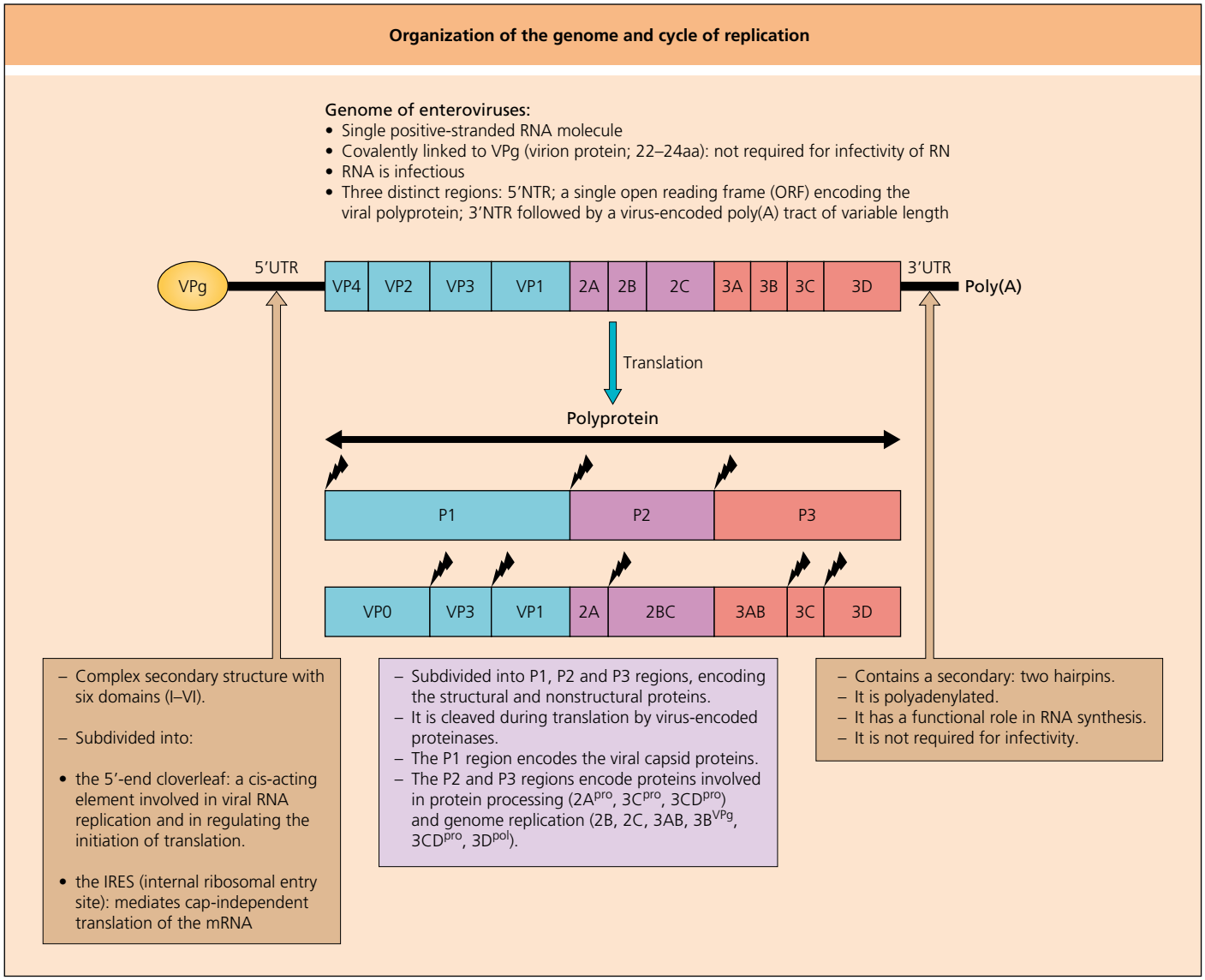


Fig. 153.1 Organization of the genome and cycle of replication. NTR, nontranslated region; UTR, untranslated region.

Table 153.4 Cell receptors for enterovirus

Virus	Receptor	Co-receptor
PV1, PV2, PV3	PVR	
CAV13, CAV18, CAV21	ICAM-1	
CAV21	Decay-accelerating factor (CD55)	ICAM-1
CAV9	$\alpha_v\beta_3$, $\alpha_v\beta_6$	
CBV1–6	Coxsackievirus–adenovirus receptor	
CBV1, CBV3, CBV5	Decay-accelerating factor (CD55)	$\alpha_v\beta_6$-integrin
E1, E8	$\alpha_v\beta_1$-integrin (VLA-2)	β_2-microglobulin
E3, E6, E7, E11–13, E20, E21, E24, E29, E33	Decay-accelerating factor (CD55)	β_2-microglobulin
EV70	Decay-accelerating factor (CD55)	
EV68	Sialic acid	

The receptor for coxsackievirus B is a 46 kDa glycoprotein that is also used as a receptor by human adenoviruses 2 and 5; thus, it is named the coxsackievirus and adenovirus receptor (CAR). For most enteroviruses, however, interaction with the receptor is not sufficient for infection as they require a second molecule, named co-receptor, for viral entry.

In general, the canyons of poliovirus and coxsackievirus are the sites of interaction with cell receptors as demonstrated by mutation analysis.

Penetration and uncoating

A series of conformational changes occur during virus entry. The interaction of poliovirus with its receptor, PVR, leads to major structural changes in the virus. The *N*-terminus of the capsid protein VP1 becomes accessible after release of the internal capsid protein VP4. These altered particles, known as A-particles, play a significant role in the more widely accepted hypothesis on poliovirus entry. The exposed *N*-terminal VP1 domain inserts into the cell membrane, forming a pore though which the viral RNA can travel to the cytoplasm. This role has been questioned by the finding that poliovirus can replicate at 25°C without formation of A-particles.

Synthesis of virus-specific protein and RNA

After the virus has been uncoated, the viral parental positive-stranded RNA serves as a template for synthesis of viral protein and RNA. After release of the VPg, viral protein synthesis takes place at the rough endoplasmic reticulum. Enteroviral mRNA has no m7G cap group. Initiation of viral protein synthesis takes place in the 5′ NTR, which houses the internal ribosome entry site (IRES). Following translation of mRNA, the viral proteins are originated by proteolytic cleavage. The first cleavage of the polyprotein is catalyzed by the 2A protease at a tyrosine–glycine dipeptide and results in release of the P1 structural segment of the genome. Subsequent cleavages of the P1 precursor into VP0, VP3 and VP1 are mediated by the 3CD protease. The cleavage of P2 and P3 precursors into stable end products is catalyzed by the 3Cpro/3CDpro at glutamine–glycine dipeptides.

The replication of viral RNA takes place at the smooth endoplasmic reticulum using intermediate negative-stranded RNA copies of the viral genome. The mature RNA polymerase 3D regulates viral transcription by interaction with viral and cellular factors through secondary structural motifs in the nontranslated regions of positive- and negative-stranded RNAs.

Virus assembly and release

Virus morphogenesis is characterized by intermediate assembly steps of the virus capsid via precursors and a procapsid. At the final stage, one molecule of positive-stranded RNA is encapsidated in the provirus and a final proteolytic cleavage of the precursor protein VP0 into VP2 and VP4 finalizes the virus maturation.

Cytopathic effects

The reproduction cycle of poliovirus is 6–8 hours. Infected cells are rounded and contain cytoplasmic protrusions. The nucleus is pyknotic and the chromatin is condensed. During the early stage of the cycle, mitosis is enhanced; at later stages, mitosis is arrested at metaphase. Two hours after infection, poliovirus shuts down synthesis of cellular protein, RNA and DNA. Cellular protein synthesis inhibition is caused by proteolytic cleavage of the cellular protein p220, the eukaryotic initiation factor eIF-4G. Analysis by scanning electron microscopy reveals that the first changes in the cell surface can be observed as early as 3 hours after infection. Eight hours after infection the changes are characterized by condensation of collapsed microvilli, formation of elongated filopodia and 'rounding up' of the cell. At this time, up to 10 000 virus particles have been synthesized in a single cell.

EPIDEMIOLOGY

Hosts

The natural host of enteroviruses is the human. Some animals are susceptible to experimental infection with human enteroviruses: nonhuman primates and CD155 transgenic mice for poliovirus; mice and some species of monkeys for coxsackievirus A and B and echovirus.

Coxsackievirus B5 is an interesting case. It is antigenically and genetically (50% homology) related to a porcine enterovirus (swine vesicular disease virus). Epidemiologic studies from outbreaks of this disease suggest that CBV5 was introduced into swine decades ago and led to establishment in this new host.[13]

The routes of enterovirus transmission are summarized in Table 153.5.

Table 153.5 Routes of transmission of enterovirus

Type	Route
Individual	• Fecal–oral route (associated with poor sanitary conditions). Except EV70, and possible EV68, all EV can be transmitted by fecal–oral route • Respiratory route (EV70 and possibly EV68) • Other proven routes of transmission: – Human breast milk – Environmental sources – Contaminated sources of water (swimming pools) – EV have been isolated from mollusks and crustaceans, demonstrating stability in sea salt waters – EV70 and CAV24 (causing hemorrhagic conjunctivitis) can be isolated from eye secretions; those causing vesicular exanthema can be spread by direct and indirect vesicular fluid. In both cases, common routes for infection are hand contact with secretions, and autoinoculation of mouth, nose and eyes
Groups	• Enterovirus household transmission • Familial outbreaks • Schools • Nosocomial transmission

Immune response

Innate immune system

The major factor affecting the outcome of an enteroviral infection is the efficacy of the immune response. The role of the innate immune system is especially important as an early determinant of pathogenesis and as a regulator of the adaptive immune response. Studies of poliovirus in wild-type CD155 transgenic mice demonstrate that the interferon response is an important determinant of tissue tropism and pathogenicity.[14] Interferons stimulate many genes that induce an antiviral state. Protection against poliovirus infection is mediated by interferon alpha (IFN-α)-induced 2′,5′-oligoadenylate synthetases and ribonuclease (RNase) L, and interferon gamma (IFN-γ)-induced double-stranded RNA (dsRNA)-dependent RNA-activated protein kinase (PKR) in coxsackievirus B-infected pancreatic islet cells.[15] Nuclear factor kappa B (NF-κB), a regulator that activates interferon beta (IFN-β) and initiates the cascade of proinflammatory cytokines, has been shown to be activated early after poliovirus infection. On the other hand, IFN-stimulated gene effects may be complex, and reductions in inflammation have been associated with a slight increase in survival in animal transgenic models.[16]

The role of innate lymphocytes and natural killer (NK) cells has been less studied and these cells may play a role, not only in protection, but also in mediating disease.

B-cell response

The key role of the humoral immune response is demonstrated by the following facts:

- high level of poliovirus neutralizing antibodies following natural infection or vaccination protect against disease, although there is no sterilizing immunity (e.g. infection of the gut can occur);
- high frequency of persistent infections in agammaglobulinemics; and
- increased susceptibility to enteroviral infection as well as increased severity of disease among neonates and infants.

The neutralization sites are generally conformational antigenic sites that correspond to exposed loops of the capsid proteins, although some regions that are internally located in the native virion can also function as neutralization sites. Only a limited number of studies have been performed to identify neutralization sites of nonpolio enterovirus; however, the results suggest similarities in the location of their antigenic sites to those found with poliovirus.

Circulation and geographic spread

Enteroviruses have a worldwide distribution. Within a given geographic locality, some serotypes may be endemic, with little or only gradual change in the range of serotypes present from year to year. In contrast, other serotypes may be introduced periodically, causing epidemics, with very few viral isolations reported in previous years. Since warm weather favors their spread, enteroviruses are more prevalent in temperate climates and in the tropics.

CLINICAL MANIFESTATIONS

Asymptomatic infections

Most enterovirus infections are silent, mild or subclinical. Since the cells of the gut epithelia normally have a high rate of turnover, it has been hypothesized that enterovirus passage through the gut may be the reason for the high incidence of inapparent infections.

Clinical syndromes

Nonpolio enterovirus

Enterovirus are implicated in a wide variety of clinical syndromes ranging from neurologic (aseptic meningitis, paralysis, encephalitis, ataxia and Guillain-Barré syndrome), muscular (pleurodynia, acute and chronic inflammatory muscle disease), respiratory infections, exanthema, herpangina and hand, foot and mouth disease (HFMD), acute hemorrhagic conjunctivitis, myocarditis and pericarditis, diabetes and pancreatitis, and neonatal sepsis-like infections (Table 153.6).

Among the nonpolio enteroviruses, two serotypes seem to be associated with particular severity of disease – enteroviruses 70 and 71 (EV70 and EV71).

EV71 is one of two serotypes most often associated with large outbreaks of HFMD and may also cause a variety of neurologic diseases, including aseptic meningitis, encephalitis and poliomyelitis-like paralysis. EV71 has caused epidemics of severe neurologic disease in Australia, Europe, Asia and the USA. Most recently, it was associated with fatal cases of brain stem encephalitis during large HFMD

Table 153.6 Clinical manifestations of enterovirus infections

Clinical disorders	Enterovirus serotypes	Characteristics
Paralysis	PV1–3 (severe paralysis) CVA4, 7, 9 E4–6 EV71 EV70	Transient mild paresis with aseptic meningitis may occur Younger children have milder disease
Aseptic meningitis	CVA2, 4, 7, 9, 10, 12, 16 CVB1–6 PV1–3 E4, 6, 7, 9, 11, 30 EV71	Benign in infants and children May involve rash or encephalitis Virus isolated from the throat, stool or cerebrospinal fluid
Acute hemorrhagic conjunctivitis	EV70 CVA24 variant	Rarely accompanied by transient radiculomyelopathy or poliomyelitis-like paralysis May produce subconjunctival hemorrhage and keratitis Pain, blurred vision, aversion to light, discharge from the eye
Hand, foot and mouth disease	CVA10, 16 (rarely CVA4, 5, 9) EV71	Vesicular exanthem, usually brief and benign, initially as a sore throat involving the tongue, followed by a rash on the hands and sometimes the feet The rash often forms small blisters, which lead to ulcers Symptoms generally resolve within a week
Rash and exanthema with fever	CVA2, 4–6, 9, 16 CVB1, 3, 4, 5 E2, 4, 9, 11, 14, 16, 19, 25	Nonpruritic, does not desquamate, on the face, neck, chest and extremities. Maculopapular, morbilliform; occasionally hemorrhagic, petechial or vesicular Summer and fall rashes in children
Respiratory disease	E4, 8, 9, 11, 20 CVA2, 10, 21, 24 CVB1–6	Fever, coryza, pharyngitis; in some patients vomiting and diarrhea Bronchitis and interstitial pneumonia are seen occasionally
Herpangina	CVA1–6, 8–10, 16, 21, 22	Palatal and pharyngeal lesions particularly severe, multiple ulcers in the throat. Swallowing becomes very painful; symptoms can persist for several weeks
Myocarditis	CVB1–6 CVA4, 16 E9	Newborns infected after birth or rarely in utero can present with sepsis with fever, lethargy, disseminated intravascular coagulation, bleeding and multiple organ failure; death may occur from circulatory collapse or hepatic failure Older children or adults may make a complete recovery
Diabetes and pancreatitis	CVB1–5 CVA9	Juvenile onset of IDDM 30% of children with IDDM have IgM antibodies to coxsackie B viruses
Chronic fatigue syndrome (myalgic encephalomyelitis)	CVBs	Persistent infection with high prevalence of antibodies against coxsackie B viruses

IDDM, insulin-dependent diabetes mellitus.

outbreaks in Malaysia in 1997 and in Taiwan in 1998. Like poliovirus, EV71 displays an affinity for anterior horn cells.

EV70 was first isolated in 1971 associated with a newly described pandemic acute hemorrhagic conjunctivitis (AHC). The epidemic of AHC had begun in 1969 in Ghana and spread across Africa to India and the Far East, with small outbreaks in Europe. It subsided, only to reappear in India in 1979, with subsequent spread to South America and the Caribbean, with isolated cases in the southern United States.

In the postpolio age, the more important causes of morbidity of enterovirus infections are the neurologic manifestations. Aseptic meningitis is a nonbacterial inflammation of the meninges associated with fever, headache, photophobia and meningeal signs in the absence of signs of brain parenchymal involvement. Meningitis and mild paresis can be induced by most nonpolio enteroviruses. Early symptoms are fever, malaise, headache, nausea and abdominal pain; these are followed by meningeal irritation with neck or back stiffness and vomiting before the onset of meningitis and mild paresis. Aseptic meningitis is very often accompanied by a rash. The manifestation of the CNS disease is usually milder and patients nearly always recover from paresis. Enteroviruses are the more frequent cause of aseptic meningitis in both children and adults in developed countries.

Encephalitis, ataxia and Guillain–Barré syndrome have been attributed to enterovirus infections. Encephalitis signifies that the brain parenchyma is infected and is not infrequently associated with a disturbed state of consciousness, focal neurologic signs and seizures, and sometimes with aseptic meningitis, resulting in meningoencephalitis. The frequency of diagnosis of viral encephalitis is low, although enteroviruses rank second to herpes simplex infection as a recognized cause of encephalitis in the USA.

Polio and acute flaccid paralysis

The infection route of polioviruses is the best understood of all enteroviruses. Following ingestion, poliovirus replicates locally at the initial virus implantation sites (e.g. tonsils, intestinal M cells, ileal Peyer's patches and the mesenteric lymph nodes) and occasionally enters the bloodstream and infects other tissues, including motor neurons.

Only a small proportion (<1%) of poliovirus infections in susceptible individuals result in paralytic poliomyelitis. Most infections are asymptomatic, although some people experience minor illness such as fever, headache and malaise or, in some cases, aseptic meningitis. Paralytic polio is preceded by 1–3 days of minor illness and a symptom-free interval, followed by the acute onset of flaccid paralysis with fever. Paralysis, usually asymmetric, progresses within a few days, and may affect skeletal muscles (spinal poliomyelitis), respiratory systems (bulbar poliomyelitis), or both (bulbospinal poliomyelitis). The loss of motor neurons is permanent and the denervated muscles atrophy.

Poliomyelitis is highly contagious and, in the past, wild viruses in endemic areas infected virtually the entire population. Paralytic attack rates vary by serotype. A small number of infected people developed recrudescence of paralysis and muscle atrophy several decades after paralytic poliomyelitis. However, postpoliomyelitis muscle atrophy cannot be assigned to persistent virus infection but results from the additive effects of physiologic aging and the prolonged loss of neuromuscular function from the earlier infection.

CONTROL OF INFECTION

Vaccines

Two different vaccines have been developed for poliovirus: Salk and Youngner's inactivated poliovirus vaccine (IPV)[17] and Sabin's attenuated oral poliovirus vaccine (OPV)[18] (Table 153.7). Both contain the three serotypes and induce humoral immunity with circulating antibodies. Both are safe and effective, each with particular advantages and disadvantages,[19] and both have played an important role in disease control.[20]

Eradication of poliomyelitis

The global effort to eradicate poliomyelitis is the largest public health initiative in history. The objectives of the Global Polio Eradication Initiative are to:

- interrupt transmission of the wild poliovirus;
- achieve certification of global polio eradication; and
- contribute to health systems development and surveillance for communicable diseases in a systematic way.

Four eradication strategies are recommended by the WHO:

- high routine infant immunization coverage with at least three doses of OPV plus a dose at birth in polio-endemic countries;
- national immunization days (NIDs) targeting all children <5 years;
- acute flaccid paralysis (AFP) surveillance and laboratory investigations; and
- mop-up immunization campaigns to interrupt the final transmission chains.

Overall, since launch of the Global Polio Eradication Initiative, the number of cases has fallen by over 99%, from an estimate of more than 350 000 cases in 1988 to 1997 reported cases in 2006. Widely endemic on five continents (125 countries) in 1988, polio is now found only in parts of Africa and South Asia (four countries: Afghanistan, India, Nigeria and Pakistan). In 1994, the WHO Region of the Americas with 36 countries (last indigenous case: Peru, 1991) was certified polio free, followed by the Western Pacific Region with 37 countries including China (last indigenous case: Cambodia, 1997) in 2000 and the European Region with 51 countries (last indigenous case: Turkey, 1998) in 2002.

The impact of the global initiative can also be monitored by the declining genetic diversity of wild poliovirus genotypes and lineages. Wild poliovirus type 2 has probably been eradicated, as it was last detected in October 1999 in Uttar Pradesh, India.[21] Wild poliovirus

Table 153.7 Characteristics of poliovirus vaccines

Properties	Inactivated poliovirus vaccine	Oral poliovirus vaccine
Antigen	Three wild reference strains: PV1 Mahoney; PV2 MEF-1 and PV3 Sauckett	Three attenuated
Administration	Injection	Orally
Induces	Circulating antibodies	Circulating antibodies and local immune response in intestinal lining
Protection	Protects individual against spread of the virus to central nervous system	Protects individual against infection and passive immunization of close contacts (risk for revertants)
Local immunity	Very low levels of local immunity (in the gut)	Induces intestinal local immunity
Viral circulation	Cannot spread poliovirus circulation	Interrupts wild poliovirus transmission

type 3 survives by steadily diminishing the chains of transmission, and in 2004 was endemic partially in five countries (India, Niger, Nigeria, Pakistan and Sudan). Wild poliovirus type 1 is highly localized in Afghanistan, Egypt, India and Pakistan, but remains more widespread in West and Central Africa following outbreaks in 2003–2004 from northern Nigeria.[22]

The endgame of poliovirus vaccination

Although OPV is safe and effective, in extremely rare cases (approximately 1 in every 2.5 million vaccine doses) the live attenuated vaccine virus in OPV can cause paralysis, either in the vaccinated child or in a close contact. Immune deficiency of the recipient may be among the possible causes. The possibility of genetic exchanges between the Sabin OPV strains and closely related enteroviruses has also come under study. Indeed, numerous vaccine-associated paralysis cases secondary to circulation of vaccine-derived poliovirus in poorly immunized communities has been documented.[23]

The recent roadblocks in India and Nigeria have led to a debate about the most appropriate vaccination protocol to achieve the goal of eradication.[24–26] In industrialized countries with no incidence of wild-type poliovirus-associated paralytic poliomyelitis, use of the OPV is avoided in favor of the safer IPV. In developing countries, however, the use of the more expensive IPV presents many logistic problems that are compounded by the fact that IPV is thought to be ineffective at producing immunity at the gut mucosal surface. As a result, it provides individual protection against polio paralysis but, unlike OPV, is less effective in preventing the spread of wild-type circulating poliovirus.

In light of these events, there is renewed interest to develop new or improved poliovirus vaccines. Three approaches have recently been proposed: attenuated vaccines where viral RNA translation efficiency is compromised by the introduction of synonymous rare codons,[27] where the 5′ IRES structure is modified to stabilize attenuation determinants[28] and where the replication fidelity is controlled by the use of a high-fidelity polymerase.[29]

Treatment

Infections caused by nonpolio enteroviruses cannot be prevented by vaccination. There are, however, several therapies (e.g. interferon and antiviral antibodies) that target early stages in the virus life cycle.[30] Pooled immunoglobulin was used with some success in enterovirus-induced CNS infections in immunocompromised patients and neonates.[31] Oral administration of WIN 54954 significantly decreased the number of upper respiratory infections. More recently, other WIN-type compounds (Pleconaril, VP63843, licensed to Schering-Plough) showed broad *in-vitro* inhibitory activity against 95% of the 215 nonpolio human and animal enteroviruses tested.[32] A clinical study – Pleconaril Enteroviral Sepsis Syndrome – is currently (2008) recruiting. All other antiviral compounds to date have been proven to show antiviral activity only in cell culture systems.[33]

DIAGNOSIS

A key factor to understanding the epidemiology of the enteroviruses, their diversity, frequent silent infections and variable clinical manifestations is virus identification and characterization. Effective diagnostic virology depends on adequate timing, collection of appropriate clinical specimens and optimal transport.

Specimens for diagnosis

For viral diagnosis, the specimens of choice will depend on the clinical manifestations observed and the type of assay available (classic or molecular), usually stool, rectal or throat swabs and cerebrospinal fluid (CSF) samples. Stool is generally the best specimen for enteroviral detection, since virus concentrations are the highest relative to the other sources (see Fig. 153.3). However, the detection of enteroviruses in stool or throat swabs might be misleading in terms of causality of the disease due to asymptomatic viral shedding. Generally, etiologic association of enterovirus with disease requires virus isolation from fluids or tissues manifesting lesions.

Thus, stools and rectal swabs are the specimens of choice for poliovirus diagnosis and AFP surveillance, CSF for neurologic disease and throat swabs for respiratory illness, together with sites of vesicular fluid for vesicular rashes, ocular secretions for hemorrhagic conjunctivitis, and tissue material from heart, muscle or brain for myocarditis, myositis or encephalitis, respectively.

Classic techniques

These involve direct detection, virus isolation, seroneutralization and serology.

Nonmolecular direct detection

Direct enterovirus detection by techniques such as immunofluorescence (IF), agglutination or ELISA are not useful due to virus antigenic diversity and the relatively low viral burden in most specimens. Electron microscopy had been used to visualize virus directly in specialized laboratories (Fig. 153.2).

Enterovirus isolation

Most enteroviruses will show a characteristic cytopathic effect (CPE) – rounded and refractile, with cell detachment in tissue culture (Fig. 153.3). It is common to use several cell lines to increase sensitivity. Viral propagation is usually attempted in Buffalo green monkey kidney (BGM) and human cell lines: rhabdomyosarcoma (RD), lung carcinoma (A-549) and embryonic fibroblasts (HEF). WHO recommends inoculation into L20B cells (a genetically engineered mouse cell line expressing PVR) for all specimens suspected to contain polioviruses. In the presence of CPE, identification of enterovirus should be done by IF.

Serotyping of enterovirus isolates

Traditional nonpolio enterovirus typing depends on neutralization with specific or pools of antisera. A standardized hyperimmune equine antiserum developed by Lim and Benyesh-Melnick (LBM) is used to identify 42 common enterovirus serotypes.[34] It consists

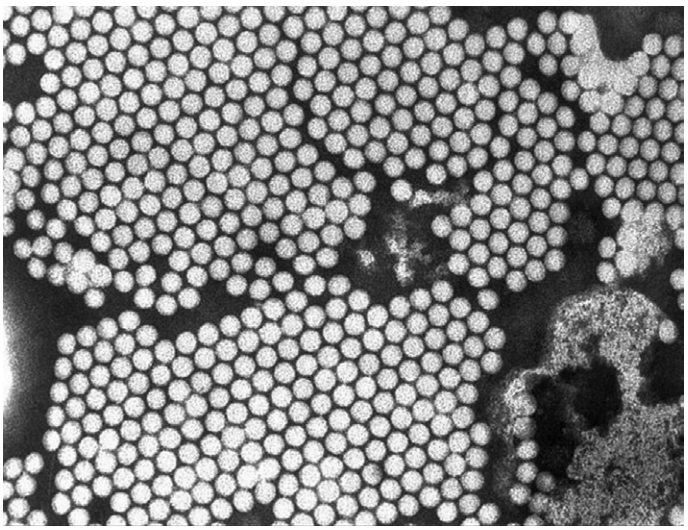


Fig. 153.2 Electron micrograph showing poliovirus particles negatively stained with 2% phosphotungstic acid.

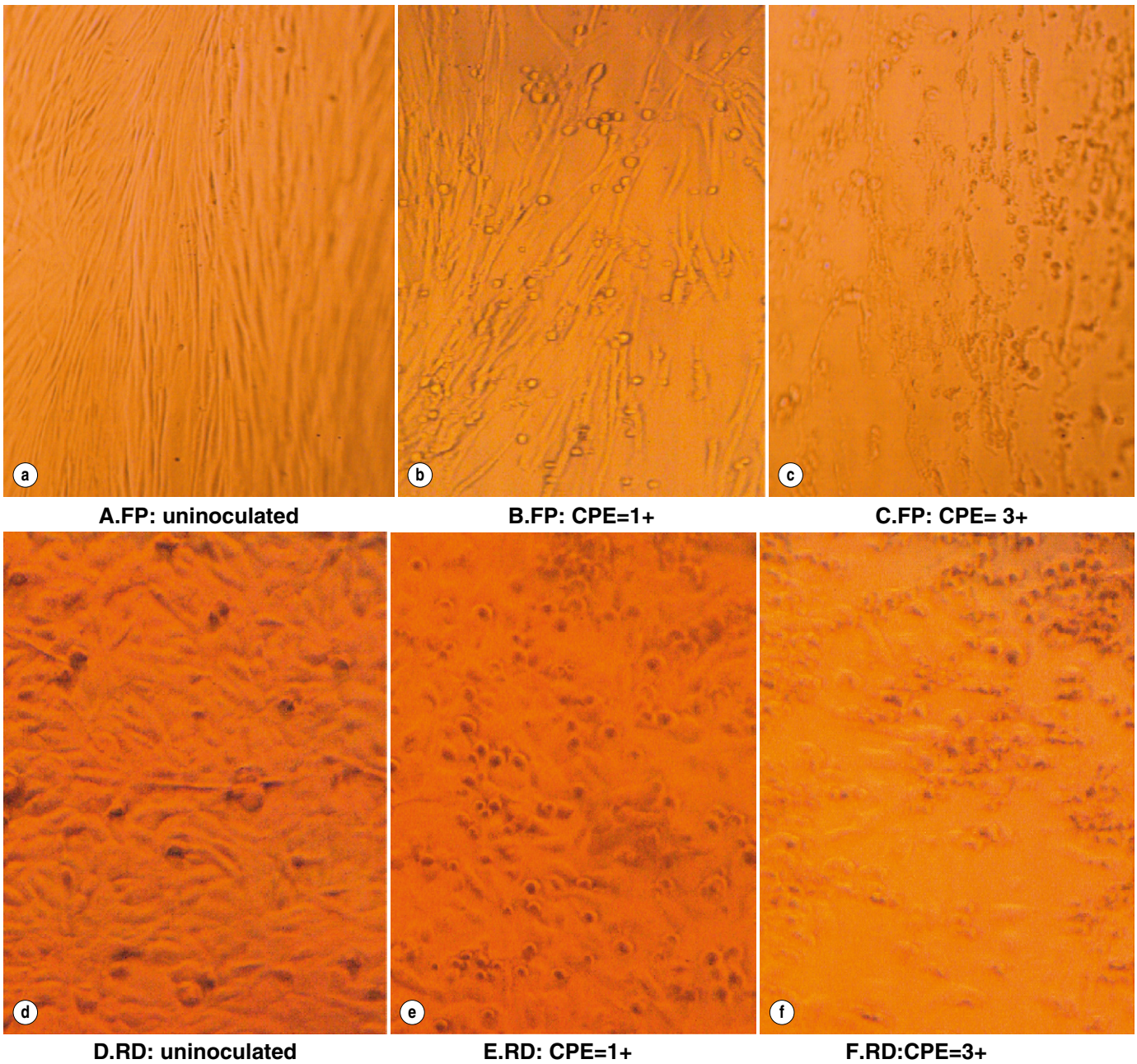


Fig. 153.3 Enterovirus (EV) cytopathic effect (CPE). Rhabdomyosarcoma (RD) cells and lung fibroblasts (FP) in culture: normal and EV infected, neither fixed nor stained. (a) Uninoculated FP monolayer. (b) FP showing typical early stage CPE by EV (25% CPE), especially rounding, indicating virus multiplication (1+). (c) FP illustrating more advanced CPE (3+); almost 100% of the cells are affected. Most of the cell sheet has come loose from the culture tube wall. The same occurs in (d), (e) and (f) with RD cells.

of a panel of eight combined pools (A–H). Enterovirus isolates are identified by their neutralization pattern against these pools (Fig. 153.4). A second panel of antisera was prepared by the National Institute of Public Health and the Environment (RIVM, Bilthoven, The Netherlands) to identify 19 coxsackievirus A serotypes.[35] After identification with these pools, further confirmation is needed by repeating the neutralization with the specific serotype antiserum. However, since such broad collections of specific reagents are rarely available in diagnostic and reference laboratories, this last step is normally ignored.

Polioviruses could be serotyped by IF with a panel of monoclonal antibodies (Chemicon, USA) or by neutralization with polyclonal antisera (RIVM). Differentiation between wild-type and Sabin vaccine-like strains is made by neutralization with monoclonal antibodies against wild-type and vaccine-like viruses or by ELISA using cross-adsorbed rabbit antisera. The latter is recommended by WHO for single serotype isolates.

Despite the use of all these techniques, some enteroviral isolates will remain unidentified because of:

- representing an untypable strain or mixture;
- problems with virus aggregation;
- extreme antigenic drift;
- microbial contamination of clinical samples, particularly throat swabs; and
- low infective viral load, thus precluding cell culture propagation.

In spite of all these drawbacks and the labor intensivity of the techniques involved, it is still important to attempt the traditional methods;

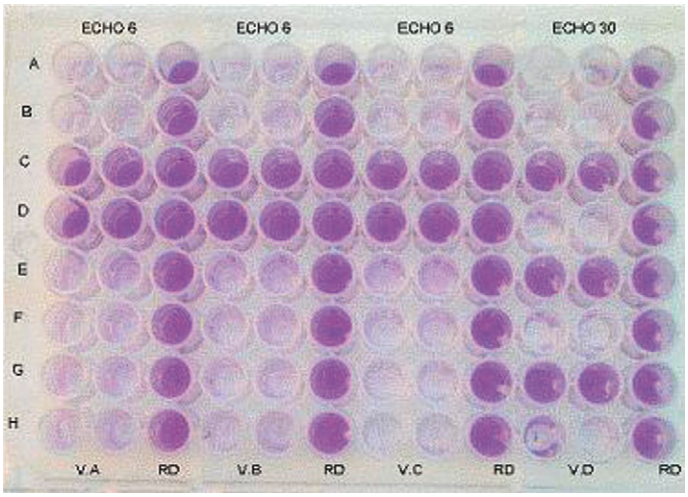


Fig. 153.4 Typing of enterovirus by seroneutralization. Rhabdomyosarcoma-stained microplate showing neutralization patterns of A, B, C and D virus with Lim Benyesh-Melnick antiserum pools (A–H). Viruses A, B and C are considered E6 as a result of neutralization with C and D pools containing specific antisera. Virus D is neutralized by C, E and G pools; thus, it is considered an E30.

pathogenesis and epidemiologic studies are often hampered by the lack of a viral specimen to continue its virulence characterization.

Serologic analysis

Several methods such as complement fixation, ELISA and immunoblotting are used to screen and detect enterovirus group-specific antibodies. However, the use of serologic techniques for primary diagnosis of suspected enteroviral illness is impractical due to virus diversity. On the other hand, these methods are useful to confirm poliovirus infection and immunization. Serologic diagnosis is performed by comparing titers in paired acute and convalescent serum specimens, in order to detect a significant fourfold antibody titer increase.

Many studies have examined the prevalence of antibodies to enteroviruses in specific populations.[36–43] The number of persons who show neutralizing antibody to any given enterovirus is large, indicating a high incidence of past infection. A high incidence of recent infection is also suggested by immunoglobulin M (IgM) surveys, which typically show 4–6% positivity. Infections with one serotype of enterovirus can boost the antibody titers to other enterovirus serotypes. The pattern of the heterotypic response varies by serotype and among individuals, and the pattern of serotype antibody prevalence varies by geographic location, by time and by age. Thus prevalence data from different years and locations are not directly comparable. These general characteristics must be considered when interpreting the findings of serologic studies of associations between enterovirus infection and disease.

Molecular techniques

Detection and typing

Application of molecular biology techniques to clinical virology has introduced significant changes in enteroviral diagnosis. Reliability has increased significantly. In addition, molecular techniques provide short detection times and diagnosis of isolates that do not readily grow in cell culture, and allow typing of enteroviruses that are difficult or impossible to identify by traditional techniques.[44]

Nucleic acid probe hybridization tests were the first application of molecular techniques to detect enteroviruses, but have been replaced by the polymerase chain reaction (PCR). In general, diagnostic assays for enterovirus are targeted to amplify conserved fragments of the 5′ NTR of the virus genome.[45]

The availability of sequence data for all members of the Enterovirus genus has enabled differentiation of viruses based on the nucleic acid sequence encoding the VP1 capsid protein.[46] Molecular identification can be performed using sequences of the reverse transcriptase PCR (RT-PCR) amplification products obtained directly from clinical specimens[47,48] or virus isolates.[48–54] Sequences surrounding the VP4–VP2 junction have also been used for molecular typing,[55–57] but this region does not always provide a reliable identification.[58] Only VP1 capsid sequence correlates with serotype, due to the high frequency of interspecies recombination among co-circulating enteroviruses (e.g. within HEV-B).[6,59–63] Several new enteroviruses, including EV73,[2,64] EV74,[3,6] EV75,[6] EV77 and EV78,[65] EV79–88, EV97, EV100 and EV101,[5] have been detected and characterized using molecular typing systems and sequencing (Fig. 153.5).

Phylogenetic studies of clinical isolates are useful in examining enteroviral evolution and to characterize enterovirus molecular epidemiology[66] (Fig. 153.6). There are two major patterns of enterovirus prevalence: endemic and epidemic.[67] The epidemic pattern is characterized by sharp peaks in numbers of isolations followed by periods with few isolations.[66,68,69] In contrast, the endemic pattern corresponds to serotypes that are isolated in about the same numbers every year, with rare peaks.[70]

Intratypic characterization of polioviruses

Differentiation between wild-type and Sabin vaccine-like strains could be performed by several molecular techniques:

- amplification of viral genomes by RT-PCR followed by restriction enzyme analysis;
- nucleic acid probe hybridization methods use digoxygenin-labeled Sabin and wild type-specific virus genotype-specific probes; or
- poliovirus Sabin strain-specific RT-PCR.

New strategies for enterovirus discovery and typing

The majority of molecular typing techniques today rely on the sequencing of partial fragments in the capsid region of the enterovirus genome. Reliability issues arise, however, when attempting to sequence samples directly without cell isolation which underestimate the circulation of enteroviruses that grow poorly or do not grow at all in cell culture.[71] As early as in 2001[48] and recently,[72] a number of strategies had been proposed to increase sensitivity via nested or semi-nested PCR. Despite moderate success, inherent limitations exist with nested PCR assays, including carry-over contamination and potential for misclassification. Since the method ability to adequately discriminate partially sequenced fragments for all enterovirus serotypes relies on the database of sequences behind it, the fact that different methods work in different regions of the genome makes comparison and sharing of the results impossible. Finally, these current typing methods do not offer the ability to detect recombination events in the enterovirus genome.

Ideally, future enterovirus typing should attempt to examine the viral genome as a whole. This would allow for greater insight into the recombination events that occur throughout the enterovirus genome, as well as provide the potential to attribute sequence information to virulence of the different serotypes. One particular platform that can be implemented for the broad interrogation of the viral genomes is DNA microarray technology. The multiplex capabilities of the DNA chip platform could expand the ability to characterize enterovirus genomes by investigating not only the capsid region, but also the noncapsid, nonstructural and untranslated regions as well. By screening the different regions of the enterovirus genome, it would be possible to observe the various enterovirus types as a whole without the need for full-length genome sequencing.

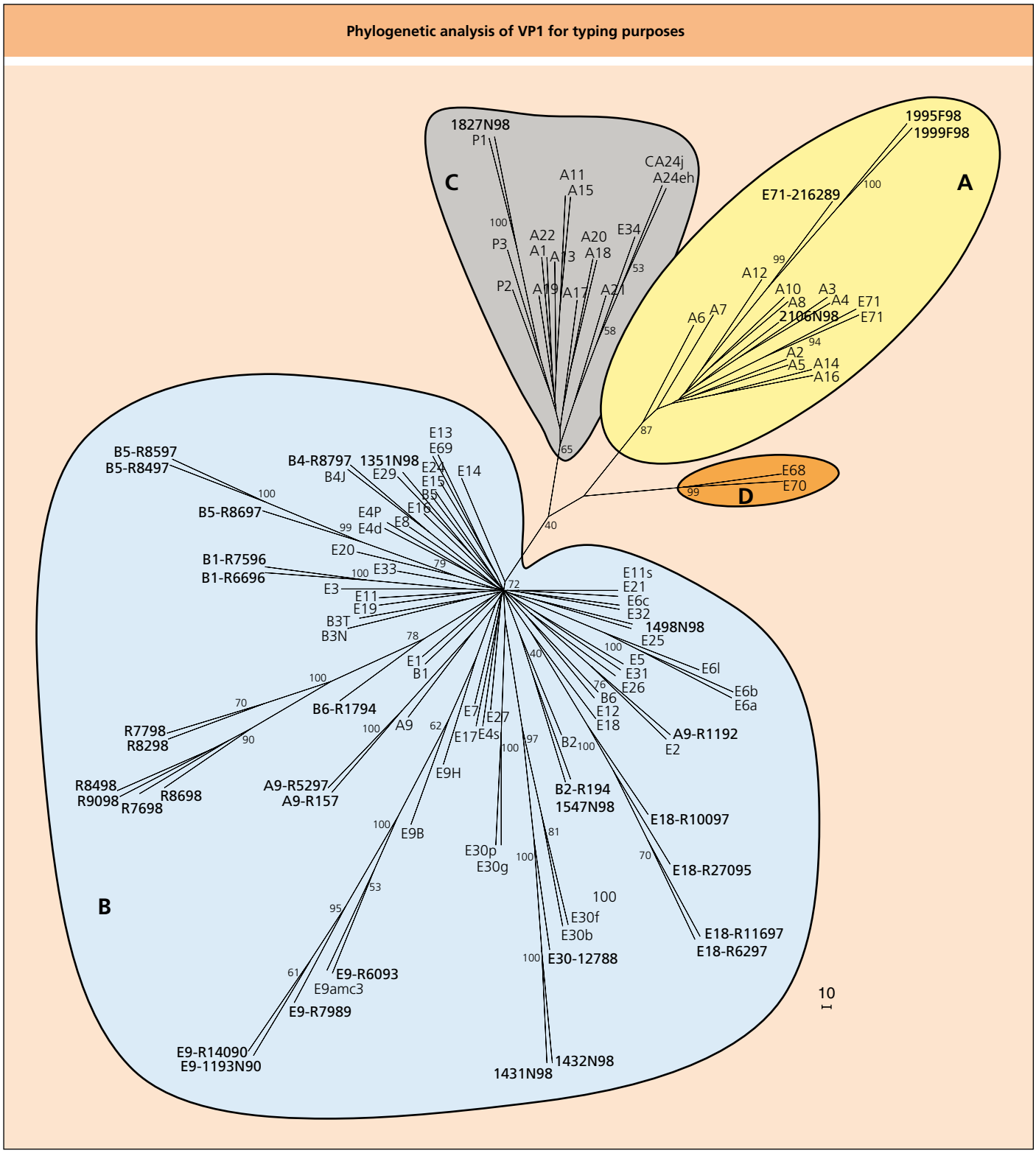


Fig. 153.5 Phylogenetic analysis of VP1 for typing purposes.

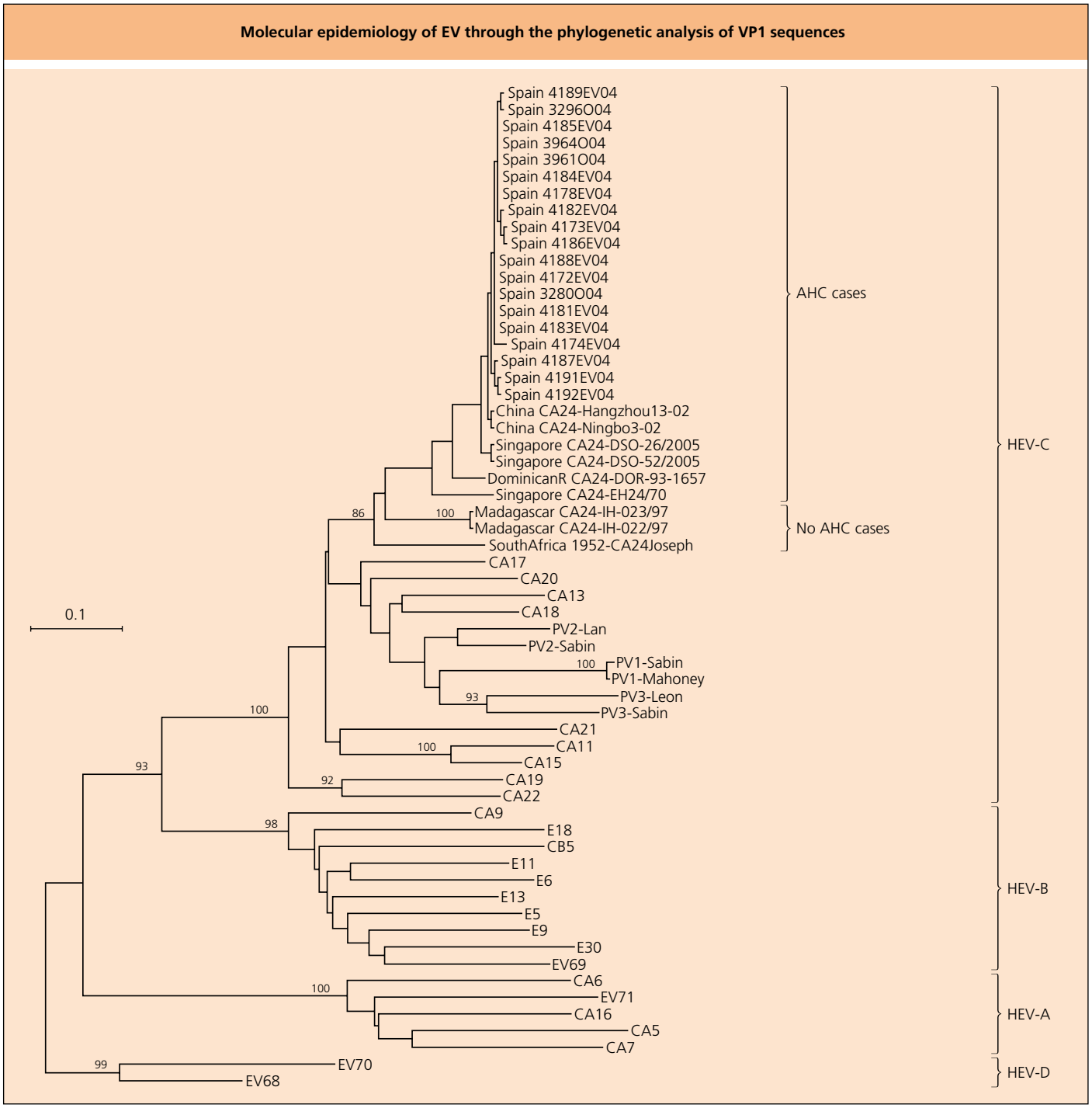


Fig. 153.6 Molecular epidemiology of enterovirus though the phylogenetic analysis of VP1 sequences. Phylogenetic tree with 28 CV-A24 sequences and other reference strains of species HEV-A to D based on 415 nucleotides within the 3'-VP1 region. HEV-D was used to root the dendrogram. The dendrograms were constructed by the neighbor-joining method, with 1000 bootstrap pseudoreplicates. Only bootstrap values >70% are shown at nodes. Genetic distances were calculated with Tamura-Nei's evolution model, and the horizontal branch lengths are drawn to scale.

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Chapter **154**

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Hepatitis viruses

INTRODUCTION

Viral hepatitis is a major public health problem throughout the world that affects several hundreds of millions of people. Viral hepatitis is a cause of considerable morbidity and mortality, both from acute infection and from chronic sequelae. In the case of infection with hepatitis B virus (HBV), hepatitis D virus (HDV) and hepatitis C virus (HCV), these chronic sequelae include chronic active hepatitis, cirrhosis and primary liver cancer.

There are five recognized pathogens, A–E; additionally the so-called non-A–E hepatitis viruses also exist, which include a range of unrelated and often unusual human pathogens (Table 154.1).

Hepatitis A Virus

Outbreaks of jaundice have been described frequently in many centuries and the term infectious hepatitis was coined in 1912 to describe the epidemic form of the disease. Hepatitis A virus (HAV) is spread by the fecal–oral route. It continues to be endemic throughout the world and hyperendemic in areas with poor standards of sanitation and hygiene.

NATURE

Classification

Electron microscopy examination of concentrates of filtered fecal extracts from patients in the early stages of infection reveals 27 nm particles typical of the Picornaviridae (Fig. 154.1). Hepatitis A virus (HAV) is a small, unenveloped, symmetric RNA virus. After the determination of the entire nucleotide sequence of the viral genome, comparison with other picornavirus sequences revealed limited homology to the enteroviruses and also to the rhinoviruses. Although the structure and genome organization is typical of the Picornaviridae, hepatitis A virus has been placed as hepatovirus within the heparnavirus genus.

Organization of the hepatitis A virus genome

The HAV genome comprises about 7500 nucleotides of positive-sense RNA. The RNA is polyadenylated at the 3′ end and has a viral polypeptide (VPg) attached to the 5′ end. A single, large open reading frame (ORF) occupies most of the genome and encodes a polyprotein with a theoretic molecular mass of M_r 252 000. An untranslated region of around 735 nucleotides precedes the ORF. Secondary structure within this region of the genome may be important for efficient translation of the RNA. There is also a short untranslated region at the 3′ end of the HAV genome (for further details see the web version of this chapter).

Hepatitis A virus is stable at low pH and is resistant to degradation by environmental conditions.

EPIDEMIOLOGY

Viral hepatitis type A (infectious or epidemic hepatitis) occurs endemically in all parts of the world, with frequent reports of minor and major outbreaks.[1] The exact incidence is difficult to determine because of the high proportion of subclinical infections and infections without jaundice, because of differences in surveillance and because of differing patterns of disease. The degree of underreporting is very high.

Specific serologic tests for hepatitis A have shown that infections with HAV are widespread and endemic in all parts of the world, that chronic excretion of HAV does not occur and that the infection is rarely transmitted by blood transfusion, although transmission by blood coagulation products has been reported. There is no evidence of progression to chronic liver disease.

The seroprevalence of antibodies to HAV has declined since the Second World War in many countries, and infection results most commonly from person-to-person contact, but large epidemics do occur. For example, an outbreak of hepatitis A in Shanghai in 1988 associated with the consumption of clams resulted in almost 300 000 cases.

PATHOGENICITY

The incubation period of hepatitis A is 3–5 weeks (Fig. 154.2), with a mean of 28 days. Subclinical and anicteric cases are common and, although the disease generally has a low mortality rate, patients may be incapacitated for many weeks. There is no evidence of progression to chronic liver damage.

Hepatitis A virus is spread by the fecal–oral route, most commonly by person-to-person contact; infection occurs readily under conditions of poor sanitation and overcrowding. Common source outbreaks are initiated most frequently by fecal contamination of water and food, but water-borne transmission is not a major factor in maintaining this infection in industrialized communities. On the other hand, many outbreaks related to food have been reported. This can be attributed to the shedding of large quantities of virus in the feces by infected food handlers during the incubation period of the illness – the source of the outbreak can often be traced to uncooked food or food that has been handled after cooking. Although hepatitis A remains endemic and common in developed countries, the infection occurs mainly in small clusters, often with only a few identified cases.

Table 154.1 The hepatitis viruses

Virus	Description	Viral group	Mode of transmission
Hepatitis A virus	Small unenveloped symmetric RNA virus	Hepatovirus, in heparnavirus genus	Fecal–oral
Hepatitis B virus	Enveloped double-stranded DNA virus	Hepadnavirus	Blood–blood Sexual
Hepatitis C virus	Enveloped single-stranded RNA virus	Related to flavivirus	Blood–blood
Hepatitis D virus	Circular single-stranded RNA virus	Related to plant viral satellites and viroids	Blood–blood
Hepatitis E virus	Unenveloped single-stranded RNA virus	Related to calciviruses; closely resembles rubella virus and a plant virus (beet necrotic yellow vein virus)	Fecal contamination of water Food-borne

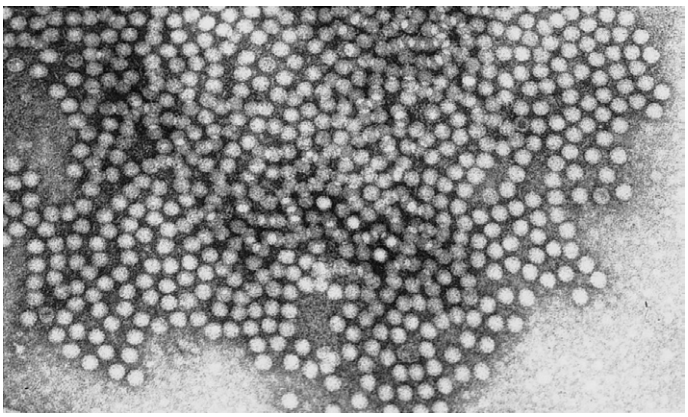


Fig. 154.1 Hepatitis A virus. Note the vast number of virus particles present in a fecal extract.

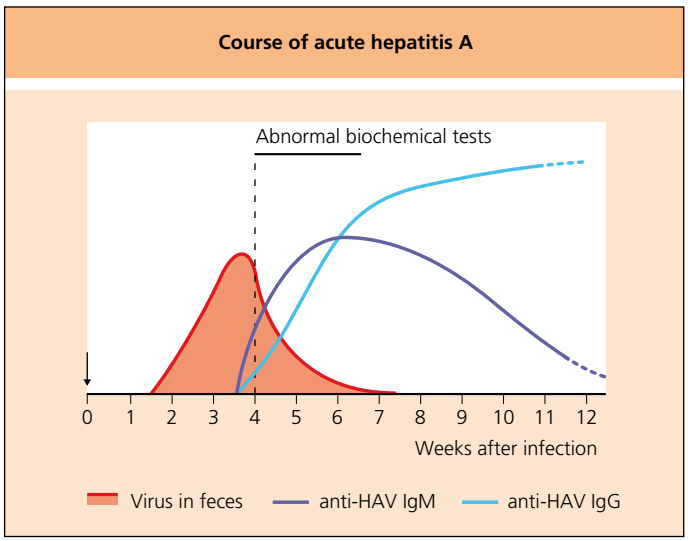


Fig. 154.2 Course of acute hepatitis A.

The clinical expression of infection with HAV varies considerably, ranging from subclinical, anicteric, mild illnesses in young children to the full range of symptoms with jaundice in adolescents and adults. The ratio of anicteric to icteric illnesses varies widely, both in individual cases and during outbreaks.

Hepatitis A virus enters the body by ingestion. The virus then spreads, probably by the bloodstream, to the liver, the target organ. Large numbers of virus particles are detectable in feces during the incubation period, beginning as early as 10–14 days after exposure and continuing, in general, until peak elevation of serum aminotransferases. Virus is also detected in feces early in the acute phase of illness but relatively infrequently after the onset of clinical jaundice. Immunoglobulin G antibody to HAV persists and is also detectable late in the incubation period, coinciding approximately with the onset of biochemical evidence of liver damage.

Hepatitis A viral antigen has been localized by immunofluorescence in the cytoplasm of hepatocytes after experimental transmission to chimpanzees. The antigen has not been found in any tissue other than the liver following experimental intravenous inoculation in susceptible nonhuman primates.

Pathologic changes induced by HAV appear only in the liver (Fig. 154.3). These include marked focal activation of sinusoidal lining cells; accumulation of lymphocytes and histiocytes in the parenchyma – these cells often replace hepatocytes that have been lost by cytolytic necrosis, especially in the periportal areas; occasional coagulative necrosis resulting in the formation of acidophilic bodies; and focal degeneration.

PREVENTION

In areas of high prevalence, most children are infected early in life, and such infections are generally asymptomatic. The later in life that infection occurs, the greater the clinical severity – fewer than 10% of cases of acute hepatitis A in children up to the age of 6 years are icteric, but this increases to 40–50% in the 6- to 14-year-age group, and to 70–80% in adults. Of 115 551 cases of hepatitis A in the USA between 1983 and 1987, only 9% of the cases, but more than 70% of the fatalities, were in those aged over 49 years. It is important, therefore, to protect those at risk because of personal contact with infected people or because of travel to a highly endemic area. Other groups at risk of hepatitis A infection include staff and residents of institutions for the mentally handicapped and day-care centers for children, sexually active male homosexuals, intravenous drug users, sewage workers, certain groups of health-care workers such as medical students on elective

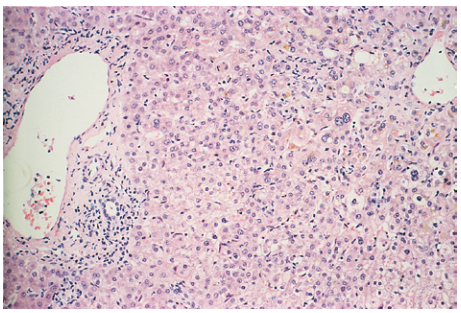


Fig. 154.3 Histologic changes in the liver of a patient with acute hepatitis A.

studies in countries where hepatitis A is common, military personnel and certain low socioeconomic groups in defined community settings. Patients with hemophilia and other blood-clotting disorders should be immunized.

Patients with chronic liver disease, especially if visiting an endemic area, should be immunized against HAV. In some developing countries, the incidence of clinical hepatitis A is increasing as improvements in socioeconomic conditions result in infection later in life, and strategies for immunization are yet to be agreed,[2] although active immunization for travelers, particularly to hyperendemic regions, is strongly recommended.

Passive immunization

Control of HAV infection is difficult. Because fecal shedding of the virus is at its highest during the late incubation period and the prodromal phase of the illness, strict isolation of cases is not a useful control measure. Spread of HAV is reduced by simple hygienic measures and the sanitary disposal of excreta.

Prevention or attenuation of a clinical illness can be achieved by the intramuscular administration of normal human immunoglobulin that contains at least 100 IU/ml of hepatitis A antibody. The dosage should be at least 2 IU of hepatitis A antibody per kilo of body weight; however, in pregnancy or in patients with liver disease, this dosage may be doubled (Table 154.2). Immunoglobulin does not always prevent excretion of HAV. The efficacy of passive immunization is based on the presence of hepatitis A antibody in normal human immunoglobulin, and the minimum titer of antibody required for protection is believed to be about 10 IU/l.

Titers of HAV antibody vary among batches of pooled normal human immunoglobulin and the titers are decreasing in batches obtained from pooled plasma of donors in industrialized countries, resulting in clinical cases despite prophylaxis with immunoglobulin. Immunoglobulin is used most commonly for close personal contacts of patients with hepatitis A and for those exposed to contaminated food. Immunoglobulin has also been used effectively for controlling outbreaks in institutions such as homes for the mentally handicapped and nursery schools. Prophylaxis with immunoglobulin is recommended for people without HAV antibody who are visiting highly endemic areas, but active immunization is recommended, particularly for travelers and for controlling outbreaks, especially if vaccine is given to all contacts immediately.

Active immunization against hepatitis A

Killed hepatitis A vaccines

The foundations for a hepatitis A vaccine were laid in 1975 by the demonstration that formalin-inactivated virus extracted from the liver of infected marmosets induced protective antibodies in susceptible marmosets on challenge with live virus. Subsequently, HAV was cultivated, after serial passage in marmosets, in a cloned line of fetal rhesus monkey kidney cells, thereby opening the way to the production of hepatitis A vaccines. Several formalin-inactivated hepatitis A vaccines are available, including combined vaccines with hepatitis B vaccine and with typhoid vaccine; other polyvalent vaccines are under development.

Table 154.2 Passive immunization with normal human immunoglobulin for travelers to highly endemic areas

Body weight	Period of stay less than 3 months	Period of stay longer than 3 months
≤55 lb (<25 kg)	50 IU anti-HAV (0.5 ml)	100 IU anti-HAV (1.0 ml)
55–66 lb (25–30 kg)	100 IU anti-HAV (1.0 ml)	250 IU anti-HAV (2.5 ml)
≥110 lb (>50 kg)	200 IU anti-HAV (2.0 ml)	500 IU anti-HAV (5.0 ml)

Live attenuated hepatitis A vaccines

The major advantages of live attenuated vaccines include ease of oral administration, relatively low cost because the virus vaccine strain replicates in the gut, production of both local immunity in the gut and humoral immunity, thereby mimicking natural infection, and the longer term protection afforded. Disadvantages include the potential of reversion toward virulence, interference with the vaccine strain by other viruses in the gut, relative instability of the vaccine and shedding of the virus strain in the feces for prolonged periods.

As with vaccine strains of polioviruses, attenuation may be associated with mutations in the 5′ noncoding region of the genome, and this affects the secondary structure of the protein compounds. There is also evidence that mutations in the region of the genome encoding the nonstructural polypeptides may be important for adaptation to cell culture and attenuation. However, markers of attenuation of HAV have not been identified and reversion to virulence may be a problem. On the other hand, there is also concern that 'over-attenuated' viruses may not be sufficiently immunogenic. Attenuated vaccines are available so far only in China, using the H2 and L-A-1 strains.

DIAGNOSTIC VIROLOGY

Various serologic tests are available for HAV, including immune electron microscopy, immune adherence hemagglutination, radioimmunoassay and enzyme immunoassay. Immune adherence hemagglutination, which has been used widely, is moderately specific and sensitive. Radioimmunoassay has been largely replaced by sensitive enzyme immunoassay techniques.

Only one serotype of HAV has been identified in volunteers infected experimentally with the MS-1 strain of hepatitis A, in patients from different outbreaks of hepatitis in different geographic regions and in random cases of hepatitis A. Seven genotypes of the virus are recognized.

Isolation of virus in tissue culture requires prolonged adaptation and it is therefore not suitable for diagnosis.

CLINICAL MANIFESTATIONS

The following description of the acute disease applies to all types of viral hepatitis. Prodromal nonspecific symptoms such as fever, chills, headache, fatigue, malaise and aches and pains are followed a few days later by anorexia, nausea, vomiting and right upper quadrant abdominal pain followed by the passage of dark urine and clay-colored stools. Jaundice of the sclera and skin develops. With the appearance of jaundice, there is usually a rapid subjective improvement of symptoms. The jaundice usually deepens for a few days and persists for 1–2 weeks. The feces then darken and the jaundice diminishes over a period of about 2 weeks. Convalescence may be prolonged (see Chapter 38).

Hepatitis E Virus

Retrospective testing of serum samples from patients involved in epidemics of hepatitis associated with fecal contamination of water supplies has indicated that an agent other than HAV or HBV was involved. Epidemics of enterically transmitted non-A, non-B hepatitis in the Indian subcontinent were first reported in 1980, but outbreaks involving tens of thousands of cases have also been documented in the former Soviet Union, South East Asia, northern Africa, Mexico

and elsewhere. A huge outbreak occurred in New Delhi in 1956–7, but tests for HAV or HBV were not available then. Infection has been reported in returning travelers, and sporadic infections have also been identified in patients who had not traveled abroad.

NATURE

Hepatitis E virus (HEV) is a nonenveloped single-stranded RNA virus that shares many biophysical and biochemical features with caliciviruses.

Morphologically the virus is spherical and unenveloped, measuring 32–34 nm in diameter with spikes and indentations visible on the surface of the particle. Confirmation that the virus has been propagated in cell culture is awaited. The virus appears similar to the caliciviruses. However, detailed morphologic studies and the lack of similarities in genome sequence between HEV and recognized caliciviruses suggest that HEV is a single member of a novel virus genus. However, HEV resembles most closely the sequences of rubella virus and a plant virus, beet necrotic yellow vein virus. These three viruses have been placed in separate but related families.

Genomic organization

Hepatitis E virus was cloned in 1991 and the entire 7.5 kb sequence is known. The genome is a single-stranded, positive-sense, polyadenylated RNA molecule, with three overlapping ORFs.

Recent studies indicate that HEV may be distributed into at least nine different groups based on sequencing.

EPIDEMIOLOGY

The epidemiologic features of the infection resemble those of hepatitis A. The highest attack rates are found in young adults and high mortality rates of 10–40% (and occasionally higher) have been reported in women infected during the third trimester of pregnancy.

All epidemics of hepatitis E reported to date have been associated with fecal contamination of water, with the exception of a number of food-borne outbreaks in China. Sporadic hepatitis E has been associated with the consumption of uncooked shellfish and has been seen in travelers returning from endemic areas. Hepatitis E virus is an important cause of large epidemics of acute hepatitis, and these, together with a high prevalence of antibody determined by serologic tests, have occurred in the subcontinent of India, South East and central Asia, the Middle East, and northern and western Africa. There have also been outbreaks in eastern Africa and Mexico.[3]

Unexpectedly, the highest prevalence of antibody to HEV is found in young adults and not in infants and children.

Hepatitis E virus has also been isolated from patients with sporadic acute hepatitis in countries not considered to be endemic for HEV such as the USA, Italy and other European countries and in individuals who had not traveled abroad. There is now evidence that HEV may have an animal reservoir and there are HEV isolates from swine with high sequence identity to human HEV strains isolated from pigs in areas without HEV epidemics. There is recent evidence of a higher prevalence of HEV antibodies among swine farmers, particularly in those with an occupational history of cleaning barns or assisting sows at birth, and also a history of drinking raw milk. Zoonotic spread of HEV may also occur from rodents.

PATHOGENICITY

Virus-like particles have been detected in the feces of infected people by immune electron microscopy using convalescent serum. Cross-reaction studies between sera and virus in feces associated with a variety of epidemics in several different countries suggest that a single serotype of virus is involved, although two distinct isolates have been recognized and designated as the Burma (B) strain and the Mexico (M) strain.

The average incubation period is slightly longer than for HAV, with a mean of 6 weeks.

The clinical spectrum of infection with HEV is similar to that caused by the other hepatitis viruses, although cholestatic features are common. Hepatitis E does not progress to chronic liver disease and persistent infection does not occur.

Infection with HEV is associated with a relatively high mortality of 1–4% of patients admitted to hospital, and fulminant hepatitis during pregnancy may lead to a mortality rate of 10–40%. Premature delivery and infant mortality of up to 33% have been recorded.

PREVENTION

The provision of safe and chlorinated public water supplies, public sanitation and hygiene, safe disposal of feces and raw sewage (including safeguarding the water supply from animal waste from farm animals) and personal hygiene are essential measures.

Passive protection with immunoglobulin prepared from pooled plasma obtained from blood donors living in endemic countries is not effective.

Several recombinant and subunit HEV vaccines have been developed and are undergoing clinical trials. A purified polypeptide vaccine preparation which was produced in *Spodoptera frugiperda* cells with recombinant baculovirus containing a truncated HEV genomic sequence encoding the capsid antigen was found to be safe and effective in the prevention of hepatitis E in a high-risk population in Nepal.[4]

DIAGNOSTIC VIROLOGY

Serologic tests are necessary to establish the diagnosis. The tests commercially available at present detect anti-HEV IgM in up to 90% of acute infections if serum is obtained 1–4 weeks after the onset of illness, with IgM remaining detectable for about 12 weeks. Anti-HEV IgG appears early and reaches a maximum titer 4 weeks after the onset of illness, falling rapidly thereafter.

Tests for HEV RNA by the polymerase chain reaction (PCR) are available in specialized laboratories.

CLINICAL MANIFESTATIONS

Individual cases cannot be differentiated on the basis of clinical features from other cases of hepatitis. In epidemics, most clinical cases will have anorexia, jaundice and hepatomegaly. Serologic tests indicate, however, that clinically inapparent cases occur (see Chapter 38).

Hepatitis B Virus

Hepatitis B virus was recognized originally as the agent responsible for 'serum hepatitis', an important and frequent cause of acute and chronic infection of the liver including up to 80% of cases of primary hepatocellular carcinoma.

NATURE

Hepatitis B virus is a member of the hepadnavirus group, which contains double-stranded DNA viruses that replicate by reverse transcription. Hepatitis B virus is endemic in the human population and hyperendemic in many parts of the world. The virus is transmitted

essentially by blood–blood contact and by the sexual route. Mutations of the surface coat protein of the virus and of the core and other proteins have been identified in recent years.[5–7] Natural hepadnavirus infections also occur in other animals, including woodchucks, beechy ground squirrels and ducks.

Structure and organization of the virus

The hepatitis B virion is a 42 nm particle comprising an electron-dense core (nucleocapsid), which is 27 nm in diameter, and an outer envelope of the surface protein (hepatitis B surface antigen, HBsAg) embedded in membranous lipid derived from the host cell (Fig. 154.4). The surface antigen, originally referred to as Australia antigen, is produced in excess by the infected hepatocytes and is secreted in the form of 22 nm particles and tubular structures of the same diameter.

The 22 nm particles are composed of the major surface protein in both nonglycosylated (p4) and glycosylated (gp27) form in approximately equimolar amounts, together with a minority component of the so-called middle proteins (gp33 and gp36) which contain the pre-S2 domain, a glycosylated 55 amino acid amino-terminal extension. The surface of the virion has a similar composition but also contains the large surface proteins (gp39 and gp42) which include both the pre-S1 and pre-S2 regions. These large surface proteins are not found in the 22 nm spherical particles (but may be present in the tubular forms in highly viremic people) and their detection in serum correlates with viremia. The domain that binds to the specific HBV receptor on the hepatocyte is believed to reside within the pre-S1 region.

The nucleocapsid of the virion consists of the viral genome surrounded by the core antigen (HBcAg). The genome, which is approximately 3.2 kb in length, has an unusual structure and is composed of two linear strands of DNA held in a circular configuration by base-pairing at the 5′ ends (for further details, see the web version of this chapter).

There are nine subtypes of HBV, with a common main antigenic determinant, *a*. The nine subtypes described, *ayw*1–*ayw*4, *ayr*, *adw*2, *adw*4, *adrq*$^-$ and *adrq*$^+$, differ in their geographic distribution. Subtyping is employed for epidemiologic studies and to trace nosocomial infection. Traditional subtyping is complemented by classification of different HBV strains into genotypes A–G.

EPIDEMIOLOGY

More than a third of the world's population has been infected with HBV, and the World Health Organization estimates that HBV results in 1 000 000–2 000 000 deaths every year.

The virus persists in approximately 5–10% of immunocompetent adults and in as many as 90% of infants infected perinatally. Persistent carriage of HBV, defined by the presence of HBsAg in the serum for more than 6 months, has been estimated to affect about 350 000 000 people worldwide.

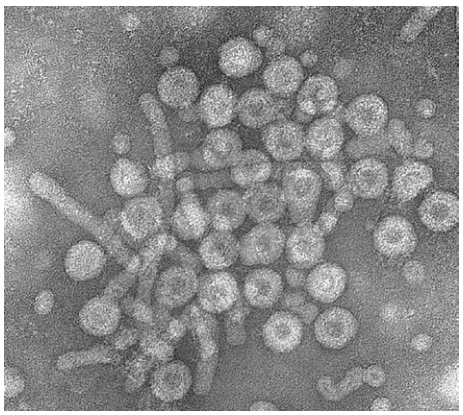


Fig. 154.4 Serum from a patient with hepatitis B. The double-shelled particle is the complete virion. Tubular structures and 22 nm HBsAg particles are present in small numbers.

Although various body fluids (blood, saliva, menstrual and vaginal discharges, serous exudates, seminal fluid and breast milk) have been implicated in the spread of infection, infectivity appears to be especially related to blood and to body fluids contaminated with blood. The epidemiologic propensities of this infection are therefore wide; they include infection by inadequately sterilized syringes and instruments, and transmission by unscreened blood transfusion and blood products, by close contact and by sexual contact. Transmission of HBV from mother to child may take place – antenatal transmission is rare but perinatal transmission occurs frequently; in some parts of the world (South East Asia and Japan) perinatal transmission is very common. Clustering of hepatitis B may occur within family groups, but it is not related to genetic factors and does not reflect maternal or sexual transmission. The mode of intrafamilial spread of HBV is not known.

PATHOGENICITY

The incubation period of hepatitis B is variable, with a range of 1–6 months.

As mentioned above, about 350 million people are carriers of HBV. The pathology is mediated by the cellular immune response of the host to the infected hepatocytes. Long-term continuing virus replication may lead to progression to chronic liver disease, cirrhosis and hepatocellular carcinoma (Fig. 154.5).

In the first phase of chronicity, virus replication continues in the liver and replicative intermediates of the viral genome may be detected in DNA extracted from liver biopsies. Markers of virus replication in serum include HBV DNA, the surface proteins (HBsAg) and a soluble antigen, HBeAg, which is secreted by infected hepatocytes. In those infected at a very young age this phase may persist for life but, more usually, virus levels decline over time. Eventually, in most infected people, there is immune clearance of infected hepatocytes associated with seroconversion from HBeAg to anti-HBe.

During the period of replication, the viral genome may integrate into the chromosomal DNA of some hepatocytes and these cells may persist and expand clonally. Rarely, seroconversion to anti-HBs follows clearance of virus replication but, more frequently, HBsAg persists during a second phase of chronicity as a result of the expression of integrated viral DNA.

Immune responses

Antibody and cell-mediated immune responses to various types of antigen are induced during the infection; however, not all of these are protective and in some instances they may cause autoimmune phenomena that contribute to disease pathogenesis. The immune response to infection with HBV is directed toward at least three antigens: HBsAg, the core antigen and the e antigen. Evidence suggests that the pathogenesis of liver damage in the course of HBV infection is related to the immune response by the host.

The surface antigen appears in the serum of most patients during the incubation period, 2–8 weeks before biochemical evidence of liver damage or onset of jaundice. The antigen persists during the acute illness and usually clears from the circulation during convalescence. Next to appear in the circulation is the virus-associated DNA polymerase activity, which correlates in time with damage to liver cells as indicated by elevated serum transaminases. The polymerase activity persists for days or weeks in acute cases and for months or years in some persistent carriers. Antibody of the IgM class to the core antigen is found in the serum 2–10 weeks after the surface antigen appears and persists during replication of the virus. Core antibody of the IgG class is detectable for many years after recovery. Finally, antibody to the surface antigen component, anti-HBs, appears.

During the incubation period and during the acute phase of the illness, surface antigen–antibody complexes may be found in the serum of some patients. Immune complexes have been found by electron microscopy in the serum of all patients with fulminant hepatitis but are seen only infrequently in nonfulminant infection.

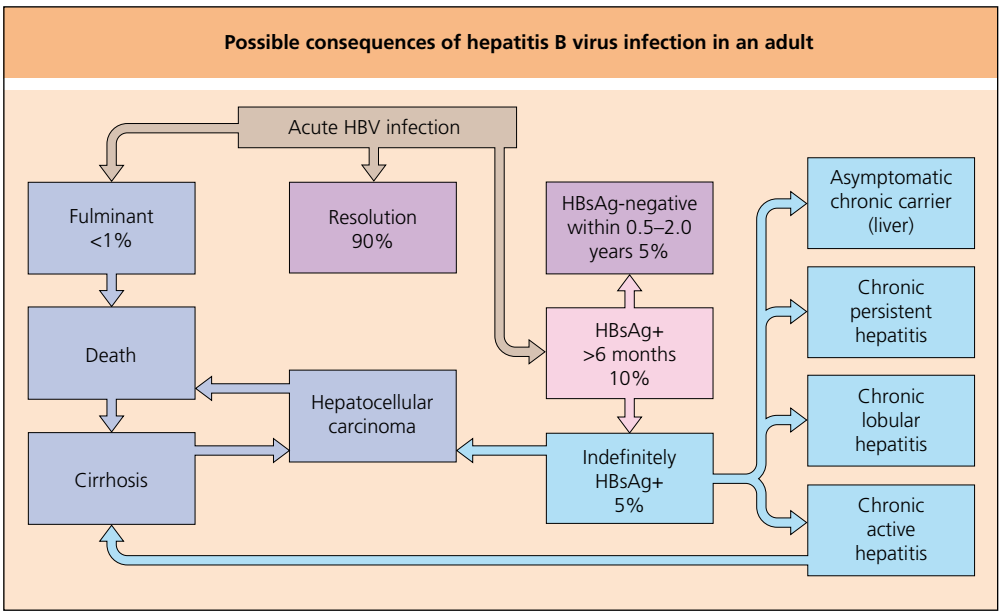


Fig. 154.5 Possible consequences of hepatitis B virus infection in an adult.

Immune complexes have been identified in variable proportions of patients with virtually all the recognized chronic sequelae of acute hepatitis B. Deposits of such immune complexes have also been demonstrated in the cytoplasm and plasma membrane of hepatocytes and on or in the nuclei; the reason why only a small proportion of patients with circulating complexes develop vasculitis or polyarteritis is, however, not clear. Perhaps complexes are critical pathogenic factors only if they are of a particular size and of a certain antigen–antibody ratio.

Cellular immune responses are known to be particularly important in determining the clinical features and course of viral infections. The occurrence of cell-mediated immunity to HBV antigens has been demonstrated in most patients during the acute phase of hepatitis B and in a significant proportion of patients with surface-antigen-positive chronic active hepatitis, but not in asymptomatic persistent HBV carriers. These observations suggest that cell-mediated immunity may be important in terminating the infection and, in certain circumstances, in promoting liver damage and in the genesis of autoimmunity. Evidence also suggests that progressive liver damage may result from an autoimmune reaction directed against hepatocyte membrane antigens, initiated in many cases by infection with HBV.

Hepatitis B virus and hepatocellular carcinoma

When tests for HBsAg became widely available, regions of the world where the chronic carrier state is common were found to be coincident with those where there is a high prevalence of primary liver cancer (Fig. 154.6). Furthermore, in these areas, patients with this tumor are almost invariably seropositive for HBsAg. A prospective study in Taiwan revealed that 184 cases of hepatocellular carcinoma occurred in 3454 carriers of HBsAg at the start of the study, but only 10 such tumors arose in the 19 253 control males who were HBsAg negative.[8]



Fig. 154.6 Hepatocellular carcinoma.

Other case-control and cohort studies and laboratory investigations indicate that there is a consistent and specific causal association between HBV and hepatocellular carcinoma, and that up to 80% of such cancers are attributable to this virus. Hepatitis B is thus second only to tobacco among the known human carcinogens. Primary liver cancer is the seventh most common cancer in males and the ninth most common in females. Hepatocellular carcinoma is one of the three most common causes of cancer deaths in males in east and South East Asia, the Pacific Basin and sub-Saharan Africa.

Southern hybridization of tumor DNA yields evidence of chromosomal integration of viral sequences in at least 80% of hepatocellular carcinomas from HBsAg carriers. There is no similarity in the pattern of integration between different tumors, and variation is seen both in the integration sites and in the number of copies or partial copies of the viral genome. Sequence analysis of the integrants reveals that the direct repeats in the viral genome often lie close to the virus–cell junctions, suggesting that sequences around the ends of the viral genome may be involved in recombination with host DNA. Integration seems to involve microdeletion of host sequences, and rearrangements and deletions of part of the viral genome also may occur. When an intact surface gene is present, the tumor cells may produce and secrete HBsAg in the form of 22 nm particles. Production of HBcAg by tumors is rare, however, and the core ORF is often incomplete; modifications such as methylation may also modulate its expression. Cytotoxic T lymphocytes targeted against core gene products on the hepatocyte surface seem to be the major mechanism of clearance of infected cells from the liver. Thus, there may be immune selection of cells with integrated viral DNA that are incapable of expressing HBcAg.

The mechanisms of oncogenesis by HBV remain uncertain. Hepatitis B virus may act nonspecifically by stimulating active regeneration and cirrhosis, which may be associated with chronicity. However, HBV-associated tumors arise occasionally in the absence of cirrhosis, and such hypotheses do not explain the frequent finding of integrated viral DNA in tumors. In rare instances, the viral genome has been found to be integrated into cellular genes such as cyclin A and a retinoic acid receptor. Translocations and other chromosomal rearrangements have also been observed. Although insertional mutagenesis of HBV remains an attractive hypothesis to explain its oncogenicity, there is insufficient supportive evidence. Transactivation by the X-gene is another possible mechanism.

Like many other cancers, the development of hepatocellular carcinoma is likely to be a multifactorial process. The clonal expansion of cells with integrated viral DNA seems to be an early stage in this process and such clones may accumulate in the liver throughout the period of active virus replication. In areas where the prevalence of primary liver cancer is high, virus infection usually occurs at an early age and virus replication may be prolonged, although the peak incidence of tumor is many years after the initial infection.

PREVENTION

The discovery of variation in the epitopes presented on the surface of the virions and subviral particles identified several subtypes of HBV, which differ in their geographic distribution. All isolates of the virus share a common epitope – *a*. This epitope is a domain of the major surface protein, which is believed to protrude as a double loop from the surface of the particle. Two other pairs of mutually exclusive antigenic determinants – *d* or *y* and *w* or *r* – are also present on the major surface protein. These variations have been correlated with single nucleotide changes in the surface ORF, which lead to variation in single amino acids in the protein. Four principal subtypes of HBV are recognized: *adw*, *adr*, *ayw* and *ayr*. Subtype *adw* predominates in northern Europe, the Americas and Australasia, and is also found in Africa and Asia. Subtype *ayw* is found in the Mediterranean region, eastern Europe, northern and western Africa, the Middle East and the Indian subcontinent. In the Far East, subtype *adr* predominates, but the rarer subtype *ayr* is occasionally found in Japan and Papua New Guinea.

Passive immunization

Hepatitis B immunoglobulin (HBIG) is prepared specifically from pooled plasma with a high titer of hepatitis B surface antibody and may confer temporary passive immunity under certain defined conditions. The major indication for the administration of HBIG is a single acute exposure to HBV, such as occurs when blood containing surface antigen is inoculated, ingested or splashed on mucous membranes and the conjunctiva. The optimal dose has not been established but doses in the range of 250–500 IU have been used effectively. It should be administered as early as possible after exposure and preferably within 48 hours. The dose is usually 3 ml (200 IU/ml) in adults. The first dose should not be administered more than 7 days following exposure. It is generally recommended that two doses of HBIG should be given 30 days apart.

Results with the use of HBIG for prophylaxis in neonates at risk of infection with HBV are encouraging if the immunoglobulin is given as soon as possible after birth or within 12 hours of birth, and the chance of the baby developing the persistent carrier state is reduced by about 70%. More recent studies using combined passive and active immunization indicate an efficacy approaching 90%. The dose of HBIG recommended in the newborn is 1–2 ml (200 IU/ml).

Active immunization

The major response of recipients of hepatitis B vaccine is to the common *a* epitope with consequent protection against all subtypes of the virus. First-generation vaccines were prepared from 22 nm HBsAg particles purified from plasma donations from chronic carriers. These preparations are safe and immunogenic but have been superseded in most countries by recombinant vaccines produced by the expression of HBsAg in yeast cells. The expression plasmid contains only the 3′ portion of the HBV surface ORF, and only the major surface protein, without pre-S epitopes, is produced. Vaccines containing pre-S2 and pre-S1, as well as the major surface proteins expressed by recombinant DNA technology, are undergoing clinical trials.

In many areas of the world with a high prevalence of HBsAg carriage, such as China and South East Asia, the predominant route of transmission is perinatal. Although HBV does not usually cross the placenta, the infants of viremic mothers have a very high risk of infection at the time of birth.

Administration of a course of vaccine with the first dose immediately after birth is effective in preventing transmission from an HBeAg-positive mother in approximately 70% of cases and this protective efficacy rate may be increased to greater than 90% if the vaccine is accompanied by the simultaneous administration of HBIG.

Immunization against HBV is now recognized as a high priority in all countries. Universal vaccination of infants and adolescents is under examination as a possible strategy to control the transmission of this infection. More than 168 countries now offer universal hepatitis B vaccine, including the USA, Canada and most western European countries.[9]

In a number of countries with a low prevalence of hepatitis B, immunization against HBV is recommended only to groups that are at an increased risk of acquiring this infection. These groups include people requiring repeated transfusions of blood or blood products, people undergoing prolonged inpatient treatment, patients who require frequent tissue penetration or need repeated circulatory access, patients with natural or acquired immune deficiency, and patients with malignant diseases. Viral hepatitis is an occupational hazard among health-care personnel and the staff of institutions for the mentally retarded and some semiclosed institutions. High rates of infection with HBV occur in narcotic drug addicts and intravenous drug users, sexually active male homosexuals and prostitutes. People working in highly endemic areas are at an increased risk of infection and should be immunized. Young infants, children and susceptible people (including travelers) living in certain tropical and subtropical areas where socioeconomic conditions are poor and the prevalence of hepatitis B is high should also be immunized. It should be noted that, in about 30% of patients with hepatitis B, the mode of infection is not known – this is a powerful argument in favor of universal immunization.

Site of injection for vaccination and antibody response

Hepatitis B vaccination should be given in the upper arm or the anterolateral aspect of the thigh and not in the buttock. There are over 100 reports of unexpectedly low antibody seroconversion rates after hepatitis B vaccination using injection into the buttock. In one center in the USA a low antibody response was noted in 54% of healthy adult health-care personnel. Many studies have since shown that the antibody response rate is significantly higher in centers using deltoid injection than centers using the buttock. On the basis of antibody tests after vaccination, the Advisory Committee on Immunization Practices of the Centers for Disease Control and Prevention in the USA recommended that the arm be used as the site for hepatitis B vaccination in adults, as has the Department of Health in the UK.

These observations have important public health implications, well illustrated by the estimate that about 20% of the 60 000 people immunized against HBV in the buttock in the USA by March 1985 had failed to attain a minimum level of antibody of 10 IU/l and were therefore not protected.

Hepatitis B surface antibody titers should be measured in all people who have been immunized against HBV by injection in the buttock, and when this is not possible a complete course of three injections of vaccine should be administered into the deltoid muscle or the anterolateral aspect of the thigh, the only acceptable sites for HBV immunization.

Apart from the site of injection there are several other factors that are associated with a poor antibody response or no antibody response to currently licensed vaccines. Indeed, all studies of antibody response to plasma-derived HBV vaccines and HBV vaccines prepared by recombinant DNA technology have shown that 5–10% or more of healthy immunocompetent subjects do not mount an antibody response (anti-HBs) to the surface antigen component (HBsAg) present in these preparations (i.e. they are nonresponders) or that they

respond poorly (i.e. they are hyporesponders). The exact proportions of each group depend partly on the definition of nonresponsiveness and hyporesponsiveness; the usual definitions are a level of less than 10 IU/l for nonresponders and 100 IU/l for hyporesponders, measured against an international antibody standard.

Hepatitis B surface antigen mutants

Production of antibodies to the group antigenic determinant *a* mediates cross-protection against all subtypes, as has been demonstrated by challenge with a second subtype of the virus following recovery from an initial experimental infection. The epitope *a* is located in the region of amino acids 124–148 of the major surface protein and appears to have a double-loop conformation. A monoclonal antibody that recognizes a region within this *a* epitope is capable of neutralizing the infectivity of HBV for chimpanzees, and competitive inhibition assays using the same monoclonal antibody demonstrate that equivalent antibodies are present in the sera of subjects immunized with either plasma-derived or recombinant HBV vaccine.

During a study of the immunogenicity and efficacy of HBV vaccines in Italy, a number of people who had apparently mounted a successful immune response and became anti-HBs positive, later became infected with HBV. These cases were characterized by the co-existence of noncomplexed anti-HBs and HBsAg, and in 32 of 44 vaccinated subjects there were other markers of HBV infection.

Furthermore, analysis of the antigen using monoclonal antibodies suggested that the *a* epitope was either absent or masked by antibody. Subsequent sequence analysis of the virus from one of these cases revealed a mutation in the nucleotide sequence encoding the *a* epitope, the consequence of which was a substitution of arginine for glycine at amino acid position 145.

There is now considerable evidence for a wide geographic distribution of the point mutation in HBV from guanosine to adenosine at position 587, resulting in an amino acid substitution at position 145 from glycine to arginine in the highly antigenic group determinant *a* of the surface antigen. This is a stable mutation that has been found in viral isolates from children and adults. It has been described in Italy, Singapore, Japan, Brunei, Taiwan, Hong Kong, India, Germany and the USA; from liver transplant recipients with hepatitis B in the USA, Germany and the UK who had been treated with specific hepatitis B immunoglobulin or humanized hepatitis B monoclonal antibody; and in patients with chronic hepatitis in Japan and elsewhere.[6–8,10]

The region in which this mutation occurs is an important virus epitope to which vaccine-induced neutralizing antibody binds, as discussed above, and the mutant virus is not neutralized by antibody to this specificity. It can replicate as a competent virus, implying that the amino acid substitution does not alter the attachment of the virus to the liver cell. Variants of HBV with altered antigenicity of the envelope protein show that HBV is not as antigenically singular as believed previously and that escape mutation can occur *in vivo*. This finding gives rise to two causes for concern: failure to detect HBsAg may lead to transmission through donated blood or organs, and HBV may infect people who are anti-HBs positive after immunization. Variation in the second loop of the *a* determinant seems especially important.

Hepatitis B virus precore mutants

The nucleotide sequence of the genome of a strain of HBV cloned from the serum of a naturally infected chimpanzee showed a point mutation in the penultimate codon of the precore region, which changed the tryptophan codon (TGG) to an amber termination codon (TAG). The nucleotide sequence of the HBV precore region from a number of anti-HBe-positive Greek patients was investigated by direct sequencing PCR-amplified HBV DNA from serum. An identical mutation of the penultimate codon of the precore region to a termination codon was found in seven of eight anti-HBe-positive patients who were positive for HBV DNA in serum by hybridization. In most cases there was an additional mutation in the preceding codon.

Similar variants were found by amplification of HBV DNA from serum from anti-HBe-positive patients in Italy and Greece. These variants are not confined to the Mediterranean region; the same nonsense mutation (without a second mutation in the adjacent codon) has been observed in patients from Japan and elsewhere, along with rarer examples of defective precore regions caused by frameshifts or loss of the initiation codon for the precore region.

Some precore variants may be more pathogenic than the wild-type virus because in many patients with severe chronic liver disease precore variants are found.

DIAGNOSTIC VIROLOGY

Direct demonstration of virus in serum samples is feasible by electron microscopy, by detecting virus-associated DNA polymerase, by assay of viral DNA and by amplification of viral DNA by various techniques. All these direct techniques are often impractical in the general diagnostic laboratory and specific diagnosis must therefore rely on serologic tests (Table 154.3).

Hepatitis B surface antigen appears first during the late stages of the incubation period and is easily detectable by radioimmunoassay or enzyme immunoassay. Enzyme immunoassay is specific and highly

Table 154.3 Interpretation of results of serologic tests for hepatitis B virus

			ANTI-HBC			
HBsAg	**HBeAg**	**Anti-HBe**	**IgM**	**IgG**	**Anti-HBs**	**Interpretation**
+	+	–	–	–	–	Incubation period
+	+	–	+	+	–	Acute hepatitis B or persistent carrier state
+	+	–	–	+	–	Persistent carrier state
+	–	+	±	+	–	Persistent carrier state
–	–	+	±	+	+	Convalescence
–	–	–	–	+	+	Recovery
–	–	–	+	–	–	Infection with HBV without detectable HBsAg
–	–	–	–	+	–	Recovery with loss of detectable anti-HBs
–	–	–	–	–	+	Immunization without or recovery from infection with loss of detectable anti-HBc

sensitive and is used widely in preference to radioisotope methods. The antigen persists during the acute phase of the disease and sharply decreases when antibody to the surface antigen becomes detectable. Antibody of the IgM class to the core antigen is found in the serum after the onset of the clinical symptoms and slowly declines after recovery. Its persistence at high titer suggests continuation of the infection. Core antibody of the IgG class persists for many years and provides evidence of past infection.

CLINICAL MANIFESTATIONS

The clinical features of acute infection resemble those of the other viral hepatitides. Acute hepatitis B is frequently anicteric and asymptomatic, although a severe illness with jaundice can occur and occasionally acute liver failure may develop. Hepatitis B, with or without its satellite hepatitis D, may be associated with persistent infection, a prolonged carrier state and progression to chronic liver disease, which may be severe. There is an etiologic association between hepatitis B and hepatocellular carcinoma (see Chapter 38).

Hepatitis D Virus

This virus requires hepadnavirus helper functions for propagation in hepatocytes and is an important cause of acute and severe chronic liver damage in some regions of the world.

NATURE

Hepatitis D virus (HDV) is an unusual single-stranded circular RNA virus with a number of similarities to certain plant viral satellites and viroids.[11]

Delta hepatitis was first recognized following detection of a novel protein, δ-antigen (hepatitis D antigen, HDAg), by immunofluorescent staining in the nuclei of hepatocytes from patients with chronic active hepatitis B. Hepatitis D virus is now known to require a helper function of HBV for its transmission. The virus is coated with HBsAg, which is needed for release of the HDV from the host hepatocyte and for entry in the next round of infection.

Two forms of HDV infection are known. In the first form, a susceptible person is co-infected with HBV and HDV, often leading to a more severe form of acute hepatitis caused by HBV. In the second form, a person who is chronically infected with HBV becomes superinfected with HDV. This may cause a second episode of clinical hepatitis and accelerate the course of the chronic liver disease, or cause overt disease in asymptomatic HBsAg carriers. Hepatitis D virus itself seems to be cytopathic and HDAg may be directly cytotoxic.

The HDV particle is approximately 36 nm in diameter. It is composed of an RNA genome associated with HDAg, surrounded by an envelope of HBsAg. The HDV genome is a closed circular RNA molecule of 1679 nucleotides and resembles those of the satellite viroids and virusoids of plants, and similarly seems to be replicated by the host RNA polymerase II with autocatalytic cleavage and circularization of the progeny genomes by way of transesterification reactions (ribozyme activity). Consensus sequences of viroids that are believed to be involved in these processes are also conserved in HDV.

EPIDEMIOLOGY

Hepatitis D is common in some areas of the world with a high prevalence of HBV infection, particularly Italy and other countries bordering the Mediterranean; eastern Europe, particularly Romania; the Middle East; the former Soviet Union; South America, particularly the Amazon basin, Venezuela, Columbia (hepatitis de Sierra Nevada de Santa Marta), Brazil (labrea black fever) and Peru; and parts of Africa, particularly western Africa. Antibody to HDV has been found in most countries, commonly among intravenous drug users, patients with hemophilia and those requiring treatment with blood and blood products. It has been estimated that 5% of HBsAg carriers worldwide (approximately 18 000 000 people) are infected with HDV. In areas of low prevalence of HBV, those at risk of hepatitis B, particularly intravenous drug abusers, are also at risk of hepatitis D.

The ratio of clinical to subclinical cases of HDV and superinfection is not known. However, the general severity of both forms of infection suggests that most cases are clinically significant. A low persistence of infection occurs in 1–3% of acute infections and in 80% or more of cases of superinfection in chronic HBV carriers. The mortality rate is high, particularly in the case of superinfection, and ranges from 2% to 20%.

PATHOGENICITY

Hepatitis B virus provides a helper function to HDV, which is a defective virus. The histopathologic pattern in the liver is suggestive of a direct cytopathic effect.

Pathologic changes are limited to the liver and histologic changes are those of acute and chronic hepatitis with no particular distinguishing features apart from severity and, in tropical areas in particular, microvesicular steatosis. It should be noted, however, that the virus was discovered by specific nuclear fluorescence in hepatocytes of patients with chronic hepatitis B.

The modes of transmission are similar to the parenteral transmission of HBV.[11]

PREVENTION

Prevention and control for HDV are similar to those for HBV. Immunization against HBV protects against HDV. The difficulty is protection against superinfection of the many millions of established carriers of HBV. Studies are in progress to determine whether specific immunization against HDV based on HDAg is feasible.

DIAGNOSTIC VIROLOGY

Laboratory diagnosis in acute infection is based on specific serologic tests for anti-HDV IgM or HDV RNA or HDAg in serum. Acute infection is usually self-limiting and markers of HDV infection often disappear within a few weeks.[11]

Superinfection with HDV in chronic hepatitis B may lead to suppression of HBV markers during the acute phase. Chronic infection with HDV (and HBV) is the usual outcome in nonfulminant disease.

CLINICAL MANIFESTATIONS

The clinical features of hepatitis D are identical to those of hepatitis A (for further details, see Chapter 38).

Hepatitis C Virus

NATURE

Hepatitis C virus (HCV) is an enveloped single-stranded RNA virus that appears to be distantly related (possibly in its evolution) to flaviviruses, although HCV is not transmitted by arthropod vectors.

Hepatitis C virus is unusual because it was identified using molecular methods rather than a conventional virologic approach.

Transmission studies in chimpanzees established that the main agent of parenterally acquired non-A, non-B hepatitis was likely to be an enveloped virus some 30–60 nm in diameter. Using infected chimpanzee plasma as a starting point, complementary DNA was used to create a library which was screened using serum from a patient with chronic non-A, non-B hepatitis. This approach led to the detection of a clone that was found to bind to antibodies present in the serum of several patients infected with non-A, non-B hepatitis. Eventually, clones covering the entire viral genome were assembled and the complete nucleotide sequence determined.[12]

Properties of hepatitis C virus

The genome of HCV resembles those of the pestiviruses and flaviviruses in that it comprises around 10 000 nucleotides of positive-sense RNA, lacks a 3′ poly-A tract and has a similar gene organization. It has been proposed that HCV should be the prototype of a third genus in the family Flaviviridae. All these genomes contain a single large ORF, which is translated to yield a polyprotein (of around 3000 amino acids in the case of HCV) from which the viral proteins are derived by post-translational cleavage and other modifications (for further details, see the web version of this chapter).

Hepatitis C virus consists of a family of highly related but nevertheless distinct genotypes – presently numbering six – and various subtypes with differing geographic distribution and a complex nomenclature. The C, NS3 and NS4 domains are the most highly conserved regions of the genome and therefore these proteins are the most suitable for use as capture antigens for broadly reactive tests for antibodies to HCV. The sequence differences observed between HCV groups suggest that virus–host interactions may be different, which could result in differences in pathogenicity and in response to antiviral therapy.

It is important, therefore, to develop group-specific and virus-specific tests. The degree of divergence that is apparent within the viral envelope proteins implies the absence of a broad cross-neutralizing antibody response to infection by viruses of different groups.

In addition to the sequence diversity observed between HCV groups, there is considerable sequence heterogeneity among almost all HCV isolates in the amino-terminal region of E2–NS1, implying that this region may be under strong immune selection. Indeed, sequence changes within this region may occur during the evolution of disease in individual patients and may play an important role in progression to chronicity.[12,13]

EPIDEMIOLOGY

Infection with HCV occurs throughout the world, and it is estimated that at present 170 million people are infected. Much of the seroprevalence data are based on blood donors, who represent a carefully selected population. The prevalence of antibodies to HCV in blood donors varies from 0.02% to 1.25% in different countries. Higher rates have been found in southern Italy, Spain, central Europe, Japan and parts of the Middle East, with as many as 19% in Egyptian blood donors. Until screening of blood donors was introduced, hepatitis C accounted for the vast majority of non-A, non-B post-transfusion hepatitis. However, it is clear that, although blood transfusion and the transfusion of blood products are efficient routes of transmission of HCV, these represent a small proportion of cases of acute clinical hepatitis in the USA and a number of other countries (with the exception of patients with hemophilia). Current data indicate that in some 50% of patients in industrialized countries, the source of infection cannot be identified, although 35% of patients have a history of intravenous drug use, and occupational exposure in the health care setting accounts for about 2% of cases. Household contact and sexual exposure do not appear to be major factors in the epidemiology of this common infection. Transmission of HCV from mother to infant occurs in about 10% of viremic mothers and the risk appears to be related to the level of viremia. The possibility of transmission in utero is also being investigated.[12,13]

PREVENTION

Difficulties in vaccine development include the sequence diversity between viral genotypes and the substantial sequence heterogeneity among isolates in the amino-terminal region of E2–NS1. Neutralizing antibodies have not been clearly defined. The virus has not been cultivated *in vitro* to permit the development of inactivated or attenuated vaccines (compared with yellow fever vaccines). Much work is in progress employing recombinant DNA techniques.[12–14]

DIAGNOSTIC VIROLOGY

Successful cloning of portions of the viral genome has permitted the development of new diagnostic tests for infection by the virus. Because the antigen was originally detected by antibodies in the serum of an infected patient it was an obvious candidate as the basis of an enzyme-linked immunosorbent assay (ELISA) to detect anti-HCV antibodies. A larger clone, C100, was assembled from a number of overlapping clones and expressed in yeast as a fusion protein using human superoxide dismutase sequences to facilitate expression. This fusion protein formed the basis of first-generation tests for HCV infection.

It is now known that antibodies to C100 are detected relatively late after acute infection. Furthermore, the first-generation ELISAs were associated with a high rate of false-positive reactions when applied to low-incidence populations and there were further problems with some retrospective studies on stored sera. Second-generation tests include antigens from the nucleocapsid and nonstructural regions of the genome. The former antigen (C22) is particularly useful and antibodies to the HCV core protein appear relatively early in the course of infection.

Positive reactions by ELISA require confirmation by supplementary testing using, for example, a recombinant immunoblot assay. Nevertheless, indeterminate results obtained by ELISA represent a significant problem that needs resolution. It should also be noted that the time for seroconversion is variable; when it can be measured more precisely (e.g. after transfusion) it is generally 7–31 weeks.

The presence of antibodies to specific antigen components is variable and may or may not reflect viremia and, in the case of interferon treatment, a correlation between response and loss of specific antibodies to the E2 component.

Detection and monitoring of viremia are important for management and treatment. Sensitive techniques are available for the measurement of HCV RNA based on reverse transcription (RT)-PCR amplification, nested PCR, signal amplification using branched DNA analytes and others.

The identification of specific types and subtypes is important, with observations suggesting that there is an association between response to interferon and particular genotypes and that different types may differ in their pathogenicity.

CLINICAL MANIFESTATIONS

Most acute infections are asymptomatic and about 20% of acute infections cause jaundice. Fulminant hepatitis has been described. Extrahepatic manifestations include mixed cryoglobulinemia, membranous proliferative glomerulonephritis and porphyria cutanea tarda (see Chapter 38).

Current data suggest that about 60–80% of infections with HCV progress to chronicity. Histologic examination of liver biopsies from asymptomatic HCV carriers (among blood donors) has revealed none with normal histology; indeed up to 70% have been found to have chronic active hepatitis, cirrhosis, or both (Figs 154.7, 154.8). Whether the virus is cytopathic or whether there is an immunopathologic element remains unclear. Infection with HCV is also associated

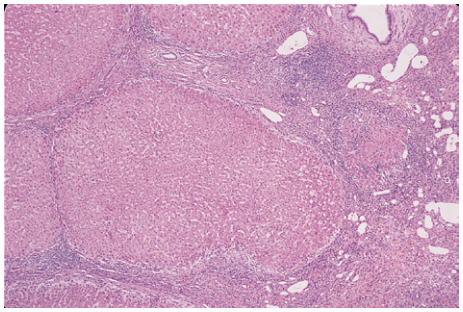


Fig. 154.7 Hepatitis C virus active cirrhosis.

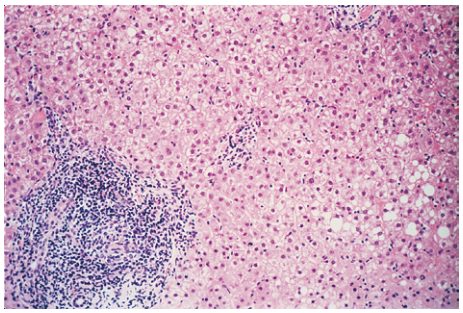


Fig. 154.8 Acute hepatitis C.

with progression to primary liver cancer. For example, in Japan, where the incidence of hepatocellular carcinoma has been increasing despite a decrease in the prevalence of HBsAg, HCV is now believed to be a major risk factor.[13]

Other Hepatitis Viruses

About 30–40 years ago, a series of transmission studies of human viral hepatitis were initiated in small South American tamarins or marmosets, which were chosen because their very limited contact with humans implied that they were unlikely to have been infected with human viruses.

Serum obtained on the third day of jaundice from a young surgeon (GB) induced hepatitis in each of four inoculated marmosets and was passaged serially in these animals. These important observations remained controversial until the recent application of modern molecular virologic techniques. Preliminary results indicate the identification of two independent viruses, GB virus A (GBV-A) and GB virus B (GBV-B), in the infectious plasma of tamarins inoculated with GB.[15]

GB virus A does not replicate in the liver of tamarins, whereas GBV-B causes hepatitis. Cross-challenge experiments showed that infection with the original infectious tamarin inoculum conferred protection from re-infection with GBV-B but not GBV-A. A third virus, GBV-C, was subsequently isolated from a human specimen that was immunoreactive with a GBV-B protein. GB virus C RNA was found in several patients with clinical hepatitis and was shown to have substantial sequence identity to GBV-A.

A series of studies, including phylogenetic analysis of genomic sequences, showed that GBV-A, GBV-B and GBV-C are not genotypes of HCV and that GBV-A and GBV-C are closely related. GBV-A–GBV-C, GBV-B and HCV are members of distinct viral groups. The organization of the genes of the GBV-A, GBV-B and GBV-C genomes shows that they are related to other positive-strand RNA viruses with local regions of sequence identity with various flaviviruses. The three GB viruses and HCV share only limited overall amino acid sequence identity.

Serologic reagents were prepared with recombinant antigens and limited testing for antibodies and by RT-PCR for specific RNA was carried out in groups of patients, blood donors and other selected people – patients with non-A,B,C,D,E hepatitis, multitransfused patients, intravenous drug users and other populations with a high incidence of viral hepatitis. Preliminary studies indicated the presence of antibody to each of the GB viruses in 3–14% of these people. The development and availability of specific diagnostic reagents will establish the epidemiology of these newly identified viruses, their pathogenic significance in humans, and their clinical and public health importance. It should be noted that the virus identified more recently as hepatitis G (HGV) as a new transfusion-transmitted agent is now believed to be identical to GBV-C.[16,17]

The blood-borne nature of GBV-C has been clearly demonstrated and there is evidence that the virus persists. The association of the virus with liver damage is illustrated by raised levels of alanine transaminase and detectable GBV-C RNA in a number of patients. However, 40–90% of viremic subjects have normal alanine transaminase levels. In a significant proportion of patients there is co-infection with HBV, HCV, or both. The primary manifestations of GBV-C infection may be extrahepatic or hepatic and these may be the result of co-infection with the hepatitis viruses or a (as yet unidentified) hepatotropic agent. At present, GBV-C appears to be a virus in search of a disease.[17]

A novel human virus was isolated in Japan in 1997 from the serum of a patient with post-transfusion hepatitis and was designated TT virus (TTV) after the initials of the patient (TTV is not an acronym for transfusion-transmitted virus as has been assumed and perpetuated in numerous publications). The virus has a circular single strand DNA, nonenveloped, of negative polarity and is 3965 nucleotides in length. There is 30% diversity in the coding region accounting for numerous genotypes believed to result mainly from recombination. The virus replicates in many tissues, particularly the liver, and is shed into the blood and feces. TT virus DNA is found in saliva (78%), breast milk, semen (60%), cervical swabs and other body fluids of infected persons. The virus is ubiquitous and is found in 20% to more than 90% of the general population and healthy blood donors. There is no evidence of involvement of TTV in acute or chronic liver disease. TT virus is found in farm animals, including chickens (19%), cows (25%), pigs (20%) and sheep (30%), and in other mammals including nonhuman primates.

TTV-like mini virus (TLMV) and several related viruses such as SANBAN, YONBAN and others have been described, but without any disease association in humans. These different viruses have been divided into at least 29 genotypes with sequence divergence of more than 30% from each other and placed into four phylogenetic groups. Since 1999, much attention has been devoted to the SEN virus (again the initials of a patient infected with a virus possibly related to TTV). The SEN virus (SENV) was isolated from an immunocompromised HIV patient with post-transfusion hepatitis of unknown etiology. Eight genotypes of this virus have been described (SENV-A to SENV-H), each differing by at least 25% in nucleotide sequence. SENV-C, SENV-D and SENV-H are supposedly associated with transfusion hepatitis and, although the prevalence of these viruses is common in patients with non-A–E liver disease, a causal association has not been demonstrated and these should not be considered as candidate hepatitis viruses.[18]

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References for this chapter can be found online at http://www.expertconsult.com



Chapter 155

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Herpesviruses

NATURE

Taxonomy

The herpesviruses are double-stranded DNA viruses belonging to Herpesvirales order.[1] More than 150 individual viruses have been described that have been found in almost all the species (both vertebrate and invertebrate) in which they have been actively sought. Their widespread occurrence suggests that they first colonized animal species at an early stage of evolution[2] and this ancient colonization has led to adaptation to their natural host so that they are generally highly host specific. This adaption is exemplified by their high rates of infection, the generally mild symptoms associated with primary infection, and their strategy for maintaining themselves in a population with a high level of immunity by establishing a latent infection.

Genetic analysis shows that the Herpesvirales fall into three distinct families.[1] The family containing the human herpesviruses, the Herpesviridae, is subdivided into three subfamilies – the Alphaherpesvirinae, the Betaherpesvirinae and the Gammaherpesvirinae based upon the biologic properties of the viruses (Table 155.1).

At present eight herpesviruses are known to infect man (Table 155.2). A further primate virus, B virus (Macacine herpesvirus 1), a virus belonging to the Simplexvirus genus of the Alphaherpesvirus subfamily, can also cause severe and sometimes fatal infections in humans.[3] The virus is enzootic among old world macaques and usually causes minimal or no morbidity in its natural host.

Structure

Herpesviruses are morphologically distinct from all other viruses. The virion often has a pleomorphic appearance when visualized by transmission electron microscopy (Fig. 155.1). Examination of herpes simplex virus type 1 (HSV-1) using the technique of cryoelectron tomography (Fig. 155.2) which permits three-dimensional visualization of the virion[4] shows that the virion has a bilayer membrane with an average diameter of 186 nm. An array of spikes protrudes from each virion, making the full diameter, approximately 225 nm.[4]

Between the capsid and envelope is the tegument. Studies with HSV-1 suggest that the tegument contains at least 20 proteins that are believed to have important functions in the early stages of virus replication following penetration of the host cell by the virion.

Physical properties

Herpesviruses are thermolabile. In cell culture media their half-life at 86°F (30°C) is between 1.5 and 14 hours. Drying in air leads to desiccation of the viral envelope and loss of infectivity. Ether in water (20%) and other lipid solvents such as 70% alcohol or chloroform rapidly and completely inactivate herpesviruses.

DNA

The genetic information of the virus is encoded by a linear molecule of double-stranded (ds)DNA and the size of this molecule varies for different herpesviruses, from approximately 80 000 to 150 000 kDa (125–245 kbp). The G+C composition of individual herpesvirus DNAs ranges from 31% to 75% and for human herpesviruses between 36% and 69%. The complete sequence of each of the human herpesviruses is known (Table 155.3) and the phylogenetic relationships of the human herpesviruses are shown in Figure 155.3.

Virion polypeptides

Herpes simplex virus type 1 virions contain about 33 virus-specific proteins, but more than double this number are found within an infected cell. The transcription of mRNAs from the genome proceeds from both strands of the genome, in either direction, with evidence of overlapping transcription and of splicing of genes and gene products.[5] Three rounds of transcription and translation are observed, the so-called:

- α phase (resulting in the production of 'immediate-early proteins');
- β phase (proteins responsible mainly for DNA metabolism); and
- γ phase (principally structural proteins).

Replication

The replicative cycle of the virus is illustrated schematically in Figure 155.4. The initial attachment of a herpesvirus to a host cell is mediated by a glycoprotein or a complex of glycoproteins projecting from the virus envelope interacting with its specific receptor on the host cell.[6]

Initial contact of the herpesviruses with the cell is usually made by low-affinity binding to glycosaminoglycans, preferentially heparan sulphate, on the cell surface. In this way viruses are concentrated at the cell surface, facilitating subsequent binding to a specific receptor. The major pathway for virus entry is fusion of the envelope with the plasma membrane, introduction of tegument proteins and viral nucleocapsid into the cell cytoplasm, and transport of the released nucleocapsid to the nuclear pore. Alternate ancillary pathways have also been identified in cell culture, including fusion within an acidic endosome and fusion within a neutral endosome.[7]

Table 155.1 Biologic properties of Herpesviridae

Common properties	Spherical enveloped virions, 150–200 nm in diameter Large, linear, dsDNA genome of 125–290 kbp contained within a T = 16 icosahedral capsid, which is surrounded by a proteinaceous matrix named the tegument and then by a lipid envelope containing membrane-associated proteins Synthesis of DNA and assembly of capsid within the nucleus, acquire envelope by budding through host cell membranes Specify a large array of enzymes involved in nucleic acid metabolism and synthesis Production of progeny virus results in destruction of the host cell Establish latency in their natural host
Alphaherpesvirinae	Variable host range Short reproductive cycle Rapid spread in cell culture Efficient destruction of infected cells Establish latency primarily but not exclusively in sensory ganglia
Betaherpesvirinae	Restricted host range (a nonexclusive property of this subfamily) Long reproductive cycle Infection progresses slowly in culture, frequently forming enlarged (cytomegalic) cells Latency in secretory glands, lymphoreticular cells, kidneys and other tissues
Gammaherpesvirinae	Experimental host range limited to family or order of natural host *In-vitro* replication in lymphoblastoid cells *In-vivo* replication and latency in either T or B cells

Table 155.2 Human herpesviruses

ICTV* name	Common name†	Subfamily	Genus
Human herpesvirus 1	Herpes simplex virus type 1	Alphaherpesvirinae	Simplexvirus
Human herpesvirus 2	Herpes simplex virus type 2	Alphaherpesvirinae	Simplexvirus
Human herpesvirus 3	Varicella-zoster virus	Alphaherpesvirinae	Varicellovirus
Human herpesvirus 4	Epstein–Barr virus	Gammaherpesvirinae	Lymphocryptovirus
Human herpesvirus 5	Human cytomegalovirus	Betaherpesvirinae	Cytomegalovirus
Human herpesvirus 6	Human herpesvirus 6	Betaherpesvirinae	Roseolovirus
Human herpesvirus 7	Human herpesvirus 7	Betaherpesvirinae	Roseolovirus
Human herpesvirus 8	Kaposi's sarcoma-associated herpesvirus	Gammaherpesvirinae	Rhadinovirus

Two variants, 'a' and 'b', of human herpesvirus 4 and human herpesvirus 6 are known.
*The International Committee on Taxonomy of Viruses (ICTV) classification scheme names the herpesviruses according to the taxon of the host that (in its natural setting) harbors the virus.[1]
†As many viruses have acquired commonly used names – for example, human herpesvirus 1 is commonly known as herpes simplex virus (HSV) type 1 – the classification is not yet rigorously applied. However, newly discovered viruses are now named in accordance with this classification scheme, for example human herpesviruses-6, -7 and -8.

The tegument proteins serve to both 'disable' the host cell and initiate viral replication. Soon after virus entry, host cell DNA synthesis is shut off, host cell protein synthesis declines rapidly and glycosylation of host cell proteins ceases. In this way the virus ensures that the metabolic machinery of the cell is fully available for virus replication. At the same time the virion nucleocapsid is transported via microtubules of the cell cytoskeleton to a nuclear pore. At the nuclear pore the viral nucleocapsid breaks down, releasing its DNA into the cell nucleus where the linear DNA molecule immediately circularizes.

Transcription of viral DNA takes place within the host cell nucleus. The first sets of viral genes to be transcribed are the immediate-early or α genes; these produce control proteins that stimulate and regulate all the subsequent steps in the replicative cycle. Their production is essential to stimulate the production of β polypeptides, which are the enzymes and other proteins involved in viral nucleic acid reproduction (e.g. DNA-dependent DNA polymerase, which reproduces the viral genome). Products of α and β genes transactivate the translation of the γ genes, which produce the late or γ proteins, the structural proteins for the virion including the viral capsid and glycoproteins.

The synthesis of nucleocapsid and all other structural proteins occurs when γ gene expression is induced by β gene products. Nucleocapsids are assembled around viral scaffolding proteins in the nucleus. Other viral proteins then interact with replicated viral DNA to allow DNA encapsidation. The DNA-filled capsids proceed to associate with tegument (matrix) proteins near the nuclear membrane. Three different models for the release of mature nucleocapsids from cells have been proposed (see Fig. 155.4). Whichever method the viruses use for their release, productive infection is fatal for the

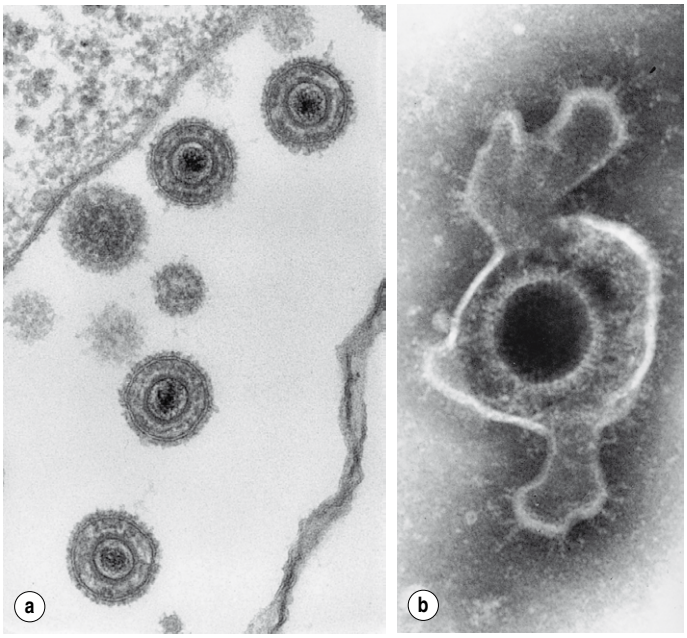


Fig. 155.1 Enveloped virus particle. (a) Thin section. (b) Negative staining. These electron microscopic views show HSV. The DNA is surrounded by a nucleocapsid comprised of 162 individual protein subunits (150 hexavalent capsomers and 12 pentavalent capsomers) arranged in the form of an icosahedron. The nucleocapsid is in turn enclosed by the tegument and virus envelope bearing glycoprotein spikes. Courtesy of Hans Gelderblom.

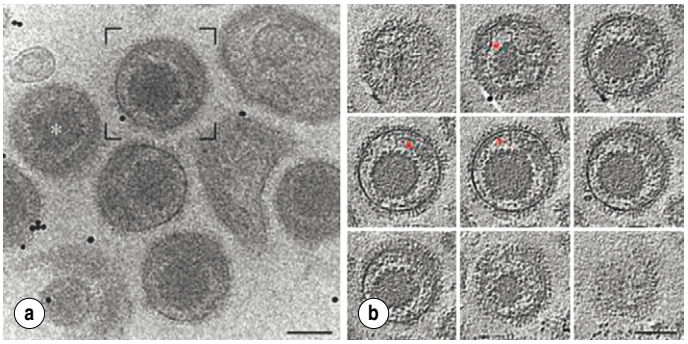


Fig. 155.2 Herpes simplex virus type 1 cryoelectron tomography. Tomographic reconstruction of HSV-1 virions in vitreous ice. (a) Zero-tilt projection from a tilt series. Black dots are 10-nm gold particles used as fiducial markers. (b) Gallery of parallel slices, 15.5 nm apart and 5.2 nm thick, through the virion framed in (a). Each slice represents the average over seven planes. Red arrowheads mark filaments in the tegument. Scale bars, 100 nm. Reproduced with permission from Grünewald, *et al.*[4]

host cell because in the process of viral DNA replication host cell chromosomes are degraded. In addition host cell metabolism is irreversibly damaged.

Latency

During the course of a primary infection a latent infection is established, with the exact site(s) of latency varying for each subfamily of herpesvirus (Table 155.4). No virions can be detected within the latently infected cell. In the latent state the virus is believed to exist as extrachromosomal circularized DNA (analogous to plasmids). The host immune response to infection rapidly eliminates virus and virus-infected cells from peripheral sites, but does not recognize latently infected cells as harboring virus since no viral antigens are expressed on the cell surface.

This establishment of latency is central to the success of herpesviruses as a pathogen, allowing the virus to persist to cause lifelong infection in the presence of a fully developed immune response. Through periodic reactivation of latent virus and the production of recurrent infection, virus shedding occurs at intervals throughout life, allowing the virus to be spread to new susceptible hosts. In order to avoid elimination by the host immune system, each herpesvirus encodes multiple proteins that combat virtually every aspect of immune recognition.[8–11] A key mechanism is to reduce or prevent expression of virus-specific peptide-MHC class I complexes at the cellular membrane, thus preventing recognition by cytotoxic T cells that would kill the infected cell.

Cell transformation

Human herpesviruses can induce mutations in host cell DNA and some human herpesviruses – HSV-1, HSV-2 and cytomegalovirus (CMV) – can transform cells in culture, albeit at a very low frequency.

Table 155.3 Properties of the human herpesviruses

Virus	Site of latency	G+C (moles %)	Genome (kb pairs)	Sequence*
HHV-1	Sensory nerve ganglia	68	152	X14112
HHV-2	Sensory nerve ganglia	69	154	Z86099
HHV-3	Sensory nerve ganglia	46	125	X04370
HHV-4	Leukocytes, epithelial cells	60	172	V01555
HHV-5	B lymphocytes	57	229	X17403
HHV-6A	T lymphocytes ($CD4^+$), epithelial cells	43	159	X83413
HHV-6B	T lymphocytes ($CD4^+$), epithelial cells	43	162	AF157706
HHV-7	T lymphocytes ($CD4^+$)	36	153	AF037218
HHV-8	B lymphocytes, epithelial cells	59	170	AF402655

*European Molecular Biology Laboratory Accession Numbers (http://www.embl-heidelberg.de).

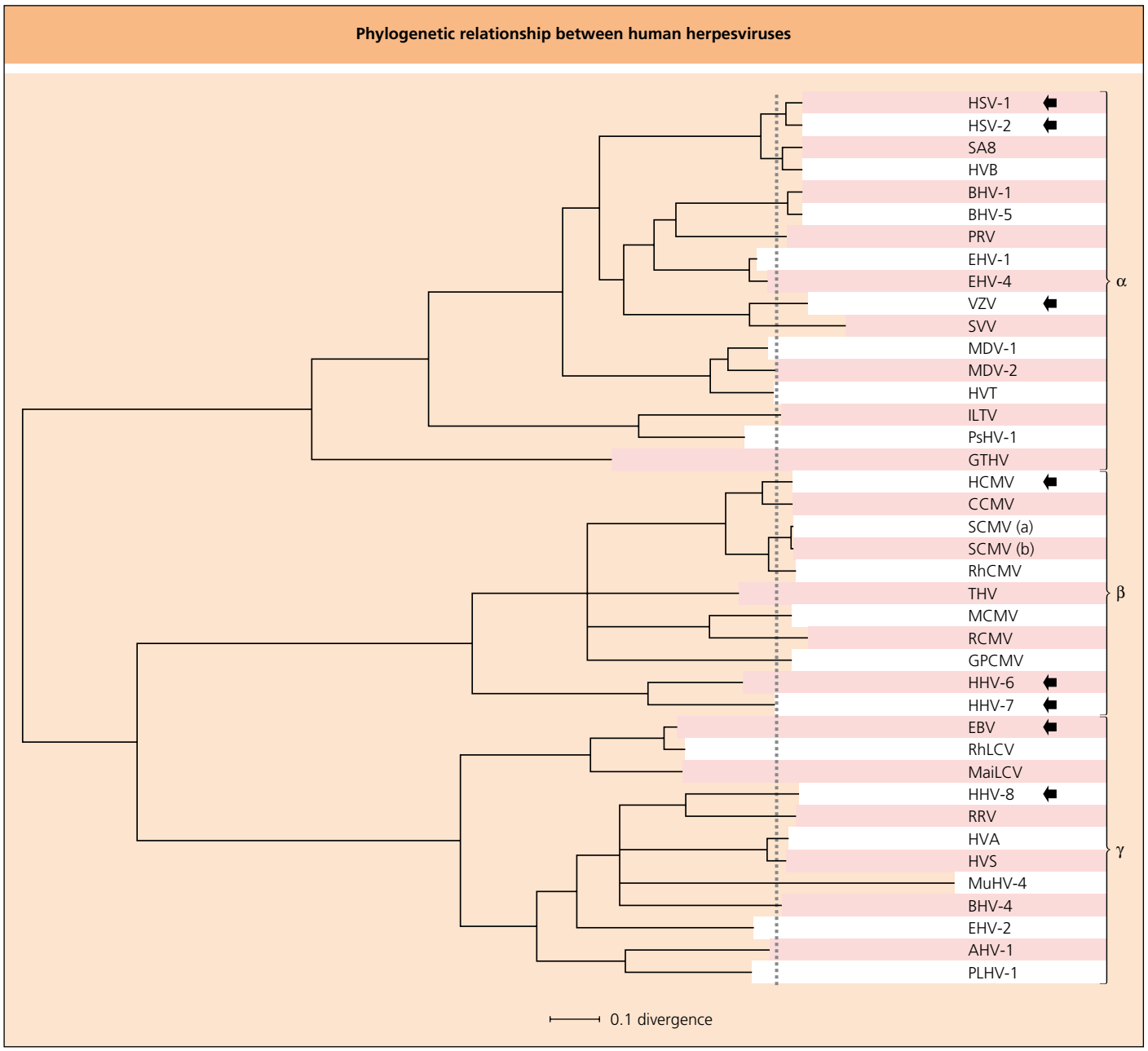


Fig. 155.3 Phylogenetic relationship between the human herpesviruses. The tree was constructed based on an alignment of amino acid sequences for six genes of HSV-1: UL15, UL19, UL27, UL28, UL29 and UL30. Reproduced with permission from McGeoch, *et al*.[5]

These viruses can also amplify persisting genomes of small tumor viruses. CMV, for example, permits the replication of the polyomavirus JC when co-transfected in human fibroblasts, providing an indirect means by which human herpesviruses could contribute to human carcinogenesis.[12] Greater oncogenic potential can be demonstrated for the human gammaherpesvirinae Epstein–Barr virus (EBV)[13] and human herpesvirus (HHV)-8.[14] EBV-infected human lymphocytes can be transformed into lymphoblast cell lines. All cells that carry the EBV genome express virus-specific nuclear antigens (EBNAs), regardless of whether mature virus is released. There are also clear epidemiologic links to several types of tumor: Burkitt's lymphoma, infectious mononucleosis that progresses to fatal B-cell lymphoma in boys with an X-linked immunodeficiency, nasopharyngeal carcinoma and post-transplant lymphoproliferative disease in immunosuppressed solid-organ transplant recipients.[13] HHV-8 similarly has strong epidemiologic links to Kaposi's sarcoma (KS) and primary effusion lymphoma. HHV-8 DNA is found in all forms of KS and infection rates are high in groups in which KS is frequent and low in groups in which KS is rare.[14]

The ubiquitous nature of herpesvirus infections and their establishment of lifelong infections make it difficult to interpret their association with tumor cells. Because of the relatively low efficiencies of transformation *in vitro* it seems likely that herpesvirus infection represents only one part of a complex sequence of events (perhaps involving a genetic predisposition and/or environmental co-factors) that ultimately leads to neoplasia.

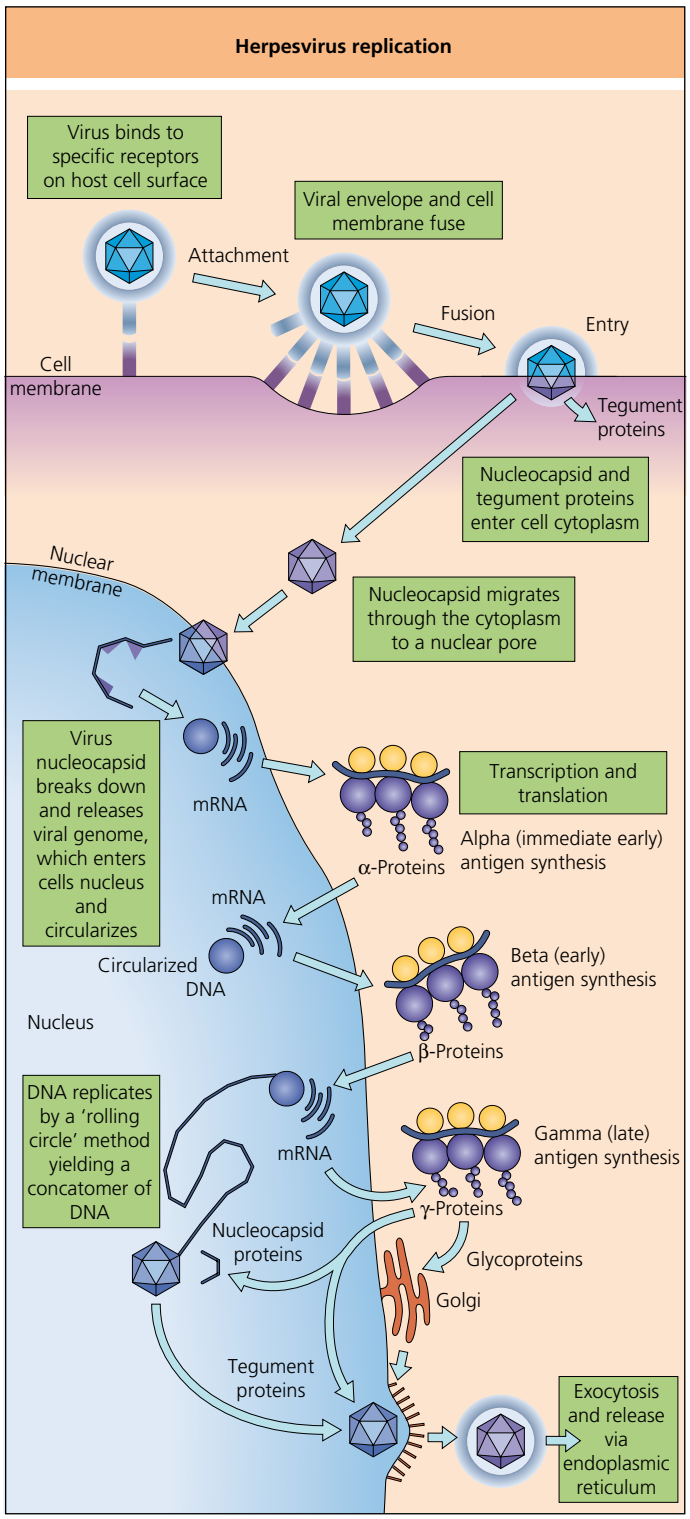


Fig. 155.4 Herpesvirus replication. The tegument proteins effect the shut-down of host cell metabolism. On entry to the nucleus the DNA circularizes and binds a tegument protein and cellular factors to initiate transcription. Transcription and translation occur in three phases: immediate-early, early and late. Capsid proteins migrate into the nucleus and the viral DNA is encapsidated. The viral glycoproteins are extensively modified post-translationally by transit through the Golgi apparatus. The glycoproteins diffuse to the nuclear envelope. The nucleocapsids are enveloped at the inner nuclear membrane and are either transported within vesicles that fuse with the plasma membrane to release the enveloped capsids into the extracellular milieu, or undergo de-envelopment at the outer nuclear membrane and re-envelopment at cytoplasmic membranes. Alternatively, nuclear pores become enlarged sufficiently to allow egress of naked capsids and the capsids then become enveloped at cytoplasmic membranes.

EPIDEMIOLOGY

Transmission pathways

Herpesviruses are relatively fragile in that they require a lipid envelope to achieve attachment to, and penetration of, the host cell. As a consequence these viruses transmit most easily on contact of warm and moist mucosal layers. Two broad groupings of virus can be distinguished:

- those where virus is transmitted most effectively by oral secretions and nongenital contact – HSV-1, varicella-zoster virus (VZV), EBV, CMV, HHV-6 and HHV-7; and
- those where the virus is transmitted most effectively by genital secretions – HSV-2 and Kaposi's sarcoma-associated herpesvirus (HHV-8).

These modes of transmission have a profound influence upon the epidemiology of the individual viruses.

Prevalence

Herpesviruses transmitted predominantly by oral secretions or nongenital contact have a peak incidence of infection in early childhood (Table 155.5). The peak incidence for infections acquired by genital transmission is in adolescence and early adulthood. The rates of infection by viruses transmitted by the genital route are not usually as high as those for viruses transmitted by a nongenital route. However, for both groups there is a further distinct relation between rates of acquisition, sexual preference and the socioeconomic status of the study population (i.e. low socioeconomic status equates with high seroprevalence).

Geographic aspects

Herpesviruses are distributed worldwide and no animal reservoirs of infection are known for any of the human herpesviruses. There is evidence that in tropical areas varicella is less prevalent in childhood than in temperate climatic areas and is more common in adults.[15] Possible explanations for this include the relative isolation of clusters of population in rural areas, epidemiologic 'interference' through infections caused by other viruses, and perhaps decreased efficiency of transmission as a result of the lability of VZV in areas where there is a high ambient temperature. Other complications of herpesvirus infection such as Burkitt's lymphoma, which is associated with EBV infection, are endemic only in tropical Africa.[13] Nasopharyngeal carcinoma is also associated with EBV infection and is endemic in Japan and Southern China.[16] A sporadic, non-HIV-associated Kaposi's sarcoma is found predominantly in countries bordering the Mediterranean and in Central Africa.[14]

Periodicity

The human herpesviruses are endemic in human populations as a result of their strategy of establishing latent infections. In addition, because of their modes of transmission there is no seasonal variation in the efficiency of transmission. For infections for which

Table 155.4 Principal sites of latency for herpesviruses

Virus	Established (most probable) sites of latency	Other possible sites of latency
HSV-1	Neurons (trigeminal ganglia)	Other sensory nerve ganglia, brain, eye
HSV-2	Neurons (sacral ganglia)	Other sensory ganglia
VZV	Neurons (dorsal, thoracic and trigeminal ganglia)	Brain, other sensory ganglia
EBV	B cells (epithelial cells of nasopharynx and submandibular salivary glands)	–
CMV	Monocytes, lymphocytes, epithelial cells	Salivary glands, renal tubule cells
HHV-6, HHV-7	T cells	Bone marrow progenitors, monocytic/ macrophage cells
HHV-8	Not firmly established	B cells

Table 155.5 Features of herpesvirus infections

Virus	PEAK INCIDENCE OF PRIMARY INFECTION		Adult seroprevalence (%)	Principal route(s) of transmission	Notes
	Childhood	Adolescence			
HSV-1	+++	+	75–95+	Oral secretions, close contact	Overall seroprevalence predominantly determined by socioeconomic status
HSV-2	–	+++	4–95	Genital secretions, close contact	Lifetime number of sex partners is predominant influence on rates of seropositivity
VZV	+++	+	90–95	Aerosol, close contact	Epidemic spread in childhood, in tropics relatively more common in adults than children
CMV	++	++	40–95+	Oral secretions, genital secretions	Infection common in infancy, but a significant proportion of women of childbearing age are susceptible; overall seroprevalence predominantly determined by socioeconomic status
EBV	++	++	70–95	Oral secretions	Second peak of incidence in early adolescence (glandular fever)
HHV-6	+++	–	>85	Oral secretions	Infection common in infancy – peak age of acquisition 2 years
HHV-7	+++	–	>85	Oral secretions	Infection common in infancy – peak age of acquisition 3 years
HHV-8	–	+++	10–25	Oral secretions, genital secretions	Homosexual men who have AIDS have highest seroprevalence

CMV, cytomegalovirus; EBV, Epstein–Barr virus; HHV, human herpesvirus; HSV, herpes simplex virus; VZV, varicella-zoster virus.

reactivation is infrequent (e.g. VZV infection) there may be 'outbreaks' of infection (mini-epidemics) when infection spreads rapidly through a nonimmune population. Outbreaks can also occur if large numbers of susceptible individuals are brought together (e.g. infectious mononucleosis among military recruits or college students).[17]

Determinants of infection

Early acquisition of infection is common for all herpesviruses transmitted by the nongenital route (see Table 155.5). In a classic study of HSV infection, Burnet & Williams[18] showed that there was a clear relationship between the age of acquisition of the virus and socioeconomic status. Populations associated with a low socioeconomic environment collectively showed earlier acquisition of HSV infection than more affluent populations, although in both groups, infection rates of 90–95% were observed by early adulthood. Primary HSV-1 infection was rare in those over 30 years of age. In recent years several seroepidemiologic studies have shown a general decrease in the overall prevalence of HSV-1 antibody in developed countries.[19,20] However, even within individual countries there are variations in seroprevalence; for example, the seroprevalence rates are generally higher

among inner city residents than among those from rural areas. Here, the major factor determining the seroprevalence is the frequency of direct person-to-person contact rather than the socioeconomic status of the individual populations.

If infection does not occur in infancy, transmission routes other than direct oral contact can constitute important risk factors. An example is found in the acquisition of CMV. Although most CMV infections occur in infancy a significant proportion (40–50% in developed countries) of women of child-bearing age are still susceptible to infection and may acquire the infection during pregnancy. Sexual transmission of infection then becomes an important mode of acquisition of infection for these women. In addition, contact with young children of less than 24 months of age is a further important risk factor.

Viruses such as HSV-2, for which the principal mode of transmission is sexual, are generally not acquired until the onset of sexual activity in adolescence and early adulthood. HSV-2 prevalence, overall and by age, varies markedly by country, region within country, population subgroup and in populations with higher risk sexual behavior.[21]

The seroprevalence of HHV-8 infection is low (<5%) in most northern, western and central European countries except for parts of Italy, Greece and to a lesser extent Spain. It is also low in the USA (0–5%). In many African countries, particularly sub-Saharan Africa, rates of 40–60% are found in adults and adolescents. In accordance with the high rates of Kaposi's sarcoma seen in HIV-infected homosexual and bisexual men, HHV-8 antibodies are more common in these groups than in the general population of the same country.

PATHOGENICITY

Molecular and cellular basis of pathogenicity

The combination of virion surface glycoproteins and the distribution of cellular receptors provide a partial explanation of the cell- and tissue-specific tropism of members of the human herpesviruses. After primary infection, herpesviruses persist in their host by a combination of lytic infectious processes (cellular persistence) and latent infections in other sites or tissues. Latent infections are characterized by restriction of viral gene expression to those required for maintenance of the latent infection and by the absence of expression of genes required for lytic infection (e.g. DNA replication and production of structural proteins). In response to various stimuli, latent virus can be reactivated to a lytic state. Virus produced during the reactivated lytic state can cause disease and provide a source of virus for transmission to susceptible individuals. In individuals who have deficits in cell-mediated immunity (CMI), infection is more poorly controlled than in those who have intact CMI. Primary infection is in consequence more severe, and reactivation of latent infection is more likely to result in asymptomatic disease.

Herpes simplex virus

In vitro, HSV can infect most types of human cells and even cells of other species, but *in vivo* it is host specific. It causes lytic infection of fibroblasts and epithelial cells, and establishes latent infection in neurons. The two biotypes of HSV, HSV-1 and HSV-2, show some predilection for infecting defined anatomic sites – oropharyngeal and genital, respectively. Both viruses are capable of infecting and producing latent infection at either site, but HSV-2 reactivation 'above the waist' and HSV-1 reactivation 'below the waist' are infrequent compared with HSV-1 reactivation 'above the waist' and HSV-2 reactivation 'below the waist', respectively.[22]

Varicella-zoster virus

Primary VZV infection begins in the mucosa of the respiratory tract and progresses via the blood and lymphatic system to cells of the reticuloendothelial system. A secondary viremia at 11–13 days disseminates virus to the skin where the characteristic vesicular lesions of 'chickenpox' are produced. The virus establishes latent infection in the cranial, dorsal root and autonomic nervous system ganglia during this viremic phase. The molecular basis of latency and reactivation is poorly understood. In contrast to studies in neurons latently infected with HSV-1 (where only the LAT transcript is found) neurons latently infected with VZV have four transcripts corresponding to VZV genes 21, 29, 62 and 63.[23]

Epstein–Barr virus

During primary infection EBV establishes a productive infection in the epithelial cells of the oropharynx. Virus is shed in the saliva and gains access to B cells in lymphatic tissue and the blood.[13] A lytic infection leads to the production of infectious virus with EBV proteins, including the early antigens, viral capsid antigen and the glycoproteins of the membrane antigen being produced by the infected cell (Fig. 155.5). Epstein–Barr virus is a B-cell mitogen, stimulating the growth and immortalization of B cells by preventing apoptosis. In EBV-immortalized cells the genome is maintained as a circular episome which is replicated by cellular DNA polymerase and is equally distributed to daughter cells when EBV-infected cells divide. The resting cell contains nine latent proteins and several noncoding

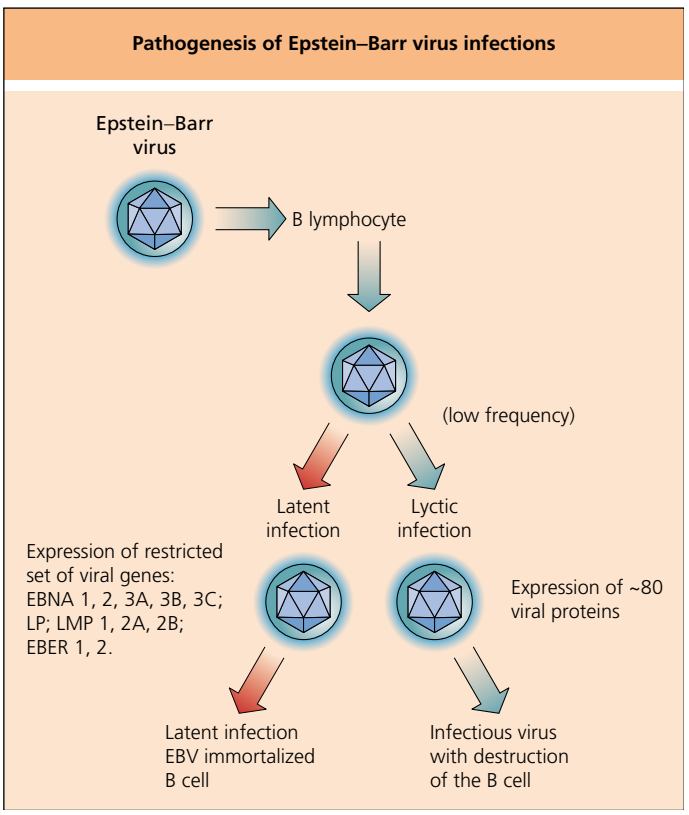


Fig. 155.5 Pathogenesis of Epstein–Barr virus (EBV) infections. Infection may result in lytic infection of the cell or cell immortalization, which can be distinguished by the production of virus and the expression of different viral proteins and antigens. T cells limit the outgrowth of EBV-infected cells. EBER, Epstein–Barr encoded small RNA; EBNA, Epstein–Barr nuclear antigen; LMP, latent membrane protein; LP, EBNA leader protein.

RNAs that are under the master control of EBNA2. These co-operate to drive the cell to resemble an antigen-activated lymphoblast. EBV-transformed proliferating lymphocytes are constantly held in check by the immune response.[8] By promoting cell growth and preventing apoptosis, the latency program of EBV is believed to assume the prime role in oncogenesis. By contrast the lytic program is not thought to be involved in oncogenesis because cells that enter a lytic program invariably die.

Cytomegalovirus

Cytomegalovirus is capable of producing lytic, productive, infection of epithelial, endothelial, smooth muscle, stromal, fibroblast and neuronal cells *in vivo*. However, viral latency only appears to occur in a restricted subpopulation of cells.[24] Hematopoietic cell precursors ($CD34^+$) are a common precursor of both lymphoid and myeloid cells. Cells of the monocyte/myeloid lineage have been shown to become latently infected by as yet undefined mechanisms.[25] In the latently infected cell there is absence of lytic gene transcription although transcripts of the major immediate early gene (IE) region of the CMV genome can be detected. The major IE transcripts IE72 and IE86 are not, however, expressed in latently infected cells. Between two and 10 copies of the CMV genome are carried in an episomal form in mononuclear cells of healthy seropositive individuals. It is presently unknown how the viral genome is maintained in dividing progenitor cells.

Human herpesviruses 6 and 7

Human herpesvirus 6 was originally isolated from T-cell cultures derived from the blood of patients who had AIDS[26] and HHV-7 was isolated from the $CD4^+$ T cells of a healthy individual. Both viruses infect and kill $CD4^+$ T cells, just like HIV, yet the outcome of infection is markedly different. In contrast to HIV infection, infection by HHV-6 or HHV-7 is rapidly controlled by the host immune response, and the virus establishes a state of latency. HHV-6 and HHV-7 establish chronic persistent infection with low-level replication in salivary, submandibular and parotid glands. Candidate sites for true latency are found in monocytes and early bone marrow progenitor cells. In HHV-6, the product of gene U94 has been shown to enable establishment and/or maintenance of latent infection in these cells.[27]

HHV-6 exists as two closely related variants: HHV-6A and HHV-6B. Although closely related, consistent differences have been observed in their biologic, immunologic, epidemiologic and molecular properties. HHV-6B is the primary etiologic agent of exanthema subitum, whereas no single disease has been definitively associated with HHV-6A. HHV-7 is responsible for a subset of exanthema subitum and other exanthema cases but like HHV-6A also remains an 'orphan' virus with no firm disease association.[28]

Human herpesvirus 8

Using representational difference analysis[29] HHV-8 was initially identified in 90% of AIDS-related Kaposi's sarcoma (KS) lesions and in 15% of non-KS tissues from people who had HIV infection. Following these observations HHV-8 sequences were identified using polymerase chain reaction (PCR) in all forms of KS, including KS from different geographic locations in individuals who had and did not have HIV infection.[14] Most KS spindle cells can be shown to be latently infected; only a small proportion (1–3%) show lytic, replicative, markers. Although HHV-8 can infect many cell types *in vitro* it is only infection of primary epithelial cell cultures that results in changes in morphology and growth. Once latency is established in these cells, dramatic elongation occurs to produce spindle-like cells. The changes can be traced to changes in the organization of the actin cytoskeleton and are related to the expression of a single viral gene – V-FLIP.[14] The modifications are not, however, related to cell immortalization and this marks a clear difference between the latency program of HHV-8 and that of EBV. A series of six proteins are known to be expressed in the latently infected KS cell; latency associated antigen (LANA), v-cyclin, v-FLIP and kaposin proteins A, B and C.

PREVENTION

In a well-controlled environment, such as that of a hospital, prevention of host-to-host transmission for most human herpesviruses is achieved by simple hygiene. In the home or other social situations prevention of transmission by avoiding contact with a person who has evidence of recurrent infection is only partially effective. This is because infectious virus is often excreted before the appearance of overt symptoms of recurrent infection and 'silent' recurrent infections also occur.

Herpesviruses are readily inactivated by a variety of physical and chemical agents (see Physical properties, above) and standard methods of sterilization, including autoclaving, dry heat, ultraviolet or gamma irradiation, and ethylene oxide sterilization are adequate for decontaminating medical equipment. Most common disinfectants (5% phenol, formaldehyde, glutaraldehyde, 1:10 000 quaternary ammonium compounds and 0.3 ppm hypochlorites) rapidly inactivate virus. Other virucidal compounds include detergents, chlorhexidine, Merthiolate, sodium azide, β-propiolactone, ethylene oxide and some proteolytic enzymes. Ultraviolet and X-ray or gamma irradiation also inactivate herpesviruses.

Active immunization

Except for VZV infection (for which a live attenuated vaccine is available) there are currently no licensed vaccines available to prevent herpesvirus infections. Varicella-zoster virus infection causes particular problems in immunosuppressed children. Primary infection in immunosuppressed patients can result in a fulminant, generalized infection or severe respiratory disease. A live attenuated VZV vaccine has been licensed for use and is administered using the same schedule as the measles, mumps and rubella vaccine. The vaccine has an excellent safety record and although breakthrough infections can occur, they usually result in mild illness. In the USA the vaccine is recommended for use for all children over 12 months of age and susceptible healthy adolescents and adults. In most other countries vaccination is only recommended for patients who are immunosuppressed or immunodeficient and who have never had chickenpox. A single dose is 80–85% effective in preventing disease of any severity and >95% effective in preventing severe varicella.[30]

Studies of the Oka-derived strain of VZV vaccines showed that these can elicit a significant increase in VZV cell-mediated immunity (CMI) in immunocompetent older adults. This led to a study of immunization of older adults with live attenuated Oka VZV vaccine (at 14 times the minimum potency of the standard vaccine) to boost their waning VZV CMI. The vaccine significantly reduced the morbidity of herpes zoster (by 61.1%) and the incidence of postherpetic neuralgia, the most common debilitating complication of herpes zoster, by 66.5%.[31]

Research on the production of effective vaccines against HSV has been actively pursued since the 1920s. A very wide range of vaccines have been explored. Two HSV-2 glycoprotein adjuvant vaccines (gB2 + gD2 and gD2) have reached phase III clinical evaluation. The overall protection rates were not significant. A gD2 vaccine formulated with an adjuvant alum and 3-deacetylated monophosphoryl lipid A showed a 73–74% protection from disease – but not infection – in women seronegative to HSV-1 and HSV-2. Gender differences and preexisting HSV-1 serologic status influence vaccine efficacy in these trials.[32]

For CMV a candidate vaccine was developed by Plotkin in the 1970s.[33] The vaccine was prepared using an isolate from a congenitally infected child (the Towne strain) after serial passage in human embryonic fibroblasts. In early studies serconversion was seen in nearly 100% of volunteers, as well as a protective effect against a low-dose challenge with CMV. However, the vaccine failed to prevent infection of

mothers in contact with CMV-excreting children, and therefore failed its primary objective: the prevention of primary infection in pregnancy. Later efforts have focused on the development of recombinant subunits, dense bodies (enveloped, replication-defective particles), peptide-based vaccines and DNA vaccines, so far with little success.[34]

Attempts to develop a prophylactic EBV vaccine have focused on the gp350 and EBNA3 antigens. Immunization with gp350 antigen has been shown to prevent infectious mononucleosis but not EBV infection in seronegative young adults.[35] Synthetic peptides based on the EBNA3 latent antigen have been the subject of small scale clinical trials. The latent antigen epitopes restricted through common MHC class I alleles can induce $CD8^+$ T-cell responses in the vaccinee. The value of this approach has been underscored by studies which show that autologous $CD8^+$ cells against EBNA3 propagated *in vivo* and then given to immunosuppressed patients at risk of post-transplant lymphoproliferative disease (PTLD), can prevent PTLD and in some cases cause regression of PTLD.[36]

Passive immunization

Varicella-zoster virus hyperimmune globulin (VZVIG) is an effective prophylaxis for babies born to mothers who develop varicella (but not herpes zoster) in the period 7 days before to 7 days after delivery.[37] VZVIG is also recommended for VZ antibody-negative infants exposed to chickenpox or herpes zoster (other than in the mother) in the first 7 days of life and VZ antibody-negative infants of any age exposed to chickenpox or herpes zoster while still requiring intensive or prolonged special care nursing. It is also given to VZ antibody-negative pregnant women exposed at any stage of pregnancy, providing VZVIG can be given within 10 days of contact. The rationale for the use of VZVIG prophylaxis in this situation is twofold: reduction in severity of maternal disease and reduction of risk of fetal infection for women contracting varicella in the first 20 weeks of pregnancy. The risk of fatal varicella is estimated to be about five times higher in pregnant than nonpregnant adults with fatal cases concentrated late in the second or early in the third trimester. Prophylactic use of VZVIG is also appropriate to control infection in pediatric units caring for immunocompromised children.

Adoptive immunotherapy

Infectious complications of human herpesvirus infection are common in immunocompromised individuals. Particular problems are found in recipients of hematopoietic stem cell transplants (HSCT) and in solid-organ transplant recipients (SOT) where conditioning regimens can ablate the recipient's immune system. In HSCT the recipient's immune system can recover after engraftment and expansion of the recipient immune system *in vivo*. In SOT the recipient's immune system never recovers its full potential because immunosuppression must be maintained life long. A process in which donor $CD4^+$ and $CD8^+$ cells are cultured *in vitro* in the presence of virus or peptides representing the virus and the stimulated cells are clonally expanded using mitogen stimulation, results in a population of cytotoxic T lymphocytes (CTLs). These may be infused into the recipient to provide virus-specific cytotoxicity that provides for prophylaxis and/or treatment of herpesvirus infection in the recipient. Successful procedures for CMV and EBV adoptive immunotherapy have been described and developments of the technique promise to accelerate the preparation of therapeutic solutions by direct selection of virus-specific CTLs from donor lymphocytes.[38]

Antiviral prophylaxis

A number of specific antiviral compounds are available for the treatment of herpesvirus infection (Table 155.6) and prophylactic use of antiviral chemotherapy to control infection is well established.

Most antiherpetic drugs are nucleoside analogues that inhibit virus-specified DNA polymerases and terminate DNA chain elongation. The first generation of these drugs – idoxuridine, vidarabine and trifluridine – had only a limited selectivity for the viral DNA polymerase due to the interaction of the phosphorylated form of these drugs with cellular DNA polymerases. The use of these compounds was associated with severe adverse effects.

Aciclovir is the prototype of the present generation of antiviral drugs (see Chapter 146). Its high specificity and therefore safety is based on its specific interaction with viral and not cellular enzymes. It is an acyclic analogue of guanosine that is activated by the viral and not the cellular thymidine kinase to serve as substrate for the viral DNA polymerase. Aciclovir is a safe, relatively nontoxic drug and is effective for therapeutic use as well as long-term suppressive treatment.

The oral bioavailability of aciclovir is relatively low and for this reason a prodrug – the L-valyl ester of aciclovir, valaciclovir – was developed. Valaciclovir hydrochloride is rapidly adsorbed from the gastrointestinal tract and rapidly and almost completely converted to aciclovir and L-valine by first-pass intestinal and/or hepatic metabolism. The mode of action, safety profile and clinical spectrum of activity of valaciclovir are believed to be identical to that of aciclovir.

Penciclovir is a further acyclic nucleoside analogue whose mode of action and safety profile is essentially identical to that of aciclovir. The drugs differ in their rate of cellular uptake, phosphorylation rate, stability of the intracellular triphosphate and inhibitory concentration for HSV DNA polymerase (100-fold higher for penciclovir triphosphate than for aciclovir triphosphate). The intracellular half-life of the active antiviral (penciclovir triphosphate) is substantially longer (7–20 hours) than that of aciclovir triphosphate (0.7–1 hour) which compensates for the slightly lower activity of the drug.

Table 155.6 Currently used antiherpesvirus drugs

Antiviral drug	Chemical class	Mechanisms of action	Target virus
Aciclovir	Guanosine analogue	Virus-activated DNA polymerase inhibitor	HSV-1, HSV-2, VZV
Valaciclovir	Guanosine analogue	Virus-activated DNA polymerase inhibitor	HSV-1, HSV-2, VZV
Penciclovir	Guanosine analogue	Virus-activated DNA polymerase inhibitor	HSV-1, HSV-2, VZV
Famciclovir	Guanosine analogue	Virus-activated DNA polymerase inhibitor	HSV-1, HSV-2, VZV
Cidofovir	Cytidylic acid analogue	DNA polymerase inhibitor	CMV, HSV-1, HSV-2
Foscarnet	Pyrophosphate analogue	DNA polymerase inhibitor	CMV, HSV-1, HSV-2
Ganciclovir	Guanosine analogue	Virus-activated DNA polymerase inhibitor	CMV (HSV-1, HSV-2)
Maribavir*	Benzimidazole riboside	Inhibits UL 97 kinase	CMV, EBV

CMV, cytomegalovirus; EBV, Epstein–Barr virus; HSV, herpes simplex virus; VZV, varicella-zoster virus.
*Scheduled to become available in 2009.

Famciclovir is the diacetyl 6-deoxy prodrug of penciclovir. Famciclovir achieves high levels of systemic bioavailability following oral administration. Following administration the drug is deacetylated and oxidized to form penciclovir. The mode of action and clinical spectrum of activity is thus identical to that of penciclovir.

The alternate antiherpetic drugs, ganciclovir (and its prodrug valganciclovir), foscarnet and cidofovir have similar mechanisms of action (i.e. inhibition of the viral DNA polymerase), but generally have a higher toxicity profile, including neutropenia and thrombocytopenia for ganciclovir, and nephrotoxicity for foscarnet and cidofovir. The newest antiviral maribavir[39] directly inhibits UL97 kinase, an early gene involved in viral DNA elongation, DNA packaging and egress of viral capsids from the cell nucleus. Maribavir does not cause nephrotoxicity or hematologic toxicity and has activity against ganciclovir-resistant CMV. The susceptibility of different herpesviruses to the various antiviral drugs is shown in Table 155.7.

Antiviral resistance has been described for all of the antiherpes compounds mentioned, particularly when antivirals are administered to immunocompromised patients over extended periods of time. Studies in these patients have shown resistance to aciclovir in 5% and to ganciclovir in 7% of patients. Antiviral resistance is due to mutations in the viral thymidine kinase or in the DNA polymerase. Resistant viruses, however, appear to be less virulent. Significant circulation in the general population has not yet been found. Because of their similar mechanisms of action, cross-resistance frequently occurs (e.g. between aciclovir, famciclovir and valaciclovir).

The nucleoside analogues are virostatic and it is thus of crucial importance that therapy is initiated very early in infection, even before laboratory confirmation of the clinical diagnosis (Table 155.8). Antiviral drug therapy is probably only effective when the disease is due to a viral rather than an immunopathologic process.

Table 155.7 Comparative susceptibility of herpesviruses to antiviral drugs

	Viral susceptibility					
Antiviral drug	**HSV-1**	**HSV-2**	**VZV**	**CMV**	**EBV**	**Main target agents***
Aciclovir, valaciclovir	+++	+++	++	–	+	HSV-1, HSV-2, VZV
Penciclovir, famciclovir	+++	+++	++	–	+	HSV-1, HSV-2, VZV
Cidofovir	+	+	++	+++	+	CMV, resistant HSV
Foscarnet	++	++	+	++	+	CMV, resistant HSV, VZV, CMV
Ganciclovir, valganciclovir	+	+	+	+++	+	CMV
Maribavir	–	–	–	+++	+++	CMV, resistant CMV

CMV, cytomegalovirus; EBV, Epstein–Barr virus; HSV, herpes simplex virus; VZV, varicella-zoster virus.
*Ganciclovir and foscarnet have *in-vitro* activity against HHV-6 and HHV-8 and have been used on a compassionate basis in severe disease associated with these agents.

Table 155.8 Indications for antiherpesvirus drug treatment

Antiviral drug	**Target virus**	**Infections**	**Possible side-effects**
Aciclovir, valaciclovir, famciclovir	HSV-1,2	Severe and/or frequent mucocutaneous HSV infection, including genital herpes; herpes encephalitis, herpes keratitis	Headache, nausea, diarrhea
	VZV	Severe cases of varicella, varicella in patients at risk for complications (immunocompromised, adolescents, adults) Severe cases of herpes zoster, including those at risk of developing postherpetic neuralgia, herpes zoster ophthalmicus, oticus	Headache, nausea, diarrhea
Cidofovir	CMV	CMV retinitis	Severe nephrotoxicity
Foscarnet	CMV, HSV-1, HSV-2, VZV; severe disease due to aciclovir-, valaciclovir- and famciclovir-resistant HSV or VZV strains	Severe CMV disease refractory to ganciclovir treatment	Nephrotoxicity, crystalluria
Ganciclovir	CMV	Severe CMV disease in immunocompromised host (e.g. retinitis, esophagitis, colitis, pneumonia, encephalitis)	Neutropenia, thrombocytopenia

CMV, cytomegalovirus; HSV, herpes simplex virus; VZV, varicella-zoster virus.

EBV and HHV-6 have shown *in-vitro* susceptibility to various antiherpesvirus drugs such as aciclovir, ganciclovir and cidofovir. However, apart from aciclovir treatment of oral hairy leukoplakia in patients who have AIDS, no significant clinical benefit has so far been demonstrated. Anecdotal use of ganciclovir or foscarnet for HHV-6, HHV-7 and HHV-8 infections has been reported. However, so far no data from well-designed clinical studies have been reported to show the effectiveness of these drugs in the treatment of these infections.

DIAGNOSTIC VIROLOGY

A key feature of the herpesviruses is their close adaption to their host. In general, primary infection is usually asymptomatic or is accompanied by nonspecific mild signs and symptoms. Consequently, most primary infections and many recurrent infections are not recognized as herpesvirus infections. Where symptoms are observed, speed in using diagnostic procedures is important because the peak of virus replication and shedding is likely to precede the appearance of symptoms. The diagnostic method chosen (Table 155.9) varies for different herpesviruses and also depends upon the type of infection (whether primary or recurrent), duration of symptoms and clinical manifestations.

Test specimens

If there are visible lesions (HSV-1, HSV-2, VZV) the base of the lesion may be sampled with a dry cotton-tipped swab, which should be placed in virus transport medium and transported to the virus laboratory as quickly as possible. If there are vesicles, vesicle fluid can be aspirated using a fine (intradermal) needle. The fluid should then be transported directly to the laboratory for virus detection by PCR testing, electron microscopy, direct immunofluorescent antibody staining or culture.

Viruria and viremia are common during both primary infection and recurrent infection with CMV, EBV, HHV-6, HHV-7 and HHV-8. Urine collected in urine transport medium is a useful specimen for detection of CMV. Blood collected in anticoagulant (EDTA) can be used in direct detection of virus. In neurologic disease, cerebrospinal fluid (CSF) and a clotted peripheral blood specimen (for CSF and blood serology) are essential. Clotted blood specimens should be collected during the acute stages of illness and again after 10–14 days.

Virus culture

While the use of virus culture has declined in many diagnostic virus laboratories it remains an essential tool in reference laboratories when live virus is required for detailed analysis. The fragility of the viral envelope presents a problem if virus is to be cultured. Collection of specimens into appropriate viral transport medium and rapid transportation of specimens to the diagnostic laboratory is essential for successful isolation in cell culture systems. Virus culture is only usually attempted for HSV-1, HSV-2, VZV and CMV.

Electron microscopy

Transmission electron microscopic examination of negatively stained vesicle fluid presents one of the most rapid methods for detection of VZV or HSV. Although now only available in few diagnostic laboratories, this procedure can be very helpful in establishing a rapid, early diagnosis. Collection of specimens for electron microscopy is a skilled procedure and several methods are available.[40] The technique is relatively insensitive and a specimen must contain at least 10^6 or more particles per milliliter to allow detection of virus.

Antigen detection

Antigen detection has a limited but important role to play in the diagnosis of most herpesvirus infections. In general, specimens may contain too little virus to allow reliable detection of antigen. Nevertheless, in a case of vesicular eruption, vesicle fluid (air dried and fixed in cold acetone) can be stained with monoclonal antibodies tagged with fluorescein isothiocyanate to provide a rapid specific diagnosis of infection. A variety of rapid immunoassay tests have also been developed

Table 155.9 Laboratory diagnosis of herpesvirus infections

Virus	Disease manifestation	Virus culture	Serology	Antigen detection	DNA amplification
HSV-1	Skin lesions CNS infection	++ –	+ ++	+ –	+++ +++
HSV-2	Genital lesions CNS infection	++ –	+ +	+ –	+++ +++
VZV	Skin lesions CNS infection	+ –	++ +	++ –	+++ +++
CMV	Mononucleosis-like illness Neonatal disease Systemic infection in immunocompromised CNS disease	– ++ + –	+++ ++ + +	– – ++ –	– +++ +++ +++
EBV	Mononucleosis-like illness Systemic infection in immunocompromised CNS disease	– – –	+++ + +	– + –	– +++ +++
HHV-6	Exanthema subitum CNS disease	+ –	+++ ++	– –	– +++
HHV-8	Kaposi's sarcoma	–	+	–	+++

CMV, cytomegalovirus; EBV, Epstein–Barr virus; HHV, human herpesvirus; HSV, herpes simplex virus; VZV, varicella–zoster virus.

for 'within the office' testing for HSV. However, the sensitivity and specificity of these tests are too low to allow their use in diagnosis for hospitalized patients. As with virus cultures, these approaches are now being replaced by rapid molecular diagnostic techniques.

Nucleic acid amplification

A number of alternative nucleic acid amplification or signal amplification techniques have been applied to the diagnosis of herpesvirus infections but it is nucleic acid amplification, in particular PCR, that is most widely applied. A wide variety of PCR techniques have been used, including single, semi-nested and nested, with product detection via gel electrophoresis, Southern blotting, ELISA-like hybridization, microarray or bead-based arrays such as the 'Luminex' procedure. 'Real-time' PCR is now the most commonly used procedure; this offers testing of high sensitivity, reduced risk of intralaboratory cross-contamination of samples from amplicon release and reduces test turnaround times by combining the detection of amplification within the thermal amplification process. The test allows direct detection of the products of amplification in 'real time', is quantitative, and can allow, for example, typing of HSV-1 and HSV-2 within the same test. Multitarget or 'multiplex' PCR procedures are also available in both real-time and conventional PCR formats. Careful optimization of these test procedures is necessary to ensure that the sensitivity of detection of each of the individual target viruses within the test is maintained when targets are combined.

Many types of clinical sample contain substances that prove inhibitory to the polymerase chain reaction which, if not efficiently removed, will result in the production of false-negative test results. The use of internal control molecules within PCR tests is used to monitor for test failure through test sample inhibition. Where internal molecules are not available the same check may be performed by 'spiking' a sample with a known amount of virus or by checking for an alternate human gene always expected to be present within a clinical sample.

Nucleic acid amplification techniques are now considered obligatory for the diagnosis of herpesvirus infections, particularly for example in HSV infection of the CNS[41] or in the diagnosis of the acute retinal necrosis syndrome.[42] Determination of the viral load in blood is an indispensable tool for the early detection, monitoring and medical management of CMV, EBV, HHV-6, and HHV-8 infections in HSTC and SOT patients.[43] Rigorous quality control and attention to detail is of course essential for routine application of any PCR technique, but in the latter application of quantitative PCR, standardization of variables through parallel comparative and proficiency testing, development of standards and of uniform units for expressing results, have all become essential to allow clinical correlation with the results of these molecular assays.

Genotypic analyses

Sequencing of products of PCR is a useful method for the comparison of strains of virus detected. For example, sequencing of viruses obtained from different bodily sites or from different persons provides a more rapid method for epidemiologic and population diversity investigation[44] than the technically demanding restriction-fragment polymorphic analyses previously applied in such studies. The widespread use of ganciclovir (GCV) to treat CMV infections in immunosuppressed patients has led to the development of drug resistance. Phenotypic assays for CMV drug resistance are presently too time-consuming to be therapeutically useful and this has led to the development of genotypic assays for GCV resistance.[45] For other human herpesviruses the genomic mutations resulting in antiviral drug resistance have not all been identified. Phenotypic tests are easier to interpret and may detect resistance that genotypic analysis cannot yet discriminate. Maintenance of the capability of cell culture in diagnostic laboratories is thus important.

Virus serology

Complement fixation and indirect immunofluorescence tests are still used in many viral diagnostic laboratories. However, their use is declining in favor of more reproducible and sensitive enzyme-linked immunosorbent assay (ELISA) methodologies. Neutralizing antibody tests for herpesvirus antibodies are not routinely performed in diagnostic laboratories and none of these viruses has hemagglutinating capability.

Immunoassays are available for the detection of IgM or IgG antibodies to HHV-1 to HHV-8. As IgM antibody is the first antibody to be produced in response to infection it is by nature a broadly reactive antibody, and cross-reactions with other viral antigens and false-positive reactions may occur. IgM antibody is also produced during both acute (primary) infection and recurrent disease (although the amount produced is generally higher during primary infection). Quantitation of virus-specific IgG antibody in serial samples (10 or more days apart) often provides a more reliable diagnostic procedure. Determination of the avidity of IgG can provide valuable information when only a single, acute phase, sample of blood is available. IgG antibody avidity matures with time after onset of infection, thus detection of IgG antibody of low avidity provides evidence of recent (primary) infection; detection of high avidity IgG antibody allows differentiation of recurrent infection from primary infection.[46]

CLINICAL MANIFESTATIONS

With the exception of VZV, primary herpesvirus infections in the immunocompetent host are usually asymptomatic or are associated with a minor illness only, with no specific symptoms. As a consequence, primary herpesvirus infections may not be recognized. When symptoms do occur, herpesvirus infections in the immunocompetent host are normally self-limiting and require only symptomatic treatment. Antiviral therapy is available for a number of herpesviruses, but is usually only indicated for those patients who have more severe disease manifestations or when it is appropriate to minimize the likelihood of complications. Herpesvirus infections in the immunocompromised host are frequently severe and sometimes life threatening. Antiviral drug therapy is required for these patients to control the infection (see Table 155.7).

Herpes simplex viruses

Oropharyngeal infection

Primary oropharyngeal HSV-1 infection usually occurs in childhood and is often asymptomatic. In symptomatic infections acute gingivostomatitis is seen accompanied by fever and submandibular lymphadenopathy. The incubation period varies from 2 to 12 days (mean 4 days). The duration of clinical illness may extend from 2 to 3 weeks, with virus excretion from the oropharynx for an average of 7–10 days.

The severity and duration of clinical illness in recurrent infection is considerably less than in primary HSV infection. Recurrent orolabial lesions ('cold sores') are heralded by a prodrome of pain, burning, itching or tingling for several hours before the development of the characteristic vesicles. The vesicular stage persists for less than 48 hours and progresses to the ulcerative and crusting stage within 3–4 days. Pain resolves quickly during the same period, and healing is generally complete within 8–10 days. Systemic illness is usually absent in recurrent infection. Recurrence rates are highly variable and the precipitating factors involved are not well defined, but include the type of HSV (HSV-1 is more likely to recur than HSV-2), fever, stress, exposure to ultraviolet light and impaired CMI in the host.

Genital infections

Primary genital herpes usually occurs in adolescents and young adults. During the last decade 40% to more than 60% of isolates of HSV from first-episode genital herpes have been found to be due to HSV-1 in studies of populations with a high socioeconomic status from the UK, Scandinavia and the USA.[21] A similar change has not been observed in populations of low socioeconomic status.

Primary genital herpes is, in the majority of cases (50–70%), asymptomatic or so mild that the infection is not recognized. Where lesions are observed the disease is characterized by the appearance of vesicular lesions – in males usually on the glans penis, on the penile shaft or in the perianal region, and in women involving the vulva, vagina, cervix and perineum. Extragenital lesions (on the buttocks and thighs) occur in 10–20% of patients (see Chapter 58)

The recurrence rate of genital HSV-2 infections is 10 times higher than that of genital HSV-1 infections. The signs and symptoms of recurrent genital herpes are usually restricted to the genital region and are relatively mild and of shorter duration than for primary disease. As for primary disease, recurrent lesions in women are more often painful and of longer duration than in men (60–90% vs 30–70%, and mean 5.9 vs 3.9 days, respectively).

Other manifestations

A wide variety of other manifestations of infection are known (Fig. 155.6) including herpes simplex virus encephalitis, neonatal herpes and keratoconjunctivitis.[21] Patients compromised by immunosuppressive therapy (e.g. organ transplant recipients) or patients who have immunodeficiency are at risk of developing a severe primary infection and more severe and frequent recurrent disease. Disease severity is directly related to the degree of immunosuppression or immunodeficiency.

Varicella-zoster virus

Varicella (chickenpox) and herpes zoster (shingles) are due to infection with the same virus, VZV. Varicella is the usual manifestation of primary infection, herpes zoster the most usual manifestation of recurrent infection.

Varicella (see Chapter 8)

Varicella is a common childhood exanthematous disease, usually affecting children in their early school years. The incubation period is 14–15 days (range 10–20 days). The rash is characterized by maculopapular lesions that vesiculate in about 3–4 days before crusting and scab formation. Scabs may remain *in situ* for up to 3 weeks. The exanthem is centripetal rather than centrifugal, with most lesions being present on the trunk and proximal extremities. Secondary bacterial infection of the lesions as a result of scratching is the most frequent complication. Occasionally cerebellar ataxia, transverse myelitis or Reye's syndrome may complicate the infection. Primary VZV infection occurring in adolescence and adult life is often associated with more severe disease including visceral complications such as pneumonitis, encephalitis and hepatitis.

Primary VZV infection may have a more aggressive course in pregnant women. This frequently includes visceral complications, notably pneumonitis. The fetus is at risk of infection and there is a low risk of severe fetal abnormalities ranging from long-lasting rash, limb or dermatomal scarring, to severe neurologic damage.[47] Severe neonatal varicella may occur when maternal varicella presents within 5 days of delivery.

As for other herpesvirus infections, primary VZV infection of immunocompromised patients such as children undergoing cancer chemotherapy or corticosteroid treatment usually has a more severe protracted course with an increased rate of complications, particularly pneumonitis.

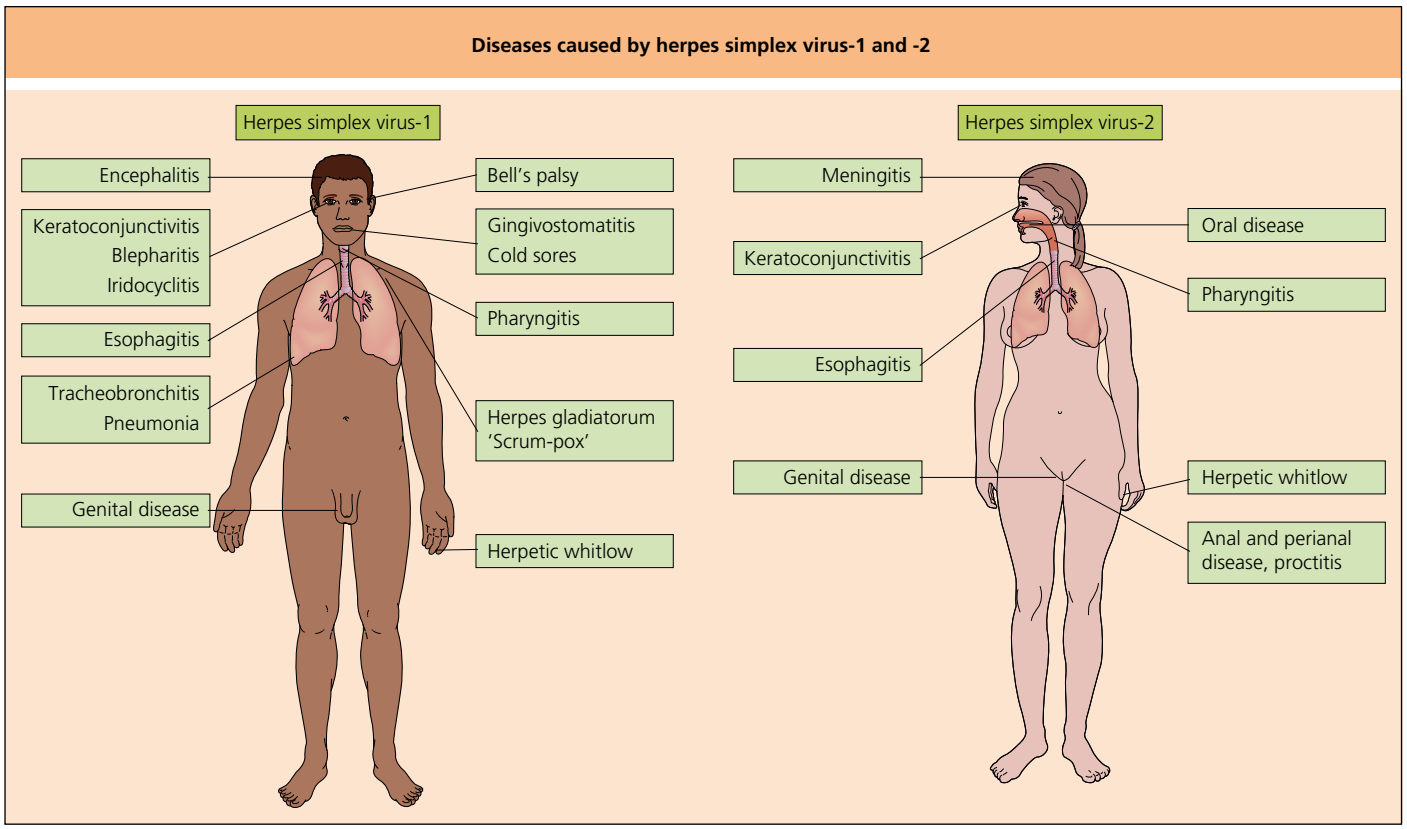


Fig. 155.6 Diseases caused by herpes simplex virus-1 and herpes simplex virus-2.

Herpes zoster

Herpes zoster is typically a disease of the elderly. Usually there is a prodrome of pain followed within a few days by the development of a unilateral vesicular rash within the dermatome served by the sensory nerve from the affected ganglion. The sites most commonly affected by herpes zoster are the dermatomes T3 through L3, often the same as those that were most affected during chickenpox. New lesions form over 2–5 days and the rash then pustulates and scabs over 2–3 weeks.

Complications including motor weakness and visceral manifestations may occur, but are unusual. However, in up to 50% of patients where herpes zoster involves the trigeminal nerve, ocular manifestations such as conjunctivitis, ulcerative keratitis, uveitis and iridocyclitis occur. Ocular complications are particularly common when the zosteriform lesions extend to the tip of the nose. Herpes zoster oticus, also called the Ramsay Hunt syndrome, consists of lesions on the ear or within the auditory canal, which are sometimes barely visible, and can result in hearing loss and facial paralysis. Persisting pain, known as postherpetic neuralgia, is the most common complication of herpes zoster. It can be severe and last for several months. The rate, severity and duration of postherpetic neuralgia are directly related to age. It is uncommon in patients under 50 years of age, but occurs in over 40% of those over 60 years of age.

Herpes zoster is frequently observed in patients who are immunocompromised. Chronic cutaneous lesions and disseminated infections have been described, particularly in patients who have HIV infection. Meningoencephalitis, pneumonitis or hepatitis can also occur in immunocompromised patients, but are rare.

Cytomegalovirus

CMV is an uncommon cause of disease in people who are immunocompetent. Primary infection may occasionally cause a glandular fever-type illness resembling EBV mononucleosis. In such patients persistent fever, myalgia and asthenia accompanied by atypical lymphocytosis and elevated liver transaminases are common signs and symptoms. Occasionally hepatitis is the presenting symptom. The presence of fever and atypical lymphocytes distinguishes CMV mononucleosis with liver involvement from infections by the usual hepatitis viruses.

Congenital and perinatal infections

CMV infections are the most common cause of congenital viral infections.[48] Both primary and recurrent infection in a pregnant woman can cause congenital and perinatal disease, but the frequency of severe disease is at least 10-fold higher after primary maternal infection. Overt clinical symptoms occur in 5–10% of cases of intrauterine CMV infection and include growth retardation, hepatosplenomegaly and thrombocytopenic purpura, and less frequently jaundice, microcephaly and chorioretinitis. The prognosis is poor for these infants. Another 10–15% of cases are neonates who are asymptomatic at birth and develop late sequelae, particularly mental retardation and sensorineural hearing loss.

Perinatal infections are nearly always asymptomatic, but neonates occasionally develop CMV pneumonitis, particularly when born prematurely. Severe disseminated disease may follow transfusion of CMV-infected blood products to premature neonates who have CMV-seronegative mothers.

Immunocompromised patients

The pathogenesis and clinical spectrum of CMV disease in patients who are immunocompromised depend upon the cause and degree of immunosuppression.[49] Treatment regimens must take into account the fact that the disease manifestations may have an immunopathologic or viral etiology.

Persistent intermittent fever is often the presenting symptom of CMV infection in patients who have had a transplant (see also Chapter 74). The infection may progress to cause pneumonitis, gastrointestinal disease, hepatitis and retinitis. In recipients of a solid-organ transplant, CMV disease most frequently occurs when the donor is CMV seropositive and the recipient is CMV seronegative. In bone marrow transplant patients, however, CMV disease most commonly results from reactivation of latent virus in the recipient, usually 20–90 days after the transplant. The most frequent manifestation is CMV pneumonitis. Unless treated very early, preferably before the appearance of respiratory symptoms, pneumonitis in these patients is often fatal.

Reactivation of latent CMV is usually the cause of CMV disease in patients who have HIV infection, frequently occurring in the later stages of the HIV infection when $CD4^+$ T-cell counts are less than 50/mm^3. The most frequent manifestation is CMV retinitis presenting with blurred vision and decreased acuity (see Chapter 91).

Gastrointestinal disease, hepatitis, encephalitis and pneumonia also occur in patients who have HIV infection. Gastrointestinal disease includes esophagitis, gastritis, enteritis and colitis and is usually associated with fever and weight loss. Although CMV is often detected in respiratory specimens from patients with AIDS who have pneumonitis, the pulmonary process is usually caused by other pathogens, particularly *Pneumocystis jirovecii*.

Epstein–Barr virus

In most individuals EBV infection does not cause overt disease.[13] Primary infections in adolescents can, however, result in infectious mononucleosis. As for CMV infection, immunocompromised hosts may develop severe manifestations during both primary and recurrent EBV infection. Such severity is exemplified by the role of EBV infection in the causation of lymphoproliferative disease.

Immunocompetent host

Infectious mononucleosis (glandular fever) usually presents as fever, fatigue and malaise accompanied by a sore throat, cervical lymphadenopathy, hepatomegaly and splenomegaly. A rash may develop, particularly in those treated with ampicillin. Disease manifestations, especially fatigue and malaise, can persist for several weeks. Relapses and a chronic course have been described but the relationship to EBV infection is uncertain (see Chapter 70).

Immunocompromised host

Immunocompromised patients are at risk of developing lymphoproliferative disorders following EBV infection. The polyclonal B lymphocyte proliferation is often associated with fever, lymphadenopathy and hepatosplenomegaly and is frequently life threatening in severely immunocompromised patients, such as bone marrow transplant patients.

Patients who have AIDS may develop oral hairy leukoplakia, which is characterized by white plaques on the lateral margin of the tongue. EBV is associated with lymphocytic interstitial pneumonia (which is common in pediatric patients who have AIDS) and a rapidly progressing, diffuse encephalitis.

Human herpesvirus 6

HHV-6, variant B, is the cause of exanthema subitum (roseola infantum) in young children. Symptomatic infection is characterized by a high fever, often associated with inflammation of tympanic membranes and sometimes associated with a mild respiratory illness and lymphadenopathy. This is followed by the appearance of a fine maculopapular rash spreading from the trunk to the extremities. Only a small proportion of these (<10%) develop exanthema subitum.[27] Recovery is usually rapid and uneventful, although a more protracted and severe course characterized by severe meningoencephalitis, fulminant hepatitis or fatal pancytopenia has been described. In adolescents and adults primary HHV-6 infection can cause a mononucleosis-like illness.

In severely immunocompromised patients, such as those who have AIDS or who have received a bone marrow transplant, reactivation of HHV-6 infection has an immunosuppressive effect, subsequent to which other pathogens may cause severe disease. In bone marrow transplant patients HHV-6 reactivation is associated with bone marrow suppression, probably as the result of viral replication in particular progenitor cells.[27] HHV-6 has also been associated with interstitial pneumonia and encephalitis in these patients.

Human herpesvirus 7

Although some cases of exanthema subitum appear to be due to HHV-7, a causal association between human disease and HHV-7 infection has not yet been established. HHV-7 has been associated with infant febrile illness as well as subsequent CNS complications. In renal transplant patients evidence has been obtained that HHV-7 may be a cofactor in the development of CMV disease.

Human herpesvirus 8

No association has been recognized between primary HHV-8 infection and specific clinical disease, but it is now generally accepted that HHV-8 has a causal role in KS, primary effusion lymphoma and multicentric Castleman's disease.[14]

Herpes B virus

Macacine herpesvirus 1 (herpes B virus) causes a benign latent infection in macaques that is analogous to HSV infection in humans. The infection is infrequently transmitted to humans. After an incubation period varying from 3 days to 3 weeks there may be localized pain, redness and vesicular skin lesions near the site of the viral inoculation, followed by localized neurologic symptoms. Encephalopathy is common and fatal in up to 70% of patients. The virus is susceptible to aciclovir and early treatment can be life saving.[49]

MANAGEMENT

As most herpesvirus infections in the immunocompetent host are self-limiting, patients usually only require supportive care. The type of supportive treatment required varies for different disease manifestations and may consist of rest, hydration, the appropriate use of antipyretics and analgesics, and treatment to soothe skin lesions and prevent secondary bacterial infection. Effective antiviral therapy is available for more severe cases of infection caused by HSV, VZV or CMV. It is stressed, however, that although antiviral drugs can help to control a herpesvirus infection they cannot eliminate the infection (see Table 155.8 and Chapter 146).

REFERENCES



References for this chapter can be found online at http://www.expertconsult.com

Chapter 156

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Papillomaviruses

NATURE

The papillomaviruses are now considered to be in a distinct taxonomic family, Papillomaviridae, and unrelated to the polyomaviruses. Papillomaviruses are widely distributed in nature; among mammals they infect humans, cattle, dogs, rabbits, monkeys and other species. Some animal papillomaviruses affect both epithelial and fibroblastic tissues and produce fibropapillomas, but human papillomaviruses (HPVs) are strictly epitheliotropic and infect the skin or the mucous membranes. Papillomaviruses cannot be propagated in cell culture. Therefore, rapid advances in the knowledge about papillomaviruses date from the 1970s, when molecular cloning of the viral genomes allowed comparisons between viruses from different species and from different sites of the same species. The cloned genomes provided reagents for examination of affected tissues for viral sequences.

The papillomavirus particle (Fig. 156.1) is about 55 nm in diameter and has a double-stranded, covalently closed, circular genome of about 8000 bp. All of the genomic information is located on one strand. The genome is divided into an early region that has eight open reading frames (ORFs E1–E8), a late region that has two ORFs (L1 and L2) and a noncoding long control region (LCR), which contains regulatory elements for viral DNA replication and transcription. The L1 ORF codes for the major L1 capsid protein, which accounts for most of the virion mass and mediates viral attachment. The L1 protein displays immunodominant type-specific neutralizing epitopes.[1] The currently available prophylactic HPV vaccines are based on the use of the L1 protein expressed by recombinant DNA technology and self-assembled as virus-like particles (VLPs). Experimental vaccines based on the minor L2 capsid protein, which displays a subdominant cross neturalizing epitope, may provide cross protection against related HPV genotypes.[2] The early region genes E6 and E7 of high-risk HPVs code for the transforming proteins of the virus that mediate the oncogenic properties of the virus. The E1 gene is required for viral DNA replication and the E2 gene modulates viral transcription. The E5 gene is a membrane protein that interacts with growth factor receptors; it is the main transforming gene for bovine papillomaviruses but not for HPVs. The E4 gene, although it is located in the early region, is expressed late in the virus cycle; it disrupts cytokeratins and probably facilitates virus exit from infected cells. The functions of E3 and E8 ORFs are not known.

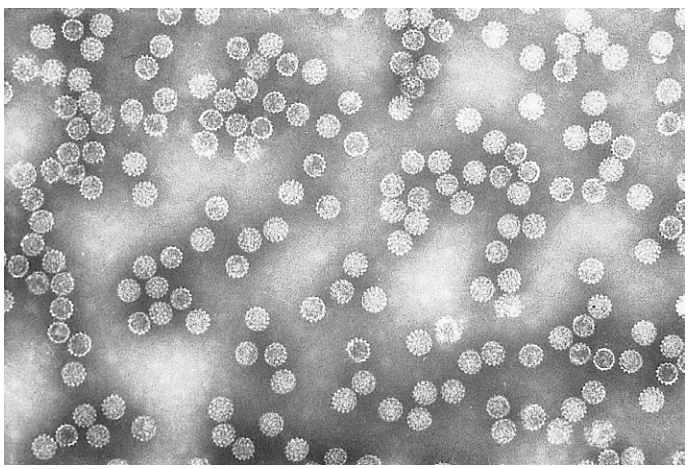


Fig. 156.1 Human papillomavirus particles. The particles are nonenveloped, have icosahedral capsids and are 55 nm in diameter. Courtesy of Dr M Reissig.

EPIDEMIOLOGY

More than 100 individual HPV types have been described to date.[3] They naturally fall into two groups, mucosal HPVs and cutaneous HPVs.

Mucosal human papillomaviruses

The genital tract is the reservoir for mucosal HPVs, except for two HPV types (HPV-13 and HPV-32) which infect the oral cavity. About 40 HPV types infect the genital tract. Genital HPV infections are the most prevalent sexually transmitted pathogens. As many as 30–40% of sexually active young women may have an HPV infection of the genital tract, as determined by sensitive DNA detection techniques. A history of multiple sexual partners, and having a male sexual partner who has many sexual partners, are the main risk factors for a woman for the acquisition of HPVs. Circumcision may decrease the risk of HPV acquisition in the male. The HPV prevalence reaches its peak in young adults and declines at older ages. HPV infections are largely asymptomatic and of 1–2 years' duration. Infection probably confers partial immunity to re-infection with the same type. The course of HPV infection is altered profoundly by HIV-induced immunosuppression.[4]

HPV-6 and HPV-11 are the etiologic agents of genital warts (condylomas), which occur in sexually active individuals, and also of recurrent respiratory papillomatosis (RRP), which may have onset in childhood or in adult life. HPV-16, -18, -31, -45 and some other types account for nearly all cervical cancers, as described in later sections of this chapter. HPV-13 and HPV-32 cause focal epithelial hyperplasia in the oral cavity. This condition is found frequently in indigenous populations, but rarely in other groups. How they are transmitted from person to person is unclear.

Cutaneous human papillomaviruses

Skin warts are transmitted by direct contact with an infected tissue or indirectly by contact with virus-contaminated objects. There is some specificity between HPV type, and site and morphology of the warts; plantar warts most often yield HPV-1, common warts HPV-2 and flat warts HPV-3 and HPV-10.

Epidermodysplasia verruciformis is a rare condition in which an extensive lifelong wart virus infection of the skin is never resolved.[5] The disease may be familial. The warts may be flat or they may be in the form of reddish-brown macular plaques. In about one-third of these patients, malignant transformation occurs in the reddish-brown plaques in areas exposed to sunlight. The epidermodysplasia verruciformis lesions contain a large number of HPV types. Types most commonly associated with skin cancers in epidermodysplasia verruciformis patients are HPV-5 and HPV-8. Low levels of HPV DNA sequences of a number of HPV types, including several novel types, have been recovered from nonmelanoma skin cancers (NMSC). HPV sequences are also found in plucked hair roots of healthy skin. It is not clear if HPVs play a role in the development of nonmelanoma skin cancers.[6] Ultraviolet (UV) light is considered the most significant risk factor of NMSCs. Cutaneous HPVs through the anti-apoptotic activity of their E6 gene may act as co-carcinogens by preventing elimination of cells with UV-induced DNA damage.

PATHOGENICITY

Papillomaviruses have a high degree of species and tissue specificity. Genital HPVs are rarely detected on skin other than that of the genital tract, but they can infect other mucosal sites in the body such as the aerodigestive tract. Cutaneous HPVs are almost never encountered in the genital tract. Papillomaviruses cannot be grown in monolayer cell cultures to yield virus particles. Full epithelial differentiation is required for the production of infectious particles.

Nearly all cervical cancers originate in the 'transformation zone', located at the lower end of the cervix, where the stratified squamous epithelium of the vagina forms a junction with the columnar cells of the endocervix. The cells in the transformation zone of the cervix must be highly susceptible to the oncogenic effects of HPVs, because cancers arise much less frequently at other sites in the genital tract (vulva, vagina, penis) that are infected with HPVs as frequently as the cervix but do not have an area similar to the transformation zone. Invasive cervical cancer is preceded by a progressive spectrum of cytologic abnormalities, classified as atypical squamous cells of undetermined significance (ASCUS), low-grade squamous intraepithelial lesions (LSILs) and high-grade squamous intraepithelial lesions (HSILs). The progression from initial HPV infection to invasive cancer may take 15–20 years.

Although almost all squamous cell abnormalities of the cervix including cervical cancer are the result of HPV infections, the probability of any one HPV infection progressing to cervical cancer is quite small (for review see[7]). Most HPV infections produce only transient cytologic abnormalities and are resolved completely without a trace. Cytologic abnormalities are seen in only about 10% of women who are positive for HPV DNA in the genital tract. A large majority of LSILs regress completely. In the USA, annually, there are tens of millions of HPV infections but only about 15 000 cases of invasive cancer. The relatively small number of cases is partly the result of treatment of LSILs and HSILs identified from Pap smear screening programs, but it is also, in a large measure, because most HPV infections do not result in significant cervical disease. In countries that have no effective Pap smear screening programs, the number of cases of cervical cancer is still a small fraction of the number of HPV infections in women.

HPVs are found in nearly all lesions spanning the entire spectrum of cytologic abnormalities from a LSIL to invasive cancer. The distribution of HPV types changes markedly with increasing severity of disease.[8] Almost all genital HPV types are represented in HPV DNA-positive cytologically normal specimens and in ASCUS and LSIL cases, but only about a dozen HPV types are found in invasive cervical cancer. Of these, four HPV types, HPV-16, -18, -45 and -31, account for nearly 80% of invasive cancers (Fig. 156.2). The genital HPV types have therefore been categorized as high-risk, intermediate-risk or low-risk types (Table 156.1) on the basis of their prevalence in invasive cervical cancer.

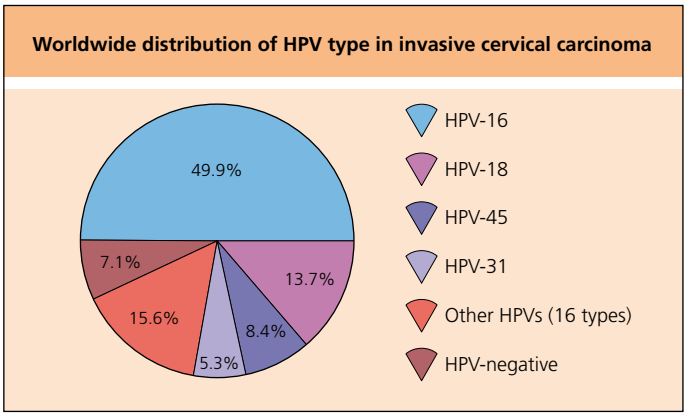


Fig. 156.2 Worldwide distribution of HPV type in invasive cervical carcinoma. The data are based on tests of over 900 cancers from different countries.[27]

Table 156.1 Major clinical associations of HPV infections

Disease	HPV types	Transmission
Cervical cancer High risk Intermediate risk Low risk	 HPV-16, -18, -45, -31 HPV-33, -35, -39, -51, -52, -56, -59, -68, -73 HPV-6, -11, -26, -42, -43, -44, -53, -54, -55, -62, -66	Sexual
Cancer of vulva, vagina, anal canal, penis	HPV-16	Sexual
Oropharyngeal cancer	HPV-16	Sexual
Anogenital warts	HPV-6, -11	Sexual
Juvenile-onset RRP	HPV-6, -11	Mother–child, at birth
Adult-onset RRP	HPV-6, -11	Unclear
Cutaneous warts	HPV-1, -2, -3, -4, -10	Nonsexual contact
Epidermodysplasia verruciformis (EV)	HPV-5, -8, -9, -12, -14, -15, -17, -19, -25, -36, -38, -47, -50	Nonsexual contact
Nonmelanoma skin cancer	EV HPVs and novel HPVs	Unclear
Focal epithelial hyperplasia of the oral cavity	HPV-13, -32	Nonsexual contact

RRP, recurrent respiratory papillomatosis.

The oncogenic potential of HPVs is mediated by the E6 and E7 proteins of high-risk HPVs. In laboratory studies, E6 and E7 of high-risk HPVs can each transform mouse 3T3 cells and, together, can immortalize human keratinocytes. Viral constructs containing E6 and E7 genes of high-risk HPVs produce HSIL-like lesions in organotypic cervical epithelium. The E6 and E7 genes are invariably expressed in cells of HPV-associated cervical cancers. The viral genome of HPVs remains episomal in infections that are not associated with cervical cytopathology, as well as in cases of LSIL and in most instances of HSIL. However, in most invasive cancers the viral genome is integrated into the cellular DNA. Integration requires linearization of the circular viral genome, which almost always occurs by a break in the E2 region of the genome. The E6 and E7 genes not only remain intact after integration, but they are released from the inhibitory effect of the E2 protein, and are expressed at high levels in invasive cancers. High levels of antibodies to E6 and E7 proteins are markers for invasive cancer.[9]

The molecular mechanisms of cellular transformation by E6 and E7 genes of high-risk HPVs are well understood and are described in detail in excellent reviews.[10] Briefly, the E6 protein complexes with tumor suppressor protein p53 and targets it for destruction via the ubiquitin pathway. The E7 protein complexes with tumor suppressor protein Rb, thereby releasing the transcription factor E2F from its complex with Rb. The E2F activates expression of *myc* and other genes that activate the cell cycle. In the normal cell cycle, tumor suppressor proteins p53 and Rb inhibit cellular proliferation. The functional inactivation of both of these cellular tumor suppressor proteins by the HPV oncoproteins results in continued cell proliferation without time for repair of DNA damage (Fig. 156.3). This leads to genetic instability and accumulation of additional cellular mutations and chromosomal changes. Thus, infections with high-risk HPVs prepare the ground for the cellular genetic alterations that underlie cervical cancer. For example, gain of 3q chromosome was found to be consistently associated with the early stages of invasive cervical carcinoma (Fig. 156.4).[11]

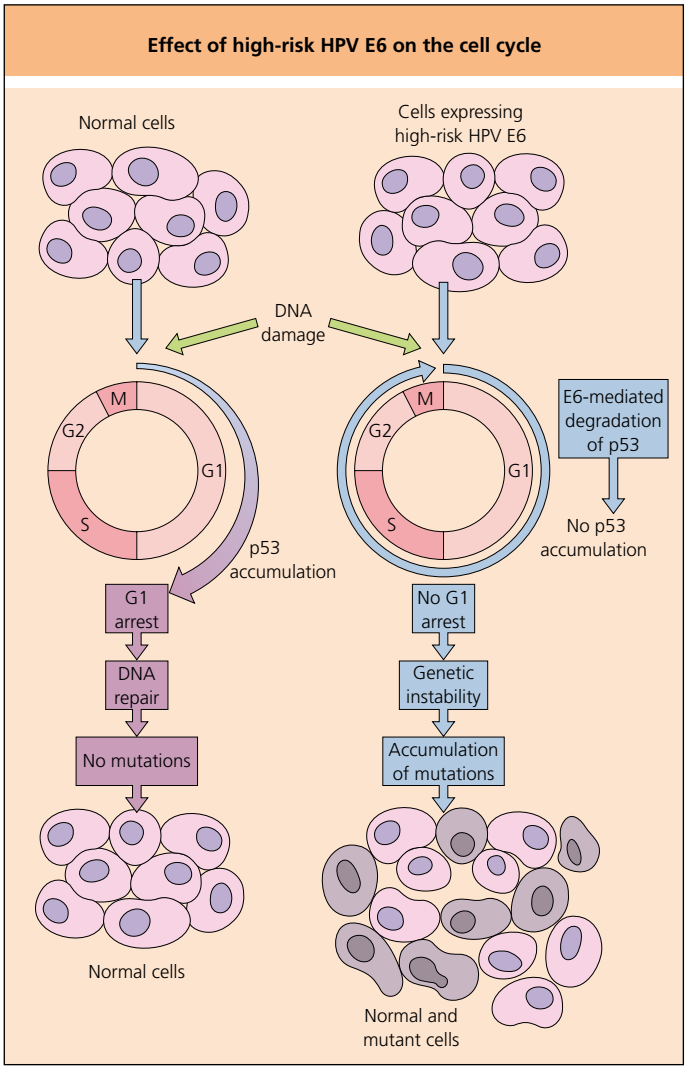


Fig. 156.3 Effect of high-risk HPV E6 on the cell cycle. In normal cells (left), DNA damage results in increased p53 production, which leads to arrest of cell cycle in G1 phase, allowing the cell time to repair DNA damage. In cells infected with high-risk HPVs (right), the HPV E6 mediates degradation of p53, so there is no accumulation of p53, no cell-cycle arrest, and continued cell multiplication. This leads to genetic instability and accumulation of cellular mutations. Courtesy of Dr TD Kessis.

PREVENTION

HPV-related diseases for which preventive strategies are being investigated are cervical and other lower genital tract cancers, tonsillar/oropharyngeal cancers, genital warts and recurrent respiratory papillomatosis.

Improved screening for cervical cancer

Implementation of Pap smear screening and treatment of cervical cancer precursor lesions identified by follow-up investigation of abnormal Pap smears have resulted in a marked reduction in the incidence of cervical cancers in the industrialized world but it has been difficult to establish effective Pap smear screening programs in the developing world. Primary cervical cancer screening by examination of cervical scrapes for DNAs of high-risk HPVs has been shown to have a sensitivity greater than that of Pap smears for the detection of HSIL and cancer.[12] Furthermore, adequate genital tract specimens can be self-collected by the women themselves, thus making it possible to avoid a pelvic examination. Assays for genital tract HPV DNAs are useful in the clinical management of mild cervical cytologic abnormalities.[13] Visual inspection of the cervix after acetic acid application (VIA) combined with treatment (if necessary) at the same visit has also been proposed as a screening method for developing countries.

Prophylactic vaccines

Virus-like particles (VLPs) that self-assemble from the L1 capsid protein when it is expressed by baculovirus or yeast recombinant DNA vectors have provided the immunogen for prophylactic vaccines. The VLPs are free of viral DNA and possess conformational epitopes of authentic virions. Immunization with VLPs of an HPV type leads to very high titers of virus neutralizing antibodies and to nearly 100% protection against subsequent HPV infection by that type.[14] Gardasil from Merck is a quadrivalent vaccine that has VLPs of high-risk HPV-16 and HPV-18 (which account for about 70% of invasive cervical cancers) and of low-risk HPV-6 and HPV-11 (which are responsible for 90% of genital warts and 100% of recurrent respiratory papillomas). Cervarix from GlaxoSmithKline is a bivalent vaccine with VLPs of HPV-16 and HPV-18. The vaccines largely provide immunity only to the VLP types included in the vaccine preparation.[15] Gardasil is commercially available in the USA and is recommended for young girls prior to sexual debut. It is not yet known if the vaccines will be effective in preventing infections in men.

Therapeutic vaccines

Several strategies are also being tried for therapeutic immunization, aimed at destroying established lesions of HSIL and invasive cancer.[16] For this purpose, the objective is to generate cytotoxic T cells directed against cells expressing the E6 and E7 proteins of high-risk HPVs.[17] Chimeric vaccines which may have both prophylactic and therapeutic properties are also under investigation.

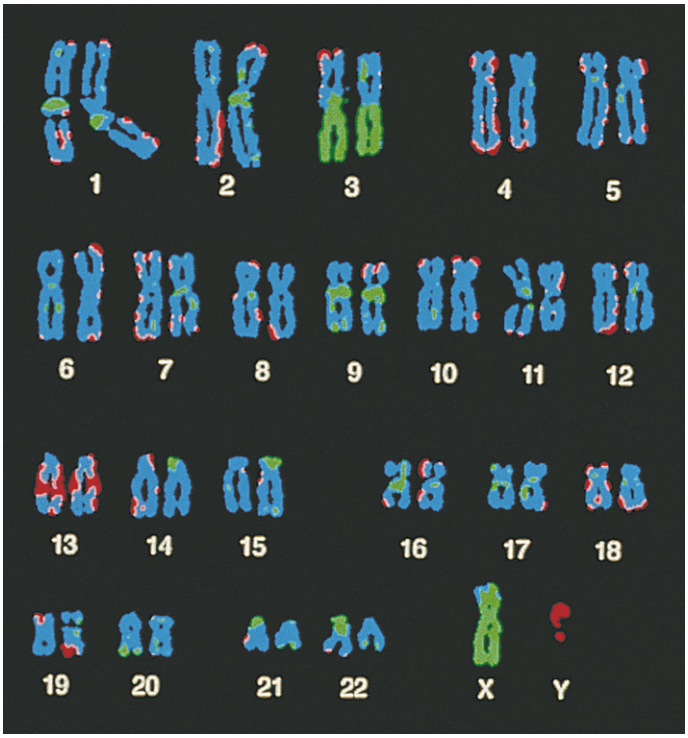


Fig. 156.4 Gain of chromosome 3q in early cervical carcinoma. The figure displays a ratio image after comparative genome hybridization, in which normal reference metaphase chromosomes are hybridized with a mixture of differentially labeled tumor DNA (green label) and normal DNA (red label). The chromosomes are ordered in a karyogram-like fashion. Chromosome 3q is gained (over-representation of green label) and chromosomal band 13q21 is lost (over-representation of red label) in this carcinoma. With permission from Heselmeyer *et al.*;[11] copyright (1996) National Academy of Sciences, USA.

DIAGNOSTIC MICROBIOLOGY

Hybridization assays

The presence of HPV in a tissue is ascertained by nucleic acid hybridization assays. The most widely used methods, especially in epidemiologic investigations, are those based on polymerase chain reaction (PCR) amplification.[18,19] Typically, consensus primers are employed to target conserved regions of the viral genome so that a large number of HPVs are amplified in a single amplification reaction. The PCR products are then screened for different HPVs by hybridization with type-specific oligonucleotide probes and by a 'generic' probe (a mixture of probes of several HPVs) that would detect many HPVs. The methods have high analytic sensitivity and a high specificity. In a modification of this assay, type-specific hybridizations are performed in a single step as line blots against probes immobilized on a strip.[20] Hybrid Capture is a signal amplification-based hybridization assay approved by the US Food and Drug Administration (FDA). In this assay, genital tract specimens are screened for HPVs in an enzyme-linked immunosorbent assay (ELISA)-type format with two pools of HPV probes, one pool consisting of high-risk HPVs and the other of low-risk HPVs.[21] This test has been found to be useful in 'triaging' women with abnormal Pap smears for colposcopy.[13]

Immunologic assays

Immunologic assays are rarely used for type-specific HPV diagnosis. In most HPV infections, viral particles and capsid proteins are present in very small amounts and so are difficult to detect with antiviral serum. Also, as cervical SILs progress toward higher grade disease and cervical cancer, synthesis of capsid proteins and infectious particles is completely shut down. Type-specific antiserum is not available for any HPV type.

Antibody response to HPVs may be measured using ELISA with VLPs.[22] Antibody response to HPV infections is low-titered and detectable in only about 40–50% of infected individuals. The proportion of infected individuals who are antibody positive increases with increased duration of infection and with greater viral load in the genital tract specimens.[23] Antibodies to E6 and E7 proteins are markers of HPV-associated invasive cancer. These antibodies may be detected by peptide-based ELISA or by radioimmunoprecipitation assays with *in-vitro* synthesized full length E6 and E7 proteins. Elevated levels of E6 or E7 antibodies are found in about 70% of patients who have invasive cancer but in less than 5% of controls.[9]

CLINICAL MANIFESTATIONS

The major clinical associations of HPVs are listed in Table 156.1. Aspects not covered in the earlier sections of this chapter are discussed below. Clinical features of these infections are also discussed in Chapter 59.

Cervical cancer

Cervical cancer is the most common female malignancy in the developing world. Approximately 500 000 cases of cervical cancer occur worldwide per year. The incidence of cervical cancer varies widely in different countries. Cervical cancer has long been known to have all the epidemiologic characteristics of a sexually transmitted disease. Observations from field, clinical and laboratory studies are mutually corroborative in building a compelling case for the HPV etiology of cervical cancer (Table 156.2), making it the first major human cancer with a single infectious etiology. The sexual behavior of the female,

Table 156.2 Evidence linking HPVs with invasive cervical cancer

Field	Evidence
Epidemiology	• HPVs present in nearly 100% of cancers in low- and high-incidence areas • HPV infection precedes cancer • HPV epidemiology and cervical cancer screening practices account for differences in worldwide incidence of cervical cancer
Pathogenesis	• HPV genome present in every cancer cell • HPV associated with entire spectrum of cervical neoplasia, from low-grade cytologic abnormalities to invasive cancer • HPV types most frequent in cancers are the most oncogenic in laboratory assays • HPV oncogenes E6 and E7 invariably expressed in cancers • HPV genome is episomal in preinvasive disease and integrates into cellular DNA in invasive cancers
Molecular mechanisms	• E6 and E7 proteins of high-risk HPV types distort cell cycle and promote genetic instability by degradation of tumor suppressor protein p53 and inactivation of tumor suppressor protein Rb • Integration of viral genome into cellular DNA enhances E6 and E7 expression

the sexual behavior of her male partners and the availability of an effective Pap smear screening program explain the wide differences in cervical cancer incidence in different countries.[24] In many high-incidence areas, male sexual behavior is the key factor for the high cervical cancer rates among relatively monogamous women.

HPV etiology of cervical cancer was proposed in 1976 and HPV vaccines which can prevent cervical cancer became available in 2006. Understanding the cause of a major human cancer and development of practical means to prevent it over a 30-year span is widely regarded as a major achievement in public health and cancer control.

Other anogenital cancers

HPV infections are responsible for the basaloid or warty vulvar cancers that occur in younger women but are not related to the more common typical keratinizing squamous cell carcinomas that occur in older women. They are strongly associated with cancers of the anal canal, vagina and penis.

Oropharyngeal/tonsillar cancer

A subset of oropharyngeal/tonsillar cancer is etiologically linked to high-risk HPVs, especially HPV-16.[25] Virologically, these tumors have a transcriptionally active HPV genome localized to tumor cell nuclei. HPV-16 accounts for more than 90% of the HPV-associated cancers at this site. As compared to other patients with head and neck cancers, these patients are less likely to have a history of alcohol and tobacco exposure, more likely to have a history of multiple sexual partners and of oral sex, and a significantly better prognosis. A majority of the estimated 51 000 annual cases of oropharyngeal cancers worldwide are due to HPV infection and are very likely vaccine-preventable.

Genital warts

Although they are benign, genital warts (condylomas) are a significant problem in sexually active populations and especially in immunocompromised individuals. Vaccines which contain HPV-6 and HPV-11 VLPs are likely to prevent a large majority of genital warts.

Recurrent respiratory papillomatosis (RRP)

This results from the transmission of HPV-6 and HPV-11 infections from the genital tract to the respiratory tract. For juvenile-onset disease, a large majority of the transmissions occur at birth, during passage of the fetus through an infected birth canal (for review of the clinical spectrum of juvenile-onset RRP, see[26]). Some reports suggest occasional intrauterine transmission. About 25% of the cases occur in the first year of life and most of these in the second 6 months. There are progressively fewer cases in each year thereafter.

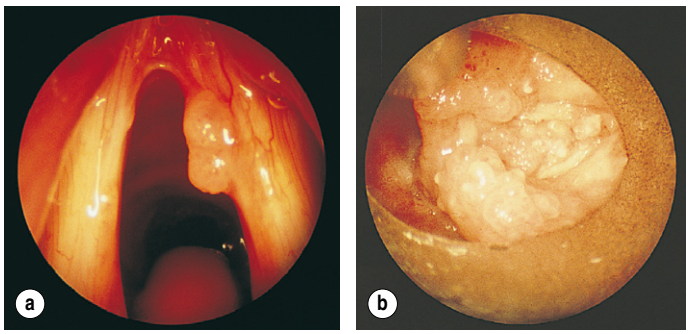


Fig. 156.5 RRP of juvenile onset. (a) A respiratory papilloma on the vocal cord of a child. (b) Papilloma obstructing the respiratory tract. Courtesy of Dr H Kashima.

The most common site of the papilloma is in the larynx on the vocal cords (Fig. 156.5). The tumors are benign but may threaten life if they grow and obstruct respiration. The tumors tend to recur after surgical removal, and in the worst cases operations may be required every few weeks. Operative procedures, especially tracheotomy, may spread the tumor by inadvertently transplanting tumor cells to other sites. Rarely, the tumor may undergo malignant transformation. Caesarian delivery when there is an active infection of the maternal genital tract will reduce the risk of juvenile-onset disease in the child. Mothers who have been immunized with HPV vaccines which have HPV-6 and HPV-11 VLPs are probably protected against having a child with juvenile-onset RRP.

MANAGEMENT

The clinical conditions associated with HPV infections are very diverse. Regular Pap smear screening and follow-up of women who have Pap smear abnormalities would greatly decrease the risk of cervical cancer. The preinvasive cervical disease is readily treated with greater than 90% cure rates. Genital and skin warts may regress spontaneously or may be treated with caustic agents (podophyllin), cryotherapy, application of an immunomodulating agent (imiquimod) or by surgical removal. Intralesional or parenteral administration of interferon has been successful in the treatment of refractory genital warts (see Chapter 59 for additional information).

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References for this chapter can be found online at http://www.expertconsult.com

Chapter 157

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Keerti V Shah

Polyomaviruses

NATURE

Polyomaviruses are small, nonenveloped viruses, which are widespread in nature. Although previously grouped with papillomaviruses in a single family, the International Committee on Taxonomy of viruses has now recognized polyomaviruses as an independent family, the Polyomaviridae. They are highly adapted to grow in the species and the tissue they infect. The first human polyomaviruses were isolated in 1971 from immunocompromised patients. JC virus (JCV) was isolated from the brain of a patient with Hodgkin's disease who died of progressive multifocal leukoencephalopathy (PML), a demyelinating disease of the central nervous system.[1] BK virus (BKV) was isolated from the urine of a renal transplant patient who developed ureteral stenosis.[2]

In 2007, genomes of two new human polyomaviruses, KI virus and WU virus, were independently detected in respiratory tract secretions of children by use of molecular techniques.[3,4] These two viruses share ~65–70% amino acid similarity with each other, but only ~15–50% similarity with JCV and BKV. The prevalence, biology and pathogenicity of these viruses are currently unknown. The viruses have been detected in respiratory specimens in the presence of other recognized respiratory pathogens and thus their role in disease is unclear. Very recently, another new human polyomavirus, Merkel cell virus (MCV), was detected by employing mass sequencing of a messenger RNA library of tumor cells and bioinformatics analysis of the data to identify nonhuman sequences with homology to known infectious agents. The viral genome was shown to be integrated within the genome of cells of a rare human skin cancer, Merkel cell carcinoma.[5] MCV is distantly related to the other known human polyomaviruses (Fig. 157.1). The prevalence of the virus in human populations is unknown and the precise role of the virus in the etiology of Merkel cell carcinoma remains to be established.

Polyomavirus virions are about 44 nm in diameter, have icosahedral symmetry and are composed of 72 capsomeres. The viral genome is a circular, covalently closed, supercoiled, double-stranded DNA of about 5000 bp. The viral genome is organized into three functional regions, the early and late coding regions and a regulatory region. The early region encodes the two viral regulatory proteins, the large T and small t antigens. The large T antigen regulates viral transcription and initiates viral DNA replication. Additionally, the large T antigen can interact with cellular proteins. Among the known properties of large T antigen is the ability to inactivate host cell tumor suppressor proteins, which contributes to the oncogenic potential of polyomaviruses. The late region codes for the major structural protein, VP1, and the two minor structural proteins, VP2 and VP3. The structural proteins assemble into capsomeres composed of five VP1 molecules and a single VP2 or VP3 molecule, which then form capsids from 72 capsomeres. The viral regulatory region spans 300–500 bp and contains elements for viral DNA replication, binding sites for cellular transcription factors, and promoters for transcription of early and late genes. The regulatory region contains repeat elements which are subject to deletions, insertions and rearrangements. As compared to strains excreted in the urine, polyomaviruses detected in nonurinary specimens often have regulatory region rearrangements, which may confer increased viral fitness in the host cell environment. Infection of cells is initiated by adsorption of virions to the cell surface by interaction with cell surface sialic acids. The protein component of the cellular receptor for BKV is unknown; however, that for JCV is the serotonin receptor $5HT_{2A}$.[6] Polyomaviruses enter the cell cytoplasm by endocytosis and are transported to the nucleus via the cytoskeleton transport machinery. Within the nucleus, viral DNA replication and formation of progeny virions takes place. New virions are thought to be released by either lysis of host cells or secretion from the plasma membrane.

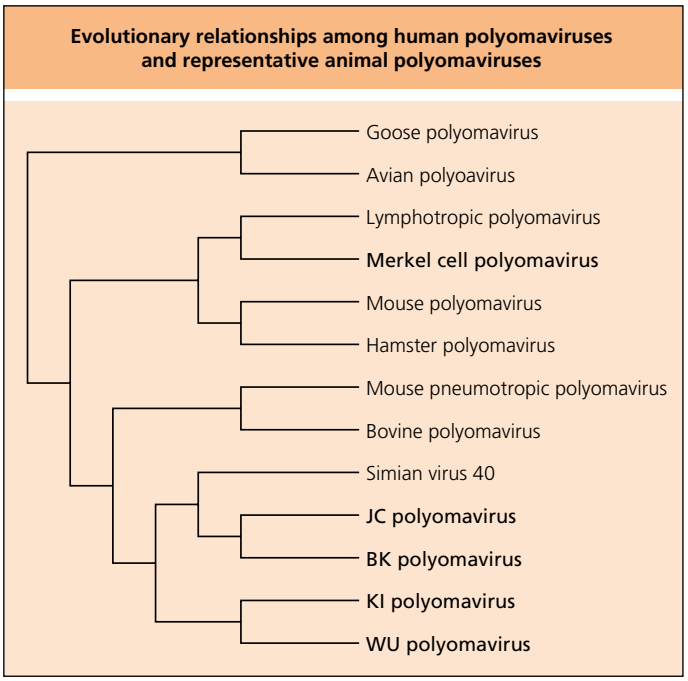


Fig. 157.1 Evolutionary relationships among human polyomaviruses and representative animal polyomaviruses. BKV and JCV are closely related to each other and to the rhesus macaque polyomavirus (SV40). KI and WU form a separate evolutionary group, which is nevertheless related to the BKV/JCV/SV40 group. The Merkel cell virus is very distantly related to the other human polyomaviruses but is closely related to a polyomavirus whose natural host is believed to be the African green monkey (lymphotropic polyomavirus).

EPIDEMIOLOGY

Both BKV and JCV infections occur in childhood, but BKV occurs at an earlier age than JCV. BKV seropositivity increases rapidly with age and reaches 98% at 7–9 years of age. JCV seropositivity increases more slowly with increasing age, reaching 50% among children aged 9–11 years and 60–70% by adulthood.[7] The most likely mode of transmission of BKV and JCV is via the respiratory tract. No serologic assays are available for the new human polyomaviruses, KI, WU and Merkel cell polyomavirus, and thus the prevalence of these viruses in human populations is unknown.

PATHOGENICITY

Primary BKV and JCV infections are either entirely asymptomatic or may be associated with nonspecific flu-like symptoms. The viruses probably multiply at the site of entry, and then reach the kidney, the main target organ, by viremia. They undergo further multiplication in the kidney and produce transient viruria. After primary infection, the viruses remain latent in the kidney for an indefinite period of time. The viruses may activate periodically and be associated with asymptomatic shedding of virus in the urine. BKV DNA can be detected in the urine of ~0–1% of healthy persons and JCV DNA can be found in ~35% of healthy individuals, with a higher incidence of JCV shedding in older individuals.[8] After primary infection the viruses also persist in B lymphocytes and JCV has been reported to persist as a latent infection of the brain.[9] Nearly all significant illnesses due to BKV and JCV occur in immunocompromised hosts, mostly as a result of reactivation of latent virus but sometimes as a primary infection in an immunocompromised host. Conditions in which viruses are reactivated include pregnancy, diabetes, organ transplantation, anti-tumor therapy, and AIDS and other immunodeficiency diseases.

PREVENTION

No attempt has been made to prevent primary BKV or JCV infection since infection is harmless in immunocompetent individuals and disease is uncommon even in immunocompromised individuals.

DIAGNOSTIC MICROBIOLOGY

BKV can be cultivated in several cell lines of human origin. JCV grows best in human fetal glial cultures. Isolation of BKV and JCV in cell culture is rarely attempted because it is inefficient and cumbersome and the needed cell types are not readily available. It is not known if WU, KI or Merkel cell polyomaviruses can be propagated in cell cultures. Cells and tissue infected with BKV and JCV can be identified by immunoperoxidase and immunofluorescence staining with antiviral antiserum. Immunization of rabbits with disrupted viral capsids or VP1 protein produces a broadly cross-reactive antiserum.

BKV and JCV DNA, as well as WU and KI virus DNA, can be readily identified by polymerase chain reaction (PCR) assays. Efforts to standardize these assays and prepare international reference samples are ongoing. Serologic diagnosis of BKV and JCV infection can be made by hemagglutination-inhibition assays or by enzyme-linked immunosorbent assays (ELISA) which use virus-like particles formed by self-assembly of VP1 protein produced in eukaryotic expression systems. Because BKV and JCV infections occur early in life and are very common,[10] serologic assays have limited utility for clinical diagnosis.

CLINICAL MANIFESTATIONS

Progressive multifocal leukoencephalopathy

Progressive multifocal leukoencephalopathy (PML) is a fatal, subacute demyelinating disease of the central nervous system that results from JCV infection of oligodendrocytes in the brain. It occurs as a complication of conditions associated with T-cell deficiency, including lymphoproliferative disorders, primary immunodeficiency diseases, prolonged immunosuppressive therapy and secondary immunodeficiency disorders such as human immunodeficiency virus (HIV) infection. Until the advent of HIV/AIDS, PML was a rare disease with onset typically in middle age or later life. At present, HIV is estimated to be the underlying cause of immunosuppression in 55–85% of cases of PML, and PML is diagnosed in approximately 5% of AIDS cases.[11] In 2006, three patients with multiple sclerosis or Crohn's disease developed PML in association with administration of natalizumab, a monoclonal antibody to alpha-4 integrin that prevents entry of inflammatory cells into brain and other tissues.[12] Why natalizumab would uniquely predispose persons to the development of PML remains unexplained.

Progressive multifocal leukoencephalopathy has an insidious onset. Early signs are impairment of speech and vision and mental deterioration. Paralysis of limbs, cortical blindness and sensory impairment occur in later stages of the disease. Patients remain afebrile and headache is uncommon. Typically, the disease is progressive, resulting in death within 3–6 months after onset. The introduction of highly active antiretroviral therapy (HAART) for HIV/AIDS has had only a modest effect on the incidence of PML, but prognosis is improved.[13] At disease onset, factors associated with better survival include higher CD4 cell count, lower JCV viral load, prior exposure to HAART, elevated myoinositol in PML lesions on MRI and higher levels of macrophage chemoattractant protein (MCP)-1 in cerebrospinal fluid (CSF).

The diagnosis of PML is established conclusively by pathologic examination of biopsy tissue. The brain shows foci of demyelination, most frequently found in subcortical white matter. Microscopically, the presence of enlarged nuclei of oligodendrocytes is the pathognomonic lesion of PML. These altered nuclei contain abundant numbers of JCV viral particles. Noninvasive techniques, particularly MRI of the brain, provide an effective means for diagnosis of PML. MRI scans are nearly always abnormal in association with PML.[14] The typical abnormalities are localized to subcortical white matter and are characterized by increased T2 signal and little contrast enhancement after gadolinium administration. CSF analysis typically shows minimal pleocytosis and modestly elevated protein. PCR of CSF for JCV DNA is the best noninvasive test for diagnosis of PML. Sensitivity of PCR is ~80% and specificity is 100%.[15]

BK virus nephropathy in kidney transplant recipients

BK virus nephropathy (BKVN), also designated polyomavirus-associated nephropathy (PVAN), has recently been recognized as an important cause of graft dysfunction and graft loss in patients with renal allografts. The incidence of BKVN is 1–10% and graft loss occurs in from 10% to 80% of cases.[16] The majority of cases occur in the first year post transplantation (Fig. 157.2). The variable incidence and outcome of BKVN most likely reflect differences across renal transplant centers in diagnostic criteria for BKVN, patient populations, immunosuppressive regimens and management strategies. The emergence of BKVN has coincided with the expanding use of tacrolimus and mycophenolate mofetil in immunosuppressive regimens to control graft-versus-host disease.

The intensity of immunosuppression appears to be a more important factor in disease than a specific drug. Bolus therapy with corticosteroids also may be a risk factor for BKVN. Host factors such as

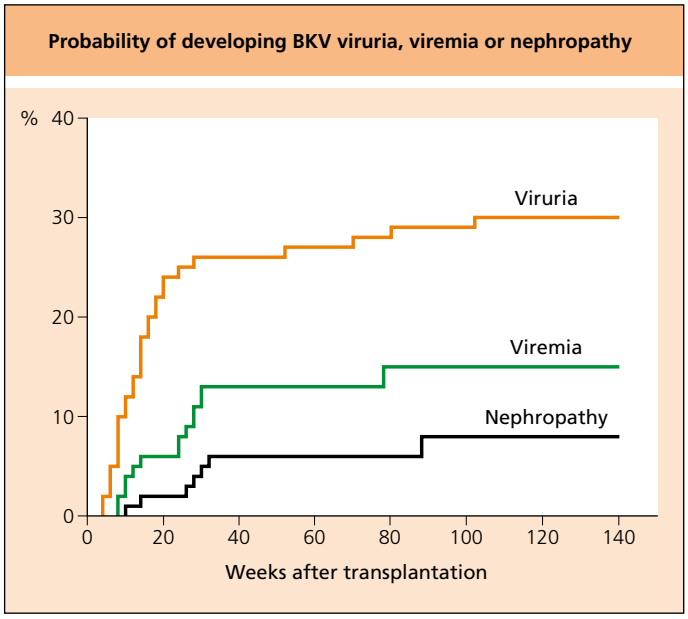


Fig. 157.2 Probability of developing BKV viruria, viremia or nephropathy in the weeks following renal transplantation. Adapted from Hirsch *et al.*[16]

age greater than 50 years, male gender, white ethnicity and underlying diabetes mellitus, and allograft factors such as number of human leukocyte antigen (HLA) mismatches between donor and recipient and prior tubular injury to the graft can increase the risk of BKVN. Apart from donor BKV seropositivity and recipient seronegativity, there is no convincing evidence that viral factors play a role in the risk of BKVN.

The definitive diagnosis of BKVN requires a renal biopsy showing polyomavirus-induced cytopathic changes in tubular or glomerular epithelial cells. Enlarged nuclei with basophilic inclusions in the tubular epithelium are the pathognomonic lesion of BKVN. Depending on the stage of disease, necrosis of cells of the tubules and collecting ducts and varying degrees of interstitial inflammation can also be observed.[17] Noninvasive diagnostic techniques can be used to screen for BKV replication and to make an early diagnosis of BKVN. Polyomavirus-bearing 'decoy cells' can easily be detected in urine with a Papanicolaou-stained cytology preparation. The positive predictive value for BKVN of the detection of decoy cells is 25–30%. BKV DNA can be detected in urine and plasma by PCR. Because of the high rate of excretion of BKV in urine of renal transplant recipients, the finding of BKV DNA by PCR in urine is of limited clinical value for diagnosis of BKVN. On the other hand, detection of BKV DNA in plasma by PCR has been shown to have a sensitivity of 100% and a specificity of ~85% for biopsy-confirmed BKVN.[18]

Hemorrhagic cystitis in bone marrow transplant recipients

BK viruria occurs in ~50% of patients after bone marrow transplantation (BMT). Hemorrhagic cystitis is the most prevalent and serious clinical manifestation of BKV infection in BMT recipients, with an incidence of 10–25%.[19] Less common clinical manifestations of BKV infection in BMT include ureteral stenosis and interstitial nephritis. Patients with hemorrhagic cystitis experience urgency, frequency of urination, dysuria, suprapubic pain and variable degrees of hematuria. BKV-associated hemorrhagic cystitis must be distinguished from noninfectious hemorrhagic cystitis resulting from direct cytotoxic effects of antineoplastic treatment. Typically, BKV infection is associated with late onset hemorrhagic cystitis and drug toxicity with early onset disease. Potential risk factors for BKV-associated hemorrhagic cystitis include acute graft-versus-host disease, intensive immunosuppressive conditional regimens, allogeneic transplant and magnitude of BKV urine viral load. BKV DNA can be detected by PCR in urine of patients with BKV-associated hemorrhagic cystitis; however, detection of BKV DNA does not have high specificity. A high urine viral load or viremia has greater specificity.

Role of polyomaviruses in human malignancies

JCV and BKV are oncogenic for laboratory animals and they transform cultured cells.[20] These viruses, therefore, have been investigated for their role in human malignancies. There are reports of finding JCV DNA in brain and colon tumors and BKV DNA in prostate, bladder and brain tumors, as well as neuroblastomas and insulinomas. However, a reproducible and consistent etiologic association of JCV or BKV with any human malignancy has not been demonstrated.

The recently described Merkel cell polyomavirus provides a more convincing example of a polyomavirus-induced human malignancy since the viral genome was found to be integrated into cellular DNA, a key event in experimental polyomavirus-induced animal tumors.[5] Additionally, the observation of integration at different chromosomal sites in virus-positive tumors suggests that viral infection precedes clonal expansion of Merkel carcinoma tumor cells.

MANAGEMENT

The majority of patients with BKV and JCV infections are asymptomatic and do not require treatment. There are no antiviral drugs with proven clinical efficacy against human polyomaviruses. The mainstay of treatment for BKV nephropathy is the reduction, change in drugs or discontinuation of immunosuppressive therapy.[21] Historically, the prognosis for PML was poor with death occurring within 3–4 months of diagnosis. However, for HIV patients, the introduction of HAART has improved survival of patients with PML. Paradoxically, in some patients with PML on HAART, a successful response to HAART is associated with an intense inflammatory response and a poor PML outcome.

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Chapter 158

Aloys CM Kroes

Parvoviruses

NATURE

The family Parvoviridae consists of remarkably small (*parvus* in Latin), nonenveloped, single-stranded DNA viruses of animals, divided into the subfamilies Parvovirinae of vertebrates and Densovirinae of arthropods. The Parvovirinae subfamily is further divided into five genuses: *Parvovirus*, *Erythrovirus*, *Dependovirus*, *Amdovirus* and *Bocavirus*. The genus *Parvovirus* consists of a large number of nonhuman mammalian viruses, including minute virus of mice, feline panleukopenia virus, Kilham rat virus and bovine, porcine, canine, goose and feline parvoviruses. The genus *Erythrovirus* consist of primate parvoviruses with the unique property of requiring erythroid precursor cells for productive infection. The important human pathogenic B19 virus belongs to this genus. Dependoviruses consist of mammalian parvoviruses that require a helper virus for replication. The typical helper virus is adenovirus, hence the name adeno-associated virus (AAV). Human AAVs are candidate gene therapy vectors because they can infect many cell types but do not replicate and are considered nonpathogenic. The *Amdovirus* genus has recently been defined to accommodate Aleutian mink disease virus, hence the name, when it appeared to differ essentially from *Parvovirus* members. Finally, there is the genus *Bocavirus*, named after the bovine and canine minute parvoviruses which share several properties. A human virus was described in 2005 with features that fit well in this genus. Since then, this human bocavirus has been confirmed to occur worldwide and to be associated with respiratory disease.

The other subfamily of the Parvoviridae, Densovirinae, infects arthropods: some species in this large and diverse group have economic importance, such as those affecting silk production.

Therefore, at this moment, two viruses belonging to the Parvoviridae are known as human pathogens, B19 virus and human bocavirus. The latter has only recently been discovered and definitive conclusions on its precise clinical significance are not yet possible. Nonetheless, as it is clear that this virus differs essentially from B19 virus, it will be dealt with separately at the end of the chapter.

Further details will now be given of the virus which is known as human parvovirus B19. With the description of another human pathogenic virus in the family, the use of the extension 'B19' has become more important and the name of the virus should preferably be abbreviated as B19 virus (B19V). This remarkable name does not refer to any systematic nomenclature but has been assigned by Yvonne Cossart, who in 1974 detected an immunoreactive antigen in human serum obtained from a donor referred to as B19. This antigen was subsequently determined to be a previously unrecognized small icosahedral viral particle on electron microscopy, with parvovirus-like features.[1] The corresponding antibodies were widely present in the human population and appeared to be increasing with age but it took some time before several important disease associations were revealed. First, infection with this virus was associated with aplastic crisis among persons with sickle cell disease,[2] subsequently with the frequent childhood exanthema known as erythema infectiosum[3] and finally with a serious complication during pregnancy, hydrops fetalis.[4] These three clinical entities are still the most relevant consequences of infection by parvovirus B19, although a number of other possible presentations have been added since then.[5]

The B19 virus appeared a typical member of the Parvoviridae family: a small (23 nm) viral particle, with a small (5.6 kb) genome consisting of single-stranded DNA (Fig. 158.1). The DNA has palindromic hairpin structures at either end and encodes only three major viral proteins, the viral capsid proteins VP1 and VP2 and one nonstructural protein, NS1, in addition to two smaller products of unknown significance. Replication of this virus is limited to human erythroid precursor cells, likely related to the presence of the cellular receptor P-antigen or globoside.

Progeny virus is assembled in the nucleus and may be observed as intranuclear inclusions. Equal numbers of B19 positive- and negative-sense DNA strands are encapsidated. The VP1 and VP2 proteins are overlapping, with the VP2 (58 kDa) fully contained in the VP1 (84 kDa) sequence. VP2 is the major constituent of the viral capsid (96%). NS1 has helicase activity and is involved in viral DNA transcription and replication. In addition, this protein has effects on the host cell,

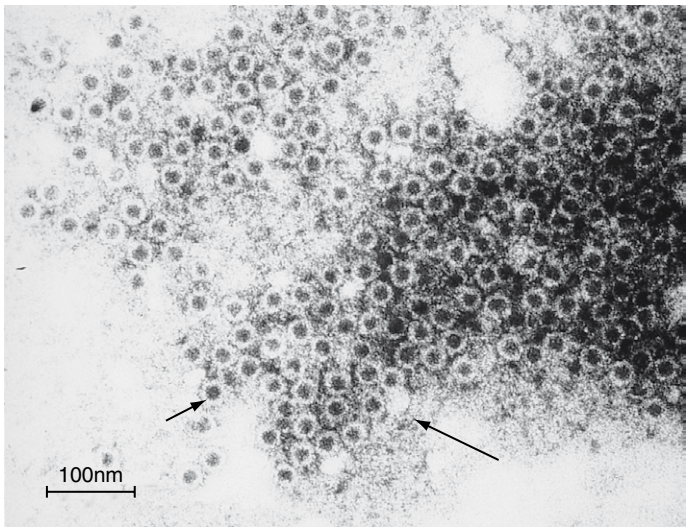


Fig. 158.1 Electron micrograph of parvovirus B19 particles showing both full particles (short arrow) and empty capsids (long arrow). Courtesy of Stanley Naides, MD.

causing cell cycle arrest and apoptosis. All further functional requirements for viral replication are derived from the host cell. Although the B19 genome is generally highly conserved, three clades are known to circulate.[6] No clear correlations are present between these three genotypes and distinctive pathologic and clinical manifestations.[7]

EPIDEMIOLOGY

Parvovirus B19 is found worldwide and infections occur in all seasons. In addition, two patterns can be recognized with regard to the occurrence of infections in time: a yearly peak in late winter and early spring in most moderate climates and a peak occurring in an approximate 4-yearly interval. This latter phenomenon of a cyclic pattern has been observed with other childhood exanthems, at least before vaccination intervened, and is related to the proportion of susceptible hosts in the population. Transmission of B19 virus occurs via nasopharyngeal secretions. Populations of children acquire B19 antibodies in an age-dependent fashion, beginning with entry into schools. Most surveys indicate that 50–70% of adults are anti-B19 virus IgG seropositive, indicating past infection and immunity. Clinical signs of infection occur after an incubation period of 7–18 days,[8] while infectivity is already present before this time. B19 virus may resist current methods of blood decontamination and therefore pooled blood products may contain infectious B19 virus.[9]

PATHOGENICITY

The particular tropism of parvovirus B19 for erythroid precursor cells plays a central role in the understanding of the pathogenesis of associated diseases. About 7–8 days after exposure to the virus, local replication in the bone marrow leads to a brisk viremia of 10^{11} or more viral particles/ml. In this same phase, the virus becomes detectable in respiratory secretions, leading to further spread. Viral replication causes maturation arrest at the giant pronormoblast stage of erythroid development, likely through viral induction of apoptosis.[10] Nonerythroid lineages may be affected as well, although these are less efficient in supporting B19 viral replication. Interestingly, in all infected individuals a temporary total disappearance of reticulocytes is observed, reflecting an arrest of erythropoiesis, which is usually too short in duration to lead to a relevant effect on hemoglobin levels. However, in individuals with shortened erythrocyte survival, as in those with hemolytic anemias, this period of areticulocytosis is extended and will often lead to severe anemia.

In recipients of recent stem cell transplantation, parvovirus B19 infection may lead to an aplastic crisis. Most likely, these complications arise as a consequence of the inability to recruit new erythroid progenitors in time, in situations of pre-existing hematopoietic stress. This also applies to the expanding hematopoietic system of the fetus, which would explain the observation of extreme fetal anemia with associated hydrops fetalis which may follow maternal infection. A few days after the peak of viremia, septic antibodies appear. In this phase the clinical signs of erythema infectiosum may develop, characteristically consisting of a skin rash and arthralgia. This temporal association is a clear indication of the likely immunopathogenetic origin of these signs.

Modern techniques have shown that there is a strong decrease in viral titers in the blood in this period which does not always immediately lead to viral clearance. Virus may remain detectable in the blood at a low level for an extended period in some hosts, possibly reflecting some steady state level of viral clearance and replication.[11] Ultimately, in normal hosts the virus will be cleared from the blood but a failure to produce sufficient antibody responses may result in persistent or recurrent viremia and chronic or recurrent bone marrow suppression.[12]

The described pathogenesis results in two clinical phases of illness, as observed in experimental infections,[8] but these may overlap

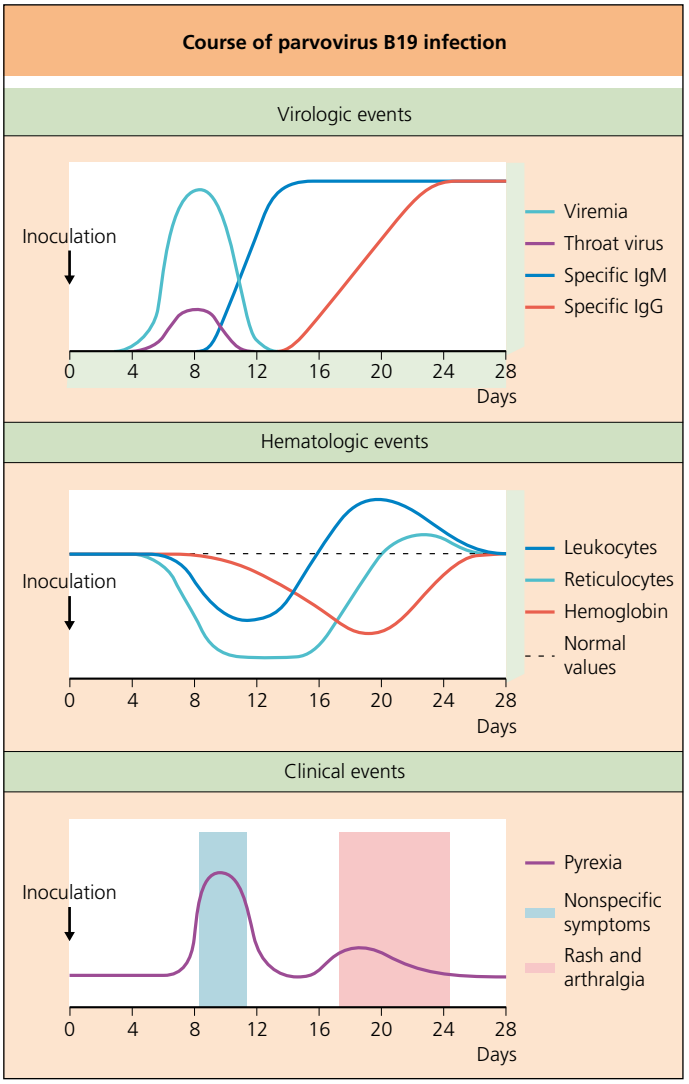


Fig. 158.2 Course of parvovirus B19 infection. Volunteers were inoculated nasally with viremic serum and virologic, hematologic and clinical events observed. Courtesy of Stanley Naides, MD.

in natural infections (Fig. 158.2). In the first phase, mild fever, malaise and myalgia may occur during the viremia, with associated areticulocytosis, leukopenia and thrombocytopenia. In the second phase, the appearance of antiviral IgM is associated with a rash and arthralgia. In an infected pregnant woman, the virus may cross the placenta, most likely during the initial extreme peak of viremia.[13] Fetal infection without an effective immune response results in a persistent arrest of erythroid maturation with a deep anemia and a high-output congestive heart failure presenting as hydrops fetalis. Theoretically, myocardial expression of the P-antigen could imply an additional role of viral myocarditis as a cause of cardiac failure but this has not been firmly established.

PREVENTION

Infected patients shed virus in nasopharyngeal secretions, at the time of, or even before, the typical signs of erythema infectiosum are apparent. This severely limits the effects of measures based on the recognition of this disease. Hygiene regarding contact with secretions as well as precautions against the spread of infectious droplets are warranted if susceptible persons are involved. Patients should be considered infectious until 1 or 2 days after onset of rash.[14] Generally, attention is

focused on pregnant women who have been in contact with (preferably proven) cases of erythema infectiosum. In these cases, it is important to determine potential susceptibility by testing for B19 virus-specific antibodies. Nonimmune exposed pregnant women should be offered further monitoring to enable a timely diagnosis of potential maternal and eventually fetal infection, which could necessitate an intrauterine transfusion.[15] No vaccine is available and there is no experience with the prophylactic administration of immunoglobulins.

DIAGNOSTIC MICROBIOLOGY

The antibody response against the virus is highly useful for diagnostic purposes. An acute infection can be confirmed by testing for IgM antibodies against B19 virus and immunity is demonstrated by the presence of IgG antibodies. Antibody determination employs recombinant capsid antigens, as the virus itself cannot be readily grown in tissue culture. The B19 virus can be demonstrated by testing for viral DNA, usually by amplification techniques, although the extreme values during the initial peak in blood also allow detection by less sensitive hybridization techniques.

Viral detection by polymerase chain reaction (PCR) is extremely useful in cases of suspected fetal infections. Maternal serology is unable to confirm such infections: maternal IgM would support the presence of this complication but may also notoriously be lacking in cases of established B19 virus-associated hydrops fetalis. Viral DNA can then be detected, often at extremely high levels, in amniotic fluid as well as umbilical cord blood samples obtained before intrauterine transfusions are given.[15] Recent experiences with the quantitative results of viral DNA detection suggested that these may provide useful additional information on the stage of the infection.[13,16–18] Maternal blood levels of viral DNA correlate with fetal viral loads but are consistently lower, suggesting a fetal origin of the extended viremia.

Detection of viral DNA is also important in the diagnosis of persisting infections in immunocompromised individuals, who may have chronic or recurrent viremia accompanied by poor IgM or IgG responses.[19] The extreme sensitivity of DNA-based techniques makes it possible to make a diagnosis in outbreak settings if minute samples are used, as obtained by finger pricks.[20]

CLINICAL MANIFESTATIONS

Although asymptomatic infections occur in an unknown but likely substantial proportion of infections, the most frequently observed clinical consequence is classic erythema infectiosum or 'fifth disease', characterized by a fairly typical exanthema described as 'slapped cheek' (Fig. 158.3). The macular or maculopapular rash spreads to trunk and extremities and may be recurrently present for some time with conditions increasing skin perfusion. The rash as a clinical presentation is not highly specific and may be easily confused with several other conditions; serologic confirmation is therefore advised to make a diagnosis.

Accompanying joint symptoms are rare in children but may be severe in adults, more often in women. These consist of an acute, symmetric, rheumatoid-like polyarthritis which usually improves within 2 weeks but occasionally may persist for months.

In individuals with chronic hemolytic anemia, such as sickle cell disease or hereditary spherocytosis, a transient aplastic crisis may develop as a consequence of parvovirus B19 infection, which may be severe.[21] Although the erythroid precursors are most severely affected, in some cases thrombocytopenia or pancytopenia may occur, rarely even without an underlying hematologic disorder. Failure to clear parvovirus B19 effectively after infection in immunocompromised hosts may lead to a chronic suppression of erythropoiesis. Persistent B19 virus infection should be suspected in cases of transplant recipients[19,22] or untreated HIV infections[23] presenting with chronic anemia. B19 virus could also complicate recovery after chemotherapy for leukemia.[24] As a consequence of infection during pregnancy, infection of the unborn child occurs in up to half of the cases. This may lead to the development of hydrops fetalis, again as a consequence of erythroid suppression. Affected fetuses present with extremely low hemoglobin values; if untreated, fetal death is often the consequence, although spontaneous recovery may occur.



Fig. 158.3 Classic 'slapped cheeks' of a child with erythema infectiosum, or fifth disease, caused by parvovirus B19. A lacy macular erythematous eruption is also present on the trunk but not shown. Courtesy of Dr K Motton.

A number of other conditions have more rarely been associated with a concomitant parvovirus B19 infection. These include hepatitis, myocarditis and a variety of neurologic complications, notably brachial plexus neuritis, and these associations are reasonably well documented. Myocarditis was recently proposed as an additional consequence of infection.[25] In general, it is difficult to provide definitive proof of the relationship between a specific clinical condition and the occurrence of this frequent infection, with a sometimes longlasting presence of IgM antibodies and viral DNA. However, it is clear that some individuals react in less typical ways to this infection. Exanthema, arthralgia and hematologic disturbances, including hydrops fetalis, remain the most relevant manifestations of parvovirus B19 infection.

MANAGEMENT

No specific antiviral therapy is available. In immunocompromised patients with persistent B19 virus infection and anemia, intravenous immunoglobulin may allow clearance of the infection and resolution of bone marrow suppression.[22,23] Patients with transient aplastic crisis may require blood transfusions. B19 virus-associated fetal hydrops requires urgent intrauterine transfusion to correct the severe anemia present with this condition and to prevent fetal death.[15] Although B19 virus in general is not considered a cause of congenital anomalies, it appeared that this correction of the anemia did not prevent some neurodevelopmental delay in these children.[26]

HUMAN BOCAVIRUS AND POSSIBLE ADDITIONAL HUMAN PARVOVIRUSES

The discovery of an additional human virus belonging to the Parvoviridae family, in specimens obtained from children with respiratory tract disease,[27,28] was followed by confirmation of the worldwide occurrence of this virus in such conditions. The delineation of associated clinical conditions is still preliminary but this virus appeared indeed to be correlated with respiratory tract disease and also with systemic infections (see also Chapter 162).

The human bocavirus was discovered as a result of random genome amplification and after sequence analysis provisionally classified the isolate in the *Bocavirus* genus of the Parvovirinae subfamily, originally only consisting of a bovine and a canine virus, hence the name. Shortly after the description of this virus, it became clear that the virus could often be detected in respiratory secretions obtained from young children, with relatively high prevalence rates of between 1% and 19%. Although in a large proportion of the cases other respiratory pathogens were also present, there clearly were cases of lower respiratory tract disease with bocavirus as the single potential

pathogen.[29] Typically, in such cases the quantity of viral DNA often appeared higher, suggesting that more common lower viral loads resulted from viral persistence for longer periods. In addition, it has been established that some infections are readily detectable in blood and therefore likely systemic in nature.[30]

Respiratory symptoms in cases of bocavirus infection are largely nonspecific, and include not only fever, cough, bronchitis, bronchiolitis and acute wheezing, but also pneumonia. Preliminary serologic findings confirmed that in particular bocavirus infections with high respiratory viral loads and viremia correlated with immune responses.[31] The virus is often detected in fecal samples but its status as a gastrointestinal pathogen is still unclear. Proper interpretation of bocavirus findings would benefit from including quantitative information or viral detection in blood to identify those infections more likely to be of clinical relevance.[32]

Another new virus belonging to the Parvoviridae was found in patients with a less clearly defined viral syndrome and has been indicated as PARV4.[33] However, in subsequent studies, no clear clinical correlates associated with detection of this virus could be defined.

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Chapter 159

R Mark L Buller

Poxviruses

NATURE

Virion morphology, structure and biochemistry

Poxviruses are the largest of all animal viruses and can be visualized under the light microscope, although the details of the virion structure remain obscure. Using negative-staining electron microscopy and/or cryoelectron microscopy, parapoxvirus virions appear ovoid with long and short axes of approximately 260 and 160 nm, respectively, whereas all other poxviruses are 'brick'-shaped, with dimensions of 350 × 250 nm on average. In clinical specimens two forms of virion have been identified: the intact 'M' (mulberry; Fig. 159.1) form is found mainly in the vesicular fluid, and the 'C' (capsule) form, which appears to be a degenerative 'M' form, is associated with dried scabs.

Electron micrographs of thin sections of poxvirus-infected tissue culture cells have revealed at least three infectious forms of the virion: intracellular mature virions, extracellular enveloped virions and occluded virus in A-type inclusion body.[1]

It has been proposed that the intracellular mature virions form from the membrane structures of the intermediate compartment, and can acquire an additional two membranes from the Golgi, one of which is lost on egress at the plasma membrane. These extracellular enveloped virions are thought to be most important in cell-to-cell spread and systemic disease. In certain poxvirus species the intracellular mature virion can also occlude in a dense protein matrix within the cytoplasm to form an acidophilic inclusion body (A-type inclusion or Downie body), which is thought to protect the virion from the environment.[2] All vertebrate poxviruses have cytoplasmic basophilic (B-type) inclusion bodies, which represent areas of the infected cells that contain large amounts of virus DNA and protein.

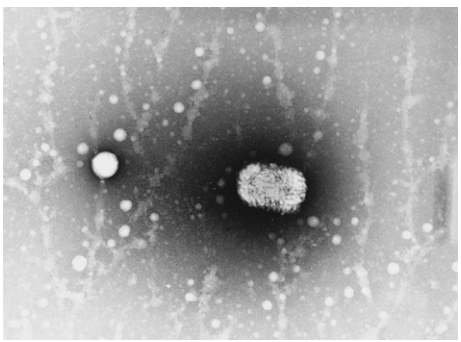


Fig. 159.1 A negative-stained M form of molluscum contagiosum virus. Molluscum contagiosum virus from lesion material.

Depending on the poxvirus, the genome can range from 130 kbp for the parapoxviruses to 300 kbp for the avipoxviruses, which is sufficient to encode more than 140 proteins. A schematic intracellular replication cycle based on the poxvirus vaccinia is presented in Figure 159.2 and described in more detail by Moss.[1] The poxvirus virion is notoriously stable in the environment, and this stability is increased by its association with the A-type inclusion bodies or scab material. Thus for poxviruses, fomites must be considered in disease transmission.

Poxviruses pathogenic for humans

Ten poxviruses infect humans (Table 159.1).[2] Except for the 'extinct' variola virus and the increasingly important molluscum contagiosum virus, the poxvirus diseases are zoonoses. With rare exception these zoonotic poxviruses fail to establish a human chain of transmission. Most human poxvirus infections occur through minor abrasions in the skin. Orf, molluscum contagiosum and monkeypox viruses cause the most frequent poxvirus infections worldwide; the incidence of molluscum contagiosum and monkeypox virus infections is on the rise, the former as an opportunistic infection in late-stage AIDS and the latter as a zoonotic infection in central Africa. Individuals with atopic dermatitis may be predisposed to poxvirus infections such as molluscum contagiosum, orf or cowpox.

PREVENTION

Because most poxvirus infections are rare zoonoses or, as in molluscum contagiosum virus, cause only superficial, 'nuisance' skin lesions in immunocompetent individuals, there are no recommended prevention strategies other than education of patients and various types of workers as to how the diseases are spread.

Molluscum Contagiosum Virus

EPIDEMIOLOGY

Geographic range

Molluscum contagiosum virus has a worldwide distribution. Restriction endonuclease analysis of genomic DNA from molluscum contagiosum virus isolates has revealed the existence of at least four virus

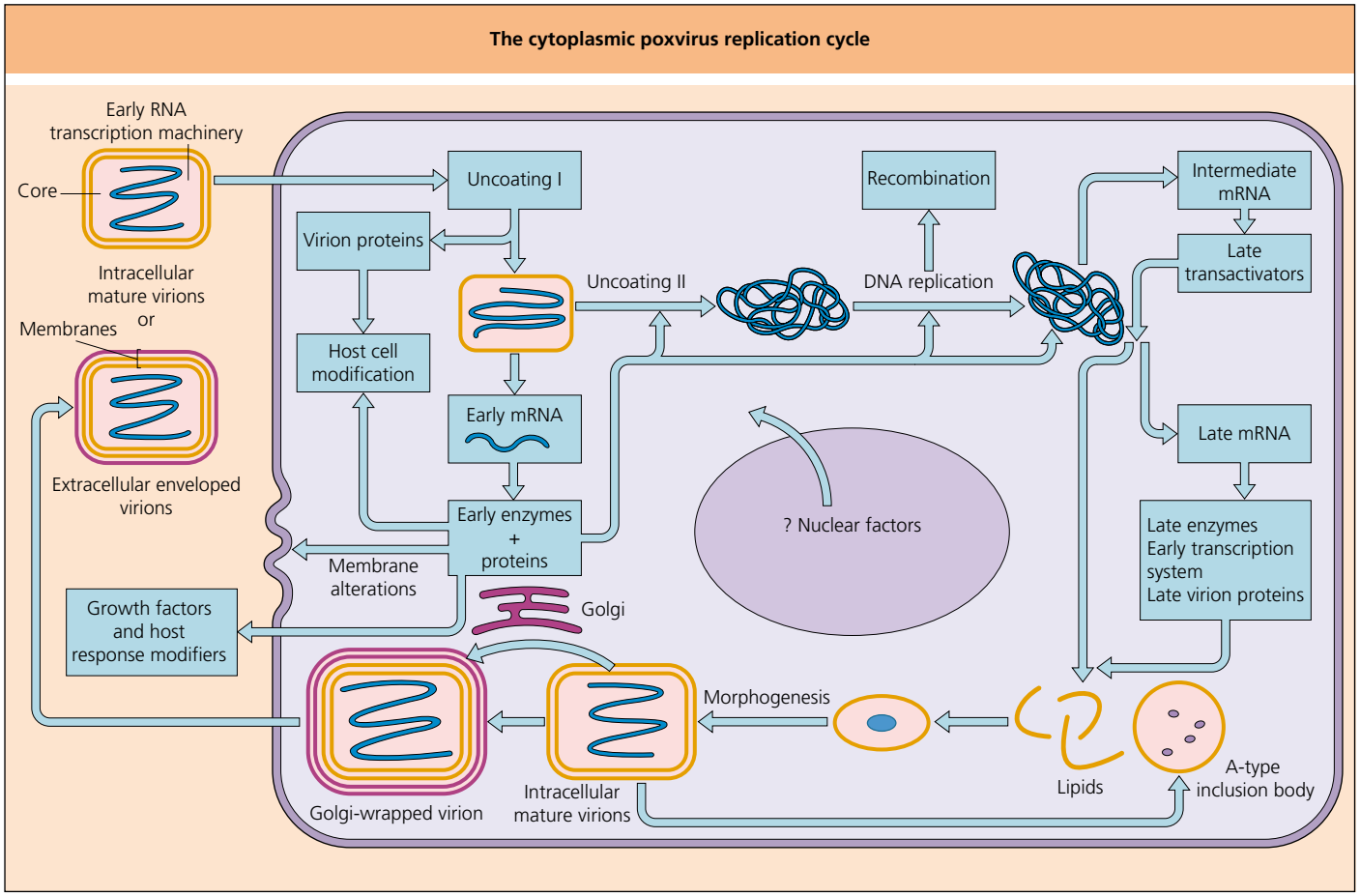


Fig. 159.2 The cytoplasmic poxvirus replication cycle. Poxvirus virions containing early RNA transcription machinery attach to, and fuse with, the plasma membrane (uncoating I). Early genes are expressed that code for a variety of functions that modify the host cell for optimal virus replication, attentuate the host response to infection and mediate virus synthetic processes. After further uncoating (II), the virus genome is replicated via concatamers, late transcription factors are expressed from intermediate genes and late gene RNA is synthesized. Late genes encode the early transcription system, enzymes and structural proteins necessary for virion assembly, which commences with the formation of membrane structures in the intermediate compartment and the packaging of resolved unit length genomic DNA. The intracellular mature virion has one membrane derived from the intermediate compartment. It may remain in the cytoplasm or (in certain virus species) become occluded in an A-type inclusion body or become wrapped by a further two membranes in the Golgi and exported from the cell with the loss of one membrane (extracellular enveloped virions). The extracellular enveloped virions are thought to be most important in cell-to-cell spread and systemic disease. This replication scheme is based on the study of the prototypic poxvirus vaccinia.[1] Other poxvirus species probably vary from this model mainly in the types of growth factors and host response modifiers encoded by the virus and the amounts of extracellular enveloped virions produced. Adapted from Moss.[1]

subtypes or strains, and one recent study suggests the distribution of subtypes can vary geographically.[3]

Prevalence and incidence

For nonsexually transmitted molluscum contagiosum, the disease is more prevalent in the tropics than in Europe. For example, molluscum contagiosum was diagnosed in 1.2% of outpatients in Aberdeen, UK between 1956 and 1963, the mean age of infection was between 10 and 12 years and spread within households and schools was infrequent. On the other hand, in Fiji in 1966, 4.5% of an entire village had the disease, mean age of infection was between 2 and 3 years and 25% of households harbored more than one case.[4,5] Prevalence of infection in New Guinea was greater than 50% in many villages. In England between 1971 and 1985 there was a 400% increase in cases of genital molluscum contagiosum; the majority of patients were aged 15–24 years, with affected women being younger than affected men.[5] In the USA between 1966 and 1983 there was a 10-fold increase in cases in patients aged 25–29 years.[5] Molluscum contagiosum is a common and sometimes severely disfiguring opportunistic infection of between 5% and 18% of patients with HIV infection, especially those with severely depressed $CD4^+$ T-cell numbers.[6] In one study 24% of patients were also diagnosed with atopic dermatitis.[7]

PATHOGENICITY

Transmission

Molluscum contagiosum is observed in children and adults, with spread within this latter group governed in part by sexual practices. Nonsexual transmission is a consequence of infection by direct contact or through fomites. Case histories have suggested transmission from surgeons' fingers, swimming pools, bath towels in gymnasia, contact between wrestlers and as a result of tattooing.[4] Transmission between persons in the absence of fomites requires fairly close contact. Lesions can be commonly observed on opposing surfaces and the virus can be further spread on the person by autoinoculation.

Table 159.1 Poxviruses pathogenic for humans. *Presented by genus in order of importance for human disease.*

Genus/species	Hosts	Geographic distribution	Disease
Molluscipoxvirus			
Molluscum contagiosum virus	Humans*	Worldwide	Molluscum contagiosum, single or multiple skin nodules
Parapoxvirus			
Orf virus (contagious pustular dermatitis virus or contagious ecthyma virus)	Sheep*, goats, dogs, bighorn sheep, thinhorn sheep, Rocky Mountain goat, chamois, reindeer, musk-ox, Himalayan tahr, Steenbok, alpaca	Worldwide	Orf, localized skin lesions
Pseudocowpox virus (milkers' nodule or paravaccinia)	European cattle*	Worldwide	Milkers' nodule, localized skin lesions
Bovine papular stomatitis virus	European cattle*	Worldwide	Localized skin lesions
Orthopoxvirus			
Cowpox virus	Rodents*, cats, cows, zoo animals	Europe, western Asia	Cowpox, localized pustular lesions
Monkeypox virus	Squirrels*, monkeys	Western and central Africa	Monkeypox, rash, generalized disease
Vaccinia virus	Natural host unknown (buffalo† and cattle)	Asia, India, Brazil and laboratory	Buffalopox, localized pustular lesions
Variola virus	Eradicated from humans in 1977	Was worldwide	Smallpox, rash, generalized disease
Yatapoxvirus			
Tanapoxvirus	Monkeys? Rodents?	Eastern and central Africa	Tanapox, localized nodular skin lesions
Yaba monkey tumor poxvirus	Monkeys?	Western Africa	Localized nodular skin lesions

*Natural reservoir.
†During the smallpox eradication program, water buffalo in India and cattle in Brazil were infected with the local vaccine strain of vaccinia virus, which apparently persists in these animals and occasionally infects humans.
?Putative host.

Lesion histopathology

Molluscum contagiosum virus has one of the narrowest cell tropisms of any virus, replicating only in the human keratinocyte of the epidermis.[8] As the virus-infected cell approaches the surface of the skin, the accumulation of progeny virions in a granular matrix in the cytoplasm forces the cell organelles, including the nucleus, to the periphery of the cell. Under light microscopy these cells stain as hyaline acidophilic masses, are referred to as molluscum or Henderson–Patterson bodies and are pathognomonic for disease (Fig. 159.3). Higher magnifications of molluscum bodies reveal them to be entirely filled with virions, partial virions and debris.

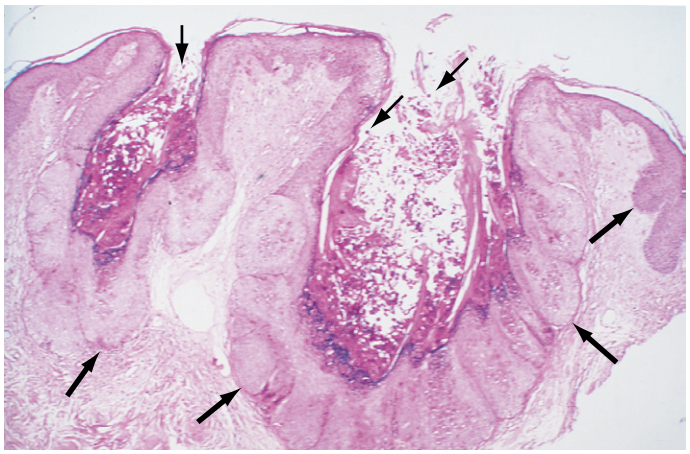


Fig. 159.3 Molluscum contagiosum lesion. In this lesion a major and minor umbilicus has formed as a result of the hypertrophy of infected cells and hyperplasia of the basal cells, which caused a severe invagination of the epidermis but no loss of integrity of the basement membrane. The molluscum bodies stain as pink to purple acidophilic hyaline masses up to 37 × 27 µm in size. Small arrows: molluscum bodies. Large arrows: epidermis–dermis boundary. (H & E.)

CLINICAL MANIFESTATIONS

Signs, symptoms and severity

Molluscum contagiosum manifests as small clusters of lesions in immunocompetent individuals. The lesions are generally painless; they appear on the trunk and limbs (rarely palms and soles) in the nonsexually transmitted disease. In children, the virus can also be fairly common in the skin of the eyelids, with solitary or multiple lesions, and can be complicated by chronic follicular conjunctivitis and later by a superficial punctate keratitis.[9] There may be an associated erythema 1–11 months after the appearance of the lesion, with no correlation to a history of allergy or eczema.[10] Lesions can persist for as little as 2 weeks or as long as 2 years. Re-infections can be common. As a sexually transmitted disease in teenagers and adults, the lesions are mostly on the lower abdominal wall, pubis, inner thighs and genitalia. As yet there is no solid correlation of virion DNA type with specific pathology or location of lesions (i.e. genital versus nongenital).[11]



Fig. 159.4 Molluscum contagiosum lesions. These are the more typical, but still large, lesions of molluscum contagiosum. Courtesy of J Burnett.

In immunocompromised individuals (especially those with HIV infection), the infection is not self-limiting, with more frequent and larger lesions present especially on the face, neck, scalp and upper body. Multiple adjacent lesions sometimes become confluent. Molluscum contagiosum can be considered a cutaneous marker of severe immunodeficiency.

Gross lesion pathology

In immunocompetent patients, molluscum contagiosum virus lesions are epidermal, flesh-colored, raised nodules of 2–5 mm in diameter. Occasionally the gross lesions may have a hypopigmented or erythematous halo. Rarely they will present as a large lesion called giant molluscum (>5 mm in diameter). Both types of lesion usually have an umbilicated center (Fig. 159.4). Although giant molluscum lesions have been reported frequently in severely immunodeficient individuals with HIV infection, they were observed in New Guinea before the introduction of HIV, and therefore may not be a consequence of immunodeficiency.[6,12]

DIAGNOSIS AND DIFFERENTIAL DIAGNOSIS

The diagnosis is usually made clinically based on the gross appearance of the lesions and their chronic nature. Confirmation is easily obtained through the detection of molluscum bodies by hematoxylin and eosin staining of a biopsy or a squash preparation of expressed material from the lesion.

Molluscum contagiosum (especially giant molluscum) can be confused with a number of other disorders such as keratoacanthoma, warty dyskeratoma, syringomas, hidrocystomas, basal cell epithelioma, trichoepithelioma, ectopic hyperplastic sebaceous glands or giant condylomata acuminata, chalazion, sebaceous cysts, verrucas, milia, lid abscess or granuloma on eyelids.[13–15] In immunodeficient patients, disseminated cutaneous cryptococcosis and cutaneous histoplasmosis can resemble typical molluscum contagiosum.[12,13] An inflamed molluscum lesion without the association of typical lesions can be mistaken for a bacterial infection.

Parapoxviruses

The parapoxviruses orf, bovine stomatitis and pseudocowpox generally cause occupational infections of humans, with orf infections being the most common. Because of the clinical similarity of the diseases caused by these agents, they have been referred to collectively as 'farmyard-pox' diseases. No human-to-human transmission of parapoxvirus infections has been reported.

EPIDEMIOLOGY

Geographic range

Orf in sheep and goat populations has been reported in Canada, the USA, Europe, Japan, New Zealand and Africa. Pseudocowpox virus and bovine papular stomatitis virus are both maintained in European-derived dairy herds in all parts of the world.

Prevalence and incidence

In a 1-year New Zealand study 500 meat workers out of a population of 20 000 at risk were infected with orf; those involved in the initial stunning, killing and hanging of the sheep had the highest risk (4%) of infection.[16] Serologic surveys of orf-infected sheep and goat herds yielded orf antibody prevalences of up to 90%. The high seroprevalence of orf antibody in herds is likely attributable to the highly stable nature of the orf virion, which contaminates the pasture.

Pseudocowpox virus and bovine papular stomatitis are probably endemic in all European-derived dairy herds.

PATHOGENICITY

Transmission

Direct transmission of orf virus has been observed as a consequence of bottle-feeding lambs, from animal bites to the hand and contact with sheep and goat products during slaughter. Fomites such as splinters, barbed wire or farmyard surfaces, including soil, feeding troughs or barn beams, have been implicated as sources of virus.

Pseudocowpox virus from lesions on teats of cows is a major source of virus for milkers' nodule of the hand.

Bovine papular stomatitis virus infection of humans occurs generally from lesions confined to the mouth, tongue, lips or nares and occasionally from the teats of infected cattle.

Lesion histopathology

Epidermis

The most striking change in the epidermis is hyperplasia in which strands of epidermal keratinocytes penetrate the dermis.[17] Generally, a mild to moderate degree of acanthosis is detected, and parakeratosis is a common feature. Cytoplasmic vacuolation, nuclear vacuolation and deeply eosinophilic, homogeneous cytoplasmic inclusion bodies often surrounded by a pale halo are also characteristic of the infection. An intense infiltrate of lymphocytes, polymorphs or eosinophils frequently involves the epidermis.

Dermis

A dense, predominantly lymphohistiocytic inflammatory cell infiltrate is present in all cases with marked edema. The most striking feature of the dermis is the massive capillary proliferation and dilatation.

CLINICAL MANIFESTATIONS

Signs, symptoms and severity

The clinical presentation of orf is usually 3–4 weeks after infection. The disease involves the appearance of single or multiple nodules (diameters of 6–27 mm),[17] which are sometimes painful, usually on the hands and, less frequently, on the head or neck. Orf infection can also be associated with a low-grade fever, swelling of the lymph nodes and/or erythema multiforme bullosum. Resolution of the disease occurs over 4–6 weeks, usually without complication; however, autoinoculations of the eye can lead to serious sequelae, and enlarged lesions can arise in humans suffering from immunosuppressive conditions, burns or atopic dermatitis.[18] Lesion healing can be complicated by bullous pemphigoid.[19] Re-infections have been documented.[16]

Pseudocowpox virus lesions usually appear on the hands and are relatively painless, but may itch. The draining lymph node may be enlarged. The nodules are gradually absorbed and disappear in 4–6 weeks.[20]

In bovine papular stomatitis, lesions occur on the hands, diminish after 14 days and are no longer evident 3–4 weeks after onset.[21]

Gross lesion pathology

The orf lesion characteristically goes through a maculopapular target stage in which a red center is surrounded by a white ring of cells which is surrounded by a red halo of inflammation (approximately 1–2 weeks after infection); however, patients usually present later when the lesion is at the granulomatous or papillomatous stages.[18]

In pseudocowpox virus infection, milkers' nodules are first observed as round, cherry-red papules; these develop into purple, smooth nodules of up to 2 cm in diameter and may be umbilicated. The lesions rarely ulcerate.[20]

The lesions of bovine papular stomatitis appear as circumscribed wart-like nodules that gradually enlarge until they are 3–8 mm in diameter.[21]

DIAGNOSIS AND DIFFERENTIAL DIAGNOSIS

Parapoxvirus disease diagnosis is by clinical (lesion morphology) and epidemiologic (recent contact with cattle or sheep) evidence, and electron microscopy of negative-stained lesion material (presence of ovoid particles).[17]

Without knowing the animal source of the infection, orf cannot easily be differentiated from milkers' nodule on the basis of clinical finding, histology or electron microscopy (i.e. disease acquired from sheep is orf and from cattle is milkers' nodule or possibly bovine papular stomatitis).[17] Atypical giant orf lesions in patients who are immunocompromised or suffering from burns or atopic dermatitis may be confused with pyogenic granuloma.[18,22] Orf can also be misdiagnosed as inflammatory vascular neoplasms.

Orthopoxviruses

Although four orthopoxviruses have been shown to cause disease in humans, only two still cause significant human infections. With the global eradication of the disease smallpox in 1977, the causative agent variola virus no longer circulates in human populations, but there is concern that it may be used as a biologic weapon by terrorist groups or rogue nations (see Chapter 71).[23]

In nature, vaccinia virus infections are limited to exposure to a vaccinia-like virus infecting milking buffalos and dairy cattle in Asia and Africa (called buffalopox) and cattle in Brazil (Cantagalo virus). Vaccinia virus (smallpox vaccine) is used also in the immunization of personnel in the laboratory, but is not recommended at this time for the general population due to the occurrence of frequent and sometimes severe complications.[23] Currently only monkeypox and cowpox viruses cause significant human infections, and the incidence of human monkeypox is increasing.

EPIDEMIOLOGY

Geographic range

Traditionally monkeypox virus is found in the tropical rain forests of countries in western and central Africa, most notably the Democratic Republic of the Congo, but its range may be expanding. In 2003 monkeypox virus was imported into the USA in a shipment of rodents destined for the pet trade, and in 2005 an outbreak was recorded in southern Sudan.[24] The reservoir of monkeypox virus in nature is most likely the African arboreal squirrels (*Funisciurus* and *Heliosciurus* spp.), other rodents and perhaps monkeys.[24] Cowpox virus is endemic in Europe and some western states of the former Soviet Union. Rodents (voles, wood mice and rats) have been implicated as reservoirs of cowpox virus; cows, zoo animals and cats are incidental hosts.[24]

Prevalence and incidence

Intensive surveillance between 1981 and 1986 in Zaire by the World Health Organization confirmed 338 cases of human monkeypox, with the greatest risk of infection to inhabitants of small villages within 100 m of tropical rain forests.[25] In recent years there has been an increase in the frequency of human monkeypox in central Africa, perhaps due in part to the cessation of vaccination for smallpox.[24]

Although humans have been infected by cows and rodents, the domestic cat is responsible for the majority of human cowpox infections. Between 1969 and 1993 there were approximately 45 human cowpox cases in the UK, three published case histories from Germany and two each from Belgium, Sweden and France.[26]

PATHOGENICITY

Transmission

The exact mode of transmission of monkeypox virus from an animal source to humans is not known but may be via the oropharynx or nasopharynx or through abrasions of the skin or oral cavity. Person-to-person transmission (like eradicated smallpox) is believed to be by the upper respiratory tract, with virus released in oropharyngeal secretions of patients who have a rash.[25] Unlike smallpox, monkeypox person-to-person transmission is very inefficient and rarely surpasses three generations.[25]

Cowpox virus is acquired usually by direct introduction of the virus from an animal source into minor abrasions in the skin; however, 30% of infections show no known risk factor.[26]

Lesion histopathology

Orthopoxvirus lesions are characterized by epidermal hyperplasia, with infected cells becoming swollen, vacuolated and undergoing 'ballooning degeneration'.[26]

CLINICAL MANIFESTATIONS

Signs, symptoms and severity

Approximately 12 days after infection with monkeypox virus, fever and headache occur. This is followed 1–3 days later by a rash and generalized lymphadenopathy. The rash (the number of lesions is variable)

appears first on the face and generally has a centrifugal distribution. The illness lasts 2–4 weeks, depending on its severity. The case fatality rate is approximately 12%.[25]

With cowpox virus infection, a lesion, usually solitary, appears on the hands or face; this can be extremely painful, and the patient can present with systemic symptoms, including pyrexia, malaise, lethargy, sore throat and local lymphadenopathy. Complete recovery takes between 3 and 8 weeks. Person-to-person transmission has not been reported. Complications can include ocular or generalized infections; the latter occurs in patients who have atopic dermatitis, allergic asthma or atopic eczema and in one case was associated with death.[26]

Gross lesion pathology

The monkeypox virus skin lesions begin as macules but rapidly progress to pustules. About 8 or 9 days after the onset of rash the pustules become umbilicated and dry up; by 14–16 days after the onset of the rash a crust has formed. Most skin lesions are about 0.5 cm in diameter.[25]

The cowpox lesion appears as an inflamed macule, progresses through an increasingly hemorrhagic vesicle stage to a pustule that ulcerates and crusts over by the end of the second week to become a deep-seated, hard black eschar 1–3 cm in diameter.[26]

DIAGNOSIS AND DIFFERENTIAL DIAGNOSIS

The diagnosis of monkeypox infection requires clinical (rash), epidemiologic (equatorial Africa) and laboratory (brick-shaped virion in scab material) findings. Although the rash with associated lymphadenopathy is usually pathognomonic, the sporadic nature of the disease makes it difficult to arrive at an accurate diagnosis solely on clinical grounds.[25]

Diagnosis of cowpox virus infection is rarely made on clinical findings (lesion morphology and systemic illness) and usually requires laboratory results (brick-shaped virion in scab material). Cowpox should be considered in patients who have had contact with cats and who present in July to October with a painful hemorrhagic vesicle or black eschar, with or without erythema, accompanied by lymphadenopathy and a systemic illness.[26]

Monkeypox can be confused with a number of other conditions that result in a rash:

- chickenpox, although its varicella-zoster lesions are more superficial, appear in crops and have a centripetal distribution;
- tanapox, except the tanapox lesions evolve slowly, are nodular and large, without pustulation; and
- syphilis, although the secondary rash of syphilis does not evolve past the papular stage.[25]

Generalized cowpox can be misdiagnosed as eczema herpeticum, whereas localized cowpox is most frequently misdiagnosed as:

- orf or milkers' nodules, although the parapoxvirus lesion is clinically distinct, and there are often no systemic disease symptoms;
- herpes virus reactivation, even though herpes lesions are not usually hemorrhagic or erythematous and the scab is not so deep-seated and of lighter color; and
- anthrax, although the anthrax lesion is painless and rapidly progresses to the eschar stage (5–6 days).[26]

DIAGNOSTIC MICROBIOLOGY

The appropriate biosafety level precautions must be taken for the handling, transport and processing of infected lesion material.[27]

Molluscum contagiosum virus

For a squash preparation the keratotic dome-shaped molluscum lesion is placed on a regular slide and under a coverslip or second slide. The lesion is flattened and can be examined using light microscopy either directly or after staining with Wright's or methylene blue stains. Round to ovoid molluscum bodies up to 37 × 27 µm are diagnostic of molluscum contagiosum.[4]

For histopathologic analysis, a biopsy specimen is fixed in 10% formal saline and submitted for wax embedding, sectioning at 5 µm and staining with hematoxylin and eosin. Microscopic examination should provide a field of view similar to that shown in Figure 159.3.

Parapoxvirus, orthopoxvirus and yatapoxvirus

Orthopoxvirus (brick-shaped), parapoxvirus (ovoid) and tanapox virus (enveloped brick-shaped) virions can be differentiated from one another by an experienced electron microscopist. Scab material or fluid from the vesicle should be processed by standard methods for negative-staining electron microscopy.[27] Polymerase chain reaction is rapidly becoming the assay of choice for laboratory diagnosis of poxvirus infections.[28]

MANAGEMENT

The management of parapoxvirus, orthopoxvirus and yatapoxvirus infections is supportive. Although at this time there are no approved systemic or topical chemotherapeutic agents commercially available for the treatment of poxvirus infections, it is anticipated that cidofovir or related compounds will be approved to treat orf virus, molluscum contagiosum virus and orthopoxvirus infections.[29,30] One new compound, ST-246, an orthopoxvirus egress inhibitor, is a promising candidate for early treatment of othopoxvirus infections.[31] Prevention of secondary bacterial infections through the use of antibiotic ointments is an option. In the case of cowpox, corticosteroids are contraindicated and may exacerbate the illness.[26] Because of the chronic nature of molluscum contagiosum lesions, one can consider curettage and cryotherapy of lesions. Removal of giant molluscum lesions, but not regular lesions, usually results in scar formation. Molluscum lesions may remit when HIV infection is controlled with anti-HIV therapy.

CURETTAGE WITH AN ANALGESIC

With children, pretreatment of molluscum lesions with lidocaine (lignocaine)–prilocaine for between 1 and 30 hours under plastic occlusion resulted in a decrease in the reported pain on lesion removal with a comedo extractor or curette.

CRYOTHERAPY

Molluscum lesions are treated for 6–30 seconds with a cotton-tipped applicator dipped in liquid nitrogen; this is repeated at 3-week intervals as needed. Cryotherapy is relatively painless, cost-effective and yields good cosmetic results. For patients with HIV infection, this treatment has the added advantage of mitigating the risk of disease transmission to medical personnel.

REFERENCES

References for this chapter can be found online at http://www.expertconsult.com

Chapter 160

Mary J Warrell
David A Warrell

Rabies and rabies-related viruses

INTRODUCTION

Rabies is a zoonosis of dogs and other mammals that is occasionally transmitted to humans, causing fatal encephalomyelitis. Dog rabies virus is universally fatal in humans and is the cause of more than 95% of human rabies deaths. However, prophylaxis can be 100% effective, so all human deaths are due to a failure of prevention.

Classic rabies virus is genotype 1 of the lyssaviruses, and genotypes 2–7 are rabies related-viruses, which may also cause fatal human infections[1] (see end of chapter). They are members of the large Rhabdoviridae family of animals and plants.

STRUCTURE

The bullet-shaped virions contain a nonsegmented negative strand of rabies RNA encoding five proteins. The RNA is coiled with a nucleoprotein, a phosphoprotein and an RNA-dependent RNA polymerase to form a helical ribonucleoprotein complex or nucleocapsid. This is covered by a matrix protein. An outer coat studded with the virus-coded, glycoprotein-bearing, club-shaped projections is acquired by budding through a host cell lipid membrane. Rabies virus strains from different vector species and geographic areas are distinguished by nucleotide sequencing and monoclonal antibody typing.

EPIDEMIOLOGY

Animal rabies

Rabies is enzootic in most parts of the world. Globally, the domestic dog is the most important reservoir species and is the dominant vector in Asia, Africa and urban areas of Latin America. Separate reservoirs of enzootic infection occur in certain wild mammalian species (Table 160.1). The sylvatic pattern of infection predominates in North America,[2,3] Europe, parts of southern Africa[4] and the Caribbean.[1] All mammals are potentially susceptible, and infection may be transmitted to other species, including domestic animals, especially cats, and to humans. Lyssaviruses have not been reported from a few areas including Iceland, Norway, Sweden, Finland, Italy, Greece, Cyprus, some other Mediterranean islands, Singapore, Sabah, Sarawak, New Guinea, New Zealand, Antarctica, Oceania, Hong Kong islands, Japan, Taiwan and some Caribbean islands. Although these countries have no apparent indigenous rabies, infected animals cross national boundaries and so infection may be imported. Genotype 1 (classic rabies) infects terrestrial mammalian reservoir species and bats in the American. Western Europe and Australia are free of genotype 1 rabies, but rabies-related lyssaviruses are found in bats (see Table 160.1). As the epizootiology changes constantly, up-to-date local advice should be sought for detailed information.

Human rabies

Estimates of human rabies mortality are notoriously unreliable in tropical areas where about 99% of the deaths occur. In Asia and Africa, 55 000 deaths annually are estimated. However, it is suspected that only 3% of cases are recorded, and the rate of underreporting may be 20 times in Asia and 160 times in Africa. Areas of high incidence include India, Bangladesh and Pakistan. There is very little surveillance in Africa.

In Europe, 5–10 deaths are reported annually, usually in Eastern Europe or in cases imported from Asia or Africa. In the USA, an average of three cases were diagnosed annually from 2000 to 2006. Up to 80% of cases are indigenous, and 90% are associated with insectivorous bat rabies virus. Although few (39%) patients remember a bat bite, possible bat contact is more often reported.

PATHOGENICITY

Transmission

Human infection is usually the result of a bite by a rabid dog or other mammal. Virus in saliva can penetrate broken skin or intact mucous membranes, and so scratches or licks by a rabid animal may cause infection.

On two occasions, human rabies may have resulted from inhalation of virus in caves in Texas that were densely populated by insectivorous bats,[5] and after laboratory accidents with aerosols of virus. The saliva, respiratory secretions and tears of rabies patients contain virus,[6] which could infect another person, but the only documented instances of human-to-human transmission have been through human tissue transplants from donors in whom rabies had not been suspected. Eight cases of infection by corneal graft have been reported. In the USA and Germany, two donors transmitted rabies to seven recipients of solid-organ transplants (kidney, liver, lung, pancreas) and one patient received only a segment of artery.[7] Several women with rabies encephalitis have delivered healthy infants. Only one case of neonatal rabies has been reported, although vertical transmission is well documented in animals.

Pathogenesis[8,9] (Fig. 160.1)

Rabies virus will attach to a variety of cellular receptors, but in a bite wound, competitive binding to acetylcholine receptors at neuromuscular junctions is a possible route of entry into the nervous system.

Table 160.1 Distribution of rabies reservoir species

Africa	Domestic dog (*Canis familiaris*) Black-backed jackals (*Canis mesomelas*) Yellow mongoose (*Cynictis penicillata*) Bat-eared fox (*Otocyon megalotis*) Frugivorous and insectivorous bats (bat lyssaviruses; see Table 160.3)	Widespread dominant reservoir Southern Africa Southern Africa Southern Africa
Asia	Domestic dog (*Canis familiaris*) Wolf (*Lupus lupus*)	Widespread dominant reservoir Middle East
Americas	Arctic fox (*Alopex lagopus*) Red fox (*Vulpes fulva*) Gray fox (*Urocyon cinereoargenteus*) Striped skunk (*Mephitis* spp.) Raccoon (*Procyon lotor*) Frugivorous bats Insectivorous bats Vampire bat (*Desmodus* spp.) Mongoose (*Herpestes* spp.) Domestic dog (*Canis familiaris*)	Alaska, North-west Canada Western Canada and north-east USA Texas, Arizona Texas, Central USA, California Eastern USA, south-east Canada South America Very widespread Mexico, Trinidad, Tobago, Isla de Margarita, northern South America Puerto Rico Mexico, parts of central and South America
Australia	Flying foxes or fruit bats (*Pteropus* spp.) Insectivorous bats (Australian bat lyssavirus; see Table 160.3)	Eastern coastal area
Europe	Red fox (*Vulpes vulpes*) Arctic fox (*Alopex lagopus*) Raccoon dog (*Nyctereutes procyonoides*) Wolf (*Lupus lupus*) Domestic dog (*Canis familiaris*) Insectivorous bats (European bat lyssaviruses; see Table 160.3)	Eastern Europe, Russian Federation Northern Russia Eastern Europe Eastern Europe, Russian Federation Turkey (act as vectors in Eastern Europe and Russian Federation) Widespread

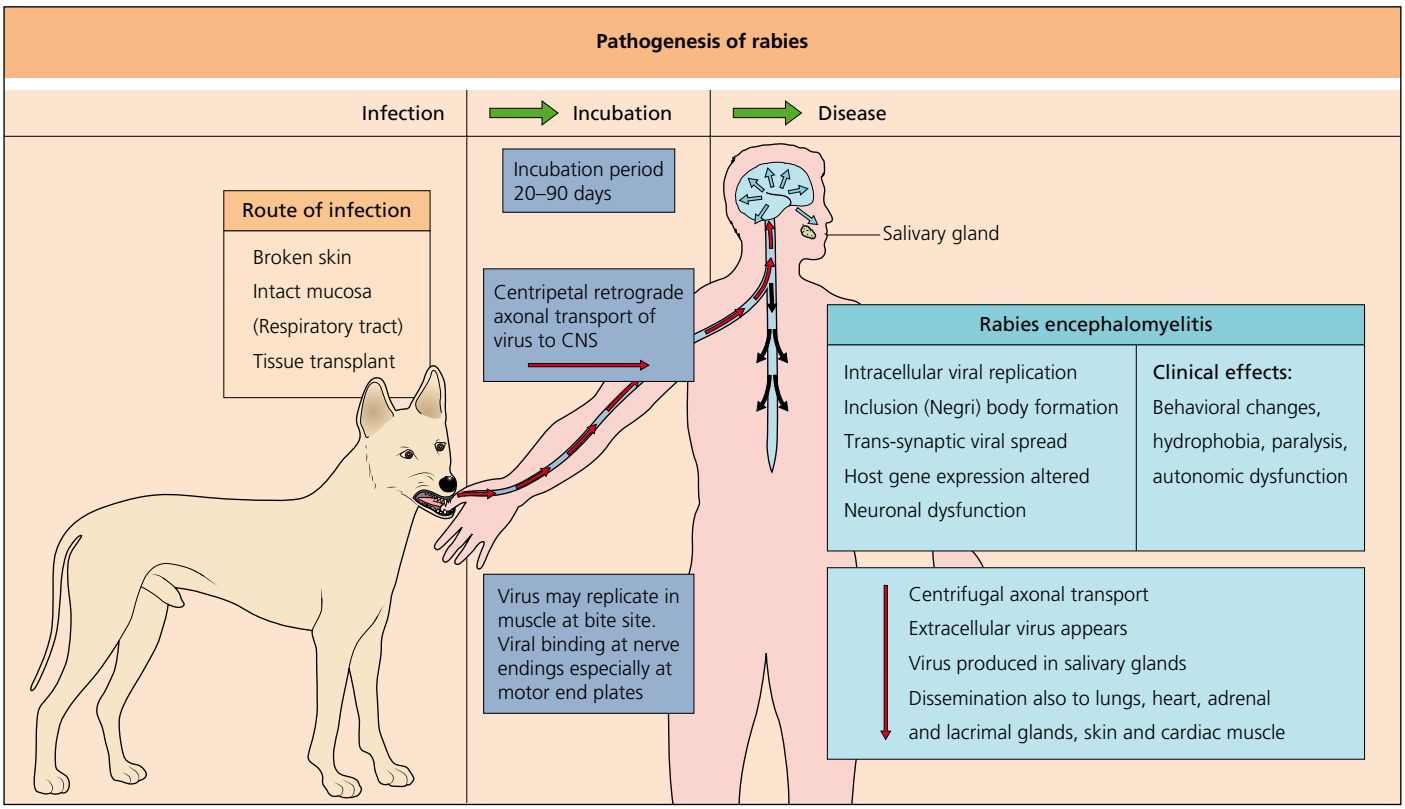


Fig. 160.1 Pathogenesis of rabies.

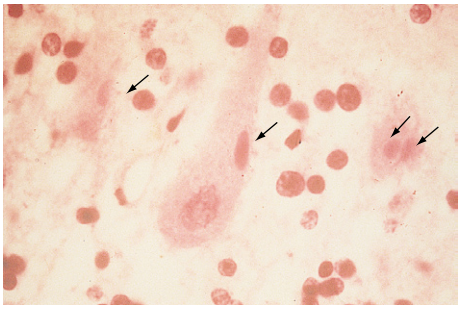


Fig. 160.2 Negri bodies in cerebellar Purkinje cells in a human victim of rabies encephalitis. The intracytoplasmic, dark-staining Negri bodies are marked with arrows. Courtesy of Armed Forces Institute of Pathology, Bethesda, USA.

During the incubation period, the virus or viral components travel to the central nervous system by retrograde axonal transport. Infection can be halted by sectioning the nerve or by disrupting microtubular function. It is presumed that viral proteins are carried by microtubule motor molecules, particularly dynein, but the means of attachment is still unknown, possibly because there is more than one mechanism.

When the virus reaches the brain, replication proceeds in neurons by budding from intracellular membranes and accumulations of viral proteins form inclusions, large examples of which are the classic Negri bodies (Fig. 160.2). The virus spreads trans-synaptically and remains intraneuronal and hidden from immune recognition. The viral glycoprotein is essential for transfer to adjacent cells.

Disruption of neuronal physiology has been observed experimentally, including neurotransmitter activity, electroencephalograph patterns and ion-channel function. The demonstrated viral effects on host gene expression are either upregulation of genes which could enhance viral spread and replication, and downregulation of genes related to cell protein synthesis. The latter will impair cell metabolism and probably contributes to the widespread dysfunction of apparently intact neurons. Viral pathogenicity correlates with low levels of rabies glycoprotein expression, inhibition of host protein synthesis, a minimal inflammatory response and little if any apoptosis. Pathologic features of diffuse encephalomyelitis may be minimal or even absent in humans.

The virus progresses centrifugally, again via peripheral nerves, to many tissues including the salivary and lachrymal glands, where replication produces extracellular rabies virus, which may initiate an immune response (see Fig. 160.1).

Immunology

Following infection, rabies virus evades the immune system[10] and no immunologic response can be detected in unvaccinated patients before signs of encephalitis develop. Rabies antibody does not appear until 1–2 weeks after the onset of symptoms, if at all, and later in the cerebrospinal fluid (CSF). Rabies-specific IgM is not detectable any earlier than IgG. The presence of antibody is diagnostic in unvaccinated patients. Specific transformation of peripheral blood lymphocytes has been found in a few patients with furious rabies, but not in paralytic cases. The reverse is found experimentally in rabid mice. The virus is immunosuppressive, which is reflected by the minimal histopathologic changes in the brain. Interferon (IFN) production in humans is very low, and high levels of IFN-α and IFN-β in animal brains do not protect animals against death. T lymphocytes are not efficient in combating rabies and, surprisingly, apoptosis of immune reactive $CD3^+$ T cells has been observed in mouse brains.[10] Clearance of a lethal virus from the brain and recovery in rodents is associated with early induction of neutralizing antibody, increased expression of viral genes, a greater inflammatory response, early appearance of IFN-γ and neuronal apoptosis. The result is limitation of viral spread, as well as an inevitable loss of neuronal tissue.[10]

CONTROL

The elimination of rabies virus from domestic dogs would prevent more than 95% of human rabies deaths, without any need of expensive prophylaxis. This has been achieved, for example, in Western Europe, North America, Japan, several urban areas of Latin America[11] and even one in India.[12] Mass killing is ineffective, but campaigns of dog vaccination and population control can be successful, if accompanied by enthusiastic education, publicity and adequate funding. Although oral vaccination of dogs is possible, it remains impracticable.

Appropriate rabies control strategies are based on surveillance to determine the principal domestic or wild reservoir species, with laboratory diagnosis to confirm the local prevalence of infection.

Sylvatic rabies has been eliminated in the red fox *Vulpes vulpes* in Western Europe by distributing oral vaccine in suitable baits around the countryside. Repeated campaigns of oral vaccination with either a live attenuated rabies virus or a vaccinia-recombinant vaccine expressing rabies glycoprotein are needed over many years. In North America, raccoons and foxes have been similarly immunized,[3] as well as jackals in southern Africa. Vaccines for skunks and mongooses are being developed. Control of vampire bat rabies,[11] which causes many deaths in cattle in Latin America, is attempted by treating cattle with small doses of anticoagulant in order to poison the bats. Vaccination of cattle is possible but expensive. No active measures are taken to control rabies in insectivorous or fruit bats in the Americas, Europe, Africa or Australia, but instructions are issued to encourage people to avoid direct contact with these animals.

CLINICAL FEATURES

The incubation period (bite to first symptom) ranges from 4 days to at least 19 years, but in about 75% of cases it is between 20 and 90 days. The only prodromal symptoms that are reasonably suggestive of rabies are itching, pain or paresthesiae, either at the site of the causative bite or radiating proximally from it. After 1–7 days of generally nonspecific symptoms, features of either furious or paralytic (dumb) rabies develop.

The pathognomonic symptom of furious rabies is hydrophobia[13] or aerophobia. Jerky and violent contractions of the diaphragm and accessory muscles of inspiration are associated with an inexplicable terror. This reflex can be provoked by attempts to drink liquid or by a draught of air on the skin. The hydrophobic spasm may end with the patient in opisthotonos, having generalized convulsions and in cardiac or respiratory arrest. Other features of furious rabies are periods of excitement, sometimes with hallucinations or aggression, interspersed with lucid intervals; fever; tachycardia and other arrhythmias; hypersalivation; lacrimation; sweating; and fluctuating temperature and blood pressure. All these features are suggestive of stimulation of the autonomic nervous system as seen in severe tetanus. Conventional neurologic examination may prove surprisingly normal, unless hydrophobia is provoked, but meningism, cranial nerve lesions, upper motor neuron lesions, fasciculation and involuntary movements are sometimes detected. Without intensive care, patients usually die, often during a hydrophobic spasm, in the first few days of their encephalitic symptoms.

Paralytic or dumb rabies is probably underdiagnosed but was recognized during an epidemic of rabies transmitted by vampire bats in Trinidad in the 1920s and 1930s. Although associated particularly with vampire bat rabies and other genotype 1 bat infections (i.e. in the Americas), it is also seen in patients bitten by dogs. After the prodromal symptoms described above, together with headache, local paresthesiae and flaccid paralysis develop, usually in the bitten limb, and ascend symmetrically or asymmetrically. There is accompanying pain and fasciculation in the affected muscles and mild objective sensory disturbances. Death follows paralysis of the muscles of deglutition and respiration; there is usually no evidence of hydrophobia or aerophobia.

Patients with rabies whose lives are prolonged by intensive care may develop a variety of respiratory, cardiovascular, neurologic and gastrointestinal complications including fatal cardiac arrhythmias, pneumothorax, cerebral edema, diabetes insipidus, inappropriate antidiuretic hormone secretion, hypo- or hyperpyrexia, diffuse axonal neuropathy and hematemesis.

Differential diagnosis

Severe and unusual neurologic symptoms, whether encephalitic or paralytic, should suggest the possibility of rabies in any unimmunized person who has had contact with mammals in a rabies-endemic area. However, children may not report animal contact and rabies patients infected by bats in the USA usually deny any exposure to a bat.

Furious rabies can be mimicked by the pharyngeal form of cephalic tetanus ('hydrophobic tetanus'); however, severe tetanus usually has a shorter incubation period than rabies and there is sustained muscle rigidity, often associated with trismus. Delirium tremens and the excitatory effects of some plant toxins and recreational drugs[7] have been confused with rabies. Paralytic rabies is indistinguishable from many other causes of an ascending paralysis, including postvaccinal encephalomyelitis complicating the use of obsolete nervous tissue rabies vaccines still used in Asia, Africa and Latin America.

Prognosis and recovery

Patients with furious rabies encephalitis usually die within 2 weeks, but some patients with paralytic rabies have survived for as long as 1 month, even without intensive care.

Only six documented cases of recovery from rabies, or survival for more than 2 years, have been reported during the virologic era.[14] No rabies virus or antigen was detected in any of them and all were diagnosed serologically. Two patients were treated after exposure with nervous tissue vaccines and recovered. The diagnosis of one of these was doubtful and the second was a 6-year-old boy bitten by a bat in Ohio, USA,[15] who began having duck embryo rabies vaccine 4 days later. He recovered completely from an encephalitic illness and lives a normal life.

Three other patients, given only pre-exposure nervous tissue vaccine or postexposure tissue culture vaccine, were left with profound neurologic deficits.

The first unvaccinated patient known to survive is a 15-year-old girl in Wisconsin, USA, bitten by a bat.[16] She developed paresthesiae of the bitten hand and features of paralytic rabies, including progressive cranial nerve paralyses and leg weakness, with fever and hypersalivation. Rabies antibody had appeared unusually early, by the sixth day after the onset of symptoms, but no virus or antigen was detected. Her intensive treatment included ketamine-induced coma and the antiviral agents ribavirin and amantadine. She has made a slow recovery over 3 years, with only minor residual neurologic deficits, and has graduated from high school. This case has features similar to the boy in Ohio. It is likely that they both had rabies antibody present at, or soon after, the onset of symptoms and had no damaging hydrophobic spasms. They are the only reported 'survivors' who were infected by an American bat virus, genotype 1, which has a different pattern of infection from the dog rabies strains experimentally. It is slower to evolve and progress and there is relatively little neuronal apoptosis. However, the treatment protocol that was apparently effective in the Wisconsin girl has not proved effective in several other patients infected by bats or dogs.[17]

DIAGNOSIS

Confirmation of the diagnosis of rabies encephalitis should be possible within a day, if ideal samples are supplied to a specialist laboratory (Table 160.2). Rapid identification of antigen can be followed by virus isolation and, in unvaccinated patients, by detecting antibody.[18]

Table 160.2 Diagnostic methods

Diagnostic sample	Aim	Method
Full thickness **skin**† punch biopsy	Antigen detection	IFA test on frozen section RT- PCR
Saliva Tears CSF	Virus isolation and antigen detection	Tissue culture Mouse inoculation test RT-PCR
Serum	Antibody test	Detectable antibody is diagnostic in unvaccinated patients Save sample for comparison later if vaccinated previously
CSF	Antibody test	Test in parallel with serum
REPEAT skin and saliva samples daily until a diagnosis is confirmed		
Brain post-mortem: needle biopsy‡ or autopsy sample brain stem and cerebellum	Virus isolation and antigen detection	Tissue culture Mouse inoculation test IFA test on impression smear RT- PCR

†Bold = most important, potentially helpful, samples
‡See text for details.

Skin, saliva, respiratory secretions, CSF, tears and brain are all suitable samples, but a full-thickness skin biopsy is the most likely to yield a rapid result. A punch biopsy is taken from a hairy area, usually at the nape of the neck. Frozen sections show the characteristic immunofluorescent staining of rabies antigen visible in nervous tissue around the base of hair follicles. With controls to ensure specificity and examination of many sections, sensitivity is 60–100%.[19] False-positive results have not been reported, whereas the corneal impression test is insensitive and false-positive results do occur. Reverse transcriptase polymerase chain reaction (RT-PCR) can be performed on the samples mentioned, although skin and saliva are the most likely to be positive. Viral isolation in suckling mice or tissue culture is the ideal method of diagnosis. Genetic analysis will indicate the likely reservoir species and geographic origin of the virus. Tests should be repeated until the diagnosis of rabies is excluded.

In unvaccinated patients, diagnostic seroconversion usually occurs in the second week of illness.[20] In vaccinated patients, very high levels of antibody both in the serum and in the CSF have been used to make a diagnosis.[15]

Post-mortem brain samples should be cultured, but antigen can be detected by immunofluorescent staining of impression smears, enzyme immunoassay or RT-PCR. A needle necropsy of brain can be taken without a full autopsy by inserting a Vim-Silverman needle via the medial canthus of the eye through the superior orbital fissure, via the foramen magnum, or via the nose and ethmoid sinus.

MANAGEMENT

No antiviral or ancillary treatments have proved effective in animal models and human rabies of canine origin remains 100% fatal. Patients should be admitted to hospital so that their agonizing symptoms can be palliated with adequate doses of analgesic and

sedative drugs.[21] Friends, family and medical staff in close contact with the patient should be given postexposure vaccination as reassurance, even though such person-to-person transmission has not been documented.

Intensive care may be appropriate for patients infected by an American bat, who present early and are already seropositive. New antiviral methods may yet be discovered against rabies, perhaps combined with an immunologic approach (e.g. IFN-γ). Until a treatment has proved effective experimentally, intensive care therapy is usually inappropriate, especially in developing countries.

PREVENTION

Postexposure prophylaxis[3,22–24]

In a rabies-endemic area, the risk of infection, and hence the decision to give postexposure prophylaxis, depends on the species of animal, its behavior and the circumstances of contact. An unprovoked attack by a known local vector suggests a high risk of exposure to rabies, especially if the animal is unvaccinated, is unusually excitable or is partially paralyzed, or if a wild animal is unusually tame. Vaccinated animals have, however, transmitted rabies. The virus gains access through any bite, scratch or contamination of broken skin or mucous membrane, but intact skin is an adequate barrier against infection. Bites or scratches by bats may pass unnoticed. The risk of infection is greatest from bites on the head, neck and hands, and multiple bites carry a higher risk than single bites.[25] Before vaccines were available, the mortality from all proven rabid dog bites in India was 35–57%.

Strenuous efforts should be made to have the biting animal tested for rabies. The routine immunofluorescent test for antigen may give a false-negative result in 1–2% of rabies cases. In addition, as the test is unreliable for rabies-related viruses, the result of viral culture is important in highly suspicious cases. Specific treatment should not await laboratory results and it should always be started immediately, irrespective of the time since the bite.

Postexposure prophylaxis aims to inactivate rabies virus in a wound and to stimulate immunity to kill the virus before it enters the nervous system, where it is protected from immune attack. Postexposure therapy includes urgent wound treatment, active prophylaxis with vaccine and passive immunization with rabies immune globulin (RIG). The complete therapy is very effective, and failures of optimal treatment started on the day of exposure are rare. Seven cases have been reported following high-risk exposure. However, many deaths occur because treatment is often delayed, unaffordable, inadequate or incomplete.

Treatment of mammal bites, scratches or licks

The treatment can be summarized as follows:

1. Scrub vigorously with soap or detergent and water, reaching the depth of the wound, and remove foreign material. Local or even general anesthesia may be necessary to allow effective wound cleaning and debridement, especially for children.
2. Swab with a virucidal agent: povidone iodine or 40–70% ethanol. (The virucidal effect of quaternary ammonium compounds is neutralized by soap, and so they are not recommended.)
3. Suturing of wounds should be avoided or delayed.
4. Tetanus prophylaxis (tetanus immune globulin or toxoid booster plus metronidazole) must not be forgotten. Antibiotic prophylaxis against other potential wound pathogens is recommended only in the case of bites on the hands.
5. If there is a risk of rabies, give specific therapy immediately.

Rabies vaccines

Vaccines of nervous tissue origin

All human rabies vaccines contain killed virus. Those produced from infected sheep or goat brain (Semple vaccines) and suckling mouse brain (SMB) are still used in Asia and Africa. SMB vaccine is also used in parts of South America. These are weak antigens and neurologic reactions still occur (1:200 Semple vaccinees).[14]

Purified tissue culture vaccines

Tissue culture-grown vaccines are used exclusively in North America, Europe, China, Thailand, Sri Lanka, India, Nepal and the Philippines and increasingly in other countries. The use of the original expensive human diploid cell vaccine (HDCV) is giving way to cheaper vaccines of equivalent efficacy and safety, including German and Indian purified chick embryo cell vaccines (PCECV) and a French purified Vero cell vaccine (PVRV), which are now widely distributed in endemic areas.

Immunologic response to vaccination

The presence of serum-neutralizing antibody is the best available indicator of protection against rabies, and it appears 7–14 days after starting a primary course of a modern rabies vaccine. A level of 0.5 IU/ml is considered satisfactory, although the protective level cannot be ascertained in humans. Only the viral surface glycoprotein molecules induce neutralizing antibody. They also stimulate helper and cytotoxic T-lymphocyte responses.

The speed and degree of the antibody response to vaccine varies, probably under genetic influence. A relatively delayed, lower level of antibody is found in about 3% of people receiving vaccine. Increasing age and immunosuppression, for example by HIV infection, also impair the response.

The induction of neutralizing antibody can be enhanced by increasing the amount of antigen (within limits) or by dividing the dose between multiple sites and injecting it intradermally.

Postexposure vaccine regimens

Intramuscular vaccine regimens

The standard regimen is one dose of tissue culture vaccine (1 ml or 0.5 ml, depending on the product) intramuscularly into the deltoid on days 0, 3, 7, 14 and 28.

Intradermal vaccine regimens

The five-dose intramuscular regimen is unaffordable by the vast majority of patients in developing countries, but other more economic regimens are as immunogenic as the standard method[26] and have proved effective. The World Health Organization recommends multiple-site intradermal (ID) economical regimens using specified vaccines – PVRV, PCECV and HDCV.[22,24] The manufacturers' instructions must be used for all other vaccines.

An eight-site regimen[22,24,27] consists of:

- Day 0: eight ID injections of 0.1 ml using a whole vial divided between the deltoids, thighs and suprascapular and lower abdominal areas
- Day 7: four doses of 0.1 ml over the deltoids and thighs
- Day 28: two 0.1 ml doses over the deltoids.

Human diploid cell vaccine or PCECV can be used, as the ampoule contains 1 ml, but it is not economic using PVRV with an ampoule of 0.5 ml. The eight-site regimen has a wide margin of safety because an adequate antibody response has been achieved with only four of the eight initial ID doses. Only three visits are needed. Patients who are anxious about the risk of rabies do not object to the method.

A two-site regimen[22,24] consists of:

- Days 0, 3, 7 and 28: two ID doses (0.1 or 0.2 ml depending on whether the intramuscular vaccine dose is 0.5 or 1.0 ml) over the deltoids.

Although it can be used economically with all three vaccines, ampoules of vaccine must be shared at each of four or five visits. The two-site regimen has proved effective in thousands of patients who usually also received RIG treatment in Thailand, the Philippines and Sri Lanka.

The eight-site regimen has induced higher levels of antibody than the two-site regimen from the earliest sample on day 7, onwards.[28] All the ID regimens require a similar amount of vaccine, less than two intramuscular doses, a saving of 60%. When one ampoule is shared between several patients, a new syringe and needle must be used for each. Diluted vaccine must be stored in a refrigerator and used within 8 hours.

These economic regimens are used in a few areas in Asia, but hardly at all in Africa due to practical and pharmaceutical difficulties. A new, simplified four-site ID regimen overcomes many of the problems, is immunogenic and can be used with vaccines of any ampoule size. It has all the advantages of the eight-site method, including the need for only three visits.[26]

A four-site regimen consists of:

- Day 0: four ID injections using a whole vial divided between the deltoid and thigh areas
- Day 7: two doses (0.1 or 0.2 ml depending on whether the vial contains 0.5 or 1.0 ml) over the deltoids
- Day 28: one dose over the deltoids.

This economical regimen is now the method of choice.

Passive immunization with rabies immune globulin

Every primary postexposure vaccine course should be accompanied by RIG to cover the first 7–10 days before vaccine-induced immunity appears. Rabies immune globulin treatment is especially important after bites on the head, neck or hands, or multiple bites. A single dose of 20 IU/kg of human RIG or 40 IU/kg of equine RIG is given at the same time as the first dose of vaccine. As much as possible is infiltrated into and around the wound, but care is needed when injecting into fingers or other tight tissue compartments. Any remaining vaccine is injected intramuscularly distant from the vaccine site. Increasing the dose of RIG may impair the response to vaccine, but if the volume is insufficient for infiltration, it can be diluted in saline two- or threefold in order to infiltrate all wounds. Skin testing with equine RIG does not predict most early (anaphylactic) reactions and is no longer recommended.[24] Adrenaline (epinephrine) should always be ready in case of very rare anaphylaxis. Reactions occur in 1.8% of equine RIG recipients and serum sickness is seen in 0.7%, but not after human RIG therapy.[29]

Postexposure treatment of previously vaccinated patients

Treatment is always urgent, but, provided that the patient has previously had a complete pre-exposure or postexposure course of tissue culture vaccine or if a neutralizing antibody level >0.5 IU/ml has been recorded at some time, only two doses of intramuscular vaccine are needed on days 0 and 3. Rabies immune globulin treatment is unnecessary.

Side-effects of tissue culture vaccines[30]

The incidence of minor symptoms is very variable. Local pain or erythema occurs in about 15% of people vaccinated and irritation is more common following intradermal injections. Generalized nonspecific symptoms are reported by about 7% of patients and transient maculopapular and urticarial rashes are occasionally seen. In the USA, 6% of late pre-exposure booster injections of HDCV have been accompanied by a mild immune complex-like disease between 3 and 13 days later. All of these patients responded to symptomatic treatment. Neurologic symptoms,[14] either Guillain–Barré-like or local limb weakness, are extremely rare, and the incidence following treatment is no greater than those following other routine vaccines. No complications have been observed in pregnancy.

Pre-exposure prophylaxis

Pre-exposure vaccination is recommended for anyone at risk of exposure to rabies virus, particularly veterinary surgeons, animal handlers, zoologists, all bat handlers, laboratory staff working with rabies, wildlife officers and people living in or traveling to rabies-endemic areas where dogs are the dominant vector species. A total of three doses of cell culture vaccine are needed on days 0 and 7 and on day 28.[22–24] The last dose can be advanced towards day 21 if time is short,[24] but the antibody level may fall more rapidly. The dose can be intramuscular or 0.1 ml ID.[22,24] Families or student groups who cannot afford intramuscular vaccine can share ampoules economically if inoculated the same day. Malarial prophylaxis with chloroquine inhibits the antibody response to this course given intradermally, so anyone on antimalarial therapy must have intramuscular injections.[31] A booster dose after 1 or 2 years prolongs the antibody response, which usually lasts 5–10 years.[32] Unnecessary boosters can be avoided if antibody is detected. As booster doses induce a reliable, prompt secondary immune response, repeated boosters are not needed for travelers who will have access to vaccine if exposed; otherwise boost after 3–5 years. Serologic testing is useful to determine the need for booster injections and is necessary if immunosuppression is suspected.

RABIES-RELATED VIRUSES

The rabies-related lyssaviruses, genotypes 2–7, are continent-specific in Europe, Australasia and Africa, and are viruses of bats (with one exception).[1] All but one, genotype 2 Lagos bat virus, have caused fatal human disease (Table 160.3). New unclassified lyssaviruses are emerging.

Africa[4,25]

Genotype 3, Mokola virus, is genetically separate from the others; it has been identified occasionally in shrews and rodents but never in bats. Clinical rabies encephalitis is not seen in humans, but Mokola virus has been associated with two doubtful infections in Nigerian children (see Table 160.3). Genotype 4, Duvenhage virus, is found rarely in bats and has caused a rabies-like encephalitis in three patients.[4,33,34] The true prevalence of these African infections is uncertain as the diagnosis of human rabies is normally made on clinical grounds and rabies-related viruses may give a weak or negative result with the routine diagnostic rabies immunofluorescent test.

Europe

There are two genotypes of European bat lyssaviruses (EBLVs):[1] genotype 5, EBLV type 1, is widely distributed in insectivorous bats across Europe and genotype 6, EBLV type 2, which is very rare. There are five reports of fatal rabies-like encephalitis following bat bites in Europe (see Table 160.3).[35–37]

Australia

Genotype 7, Australian bat lyssavirus,[38] was identified in 1996 in flying foxes (*Pteropus* spp.) and other bats, and has caused two fatal human infections indistinguishable from rabies encephalitis.[39] Lyssavirus seropositive bats have also been found in the Philippines, Thailand, Cambodia and Bangladesh.

Table 160.3 Rabies-related lyassaviruses

Genotype	Virus found*	Animal reservoirs (potential vectors)	Disease in humans
3 Mokola	South Africa, Nigeria, Cameroon, Ethiopia (rare)	Shrews, rodents (cat, dog)	• Child febrile convulsion;† recovered[1,25] • Child encephalitic signs;‡ died[1,25] • Vaccinated laboratory worker, mild illness; recovered.
4 Duvenhage	South Africa, Zimbabwe, Kenya (very rarely identified)	Insectivorous/fruit bats (cats)	• Man bitten by a bat, signs of typical furious rabies; died[1,25] • Man scratched by a bat, signs of classic rabies encephalitis; died[33] • Woman scratched by a bat, signs of rabies encephalitis; died[34]
5 European bat lyssavirus (EBLV) EBLV type 1a EBLV type 1b	 Northern and Eastern Europe Western Europe	Insectivorous bats	• Girl had bat bite, had acute ascending paralysis and encephalitis; died (clinical diagnosis only) • Girl had bat bite, signs of furious rabies; died of EBLV 1a[35] • Man had bat bite, signs of furious rabies; died (clinical diagnosis only)
6 European bat lyssavirus EBLV type 2a EBLV type 2b	 Netherlands, UK Switzerland (very rare)	Insectivorous bats (*Myotis* spp.)	• Bat conservationist bitten by bats, signs of classic rabies encephalitis; died of EBLV 2a[36] • Zoologist bitten by sick bat, signs of furious rabies; died of EBLV 2b[37]
7 Australian bat lyssavirus	Australia	Fruit bats (*Pteropus* spp.), insectivorous bats	• Woman bat carer, scratched by bats, signs of classic rabies encephalitis; died • Woman, bitten by a fruit bat, signs of paralytic rabies; died[39]

*There is serologic evidence of lyssavirus infection of bats in the Philippines, Cambodia, Thailand, Bangladesh and China.
†Doubtful diagnosis;
‡Alternative diagnosis possible.

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References for this chapter can be found online at http://www.expertconsult.com

Chapter 161

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Influenza viruses

INTRODUCTION

Influenza is a seasonal respiratory viral infection that results in some 250 000–500 000 deaths globally each year.[1] The illness is characterized by a sudden onset of high temperature and debilitating systemic symptoms. In healthy individuals, infection is typically self-limited; however, complications often occur in certain high-risk groups such as the elderly or the immunocompromised. Despite the availability of preventive vaccines and anti-influenza therapeutics, seasonal influenza remains a major global health problem. Moreover, as well as direct medical costs, the indirect social and economic costs remain significant.

In addition to the seasonal influenza epidemics which usually occur annually in the autumn and winter, global pandemics also occur, albeit much more infrequently. At the time of writing, the world is in the midst of the first influenza pandemic of the 21st century. The virus involved is a novel H1N1 virus of swine origin that originated in Mexico in the spring of 2009 and has now spread globally. To date, there have been more than 145 000 reported cases worldwide with over 1000 deaths. The emergence of this new pandemic serves to secure influenza's place in history as 'the last great uncontrolled plague of mankind'.[2]

NATURE

Virus structure

Influenza viruses are enveloped, single-stranded, negative-sense RNA viruses of the family Orthomyxoviridae, and there are three major antigenic types, A, B and C. These can be differentiated not only on the basis of antigenic differences in their nucleocapsid and matrix proteins, but also with respect to the number of gene segments and viral proteins, host range and capacity to cause disease.[3]

Influenza viruses possess segmented genomes: influenza A and B contain eight RNA segments and influenza C seven. Influenza A, the predominant pathogen in seasonal influenza and the cause of pandemic influenza, will provide the main focus for the remainder of this section. The influenza A genome encodes 11 viral proteins:

- haemagglutinin (HA), which is divided into two domains or subunits (HA1 and HA2);
- neuraminidase (NA);
- two matrix proteins (M1 and M2);
- the heterotrimeric RNA-dependent RNA polymerase, composed of one polymerase acidic (PA) and two polymerase basic (PB1 and PB2) subunits and the alternatively transcribed proapoptotic peptide, PB1-F2;
- nucleoprotein (NP); and
- two nonstructural proteins (NS1 and NS2; NS2 is also known as NEP, or nuclear export protein).[3–5]

The virus particle (virion) has an irregular spherical shape with a lipid envelope, approximately 80–120 nm in diameter. The surface is covered with spike-like projections composed of the two primary viral glycoproteins, HA and NA, which are involved in host cell attachment and host cell exit, respectively. In addition, M2 (a transmembrane ion channel) is also present on the external surface of the virus. M1 is found within the lipid bilayer, which surrounds the virus core. The core is a ribonucleoprotein (RNP) complex which consists of the viral RNA segments and the NP and the RNA polymerase (Fig. 161.1). Influenza A is currently classified on the composition of the HA and NA proteins. At present, 16 different HA (H1–H16) and 9 different NA (N1–N9) molecules are known to exist.[5]

Virus replication

At the initiation of human infection, the HA binds to receptors on the host cell surface that contain α2,6-linked sialic acid residues, and virion entry occurs via receptor-mediated endocytosis. In the host cell cytoplasm, the vesicle containing the virus undergoes an acidification process through fusion with intracellular endosomes. This triggers a conformational change in the HA, exposing a fusion peptide and permitting exit from the endosome; in addition, hydrogen ions are pumped from the endosome through the M2 ion channel into the virus particle. This allows the viral RNPs to be released, and they are then actively transported into the nucleus through the nuclear pore complex.[3] The M2 inhibitor class of antiviral agents, the adamantanes, inhibits this acidification process.

Transcription of the influenza viral genomic RNA (vRNA) into messenger RNA (mRNA) takes place in the nucleus. Following transcription, viral mRNA strands are exported to the cytoplasm where new viral proteins are synthesized. Newly synthesized viral RNAs are exported via a vRNP–M1–NEP protein complex. Packaging of progeny virions occurs at the host cell surface where the RNP–M1–NEP complex assembles at the cytoplasmic membrane under regions containing viral glycoproteins. As the new viruses bud and are released from the host cell, the NA is responsible for cleaving sialic acid residues that would cause the virus to remain bound to either the cell surface or other viral particles (Fig. 161.2). The neuraminidase inhibitor class of antiviral agents prevents this cleavage process and thereby the release of virus and subsequent infection of new host cells.

Structure of the influenza virion

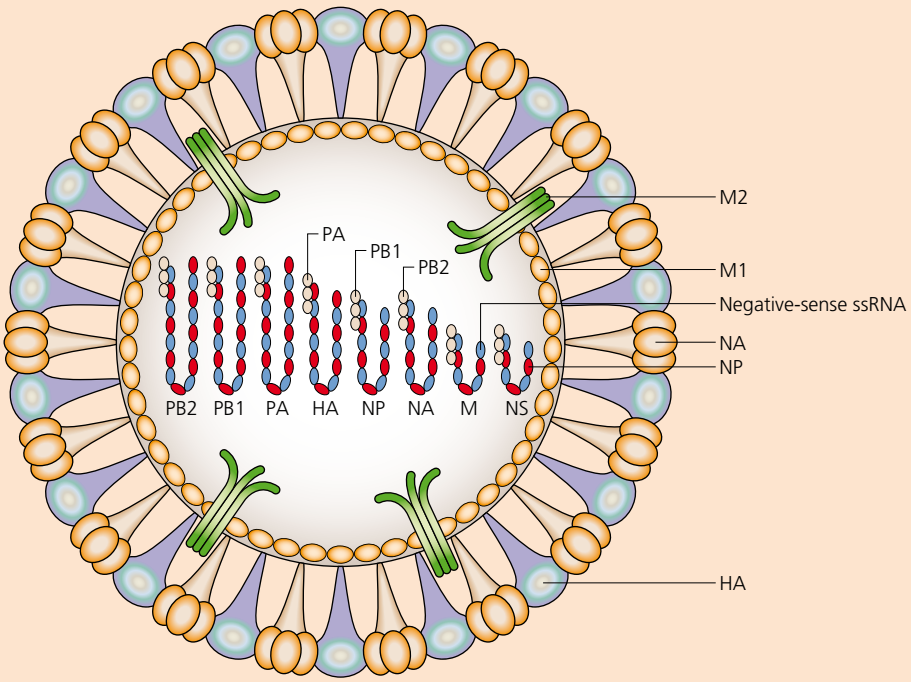


Fig. 161.1 Structure of the influenza virion. The genome of the influenza A virus is composed of eight genomic segments and each segment contains a region that encodes one or two proteins. In all, the eight segments encode 11 proteins. Details of the proteins, their biologic functions and their roles in virus replication are summarized in the text. Reproduced with permission from Nelson & Holmes.[6]

EPIDEMIOLOGY

Natural reservoirs of influenza viruses

Wild aquatic birds are the natural reservoir of all influenza A viruses.[7,8] Influenza A viruses in humans are maintained by human-to-human transmission during acute infection; however, other animals including primates, swine, ferrets, horses, seals, whales and mink can also become infected.

Antigenic variation – antigenic drift and shift

The influenza A viruses evolve rapidly primarily because of the absence of a proofreading mechanism during the copying of the vRNA to mRNA. The antigenic variation in influenza viruses occurs as a result of small changes, *antigenic drift*s, over time within the RNA segments encoding the surface glycoproteins NA and, in particular, HA. Antigenic drift leads to positive selection of these escape mutants from neutralizing antibody responses elicited by previous exposure(s) to wild-type viruses.

Human influenza virus HA molecules preferentially bind α2,6-linked sialic acid expressed on respiratory epithelia, whereas the HA of avian viruses binds predominantly α2,3-linked sialic acid receptors. Reassortment of influenza vRNA segments can occur if different viruses infect a single cell at the same time, allowing RNA exchange. This can result in the production of a novel progeny virus and is known as *antigenic shift*. Reassortment may occur in an intermediate host species such as swine (the mixing vessel hypothesis) which can be infected with both human and avian viruses as swine epithelia possess both α2,3- and α2,6-linked sialic acid receptors. Antigenic shift can lead to the introduction of a new influenza subtype into an immunologically naive population, and has the potential for pandemic spread.

Influenza pandemics

During influenza pandemics, virus spreads rapidly, resulting in significant morbidity and mortality. The 'Spanish influenza' pandemic of 1918–19 was caused by a highly virulent influenza A, subtype H1N1 virus that infected approximately one-third of the human population, leading to the deaths of more than 50 million people.[9,10] One striking feature of the 1918–19 pandemic was the disproportionately high numbers of healthy young individuals who succumbed to infection that has been attributed to an uncontrolled immune response with hyperproduction of proinflammatory cytokines, the so-called 'cytokine storm'. In 1957–58, the 'Asian' influenza pandemic arose after genetic shift of the circulating H1N1 virus to an H2N2 subtype.

The H2N2 viruses were subsequently supplanted by the H3N2 'Hong Kong' influenza pandemic of 1968. Lindstrom and co-workers performed a whole genome phylogenetic analysis of human influenza H2N2 viruses isolated from 1957 to 1968 and human H3N2 viruses isolated from 1968 to 1972.[11] The data suggest that H2N2 viruses continued to circulate for some time after 1968 and that the subsequent establishment of human H3N2 was associated with multiple reassortment events that increased the genetic diversity. In 1977 an outbreak of H1N1 occurred by an unknown mechanism involving a virus which was identical to that circulating in humans in the 1950s. Since then, seasonal influenza outbreaks have involved two main subtypes, H1N1 and H3N2, and these viruses continue to undergo antigenic drift.

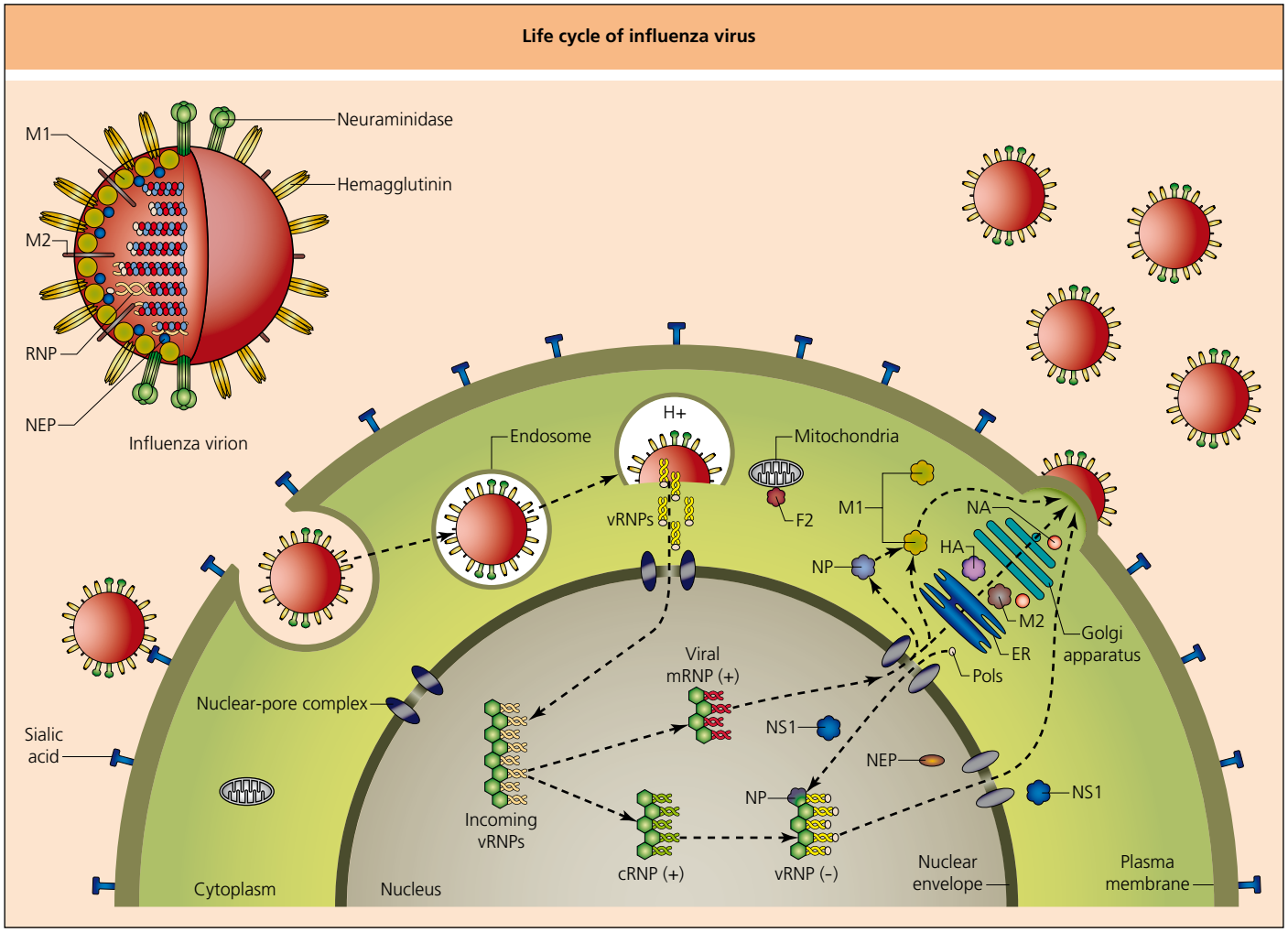


Fig. 161.2 Life cycle of the influenza virus. Virus particles initially associate with the host cell by binding to sialic acid-containing receptors on the cell surface. The bound virus is endocytosed, with the eventual release of the uncoated viral ribonucleoprotein (vRNP) complex into the cytoplasm. The ribonucleoprotein complex is transported through the nuclear pore complex into the nucleus. The incoming viral RNA (vRNA) is transcribed into messenger RNA (mRNA). The viral proteins are expressed and processed and eventually assemble with vRNAs at budding sites within the host cell membrane. The viral protein complexes and ribonucleoproteins are assembled into viral particles that bud from the host cell. Further details are provided in the text. Reproduced with permission from http://www.reactome.org.

Transmission of avian influenza to humans

Until 1997, the difference in HA receptor-binding specificities was thought to provide a host range barrier such that direct infection of humans by an avian-origin virus seemed highly unlikely. However, in 1997 avian influenza A, subtype H5N1 virus was transmitted to humans from infected chickens in poultry markets in Hong Kong, resulting in the deaths of 6 of the 18 patients infected.[12] The high case fatality rate and the subsequent global spread of highly pathogenic H5N1 in birds raised the possibility of a significant future pandemic. The cumulative number of confirmed human cases from 2003 to 2009 has been reported as 423 with 258 deaths, a 61% case fatality rate.[13] In most cases, infected individuals have had close contact with infected poultry. On rare occasions human-to-human transmission has been reported in Thailand, China, Vietnam, Indonesia and Azerbaijan; however, fortunately, there has been no sustained human-to-human transmission.[14–20] In March 1999, influenza A, subtype H9N2 viruses infected two children in Hong Kong; however, these two cases were mild and self-limiting.[21] Subsequently, an outbreak of highly pathogenic avian influenza A H7N7 occurred in early 2003 in commercial poultry farms in the Netherlands which resulted in 89 human infections of whom 83 developed conjunctivitis. Notably, one fatality occurred in a veterinarian who developed acute respiratory distress and fatal pneumonia.[22]

Swine influenza (H1N1v) pandemic 2009

In March 2009, public health authorities in Mexico City observed a large increase in the number of patients presenting with influenza-like illness. Subsequent testing in Canada and the USA confirmed that a previously undescribed influenza A virus was responsible. In April 2009, a novel swine-origin influenza A virus (H1N1v) infection was reported to the World Health Organization (WHO) by the Centers for Disease Control and Prevention (CDC) in Atlanta in two children presenting with a febrile respiratory illness from neighboring counties in southern California.[23] These cases were not epidemiologically linked and neither child had exposure to swine. Phylogenetic characterization of the virus from the US index case (A/California/04/2009) showed that the virus had a unique gene constellation not previously seen in humans or other animal reservoirs. Six genes (PB2, PB1, PA, HA, NP and NS) were similar to viruses previously identified in triple-reassortment swine influenza viruses in North American pigs and the remaining two gene segments (NA and M) were derived from Eurasian swine influenza viruses (Fig. 161.3).[25,26]

By the end of May 2009, Mexico had documented 4910 laboratory-confirmed cases with 85 deaths.[27] At the time of writing, Mexico has also reported the largest number of cases with severe clinical presentations, in contrast to other countries which have reported

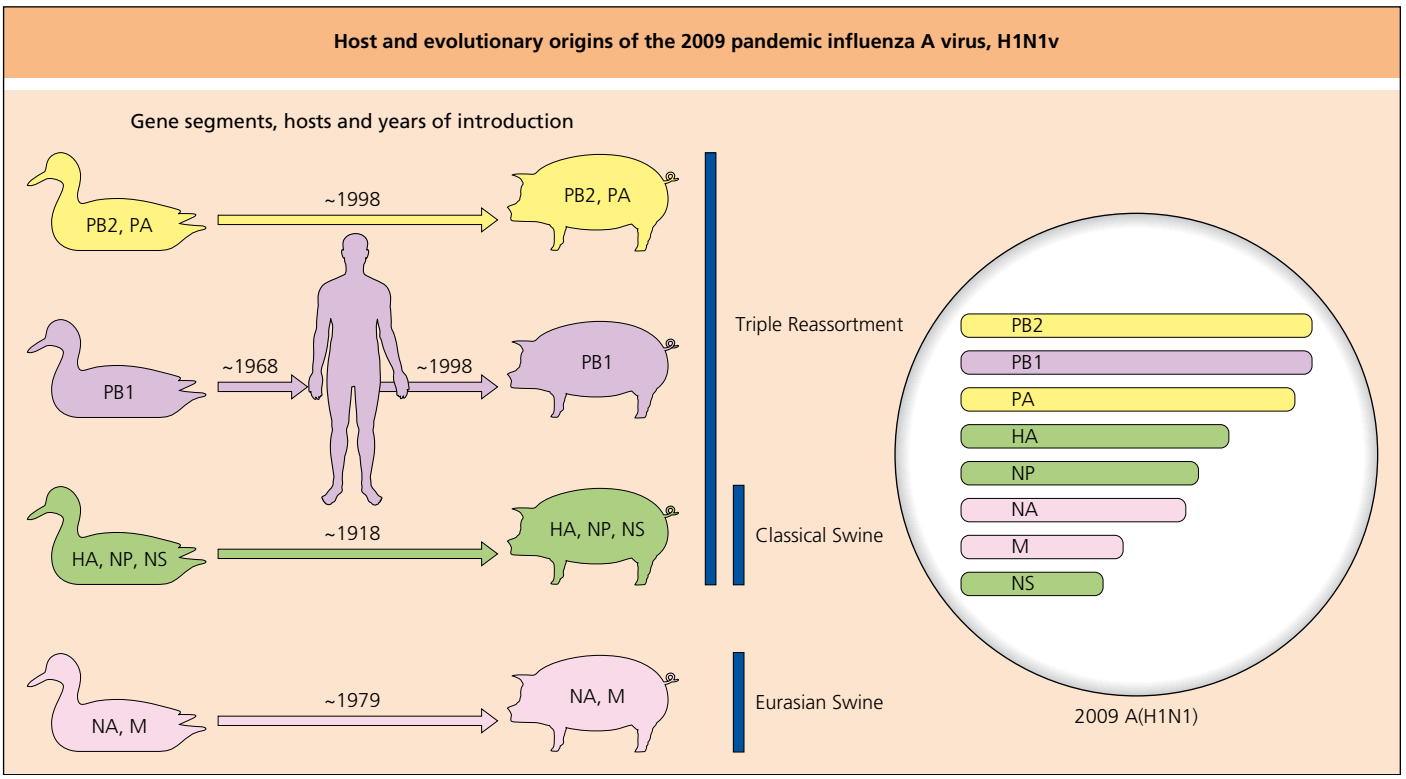


Fig. 161.3 Host and evolutionary origins of the 2009 pandemic influenza A virus, H1N1v. Host and lineage origins for the gene segments of the 2009 A (H1N1) virus: PB2, polymerase basic 2; PB1, polymerase basic 1; PA, polymerase acidic; HA, hemagglutinin; NP, nucleoprotein; NA, neuraminidase; M, matrix gene; NS, nonstructural gene. Color of gene segment in circle indicates host. Reproduced with permission from Garten *et al.*[24]

predominantly mild cases of a self-limiting influenza-like illness. However, deaths have occurred in individuals with underlying medical conditions. Since the original identification of H1N1v, sustained human-to-human transmission has occurred globally and on June 11, 2009, the WHO declared the first pandemic of the 21st century. In addition to the rapid global spread, a major concern is the continued co-circulation – and potential reassortment – of the novel H1N1v with seasonal H1N1 (which has acquired oseltamivir resistance approaching 100%) and the highly pathogenic H5N1 avian virus.

PATHOGENESIS

Influenza virus is classically acquired by the inhalation of infectious droplets. However, transmission by airborne droplet nuclei, small-particle aerosols, or self-inoculation (via virus-contaminated hands or fomites) may also occur. Following inhalation, the virus attaches to specific α2,6-linked sialic acid receptors expressed on respiratory epithelial cells. Although these receptors are widespread, the virus attaches preferentially to tracheal and bronchial epithelium.[28] In contrast, human infection by avian influenza viruses preferentially involves the distal airways.[29]

In uncomplicated influenza, histologic examination reveals diffuse mucosal inflammation with edema of the upper airways: epithelial cells become vacuolated and edematous and lose their cilia; this is accompanied by hyperemia and infiltration with primarily lymphocytes and histiocytes. Virus replication peaks at about 48 hours and the level of viral shedding correlates directly with severity of symptoms.[3] In the more severe primary viral pneumonia, the pathologic process extends distally to the lung causing an interstitial pneumonitis. Damage to the alveoli with formation of hyaline membranes allows exudates and hemorrhage from alveolar capillaries into the alveolar lumina, resulting in impaired gas exchange and severe respiratory dysfunction.

The host immune response against influenza virus is complex. Following infection of the respiratory epithelial cells, proinflammatory cytokines – primarily interleukin (IL)-6 and interferon alpha (IFN-α) – are produced by, and secreted from, infected cells. Cytokine production typically peaks by day 2 after infection, and corresponds with the peak of systemic symptoms.[30] In addition to cytokines, neutralizing antibodies (predominantly of the IgA subclass) are also produced at the site of infection. However, it is serum antibody levels (to HA and NA) that correlate with resistance to illness and with restriction of infection. Serum antibodies (IgM, IgG and IgA) are present within 1 week of the onset of illness, and may provide immunity and protection against re-infection by homotypic viruses for many years.[31] Cell-mediated immune responses are detectable before humoral responses and involve the activity of both $CD8^+$ cytotoxic T cells and $CD4^+$ helper T cells. Although these two classes of T cells closely interact, animal models have demonstrated that the response of either alone is capable of clearing infection.[32]

PREVENTION

Vaccination

Vaccination remains the cornerstone of the prevention of seasonal influenza. Indeed, some 80% of the population in the USA are now included in groups for whom seasonal influenza vaccination is recommended by the Advisory Committee for Immunization Practices (ACIP) (Table 161.1).[33]

As a result of antigenic drift, the process of candidate vaccine selection is complex. The WHO is responsible for decisions as to which influenza strains should be included in the vaccine, and separate recommendations are made annually to regional health authorities in the Northern and Southern hemispheres. The WHO conducts global surveillance programs through a network of national and regional

Table 161.1 Summary of seasonal influenza vaccination recommendations

Children and adolescents aged 6 months to 18 years

All children aged 6 months to 18 years should be vaccinated annually.

Children and adolescents at higher risk for influenza complications should continue to be a focus of vaccination efforts as providers and programs transition to routinely vaccinating all children and adolescents, including those who:

- are aged 6 months to 4 years (59 months);
- have chronic pulmonary (including asthma), cardiovascular (except hypertension), renal, hepatic, cognitive, neurologic/neuromuscular, hematologic or metabolic disorders (including diabetes mellitus);
- are immunosuppressed (including immunosuppression caused by medications or by HIV);
- are receiving long-term aspirin therapy and therefore might be at risk for experiencing Reye's syndrome after influenza virus infection;
- are residents of long-term care facilities; and
- will be pregnant during the influenza season.

Note: Children aged <6 months cannot receive influenza vaccination. Household and other close contacts (e.g. day-care providers) of children <6 months, including older children and adolescents, should be vaccinated.

Adults

Annual vaccination against influenza is recommended for any adult who wants to reduce the risk of becoming ill with influenza or of transmitting it to others. Vaccination is recommended for all adults without contraindications in the following groups, because these persons either are at higher risk for influenza complications or are close contacts of persons at higher risk:

- persons aged ≥50 years;
- women who will be pregnant during the influenza season;
- persons who have chronic pulmonary (including asthma), cardiovascular (except hypertension), renal, hepatic, cognitive, neurologic/neuromuscular, hematologic or metabolic disorders (including diabetes mellitus);
- persons who are immunosuppressed (including immunosuppression caused by medications or by HIV);
- residents of nursing homes and other long-term care facilities;
- health-care personnel;
- household contacts and caregivers of children aged <5 years and adults ≥50 years, with particular emphasis on vaccinating contacts of children <6 months; and
- household contacts and caregivers of persons with medical conditions that put them at higher risk for severe complications from influenza.

From Advisory Committee for Immunization Practices[33]

virology reference laboratories which provide information on circulating viruses. In parallel, serologic surveys are performed on recipients of the previous year's vaccine to evaluate immune responses to the emerging strains of the current season.[34]

Since 1977, seasonal influenza has been caused by two co-circulating influenza A viruses (H1N1 and H3N2) and influenza B, and all three viruses are included in seasonal vaccines. During influenza outbreaks where a close match exists between circulating virus strains and those in the vaccine, efficacy has been estimated at 80%. In seasons with a poor match, this can fall to as low as 50%.[35]

Seasonal influenza vaccines may comprise either inactivated virus particles or live attenuated virus. Both vaccine formulations have been shown to be safe and efficacious in healthy children and adults, although, in general, the immune responses tend to be poorer among the elderly and young children. Live attenuated influenza virus vaccine (LAIV) is presently only licensed for use in healthy children aged 2 years and older, and in healthy nonpregnant adults through the age of 49 years. Trivalent inactivated influenza vaccine (TIV) is licensed for use in all ages and all high-risk groups. Studies have shown that both vaccine types have equivalent efficacy.[35]

Seasonal influenza vaccination is both clinically and cost-effective. It results in lower rates of laboratory-confirmed influenza cases; lower rates of clinical influenza-like illness; reduced numbers of physician visits, days of illness, days of work loss, hospitalization rates and mortality. From a logistics perspective, LAIV has the advantage of intranasal administration, thus avoiding the need for injections. As patient surveys have indicated a preference for intranasal immunization over parenteral, more widespread use of the former would be expected to lead to an increased uptake of seasonal vaccine.[34]

In general, vaccination is well tolerated. Local reactions to TIV (discomfort, erythema and swelling) are typically mild. Systemic symptoms, which include fever, malaise and myalgia, may also occur. Despite widespread concern following an association between swine influenza vaccine and Guillain–Barré syndrome (GBS) in the USA in 1976, no definitive association between seasonal influenza vaccination and GBS has been demonstrated.[36]

Chemoprophylaxis

According to the Infectious Diseases Society of America, 'influenza vaccination is the primary tool to prevent influenza and antiviral chemoprophylaxis is not a substitute for vaccination'.[37] However, antiviral chemoprophylaxis is effective and should be considered in the following groups:

- high-risk individuals within 2 weeks of influenza vaccination (before an adequate immune response develops);
- high-risk individuals for whom vaccination is contraindicated, unavailable or expected to have low effectiveness (significantly immunocompromised);
- high-risk individuals who have not yet been vaccinated (influenza vaccine should be also administered);
- those who are in close contact with people at high risk of developing influenza complications; and
- all residents (vaccinated and unvaccinated) of institutions experiencing influenza outbreaks.

The adamantane M2 inhibitors amantadine and rimantadine, and the neuraminidase inhibitors oseltamivir and zanamivir, are recommended for the chemoprophylaxis of influenza A virus infections; only the neuraminidase inhibitors are recommended for chemoprophylaxis of influenza B.[37] Chemoprophylaxis is estimated to be 70–90% effective in preventing illness in healthy adults. However, decisions regarding choice of antiviral should take into account drug resistance in circulating influenza viruses. Thus, oseltamivir is no longer

appropriate for seasonal H1N1 chemoprophylaxis and the adamantanes should not be used for H3N2 chemoprophylaxis.[37] In a situation where influenza A virus infection has been diagnosed but subtype information is not available, then either zanamivir or a combination of oseltamivir and rimantadine should be employed. In hospital or residential institution settings, strategies for the prevention of nosocomial transmission of influenza should be implemented. These include staff and patient vaccination programs, chemoprophylaxis if appropriate, and compliance with droplet isolation precautions.

DIAGNOSTIC MICROBIOLOGY

Laboratory diagnosis plays a crucial role in the management of influenza. The development of highly sensitive and specific methods for virus detection allows the laboratory to provide a rapid definitive diagnosis, enabling clinicians to initiate prompt and appropriate management.

Virus isolation in culture

Influenza viruses can be isolated in culture from a range of specimens taken from the upper and lower respiratory tract; preferred specimen types are nasopharyngeal or nasal swabs and nasal washes or aspirates. Recently, the capital costs and longer turnaround times of culture compared to other more rapid detection systems have called into question the relevance of culture isolation methods.[38] However, it is clear that virus isolation remains essential for phenotypic antiviral susceptibility testing. In addition, the isolation process can be accelerated using centrifugation-enhanced ('shell vial') techniques.[39]

Serology

Serologic assays, such as hemagglutinin inhibition and complement fixation, require an acute-phase serum to be collected within 7 days of the onset of symptoms and compared to a convalescent serum specimen taken 14–21 days later to demonstrate a greater than fourfold rise in strain-specific, antibody titer. Because of this time frame, serology is of little use for the diagnosis of acute influenza; however, it remains important in basic research, epidemiologic investigations and the evaluation of antibody responses to vaccination.[40]

Rapid antigen testing

Rapid antigen or point-of-care (POC) testing has become increasingly important as timely results can lead to the prompt initiation of antiviral therapy, implementation of outbreak control measures and a reduction of inappropriate antibiotic usage.[41] The POC testing devices are simple to use and easily interpretable. However, whilst they have high specificities for virus detection, they have a lower sensitivity than 'gold standard' methods such as virus culture and reverse transcriptase polymerase chain reaction (RT-PCR).[42–46] In general, the sensitivity of POC tests in adults is lower than in pediatric patient populations, attributable to the higher viral loads and antigen shedding in younger age groups.[47] Thus, a negative test may not definitively exclude a positive diagnosis.

Immunofluorescence microscopy

The detection of influenza virus by direct fluorescent antibody (DFA) or immunofluorescent antibody (IFA) testing is extremely rapid and employs specific monoclonal antibodies to influenza virus antigens and visualization by fluorescence microscopy. The specificity and the sensitivity of this approach depend on sample collection and the presence of infected cells in the specimens. This is widely employed and is an excellent first-line choice for clinicians requesting a laboratory confirmation of POC positives already identified in the clinical setting.

Molecular detection

Molecular diagnostic approaches are increasingly replacing virus isolation and to a lesser extent immunofluorescence and rapid antigen testing for the detection of influenza due to their rapidity, cost-effectiveness and standardization. Molecular detection of nucleic acids extracted from respiratory specimens, employing real-time RT-PCR, is the method of choice. In addition, multiplex PCRs have been developed for the identification of multiple viral and bacterial respiratory pathogens, and are being incorporated into diagnostic algorithms for the differential diagnosis of respiratory tract infections.[48–50] Rapid real-time influenza subtyping approaches have also been developed and these can be employed downstream of universal influenza molecular detection assays, allowing the differentiation of seasonal human influenza, H1N1 and H3N2, avian H5N1 subtypes and novel swine H1N1v.[51] With the spread worldwide of seasonal human influenza A H1N1 with oseltamivir resistance (the H275Y mutation in the NA protein), several molecular methods have been developed to detect the single nucleotide polymorphism underlying resistance.[52,53] More recently, pyrosequencing ('sequencing by synthesis') has also been applied for the detection of drug resistance.[54]

CLINICAL MANIFESTATIONS

Seasonal influenza

Influenza A and B cause primarily an acute respiratory illness, with fever, cough, coryza and headache. However, it is the associated systemic symptoms that distinguish influenza from other respiratory virus infections, such as rhinovirus or respiratory syncytial virus. The incubation period for influenza infection averages 2 days (range 1–4 days). The classic illness has an abrupt onset with headache, high-grade fever, chills, dry cough, myalgia and malaise. Rhinorrhea, nasal congestion and pharyngitis may also be present. Fever typically peaks within the first 24 hours of illness. It begins to decline on the second or third day and is usually resolved by day 6; however, fever may be continuous or intermittent. Systemic symptoms usually resolve within a similar timescale. Weakness and fatigue, which on occasions can be accompanied by cough, can persist for an additional 1–2 weeks. Influenza attack rates are higher in children compared to adults, with fever, cervical lymphadenopathy, nausea and vomiting being frequent manifestations; bronchiolitis and croup (laryngotracheitis) may also occur.

Subclinical or asymptomatic infection can also occur, particularly in individuals with pre-existing immunity to the circulating strains of virus. In contrast to influenza A and B, influenza C typically causes only a sporadic and usually afebrile upper respiratory tract illness.

Complications

Complications occur not infrequently. Whilst the most common complications are bronchitis in adults and otitis media in children, exacerbations of pre-existing chronic medical conditions, such as asthma, chronic obstructive pulmonary disease and congestive heart failure, have been well described.

Pulmonary

Three distinct syndromes of severe pneumonia can occur after influenza infection in children or adults:

- primary viral pneumonia;
- combined viral–bacterial pneumonia; and
- secondary bacterial pneumonia.

Pneumonia complicates influenza predominantly in the elderly, patients with chronic cardiopulmonary disease, pregnant women and immunocompromised individuals.

Primary influenza virus pneumonia develops abruptly after the onset of symptoms and progresses within 24 hours to a clinical picture resembling severe acute respiratory distress syndrome (ARDS)

associated with hypotension, hypoxia and cyanosis. Fatality rates are as high as 50%, with death typically occurring within 4 days.

Combined viral–bacterial pneumonia is more common than primary viral pneumonia, with *Streptococcus pneumoniae*, *Staphylococcus aureus* and *Haemophilus influenzae* being predominantly involved. The clinical syndrome is indistinguishable from primary viral pneumonia. However, the later onset and the detection of both virus and bacteria in clinical samples confirm the diagnosis. The case fatality rate for combined pneumonia is some 10%, although this is frequently higher in cases of *Staph. aureus* co-infection.

Secondary bacterial pneumonia typically manifests after the patient has started to improve clinically from the original influenza illness. The onset of new respiratory symptoms is accompanied by localized clinical and radiologic findings. Bacteria may be isolated from clinical specimens, but usually virus is no longer detectable. The case fatality for this group of patients has been reported at around 7%.

Extrapulmonary

A variety of extrapulmonary manifestations has been described. Central nervous system (CNS) involvement, ranging from irritability and drowsiness to seizures and coma has been reported, although the pathogenesis remains unclear. Influenza-associated acute encephalopathy (IAAE), which is associated histologically with diffuse cerebral congestion and edema, is a recognized, uncommon, but potentially fatal neurologic syndrome generally occurring in children and adolescents and typically presenting during the early phase of infection.[55] A post-influenza encephalitis may also occur (albeit rarely) 2–3 weeks following recovery from the acute illness; fortunately, recovery occurs in the majority of cases. Reye's syndrome has also been reported following influenza (B more frequently than A) infection. Though the etiology is unknown, the risk of Reye's appears to be increased by the administration of therapeutic doses of salicylates and their use is now contraindicated in influenza infection.

Myocarditis has also been reported but the pathogenesis is poorly understood and is probably due to a combination of viral and host factors. Clinically, myocarditis may be associated with heart failure, pericardial effusion or conduction system disorders. Myositis or myopathy has also been described. While most cases recover, this may be so severe that acute renal failure secondary to myoglobinuria can occur. Toxic shock syndrome may also present in the context of influenza virus infection. It is believed that this is the consequence of bacterial exotoxin (TSST-1) secreted by colonizing or co-infecting *Staph. aureus* strains.

Infection in specific groups

In the immunocompromised host, influenza virus infection is associated with an increased risk of complications, hospitalizations and death. While the observed illness typically reflects that seen in the healthy individual, the clinical presentation may be atypical in the elderly (e.g. confusion, lethargy, low-grade fever) or in the transplant population (e.g. pneumonia, rejection). A prolonged disease course with viral shedding and the rapid emergence of antiviral resistance are also features of influenza in the immunocompromised.

Pregnant women in the second or third trimester have an increased risk of mortality from influenza. Although this is more commonly seen in the pandemic setting, it is also a feature of seasonal influenza, and the cause of death is usually overwhelming pulmonary disease. There are no clear explanations for the increased risk of pulmonary disease in pregnancy; however, it has been postulated that the increased pulmonary blood flow in pregnancy may predispose to pulmonary edema when alveoli are damaged.[56] Although maternal influenza infection has been associated with an increased risk of congenital anomalies and fetal loss, no specific teratogenic effect has been attributed to influenza virus.

Avian influenza (H5N1)

H5N1 avian influenza, in contrast to seasonal influenza and H1N1v, is highly pathogenic in humans with high morbidity and mortality. Although a definitive explanation for the severity of H5N1 infection remains to be established, much is known.[57] The virus is capable of infecting pulmonary epithelial cells, thereby causing diffuse alveolar damage and hemorrhage (this process is not dissimilar to primary viral pneumonia as described above). In addition, H5N1 (unlike seasonal influenza) disseminates beyond the respiratory tract during infection. The virus also has the capacity to impair cytotoxic T-cell activity *in vitro*, thereby reducing the capacity of the host to clear infection. Studies indicate that aberrant production of proinflammatory cytokines may also contribute to the pathogenesis of H5N1 influenza.

Pandemic influenza (H1N1v)

In general, the clinical presentation of pandemic influenza is similar to that described above, although some symptoms atypical for seasonal influenza may be more prominent. This is the case in the present H1N1v pandemic with diarrhea and/or vomiting being reported in some 38% of patients. At the time of writing (July 2009), and it should be stressed that this situation may change over time, 'this pandemic has been characterized by the mildness of symptoms in the overwhelming majority of patients'.[58] However, despite mild disease, the hospitalization rate is significant, and recent reports from Australia have indicated this may be in the region of 10% of 'laboratory-confirmed' cases.[59] Furthermore, severe respiratory disease and death have been reported, both in previously healthy individuals and those with underlying chronic disease.[27] Pregnant women, those with a body mass index (BMI) of >40 and those with pre-existing pulmonary disease may be particularly at risk of severe respiratory distress following H1N1v infection.[60,61] Notably, initial serologic studies suggest that a degree of pre-existing immunity to H1N1v exists in adults >60 years.[62]

MANAGEMENT

The majority of individuals infected with influenza virus have a self-limited, uncomplicated illness. However, in those patients with severe illness, early treatment with antiviral therapy may reduce the severity and duration of symptoms, the number of hospitalizations, the incidence of complications, the use of outpatient facilities, and in some cases the inappropriate use of antibiotics.[37]

Antiviral therapy for influenza has been covered in detail elsewhere in this text. However, in brief, there are two classes of agents with anti-influenza activity: the adamantane M2 inhibitors (amantadine and rimantadine) and the neuraminidase inhibitors (oseltamivir and zanamivir). All four agents are licensed for the treatment of influenza A, while the latter two are licensed for influenza B. Three of the four are administered orally, whereas zanamivir is delivered by inhalation. To be effective, antiviral therapy must be administered within 24–48 hours of the onset of symptoms. In general, both classes of agents are well tolerated, although minor CNS complaints are not uncommon in the case of the M2 inhibitors. With regard to the neuraminidase inhibitors, behavioral abnormalities have been described in children following oseltamivir administration. This has been reported primarily from Japan and may well reflect a genetic predisposition; however, patients should be advised accordingly. Zanamivir-induced severe bronchospasm has also been described, particularly in individuals with a history of asthma.

In recent years, antiviral drug resistance has become prevalent for both classes of antivirals and therefore local resistance data should inform their use. With regard to the two current co-circulating human seasonal influenza viruses, widespread resistance to the M2 inhibitors has been documented in the H3N2 virus, and therefore these should not be used to treat influenza unless the virus has been definitively identified as H1N1. Conversely, the prevalence of oseltamivir resistance in the H1N1 virus is now greater than 90%, and this should only be used to treat H3N2 virus infection.[37]

At the time of writing, rare oseltamivir resistance (H275Y mutation in NA) has been reported by the WHO in novel H1N1v from treated

patients but also notably in one case without exposure to the drug; furthermore, resistance to the adamantanes has been reported in all H1N1v viruses studied to date. However, the virus remains sensitive to zanamivir.

The following groups should be considered for antiviral therapy if influenza virus infection is documented: unvaccinated infants aged 12–24 months and individuals with any of the following: chronic pulmonary disease; hemodynamically significant cardiac disease; HIV infection; sickle cell anemia or other hemoglobinopathies; disease requiring long-term aspirin therapy; chronic renal dysfunction; cancer; chronic metabolic conditions; neuromuscular disorders; and seizure disorders. In addition, all adults over 65 years of age and all residents of nursing homes (or other long-term care facilities) should be considered for treatment.[37]

REFERENCES


References for this chapter can be found online at http://www.expertconsult.com

Chapter 162

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Respiratory viruses

INTRODUCTION

Respiratory viral infections are among the most common diseases of humans. These illnesses are caused by a heterogeneous group of viruses commonly known as human respiratory viruses (RVs). Over the last decade new molecular diagnostics have expanded the range of viruses detected in respiratory samples and several novel RVs have been identified. Real-time polymerase chain reaction (PCR) has been established as a powerful tool to diagnose and quantify RVs. The characteristic illnesses associated with the different respiratory viruses and the populations at higher risk are presented in Table 162.1. Other viruses, such as some of the enteroviruses, influenza viruses and herpesviruses, can also cause respiratory disease but they are discussed in separate chapters.

Respiratory viral infections are largely benign and self-limited, although newer studies using molecular amplification approaches have highlighted the importance of respiratory viruses as common pathogens in adults with pneumonia. They can exacerbate underlying chronic cardiopulmonary diseases, increase visits to health-care providers and result in unnecessary prescription of antibiotics. Among patients at the extremes of age with underlying cardiopulmonary diseases or those with primary or secondary immunodeficiencies, these illnesses can be associated with serious, potentially fatal complications.

The most common methods for the laboratory diagnosis of viral respiratory infections are summarized in Table 162.2 and Figures 162.1–162.5.

ADENOVIRUS

Nature

Adenoviruses were first isolated in the 1950s from human adenoids and later from respiratory secretions of military recruits with acute febrile respiratory illnesses. Human adenoviruses belong to the family Adenoviridae. More than 50 serotypes have been described and are being grouped into six species (A–F). Approximately one-half of these serotypes are known human pathogens. Virions are non-enveloped icosahedral particles measuring 80–100 nm in diameter and containing a linear double-stranded DNA genome. The capsid consists of 252 capsomers, of which 240 are hexons and 12 are pentons (at the vertices). A fiber with a knob at the end projects from every penton.

Epidemiology

Although epidemiologic characteristics of the adenoviruses vary by type, the route of transmission is either via the respiratory route, fecal–oral or water-borne. Infections are most common during early childhood and nearly 100% of adults have serum antibodies against multiple serotypes. Adenoviruses have been found to account for 3–5% of acute respiratory infections in children and up to 2% in adults. Close contact in crowded institutions increases the risk for adenovirus infections and outbreaks have been described in day-care centers, hospitals, shipyards and military quarters. Adenovirus infections can occur throughout the year but outbreaks of adenovirus-associated respiratory disease have been more common in the late winter, spring and early summer.

Pathogenesis

Adenoviruses causing respiratory disease can be spread via contact transmission (direct or indirect) and droplet transmission. Droplet transmission occurs when droplets containing infectious viral particles generated from the infected person are propelled a short distance (<1 meter) and deposited on the host's conjunctivae, nasal mucosa or mouth. Droplet particles (>5 µm diameter) are produced during coughing, sneezing or talking and during the performance of certain procedures such as suctioning and bronchoscopy.

Virions attach to human cells via interaction of the fibers with the CAR receptor (coxsackievirus and adenovirus receptor) on the cell surface and enter the cell via endocytosis. Virus uncoating occurs in the cytoplasm and the virus core is imported into the nucleus. The transcription of viral DNA depends in part on host cell machinery.

The replicative cycle in the host cells may be lytic or may result in the establishment of a latent infection, primarily in lymphoid tissue. The symptoms of adenovirus disease are due to the lysis of infected epithelial cells and the resulting inflammatory response. The initial immune response is probably the recognition of infected cells by natural killer cells and monocytes, which evokes a cytokine response, followed by induction of cytotoxic T cells and B cells. Prolonged shedding of adenoviruses in stools has been recognized even in immunocompetent individuals. Some adenovirus serotypes (especially 12, 18 and 31) can induce oncogenic transformation in cultured cell lines. In recent years, intensive research has focused on the use of adenoviruses as gene vectors.[1]

Prevention

An effective oral enteric-coated vaccine has been used to prevent adenovirus serotypes 4 and 7 infections in military recruits; however, this vaccine is not used in the general population. Contact and droplet precautions are indicated for controlling nosocomial transmission of adenovirus infection. Contact precautions include the isolation or cohorting of infected patients, the use of personal protective equipment (gloves, gowns), hand washing and the use of dedicated patient

Table 162.1 Human respiratory viruses: characteristic illnesses and populations at higher risk

Virus	Associated illnesses [most common virus subtype]	Patient population at higher risk
Adenovirus	URI [1–3, 5–7] Pharyngoconjunctival fever [3, 7, 14] URI, pneumonia [3, 4, 7, 14, 21] Pertussis-like syndrome [5] Pneumonia [1, 2, 3, 4, 7]	Infants, young children School-aged children Military recruits Infants, young children Infants, young children, immunocompromised
Coronavirus	Common cold Otitis Pneumonia Exacerbation of asthma-COPD Severe acute respiratory syndrome	All ages Children Military recruits, elderly, immunocompromised Persons with asthma-COPD All ages
Reovirus	Common cold (?) Enteritis (?)	All ages Children
Rhinovirus	Common cold URI Exacerbation of asthma-COPD Pneumonia	All ages All ages Persons with asthma-COPD Infants, young children, elderly, immunocompromised
Respiratory syncytial virus	URI Pneumonia Bronchiolitis Otitis	All ages Infants, young children, elderly, immunocompromised Infants, young children Children
Parainfluenza virus	URI Croup Otitis media Pneumonia	All ages Children Children Children, immunocompromised
Metapneumovirus	URI Pneumonia Bronchiolitis	All ages Infants, young children, elderly, immunocompromised Infants, young children
Human bocavirus	URI (?) Pneumonia (?)	Children Children

COPD, chronic obstructive pulmonary disease; URI, upper respiratory tract infection.

Table 162.2 Human respiratory viruses

Most common methods for laboratory diagnosis: culture, direct assay and serology

Virus	Culture cell lines	Characteristic findings	Direct assay	Serology
Adenovirus	Primary human embryonic kidney (HEK) and the human lung carcinoma (A549) cell lines are sensitive for a broad range of adenoviruses. Some adenoviruses may require up to 28 days to grow. The continuous epithelial lines, such as Hep-2 and HeLa, are sensitive but more difficult to maintain for a long period of time as required by some serotypes	Enlarged, refractile, rounded cells forming grape like clusters (Fig. 162.1), with some isolates forming a lattice-type arrangement of rounded cells	IF and EIA	Genus-specific EIA
Coronavirus	Culture not performed in most laboratories. SARS-CoV and HCoV-NL63 can be cultured in monkey epithelial cells	Cytocidal coronavirus infections may form multinucleated syncytia, lysis or both	EIA used in research settings	IF, EIA
Reovirus	Rarely isolated in the routine diagnostic setting. PRMK, LLC-MK2, HNK, HeLa, MDBK and H292 cell lines may support the growth of this virus	CPE is slow to develop. CPE may be nonspecific with increased granularity and progressive degeneration. HI and IFA can be used for typing	IF used in research settings	CF, NT, EIA used in research settings

(Continued)

Table 162.2 Human respiratory viruses—cont'd

Most common methods for laboratory diagnosis: culture, direct assay and serology

Virus	Culture cell lines	Characteristic findings	Direct assay	Serology
Rhinovirus	Human embryonic kidney and human fibroblast cell lines have been used most extensively: WI-38, HFF and MRC-5. HeLa is also sensitive	CPE is usually detected within the first week. CPE consists of rounded, highly refractile cells in loose clusters (Fig. 162.2). CPE permits a presumptive diagnosis of picornavirus infection. Demonstration of lability at pH 3 is used for differentiation from enteroviruses	EIA used in research settings	NT, CF, EIA used in research settings
Respiratory syncytial virus	Hep-2 is the preferred cell line. Alternatives include HeLa, A549, MRC-5, RhMK and Vero. Calcium and glutamine in the culture medium are important for optimal replication and cytopathic effect	CPE requires 3–7 days or more. Syncytia develop along with nonspecific granular degeneration (Fig. 162.3). Confirmation by means of IF (Fig. 162.3) or EIA	IF (Fig. 162.3), EIA, IP	CF, IF, EIA, NT
Parainfluenza virus	PRMK or MDCK are the preferred cell lines. LLC-MK2, a rhesus kidney heteroploid cell line and NCI_H292, a human lung carcinoma line, are also sensitive	CPE is variable and nonspecific. HPIV-2 and HPIV-3 may induce syncytia formation. Growth may be detected by means of HAd (Fig. 162.4) with confirmation and typing (Fig. 162.5), EIA, HAdI or HI	IF, EIA	HI, CF, EIA, NT
Metapneumovirus	Many strains replicate in tertiary monkey kidney cells/LLCMK-2 cells. Vero cell clone 118 seems to be permissible for all four lineages	CPE usually occurs after 10–21 days as syncytia formation or rounding of cells	IF	EIA
Human bocavirus	No cell culture model available	–	–	–

CF, complement fixation; CPE, cytopathic effect; EIA, enzyme immunoassay; HAd, hemadsorption inhibition; HAdI, hemadsorption assay; HI, hemagglutination inhibition; IF, immunofluorescence; IP, immunoperoxidase staining; LA, latex agglutination; NT, neutralization test; SARS, severe acute respiratory syndrome; PRMK, primary rhesus monkey kidney.

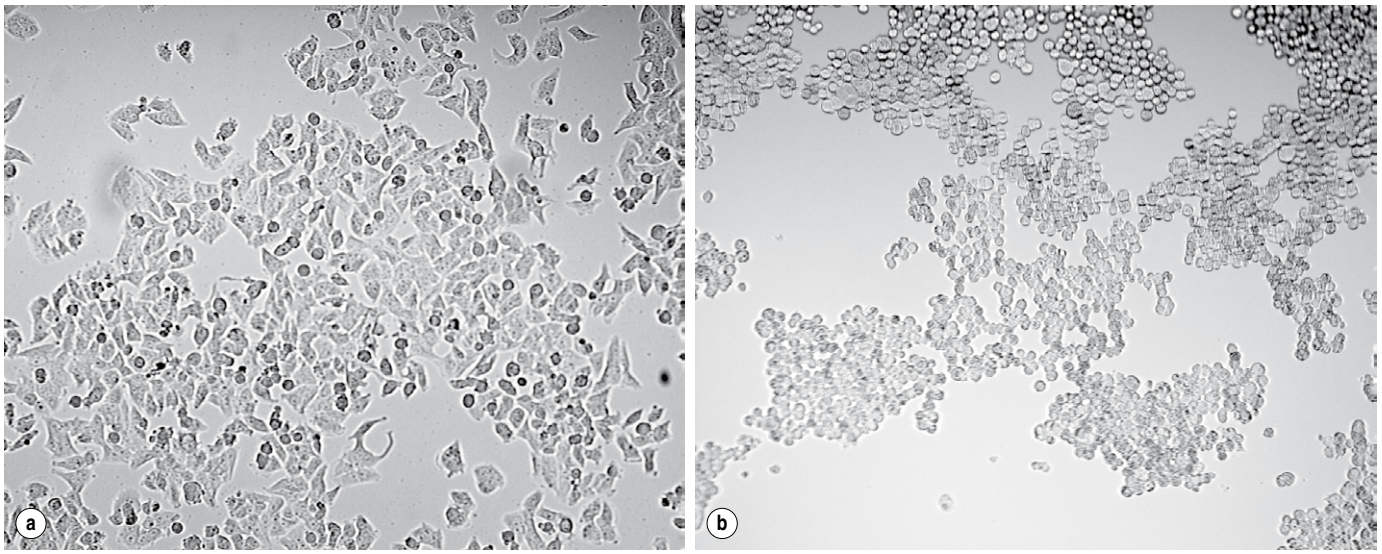


Fig. 162.1 Cytopathic effect caused by adenovirus on Hep-2 cell line culture. (a) Uninoculated cell line. (b) Enlarged, refractile, rounded cells forming grape-like clusters.

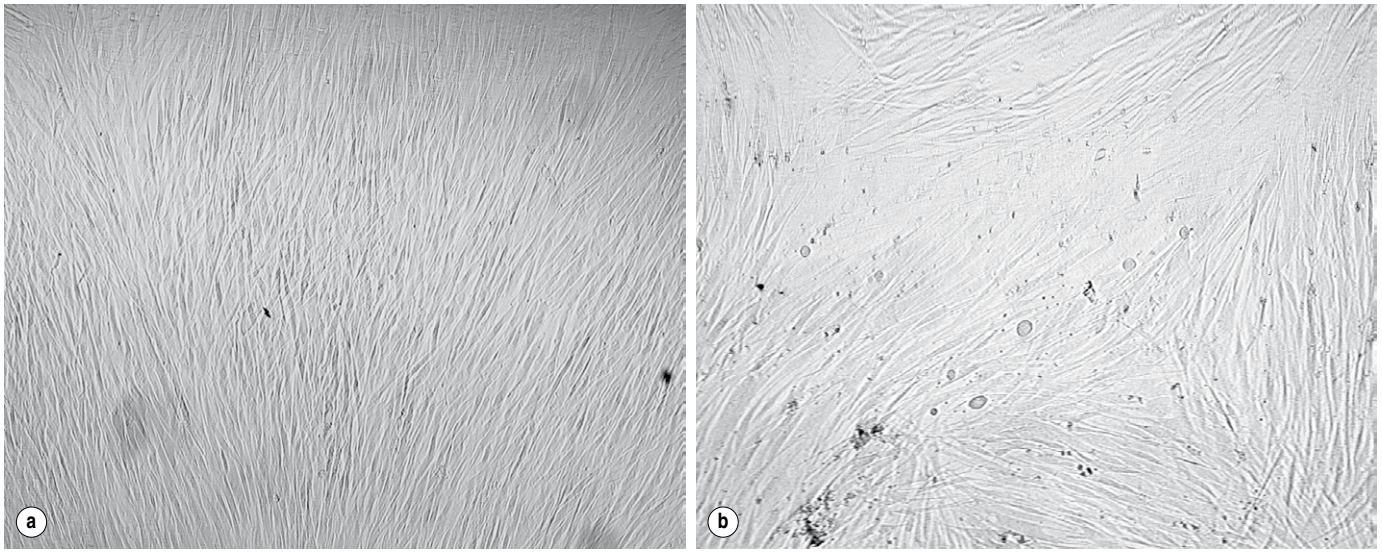


Fig. 162.2 Cytopathic effect caused by rhinovirus on human foreskin fibroblasts (HFF) cell line culture. (a) Uninoculated cell line. (b) Formation of small teardrop- to oval-shaped highly refractile cells.

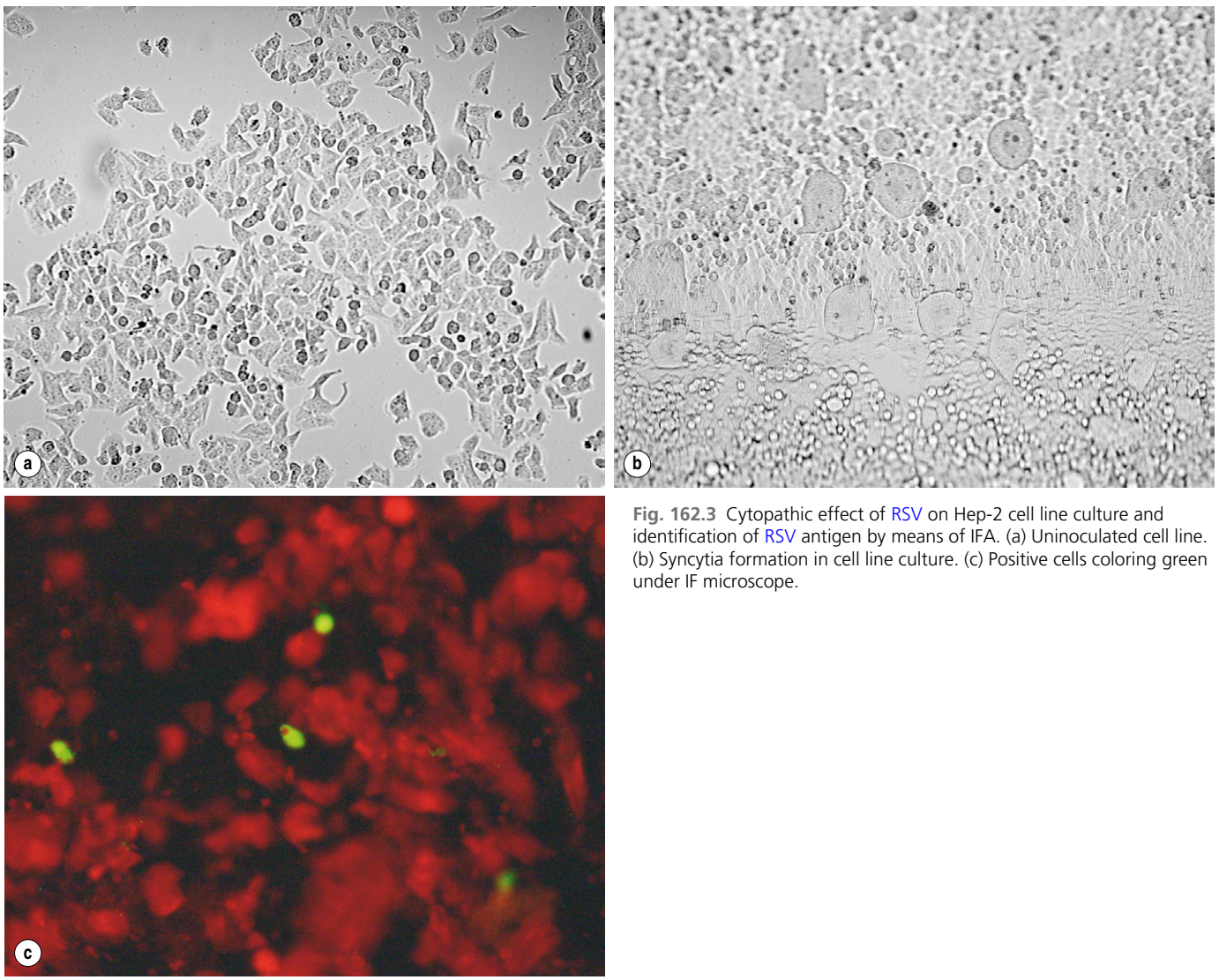


Fig. 162.3 Cytopathic effect of RSV on Hep-2 cell line culture and identification of RSV antigen by means of IFA. (a) Uninoculated cell line. (b) Syncytia formation in cell line culture. (c) Positive cells coloring green under IF microscope.

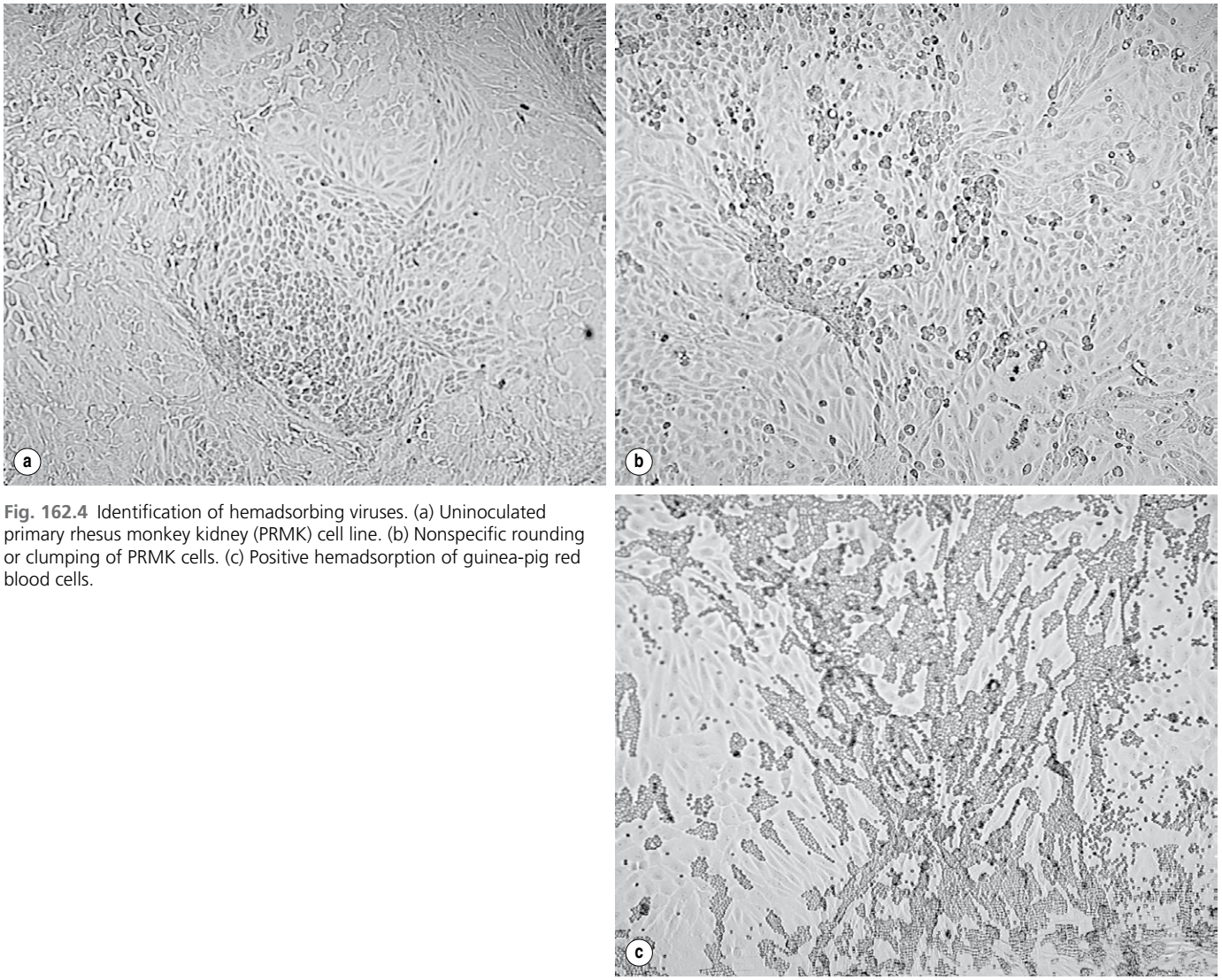


Fig. 162.4 Identification of hemadsorbing viruses. (a) Uninoculated primary rhesus monkey kidney (PRMK) cell line. (b) Nonspecific rounding or clumping of PRMK cells. (c) Positive hemadsorption of guinea-pig red blood cells.

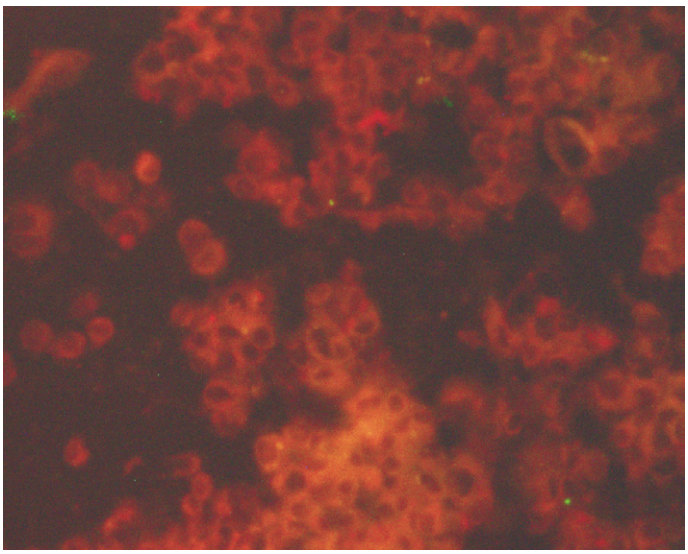


Fig. 162.5 Differentiation of hemadsorbing viruses by means of IFA. Negative control.

care equipment when possible. Droplet precautions include the additional use of masks by health-care workers within the patient's room and the use of masks by the patient when outside the room. Maintaining adequate levels of chlorination is necessary to prevent swimming pool-associated outbreaks of adenovirus conjunctivitis.

Diagnostic microbiology

Virus isolation, antigen detection, PCR assay and serology can be used to identify adenovirus infections. Adenovirus serotyping is usually accomplished by hemagglutination inhibition and/or neutralization with type-specific antisera. The isolation of adenoviruses from body secretions is not sufficient to establish a diagnosis of adenovirus disease as prolonged asymptomatic shedding of adenoviruses can occur. New molecular diagnostics such as quantitative PCR have improved the rapid diagnosis of adenovirus infections and monitoring of viral loads in blood has been shown to correlate with clinical progression of disease.[2]

Clinical manifestations

Respiratory illnesses associated with adenovirus infections include undifferentiated upper respiratory illness, pharyngoconjunctival fever, pertussis-like syndrome and pneumonia. The various adenovirus

serotypes associated with respiratory disease are shown in Table 162.1. In addition, adenoviruses may also cause gastroenteritis, keratoconjunctivitis, cystitis, meningoencephalitis and hepatitis, depending on the serotype. The incubation period for the endemic serotypes ranges from 5 to 10 days and the symptoms last approximately 1 week. Chronic lung damage in the form of bronchiolitis obliterans has been reported after infections with serotypes 1, 3, 4, 7 and 21.

The incidence of severe adenovirus disease is increasing with the rising number of immunomodulatory treatments used in modern medicine. Pediatric patients are more susceptible to adenovirus infection and disease than adults, probably due to the higher rate of primary infections occurring among children. However, cases of severe adenovirus disease have also been reported in apparently normal hosts. Immunocompromised patients, particularly hematopoietic stem cell transplant (HSCT) and solid-organ transplant recipients, are susceptible to severe and potentially fatal adenovirus disease,[3–5] such as protracted hemorrhagic cystitis, encephalitis, nephritis, hepatitis, enteritis, pneumonia or a combination of these illnesses (disseminated disease).

Management

Most infections are mild and require only symptomatic treatment. Ribavirin has *in-vitro* activity against several adenovirus serotypes but the clinical experience in immunocompromised individuals has produced conflicting results (see Chapter 147).

CORONAVIRUS

Nature

Human coronavirus (HCoV) was first isolated in 1965 from a patient with symptoms of the common cold. Three new coronaviruses have been detected since 2001: the severe acute respiratory syndrome (SARS)-associated coronavirus in 2003, coronavirus NL63 in 2004 and coronavirus HKU1 in 2005. Coronaviruses are classified into three groups: group 1 consists of HCoV-229E and HCoV-NL63, group 2 harbors HCoV-OC43 and HCoV-HKU1, and SARS-CoV is classified independently and is thought to be of animal origin.[6] Virions are pleomorphic and measure 80–200 nm in diameter. The outer envelope bears distinctive 'club-shaped' peplomers that correspond to the S (long spike) glycoprotein. The resulting 'crown-like' appearance under electron microscopy gives the family its name. The S glycoprotein is involved in receptor binding and cell fusion and is a major target for neutralizing antibodies. Other structural proteins include the M (matrix) protein, the E (envelope) protein and the N (nucleocapsid) phosphoprotein. In some types, there is also a glycoprotein HE (hemagglutinin-esterase, short spike). Entry occurs via endocytosis and membrane fusion. Replication of the linear single-stranded positive-sense RNA genome takes place in the cytoplasm.

Epidemiology

HCoVs are ubiquitous. Serologic studies with the initial two coronaviruses OC43 and 229E have suggested that these viruses are a major cause of respiratory disease and account for 10–30% of all common colds. Serologic studies have also suggested that one-half of the infections with coronaviruses are asymptomatic.[7] The identification of a coronavirus as the underlying pathogen for SARS was published in 2003 and this was the first time that a coronavirus was definitely demonstrated to cause severe human disease.[8,9] The SARS epidemic was contained through global infection control efforts organized by the World Health Organization (WHO) and currently there is no evidence that SARS coronavirus is active in human populations. More than 8000 cases were reported in the course of the SARS epidemic in 29 affected countries, leading to more than 800 deaths worldwide.[10] Since the discovery of SARS two newer coronaviruses HKU1 and NL63 have been identified. These HCoVs are similar to their older cousins in being mostly associated with common cold symptoms but severe clinical disease can be caused in young children, the elderly and in immunocompromised patients.[11]

Pathogenesis

Intranasal inoculation of volunteers with these viruses results in common colds with disruption of the ciliated epithelium and ciliary dyskinesia.[12] Aminopeptidase N (APN), the receptor for HCoV-229E, is expressed on the apical surfaces of respiratory and intestinal epithelium, myelocytic cells, kidney tubular epithelium and synaptic junctions. HCoV-OC43 binds to cells by means of the HE glycoprotein or the viral S glycoprotein, which can recognize 9-*O*-acetylated sialic acid on the cell surface.

Prevention

No specific antiviral drugs or vaccines are currently available. The major transmission modes for SARS are the contact and/or droplet route, although airborne transmission over a limited distance had been suggested.[13] The outbreak of SARS was successfully controlled through early detection of cases by screening of persons with symptoms of a respiratory infection and implementing strict contact and droplet precautions. The Centers for Disease Control and Prevention (CDC) currently recommends contact precautions and airborne precautions with N95 or higher respirator masks[13] although in Hong Kong the use of contact and droplet precautions alone was sufficient to protect health-care workers.[14]

Diagnostic microbiology

Diagnosis of infection is achieved by nucleic acid extraction, reverse transcription and PCR. Coronaviruses are included in multiplex assays that have been developed for the diagnosis of respiratory viral infections. Some of the coronaviruses can be cultured in monkey epithelial cells (SARS-CoV and NL63). Most of the population studies of HCoVs have used serologic methods, including complement fixation, hemagglutination inhibition (OC43 only) and recently enzyme immunoassay (EIA).

Clinical manifestations

Apart from SARS-HCoV, HCoVs are predominantly associated with mild upper respiratory tract infections and occasionally severe disease is observed in immunocompromised patients. In a study of adult volunteers the mean incubation period was 3.3 days, with a range of 2–4 days. The symptoms lasted for a mean of 7 days, with a range of 3–18 days.[15] In contrast, SARS-HCoV was capable of causing a multisystem disease with infection of the gastrointestinal tract, liver, kidney and brain.[16]

The clinical course is mainly characterized by fever, dyspnea, lymphopenia and lower respiratory tract infection; 30–40% of patients had diarrhea.[10] Compared to the other coronaviruses SARS was causing a much more severe disease with a mortality rate of nearly 10%.[17] Coronavirus particles have also been seen (via electron microscopy) in the stools of children and adults with diarrhea and gastroenteritis. Earlier speculations that coronavirus could participate in the etiology of multiple sclerosis were not confirmed.[10] However, the importance of human coronaviruses as a cause of diseases outside the respiratory tract remains to be determined.

Management

No effective antiviral therapy against coronaviruses, including SARS, has been found so far. *In-vitro* ribavirin is capable of inhibiting viral replication but there are no *in-vivo* data supporting its therapeutic

efficacy.[10] Steroids were used to treat SARS-infected patients with the aim of modulating cytokine levels. Interferons were found to possess antiviral activity *in vitro* and in animal models.[10] A protective vaccine against SARS has not yet been generated.

REOVIRUS

Nature

Isolation of the first reovirus was made in 1951, but it was in 1959 that Sabin coined the term reovirus to emphasize the fact that these viruses were isolated from the respiratory and enteric tracts and that they were not associated with any known human disease (Respiratory Enteric Orphan viruses). Reoviruses are nonenveloped viruses of about 65–75 nm in diameter. Viruses of the genus *Orthoreovirus* are composed of two concentric icosahedrically symmetric protein capsids, containing 10 discrete segments of genomic double-stranded RNA. Orthoreoviruses have been isolated from a wide variety of mammalian, avian and reptilian hosts, and over recent years several previously unknown reoviruses (especially of bat origin) have been reported.[18]

Epidemiology

A study using enzyme linked immunosorbent assay (ELISA) as the diagnostic method showed that the prevalence of antibodies against reoviruses increased steadily throughout life from about 35% in infants to 60% in teenagers and to more than 85% among those older than 60 years of age.[19]

Diagnostic microbiology

Trying to diagnose reovirus infection on a routine basis is not recommended. In the research setting, a variety of techniques are available.

Clinical manifestations

In a study of adult volunteers, intranasal inoculation of reovirus was followed by an increase in serotype-specific antibodies, enteric virus isolation and development of respiratory illness in a third of the individuals infected with reovirus T1L.[20] Two newer reoviruses, Melaka virus and Kampar virus, were isolated from patients with acute respiratory tract disease, raising the possibility that the clinical importance of this group of viruses was underestimated in the past.

RHINOVIRUS

Nature

Human rhinovirus (HRV) was first isolated in the 1950s from nasopharyngeal secretions of patients with the common cold. HRV belongs to the Picornaviridae family, which includes the genera *Enterovirus* and *Hepatovirus* (hepatitis A virus). Rhinoviruses are small (30 nm), nonenveloped particles that contain a linear single-stranded positive-sense RNA genome. The icosahedral capsid is composed of 60 subunits arranged as 12 pentameres. Each subunit is composed of one of each of four structural proteins (VP1 to VP4). VP1–3 have exterior projections that are major epitopes for neutralizing antibodies.

More than 100 serotypes have been identified to date. Phylogenetic analysis of genome sequences revealed two different species of rhinovirus A and B, HRV-A and HRV-B. Recently, a third genetic cluster was proposed (HRV-C) by analysis of viruses amplified from children with exacerbations of asthma.[21]

Epidemiology

Rhinoviruses are ubiquitous and infections occur in all age groups. They cause about half of all common colds and over the last few years an increasing number of publications have linked HRVs to more severe upper and lower respiratory tract infections in children, the elderly and immunocompromised patients.[22–24] HRV infections occur throughout the year, with peaks in the fall and spring, and with multiple serotypes circulating simultaneously in a given geographic location. Preschool children commonly introduce the virus to the rest of the family, with a secondary attack rate of about 50% after an interval of 2–5 days. Extensive studies in susceptible individuals have shown that 95% of challenged subjects become infected and about 75% of them become ill. The major factor determining the risk of illness is the prechallenge level of neutralizing antibodies.

Pathogenesis

In spite of the extensive literature on the transmission of rhinoviruses, controversy still exists about the predominant mode of transmission. Most data support the notion that contact transmission, primarily as hand shaking followed by inoculation of the nasal mucosa and lacrimal canals, is the most efficient mode of transmission. HRVs can survive from a few hours to as long as 4 days on nonporous surfaces and for over 2 hours on human skin. Although droplet or airborne transmission of rhinovirus infection is possible, prolonged and close exposure is apparently required. There is no convincing clinical evidence to support the common notion that exposure to cold plays a role in the genesis of HRV illness.

Rhinoviruses attach to respiratory epithelium and spread locally. The leukocyte binding protein CD54/intercellular adhesion molecule 1 (ICAM-1) is the receptor for the majority of rhinovirus serotypes. The receptor-binding site resides in a depression of the capsid surface that is not accessible to antibodies. After endocytosis, a conformational change of the capsid allows the release of the viral RNA into the host cell cytoplasm, where viral replication and assembly take place. A polyprotein precursor is processed mainly by two viral proteases designated 2A and 3C. The 2A protease makes the first cleavage between the structural and nonstructural proteins, while the 3C protease catalyzes most of the remaining internal cleavages.

Infection is followed by activation of several inflammatory mechanisms including the release of cytokines, particularly interleukin (IL)-8, bradykinins, prostaglandins and histamine, as well as stimulation of parasympathetic reflexes.

The resulting process includes vasodilatation of nasal blood vessels, transudation of plasma, glandular secretion and stimulation of nerve fibers, triggering sneeze and cough reflexes. Nasal mucociliary transport is reduced markedly during the illness and may be impaired for weeks. Immunity to individual rhinoviruses is type specific and not long lasting. Protection correlates more with the level of locally synthesized IgA antibodies, which decline within months of infection, rather than with IgG levels in serum, which may persist for a few years.

Prevention

Hand washing after personal contact with a symptomatic person or after touching potentially contaminated objects is strongly recommended. Alcohol-based antiseptics are also effective for this purpose. Symptomatic patients should be advised to wash hands frequently and to use disposable tissues. The CDC recommends droplet precautions for the prevention of nosocomial HRV infections.

The diversity of serotypes and the specificity of the immune response have precluded the development of an effective vaccine. A number of drugs have been studied for the prophylaxis of HRV infection, including interferon alpha (IFN-α), pirodavir and the recombinant soluble CD54/ICAM-1 molecule. However, the practical utility of these drugs remains to be demonstrated.

Diagnostic microbiology

In clinical practice HRV infections are diagnosed by viral RNA detection with RT-PCR. RT-PCR assays are more rapid and sensitive than isolation techniques for the detection of HRV RNA in clinical specimens. Historically, HRV infections have been detected by virus isolation in cell culture followed by acid lability testing to distinguish them from enterovirus infections. Virus isolation typically requires 2–14 days. Presumptive identification of a cell culture isolate as rhinovirus is based on a combination of enterovirus-like cytopathic effects and clinical information. Further differentiation from enteroviruses is based on the diminished replication of rhinoviruses at 98.6°F (37°C) compared to growth at 91.4°F (33°C) and at pH 3.0 compared with pH 7.0, respectively

ELISA has been developed for the detection of rhinovirus antigens. The use of serologic assays is limited by the diversity of serotypes and the lack of a group-specific antigen.

Clinical manifestations

Symptoms of the common cold include profuse watery rhinorrhea, nasal congestion, sneezing and quite often headache, sore throat and cough. The symptoms begin within 8–10 hours of infection and peak around 1–3 days. There is little or no fever. By days 3–5 of the illness, nasal discharge may become mucopurulent from polymorphonuclear leukocytes that have migrated to the infection site in response to IL-8. Resolution of symptoms generally occurs within a week. Complications include sinusitis, otitis media and exacerbation of asthma or chronic obstructive pulmonary disease (COPD). Secondary bacterial infections may also occur. Studies using CT of the sinuses have shown that HRV infections can cause the accumulation of secretions in the sinuses, possibly from the passage of nasal secretions into the sinuses during nose blowing.[25,26]

Recent published evidence has confirmed the association of HRVs with severe airway disease in immunocompromised patients, children and the elderly.[21,22] A novel HRV species (HRV-C) has been found in a significant proportion of children with exacerbations of asthma.

Management

Symptomatic treatment with decongestants, antihistamines and antitussives is currently the mainstay of therapy. The efficacy of zinc lozenges is controversial and their practical utility is limited because of their metallic taste. A variety of candidate drugs have been studied.[27]

Interferon alpha 2b has been found to be effective for the prevention of rhinovirus-associated colds but ineffective for the treatment of established colds.[28] Recent therapeutic advances include the development of protease 3C inhibitors, recombinant soluble ICAM-1 antagonist and capsid-function inhibitors (pleconaril), all of which exhibit potent antirhinoviral activity *in vitro* and varying activity in clinical trials.[27,29] Recombinant soluble ICAM-1 molecule (Tremacamra) has been found to be effective in reducing the symptoms of experimental common colds in randomized controlled trials when given either as a preventative or a therapeutic agent.[30]

Pleconaril binds to a hydrophobic pocket in the viral capsid, inducing conformational changes that lead to altered receptor binding and viral uncoating. Pleconaril is orally bioavailable and achieves serum concentrations in excess of those required to inhibit 90% of clinical rhino- and enteroviral isolates *in vitro*. In a placebo-controlled study of rhinovirus infections, pleconaril-treated patients had a 1.5-day reduction in time to resolution of symptoms, significant reduction in symptom severity within 12–24 hours after initiation of therapy and a significant reduction in median viral titers in nasal mucus.[31] The US FDA Advisory Committee has not yet recommended the approval of pleconaril for treatment of the common cold.

RESPIRATORY SYNCYTIAL VIRUS

Nature

Respiratory syncytial virus (RSV) was first isolated in the mid-1950s from a symptomatic laboratory chimpanzee during an outbreak of illness resembling the common cold. This virus derives its name from the characteristic formation of multinucleated giant cells in tissue culture.

RSV is a linear single-stranded, negative-sense genomic RNA of the Paramyxoviridae family of viruses. Virions are pleomorphic, with both spherical and filamentous forms averaging 120–300 nm in diameter. The envelope consists of host-derived plasma membrane and three virally encoded transmembrane surface glycoproteins: the attachment protein G, the fusion protein F and the small hydrophobic SH protein. The F protein can mediate fusion with neighboring cells to form syncytia. The G glycoprotein plays a major role in the process of attachment. Two major types of RSV – A and B – are distinguished serologically based on the antigenic characteristics of the surface glycoprotein G. Other viral proteins include the matrix proteins (M and M2), the major nucleocapsid (N) protein, a phosphoprotein (P) and the major polymerase (L).

Epidemiology

RSV is the most common cause of bronchiolitis and pneumonia among infants and young children worldwide.[32] Virtually all children are infected by the time they reach 3 years of age. Natural immunity is transient and re-infection is common. Crowding in households and day-care centers increases the attack rate of RSV. A large CDC-initiated study found that among children with acute respiratory infection admitted to hospital or attending outpatient services, 18% of specimens were positive for RSV.[33] Extrapolating this figure to the entire US population, 2 million children under 5 years of age would require medical attention due to RSV infection on a yearly basis.[33] In older children and immunocompetent adults, RSV infections are seldom severe and are usually manifested as upper respiratory infection (URI) or tracheobronchitis. Individuals at high risk for severe RSV infection include premature infants (particularly those born at less than 34 weeks of gestation), young infants with chronic lung disease or a hemodynamically significant congenital heart defect, elderly individuals and immunocompromised subjects. Recent studies indicate that the disease burden caused by RSV in elderly and high-risk adults had previously been underestimated and is currently thought to be similar to that of endemic influenza A.[34] Development of an effective RSV vaccine is therefore required for both young children and the elderly.

RSV activity follows a seasonal pattern in temperate zones of the world. In urban centers, annual outbreaks occur during the fall, winter and early spring. In the northern hemisphere most outbreaks peak in February or March. In tropical or subtropical areas, epidemics peak during the rainy season.

Pathogenesis

RSV is present in large numbers in the respiratory secretions of symptomatic infected persons and viral titers remain high for about 1 week, followed by a gradual decline over 3–4 weeks. The portal of entry is usually the conjunctiva or the nasal mucosa. Although transmission can occur via large droplets, contact transmission predominates.[35] Virus can be viable for approximately 30 minutes on impervious surfaces such as bed rails. Thus, caregivers can contaminate their hands during routine care and transmit the virus by contact to other patients. Neutralizing antibodies to the surface glycoproteins F and G correlate with resistance to re-infection, but protection is not complete and is of short duration.[36]

The pathology of RSV bronchiolitis is characterized by necrosis of ciliated bronchiolar epithelial cells, mononuclear infiltrates and edema of the peribronchial space. Bronchiolar obstruction from increased mucus production results in air trapping and total obstruction results in atelectasis. Pathologic examination of lungs from patients with RSV pneumonia has revealed marked interstitial inflammation and edema of the lung parenchyma, along with the findings of bronchiolitis.

Prevention

In the household setting, the transmission of RSV may be decreased by frequent hand washing and not sharing household items such as cups, glasses and utensils. The CDC recommends contact precautions for the prevention of nosocomial RSV infections. Some institutions also observe droplet isolation precautions. Implementation of multifaceted infection control strategies resulted in a reduction of the incidence of nosocomial RSV infections.[37] Passive immunoprophylaxis is indicated for high-risk children.

RSV intravenous immunoglobulin (RSV-IVIG, RespiGam) is a human polyclonal immunoglobulin with high RSV microneutralization titers. In placebo-controlled studies, monthly intravenous doses of 750 mg/kg during the RSV season significantly reduced the incidence of RSV hospitalizations and the severity of illness among premature infants and infants with bronchopulmonary dysplasia.[38,39] Palivizumab is a humanized monoclonal antibody that specifically inhibits an epitope at the A antigenic site of the F protein of RSV subtypes A and B. In a large multicenter placebo-controlled trial involving high-risk infants, palivizumab prophylaxis resulted in a 55% reduction in hospitalization rates and number of hospital days ascribed to RSV infection.[40] Both agents have been well tolerated, with few adverse effects; however, their high cost necessitates strict guidelines for their use. RSV-IVIG or palivizumab is indicated for the prophylaxis of RSV infections among infants with chronic lung disease (formerly designated bronchopulmonary dysplasia) and premature infants. In children with a hemodynamically significant congenital heart defect only palivizumab is indicated for prophylaxis.[41] Cost-saving benefit has not been observed uniformly,[42–46] perhaps as a result of major differences in the prevalence of RSV lower respiratory tract infections in different countries and regions, suggesting the need for locally adapted recommendations. Palivizumab is easier to administer and does not interfere with the measles, mumps, rubella (MMR) vaccine or varicella vaccine. RSV-IVIG, however, provides additional protection against other respiratory viral illnesses and may be preferred for children receiving replacement IVIG because of underlying immune deficiency.[40]

The role of passive immunoprophylaxis for the prevention of RSV infections in high-risk adults remains to be defined. Development of an RSV vaccine is ongoing but none is yet available.

Diagnostic microbiology

The gold standard diagnostic method is isolation of the virus from respiratory secretions, but increasingly nonculture diagnostic methods are used for RSV.

RSV was the first respiratory virus to be readily identified by means of rapid diagnostic tests based on antigen detection. Current methods for rapid detection of RSV antigen include EIA, immunoperoxidase staining, direct and indirect immunofluorescence tests and RT-PCR. Serologic diagnosis requires a convalescent-phase RSV IgG antibody titer at least four times higher than the acute-phase titer.

Clinical manifestations

The incubation period for RSV ranges from 2 to 8 days. Illness begins most frequently with rhinorrhea, sneezing and cough. When fever is present it is generally low grade. Most children experience recovery from illness after 8–15 days. During their first RSV infection, 25–40% of infected infants and young children have signs or symptoms of bronchiolitis or pneumonia and 0.5–2% require hospitalization. In contrast, hospitalization will be required in up to 25% of high-risk children such as those with a history of prematurity or chronic lung disease. RSV bronchiolitis or pneumonia should be suspected when an infant or young child presents with progressive cough, wheezing and dyspnea during the RSV season.

Radiographic findings in patients with bronchiolitis include hyperlucency, diaphragmatic flattening and outward bowing on the intercostal spaces. Findings compatible with pneumonia include interstitial infiltrates and, less frequently, consolidation, particularly in the right upper and middle lobes.

In neonates, the clinical presentation may be atypical with lethargy, irritability or poor oral intake. RSV has been detected in lung tissue of infants with sudden death syndrome, but a causal relationship has not been confirmed. Prolonged pulmonary function deficit and airway hyperreactivity have been reported following RSV infection but it remains unclear whether these abnormalities were present before the infection.[47] Otitis media has also been associated with RSV infection.

Among immunocompetent adults, RSV infection usually manifests with rhinorrhea, pharyngitis, cough, bronchitis, headache, fatigue and fever. In older persons, particularly the institutionalized elderly and those with chronic cardiopulmonary illnesses, severe pneumonia may occur, leading to respiratory failure. Among immunocompromised individuals, RSV has been the most frequently isolated viral respiratory pathogen in surveillance studies performed at large cancer centers.[48] In this setting RSV infection typically begins as a URI and may evolve into lower respiratory tract disease in up to 50% of cases. A particularly high rate of progression to pneumonia (70–80%) has been reported among adult HSCT recipients developing RSV infection within the period prior to engraftment. In such settings RSV pneumonia resulted in 60–80% mortality, regardless of whether antiviral drugs were used for treatment.

Management

For patients with mild disease, only symptomatic treatment is necessary. Children with severe disease may require hospitalization for supplemental oxygen therapy, hydration and nutrition, or for mechanical ventilation. Improvements in supportive care have made a significant impact on the mortality from RSV bronchiolitis and pneumonia. The use of bronchodilators is likely beneficial for patients with significant bronchospasm. Corticosteroids are commonly used as anti-inflammatory therapy, although there is no supportive data demonstrating the benefits of such intervention. Aerosolized ribavirin (Virasole) was approved in 1986 for the treatment of infants and children with severe RSV disease. The treatment is cumbersome to deliver and its impact on mortality remains controversial.[49,50]

A combination of immune therapy and ribavirin has been reported to be well tolerated and to improve the outcome of severe RSV infection among bone marrow transplant recipients.[51,52] In one open trial the mortality was only 22% among nine adults with pneumonia in whom therapy was initiated prior to the onset of profound respiratory failure.[52] Pre-emptive treatment strategies are being investigated primarily in immunocompromised individuals. Delay of immunosuppressive chemotherapy should be considered when severely immunosuppressed individuals, such as HSCT recipients, develop acute RSV infection.

PARAINFLUENZA VIRUS

Nature

Human parainfluenza viruses (HPIVs) were first described in the 1950s, originally as croup-associated viruses; they belong to the Paramyxoviridae family.

The virions are pleomorphic and range in average diameter from 150 to 200 nm. They are enveloped particles with a linear single-stranded, negative-sense RNA genome. The HPIVs encode two surface

glycoproteins: an attachment protein called HN (hemagglutinin-neuraminidase) that binds to sialic acid-containing receptors on host cell surfaces, and a fusion protein F that is involved in the fusion of the viral membrane with the cellular plasma membrane. The HN protein is a multifunctional molecule with binding activity (binding to sialic acid containing surface glycoproteins and glycolipids), neuraminidase activity and fusion promotion activity. The attachment activity can be measured by hemagglutination assay. The neuraminidase activity prevents aggregation of virus particles by removing sialic acid from the virion glycoproteins and facilitates infection in the respiratory tract by digesting the mucin coating of epithelial cells.

These viruses also encode three nucleocapsid associated proteins, NP, P and L, and the nonglycosylated internal protein M. The four HPIVs are segregated into two genera, the *Respirovirus* (HPIV-1 and HPIV-3) and the *Rubulavirus* (HPIV-2 and HPIV-4), based on antigenic and structural characteristics, including additional accessory proteins (V, D, W, I, X, C and SH).

Epidemiology

HPIVs are distributed worldwide. Each of the four HPIVs can cause a full spectrum of acute respiratory tract illnesses. HPIV-1 and HPIV-2 are the most common pathogens associated with croup or laryngotracheobronchitis.[53] HPIV-1, -2 and -3 are also important causes of bronchiolitis and pneumonia among infants and young children;[53,54] less is known about HPIV-4. Most children have serologic evidence of infection by 5 years of age. Most HPIV infections are detected during seasonal epidemics. In a study on an outpatient pediatric population,[55] HPIV-1 occurred in the fall of odd-numbered years, HPIV-2 was less predictable and HPIV-3 appeared yearly with peak activity in spring or summer.

Pathogenesis

Although droplet transmission can occur, contact is thought to be the primary mode of transmission of HPIV infection. This can be direct person-to-person contact or indirect through intermediate objects. Inoculation is via the nasopharyngeal or oral mucosa. The pathogenesis of the ensuing laryngotracheobronchitis and croup is not completely understood. The immune response after HPIV infection is complex and includes the production of serum-neutralizing antibodies directed towards epitopes of the HN and F proteins. The mucosal immune response plays an important role in the protection against HPIVs infection, primarily through the production of IgA antibodies. In animal model studies, $CD8^+$ T cells were critical for virus clearance.

Prevention

The CDC recommends contact precautions for the prevention of nosocomial HPIV infections. Some institutions also observe droplet isolation precautions. No specific vaccine is yet available and no drugs have been tested for prophylactic use.

Diagnostic microbiology

Definite diagnosis relies on the isolation of the virus from respiratory secretions. Other diagnostic methods include detection of viral antigens or nucleic acid, and serologic analysis. Primary isolation and identification by hemadsorption assay can be followed by typing of the virus through immunofluorescence or hemadsorption inhibition. PCR assays are more sensitive than isolation and antigen detection methods.[56]

Clinical manifestations

Infection in children is associated with an acute febrile illness in up to 80% of cases. Initial symptoms include coryza, sore throat, hoarseness and dry cough. In croup, a brassy or barking cough may progress to stridor and occasionally to airway obstruction. The anteroposterior radiograph of the neck shows glottic and subglottic narrowing ('steeple sign') which differentiates croup from epiglottitis. In cases of bronchiolitis and pneumonia, progressive cough is accompanied by wheezing, tachypnea and hypoxemia. Chest X-ray examination may reveal air trapping and interstitial infiltrates. Otitis media is a common finding in patients with HPIV infections.

In older children and adults, HPIV infections tend to be milder and present as URI. However, HPIV-1 and -3 have been diagnosed by serology in a significant number of adults admitted with acute respiratory illness.[57] A few cases of HPIV meningitis have been described.[58] Immunocompromised pediatric and adult individuals such as HSCT,[59–64] lung[65] and renal[66] transplant recipients may develop severe HPIV infections with a high rate of progression to pneumonia. The use of corticosteroids was linked with a higher risk of HPIV-3 pneumonia among HSCT recipents.[61]

Management

Symptomatic treatment of croup usually includes humidification of air by ultrasonic nebulizer and inhalations of racemic adrenaline (epinephrine). Adrenaline (epinephrine) provides rapid but transient relief of the airway obstruction. The anti-inflammatory effect of systemic corticosteroids is advocated to prevent or shorten the period of intubation in severe croup. Patients with bronchiolitis are additionally treated with bronchodilators. Ribavirin has exhibited *in-vitro* activity against HPIVs[67] but data from controlled clinical trials are lacking.

HUMAN METAPNEUMOVIRUS

Nature

In June 2001, it was reported that a new respiratory virus had been isolated from nasopharyngeal aspirates of 28 young children in the Netherlands. Based on virologic data, sequence homology and gene constellation, the virus was classified as a member of the *Metapneumovirus* genus of the Paramyxoviridae family and was named human metapneumovirus (HMPV).[68] HMPV is the first known mammalian metapneumovirus causing disease. Serologic analysis detected antibodies against the virus being present in all individuals older than 8 years of age in specimens collected since 1958.[68]

The genome of HMPV consists of negative-sense, nonsegmented RNA. Similar to other paramyxoviruses, virions are pleomorphic with a mean diameter of 200 nm. The lipid envelope, which is derived from the plasma membrane of the host cell, contains three viral glycoproteins, the attachment (G), fusion (F) and small SH proteins. The functions of these proteins are similar to those of their counterparts found in other paramyxoviruses (see above). There are two major genotypes of HMPV, A and B.[69] Both of these major genetic lineages are further divided into two genetic subgroups termed A1 and A2 and B1 and B2. Current evidence suggests that the two genetic lineages are not distinct antigenic subtypes.[70] HMPV replicates slowly in cell culture with characteristic syncytia formation

Epidemiology

HMPV infections have been diagnosed worldwide in patients of all age groups with respiratory disease. In temperate climates the virus is predominantly transmitted in late winter and spring, whereas in the subtropics infections culminate in spring/summer. Infection rates peak at the same time or shortly after the RSV infection peak.[71] HPMV has been associated with upper and lower respiratory tract infections in young children and is the second most frequently isolated viral pathogen after RSV to cause bronchiolitis in early childhood.[72–75]

Pathogenesis

Transmission most likely occurs in a fashion similar to RSV via droplets and contaminated fomites; nosocomial transmission has been reported.[76] Human respiratory infection was associated with an increase of IL-8 in respiratory secretions and with chronic inflammatory changes in the airways. Levels of inflammatory cytokines (IL-12, tumor necrosis factor, IL-6, IL-1β) found in HMPV disease were lower than levels observed with RSV infection.[77]

Prevention

Both droplet precautions and direct contact precautions should be observed to prevent transmission in the health-care setting.

Diagnostic microbiology

The virus is difficult to grow in cell culture. Most strains show cytopathic effect in LLC-MK2 (tertiary monkey kidney) cells; recently a Vero cell clone was reported as being permissible for all four viral lineages.[70] Cytopathic effects usually occur after 10–21 days and vary from syncytia formation to rounding of cells. Direct immunofluorescence assays with commercially produced virus-specific antibodies are available, although they are probably less sensitive than RT-PCR for the detection of virus. Molecular methods have become the method of choice for the detection of the HMPV genome. Real-time RT-PCR targeting the N and/or L gene has been found to be sensitive, specific and rapid in detecting the virus in clinical specimens.[78] Serologic diagnosis can be achieved by ELISA methods demonstrating a fourfold increase in antibody titers or seroconversion.

Clinical manifestations

HMPV has been associated with respiratory tract infections in all age groups: severe lower respiratory tract infections have been observed in very young children, the elderly and immunocompromised patients. HMPV infection in adults usually presents with flu-like symptoms such as rhinorrhea, sore throat and cough. Children are at higher risk for the development of lower airway disease (bronchiolitis, pneumonia), especially infants younger than 2 years of age.[79] The severity varies from self-limiting mild respiratory illness to respiratory failure requiring mechanical ventilation. Clinical disease associated with HMPV is similar to RSV disease and 12% of outpatients with acute lower respiratory tract infections were harboring HMPV which was second only to RSV.[72] Heikkinen *et al.* reported that 60% of children less than 3 years of age developed acute otitis media due to HMPV infection.[79]

Management

Several vaccine candidates have shown promising results in animal models but their safety and efficacy in humans remains to be determined. Antiviral treatment strategies for severe disease are derived from experiences obtained with RSV infections. Ribavirin shows equivalent antiviral potential against HMPV to that observed with RSV[80] and an HMPV-neutralizing monoclonal antibody for prophylaxis in high-risk infants (similar to palivizumab in RSV) is likely to be developed.

HUMAN BOCAVIRUS

Human bocavirus (HBoV) was identified in 2005 by random amplification and cloning of DNA followed by large scale sequencing and bioinformatic analysis.[81] By sequence analysis it has been provisionally classified as a parvovirus. Since then multiple studies have confirmed the worldwide presence of HBoV in the respiratory tract of children, usually under 2 years of age.[82] HBoV is often present as a co-infection with other respiratory viruses (such as RSV) raising the issue of disease association.

Determination of the pathogenicity of HBoV is complicated by the lack of an *in-vitro* culture system and a suitable animal model. High viral loads in respiratory specimens and detection of viral DNA in blood seem to be associated with respiratory tract symptoms, whereas the presence of low viral loads is of unclear significance.[83,84] The first serologic studies analyzing the antibody response in humans against HBoV structural proteins have now been published.[85,86] Antibodies against HBoV were detected in 71.1% of serum samples collected from people from infancy to 41 years in Japan.[85] High viral load in the nasopharynx and viremia correlated with a serologic diagnosis of primary infection (IgM antibodies and/or an increase in IgG antibody level).[86] Further studies are needed to clarify the pathogenesis and the natural course of infection of HBoV.

CONCLUSION

A better understanding of the profound impact that human respiratory viruses have on human morbidity and mortality has resulted in more resources being devoted to their study. More efficient diagnostic tools, new antiviral agents, the use of passive immunoprophylaxis, improvements in vaccination programs and in strategies to control the nosocomial transmission of these infections are among the most significant accomplishments.

Acknowledgment

Illustrations are contributed by Joseph Yarsa BS, MT(ASCP) and Xiang-Yang Han MD, PhD from the Microbiology Laboratory at The University of Texas MD Anderson Cancer Center, Houston, Texas, USA.

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Chapter 163

Charles AB Boucher

Retroviruses and retroviral infections

INTRODUCTION

The Retroviridae constitute a large family of viruses that predominantly infect both human and animal vertebrates. Retroviral infections can cause a wide spectrum of diseases ranging from malignancies to immune deficiencies and neurologic disorders. However, most retroviral infections occur without any detectable deleterious effect to the host. In spite of this diversity of associated pathology and the broad range of hosts, all retroviruses share a similar virion structure and genome organization and a common mode of replication.

This chapter discusses the general features of human retroviruses, such as virion structure and genome organization, as well as the procedures used in the laboratory for diagnosis and treatment evaluation. During the past decades, the AIDS pandemic has resulted in an upsurge of scientific interest in retroviral infections and related pathology in humans. Several human retroviruses have been identified, of which only the human immunodeficiency viruses (HIVs) and the human T-lymphocyte leukemia viruses (HTLVs) are known to cause clinical manifestations and are discussed in this chapter.

NATURE

The electron microscopic view of retroviral particles shows spherical particles approximately 100 nm in diameter with an electron-dense core (Fig. 163.1). Retroviruses are enveloped viruses (Fig. 163.2).

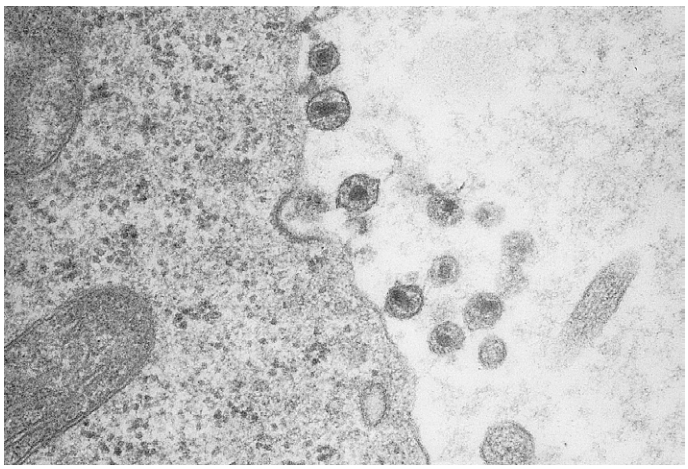


Fig. 163.1 Typical electron microscopic view of retroviruses (HIV) showing an electron-dense core. Courtesy of Dr Piet Joling.

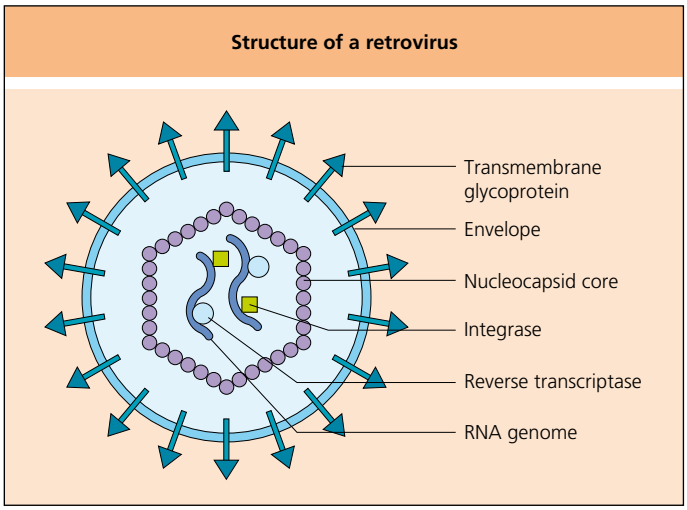


Fig. 163.2 Structure of a retrovirus.

The envelope consists of a phospholipid double layer derived from the plasma membrane of the host cell. Viral-encoded transmembrane glycoproteins are inserted into the envelope, enabling the virus to attach to the host cell. The envelope surrounds the nucleocapsid core, which contains the viral genome: two identical RNA molecules, having the same polarity as mRNA, with which an RNA-dependent DNA polymerase enzyme, reverse transcriptase (RT), is closely associated. Also present in the core is the integrase enzyme.

The genome of retroviruses is approximately 10 000 base pairs (bp) in length. Each end of the genome consists of repeating nucleotide sequences of 4–6 bp, called the long terminal repeats (LTRs). The LTRs flank the three genomic regions that encode for sets of structural genes:

- the group antigen (*gag*) region, which codes for the core antigens;
- the polymerase (*pol*) region, which codes for the enzymes protease, RT and integrase; and
- the envelope (*env*) region, which codes for glycoproteins of the envelope.

The structural genes always appear in the same order: *gag-pol-env* (Fig. 163.3).[1] The genomes of so-called simple retroviruses only contain a *gag*, *pol* and *env* gene. There are several retroviruses that contain additional genes that encode for viral proteins involved in the regulation of several processes in the replication cycle of the virus, such as the rate of transcription, the splicing of mRNA, the transport of mRNA from the nucleus to the cytoplasm and the release of progeny virus. Retroviruses that contain additional genes are called complex retroviruses; examples of these are the HIVs and the HTLVs.

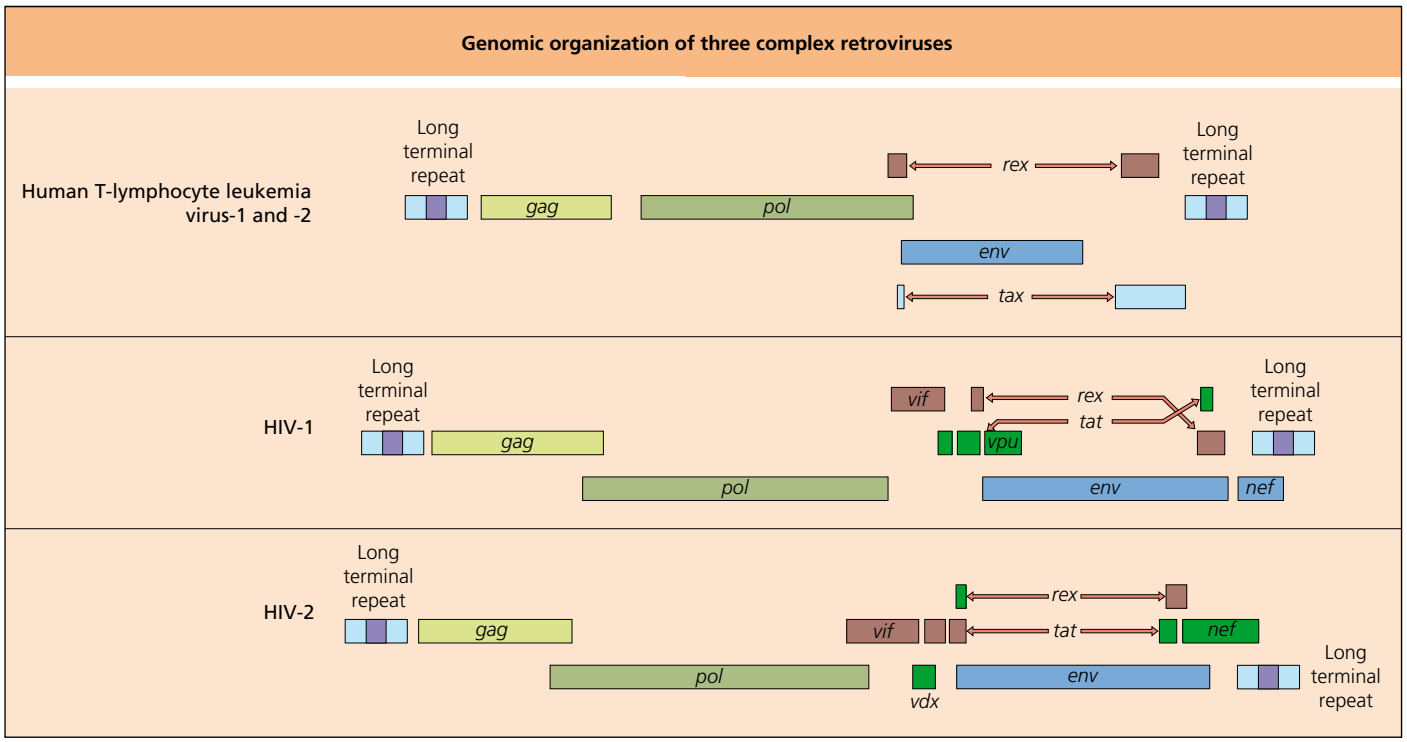


Fig. 163.3 Genomic organization of three complex retroviruses. The diagram shows HIV-1, HIV-2 and HTLV. The components of the long terminal repeats are shaded. The open reading frames that encode viral proteins are depicted by unshaded boxes. Adapted from Galasso *et al.*[1]

The first step in the replication cycle (Fig. 163.4) of retroviruses is attachment of the virus to a host cell through interaction of the envelope glycoprotein with a receptor molecule on the host cell surface. This interaction between virus and host cell largely determines the host range of the virus (i.e. which species or cell type the virus can infect). After attachment, fusion of the envelope membrane with the host cell membrane takes place and the nucleocapsid core is released into the cell. However, some retroviruses enter the cell by receptor-mediated endocytosis followed by fusion of the viral envelope with the endosomal membrane.[2]

Following the entry of the nucleocapsid core into the cytoplasm of the host cell, the viral RNA is transcribed into double-stranded DNA (dsDNA). This process is catalyzed by the viral enzyme RT. The viral dsDNA, in association with RT and the viral enzyme integrase, is transferred to the nucleus. Subsequently the dsDNA is integrated into the chromosomal DNA of the cell by the action of the viral enzyme integrase. Once integrated the viral DNA copy is called a provirus.

Using the proviral DNA as template, cellular DNA-dependent RNA polymerases synthesize viral mRNA molecules. Some of them are spliced and transported to the cytoplasm, where they are translated and yield full-length viral precursor polyproteins. These polyproteins, combined with two copies of genomic viral RNA, are then assembled into immature virus particles. By budding through the plasma membrane the immature particles acquire a bilipid layer envelope. Maturation into infectious and replication-competent virions is accomplished after release of the immature particles from the host cell; this process requires processing of the precursor polyproteins by the viral-encoded protease. Then, a new cycle of replication may start.

A particular feature of retroviruses is that the retroviral DNA becomes integrated in the host chromosomal DNA and can be inherited into the progeny of the infected cell. When retroviruses infect germline cells (sperm or egg cells) and the viral DNA is integrated into the chromosomal DNA of these cells, the retrovirus is passed from parent to offspring of the infected host. Such a virus is called an endogenous retrovirus. Most vertebrates carry the remnants of retroviral DNA in their chromosomes as a result of common ancestors who were infected with endogenous retroviruses.

Retroviruses that are not integrated in germline cells of their host are called exogenous retroviruses. The transmission route of exogenous retroviruses is both horizontally and vertically via blood and saliva and by sexual intercourse.

HUMAN ENDOGENOUS RETROVIRUSES

Approximately 1% of the human genome consists of sequences of human endogenous retroviruses (HERVs).[3] Full-length HERVs contain sequences that are homologous to the *gag*, *pol* and *env* genes of infectious exogenous retroviruses. The biologic properties and functions of endogenous retroviruses are still under investigation.

Nine different HERV families have been described, some of which have as many as 50 copies in the human genome.[3] In general, HERVs are defective proviruses.[3,4] Expression of HERV mRNA and HERV proteins has been demonstrated in several normal and pathologic tissues, and HERV retroviral particles have been detected by electron microscopy in these tissues.

Because it was demonstrated that HERV mRNA and proteins can be expressed, it has been postulated that HERVs might be etiologically involved in human disease. The possible role of HERVs in human disease has been reviewed,[4] but more research is required to elucidate the general role of HERVs and their specific role in human disease.

Human Foamy Virus

In 1971, a human foamy virus (HFV) was the first human exogenous retrovirus to be successfully isolated from human tissue.[5] Human foamy virus is a member of the family of Spuma retroviruses. A clear relationship between HFV and human disease has never been established. These viruses have been associated with a variety of diseases, such as subacute

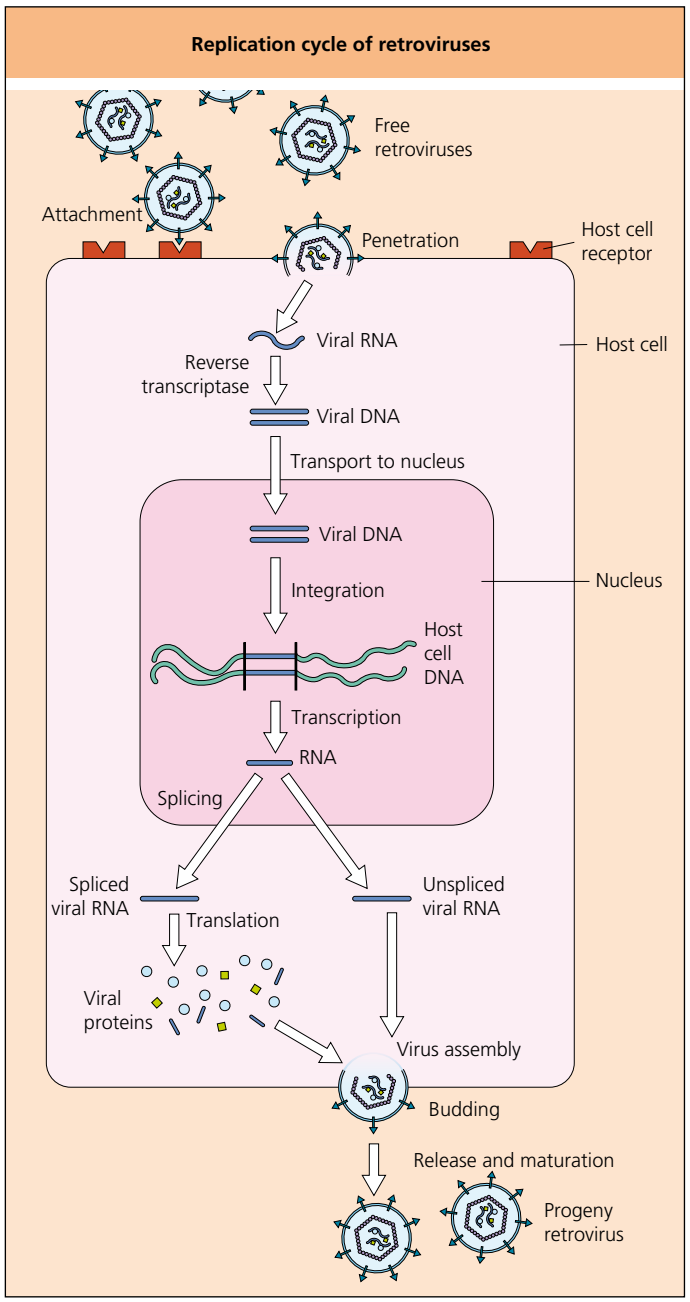


Fig. 163.4 Replication cycle of retroviruses.

thyroiditis, chronic fatigue syndrome, hemodialysis encephalopathy, multiple sclerosis, Guillain–Barré syndrome, amyotrophic lateral sclerosis and Kawasaki's disease. However, no definitive evidence has been found to support involvement of HFV in any of them.

Human T-Lymphocyte Leukemia Viruses

NATURE

Human T-lymphocyte leukemia virus-1 and HTLV-2 are complex retroviruses. In addition to a *gag*, *pol* and *env* gene, they contain four open reading frames in the pX region; these open reading frames have regulatory and accessory functions.[6,7] Two of these proteins, *rex* and *tax* (see Fig. 163.3), play an important role in regulation of viral replication. The *rex* protein is involved in the stabilization of viral mRNA and the transport of viral mRNA from nucleus to cytoplasm. The *rex* protein also regulates the splicing and processing of viral mRNA. The *tax* protein is a transactivator protein; by acting on the LTR located 5′ to the viral *gag* gene, *tax* induces the transcription of viral mRNA and is also believed to play a role in oncogenesis.

EPIDEMIOLOGY

Seroepidemiologic studies have shown that the highest incidence of anti-HTLV-1 antibodies is found in south-western Japan, ranging from 5% to 35% in endemic areas. Other areas with high incidence are the Caribbean islands, some regions of South and Central America, the south-west Pacific and Papua New Guinea.[2] The general incidence in the USA and Europe is low (0.05%), although an increasing incidence has been reported among homosexuals and intravenous drug users.

The epidemiology of HTLV-2 has been less well studied. A high incidence of HTLV-2 has been found among native Americans in Panama and New Mexico and among intravenous drug users in the USA and Italy.

PATHOGENESIS

HTLV-1 is transmitted by infected lymphocytes and not as free virus in cell-free body fluids. Three transmission routes for HTLV-1 have been described.

- transmission of HTLV-infected lymphocytes via the placenta, during birth or after birth through the mother's milk;
- transmission from male to female during sexual intercourse; and
- blood transfusion.

Human T-lymphocyte leukemia virus-1 is a lymphotropic virus that preferentially infects $CD4^+$ T cells. Usually, the leukemic cells in adult T-lymphocyte leukemia (ATL) have the following phenotype: $CD2^+$, $CD3^+$, $CD5^+$, $CD7^-$, $CD4^+$ and $CD8^-$; they also express the activation surface markers CD38 (the interleukin (IL)-2 receptor), CD30 and major histocompatibility complex class II.

The molecular mechanisms by which HTLV-1 is able to induce cell transformation have not been unraveled. Human T-lymphocyte leukemia virus does not contain a typical oncogene. Several hypothetical mechanisms of HTLV-induced oncogenesis have been formulated. However, these hypotheses do not explain why it takes an average incubation time of 20–30 years to develop ATL or why only a small number of HTLV-1-infected people develop clinical manifestations of ATL.

DIAGNOSTIC MICROBIOLOGY

Infection with HTLV-1 and HTLV-2 can be diagnosed using serologic assays (enzyme-linked immunosorbent assays and rapid tests) to detect antibodies against the virus. Confirmation and distinction between HTLV-1 and HTLV-2 can be done through polymerase chain reaction (PCR) detection of proviral DNA in lymphocytes.

CLINICAL FEATURES

Human T-lymphocyte leukemia virus-1

Adult T-lymphocyte leukemia

Several pieces of evidence established the causal relationship between HTLV-1 and ATL:[8]

- ATL has an identical geographic distribution to that of HTLV-1, having a high incidence in south-western Japan, as was shown in seroepidemiologic studies.

- All ATL tumor cells contain one or more copies of the HTLV-1 provirus in their genomic DNA.
- *In-vitro* infection of human T cells with HTLV-1 results in T-cell immortalization.
- HTLV-1 has been demonstrated to be oncogenic in animals.

The lifetime chance of an infected person developing ATL is very low (about 1%). The first clinical manifestations of ATL generally occur 20–30 years after infection with HTLV-1.[9] The median age of ATL onset is 52.7 years.

Tropical spastic paresis

Infections with HTLV-1 have also been associated with a neurologic syndrome affecting the pyrimidal tract called tropical spastic paresis or HTLV-1-associated myelopathy (HAM). This disorder is characterized by a slowly progressive symmetric myelopathy combined with high titers of antibodies to HTLV-1 in plasma and cerebrospinal fluid (CSF). The myelopathy primarily affects the pyramidal tract. The mechanism by which HTLV-1 infection causes HAM is unclear.

Ophthalmologic complications and arthopathy

Human T-lymphocyte leukemia virus-1 infections have also been associated with ophthalmologic complications and arthropathy.[2]

Human T-lymphocyte leukemia virus-2

Human T-lymphocyte leukemia virus-2 infections have been associated with T-cell malignancies, predominantly hairy T-lymphocytic leukemia and chronic lymphatic leukemia. Neurologic complications similar to those seen in tropical spastic paresis have been described.

Human Immunodeficiency Viruses

NATURE

The human immunodeficiency viruses HIV-1 and HIV-2 are members of the family of Lentiviruses. Both HIV-1 and HIV-2 are the causative agents of AIDS. For detailed information regarding the clinical manifestations of HIV infection and AIDS, see Chapters 84–99; antiretroviral therapy is discussed in Chapters 100 and 145. This chapter describes viral structure, replication, epidemiology and diagnostic procedures.

Virion structure

The electron microscopic view of human immunodeficiency retroviruses shows an electron-dense, cone-shaped core[10] (see Fig. 163.1). The viral p24 capsid protein functions as a shell for the condensed core, which contains the two identical genomic RNA strands in close association with RT (Fig. 163.5). The viral genome is approximately 9200 bases long and contains nine genes[11] (see Fig. 163.3). The viral proteins Vpr (in HIV-1) and Vpx (in HIV-2) and the p6 protein are found in the virion and outside the core. The Vif protein may also be present in the viral particle in close association with the viral core. The inner part of the viral membrane is covered with a myristylated p17 core protein, which provides the matrix for the viral structure. Glycoprotein spikes project from the virion surface and are anchored into an envelope that consists of a phospholipid bilayer derived from the host cell membrane. The spikes contain two viral glycoproteins (gp), gp120 and gp41. The gp41 acts as a transmembrane protein and its central portion binds to the external surface gp120. In addition to gp41 and gp120, the viral envelope can contain various proteins derived from the host cell.

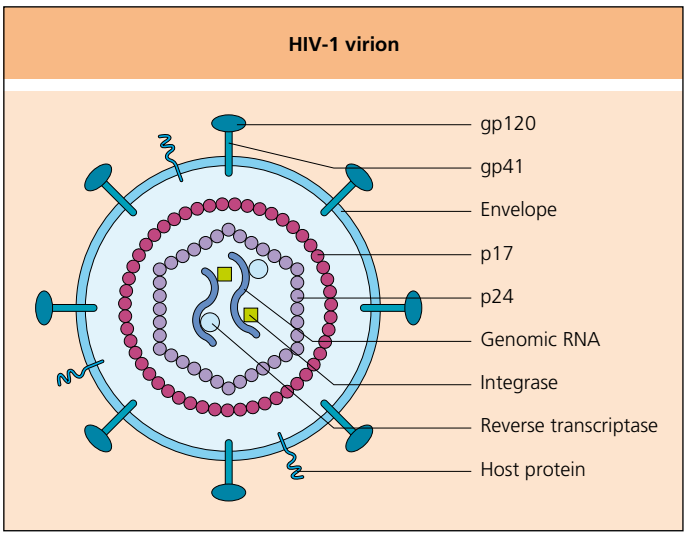


Fig. 163.5 HIV-1 virion.

Replication cycle (see Fig. 163.4)

Infection starts with the binding of gp120 to the cellular surface CD4 molecule, which acts as a high-affinity receptor. Two secondary or co-receptors can be used: CXCR4 (also known as fusin) and CCR5.[12] These co-receptors are members of a cellular transmembrane chemokine receptor family. All HIV strains tested to date can use either CXCR4 or CCR5, or both (dual tropic). However, several other co-receptors for HIV have also been identified. Co-receptor usage determines the cell tropism of the HIV strain. The CCR5 co-receptor is used by HIV strains that *in vitro* do not cause cell fusion (syncytia) in T-cell lines (non-syncytium-inducing (NSI) strains). Syncytium-inducing (SI) HIV variants use both co-receptors CXCR4 and CCR5. In general, NSI (CCR5) variants are found throughout the complete course of HIV infection, whereas SI (CXCR4) variants emerge only in a later stage of HIV infection. The appearance of SI (CXCR4) variants in a patient is associated with a more rapid CD4 decline and faster disease progression (see also Chapter 88).

After binding, HIV enters the cell through fusion of the viral and host cell membranes. Subsequently the viral core, containing the genome, is released in the cytoplasm of the cell; this involves a process of internalization and uncoating.

During the replication cycle, the first synthesized transcription products are the viral regulatory proteins *nef*, *tat* and *rev* (for a review see Greene *et al.*[13]). The *tat* protein will bind to the transactive response element region on the proviral DNA and, as a consequence, high-level expression of all viral genes occurs. The *nef* protein is required for high-level viral replication *in vivo* and it decreases CD4 on the cell membrane. The *nef* protein may also enhance the efficiency of reverse transcription.

Through the action of *rev* a switch may occur toward the synthesis of late regulatory and viral structural proteins. The *rev* protein facilitates the nuclear transport of unspliced and incompletely spliced RNA transcripts that contain the *rev*-responsive element, located in the *env* gene. In addition it promotes translation of messages containing the *rev*-responsive element. It remains unclear to what extent the relative amounts of each of these regulatory proteins and their interaction with cellular factors can determine whether the infection of a cell results in a productive cycle or merely a viral latent state.

The *gag* and the *pol* open reading frames (ORFs) overlap. The *pol* ORF is positioned in the –1 frame relative to the *gag* ORF. In the unspliced, full-length mRNA, both reading frames are present. Translation starts at the beginning of the *gag* ORF and continues in most cases until the end of the ORF, and a 55 kDa *gag* precursor protein (p55), containing p17, p24, p7, p2, p1 and p6, is made. During

translation of full-length mRNA, approximately 5–10% of the ribosomes slip back one nucleotide (frame shift) and gain access to the *pol* ORF, and translation continues. A partial *gag* precursor protein is fused to the *pol* polyprotein, resulting in a *gag-pol* polyprotein. The *pol* gene encodes for three proteins: protease, RT and integrase. Cleavage of the *gag-pol* protein generates protease, RT and integrase. The 99-amino acid protease is coded for at the 5′ end of the *pol* gene. HIV protease is active only as homodimer, which is generated by an autolytic process. It is possible that aggregation of two *gag-pol* proteins is required to generate the active form, thereby limiting activation to the budding site. Both *gag* and *gag-pol* polyproteins are post-translationally modified by a covalent attachment of a lipophilic myristyl group onto their aminoterminal glycine by a host cell-derived myristyl transferase. Myristylation may be required for intracellular transport to the cell membrane because, in the absence of myristylation, no infectious particles are produced.

The *gag* precursor protein is cleaved by viral protease into four structural proteins (p17, p24, p7 and p6) and two smaller proteins (p2 and p1). The p2 protein may be important for proper assembly of the virions. The *gag* precursor protein may also play a role in the packaging of viral RNA into the virus particle, specifically through the action of the nucleic acid binding protein p7. This protein binds to a sequence at the 5′ end of the viral genome, possibly through the interaction of so-called cystine-histidine motifs. The p6 protein has been shown to be rich in proline, and through binding to p6, Vpr is incorporated in the viral particle. Viruses with a mutation in p6 are not released from the cell surface.

The accessory genes *vif*, *vpr* and *vpu*/*vpx* influence the viral assembly and budding process, and alterations in these genes lead to a decrease in infectivity. These proteins may be more important for replication in macrophages and primary lymphocytes than for T-cell lines. The protein Vpr can cause cell cycle arrest in the G2 phase, and it is as yet unclear whether this phenomenon plays a role in viral replication. The protein Vpu interacts with CD4 and may cause CD4 degradation.[2] The Vif protein is considered to enhance infectivity.

TRANSMISSION AND EPIDEMIOLOGY

A large variety of nonhuman primates in Africa have been reported to be infected with the retrovirus simian immunodeficiency virus (SIV). Two of these viruses are the cause of AIDS in humans. It has been estimated that they have been transmitted to humans on at least seven different occasions.[14]

Transmission

The three major routes of transmission of HIV are by sexual contact or blood or blood products or vertically through maternal-fetal transmission. The first step in infection is binding of the viral particles to submucosal dendritic cells. These cells, which normally process antigens and present them to immune cells, express a protein termed DC-SIGN. The viral gp120 binds to DC-SIGN with high affinity; subsequently the viral particles are internalized and presented again at the cell surface after the dendritic cells have migrated to the regional lymph tissues and present viral particles to the T cells (see Chapter 88).

The amount of infectious HIV in the inoculating material is the most important factor determining the risk of transmission. The amount of virus is, at least in the blood of the donor, determined by disease stage. The relation between the amount of HIV in semen and disease stage has not been fully explored. Individuals with a primary infection are extremely infectious, most likely because they have a very high amount of virus in their body fluids; given the recent infection most of the circulating viruses are closely related to the inoculating strain and therefore infectious themselves.

The risk of heterosexual transmission correlates to the HIV RNA concentration in the plasma of the positive partner and the stage of disease.[15,16] The rate of transmission may also be determined by biologic properties of the virus and the integrity of mucocutaneous membranes of the urogenital tract. Among heterosexual couples the risk of transmission increases when the negative partner has a genital infection.[16]

Studies addressing transmission from mother to baby have also shown that the risk of vertical transmission increases with a higher viral load in the mother at the time of delivery (as measured as by HIV p24 antigen, HIV-1 RNA, proviral DNA or peripheral blood mononuclear cell viral titer) and with lower $CD4^+$ lymphocyte counts.[17] The current opinion is that the majority of infections of the child occur late during pregnancy and most commonly during delivery, because elective Caesarian section and antiretroviral treatment of the mother around the time of delivery lowers the transmission rate significantly.[17–19]

The global HIV-1 epidemic is seen as a composite of infections from at least nine genetically distinct subtypes or clades of virus, designated A to H.[20] Together they form the M group. Subtypes can be distinguished on the basis of sequence information (*env* and *gag* genes and RT). Two isolates that are highly divergent from group M have been fully sequenced. The results show that viruses from the new group O are almost as close to HIV-2 as to HIV-1. The distribution of certain HIV-1 subtypes is still geographically limited, but geographic dispersion will contribute to the growing pandemic (see Chapters 84 and 96). The distribution of subtypes within countries as well as throughout the world shows that there is no evidence for host specificity for certain subtypes. Dual infection with two subtypes and hybrids between two subtypes have been described in infected individuals.

Five distinct subgroups have been identified for HIV-2; in analogy with HIV-1 these were designated A to E. The observed viral variation has clear implications not only for vaccine development but also for drug development and treatment of patients.

The non-nucleoside reverse transcriptase inhibitors (NNRTIs) inhibit RT activity through binding various amino acids of RT, the most important amino acid being the tyrosine residue at codon 181 for HIV-1 RT. Selection of drug-resistant HIV-1 isolates both *in vitro* and *in vivo* results in a substitution at codon 181 of a cysteine. The cysteine substitution gives a considerable increase in the 50% inhibitory concentration for all NNRTIs. Sequence analysis of several HIV-2 RT genes has shown that at codon 181 the cysteine is normally present. The presence of the cysteine variant makes HIV-2 viruses naturally resistant to the NNRTIs.

The *env*-based classification of widely divergent HIV-1 strains into sequence subtypes is also supported by nucleotide sequence analysis of the *gag* genes. The distinction between SI and NSI viruses, conferred by gp120 described in clade B, is also present among other HIV-1 subtypes. Detailed information is available on the prevalence of the different subtypes for certain areas in the world. Information on the prevalence of different clades in a region may give interesting information on the spread of the infection and the possible transmission routes. For instance, in Thailand it has been shown that type A is mainly spreading through sexual intercourse, whereas type B is transmitted among intravenous drug users. Monitoring the prevalence of different clades around the world and searching for possible new clades is important for the use and development of diagnostic procedures. For instance, some screening assays for HIV antibodies have been shown to be relatively insensitive for clade O viruses. The use of diagnostic PCR procedures, especially in children born to mothers who have HIV infection, may be complicated by sequence variation in the region where the primers have to anneal to their substrate.

DEVELOPMENT OF RESISTANCE DUE TO ANTIRETROVIRAL THERAPY (SEE ALSO CHAPTERS 99 AND 100)

After the introduction of (partial active) monotherapy in the mid-1980s, drug-resistant viruses were selected in most treated patients. Initially a large pool of patients who failed therapy harbored nucleoside-resistant viruses. Some of these patients infected other individuals

with these drug-resistant viruses. After the first report, it became apparant that transmission of resistant viruses was a relatively frequent event, occurring at 10–20% in the USA and Europe.[21–23] Subsequent introduction of the NNRTIs and the first generation of protease inhibitors (PIs) in these nucleoside analogue failing patient populations did not result in complete virus suppression in most patients; indeed, a second wave of viruses now also resistant to the NNRTIs and/or PIs were selected. These viruses were also transmitted, albeit with a lower frequency.

With the introduction of second-generation boosted PIs and the availability of novel classes of fusion inhibitors and integrase inhibitors it became possible to finally fully suppress virus replication in those patients previously failing. Moreover, these novel highly active retroviral therapy (HAART) regimens, when used as first-line regimens, rarely select for resistance virus. These developments will most likely significantly reduce the future rate of transmission of drug-resistant virus in the USA and Europe.

DIAGNOSTIC TOOLS FOR HIV

Serologic assays

Assays have been developed for detection of HIV antibodies in serum, whole blood, saliva, urine and dried blood collected on filter paper.

Enzyme-linked immunosorbent assay

Current antigen sandwich enzyme-linked immunosorbent assays (ELISAs) have a sensitivity and specificity that approach 100%. In contrast to earlier tests, which detected only IgG antibodies, third-generation HIV ELISAs detect all classes of anti-HIV antibodies, considerably shortening the time to diagnosis following acute infection. However, these assays may fail to detect antibodies to the highly divergent HIV-1 subtypes from groups N and O.

Circulating HIV-1 capsid (p24) antigen can be detected by qualitative or quantitative antigen-capture ELISA. Qualitative assays for p24 antigen are useful in diagnosing HIV-1 infection prior to seroconversion, but quantitative assays have been replaced by more sensitive viral RNA assays. The newer generations of ELISA combine detection of antibodies with the detection of antigen in the plasma. These ELISAs have shortened the window between the moment of infection and the first positive ELISA considerably because samples obtained early after infection (a couple of days) contain unbound HIV antigen while antibodies at that stage may not be present.

Rapid tests

Diagnostic tests based on red cell or particle agglutination as well as dot-blot assays have been developed that permit rapid diagnosis of HIV-1 infection. In laboratory comparisons, sensitivity and specificity are similar to those of the ELISA, but performance may be somewhat lower in the field. The simplicity and wide operating temperature of these tests make them suitable for use in resource-poor settings, for women during labor and delivery, and for source patients following an occupational needlestick injury.

Western blot

Despite a specificity of 99%, a reactive HIV ELISA has a relatively low positive predictive value when applied to populations at low risk for HIV infection. Thus, a confirmatory test is essential to exclude false-positive results. The Western blot (WB) method is currently the most widely used. In a WB, viral proteins are separated by polyacrylamide gel electrophoresis and transferred by blotting onto a nitrocellulose strip. The strips are then reacted with the test serum to determine which, if any, viral proteins are recognized by patient antibodies. Figure 163.6 shows a typical WB positive for HIV-1, with the different

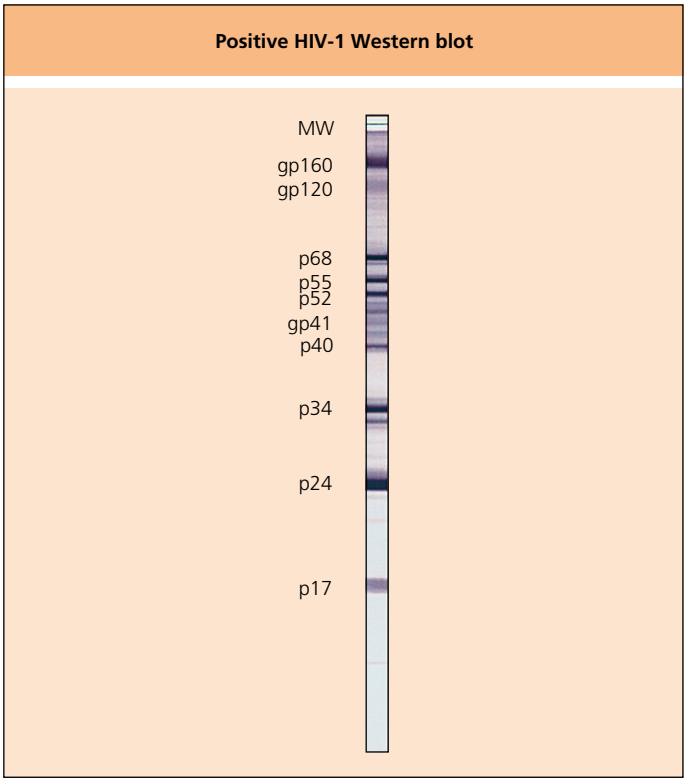


Fig. 163.6 Positive HIV-1 Western blot. The binding of the patient's antibodies to viral antigens coated on the strip is revealed by an enzyme-labeled antihuman globulin. gp160, gp120 and gp41 are *env* gene products. p55, p24 and p17 are *gag* gene products. p68, p52 and p34 are *pol* gene products. MW, molecular weight of the viral proteins.

reactive proteins, and in Figure 163.7 the pattern as observed in an HIV-1 seroconverter is shown. Serologic differentiation between HIV-1 and HIV-2 can be done using detection of specific antibodies against the HIV-2 transmembrane protein (gp36).

HIV isolation

HIV can be isolated from blood, plasma, CSF, genital secretions or tissue by co-culture with lymphocytes from a seronegative donor that

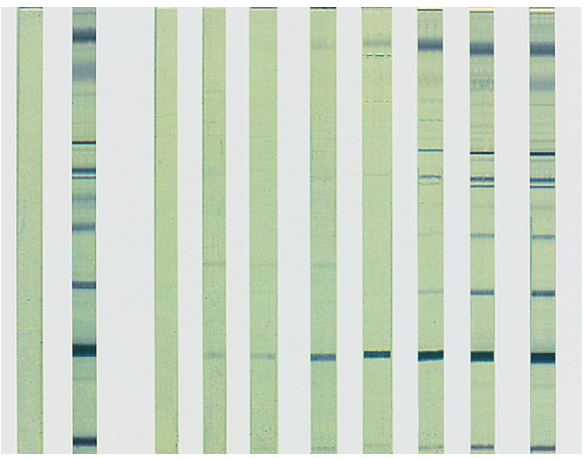


Fig. 163.7 Western blot reactivity in one HIV-1 seroconverter. Lanes 1 and 2, negative (NC) and positive controls (PC). Lanes 3–10, serial samples collected on days (D) 0, 2, 3, 5, 7, 12, 22 and 30. Day 0 corresponds to the first collected sample. Anti-p24 was the first antibody detected, rapidly followed by anti-gp160, p55, p40 and gp120. Later, gp41 and p18 are weakly reactive.

have been stimulated with phytohemagglutinin and interleukin-2 before use. Virus isolation is successful in more than 95% of individuals infected with HIV-1, but sensitivity is lower in patients with higher CD4 cell counts

Viral nucleic acid detection

HIV-1 DNA polymerase chain reaction

HIV-1 DNA PCR assays are used almost exclusively for early diagnosis of infection in neonates. These tests have limited utility in adults, but occasionally may be useful in resolving cases in which results of serologic tests are ambiguous.

Quantitative HIV-1 RNA assays

Use of these assays is now standard in the management of HIV-1-infected patients in the developed world. Several different assay formats have been developed for HIV-1 RNA quantification. In PCR-based assays HIV-1 RNA is converted into DNA by reverse transcription followed by PCR amplification of the DNA. The PCR product is detected by hybridization to an enzyme-conjugated probe specific for HIV-1, and quantified by reacting bound probe with a substrate that undergoes a color change, as in an ELISA. Branched DNA assay uses nonenzymatic means to amplify the signal from HIV RNA. In this assay, viral RNA is 'captured' by hybridization to complementary oligonucleotides that are bound to the wells of a microtiter plate. The captured viral RNA target is then hybridized to branched oligonucleotides (hence the name 'branched' DNA assay), which in turn are hybridized to enzyme-conjugated oligonucleotides that can be quantified as above. Performance characteristics of most commercially available assays are similar. Most assays have a lower limit of quantification of approximately 50–80 copies/ml. Assay sensitivity can be extended by concentrating plasma virus from larger sample volumes. Although detection limits of 3–5 copies/ml are achievable by such means, these assays are much less precise at plasma HIV-1 RNA titers below 200 copies/ml. Once infection becomes established, steady-state plasma virus levels are relatively stable, varying by 0.3–0.4 $\log_{10}$ copies/ml over weeks to months. Given these factors, changes of greater than 0.5–0.7 $\log_{10}$ (three- to fivefold) are likely to reflect significant changes in HIV-1 replication.[24]

Plasma HIV-1 RNA levels that appear to be lower than expected in a patient with advanced disease can be a clue to infection with a nonsubtype B strain. Incorporation of alternative primer sets in the latest versions of the HIV-1 molecular quantitative assays has improved the ability of these assays to detect diverse HIV-1 subtypes. The kinetics of viral markers as they occur during primary infection are shown in Figure 163.8.

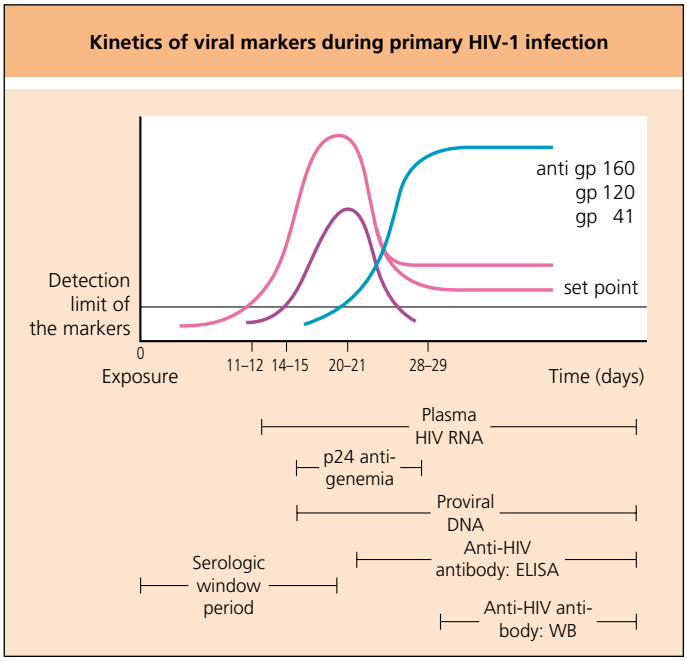


Fig. 163.8 Kinetics of viral markers during primary HIV-1 infection. The first positive viral marker is plasma RNA 11–12 days after infection. p24 Antigenemia is detectable on day 14 or 15. The first anti-HIV antibodies are detectable by third-generation ELISAs on days 20–21. (Pink, plasma HIV RNA; purple, p24 antigenemia; blue, anti-HIV antibody.)

Drug resistance assays

A variety of assays are available for assessing drug resistance which now has become routine practice in Europe and the USA[25,26] based on the results of clinical studies[27–29] including:

- genotypic assays, in which nucleotide sequencing of viral genetic material is used to detect the presence or absence of critical drug resistance mutations; and
- phenotypic assays, in which the concentration of drug necessary to inhibit virus replication *in vitro* is estimated in a drug susceptibility assay.

Each method has potential advantages and disadvantages. An important limitation of both approaches is that they provide a measure of the characteristics of the predominant viral species but do not indicate the presence of minor species that may emerge as resistant variants during subsequent treatment (Table 163.1).

Table 163.1 Comparison of genotypic and phenotypic drug resistance tests for HIV

Type of assay	Advantages	Disadvantages
Genotypic assays	Rapid turn-around time Appearance of resistance mutations may precede change in phenotype Widely available	Genotype may not correlate with phenotype Require expert/database interpretation Fail to detect minor species Unable to assess mutational interaction
Phenotypic assays	Direct measure of viral drug susceptibility Assess net effect of mutational interactions and cross-resistance patterns	Cost Longer turn-around time Fail to detect minor species

Genotypic tests of HIV-1 drug resistance

Several approaches to genotyping are available, ranging from full-length sequencing of the target gene to point mutation assays, which focus only on a particular mutation of interest. The most commonly used genotypic assays rely on automated DNA sequencing. Using this technique, the nucleotide sequence of some or all of the gene of interest (e.g. protease or reverse transcriptase or integrase) is obtained, then translated into the predicted amino acid sequence in order to determine whether specific mutations are present or absent. Automated sequencing offers the most complete data on viral genotype, but generates more information than is needed for most clinical purposes. For example, HIV-1 RT has 550 amino acids, but mutations at only a small number of these positions are implicated in drug resistance. Therefore, interpretation of the genotype is needed in order to help distinguish which changes are merely polymorphisms and which might be significantly associated with drug resistance.

In most commercially available genotypic tests, viral RNA is extracted from a sample of plasma and reverse transcribed into complementary DNA in the laboratory. The protease and RT or integrase coding regions of the cDNA are then amplified by PCR, and the nucleotide sequence of the PCR product is determined on an automated DNA sequencer. Some laboratories use specific diagnostic kits that provide standardized reagents needed for the RT-PCR and DNA sequencing steps. Generally, these kits are part of an HIV-genotyping system that includes equipment for running the sequencing assays and software for interpreting assay results.

The frequency of false-positive and false-negative results is 0.1% for genotypic assays when assessed using samples that carry predominantly mutant or predominantly wild-type virus populations. However, sensitivity for detecting presence of a mutation is variable when both wild-type and mutant viruses are present as a mixture. In general, mutant species must constitute 10–20% of the population to be detected by standard sequencing methods. Some mutations may go undetected unless they are present as the majority species. Interpretation of genotyping results requires expertise and the access to databases relating the presence of mutations to therapy outcome and drug activity (see Table 163.2 for useful websites).

Phenotypic tests of HIV-1 drug resistance

Drug susceptibility tests with HIV-1 are usually performed using a recombinant virus assay, in which the viral genes of interest (e.g. protease and reverse transcriptase) are introduced into a plasmid that carries all of the other viral genes needed for replication in cell culture (Fig. 163.9).[30,31] Modification of the assay allows introduction of the integrase (*in*) or envelope (*env*) genes in order to determine susceptibility to integrase inhibitors and entry inhibitors, respectively. Using these assays, small differences in susceptibility can be detected (approximately two- to fourfold compared with control). Phenotypic assays are more complex and labor intensive than genotypic assays. Automation makes it possible to test many samples simultaneously, and allows for high throughput. However, the cost and complexity of the automation limit availability of these assays to a few reference laboratories.

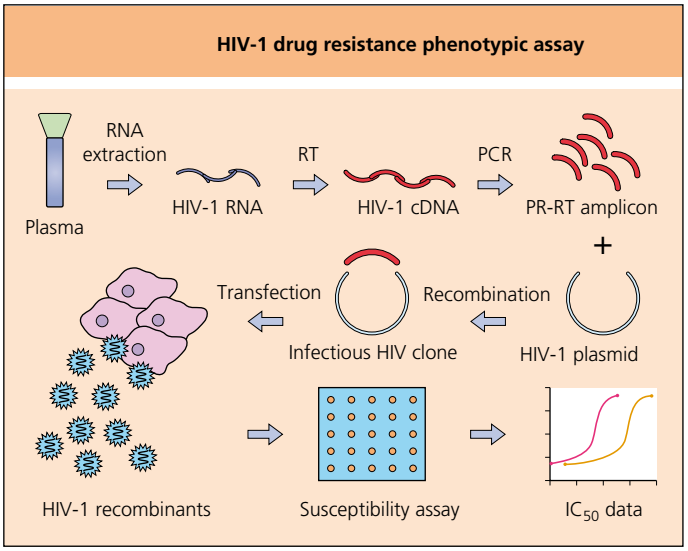


Fig. 163.9 HIV-1 drug resistance phenotypic assay. RT, reverse transcription reaction; cDNA, complementary DNA; PR-RT, protease and reverse transcriptase gene; IC_{50}, 50% inhibitory concentration.

Table 163.2 Useful websites for interpreting HIV-1 genotypic resistance tests

International AIDS Society-USA	http://www.iasusa.org
Stanford HIV RT and Protease Sequence Database	http://hivdb.stanford.edu
Los Alamos National Laboratory	http://hiv-web.lanl.gov

REFERENCES

References for this chapter can be found online at http://www.expertconsult.com

Chapter 164

David Brown
Graham Lloyd

Zoonotic viruses

INTRODUCTION

Zoonotic infections are infections of animals that can naturally infect humans. More than 400 viruses cause clinically important zoonoses and infection is acquired by several different routes. Humans can be infected directly from the host animal species, for example following the bite of a rabies-infected animal, through inhalation of rodent urine (hantavirus) or by direct contact with rodent urine (Lassa fever). However, the majority of zoonotic infections are spread by arthropod vectors and are classified as arboviruses.

The geographic distribution of the infections described in this chapter depends on many factors and changes over time. Recent examples include West Nile fever, which has recently spread to and become established across the USA. Nipah and Hendra viruses, newly recognized causes of encephalitis in humans, have been identified in Australia, Malaysia, Singapore and India. Congo-Crimean hemorrhagic fever has re-emerged in Kosovo, following social dislocation resulting from conflict. Other important zoonotic infections include DNA viruses, such as poxviruses and herpes B virus, and RNA viruses, such as rabies. A number of rare occupational viral zoonoses have been described, including foot and mouth disease, vesicular stomatitis virus and swine vesicular disease.[1,2]

ARBOVIRUSES

Over 400 viruses that cause human disease are classified as arboviruses. These are distributed worldwide because of their close association with lower vertebrates and insects and their wide host range. Most arbovirus infections are caused by RNA viruses and include members of the togaviruses, bunyaviruses and reoviruses.

Many arboviruses that infect humans cause nonspecific symptoms such as acute fever. Three broad clinical patterns are recognized with arbovirus infection:

- fever, rash and arthritis or retinitis;
- encephalitis; and
- viral hemorrhagic fevers (VHFs).

Several comprehensive reviews are available.[3,4]

Most arboviruses are maintained in complex life cycles that involve nonhuman vertebrate hosts with or without a primary vector. Mosquitoes are the most important arbovirus vector, followed by ticks, sandflies (*Phlebotomus* spp.) and midges (*Culicoides* spp.). These arbovirus life cycles only become evident when humans are bitten directly by the natural enzootic focus, or when they are exposed to a virus that has escaped the primary cycle via a secondary vector or vertebrate host. Many arboviruses have several vertebrate hosts and many can be transmitted by a number of vectors.

Viruses such as urban dengue, urban yellow fever and occasionally urban St Louis encephalitis cause significant viremia and represent the simplest form of epidemic transmission cycle, in which the virus is transmitted directly between mosquitoes and humans (see Fig. 164.1). In some cases the introduction of a secondary vector may allow the transmission of the virus to other nonhuman vertebrate reservoirs (sylvatic cycle), many of which can be regarded as blind-end hosts (Fig. 164.2). Prime examples of sylvatic cycles with multiple hosts are the encephalitides such as Western equine encephalitis, eastern encephalitis and St Louis encephalitis. Many arboviruses are maintained in arthropods, through transovarial transmission. Amplification of the cycle occurs by spread to and from small mammals (Fig. 164.3). The flavivirus infections are discussed in Chapter 18. The key features of the clinically important arboviruses transmitted to humans by hematophagous arthropod vectors, such as mosquitoes, ticks and *Phlebotomus* flies (sandfly), are given in Table 164.1. Yellow fever is an example of a mosquito-borne zoonotic virus infection.

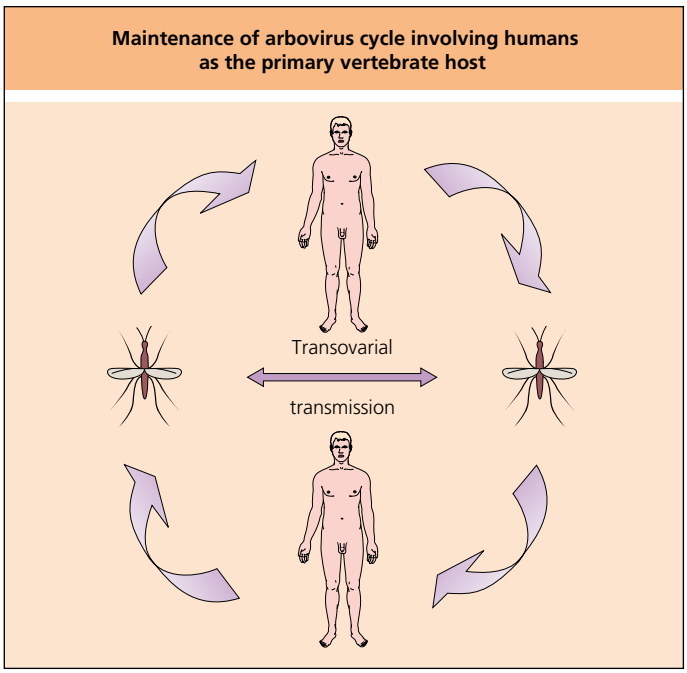


Fig. 164.1 Maintenance of arbovirus cycle involving humans as the primary vertebrate host (e.g. urban dengue and urban yellow fever).

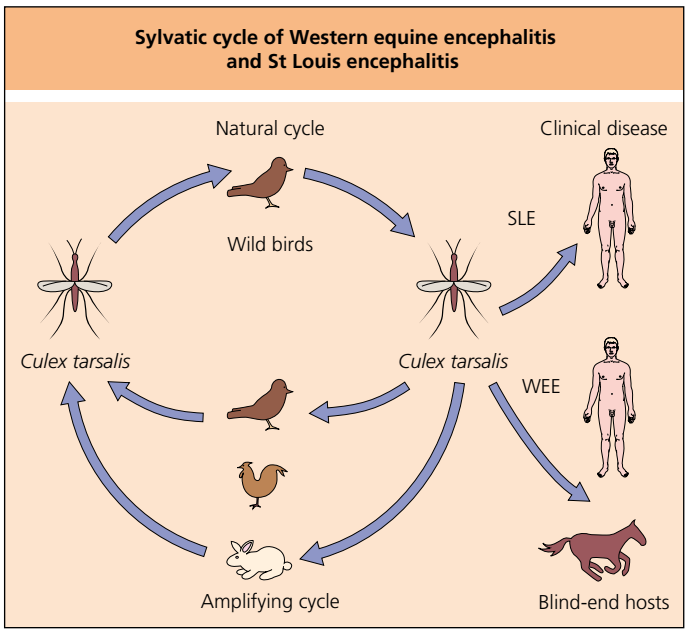


Fig. 164.2 Sylvatic cycle of Western equine encephalitis (WEE) and St Louis encephalitis (SLE).

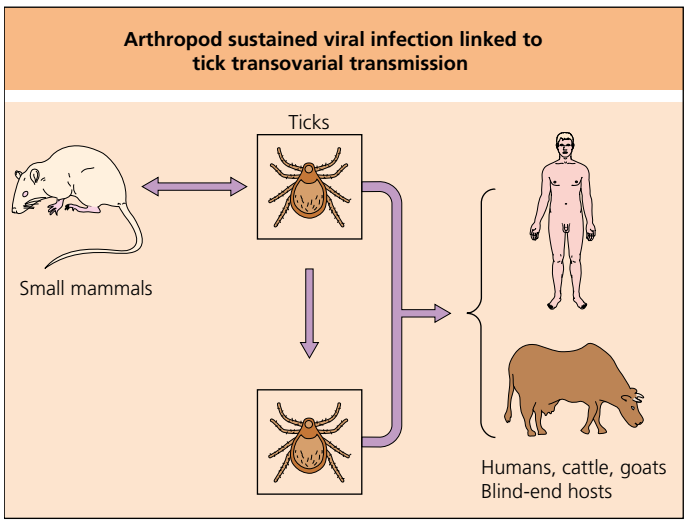


Fig. 164.3 Arthropod sustained viral infection linked to tick transovarial transmission.

YELLOW FEVER

Nature

Yellow fever has historically been one of the great killers of humans. Regrettably, this mosquito-borne virus continues to exact a considerable toll on human populations living in endemic areas even today. Yellow fever was principally responsible for the failure of the French to successfully complete the Panama Canal in the 1880s as the workforce was decimated by epidemics of yellow fever. Yellow fever was the first virus shown to be transmissible by a mosquito vector. Pioneering work by Carlos Finlay and Walter Reed are credited with this important discovery, which led to mosquito control efforts and dramatic reductions in the incidence of yellow fever. Yellow fever remains a considerable public health risk to travelers and inhabitants in endemic regions of South America and Africa.

Epidemiology

The World Health Organization (WHO) reported that between 1987 and 1991 there were 18 735 cases of yellow fever worldwide, with 4522 deaths.[5] This represented the greatest number of reported cases over a 5-year period since active reporting began in 1948. Yellow fever primarily occurs in jungle regions of the world and it is estimated that the incidence of yellow fever is grossly under-reported. The WHO estimates that perhaps as many as 200 000 cases of yellow fever occur annually.[6] Countries reporting active cases of yellow fever as of January of 1999 are indicated in Figure 164.4.[7] Yellow fever has caused significant outbreaks in recent years; in Guinea in 2000, Peru in 2001 and Senegal in 2002. The value of mass vaccination for controlling an outbreak was demonstrated in Guinea.[8]

There are two transmission cycles of yellow fever. The first and most common today is the jungle (or sylvatic) cycle of yellow fever. The jungle transmission cycle consists of a natural reservoir of disease in nonhuman primates with transmission by forest-dwelling mosquitoes. In South America the mosquito primarily responsible for yellow fever transmission to humans is the *Haemagogus* sp. mosquito. In sub-Saharan Africa yellow fever is transmitted in rural areas primarily by *Aedes africanus*. The urban (or epidemic) cycle of yellow fever is related to urban outbreaks transmitted by the human-adapted mosquito *Aedes aegypti*.

All susceptible populations appear to be equally at risk for disease yet most cases occur in children or young men (particularly those involved in the logging industry or construction in heavily forested areas). The increased incidence of yellow fever recently may reflect population expansion and economic development of rainforest areas and inadequate distribution of yellow fever vaccine in regions of the world where yellow fever remains endemic. Expanding commercial trade and international travel increases the risk of yellow fever outbreaks in nonendemic regions of the world in which the mosquito vector is found. Competent vectors for yellow fever are found in southern USA where the risk for yellow fever outbreaks remains a public health concern.[9]

Pathogenesis

Yellow fever is a prototypic mosquito-borne viral hemorrhagic fever. The virus is a 38 nm ssRNA virus with a genome of approximately 4×10^6 Da. The virus is injected into humans during the course of a mosquito bite as the salivary contents of the mosquito contaminate the bite site. Local replication of the virus takes place in human skin and regional lymph nodes, followed by viremia and dissemination to multiple organs of the body.

Major pathologic findings are found in the liver, kidney, heart and gastrointestinal tract. The virus infects the liver and causes massive hepatic necrosis. The characteristic pathologic finding is that of mid-zone necrosis with sparing of hepatocytes around the central vein and portal triads. Another characteristic finding is that of Councilman bodies within degenerating hepatocytes. Councilman bodies are acidophilic cytoplasmic deposits and not viral inclusion bodies. Diffuse areas of petechial hemorrhage are found throughout the body with the most marked changes found in the brain, kidney and gastrointestinal tract.

Prevention and control

Control of the mosquito vector population within yellow fever endemic areas is a key control measure. This can be accomplished by environmental control measures that remove mosquito habitats from areas of human habitation.

There is a highly effective preventive vaccine against yellow fever. This is a live attenuated vaccine using strain 17-D.[10] The vaccine is highly effective and one dose of 1000 mouse LD_{50} units results in immunity in >95% of recipients and neutralizing antibody that persists for >10 years. Adverse events following yellow fever vaccine are

Table 164.1 Important arbovirus infections that cause human disease

		GEOGRAPHIC DISTRIBUTION									
Genus	**Virus**	**Afr.**	**Asia**	**Aust.**	**Eur.**	**N. Am.**	**C. Am.**	**S. Am.**	**Pac.**	**Main hosts**	**Clinical diseases**
Mosquito vector											
Alphavirus	Western equine encephalitis	–	–	–	–	+	–	+	–	Rodents, birds, marsupials, equines	Encephalitis
	Eastern equine encephalitis	–	–	–	–	+		–	–	Wild birds, bats, rodents, equines, reptiles	Encephalitis
	Venezuelan equine encephalitis	–	–	–	–	–	+	+	–	Rodents, birds, marsupials, equines	Encephalitis
	Chikungunya	+	+	–	+	–	–	–	–	Primates, birds, bats, squirrels	Fever, rash, myalgia, polyarthritis
	Ross River	–	–	+	–	–	–	–	+	Large mammals, marsupials	Fever, rash, myalgia, arthralgia, hemorrhagic fever
Flavivirus	Dengue	+	+	+	–	–	+	+	+	Nonhuman primates	Encephalitis
	Japanese B encephalitis	–	+	+	–	–	–	–	–	Birds, pigs	Encephalitis
	St Louis encephalitis	–	–	–	–	+	–	+	–	Rodents, mammals	Encephalitis
	Murray Valley encephalitis	–	+	+	–	–	–	–	–	Birds	Encephalitis
	West Nile fever	+	+	–	+	+	+	+	–	Birds	Fever, rash, myalgia, polyarthritis, encephalitis
	Yellow fever	+	–	–	–	–	+	+	–	Primates, marsupials	Hemorrhagic fever
Phlebovirus	Rift Valley fever	+	–	–	–	–	–	–	–	Wild mammals, domestic livestock	Hemorrhagic fever, retinitis, encephalitis
Tick vector											
Flavivirus	Central European encephalitis	–	–	–	+	–	–	–	–	Rodents	Encephalitis
	Russian spring-summer encephalitis	–	–	–	+	–	–	–	–	Rodents	Encephalitis
	Omsk hemorrhagic fever	–	+	+	–	–	–	–	–	Water voles, muskrats	Encephalitis
	Powasson	–	–	–	–	+	–	–	–	Rodents	Encephalitis
Nairovirus	Crimean-Congo hemorrhagic fever	+	+	–	+	–	–	–	–	Wild and domestic mammals	Hemorrhagic fever
Coltivirus	Colorado tick fever virus	–	–	–	–	+	–	–	–		Febrile illness
Sandfly vector											
Phlebovirus	Sandfly fever Naples	+	+	–	+	–	–	–	–	Rodent	Febrile illness, myalgia, conjunctivitis
	Sandfly fever Sicilian	+	+	–	+	–	–	–	–	Rodent	Febrile illness, myalgia, conjunctivitis
	Toscana	–	–	–	+	–	–	–	–	Rodent	Febrile illness, myalgia, conjunctivitis

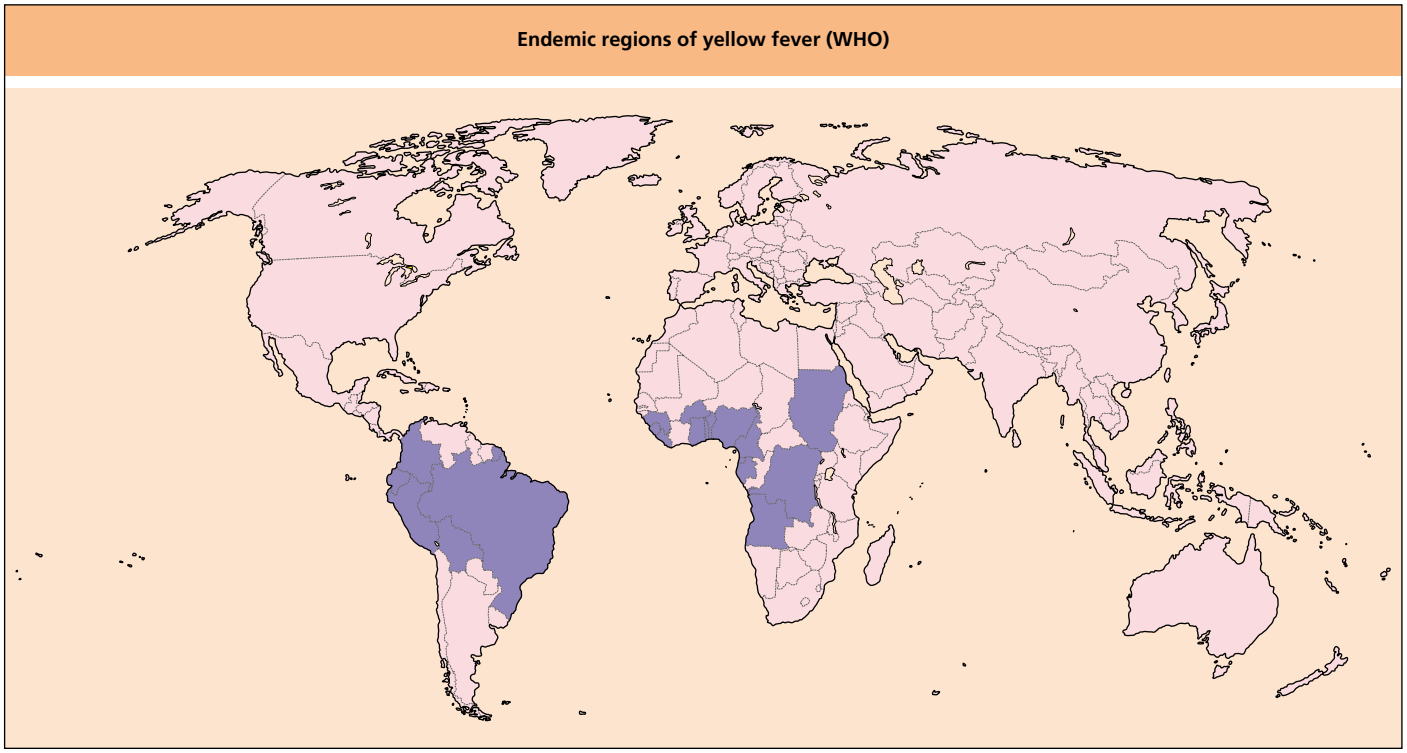


Fig. 164.4 Endemic regions of yellow fever (WHO), 1 January 1999. Dark purple areas show the worldwide distribution of reported cases.

generally mild. Recently, a new pattern of yellow fever vaccine-associated neurologic adverse disease (YEL-AND) has been recognized which resembles severe yellow fever. Risk is estimated to be 4 per million doses and is higher in recipients aged more than 60 years. Travelers to yellow fever endemic areas and residents within endemic areas should be considered for vaccination.[11,12]

Diagnostic microbiology

The virus can be isolated from a number of cell lines including Vero cells and AP-61 cells. Virus isolation represents an extreme biohazard and should only be attempted in specialized laboratory facilities. Serodiagnosis is possible within 1 week of onset of infection, with IgM antibodies detectable using enzyme-linked immunosorbent assay (ELISA), hemagglutination inhibition or plaque reduction neutralization tests. Virus detection can also be accomplished using reverse transcriptase semi-nested polymerase chain reactions (PCRs) from blood as well as tissue samples.[13]

Clinical manifestations

Human infection with yellow fever follows three discrete phases. The first phase of infection lasts approximately 72 hours and is initiated by headache, malaise, weakness, nausea and vomiting. The virus is replicating rapidly and high titers of infectious virus are found in the body fluids of patients at this point. This initial period of infection is followed by a brief period of remission where fever and symptoms remit. After 24–48 hours, symptoms recur and are dominated by symptoms of acute hepatic failure and renal failure. Patients develop jaundice and very high fever (hence the name yellow fever) and begin developing clinically overt signs of a hemorrhagic diathesis with petechiae, mucosal hemorrhages and gastrointestinal bleeding. A characteristic physical finding is marked bradycardia in the face of high fever (Fagetis' sign). Patients develop delirium, seizures, coma, acute renal failure and death within 7–10 days of illness. The mortality rate may be as high as 50% in some outbreaks and as low as 5% if optimal medical supportive care is available.[14]

Management

The management of yellow fever remains primarily supportive. Intravenous fluids, blood product support and management of hepatic and renal insufficiency are the mainstays of therapy. Ribavirin does not appear to be effective and no other antiviral agent has been shown to be useful to treat yellow fever thus far. Transmission to health-care workers, particularly after needlestick injuries, has been described. Patients should be isolated as their blood and body secretions may contain infectious virus.

WEST NILE VIRUS

Recently human and horse West Nile virus (WNV) infection have been identified in new temperate regions of Europe[15] and North America. Clinical presentation is variable, but severe infection is characterized by encephalitis and a significant mortality rate in humans and horses as well as certain domestic and wild birds. The virus was first isolated from the blood of a febrile women in the West Nile district of Uganda in 1937[16] and was subsequently isolated from patients, birds and mosquitoes in Egypt in the early 1950s.[17] The WNV was first recognized as a cause of human meningoencephalitis in Israel in 1957 and as a cause of equine disease in Egypt and France in the early 1960s.

Virology

The WNV is an RNA virus belonging to the family Flaviviridae (genus flavivirus). It is a member of the Japanese encephalitis complex, which also includes Kunjin, Murray Valley encephalitis and St Louis encephalitis. The WNV strains can be divided genetically into two lineages. Only members of lineage 1 have been associated with clinical human

encephalitis. Lineage 1 has been isolated from Africa, India, Australia, Europe, Asia and North America. Among lineage 1 WNV causing the recent human and equine outbreaks throughout Europe and Asia are closely related to the WNV (strain ROM96) first isolated in Romania and subsequently in Kenya in 1998. The virus responsible for the outbreak in the USA (NY99) is genetically distinguishable from ROM96, but it is closely related to the virus circulating in Israel from 1997 to 2000 (Isi98). Only the USA and Israel have reported illness and death in humans and animals caused by the WNV strains Isi98 and NY99. The close relations between these strains suggest that the virus causing the outbreak in the USA was imported from the Middle East.

Epidemiology

Since the original isolation of WNV, outbreaks have occurred infrequently in humans, those in Israel (1951–4 and 1957) and South Africa (1974) being the most noticeable. Since the mid-1990s there have been three distinct epidemiologic trends.

First, there has been an increase in disease frequency in humans, notably in Romania,[18] the Czech Republic, Russia[19] and the USA.[20] Epizootics of disease in horses have occurred in Morocco where 42 of 94 affected horses died, Italy (14 cases in 1998, six died), USA (1999–2002) and the Camargue region of France (2003, 58 confirmed cases, 20 deaths).

Second, there has been a noticeable increase in severity of human disease, with large outbreaks with significant mortality rates, especially in temperate urban areas. They include Bucharest, Romania (1996, over 400 cases, 17 deaths); Volgograd, Russia (1999, over 800 human cases, 40 deaths); New York City (1999–2000, 83 human cases, nine deaths); and Israel (2000, over 300 human cases, 29 deaths).[21] Since the introduction of WNV into the USA in 1999[17] it has been responsible for the identification of an increased number of cases (1999–2001, 149 human cases, 18 deaths; during the first 9 months of 2002, 2369 human cases, 139 deaths). The WNV is responsible for the spread of human disease westward, found in all states of the USA in less than 6 years and reported as far south as Argentina.[22]

Finally, high avian death rates accompanying human outbreaks have been a significant feature of WNV outbreaks in the USA and Israel.

The principal vectors are mosquitoes, predominantly of the genus *Culex* (*C. pipiens* and *C. restuans*), and the principal amplifying hosts are wild birds. Humans and horses are considered 'end-hosts' or 'incidental hosts'. A striking feature of the initial human epidemic in New York City in 1999 was the high number of avian deaths among American crows (*Corvi brachrhynchos*) and other corvids.

Clinical manifestations

The incubation time in humans is reported to be 3–14 days. Symptoms generally last 3–6 days. Reports from earlier outbreaks describe a mild form of WNV infection presenting as a febrile influenza-like illness with sudden onset. This is often accompanied by malaise, anorexia, nausea, vomiting, rash, myalgia, lymphadenopathy and retro-orbital pain. A small proportion of cases (<1%) develop severe illness, such as acute encephalitis, aseptic meningitis or Guillain–Barré syndrome. In the recent outbreak in the USA those patients hospitalized with severe disease reported fever, weakness, gastrointestinal symptoms and flaccid paralysis. Persons of all ages appear to be equally susceptible to WNV infection, but it is reported that the incidence of neuroinvasive WNV disease and death increases with age, especially those between 60 and 89 years of age.[20] A minority of patients with severe disease develop a maculopapular rash or morbilliform rash involving the neck, trunk, arms or legs. Neurologic presentations include ataxia and extrapyramidal signs, cranial nerve abnormalities, myelitis, optical neuritis and seizures, and are more common in those over 50 years of age and in young children. New modes of transmission through blood donations, organ transplants and the intrauterine route have been reported in the USA[23,24] and Italy.[25]

Diagnostic microbiology

Diagnosis of patients with WNF encephalitis or meningitis is made by the detection of IgM antibody to WNV in serum or cerebrospinal fluid (CSF) collected within 8 days of illness using the IgM antibody capture ELISA (MAC-ELISA). As IgM does not cross the blood–brain barrier, IgM antibody in the CSF strongly suggests central nervous system (CNS) infection. Roehrig and colleagues described the long-term persistence of IgM antibody in patients with West Nile (WNV) infection. Care must therefore be taken since the IgM test cannot differentiate between recent and past infections.[26] As such, the laboratory diagnostic confirmation strategy involves the serologic detection of IgM or IgG ELISA in CSF and serum with subsequent plaque reduction neutralization test (PRNT) confirmation. In addition, highly sensitive reverse transcriptase (RT)-PCRs have been described. Virus isolation on acute CSF samples provides an alternative approach. Antigenic cross-reactivity with other closely related flaviviruses is a major problem in serologic testing, and this underlines the need for confirmation with PRNT. It should be noted that samples from patients recently vaccinated against or recently infected with related flaviviruses (e.g. yellow fever, Japanese encephalitis) may give positive WNV MAC-ELISA results.

Management

There is no specific therapy or vaccine available and management is supportive.[27]

ARENAVIRIDAE

Nature

Members of the Arenaviridae have been isolated from diverse species of rodents, which are their natural hosts, in a wide range of geographic locations. Two arenavirus complexes are recognized – the complex of lymphocytic choriomeningitis virus and Lassa virus (LCMV-LASV), or old world arenaviruses, and the Tacaribe complex, or new world arenaviruses.[28] This broad antigenic classification is supported by more detailed phylogenetic comparisons. A wide range of arenaviruses have been described in their host species; several cause human infection, of which the most important human pathogens are listed in Table 164.2.

Arenavirus virions range from spheric to pleomorphic and have a mean diameter of 110–130 nm. Particles are characterized by a variable number of electron-dense ribosomes (diameter 20–25 nm) within the virus particles. They have a lipid membrane with glycoprotein spikes that project 8–10 nm from the surface.

The genome consists of two ssRNA molecules, L (large) and S (small). The L and S RNAs of arenaviruses have an ambience coding arrangement; nucleocapsid (N) is encoded in the viral-complementary sequence corresponding to the 5′ half of segment S, whereas the viral glycoprotein precursor (GPC) is encoded in the viral-sense sequence corresponding to the 3′ half of S.[28]

The N protein, which is the most abundant polypeptide, is nonglycosylated and forms a ribonucleoprotein (RNP) complex with the genomic RNA. Two glycosylated proteins, GP-1 and GP-2, are derived by post-transitional cleavage from the GPC. The L segment codes for the L protein, which is an RNA polymerase, and the Z protein, a putative zinc binding protein. α-Dystoglycan has recently been identified as a receptor for LCMV and Lassa fever virus.[29] This belongs to a highly conserved family of proteins found in epithelial, muscle and neurologic cells in humans.

Pathogenesis

In their natural rodent hosts, arenavirus infections occur in two distinct patterns: during the neonatal period, a chronic viremic infection

Table 164.2 Arenaviruses known to cause human disease

Virus	Host in nature	Geographic distribution	Main features of human disease
Lymphocytic choriomeningitis virus (LCMV)	*Mus domesticus, Mus musculus*	Europe, Americas, perhaps elsewhere	Isolated 1933; causes lymphocytic choriomeningitis, which usually presents as an aseptic meningitis with a mortality rate <1%
Lassa fever	*Mastomys* spp.	West Africa	Isolated 1969; causes Lassa fever, a severe systemic illness; severe cases suffer shock and hemorrhages; mortality rate 16%
Junin virus	*Calomys musculinus*	Argentina	Isolated 1958; causes Argentine hemorrhagic fever (AHF), which causes a similar illness to Lassa but hemorrhage and CNS disease more frequent; mortality rate up to 30%
Machupo	*Calomys callosus*	Beni region of Bolivia	Isolated 1963; causes Bolivian hemorrhagic fever; similar clinical picture to AHF; mortality rate 25%
Guanarito virus	*Zygodontomys brevicauda: Sigmodon aistoni*	Venezuela	Isolated 1990; similar to AHF; mortality rate 25%
Sabia virus	Unknown	Brazil	Isolated 1990; only three human cases described, one fatal; clinical picture probably similar to AHF

is established; in adult rodents the infection is transient and self-limiting. This lifelong infection does not have major clinical sequelae for the infected rodent.[28]

In humans several arenavirus infections cause severe illness, including meningoencephalitis and hemorrhagic fever, with significant mortality rates. The pathogenesis is not fully understood, and microscopic changes found at autopsy are modest and do not account for the severity of illness. Petechial hemorrhages of the skin are found, and multiple hemorrhages and focal necrosis may be present in internal organs. Serous effusions and interstitial pneumonia have been described. Hemorrhages are more common with South American arenavirus infections. In contrast to these modest pathologic lesions, the physiologic changes are extensive. The state of shock characteristic of severe disease reflects an increase in vascular permeability. The mechanism involved is not fully understood, but may be an indirect effect of immune mediators on endothelial cells produced by infected macrophages and monocytes, rather than direct viral damage.

Epidemiology

The epidemiology of individual arenaviral disease in humans is dependent on the geographic distribution of infected rodents and the nature of their contacts with humans (Fig. 164.5).

Several distinct patterns are seen. In Lassa fever, which is a common human infection, the reservoir host is *Mastomys* spp., which is a peridomestic rodent found in sub-Saharan Africa. The rodents are persistently infected and contaminate the environment with the virus, which generates infectious aerosols on drying. Other routes of transmission include direct contact with rodent excretions during the capture and killing of these animals for consumption. The host of junin virus, the cause of Argentine hemorrhagic fever, is *Colomys musculinus*. The natural habitat of this rodent is the hedgerow and transmission occurs at harvest time, presumably because of aerosol transmission during mechanical harvesting.

Infection by LCMV is found worldwide, wherever its natural hosts *Mus domesticus* or *Mus musculus* are infected. The burden of disease is not well established, but LCMV infection is linked with up to 10% of CNS disease of suspected viral origin. Antibody studies show a prevalence of 5% in urban populations in the USA,[30] 9% in urban populations in Germany and 2.2% in Argentina. An increase in the number of cases is reported in the autumn, which may result from the reservoir host moving into homes for the winter. The potential for LCMV to cause human disease was recently highlighted by a case linked to transplantation from an infected blood donor.[31]

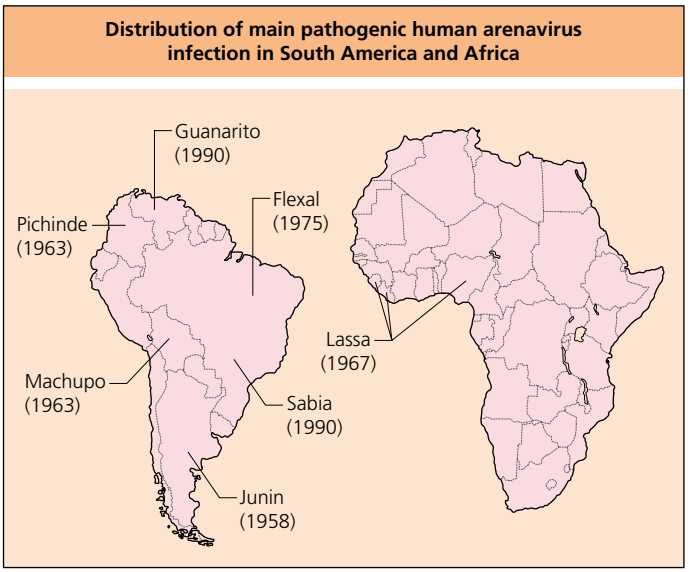


Fig. 164.5 Distribution of main pathogenic human arenavirus infection in South America and Africa, and year of first isolation of each virus.

Lassa fever is a serious public health problem in West Africa, in two distinct hyperendemic areas – Sierra Leone, Guinea and Liberia and Nigeria. Identification of a recent case in Côte d'Ivoire indicates that infection may be distributed more widely in West Africa.[32] Infections are found throughout the year, but a peak incidence is seen in the dry season. Lassa fever is a more common infection than previously thought, with estimates of up to 400 000 human cases annually and an overall mortality rate of 2%.[33] More severe hospitalized cases have a mortality rate of 16%.[34]

Several hundred cases of Argentine hemorrhagic fever are identified each year, and are associated with the harvest. Most cases occur in adult males.

Bolivian hemorrhagic fever was first identified in 1963–4, when it caused an extensive outbreak in San Joaquin that resulted in 113 deaths.[35] This followed the rodent host entering the small town.

However, the disease has since been uncommon, with occasional cases reported in rural communities in those more likely to come into contact with the infected rodent host.

Prevention

Two strategies have been proposed to prevent arenaviral disease in humans – rodent control and vaccination. Rodent control was important in controlling the Bolivian hemorrhagic fever outbreak, by the eradication of *Colomys callosus*. The house mouse, *Mus domesticus*, can be effectively eliminated from homes, thus reducing exposure to LCMV. However, its value in controlling other arenavirus infections has not been established. *Mastomys natalensis* is widespread and it has not proved practical to control rodent populations with living conditions found in West Africa. Both *C. callosus* and *C. musculinus* inhabit rural areas and control is not feasible.

A live, attenuated junin vaccine (Candid–1) has been developed and its efficacy demonstrated in a trial of 6500 volunteers.[36] It is now used for high-risk workers.

Lassa fever is the most important human arenavirus infection and vaccination seems to be the best hope for control. Killed and live vaccines have been developed and evaluated in animal models, and the most promising candidate vaccine is a vaccinia recombinant containing the Lassa virus glycoprotein gene. In challenge experiments this vaccine prevented death from Lassa challenge in guinea pigs and nonhuman primates. No human studies have been performed.[28]

Diagnostic microbiology

All arenavirus infections present similar diagnostic problems; the virus can be isolated early in the disease and this is followed by the later development of IgM and IgG antibody responses. There has been relatively little development of diagnostic assays for these agents because they are relatively rare and geographically localized.

Infection by LCMV is generally diagnosed by the detection of specific antibody. Usually immunofluorescence is used, but an ELISA test has been described. For virus isolation, blood and CSF samples are the most useful. Mice and guinea pigs are the most sensitive culture system, but isolation in Vero cells is more practical.

Lassa fever and several of the South American hemorrhagic fevers cause severe infections and can be transmitted by aerosol. Consequently, they have been classified as biohazard level 4 viruses and work on these agents is confined to the few high-containment laboratories worldwide.

The early clinical diagnosis of Lassa fever is difficult, and because ribavirin is an effective treatment if started early in the disease course, laboratory confirmation of infection is important. For rapid diagnosis either virus culture in Vero E6 cells or genome detection by RT-PCR is used on acute blood samples. Now RT-PCR is the first-line diagnostic test because it enables same-day diagnosis, and the use of guanadinium to extract RNA also inactivates viral infectivity, which makes the sample safe to work on outside the containment laboratory. In a recent study all Lassa fever patients were diagnosed by RT-PCR within 3 days of admission.[37] Detection of Lassa-specific antibody by immunofluorescence is widely used, but antibody rises late in clinical disease and is often not detectable until the second week of illness, which is often too late to effect patient management. Baculovirus-expressed nucleoprotein (NP) has been used for serologic diagnosis and avoids the need to culture virus. Quantitative real-time PCR has now been applied to the diagnosis of Lassa fever. The ability to quantify and monitor viral load may have prognostic value and help to guide treatment, but substantive studies are required.

The South American arenaviruses can be cultured from acute blood samples in newborn mice and hamsters or Vero cells. The detection of virus-specific IgG and IgM by immunofluorescence is widely used, and more recently ELISA using baculovirus-expressed viral proteins to detect antibody and RT-PCR tests have been described.

Clinical features

Arenavirus infections are often mild or subclinical. Even in severe infections, early illness is characterized by nonspecific symptoms, such as fever and myalgia, before the onset of severe systemic disease.

In LCMV infection headache, nausea or vomiting accompany the febrile prodrome. In the second week of illness CNS disease develops in a minority of cases and usually presents as an aseptic meningitis. In severe cases encephalitis can develop and this accounts for a significant minority of hospitalized cases. Full recovery is the most common outcome, but occasional deaths have been reported.

The incubation period of Lassa fever is about 10 days. It generally begins with a gradual onset of fever and malaise. As the disease progresses, abdominal pain, nausea and vomiting become more pronounced and an exudative pharyngitis and conjunctivitis are common. More pathognomonic signs and symptoms develop during the second week of illness, including facial edema, pleural edema, encephalopathy and shock. In severe cases illness is progressive, with shock followed by death. In survivors, defervescence is followed by recovery after 2–3 weeks. Hemorrhagic complications are not uncommon in severe cases and generally present with epistaxis, bruising and conjunctival hemorrhage. In the convalescent period pericarditis has been described. Deafness is now recognized as a late complication.[38] It can be unilateral or bilateral and is not related to the severity of disease; it may be immune mediated.

Argentine and Bolivian hemorrhagic fever present with a similar clinical picture. The progression of illness is more rapid than in Lassa fever, with severe prostration developing 3–4 days after onset. In more severe cases mucous membrane hemorrhage and bruising at intravenous injection sites are seen, which indicate a poor progress. In a few cases substantial neurologic involvement leads to convulsions. Recovery usually starts at the end of the second week of illness.

Management

Lassa fever has caused nosocomial infections in Africa and is the biohazard level 4 agent most commonly imported into nonendemic countries. Consequently, many countries have developed containment guidelines for the management of suspected Lassa fever cases to prevent secondary transmission.[39,40] Transmission is through direct contact with infected body fluids and little evidence suggests that airborne transmission is important. The guidelines follow a similar approach based on an initial patient risk assessment, which leads to different levels of investigation, containment and monitoring of close contacts, depending on risk (see also Chapter 126).

Ribavirin, a guanosine analogue, is effective *in vitro* against arenaviruses, but its mode of action is unknown. Ribavirin has been evaluated for the treatment of severe Lassa fever cases and dramatically reduces the mortality rate provided it is given early in the illness.[41] Its use for prophylaxis of contacts with Lassa fever cases and for the treatment of other arenavirus infections has been proposed, but no trial reports have been published to date.

Treatment of Argentine hemorrhagic fever infections with convalescent human plasma is effective in reducing the mortality rate from 15–30% to less than 2% if given during the first 8 days of illness. Convalescent plasma could potentially play a role in Lassa fever treatment, but this has not been established in practice. Patient management of arenaviral infections requires general supportive care, with particular attention to fluid balance.

HANTAVIRIDAE

Nature

Hantavirus is the most recently described genus in the Bunyaviridae family and comprises a growing number of isolated viruses, including those that cause hemorrhagic fever with renal syndrome (HFRS) and

Table 164.3 Characteristics of some known hantaviruses found in Europe, Asia, Russia and India

Species	Human illness	Pathology	Mortality rate (%)	Reservoir	Geographic virus distribution
Hantaan (HTN)	Severe: HFRS, EHF, KHF	Renal	5–15	*Apodemus agrarius* (striped field mouse)	China, Russia, Korea
Dobrava/Belgrade (DOB)	Severe: HFRS	Renal	5–15	*Apodemus flavicollis* (yellow neck mouse)	Balkans
Seoul (SEO)	Moderate: HFRS	Renal	1	*Rattus norvegicus* (Norway rat)	Worldwide
Puumala (PUU)	Mild; NE	Renal	1	*Clethrionomys glareolus* (bank vole)	Europe, Russia, Scandinavia
Khabarovsk (KHB)	?	?	?	*Microtus fortis* (reed mole)	Russia
Tula (TUL)	?	?	?	*Microtus arvalis* (European common vole)	Europe
Thailand (THAI)	?	?	?	*Bandicota indica* (bandicoot rat)	Thailand
Thottapalayan (TMP)	?	?	?	*Suncus murinus* (musk shrew)	India
Topografov (TOP)*	?	?	?	*Lemmus sibiricus* (Siberian lemming)	Siberia

EHF, epidemic hemorrhagic fever; HFRS, hemorrhagic fever with renal syndrome; KHF, Korean hemorrhagic fever; NE, nephropathia epidemica.
?Not yet documented.
*Virus not yet isolated/molecular analysis suggests they are probable hantavirus species.

hantavirus pulmonary syndrome (HPS).[42,43] Some recently recognized probable species that have been identified by molecular genetic analysis have not yet been isolated (Table 164.3). Hantaviruses contain a tripartite, single-stranded, negative-sense RNA genome. The three segments are designated as small (S), medium (M) and large (L), and encode for the nucleocapsid (N) protein of 50–53 kDa, the two envelope proteins (G1 and G2) of 65 and 74 kDa, respectively, and an associated virus of 200 kDa.[44]

Epidemiology

The epidemiology of hantavirus disease in humans is dependent on the geographic distribution of persistently infected rodent hosts.[45] The hantaviruses linked with HFRS are associated with specific rodent hosts, each named for the region in which they were first isolated:

- *Apodemus agrarius* (the striped field mouse) for the hantavirus;
- *Rattus norvegicus* (the Norway rat) and *Rattus rattus* (the black rat) for the Seoul virus; and
- *Apodemus flavicollus* (the yellow necked field mouse) for the Dobrava virus.

The hantavirus from Korea, Russia and China and the Dobrava virus (DOBV) from the Balkan countries of Europe (Bosnia, Croatia, Yugoslavia, Albania, Slovenia, Greece, Bulgaria and Russia) are associated with the severe form of HFRS, with an estimated mortality rate of 5–15% (see Table 164.3).[46,47] DOBV appears to be the most life-threatening hantavirus in Europe; a moderate form of HFRS is caused by the Seoul virus, which (along with its host) is found virtually worldwide. Serologic evidence for infection with Seoul-like hantaviruses has been found in rodents in major cities within the USA.

A mild form of HFRS, caused by the Puumala virus (PUUV), is responsible for nephropathia epidemica (NE) in Scandinavia, the European regions of the former USSR and south of this through much of Europe to France, Spain and Portugal.

The hantavirus associated with HPS, Sin Nombre virus (SNV), has been recognized throughout the USA since its discovery in 1993.[48] Molecular biologic studies have identified similar viruses – Black Creek Canal (BCC), New York, Bayou (BAY) and Andes – that are also associated with HPS illness, that extend their geographic distribution from North America to Mexico and South America (Table 164.4). The distribution of specific host species throughout the Americas suggests a wider distribution of hantaviruses which potentially could cause further clinical cases. Recently, two novel hantaviruses, with unknown pathogenic potential, were found in Africa.[49–51]

All rodent hosts are persistently infected with specific hantavirus strains that apparently cause no illness. In wild rodent populations, most infections occur through age-dependent horizontal routes, predominately in mature males. Horizontal transmission among cage mates was experimentally demonstrated via the aerosol route at median infectious doses of 1.0 plague forming units (pfu) for each virus tested. However, although rodents are susceptible to hantavirus through the aerosol route, their susceptibility is increased by inoculation (median infectious doses 0.003–0.016 pfu). Vertical transmission from dam to pup has proved negligible or absent, both in wild and experimental settings.

Hantavirus disease, irrespective of strain, is a seasonal disease. This seasonality is associated with population dynamics of the rodent hosts that maintain each virus and their interaction with local human populations. Rodent population cycles vary according to climatic changes and interspecies competition. Rural-associated HFRS in Asia and Europe is linked to human rodent contact during the planting and harvesting of crops (late autumn and early winter). Similarly, in Scandinavia Puumala virus causes most cases of NE during the same period. Both Puumala outbreaks in Scandinavia and the original HPS outbreak in America were associated with increases in the natural rodent population followed by rodent migration from the fields to buildings in response to adverse weather conditions. Alternatively, in Greece, HFRS is observed predominately in the warmer months as a result of increased human outdoor activities, including camping. These cases point to higher risk being experienced by people such as forestry workers and shepherds. In China HFRS induced by Seoul virus appears to be most common in the spring.

The aerosol route of infection is regarded as the most common means of transmission among rodents and to humans. Rodent bites cannot be excluded as a way of maintaining the disease among

Table 164.4 Some known hantaviruses found in North and South America

Species	Human illness	Pathology	Mortality rate (%)	Reservoir	Geographic virus distribution
Sin Nombre virus (SNV)	Severe: HPS	Pulmonary	50	*Peromyscus maniculatus* (deer mouse)	USA, Canada, Mexico
New York (NY)	HPS	Pulmonary	Not recorded	*Peromyscus leucopus* (white-footed mouse)	USA
Black Creek Canal (BCC)	HPS	Pulmonary	Not recorded	*Sigmodon hispidus* (cotton rat)	USA
El Monro Canyon (ELMC)	?	?	?	*Reithrodontomys megalotis* (Western harvest mouse)	USA, Mexico
Bayou (BAY)*	HPS	Pulmonary/renal	?	*Oryzomys palustris* (rice rat)	USA
Andes (AND)*	HPS	Pulmonary	50	*Oligoryzomys longicaudatus* (long tailed pygmy rice rat)	Argentina
Unnamed*	HPS	Pulmonary	?	*Calomys laucha* (vesper mouse)	Paraguay
Rio Segundo (RIOS)*	?	?	?	*Reithrodontomys mexicanus* (Mexican harvest mouse)	Costa Rica
Rio Manore (RIOM)*	?	?	?	*Oligoryzomys microtis* (small-eared pygmy rice rat)	Bolivia
Isla Vista (ISLA)*	?	?	?	*Microtus californicus* (California vole)	USA
Bloodland Lake (BLL)*	?	?	?	*Microtus ochrogaster* (prairie vole)	USA

HPS, hantavirus pulmonary syndrome.
?Not yet documented.
*Virus not yet isolated/molecular analysis suggests they are unique hantavirus strains.

rodents or occasionally resulting in human infection. Epidemiologic investigations link virus exposure to farming activities, sleeping on the ground and military exercises. Indoor exposure has been linked to rodent infestation of homes during cold weather or to nesting rodents in or near dwellings. Many hantavirus infections are identified among people who live in poor housing conditions or in those who pursue recreational activities such as camping and various water sports. Human-to-human transmission is rare and consequently hantaviruses do not pose a risk to hospital workers. However, recent outbreaks of HPS in South America suggested that minimal exposure to body fluids of infected patients resulted in apparent person-to-person spread.

The annual incidence of HFRS involving hospitalization throughout the world is estimated to be between 150 000 and 200 000 cases. More than half these cases predominate in the endemic center of epidemic hemorrhagic fever in the Chinese provinces of Hubei, Heilongjiang, Jiangxi, Jilin and Shanxi. Annual incidence rates here range from 0.05 to 3.0/1000. Russia and Korea also report hundreds to thousands of cases of HFRS each year. Most of the remaining cases (a few hundred) are reported from Japan, Finland, Sweden, Belgium, The Netherlands, Greece, Hungary, France and the Balkan countries formally constituting Yugoslavia. Mortality rates range from less than 0.1% for HFRS caused by Puumala to approximately 5–10% for HFRS caused by hantavirus.

Several hundred cases of HPS have been reported throughout North and South America (see Table 164.4).[52] Most of the identified cases in America and Canada were caused by SNV. Cases of HPS in southwestern USA and South America are caused by molecularly identified strains that include BAY, BCC and Andes viruses.[53]

A number of hantavirus infections have caused several laboratory-associated outbreaks of HFRS, all of which were traced to persistently infected rodents obtained from breeders, to wild-caught naturally infected rodents or to experimentally infected rodents. No illness has been associated with laboratory workers using cell-culture adapted viruses, although asymptomatic seroconversions have been documented.

Pathogenesis

The process by which hantaviruses cause multisystem organ dysfunction syndrome is unclear. Thrombocytopenia, defects in platelet function, transient disseminated intravascular coagulation (DIC) and increased vascular fragility are all thought to play a key role in the early stages of the disease. Hantavirus disease is mainly microvascular in nature and endothelium is the predominant cell type involved. The presence of hantavirus antigen has been demonstrated by immunochemistry in vascular endothelial cells of fatal HFRS infections, experimentally infected rodents and endothelium of lung capillaries in the highly lethal form of HPS.[54] Endothelial damage or dysfunction leads to capillary engorgement, leakage of erythrocytes and increased permeability.

Recent studies implicated the disease process as being immunologically based, with lymphocytes playing a key role, especially T cells. The CD4:CD8 ratio is greatly decreased, although abundant $CD4^+$ and $CD8^+$ lymphocytes have been reported in the lung interstitium of HPS patients.[54] Lymphocyte induction of migrating macrophages and other inflammatory cells results in the production of cytokines, such as tumor necrosis factor (TNF), interleukin (IL)-1 and IL-2, and interferon gamma (IFN-γ), which in turn increases vascular permeability.

Prevention

Prevention of hantavirus infection is dependent upon reducing contact between humans and infected rodents. However, many rodent control programs in known highly endemic areas, such as China, have been ineffective. Guidelines in the USA to control HPS infections have been aimed at reducing rodent access to homes, clean-up recommendations of buildings with heavy rodent infestations, and precautions for workers exposed to rodents and for campers and hikers in infected areas.

Although a number of candidate (inactivated, recombinant) vaccines against hantaviruses are under development, none is currently available.

Both wild-caught rodents introduced into laboratories and laboratory-bred colonies have caused a number of laboratory-based infections. Current recommendations directed at the manipulation of hantaviruses call for handling at biosafety containment levels 2 and 3, dependent on the strain. Work with infected laboratory rodent colonies that may lead to the generation of infectious aerosols in urine or feces should be undertaken in facilities that have suitable room ventilation and high-efficiency particulate air (HEPA) filtration, primary containment of animals, and personal protective equipment at a minimum of biosafety containment level 3. Precautions should also ensure suitable decontamination of animal bedding, cages and animal waste.

It is prudent to introduce hantavirus screening of laboratory-bred and wild-caught rodents and cell lines that originate from rodent tissue before use. Such animals and cells should be segregated from other animals, with care being taken to prevent possible aerosolized spread of infectious virus.

Diagnostic microbiology

Diagnosis of hantavirus infection relies upon the recognition of characteristic clinical features, a history consistent with the epidemiology of the disease and serologic confirmation.[52]

Direct isolation of hantavirus from acutely ill patients is prolonged and often unsuccessful and consequently of little diagnostic value. Similarly, the success of antigen-capture ELISA or the application of RT-PCR on acute blood samples is of no practical value for the diagnosis of human infection.

Although the immunofluorescence assay is regarded as a useful diagnostic tool, the method of choice to diagnose acute hantavirus infection is an IgM antibody assay of high sensitivity and specificity.[55] Currently two alternatives are available:

- M antibody capture IgM enzyme immunoassay (EIA) based on the use of cell-culture grown hantavirus antigen; and
- M antibody capture IgM EIA based on baculovirus or *Escherichia coli* expressed full-length nucleocapsid protein.[56]

As IgG antibodies persist for life in both humans and rodents, the presence of IgG seropositivity may not reflect a recent infection. In addition, a rise in IgG has not always been observed in recent clinical cases. The IgG ELISA or immunofluorescent antibody (IFA) test is well suited for epidemiologic studies, whereas determination of the specific infecting virus is best achieved by focus reduction neutralization tests.

Other methods are also under development to investigate hantavirus:

- hantavirus recombinant immunoblot assay (RIBA), which uses recombinant and synthetic peptides; and
- application of RT-PCR and immunohistochemical staining of antigen contained in formalin-fixed autopsy tissues.[57]

Clinical manifestations

Although HFRS has been clinically recognized and reported in Asia, Russia and Scandinavia for the past century, the causative agent, hantavirus, was not isolated until 1978.[58] The HFRS complex comprises three distant clinical diseases, each infection being caused by a specific hantavirus.[45,46] The spectrum of disease ranges from nonapparent infection to fulminant hemorrhagic fever with renal failure (sometimes having a fatal outcome). Depending on which hantavirus is responsible for the illness, HFRS can appear as a mild, moderate or severe disease. Fatality rates range from less than 1% for HRFS caused by Puumula virus to approximately 5–10% for HFRS caused by hantavirus (Table 164.5).

Table 164.5 Main characteristics of the two severe forms of hantavirus disease

Characteristics	Hemorrhagic fever with renal syndrome	Hantavirus pulmonary syndrome
Primary target organ	Kidney	Lung
Acute phase	Febrile	Febrile 'prodrome'
Later phases	Shock, hemorrhage	Shock, pulmonary edema
Disease progression	Hypotensive, oliguric, diuresis, convalescence	Diuresis, convalescence
Other clinical and laboratory features	Thrombocytopenia, leukocytosis, proteinuria, hematuria, creatinine >100 mmol/l, hemoconcentration, raised transaminases	Thrombocytopenia, leukocytosis, hemoconcentration, shortness of breath, abnormal respiratory rate, lung infiltrates
Mortality rate (%)	1–15	≥50

The clinical course of pathogenic hantavirus infection comprises an acute febrile illness characterized by variable degrees of hemorrhagic and renal dysfunction. More severe disease involves five overlapping stages – febrile, hypotensive, oliguric, diuretic and convalescent.

The onset of the disease is abrupt and characterized by high fever of 102.2–104°F (39–40°C), chills, intensive headache, malaise, myalgia, dizziness and anorexia. A petechial rash may also appear on the face, neck and trunk. The main laboratory findings include normal-to-elevated white blood count (WBC), decreasing platelets, rising hematocrit and rising proteinuria.

As the febrile phase ends, hypotension can abruptly develop and last for a few hours or days. Prominent features are tachycardia, falling arterial pressure and narrowing pulse pressure, and in severe cases classic shock. Laboratory findings include leukocytosis with a left shift, thrombocytopenia and prolonged bleeding times. Urinalysis shows heavy proteinuria, mild hematuria and hyposthenuria. About one-third of fatalities occur during this phase.

The oliguric phase lasts 3–7 days, during which blood pressure returns to normal or is slightly raised because of hypervolemia. Laboratory findings include normal WBC and platelet counts, initially normal and then depressed hematocrit, continual marked hematuria and elevated blood urea nitrogen and creatinine. Hyponatremia, hyperkalemia and hypocalcemia result from renal failure. Pulmonary edema may occur and fluid management should be handled with care. Almost half of the deaths occur during this phase, often from pulmonary edema or infection, electrolyte imbalance, late shock or hemorrhage into the brain.

Clinical recovery is signaled by the diuretic phase, with improved renal function and normal clotting. Diuresis over a few hours or days is evident, with an output of 3–6 liters. Fluid management must be maintained to prevent negative fluid balance that may lead to shock. The final (convalescent) phase can last weeks to months before recovery is complete.

Seoul virus infections (mild HFRS) are less severe than those caused by hantavirus. Typically the phases are shorter and difficult to recognize or even absent. The disease is characterized by fever, anorexia, chills and nausea, and vomiting. Palatal injection is common, although other hemorrhagic signs (epistaxis, melena, hematemesis) are observed in fewer than 30% of patients. Laboratory findings include lymphocytosis, thrombocytopenia, microscopic hematuria and proteinuria, and elevation of serum transaminase. Renal involvement is less severe and fatalities are uncommon.

Puumula virus infection (NE) is characterized by a sudden onset of fever accompanied by headache. Characteristically, by the fourth day of illness nausea, vomiting, petechiae in the throat and soft palate, and facial flush and petechial rash have occurred. A mild thrombocytopenia can be observed. Hypotension may be found, but evidence of shock is rare. Onset of oliguria or recognition of renal failure around the sixth day of disease is the main cause of hospital admission. Serum creatinine levels rise and dialysis may be required in 10% of cases. As with other hantavirus infections, the onset of polyuria indicates the recovery process.

First recognized as a severe, often fatal respiratory illness in adults, HPS was characterized as a severe, systemic illness with a nonspecific onset, fever, myalgia, cough or dyspnea, gastrointestinal symptoms and headache that typically lasted 3–5 days.[59,60] Rapid and abrupt onset of noncardiogenic edema, hypotension (systolic blood pressure ≤80 mmHg) then follow, resulting in shock and death in over half of the recognized cases (see Table 164.5). Laboratory findings indicate leukocytosis, an increase in polymorphonuclear leukocytes, left shift, increased hematocrit, thrombocytopenia, prolonged prothrombin and partial thromboplastin time, elevated serum lactate dehydrogenase, decreased serum protein concentrations and proteinuria. Increases in hematocrit and thromboplastin time are considered to be predictors of death. Radiographic examinations show bilateral pulmonary infiltrates and evidence of pleural effusions in the majority of hospitalized patients. Histopathologic features seen in lung tissue reveal mild-to-moderate interstitial pneumonitis with variable degrees of congestion, edema and mononuclear infiltrates. The virus has been identified extensively within endothelial cells of the pulmonary microvasculature, spleen and lymph nodes.

Current understanding of the disease indicates little evidence of renal damage as a feature of this hantavirus infection. The extensive pulmonary endothelial involvement and severe pulmonary edema make clinical management difficult.

Management

Effective management of HFRS and HPS requires early diagnosis, knowledge of disease course and prompt hospitalization. Aggressive clinical management is essential to improve survival. Care should be phase specific, with special attention given to fluid management. Ribavirin may be efficacious in the treatment of HFRS patients, but its treatment of HPS is not proved.

FILOVIRIDAE

Nature

The family Filoviridae consists of two distinctive species, Marburg and Ebola, which cause severe and often fatal hemorrhagic disease in humans and monkeys (see Chapter 126). The viruses have a distinctive filamentous morphology under the electron microscope with a genome that consists of a nonsegmented, negative stranded RNA approximately 19 kb in length. Several features of their molecular organization and structure have linked these viruses to members of the Paramyxoviridae and Rhabdoviridae under the taxonomic order Mononegavirales.[57] The virions are composed of a central core formed by an RNP complex, which is surrounded by a lipid envelope derived from the host cell plasma membrane. The RNP is composed of a genomic RNA molecule bound by the NP, virion structural protein 30 (VP30), VP35 and the L protein (RNA transcriptase polymerase). The three remaining structural proteins are membrane associated – GP, VP24 and VP40 are located on the inner side of the membrane. Among the newly isolated Ebola viruses are differences in molecular structure, pathogenicity and virulence in humans.[61]

Marburg and Ebola were first detected in 1967[62] and 1976,[63,64] respectively, although human infections since then have been rare. Fresh outbreaks of Ebola infections were identified in Côte d'Ivoire (1994, 1995), Republic of Congo (formerly Zaire; 1995, 2001, 2002, 2003, 2007),[65] Gabon (1994, 1996, 1997, 2001) and Uganda (2000, 2008).[66] In addition, importation of Ebola-infected monkeys into the USA (1989–1990, 1996) and Italy (1992) attracted extensive worldwide media coverage and raised public concerns about the potential public health threat of these pathogens through international travel and commerce.[67,68]

Pathogenesis

The precise mechanisms by which filoviruses cause the most severe forms of VHF are unclear, but there is marked hepatic involvement, DIC and shock, producing extremely high fatality rates (30–90%). In the early stages of infection laboratory findings, such as high aspartate transaminase/alanine transaminase (AST/ALT) ratios, and marked lymphopenia followed by a marked neutropenia with left shift, suggest other extrahepatic targets affected by the virus. As with other VHF infections (HFRS, Lassa, dengue) fluid imbalance and platelet abnormalities indicate endothelial cell and platelet damage or dysfunction. Human monocytes and/or macrophages, fibroblasts and endothelial cells support virus replication. Infected monocyte and/or macrophages have been shown in *in-vitro* models to secrete the cytokine TNF which has the ability to increase vascular endothelial cell damage.[69] The data currently available support mediator-induced vascular damage that leads to increased permeability and shock observed in severe cases. Hemorrhage is likely to be caused by reticuloendothelial system damage that cannot be repaired because of platelet and coagulation malfunctions.

Epidemiology

The natural history and reservoirs of filoviruses remain to be established, but recently the potential role of bats has been highlighted.[70] In 1967, a fulminating hemorrhagic fever, Marburg disease, occurred in Marburg and Frankfurt, Germany, and Bosnia, Croatia and Yugoslavia among laboratory workers handling blood and tissue from a shipment of African green monkeys (*Cercopithecus aethiops*) soon after being imported from Uganda via London. Among the 31 human cases, 25 of which were primary infections, there were seven (23%) deaths.[62] None of the secondary cases died. Since then, sporadic cases have been reported from South Africa and Kenya (Fig. 164.6). The geographic spread widened with a major outbreak of Marburg in the Uige Providence of Angola during 2004–5 where 252 cases were identified with 227 deaths (case fatality rate 90%).

In 1976, the first known cases of Ebola hemorrhagic fever were identified in southern Sudan (Table 164.6) and in a simultaneous outbreak in northern Republic of Congo (formerly Zaire), with fatality rates of about 60% and 90%, respectively.[63,64] A further smaller outbreak occurred in the same region of Sudan in 1979, when 22 (65%) of 34 infections proved fatal. Lack of community co-operation hampered control efforts and investigations during an outbreak of Ebola in the Republic of Congo during 2002. Recent instances of Ebola infection were the severe illness of a Swiss zoologist working with infected chimpanzees (*Pan troglodytes verus*) in the Taï National Park in Côte d'Ivoire, West Africa, in late 1994 and a major epidemic in Kikwit, Bandundu Province, Republic of Congo (formerly Zaire), in 1995. Of 315 recorded cases of Ebola hemorrhagic fever a total of 244 died (77% case fatality rate).[64] A further four outbreaks between 2001 and 2007 in the D.R Congo recorded over 499 cases where 387 died (77.5% case

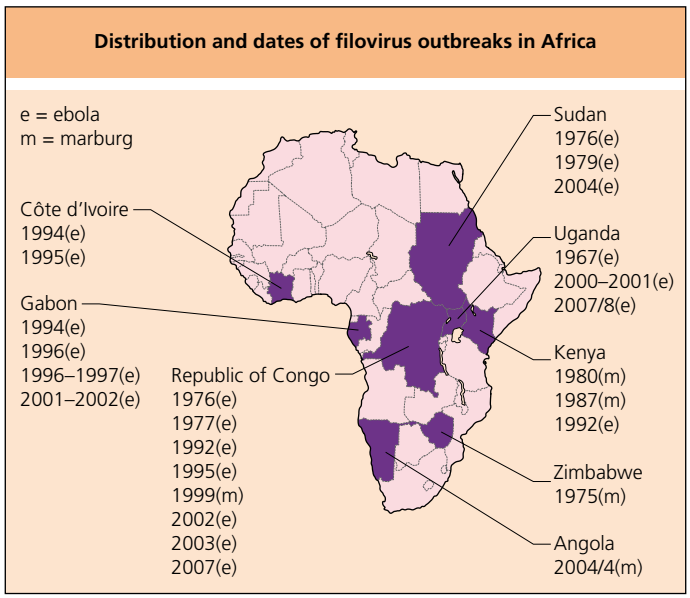


Fig. 164.6 Distribution and dates of filovirus outbreaks in Africa.

fatality rate). In the later part of 1995 further cases were identified in the Côte d'Ivoire among refugees who originated in Liberia. Finally, three reportedly independent human outbreaks of Ebola have occurred in the forested areas found in north-eastern Gabon (1994, February 1996, July 1996, December 2001). Subsequent investigations of the 2001–2 outbreak suggested that cases in villages north of Mekambo resulted from contact with a gorilla whose remains were found to be positive for Ebola virus. A severe outbreak occurred between August 2000 and January 2001 in Uganda (Gulu and Masindi districts) that recorded 425 cases with 224 fatalities.[69] A case from the 1996 infection was treated in South Africa and the source of a fatal nosocomial transmission to a health-care worker. All three outbreaks were associated with the deaths of chimpanzees, also thought to be from Ebola virus infection. In 2008, a newly discovered Ebola virus was found responsible for two cases in young men working lead and gold mines in Kamwenge District, Uganda.[70]

Hence, since 1994 West Africa has been considered to be part of the African filovirus map (see Fig. 164.6). Although filovirus outbreaks are rare events, the public health importance lies in their high mortality rate rather than the total number of infections in the 30 years since they were first identified (Tables 164.6 and 164.7).

Ebola virus has also appeared outside Africa, among shipments of imported cynomolgus monkeys in Reston, Philadelphia and Texas, USA, in 1989, 1990 and 1996, respectively, and Sienna, Italy, in 1992.[66] The monkeys involved in each epizootic event were imported from the Philippines and traced to the same handling facility, where the presence of the virus was also documented. The epizootics indicate that monkeys acutely infected with filoviruses present a major veterinary emergency and possibly could pose a serious hazard to other animals or humans if not recognized in the future. Although an Asian origin of these virus strains cannot be discounted, molecular biologic and serologic studies carried out to date suggest a close similarity with the original Ebola viruses isolated from the 1976 African outbreaks. Current interest in this strain of Ebola virus has increased with the description of Ebola-Reston in samples originating from domestic pigs originating in the Philippines.

Prevention

The imposition of ecologic controls to prevent outbreaks is impossible until the natural history of the viruses has been discovered. It is important that health-care workers in areas where hemorrhagic fever exists are aware of the high risk of nosocomial spread if patients are not recognized early and placed in complete isolation.[71] In nonendemic areas an awareness of the changing epidemiology associated with viral re-emergence or emergence should always be considered, bearing in mind the threat and consequences of importation.[72] High-risk patients are those who during the 3 weeks before illness had:

- traveled into areas where VHF occurred recently;
- been in contact with body fluids from a person with VHF; or
- worked in a laboratory environment handling the virus.

(See also Chapter 126.)

Importantly, filovirus illness is a rare event if patients do not meet any of these criteria.

As nonhuman primates are responsible for the introduction of Marburg into Europe, and of Ebola into the USA and Italy, the management of transportation, quarantine facilities and animal husbandry must ensure that personnel understand the hazards of handling nonhuman primates, and thus reduce the risks of future human outbreaks.[73]

Clinical manifestations

The incubation period for Ebola ranges from 4 to 10 days and for Marburg from 3 to 9 days.[62] The shorter times have been associated with exposure to contaminated needles.[74]

Filoviruses enter the host through close contact with contaminated body fluids. Epidemiologic studies in humans indicate that infection is not readily transmitted by the aerosol route. Although studies in nonhuman primates have established that aerosol transmission can cause infection,[75] extra care must be taken with contaminated body fluid, tissue, hospital material and waste.

Both Marburg and Ebola virus infections follow a similar pattern of illness. Following exposure, initial symptoms include an abrupt onset with fever, severe frontal headache, malaise and myalgia. Such symptoms are similar to those of many viral illnesses that originate locally and in tropical areas. The initial diagnosis of a filovirus infection is very difficult. Disease progression is characterized by pharyngitis, nausea, severe vomiting of blood and production of bloody stools. Uncontrolled bleeding follows, which can be seen from under the skin, from venepuncture sites and from other orifices of the body. A characteristic maculopapular rash occurs 7–10 days (range 1–21 days) after onset of the clinical disease. If fatal, death occurs 6–9 days after onset of the clinical disease and results from severe blood loss and shock. Convalescence is slow, taking many weeks, and is marked by weight loss, prostration and amnesia of the acute phase of illness.[76]

Clinical laboratory findings in the early acute phase include lymphopenia followed by neutrophilia, marked thrombocytopenia and abnormal platelet aggregation. Serum AST and ALT are elevated and characterized by an AST/ALT ratio of between 10 and 3:1.

Diagnostic microbiology

The clinical diagnosis of Ebola or Marburg should be considered in patients who show acute, febrile illness and have traveled in known epidemic or suspected endemic areas of rural sub-Saharan Africa and Asia, particularly when hemorrhagic signs are present. As the differential diagnosis in the early acute phase of illness is difficult, other causes (malaria, typhoid) should not be excluded and treatment delayed. Laboratory diagnosis carried out on patient specimens is undertaken by more than one procedure to guard against false-positives.

Care should be taken when drawing or handling blood specimens at the acute stage of illness because they contain large amounts of infectious virus – the virus is stable for long periods at room temperature at this stage.

Although their morphologic appearance is similar by electron microscopy, Marburg and Ebola are immunologically distinct. The basic diagnostic tool for filovirus infection and the only one to date that has widespread acceptance for the diagnosis of human filovirus disease is IFA, although some epidemiologic serology based on this

Table 164.6 Characteristics of some known filovirus infections in Africa

Date	Filovirus Infections	Documented fatalities/total cases	Main characteristics
Marburg			
1967	Marburg, Germany	5/23	Infected through contact with African green monkeys imported from Uganda
1967	Frankfurt, Germany	2/6	Infected through contact with African green monkeys imported from Uganda
1967	Belgrade, Yugoslavia	0/2	Infected through contact with African green monkeys imported from Uganda
1975	Zimbabwe	1/3	Contracted by travelers entering region – source unknown
1980	Mount Elgon, Kenya	1/2	Contracted by travelers entering region – source unknown
1987	Mount Elgon, Kenya	1/1	Contracted by travelers entering region – source unknown
1998–2000	D.R. Congo	128/154	Gold miners near Durba
2004–05	Angola	227/252	Outbreak started in Uige Province
2007	Uganda	1/2	Contracted infection in mine
2008	Netherlands	1/1	Contracted illness in Uganda
Total mortality rate		**367/446 (82.3%)**	
Ebola (Africa)			
1976	Yambuku, D.R. Congo	280/318	Virus when introduced into hospital amplified disease
1976	Maridi, Sudan	151/284	Virus when introduced into hospital amplified disease
1976	England	0/1	Laboratory acquired infection
1977–8	Tandala, D.R. Congo	1/1	Child infected
1979	Nzara and Yambio, Sudan	22/34	Amplified by nosocomial transmission in hospital
1994	Taï Forest, Côte d'Ivoire	0/1	Worker infected after autopsy of chimpanzee – repatriated to Switzerland
1994	Gabon	31/52	Origin gold mining camps at Mekokou and Andcock. Primarily reported as yellow fever
1995	Kikwit, D.R. Congo	250/315	Included laboratory confirmed cases. Rapid transmission among unprotected health-care workers
1995	Taï Forest, Côte d'Ivoire	0/1	Case originated in Liberia? four additional cases in Liberia
1996 (January-March)	Mayibout, Gabon	21/37	Contact with dead chimpanzees
1996 July & anuary 1997	Booué and Libreville, Gabon	45/60	Index case hunter living in forest area – infected chimpanzees identified
1996	South Africa	1/2	Epidemiologically linked with outbreak in Gabon. Fatal case – nosocomial infection of nurse by patient transferred from Gabon
2000–1	Uganda	224/425	Occurred in Gulu, Misindi and Mbarara districts of Uganda. Spread associated with attending funerals, community nursing and medical care without personal protective measures
2001–2	Gabon	53/65	Community outbreak evidence suggests contact with dead nonhuman primates (gorilla)
2001–2	D.R. Congo	43/57	Outbreak occurred over the border of Gabon with Republic of the Congo
2002–3	D.R. Congo	128/143	Outbreak in Mbomo and Kelle
2003	D.R Congo	29/35	Outbreak in Mbomo and Mbandza villages
2007	D.R. Congo	187/264	Outbreak in Kasai Province
2007–8	Uganda	37/149	Outbreak in Western Uganda
Total mortality rate		**1503/2244 (66.9%)**	

Table 164.7 Recorded filovirus (Ebola (Reston)) infections

Date	Filovirus infections	Documented fatalities/ total cases	Main characteristics
1989	Richmond, Virginia, USA	0/4	Reston strain isolated from monkeys imported from Philippines. Serologic evidence of asymptomatic infection among animal workers
1990	Manila, Philippines	0/12	Serologic evidence of asymptomatic infection among animal workers
1992	Siena, Italy	No cases	Reston strain isolated from monkeys imported from Philippines
1996 (April)	Alice Texas, USA	No cases	Reston strain in monkeys imported from Philippines
1996	Philippines	No cases	Virus identified in primate export facility
2008	Philippines	No cases	Virus identified in pigs
Total mortality rate		**0/16 (0%)**	

assay has been regarded as nonspecific and unreliable. A rising antibody in paired serum or a high IgG titer (>64) and the presence of IgM antibody together with clinical symptoms compatible with hemorrhagic fever are consistent with a diagnosis of filovirus infection.

Filoviruses can be readily isolated from fresh or stored (–94°F) specimens of blood or serum collected during the acute phase of illness. Vero cells (clone E6) and MA104 have proved the most sensitive for the propagation and assay of Marburg and Ebola viruses. As primary isolation using tissue culture rarely produces a specific cytopathic effect, evidence of infection is confirmed by immunofluorescence staining using antivirus-specific monoclonal or polyclonal antibodies. Some strains of Ebola, such as Ebola Sudan, proved difficult to grow in tissue culture and success was improved through interperitoneal inoculation of young guinea pigs. The resultant febrile response coincides with high levels of virus in the blood, which can be easily recovered by tissue culture or examined directly by electron microscopy. The isolation or propagation of filoviruses should not be attempted outside a biosafety containment level 4 laboratory.

During recent epizootic and human African outbreaks, early detection of filovirus infections was considerably improved by the development of an antigen-capture ELISA, amplification of filovirus RNA by RT-PCR, and immunohistochemistry identification of Ebola in skin biopsies. Skin biopsies fixed in formalin can be safely transported to reference facilities for confirmation without the need for low temperature preservation. Recent advances in molecular biology have expressed the NP gene of Ebola virus in a baculovirus expression system. The noninfectious N protein can replace the need for inactivation of infectious virus used in many serologic assays (IFA and ELISA). The diagnostic capabilities of such a system are currently being evaluated.

Management

No specific treatment is available. Treatment is limited to the provision of intensive nursing and effective control of blood volume and electrolyte balance. Shock, renal failure, depletion of blood clotting factors, severe bleeding and oxygen depletion must be managed. Human interferon and human convalescent plasma have been used to treat patients, but their efficacy is not proved. Ribavirin has no therapeutic value.

Patient management is further complicated by the need to isolate the patient and protect medical and nursing staff.[67,71] Use of patient isolators is a requirement in many countries, whereas strict barrier nursing techniques are acceptable in others. The latter can be supplemented using high efficiency particulate air filter respirators for protection against aerosols if considered feasible. Particular emphasis should be placed on ensuring that high-risk procedures (such as handling blood, secretions, catheters and introducing intravenous lines) are undertaken under barrier nursing conditions. It is recommended that patients who die from the disease should be buried or cremated promptly.

NIPAH AND HENDRA VIRUSES

Epidemiology

Both Hendra virus (HeV; formerly called equine morbillivirus) and Nipah virus (NiV) are newly recognized hazard group 4 zoonotic viruses.

Two outbreaks of a new zoonotic disease affecting horses and animals in Australia were reported within 1 month of each other in Brisbane (southeast Queensland) and Mackay (central Queensland) in 1994. A third event involving a single fatal equine case occurred near Cairns (North Queensland) in 1999. To date, two humans and 16 horses have died from this disease with acute respiratory failure.[77–79] Within the subfamily Paramyxovirinae, the extent of nucleotide homology in the N gene between different viruses in the same genus ranges from 56% to 78%, whereas the extent of nucleotide similarity between viruses from different genera is 39–78%. The N genes of HeV and NiV have 78% nucleotide homology, but the two viruses have no more than 49% similarity with any other members of the Paramyxovirinae.[80] Phylogenetic analysis of the N gene sequences show that HeV and NiV form a distinct cluster within the subfamily Paramyxovirinae. Both NiV and HeV are identified as henipaviruses, a new family of emerging paramyxoviruses.[80]

Another member of the Paramyxoviridae (NiV) was responsible for an outbreak of severe febrile encephalitis in humans in Malaysia in 1998–9. The outbreak was associated with respiratory illness in pigs and was initially considered to be Japanese encephalitis. Of a total of 256 cases, 105 were fatal. In March 1999, a cluster of 11 cases (one fatality) was described in Singapore in abattoir workers who handled pigs imported from the outbreak regions in Malaysia.[81] Measures to control the concurrent outbreak of respiratory disease in pigs resulted in the culling of over one million pigs (almost half the national pig herd) and had major domestic and international trade implications.

Seven outbreaks of NiV also occurred in Bangladesh between 2001 and 2008, the last outbreak affecting two villages in the Rajbari district,[82] 70 km west of the city of Dhaka. Of the 12 identified cases, there were 10 deaths, resulting in a case fatality rate of 83%.[83] The original outbreaks in 2001 and 2003 involved primary men and women more than 25 years of age; the last outbreak involved boys, 15 years of age. Further NiV infections were identified in Siliguri, West Bengal, India.[84]

The geographic distribution of both HeV and NiV is currently undefined although Australia and South East Asia are considered to be endemic areas on the basis of the known incidents reported in the literature. Fruit bats of the genus *Pteropus* are considered to be the reservoir hosts of HeV and NiV[85] and are found in north, east and southeast areas of Australia, Indonesia, Malaysia, Philippines, the Indian

subcontinent and some of the Pacific Islands.[86] Pigs were the apparent source of infection among most human cases in the Malaysian outbreak of NiV, but other sources, such as infected dogs and cats, cannot be excluded.[87,88] Unpublished laboratory data from Bangladesh have not supported the presence of an intermediate or primary reservoir host other than *P. giganteus*. Available data suggest that direct transmission of NiV to humans is through contact with bat secretions (saliva, urine, guano, partially eaten fruit) during fruit-tree climbing.[89]

Virology

Nipah and Hendra are closely related and difficult to distinguish serologically, although they have a diverse geographic distribution.[79,90] Both NiV and HeV share a nonsegmented, negative-stranded RNA genome and similar genome organization, replication strategy and domain structure in the polymerase proteins to members of the Filoviridae, Paramyxoviridae, Rhabdoviridae and Bornaviridae. While being related to these families they are closely related to the Paramyxoviridae and more specifically the morbilliviruses. The genome organization of NiV and HeV is the same as for the rest of the Paramyxoviridae. There are a total of six transcription units encoding six structural proteins. These are the nucleocapsid protein (N), phosphoprotein (P), matrix protein (M), fusion protein (F), glycoprotein (G) or attachment protein, and large protein (L) or RNA polymerase. They are found on the genome in the order 39-N-P-M-F-G-L-59. Evidence suggests that NiV and HeV form a distinct group of viruses within the subfamily Paramyxovirinae.[90]

Clinical features

The emergence of NiV and HeV has raised clinical concerns in the management of individuals returning from Australia and South East Asia presenting with pyrexia and other nonspecific signs and symptoms. The incubation period for NiV and HeV is generally from 4 to 18 days. Onset of disease is usually influenza-like with high fever and myalgia. Sore throat, dizziness, and drowsiness and disorientation have been described. The case fatality rate for clinical cases is about 50% and subclinical infections may be common.[91]

Experimental studies have confirmed the possibility of transmission through close contact with infected body fluids and that aerosol transmission does not seem to be significant. Human-to-human transmission has been reported in a study within the Bangladesh community[92] and the risk of transmission from horse to human has not been established. The mode of transmission from animal to animal, and from animal to human is uncertain.

Laboratory diagnosis

Virus isolation has been an important primary diagnostic tool in early outbreaks.

Both NiV and HeV grow well in Vero cells. A cytopathic effect (CPE) usually develops within 3 days but two 5-day passages are recommended before virus isolation is considered to be unsuccessful. However, isolation should only be attempted in a containment level 4 laboratory.

Virus neutralization tests are considered to be the reference standard in the serologic identification of NiV and HeV infection. This procedure needs to be carried out at a containment level 4 laboratory. Sera are added into the media covering virus-inoculated Vero monolayers in a 96-well plate format. Positive results are demonstrated by the inhibition of CPE production by sera.[93]

To reduce the dependence on high containment facilities (CL4), both the indirect IgG and capture IgM ELISA NiV and HeV systems can use irradiated viral antigens purified from cell culture. The level of sample processing involved to make the antigen safe to use outside Advisory Committee on Dangerous Pathogens (ACDP) 4 containment reduces the specificity and sensitivity of the assay.

NiV-specific primers that amplify a 228 bp segment of the N gene region of the virus will form the basis of the RT-PCR detection system that has proven of diagnostic value when used in conjunction with specimens (sera, tissue from humans and animals) implicated in HeV and NiV outbreaks.[94] In addition, another published primer pair that amplifies a 200 bp region of the matrix (M) protein will form a proven alternative primer set for the RT-PCR detection of HeV originally designed by Halpin *et al*.[85]

Management

As there are neither vaccine nor antivirals for treatment and because the viruses are potentially able to transmit by the aerosol route, [95] both HeV and NiV have been classified as hazard group 4 agents. Working with these agents requires the use of ACDP containment level 4 laboratories. Therefore, with the increasing movement of horses and travelers to endemic areas, both clinicians and veterinarians have increasingly expressed a need to consider these infections as part of the current differential diagnosis. Care in the management of patients should be exercised as NiV has been isolated in the respiratory secretions and urine of patients identified as having Nipah virus encephalitis in Malaysia[94] and nosocomial risks identified in a Bangladesh hospital.[95]

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Chapter 165

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Staphylococci and micrococci

GENERAL CLASSIFICATION

Classification

Historically, based on morphologic similarities, the genera *Staphylococcus*, *Micrococcus*, *Planococcus* and *Stomatococcus* were placed together in the family Micrococcaceae. More recently, however, based on DNA/rRNA analysis and guanine–cytosine (GC) content, the genus *Staphylococcus* has been classified together with the genera *Bacillus*, *Brochothrix*, *Gemella*, *Listeria* and *Planococcus*, in the family Bacillaceae of the broad *Bacillus–Lactobacillus–Streptococcus* cluster of Gram-positive bacteria with a low GC-content.

The genus *Micrococcus* currently contains three species, *M. luteus*, *M. lylae* and *M. antarcticus*,[1,2] and resides in the family Micrococcaceae of the order of the Actinomycetales;[3] a fourth species, *M. flavus*, has been proposed. *Stomatococcus mucilaginosus*, the sole species in the former genus *Stomatococcus*, was recently reclassified as a member of the genus *Rothia* (family of the Micrococcaceae) and renamed *Rothia mucilaginosa*;[4] *R. dentocariosa* is the only other *Rothia* spp. isolated from humans.

Staphylococci, in particular *Staphylococcus aureus*, are frequent causes of infection in humans. Next to *S. aureus*, the foremost pathogenic staphylococcal species are *S. lugdunensis*, *S. schleiferi*, *S. epidermidis*, *S. haemolyticus* and *S. saprophyticus*. *S. lugdunensis* and *S. schleiferi* may cause infections similar to those caused by *S. aureus*, including abscesses, endocarditis and wound infections. *S. epidermidis* is primarily responsible for foreign body infections, and both prosthetic and native valve endocarditis. *S. saprophyticus* is a cause of urinary tract infections. Multiple animal species can be colonized or infected by different staphylococcal species. *S. hyicus* is the main causative agent of infectious dermatitis and arthritis in swine, *S. aureus* causes bovine mastitis, and has also been reported in pigs, pigeons, cats and dogs. *S. intermedius* causes infections in dogs, foxes, mink, pigeons and horses.

This chapter will focus mainly on the staphylococci. *R. mucilaginosa* and *Micrococcus* spp. colonize humans but rarely cause disease; these bacteria will be briefly discussed. *R. dentocariosa* is discussed in Chapter 167.

Staphylococci

NATURE

History

The name 'staphylococcus' (derived from the Greek σταφυλη, a bunch of grapes) was introduced by Alexander Ogston, a Scottish surgeon who in 1881 described the presence of grape-like clusters of spherical micro-organisms in pus from abscesses.[5] In a series of laboratory experiments and clinical observations he subsequently described staphylococcal disease and its role in sepsis and abscess formation. The first to isolate and culture staphylococci was the German surgeon Friedrich Rosenbach. Rosenbach distinguished two different species of staphylococci based on colony color: a species with yellow/orange/golden colonies which he named *Staphylococcus aureus*, and a species with white colonies which he called *Staphylococcus albus* that was later renamed *Staphylococcus epidermidis*.

Staphylococcal infections appeared to play a major role in wound infections. As the cause of postinfluenza necrotizing pneumonias, *S. aureus* was considered responsible for many deaths during the Spanish influenza pandemic of 1917–1918. It is estimated that half of the casualties in the trenches of the First World War were due to septic wound infections with *S. aureus*. Such was the impact of *S. aureus* infections on military campaigns that during the Second World War the production process for penicillin (then still universally active against the bacterium) was considered a military secret by the allied forces. Even now, in the antibiotic era, *S. aureus* continues to be a major community-acquired pathogen and the single most relevant cause of nosocomial infections.

Other staphylococci, mainly *S. epidermidis*, are important pathogens in immunocompromised patients and patients with prosthetic materials and indwelling catheters.

Microbiology

Staphylococci are Gram-positive spherical bacteria about 1 micrometer in diameter, which divide in two planes and, therefore, grow in clusters; they are nonmotile, nonspore-forming, and have a genome size of between 2000 and 3000 kbp, with a 30–39% GC-content. In 2007, 39 species were recognized within the genus *Staphylococcus*, 17 of which were isolated from humans; new species continue to be discovered (http://www.bacterio.cict.fr) (Fig. 165.1). Most staphylococcal species demonstrate catalase activity and are facultatively anaerobes. Further characteristics of the genus include susceptibility to furazolidone, resistance to bacitracin, and production of acid from glucose under anaerobic conditions or in the presence of erythromycin.

The main constituents of the staphylococcal cell wall are peptidoglycan, which constitutes 50% of the dry cell mass (Fig. 165.2), and teichoic acid (40% of the dry cell mass). The glycan chains of the peptidoglycan layer are built with approximately 10 alternating subunits of *N*-acetylmuramic acid and *N*-acetylglycosamine. Pentapeptide side chains are attached to the *N*-acetylmuramic acid subunits; the glycan chains are then cross linked with peptide bridges between the side chains. The teichoic acids are macromolecules of phosphate containing polysaccharides (Fig. 165.3). Teichoic acid is bound both to the peptidoglycan layer and to the cytoplasmic membrane. The polysaccharides are species specific; ribitol teichoic acids are present in *S. aureus* cell walls and glycerol teichoic acids are present in *S. epidermidis*.

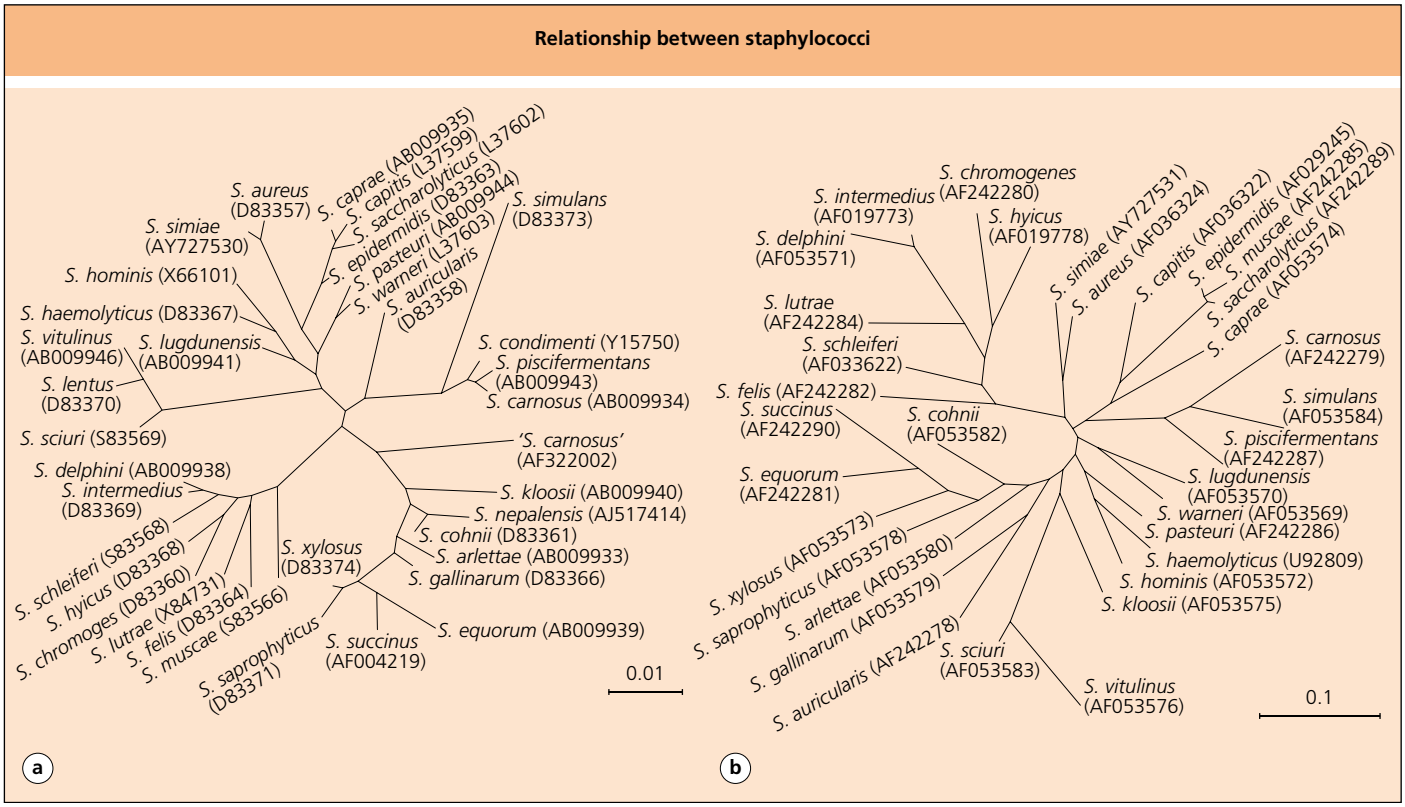


Fig. 165.1 Unrooted phylogenetic trees constructed on the basis of 16S rRNA gene sequences, showing the relationship between staphylococci. The GenBank accession numbers of the sequences of the organisms used are shown in parentheses. Redrawn from Pantcek R, Sedlácek I, Petrás P, *et al*. *Staphylococcus simiae* sp. nov., isolated from South American squirrel monkeys. Int J Syst Evol Microbiol 2005;55:1953–8.

PATHOGENICITY OF *STAPHYLOCOCCUS AUREUS*

The broad spectrum of diseases caused by *S. aureus* is associated with its production of many surface-bound and extracellular virulence factors. The bacterium has a large variety of molecules that specifically counteract the host defense mechanisms, and it excretes a number of toxins with membrane damaging, pyrogenic or epidermolytic actions (Table 165.1).

Adherence to host cells and tissues

Staphylococcus aureus expresses a number of adhesion molecules that facilitate interactions with host cells and extracellular matrix (ECM) components allowing effective colonization. These 'microbial surface components recognizing adhesive matrix molecules' (MSCRAMMs) are surface-anchored molecules that bind host molecules like collagen, laminin, fibronectin, elastin, vitronectin and fibrinogen.[6] The collagen-binding adhesin, for instance, recognizes collagen type I and IV and was shown to play an important role in the pathogenesis of septic arthritis induced by *S. aureus*. MSCRAMMS are also involved in the attachment of *S. aureus* and *S. epidermidis* to foreign body materials and indwelling devices: coating of the biomaterials with a mixture of host proteins and platelets subsequently leads to biofilm formation[7] (Fig. 165.4).

Blocking host defenses

The immune response against *S. aureus* largely depends on the innate immune system: antimicrobial peptides, the complement system and phagocytes. The bacterium, in response, produces highly specific, small, soluble proteins that enable it to suppress the innate immunity and survive in the human body.

Resistance to antimicrobial peptides

In response to infectious stimuli, skin keratinocytes, mucosal epithelial cells and neutrophils produce high levels of antimicrobial peptides (AMPs) known as cathelicidins (LL-37) and defensins. The *S. aureus* metalloproteinase aureolysin cleaves LL-37, while staphylokinase (SAK) inhibits the bactericidal effect of α-defensins.[8] Furthermore, modification of cell wall teichoic acids promotes *S. aureus* resistance to AMPs.[9]

Complement evasion

The complement cascade serves three major functions in innate immunity:

- to opsonize bacteria (through C3b) (Fig. 165.5);
- to attract phagocytes (through C3a and C5a); and
- to perturb bacterial membranes of Gram-negative bacteria (C5b–9, the membrane attack complex).[10,11]

Complement activation is initiated by three different pathways (classical, lectin or alternative) that result in the formation of C3 convertases, bimolecular enzymes that cleave the central complement protein C3 (Fig. 165. 6). The C3 cleavage product C3b covalently binds to the bacterial surface and is recognized by phagocytic cells expressing complement receptors. Furthermore, C3b associates with C3 convertases to form a C5 convertase that cleaves C5 into C5a (a potent chemoattractant) and C5b (part of the membrane attack complex). *S. aureus* produces a variety of molecules that interfere with the complement cascade:

- the secreted staphylococcal complement inhibitor (SCIN) blocks C3 convertases through stabilization of these complexes;[12]

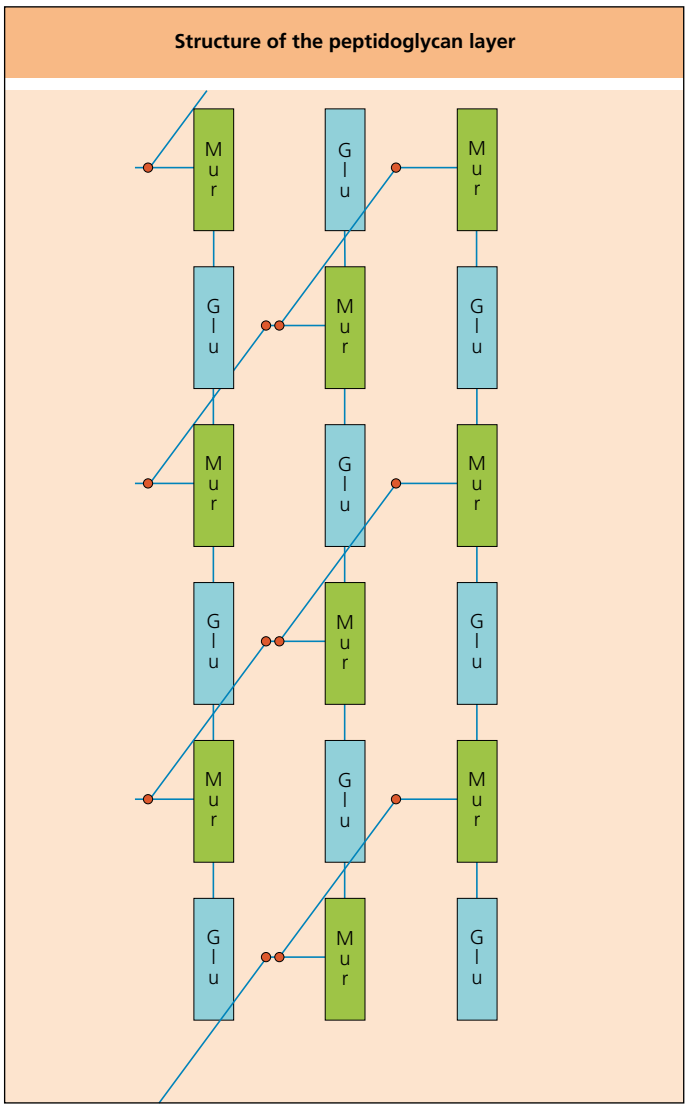


Fig. 165.2 Structure of the peptidoglycan layer. The peptidoglycan layer consists of three integral parts. The glycan chains are built with 10–12 alternating *N*-acetylglucosamine (Glu) and *N*-acetylmuramic acid (Mur) subunits jointed with β-1,4 glycosidic bonds. Vertical pentapeptide side chains are linked to the muramic acids subunits, and the side chains are in turn cross linked with diagonal intrapeptide bridges. For example, the glycan chains in *Staphylococcus aureus* are crosslinked with pentaglycine bridges attached to L-glycine in one pentapeptide chain and D-alanine in an adjacent chain. Courtesy of Jan Verhoef, Ad C Fluit and Franz-Josef Schmitz.

- extracellular fibrinogen binding protein (Efb) and extracellular complement binding protein (Ecb) target surface-bound C3b-containing convertases,[13] inhibiting C3b deposition via the alternative pathway and C5a formation via all pathways;
- the staphylococcal cell wall-associated protein Sbi has two extracellular complement-inhibitory domains that bind C3; and
- staphylococcal superantigen-like (SSL) protein 7 specifically binds to C5, preventing cleavage by C5 convertases and subsequent formation of C5a.[14]

Inhibition of neutrophil recruitment

Effective eradication of *S. aureus* depends on phagocytosis and intracellular killing by cells of the monocyte and granulocyte lineage, mainly neutrophils.[15] This critical role is reflected by the increased risk for *S. aureus* infections in patients with defects in granulocyte function, both inherited (e.g. chronic granulomatous disease, myeloperoxidase deficiency, leukocyte adhesion deficiencies) and acquired (e.g. diabetes mellitus, rheumatoid arthritis, HIV). During an infection, neutrophils are rapidly recruited from the circulation to sites of microbial invasion by host stimuli (complement fragments C3a and C5a, interleukin 8, leukotriene B4) and pathogen-derived stimuli (fMLP, phenol-soluble modulins).[16] These chemotactic factors activate neutrophils, increase vascular permeability and induce expression of adhesion molecules on endothelial cells. Neutrophils express selectins and integrins that bind these adhesion molecules; the cells start to roll on the endothelial lining and firmly adhere to it.[17] Subsequently, the neutrophils migrate through the endothelial cell layer (diapedesis) and move towards the site of infection under a gradient of chemoattractant substances, the foremost being C5a (chemotaxis).

The staphylococcal superantigen-like 5 (SSL5) inhibits the interaction between P-selectin on endothelial cells and P-selectin glycoprotein ligand-1 (PSGL-1) on neutrophils, thereby inhibiting neutrophil rolling on the endothelium.[18] Extracellular adherence protein (Eap) of *S. aureus* inhibits the next steps in neutrophil extravasation by its interactions with the intercellular adhesion molecule 1 (ICAM-1), fibrinogen or vitronectin.[19] Finally, *S. aureus* secretes two molecules that specifically block chemotactic movement of phagocytes: the chemotaxis inhibitory protein of *S. aureus* (CHIPS) which binds the formylated peptide receptor and the C5a receptor,[20] and the FPRL1 inhibitory protein (FLIPr) which binds the formylated peptide receptor-like 1.[21]

Resistance to phagocytosis and intracellular killing

Neutrophil ingestion of opsonized micro-organisms in phagosomes depends on the interaction between opsonic ligands on the bacterium and receptors on the phagocyte membrane. Upon bacterial uptake, the interaction of opsonic ligands with receptors triggers the release of oxidative products and granule contents (e.g. myeloperoxide, defensins) into the phagosome, destroying the ingested particle. *S. aureus* is most efficiently phagocytosed after opsonization by both complement and antibodies.

Staphylococci are often surrounded by a loose-fitting polysaccharide capsule (Fig. 165.7) that interferes with opsonization, either by shielding the cell wall from reacting with antibodies and complement, or by hindering the binding of surface-bound complement factors to phagocyte receptors. Effective opsonization of encapsulated *S. aureus* therefore requires anticapsular antibodies, largely directed against the O-acetyl group of the capsular polysaccharide. Eleven putative capsular serotypes have been proposed; expression of type 5 and type 8 capsules (75% of all capsulated strains) is associated with increased virulence in animal infection models.[22]

Staphylokinase (SAK), a plasminogen-activating molecule, generates surface-bound plasmin. This, in turn, cleaves the major opsonins IgG and C3b, preventing their recognition by phagocytes. The *S. aureus* surface protein A (SpA) binds the Fc terminal of human IgG, covering the bacterial surface with outward-facing IgG molecules that cannot be recognized by Fc receptors on phagocytes (Fig. 165.8). SSL7 interacts with human IgA1 and IgA2 to interfere with IgA-dependent cellular effector functions.[23]

Once phagocytosed, staphylococci may inhibit killing and travel through the bloodstream within neutrophils. The golden pigment (for which *S. aureus* is named) is a carotenoid molecule with antioxidant properties that scavenges free oxygen radicals.[24] Furthermore, *S. aureus* can resist oxidative stress by two superoxide dismutase enzymes that remove superoxide.

Cytolytic toxins

Cytotoxins secreted by *S. aureus* lyse host cells by forming β-barrel pores in cytoplasmic membranes of target cells. Well-known examples are α-toxin and the bi-component leukotoxins γ-toxin and the exfoliative toxins (ETA and ETB) that cause separation of the

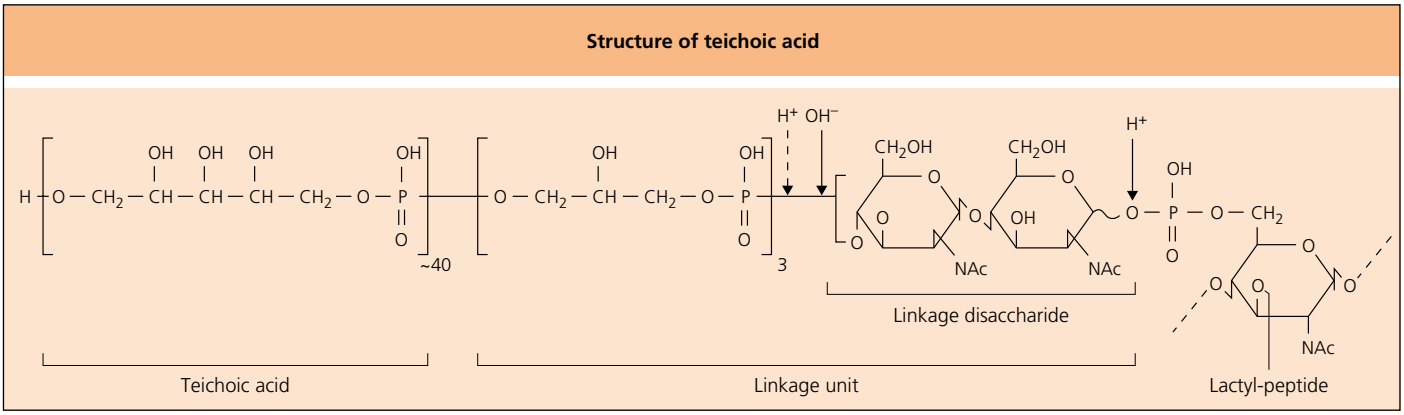


Fig. 165.3 Structure of teichoic acid and linkage unit attaching teichoic acid to peptidoglycan in *Staphylococcus aureus.* The C2 and C4 positions of the ribitol residues are substituted by D-alanyl and *N*-acetylglucosamine residues. Adapted with permission from Crossley KB, Archer GL, eds. The staphylococci in human disease. New York: Churchill Livingstone; 1997.

Table 165.1 Virulence factors of *Staphylococcus aureus*

Immune evasion mechanisms		
Virulence factor	**Acronym or gene**	**Activity**
Clumping factor (bound coagulase)	ClfA, ClfB	Binds fibrinogen, coating the bacterial cell and inhibiting phagocytosis
Chemotaxis inhibitory protein of *S. aureus*	CHIPS	Downregulates the C5a receptor and the formylated peptide receptor (FPR) on neutrophils; inhibits chemotaxis
Extracellular adherence protein	EAP	Binds to ICAM-1, fibrinogen, vitronectin Blocks leukocyte adhesion, diapedesis and extravasation
Extracellular fibrinogen-binding protein/ extracellular complement binding protein	Efb/Ecb	Binds to the C3d part of C3 molecules, inhibiting C3b-containing convertases Efb also binds fibrinogen
Staphylococcal complement inhibitor	SCINSCIN-C	Binds and stabilizes C3 convertases, inhibiting C3b deposition and phagocytosis
Staphylokinase	SAK	Activates human plasminogen at the bacterial surface, leading to degradation of IgG and C3b; inhibits bactericidal effect of α-defensins
Polysaccharide capsule		Antiphagocytic function
FLPR1 inhibitory protein	FLIPr	Impairs neutrophil responses to formylated peptide receptor-like-1 agonists
Polysaccharide intercellular adhesin	PIA	Holds multilayered cell complexes that form biofilms together, decreases susceptibility to defensins
Catalase		Inhibits bacterial killing by inactivating hydrogen peroxidase and free radicals formed by the myeloperoxidase system within phagocytic cells
Protein A	SpA	Binds Fc part of human IgG and prevents phagocytic uptake by Fc receptors; stimulates B lymphocytes
Free coagulase	*coa*	Coats the bacterial cell with fibrinogen and inhibits phagocytosis
Staphylococcal superantigen-like 5	SSL5	Binds PSGL-1 and inhibits P-selectin-mediated neutrophil rolling
Staphylococcal superantigen-like 7	SSL7	Binds IgA and blocks FcαRI-mediated responses. Binds C5 and blocks C5 cleavage into C5a and C5b
Aureolysin		Metalloproteinase that cleaves LL-37
Staphylococcal immunoglobulin-binding protein	SBI	Binds IgG and C3. Blocks complement activity

(Continued)

Table 165.1 Virulence factors of *Staphylococcus aureus*—cont'd

Invasion mechanisms		
Virulence factor	**Gene**	**Activity**
α-Hemolysin	*hla*	Multimerizes on eukaryotic membranes to form lytic pores
β-Hemolysin	*hlb*	Sphingomyelinase; damages eukaryotic cell membranes containing sphingomyelin by enzymatic alteration of their lipid content; causes lysis of sheep erythrocytes on blood agar
γ-Hemolysin	*hlgA, hlgB*	Consists of two proteins which assemble to form membrane-perforating complexes; toxic to PMNLs, monocytes and macrophages, lytic for red blood cells
Panton–Valentine leukocidin	*lukS (lukS-PV, lukF-PV)*	Homologue of γ-hemolysin encoded by mobile phage, lytic to leukocytes; associated with furunculosis and necrotizing pneumonia
Leukocidin E-D	*LukED*	Lytic to leukocytes
δ-Hemolysin	*hld*	Variety of attributed actions: multimerizes on eukaryotic membranes to form lytic pores; possible mediator of staphylococcal membranous enterocolitis; increases vascular permeability in guinea pig skin, inhibits water absorption and activates adenylate cyclase; activation of membrane phospholipase A_2, stimulation of prostaglandin synthesis, release of lysozyme and β-glucuronidase from PMNL granules, activation of the acetyltransferase
Exfoliative toxins	*eta, etb*	Toxins with protease activity, sequence similarity to serine protease of *S. aureus*; epidermolytic effect on stratum granulosum of the keratinized epidermis; causes staphylococcal scalded skin syndrome
Fibrinolysins		Break down fibrin clots
Hyaluronidase	*hysA*	Hydrolyzes intercellular matrix of mucopolysaccharides
DNAse/thermonuclease	*nuc*	Hydrolyzes RNA and DNA, frees nutrients
Lipase	*geh*	Facilitates spread in subcutaneous tissues; associated with furunculosis
Superantigens/pyrogenic exotoxins		Stimulate T cells nonspecifically to cytokine release
Enterotoxins A, B, C, D, E, G, H, K (and others)	*sea, seb, sec, sed, see, seg, seh, sek*	Cause staphylococcal food poisoning and half of the cases of nonmenstrual toxic shock syndrome (TSS)
Toxic shock syndrome toxin	TSST-1/*tst*	Responsible for about 75% of cases of TSS, including all cases of menstrual TSS
Microbial surface components recognizing adhesive matrix molecules		
MSCRAMM	**Gene**	**Activity**
Fibronectin-binding protein	*fnbpA*, *fnbpB*	Binds fibronectin, fibrinogen and elastin
Collagen-binding protein	*cna*	Binds collagen/cartilage
Clumping factor	*clfA, clfB*	Binds fibrinogen

ICAM-1, intercellular adhesion molecule 1; PMNLs, polymorphonuclear leukocytes; PSGL-1, P-selectin glycoprotein ligand-1.

dermis at the granular cell layer resulting in extensive scalding (staphylococcal scalded skin syndrome).

Panton–Valentine leukocidin (PVL) is a cytotoxin associated with furunculosis and hemorrhagic pneumonia.[15] In the last decade community-acquired infections, mainly skin and soft tissue infections, have been increasingly caused by PVL-positive community-acquired methicillin-resistant *S. aureus* (MRSA) strains (CA-MRSA). Although PVL is found in 69–98% of CA-MRSA clinical isolates,[25] the exact role of PVL in the pathogenesis of these infections is not completely clear and studies of its pathogenicity in animal models have yielded conflicting results.

Phenol-soluble modulins are a class of secreted staphylococcal peptides that recruit, activate and subsequently lyse human neutrophils, thus eliminating the main cellular defense against *S. aureus* infection. A recent study revealed a contribution of these factors to the virulence of CA-MRSA.[16]

Immunostimulatory molecules

Superantigens (or pyrogenic exotoxins) are the agents responsible for toxic shock syndrome (TSS) (Fig. 165.9). These extracellular proteins bind to the exterior surface of major histocompatibility complex (MHC) class II molecules on antigen-presenting cells (APCs), and link them to receptors on the surface of T-helper cells, activating them without the need for antigen presentation by the APCs. Up to a third of the T cells may be stimulated to proliferate and release cytokines. Due to this nonspecific activation of T cells, the immune response

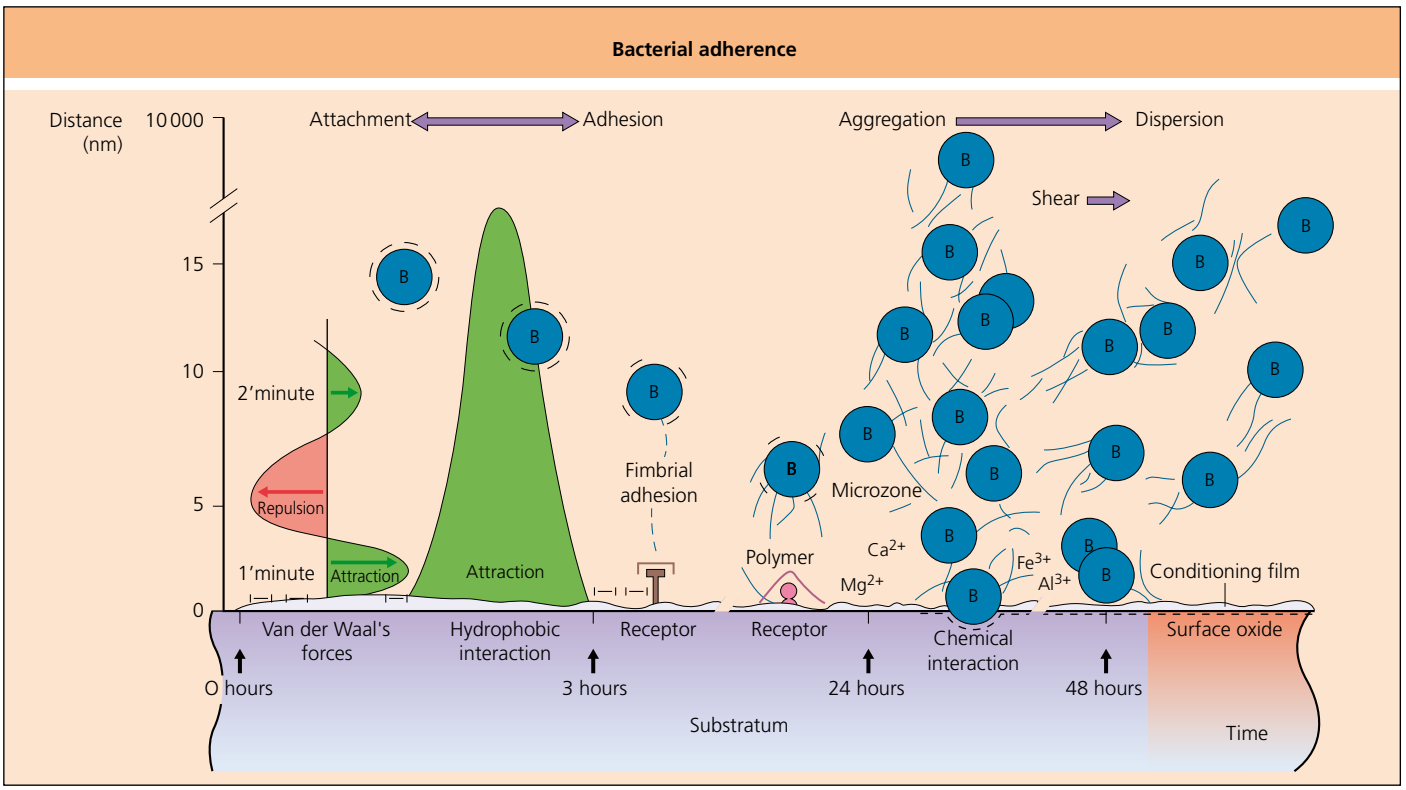


Fig. 165.4 Bacterial adherence. The figure illustrates the events associated with bacterial (B) adherence to a biomaterial in relation to time and the molecular sequence in bacterial attachment, adhesion, aggregation and dispersion at substratum surface. A number of possible interactions may occur depending on the specificities of the bacteria or substratum system, the distance from the biomaterial and the stage of adherence. The attachment stage is mediated by nonspecific forces. Adhesion is driven by specific adhesin–receptor interactions. The final aggregative step results in a bacterial macrocolony on the biomaterial surface in which the bacteria are firmly adherent to the biomaterial and each other. Bacterial exopolysaccharide blankets the macrocolony and may serve to improve the nutritional microenvironment and protect the bacteria from host defenses. In the dispersion phase, bacteria disaggregate, break loose from the macrocolony and drift free into the bloodstream. Adapted with permission from Gristina AG. Biomaterial centered infection: microbial adhesion versus tissue integration. Science 1987;237:1588.© 1987 American Association for the Advancement of Science.

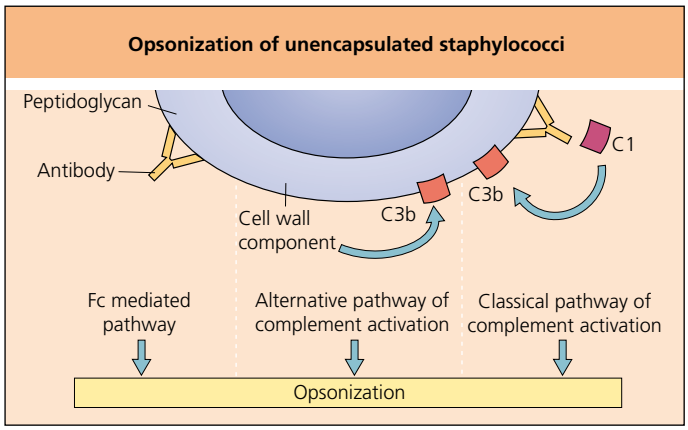


Fig. 165.5 Opsonization of unencapsulated staphylococci. Opsonization through complement activation is primarily a function of C3b and iC3b. When antibody (ab) molecules bind to antigen, the antigen–antibody complex activates the first complement component, C1. C1 is then converted into an esterase, initiating the classical pathway. Additionally, some cell-wall components can activate the alternative pathway. Courtesy of Jan Verhoef, Ad C Fluit and Franz-Josef Schmitz.

against superantigenic toxins is impaired and patients suffering TSS often lack specific antibodies against superantigens.[15]

Toxic shock syndrome toxin 1 (TSST-1) causes most cases of TSS, including all cases of tampon-associated TSS; approximately one-fourth of the cases are caused by enterotoxins. Apart from their superantigenic activity, when ingested orally the enterotoxins may also cause gastrointestinal disease (*S. aureus* food poisoning), characterized by emesis with or without diarrhea. The target responsible for initiating the emetic reflex is located in the abdominal viscera, where putative (unidentified) cellular receptors for the enterotoxins exist. Staphylococcal protein A (SpA) binds to the surface of B lymphocytes, where it also exerts a potent immunostimulatory activity.[26]

Interactions with the coagulation system

S. aureus produces extracellular coagulase which binds to prothrombin to form a complex called staphylothrombin, thereby activating the protease activity of thrombin. The activated thrombin converts fibrinogen to fibrin, causing localized clotting and shielding the bacteria from host defenses.[27] In addition, most strains express a fibrin/fibrinogen binding protein (clumping factor) which promotes attachment to blood clots and traumatized tissue.

Genetic location and regulation of virulence factors

S. aureus virulence factors can be chromosomally encoded and uniformly present, or located on mobile genetic elements such as insertion sequences, bacteriophages, plasmids, transposons and pathogenicity islands. The genes for exfoliative toxins A and B are located on a bacteriophage and a plasmid respectively, and have been demonstrated in 0–2% of strains. PVL is located on a bacteriophage and was present in only 2% of isolates. The pathogenicity island harboring TSST-1 is found in 14–24%. The immune modulators CHIPS, SCIN,

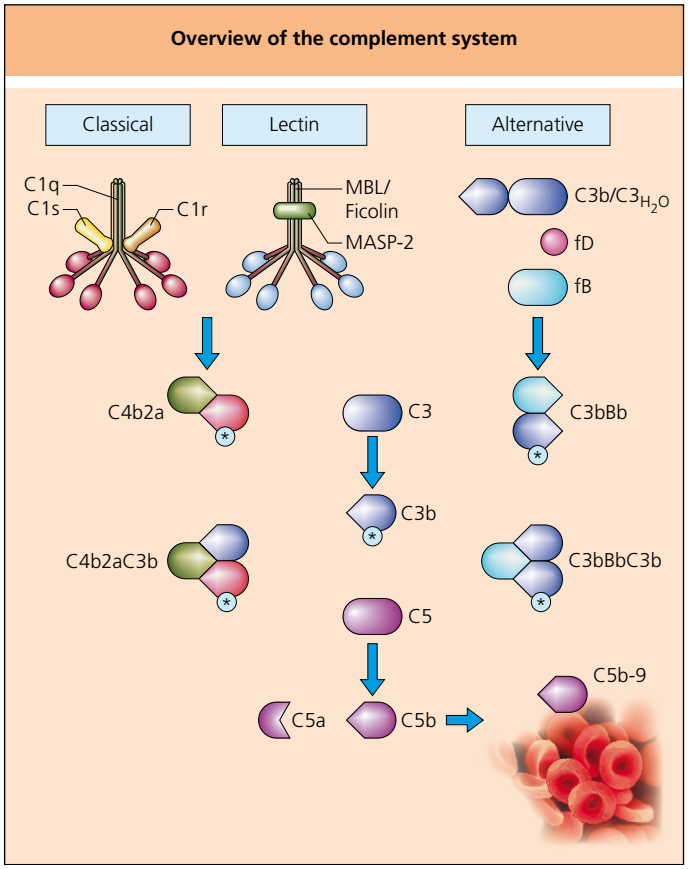


Fig. 165.6 Schematic overview of the complement system. Complement activation can occur via three different pathways. The antibody-dependent classical pathway starts when C1q in the C1q–C1r_2–C1s_2 complex recognizes antibodies that are bound to the microbial surface. In the lectin pathway, mannose binding lectin (MBL) and ficolins recognize microbial sugar patterns and activate the MBL-associated serine protease 2 (MASP-2). Both C1s and MASP-2 can cleave complement proteins C4 and C2 to generate the CP/LP C3 convertase, C4b2a. Within this complex, C4b is covalently (*) attached to the microbial surface. The alternative pathway C3 convertase (C3bBb) is generated after binding of factor B (fB) to surface-bound C3b or fluid-phase C3(H_2O). Factor B is subsequently cleaved by factor D (fD) to generate C3bBb. Both C3 convertases C4b2a and C3bBb cleave C3 into covalently bound C3b (*) and an anaphylatoxin C3a. C3b contributes to phagocytosis, antigen presentation and formation of C5 convertases, C4b2a3b and C3bBb3b. C5 convertases cleave C5 into an anaphylatoxin C5a and C5b, which forms a complex with complement proteins C6, C7, C8 and C9 to generate the membrane attack complex (MAC) and mediate microbial lysis.

SAK and SEA are clustered together on a β-hemolysin converting bacteriophage present in 90% of clinical *S. aureus* isolates.[20]

The expression of virulence factors in *S. aureus* is controlled by a complex system of regulatory mechanisms. A well-studied response regulator is the accessory gene regulator (*agr*), a two-component quorum sensing system which, through a positive feedback loop, switches from the preferential expression of surface adhesins during the exponential phase of growth to the expression of exoproteins during the postexponential and stationary growth phases.[28] When bacterial density increases above a certain level, *agr* drives the transcription of RNAIII, an RNA molecule that modulates virulence factor expression both at the transcriptional and translational levels. *Agr* downregulated gene products include protein A (*spa*) and fibronectin-binding protein (*fnb*); the hemolysins, enterotoxins, exfoliatins and phenol-soluble modulins are examples of virulence factors which are upregulated by *agr*. Most other currently known regulatory mechanisms interact at some level with *agr*, either synergistically or reciprocally.[29]

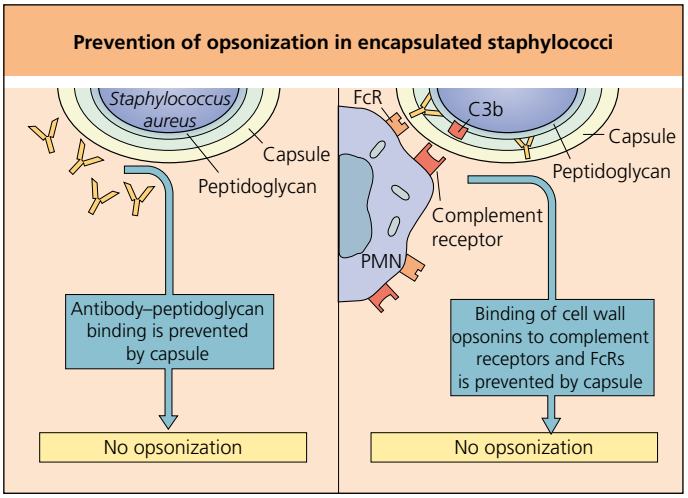


Fig. 165.7 Prevention of opsonization in encapsulated staphylococci. (Left) The capsule of *Staphylococcus aureus* prevents binding of antibodies to peptidoglycan: no opsonization. (Right) The capsule prevents binding of opsonins on the cell wall of *Staphylococcus aureus* to complement and Fc receptors on PMNLs. Courtesy of Jan Verhoef, Ad C Fluit and Franz-Josef Schmitz.

PATHOGENICITY OF *STAPHYLOCOCCUS EPIDERMIDIS*

Most of the pathogenicity studies in coagulase-negative staphylococci (CoNS) have focused on virulence factors involved in foreign-body infections by the most common and relevant species, *Staphylococcus epidermidis*. These infections are characterized by the formation of biofilms: first the bacteria adhere to the foreign body or indwelling device, followed by an accumulation phase in which the bacteria form multilayered cell clusters embedded in extracellular material.

Hydrophobic interactions and Van der Waal's forces play a role in initial adherence of the bacteria to the foreign body (more often than not a polymer), as do a number of surface proteins. On insertion or implantation, the material is rapidly coated with plasma proteins and extracellular matrix proteins (e.g. fibronectin, fibrinogen, vitronectin, von Willebrand factor), providing additional attachment sites. Molecules which mediate attachment to polymers include the staphylococcal surface proteins SSP-1 and SSP-2, the surface-associated autolysin AtlE, biofilm-associated protein (Bap) and the capsular polysaccharide/adhesin (PS/A). Molecules which bind to extracellular matrix proteins include fibrinogen-binding protein (Fbe, a protein with similarity to ClfA in *S. aureus*), cell-wall techoic acid (attachment to fibronectin) and AtlE (binds to vitronectin). A number of factors involved in the accumulation phase have been identified: the polysaccharide intercellular adhesin (PIA), also known as slime-associated antigen (SAA); the capsular polysaccharide/adhesin (PS/A); biofilm-associated protein (Bap); and accumulation-associated protein (AAP).[30] Elastases, proteases, lipases and fatty-acid modifying enzymes have been identified in *S. epidermidis* and are considered possible virulence factors.[30]

EPIDEMIOLOGY AND CLINICAL PRESENTATION OF *STAPHYLOCOCCUS AUREUS*

Epidemiology

In healthy humans, carriage (or colonization) of *S. aureus* may occur on multiple sites of the skin and mucosal surfaces (including the intestine and vagina), the main reservoir being the anterior nares (vestibulum

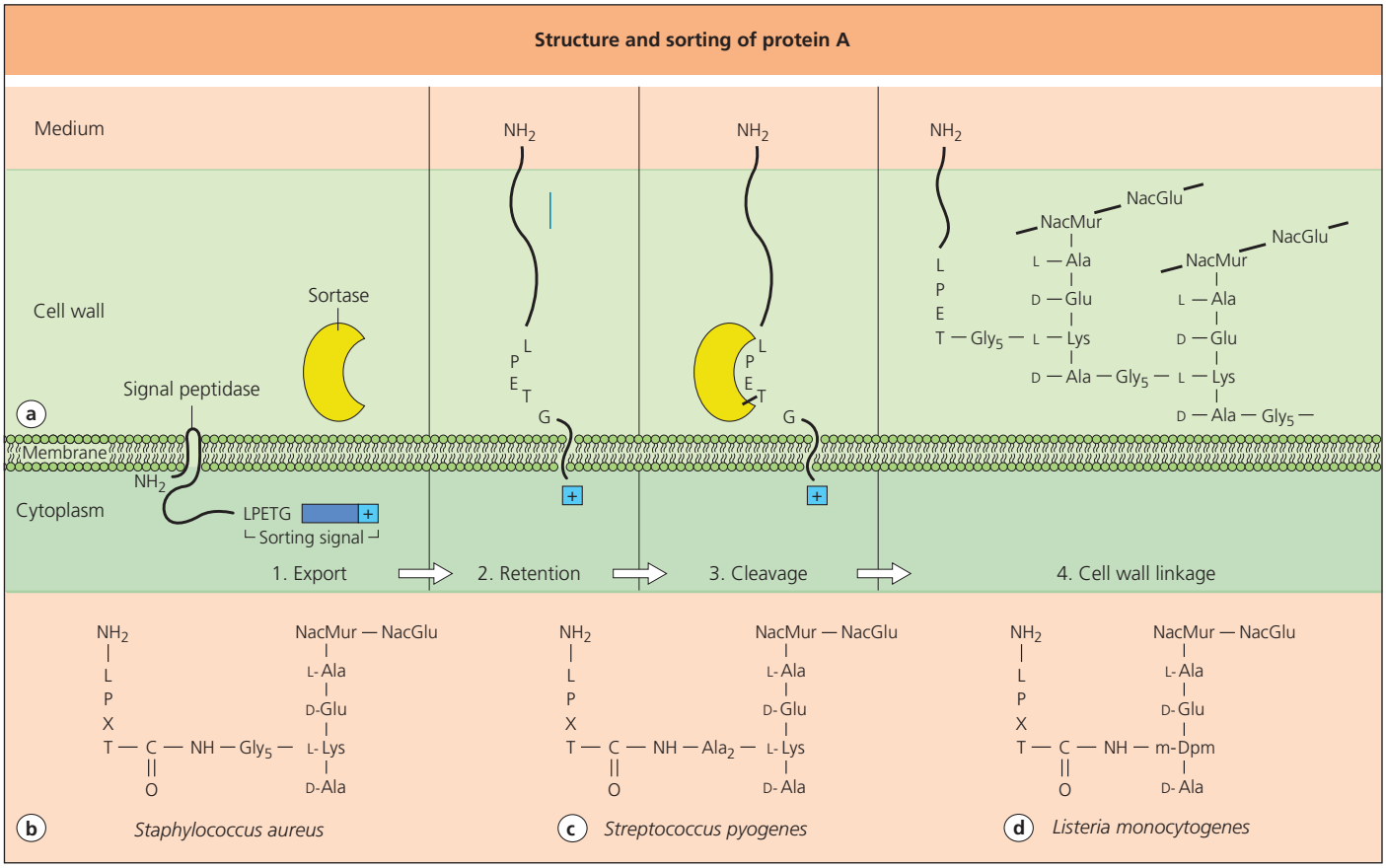


Fig. 165.8 Structure and sorting of protein A. Structure of the cell wall anchor of surface proteins in *Staphylococcus aureus* and other Gram-positive bacteria. (a) Cell wall sorting of surface proteins consists of four distinct steps, which lead to the proteolytic cleavage of the polypeptide chain between threonine (T) and glycine (G). The carboxyl of threonine is subsequently amide-linked to the free amino group in the pentaglycine cross-bridge of the staphylococcal cell wall. The cell wall linkage of surface proteins in *S. aureus* (b) is compared with that proposed for other Gram-positive bacteria such as *Streptococcus pyogenes* (c) and *Listeria monocytogenes* (d). NacGlu, *N*-acetylglucosamine; NacMur, *N*-acetylmuramic acid. Adapted with permission from Crossley KB, Archer GL, eds. The staphylococci in human disease. New York: Churchill Livingstone; 1997.

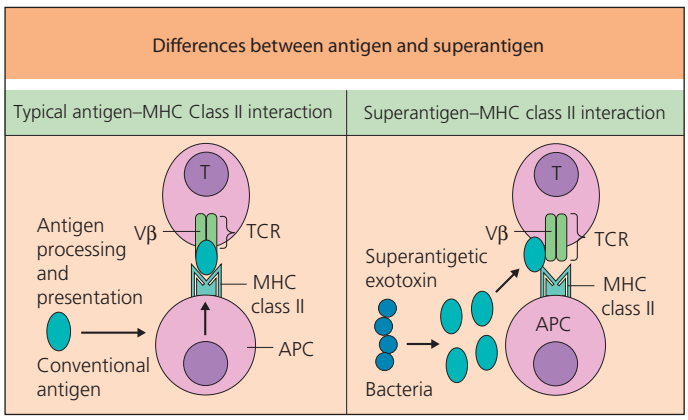


Fig. 165.9 Differences between antigen and superantigen. Staphylococcal enterotoxin and TSST-1 act as superantigens, binding directly to MHC class II and the Vb chains of the T-cell receptor (TCR) without the need for normal antigen processing. Courtesy of Jan Verhoef, Ad C Fluit and Franz-Josef Schmitz.

nasi/nostrils). Generally, the established flora of the nose prevents the acquisition of new strains. Person-to-person spread is believed to occur mainly by direct hand/skin contact, in a hospital setting primarily mediated by health-care workers. Furthermore, up to 10% of healthy *S. aureus* carriers disperse the bacterium into the air. Under normal circumstances, when airborne dispersers are at rest, they are surrounded by 0.01–0.1 colony-forming units/m^3 but up to 0.3 cfu/m^3 in selected cases. However, the bacterial density may increase 40-fold with movement (due to release of bacteria from the clothing) and with respiratory tract infections. A number of outbreaks have been attributed to single airborne spreaders.[31] Although acquisition occurs primarily on the skin, *S. aureus* can only persist in the long term if the nares or perineum become colonized.

Cross-sectional prevalence rates of colonization among healthy subjects differ extensively; from as low as 14% in African American job applicants, to as high as 64% in UK hospital personnel.[32] Persons can be subdivided into 'persistent carriers', 'intermittent carriers' and 'non-carriers', based on the proportion of positive nasal swabs as well as the quantity of isolates. Based on different definitions and different populations of healthy individuals, 2–71% are considered non-carriers, 19–70% are considered intermittent carriers and 9–37% are considered persistent carriers.[32] Groups with documented increased risks of colonization include patients with type 1 diabetes, patients undergoing hemodialysis, surgical patients, intravenous drug users and HIV patients. Heavy antibiotic pressure may lower (detectable) colonization rates.

Nasal carriage of *S. aureus* is a risk factor for subsequent infection. Colonized surgical patients had an absolute risk of wound infection of roughly 5–15%, which was two to eight times the risk of control patients without *S. aureus* carriage preoperatively. A relation between colonization and (bacteremic) infection has also been demonstrated for continuous ambulatory peritoneal dialysis (CAPD) patients, hemodialysis patients, HIV patients and hospitalized patients colonized

with MRSA. Infection risk was even higher in patients who had both nasal and rectal *S. aureus* colonization than in those who only had nasal colonization, suggesting a relation between the bacterial load and the chance of subsequent infection.[33] When linking *S. aureus* isolates from blood and previously obtained specimens from the anterior nares of 219 patients with *S. aureus* bacteremia, genotypical identity was demonstrated in 180 of 219 patients (82.2%).[34] In another study patients colonized with *S. aureus* in the nares upon hospital admission and subsequently developing nosocomial *S. aureus* bacteremia (with identical strains) had a lower mortality than those developing *S. aureus* bacteremia while not already being colonized when admitted.[35] It was suggested that colonization may offer protection against an overexaggerated inflammatory response during infection.

S. aureus has been reported to persist in dust and on fomites for up to 7 months. It has been isolated from door handles, desk tops, water bowls, computer keyboards, faucet handles and blood pressure cuffs. Although (methicillin-resistant) *S. aureus* was isolated from inanimate surfaces in several documented outbreaks, the role of environmental contamination or airborne transmission is controversial.

Clinical presentation

S. aureus is an invasive micro-organism with a propensity for abscess formation. Community-acquired infections mostly involve skin and soft tissue infections such as cellulitis and furunculosis, but also pneumonia (typically post-influenza), osteomyelitis and acute endocarditis. Staphylococcal toxins may be responsible for food poisoning, staphylococcal toxic shock syndrome (TSS) and staphylococcal scalded skin syndrome (SSSS).[36]

In nosocomial settings *S. aureus* is the main causative agent of postoperative wound infections, often leading to abscess formation. It is notorious for infecting prosthetic materials, such as prosthetic joints, prosthetic heart valves and internal pacemakers. Furthermore, it is one of the main causes of intravascular catheter-associated bloodstream infections, hospital-acquired pneumonia and ventilator-associated pneumonia. *S. aureus* bacteremia (SAB), although more a symptom than a disease, is often regarded as a specific clinical entity due to its associated mortality risk and high rate of relapses and complications.[37]

S. aureus infrequently causes urinary tract infections, predominantly in patients with recent urinary tract surgery or other manipulations, and in patients with urinary tract obstruction.[38]

EPIDEMIOLOGY OF ANTIBIOTIC RESISTANCE

Although penicillin was initially considered a miracle drug for *S. aureus*, the first cases of penicillin resistance, due to β-lactamase production, were reported as early as 1944. By 1950 approximately 80% of hospital-acquired infections were caused by these penicillinase producers. Shortly after the introduction of the β-lactamase-stable antibiotics (e.g. methicillin) in the early 1960s, the first reports of MRSA appeared. Methicillin resistance resulted from the production of an alternative penicillin-binding protein, PBP2A (or PBP2′), encoded by the *mecA* gene on the staphylococcal cassette chromosome mec (SCCmec), a mobile genetic element.[39]

In most countries colonization with MRSA is now endemic within hospital populations. In the USA, for instance, the proportion of MRSA infections among patients with nosocomial *S. aureus* bacteremia increased from 2.4% in 1975 to 29% in 1991, and in American intensive care units the proportion of MRSA infections had risen to nearly 60% by 2003.[40] A few countries, such as the Netherlands and the Scandinavian countries, have succeeded in containing the nosocomial spread of MRSA by using extensive infection control measures and restrictive antibiotic policies.

An epidemiologic characteristic of MRSA has been the almost complete absence of patient-to-patient transmission outside the hospital. Recently, though, MRSA strains with clear potential for transmission between healthy subjects have emerged. The most prominent example of these so-called community-acquired MRSA (CA-MRSA) is the USA300 genotype (based on pulsed-field gel electrophoresis analysis).[41] Outbreaks have occurred in communities of men who have sex with men, homeless populations, inmates in correctional facilities, military recruits, sports teams and children in day-care centers. Recently, percentages of CA-MRSA infections have been reported as high as 76% by different American health-care institutions. In contrast to hospital-acquired MRSA (HA-MRSA), young age and absence of co-morbidity are associated with CA-MRSA infections.[42] Apparently, USA300 has acquired a number mobile genetic elements that encode resistance and virulence determinants that could enhance fitness and pathogenicity. In several European countries, MRSA also appears to be widely spread among animals, such as pigs and calves, with subsequent transmission of MRSA to caretakers.

Based upon multilocus sequence typing (MLST) the population structure of MRSA is characterized by five major clonal complexes (CCs): CC5, CC8, CC22, CC30 and CC45. Within these five clonal complexes different SCCmec types are found, indicating that MRSA clones emerged by multiple independent introductions of the *mecA* gene.[43] Four of the five major CCs represent pandemic clones of HA-MRSA: CC5 (New York/Japan clone, pediatric clone), CC8 (Vienna clone, Brazilian, Portuguese, Irish-1 and Iberian clone), CC22 (Barnim clone) and CC45 (Berlin clone). USA300 belongs to CC8 and the nontypeable MRSA linked to the animal reservoir in Europe is ST398, which is not phylogenetically linked to any of the major MRSA CCs.

The high prevalence of MRSA in hospitals necessitated extensive use of the glycopeptide vancomycin, which has been the 'last resort' antibiotic for MRSA infections for many years. The first *S. aureus* strain with reduced vancomycin was isolated in Japan in 1997 (the Mu50 strain) and has now emerged among all five major hospital MRSA-lineages.[44] In 2005 0.2% of 240 000 *S. aureus* isolates in the Surveillance Network data from US laboratories were vancomycin-intermediate *S. aureus* (VISA), defined by a minimum inhibitory concentration (MIC) of >2 and ≤8 mg/l.[45] Intermediate resistance to glycopeptides is associated with thickening of the bacterial cell wall, but the exact mechanism remains to be elucidated. Unfortunately, reduced susceptibility to vancomycin appears to be associated with reduced susceptibility to new drugs such as linezolid and daptomycin.[46]

High-level vancomycin-resistant *S. aureus* (VRSA) (MIC ≥32 mg/l) can result from acquisition of the enterococcal *vanA* resistance gene by *S. aureus*.[47] As of September 2007, seven cases of *vanA*-mediated VRSA have been reported, all from the USA and five of them from Michigan.

EPIDEMIOLOGY OF COAGULASE-NEGATIVE STAPHYLOCOCCI

Shortly after birth colonization with CoNS occurs, the normal habitats of these staphylococci being the skin and the mucous membranes. *S. epidermidis* is the predominant species; other frequent colonizers include *S. hominis*, *S. haemolyticus* and *S. warneri*. Although in general CoNS are nonpathogenic colonizers, their propensity to adhere to biomaterials and form biofilms makes them important causative agents of foreign body-related infections. CoNS are the main pathogens isolated in catheter-associated bloodstream infections and drain-associated meningitis. They are amongst the foremost causes of prosthetic joint infections and prosthetic heart valve endocarditis, and they cause 7.8% of all cases of native valve endocarditis in patients without a history of intravenous drug use.[48] Furthermore, *S. saprophyticus* causes urinary tract infections (see Chapter 53) and *S. lugdunensis* and *S. schleiferi* may cause infections very similar to those of *S. aureus*.

The colonizing CoNS flora may be influenced by antibiotic therapy. In hematology and neonatology wards with high antibiotic pressure, a population of more resistant and/or more virulent CoNS strains may be selected and become epidemic among patients and health-care workers: multiple reports describe outbreaks of *S. epidermidis* clones causing intravascular catheter-related bloodstream infections.[49]

PREVENTION

Prevention of MRSA/spread

Several countries have implemented nationwide 'search-and-destroy policies' to limit the spread of MRSA within hospital settings. These strategies were implemented in the late 1980s, when carriage of MRSA among hospitalized patients was still extremely low. The cornerstone of the search-and-destroy policies is that colonized patients are treated in strict isolation; admitted patients with an increased risk of MRSA carriage are screened (see below) and isolated until culture results rule out MRSA carriage. Finally, contact patients and health-care workers are screened for MRSA carriage in case of unexpected detection of MRSA in a hospitalized patient.

More detailed information on infection control is provided in Chapter 6.

Prevention of hospital-acquired infections

Decolonization

Eradication of *S. aureus* carriage preoperatively has been evaluated in several studies. The use of oral rifampin (rifampicin), which appeared effective in a cohort of hemodialysis patients, has been largely abandoned, due to increasing rifampin resistance and associated toxic effects of this agent. Mupirocin is highly effective in short-term nasal eradication of carriers (87–94% of patients are negative after 1 week), but high recurrence rates after 6 months were found in one study. Two randomized placebo-controlled trials with 4030 and 614 surgical patients failed to demonstrate a statistically significant reduction in postoperative surgical site infections when using decolonization with mupirocin. However, in the larger of these trials, there was a significant difference in nosocomial *S. aureus* infections observed among *S. aureus* carriers (4% vs 7.7%).[50,51] In smaller populations, such as CAPD patients, hemodialysis patients and patients with recurrent skin infections, mupirocin treatment was associated with significant reductions in *S. aureus* infections.[52]

DIAGNOSTIC MICROBIOLOGY

Isolation and determination

Most staphylococcal lesions contain numerous polymorphonuclear leukocytes (PMNLs) and large numbers of *S. aureus*, which may readily be demonstrated by a direct Gram smear of pus (Fig. 165.10). Direct

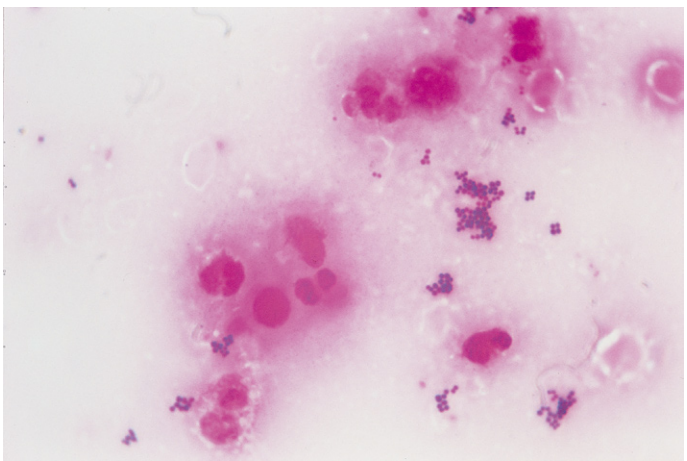


Fig. 165.10 *Staphylococcus aureus* in a Gram stain of pus. Courtesy of Jan Verhoef, Ad C Fluit and Franz-Josef Schmitz.

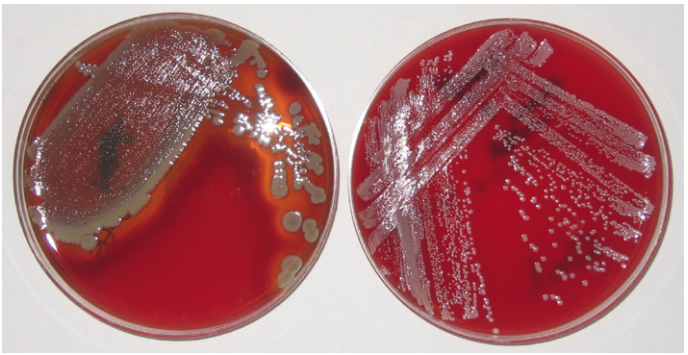


Fig. 165.11 Growth of *Staphylococcus aureus* (left) and *Staphylococcus epidermidis* (right) on trypticase soy agar with sheep blood.

Gram smears of sputum samples may also assist in rapid identification of staphylococcal pneumonia.

Staphylococci generally grow overnight on most conventional bacteriologic media. The preferential medium for isolation is (sheep) blood agar, on which they form colonies of 2 mm or more in diameter (Fig. 165.11). Blood cultures from untreated bacteremia patients are usually positive after overnight incubation. Staphylococci may grow at a temperature range of 15–45 °C and at NaCl concentrations as high as 15%. Differentiation from other Gram-positive cocci may be aided by the determination of a couple of characteristics (Table 165.2). The fermentation of mannitol by *S. aureus* is used in mannitol salt agar to screen for this bacterium in clinical and environmental samples.[53]

S. aureus colonies on blood agar can be differentiated from other staphylococci by their yellowish (gold-colored) pigment. Confirmation tests include latex agglutination assays that detect protein A and clumping factor ('bound coagulase') on the cell surface of *S. aureus* (Fig. 165.12), testing for free coagulase and for DNAse/thermostable endonuclease. However, nonoptimal sensitivity of these tests has been reported, especially in identifying MRSA. Most CoNS species can be determined with carbohydrate utilization tests and enzyme tests (e.g. phosphatase, urease, nitrate reduction). *S. saprophyticus* from urine samples may be identified by demonstrating novobiocin resistance.

S. aureus can also be identified by molecular techniques demonstrating specific genes such as nuclease (*nuc*), coagulase (*coa*), protein A (*spa*), surface-associated fibrinogen-binding protein or the sa442 gene; however, none of these techniques is 100% sensitive or specific.

Susceptibility testing

S. aureus susceptibility testing can be performed by disc diffusion or E-test on several standard bacteriologic media, or by microbroth and macrobroth dilutions. Guidelines and breakpoints are available from the Clinical and Laboratory Standards Institute (CLSI), the European Committee on Antimicrobial Susceptibility Testing (EUCAST) and the International Organization for Standardization (ISO). A number of automated systems are available for broth dilution susceptibility testing. These tests are adequate for most antibiotics, but certain special considerations apply.

Clindamycin susceptibility testing

Methylation of the ribosomal target, usually encoded by *ermA* or *ermC*, is the main mechanism of resistance against clindamycin, and also results in cross-resistance to macrolides, lincosamide and streptogramin B (MLS_B).[54] The methylase genes are associated with clinical failure of clindamycin therapy, but as clindamycin does not induce expression of these genes *in vitro*, tested strains may wrongly appear susceptible to the antibiotic. Erythromycin *is* a potent inducer of methylases *in vitro*, and erythromycin resistance is an initial clue to MLS_B resistance. An induction test (with an erythromycin and clindamycin

Table 165.2 Differentiation of staphylococci from other Gram-positive cocci

	Staphylococcus spp.	*Micrococcus* spp.	*Kocuria kristinae*	*Rothia mucilaginosa*
Gram stain	Gram-positive cocci, in clusters	Gram-positive cocci, in clusters	Gram-positive cocci in tetrads	Gram-positive cocci in pairs or clusters with capsules
Color	Cream colored to yellow/white	Cream colored to canary yellow	Cream colored to canary yellow	Clear to white
Mupirocin	Susceptible	Resistant	Resistant	–
Bacitracin	Resistant	Susceptible	Susceptible	Susceptible
Growth in 6.5% NaCl	Yes	Yes	Yes	No
Oxidase	Negative	Positive	Positive	Negative
Catalase	Positive	Positive	Positive	Weakly positive or negative

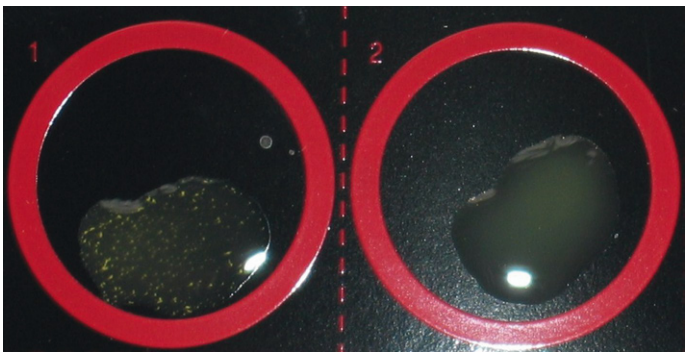


Fig. 165.12 Slide coagulase test. Latex particles coated with fibrinogen and IgG agglutinate when a colony of *Staphylococcus aureus* is suspended in the solution (left), and negative control (right).

disk placed 20–26 mm from each other) should, therefore, be performed on erythromycin-resistant *S. aureus* strains. After overnight incubation, MLS_B resistance will be induced by erythromycin, creating a characteristic D-shaped inhibition zone around the clindamycin disk. Such strains should also be considered resistant to the streptogramin B antibiotic quinupristin–dalfopristin. Enzymatic inactivation of clindamycin is another potential resistance mechanism, but this occurs much less frequently.

Isoxazolyl penicillins

Identifying methicillin-resistant strains in the bacteriologic laboratory is challenging. The gold standard for identification of MRSA is the detection of the *mecA* gene, which encodes the altered penicillin-binding protein PBP2a. Although all MRSA strains harbor the *mecA* gene, in heterogeneous MRSA populations expression of PBP2a is suppressed in most colony-forming units and may not always be detected by disk diffusion with oxacillin or by automated (microbroth dilution) systems. A screening assay with 30 µg cefoxitin disks has the highest sensitivity for MRSA detection, with specificity being comparable to other susceptibility assays.[55] Rapid latex agglutination assays which react to PBP2A may confirm MRSA, but these tests are less sensitive than cefoxitin screening.[55]

Screening for MRSA colonization is performed on selective media (both liquid and solid) which contain either oxacillin or cefoxitin. Several rapid MRSA detection media are available (both commercially and in-house produced media) which contain an indicator agent to distinguish *S. aureus* from CoNS. Sensitivity and specificity of most of these tests are reported to be higher than 90–95%.[56]

Recently, rapid molecular detection tests for MRSA screening have been introduced. Polymerase chain reaction (PCR) targets are usually the *mecA* gene in combination with a specific *S. aureus* gene (e.g. the sa442 gene, the coagulase gene or the nuclease gene). PCR-based techniques detect MRSA in screening specimens within 4–6 hours and appear to be highly specific and sensitive.[57] However, performance may vary; depending on the primer set used, certain MRSA clones will not be detected. At present, cultures remain essential in MRSA screening, especially to confirm positive PCR results.

Typing methods

The epidemiology of (methicillin-resistant) *S. aureus* may be studied by typing the isolated strains. Numerous typing methods are available, differing in reproducibility, cost, ease, speed and discriminatory capacity. The currently most widely employed techniques are discussed below.

Pulsed field gel electrophoresis

Pulsed field gel electrophoresis (PGFE) is based on the digestion of bacterial DNA with restriction endonucleases (for MRSA usually *smaI*), generating large fragments of DNA (10–800 kb). A sharper resolution of the fragments is obtained by alternating the direction of the electric current on the separation gel. PFGE has a high discriminatory power and the results are highly reproducible.[58] As such, the technique has been proposed as the gold standard for MRSA typing. However, there are limitations to its use, such as the long time interval until the final results are obtained, and the cost of reagents and specialized equipment.[58] Furthermore, even though relatively few bands are generated, small differences in electrophoretic conditions can alter the distance traveled by each band and complicate the comparison between isolates submitted to electrophoresis in different gels.

Multilocus sequence typing

Multilocus sequence typing (MLST) characterizes bacterial isolates by using the sequences of internal fragments of seven housekeeping genes.[59] Every polymorphism of a housekeeping gene is assigned a number, yielding a code consisting of seven numbers for each bacterial isolate; subsequently, each new code receives a sequence type (ST) number. Advantages of MLST include its unambiguous nomenclature, easy global exchange of typing data and the possibility for population structure and evolutionary analyses. On the downside, MLST is less discriminatory than PFGE and quite expensive.

Spa typing

Spa typing is a single-locus sequence typing technique for *S. aureus*, based on the polymorphic region X of the protein A gene.[60] Spa typing is highly reproducible and easy to interpret. It has less discriminatory power than MLST and PFGE, but is less costly and easier to perform. A web-based reference database (http://www.spaserver.ridom.de), which uses a standardized spa-type nomenclature, permits global epidemiologic comparison of isolated MRSA strains.

MANAGEMENT OF *S. AUREUS* INFECTIONS

Management of *S. aureus* infections often includes the combined use of source control and antibiotic therapy. Uncomplicated wound or skin and soft tissue infections should be treated locally by drainage (after incision in case of abscess formation or necrotectomy in case of necrosis). Systemic antibiotics may be required if there is severe cellulitis or associated deep tissue infection.

β-Lactam antibiotics are the agents of first choice in the treatment of (severe) systemic methicillin-sensitive *S. aureus* (MSSA) infections. Comparative studies between different β-lactam antibiotics are lacking, as are studies evaluating different durations of treatment. Isoxazolyl penicillins (cloxacillin, dicloxacillin, flucloxacillin), penicillin/β-lactamase inhibitor combinations (amoxicillin–clavulanic acid, piperacillin–tazobactam), first- and second-generation cephalosporins and carbapenems are considered equally effective in the treatment of MSSA infections. Clinical experience with the isoxazolyl penicillins and their narrow spectrum of activity makes them the first choice of therapy. Vancomycin, a glycopeptide, is the antibiotic of choice for (severe) systemic infections with MRSA and in patients with β-lactam allergy. The glycopeptides are, however, significantly less active than the β-lactams.[61] CA-MRSA strains are mostly still susceptible to several antibiotic classes and can often be treated with co-trimoxazole (trimethoprim–sulfamethoxazole) or clindamycin (used especially in the treatment of abscesses, for its high tissue penetration). Other antibiotics with antistaphylococcal activity include the glycopeptide teicoplanin, the macrolides, the newer classes of the fluoroquinolones, rifampin, fusidic acid, tigecycline, linezolid and daptomycin.

Because of the severe complications of *S. aureus* bacteremia and its propensity to relapse,[37] treatment with systemic antibiotic therapy for a minimum of 2 weeks is recommended.[62] *In vitro*, aminoglycosides act synergistically in *S. aureus* killing, and the addition of an aminoglycoside (most often gentamicin) may shorten the duration of fever and bacteremia, although improved outcome with this combined therapy has not been demonstrated. In any case, because of nephrotoxic side-effects, it is advised to limit the duration of aminoglycoside therapy to 3–5 days. Because of the high a priori risk of endocarditis in patients with *S. aureus* bacteremia (12% in a large observational study[37]), transesophageal echocardiography should always be considered in these patients and is mandatory in all patients who fail to improve or maintain positive blood cultures under adequate antibiotic therapy.[62]

Intravascular catheters colonized with *S. aureus* are associated with a high risk of subsequent *S. aureus* bacteremic complications, even when there were no signs of catheter site infection at the time of line removal or negative blood cultures. Antistaphylococcal antibiotic therapy in such cases was associated with a markedly reduced incidence of complications and is, therefore, advised.[63]

Recently, several new antistaphylococcal drugs (linezolid, quinupristin–dalfopristin and daptomycin) have been approved. Televancin and dalbavancin, two lipoglycopeptides, are pending registration, and several cephalosporins and carbapenems with anti-MRSA activity are in phase III studies. Clinical trials evaluating new drugs are usually manufacturer-sponsored studies intended for registration, designed as non-inferiority studies, and underpowered to demonstrate superiority of new drugs when compared to standard therapy. Thus far, in randomized controlled trials, none of the new agents has been proven superior to standard therapy in regard to mortality and clinical cure, although trends towards superiority are sometimes observed, and meta-analysis may yield significant differences. At present there is clearly no such thing as the 'ideal anti-MRSA antibiotic'. Prolonged therapy with linezolid (one of the few new anti-MRSA drugs available in an oral formulation) may cause central (optic) neuropathy and bone marrow depression (especially thrombocytopenia) and it is therefore advised not to extend therapy beyond 28 days. Daptomycin is inhibited by pulmonary surfactant, and should not be used in case of a clinical suspicion of pneumonia.[64]

One thing that *has* become clear from all the clinical trials conducted to evaluate new antibiotics is that intravenous administration of vancomycin, the predominant comparator antibiotic for new anti-MRSA agents, is safe with limited nephrotoxic effects. The nephrotoxicity formerly attributed to vancomycin probably resulted from either contamination of previously used vancomycin formulations (these suspensions were known as 'Mississippi mud' for their turbid, dark brown appearance), the underlying illness of patients, or co-medication.[65] Vancomycin is believed to enhance aminoglycoside nephrotoxicity but the evidence for this effect is limited and contradictory.[66] Nevertheless, it seems prudent to avoid the combination of these drugs as much as possible, especially when longer treatment periods are indicated. Vancomycin trough levels should be monitored to ensure adequate (high enough) dosing in patients with severe infections, especially when patients fail to respond to treatment.

Rifampin and fusidic acid, both available in oral and parenteral formulations, are regularly used in combination therapy for particular *S. aureus* infections. Both agents are unsuitable as monotherapy due to the rapid development of resistance by *S. aureus*. Because of its penetration in biofilms and its activity on slowly dividing bacteria, rifampin is often part of an antibiotic regimen to treat foreign body infections (e.g. prosthetic joints) and endocarditis, in particular endocarditis involving prosthetic heart valves.[67] Fusidic acid is used in combination therapy for the treatment of MRSA infections, particularly bone and joint infections and in *S. aureus* endocarditis.[67] Unfortunately, clinical evidence regarding the use of these two drugs remains scarce, and further studies are needed to determine their exact place in clinical practice.

MANAGEMENT OF COAGULASE-NEGATIVE STAPHYLOCOCCAL INFECTIONS

Vancomycin is usually the agent of choice in the treatment of CoNS infections, but when thorough laboratory testing indicates that a CoNS is methicillin susceptible, the isoxazolyl penicillins or first-generation cephalosporins are preferred. Other antibiotics which may be considered include teicoplanin, linezolid, daptomycin, co-trimoxazole and quinupristin–dalfopristin. Since CoNS are often multiresistant, therapy should be guided by the susceptibility test results.

In intravascular catheter-associated bloodstream infections, removal of the intravascular catheter is the foremost part of the treatment, and this may even suffice (without antibiotic therapy) in patients who are not immunocompromised, do not have prosthetic implants, without underlying valvular heart disease and whose CoNS are not *S. lugdunensis* or *S. schleiferi*; no studies have been conducted which indicate that antibiotic treatment has additional value in these patients. Nonetheless, some experts advise a standard treatment of 5–7 days of systemic antibiotic therapy for all patients.[62] If removal of the intravascular catheter is not possible, systemic antibiotic therapy should be initiated, preferably in combination with antibiotic lock therapy.

The treatment of CoNS endocarditis does not differ in principle from the treatment of *S. aureus* endocarditis.[67]

Micrococci

Micrococci are human commensals that colonize the skin, mucosa and oropharynx. Based on phylogenetic and chemotaxonomic data, the genus *Micrococcus* was, in 1995, split into five separate genera: *Micrococcus*, *Kocuria*, *Nesterenkonia*, *Kytococcus* and *Dermacoccus*.[68] The only clinically relevant species within the genus *Micrococcus* is *M. luteus*, which occasionally may cause invasive disease, usually in neutropenic patients. Micrococci have occasionally been reported as the cause of pneumonia, meningitis associated with ventricular shunts, septic arthritis, bacteremia, catheter-associated bloodstream infection and endocarditis.

Other former micrococci which have been reported to cause (opportunistic) infections include *Kocuria rosea*, *Kocuria kristinae* and *Kytococcus schroeteri*.

DIAGNOSIS

Micrococci are catalase-positive, oxidase-positive, strictly aerobic Gram-positive cocci that grow in clusters. On sheep blood agar they form cream-colored to yellow colonies. Resistance to mupirocin and staphylolysin, and susceptibility to bacitracin and lysozyme differentiate them from the staphylococci. Micrococci isolated from clinical specimens usually represent contamination, either from the skin and mucous membranes or from the environment. *M. luteus* may be differentiated from *K. kristinae* and *K. rosea* by testing for carbohydrate usage. *Kytococcus* differs from micrococci by resistance to penicillin and methicillin and by arginine dihydrolase activity. *Kocuria* and *Kytococcus* spp. usually appear as tetrads in Gram smears.

MANAGEMENT

A study with 188 micrococci, identified only to the genus level, demonstrated MICs at achievable concentrations for most β-lactams, aminoglycosides, glycopeptides, clindamycin and the most active drug *in vitro*, rifampin. Fosfomycin, erythromycin and fusidic acid should be considered inactive.[69]

Clinical data on infections with micrococci are too scarce to formulate any clear therapeutic recommendations. In case of infections of prosthetic materials, combination therapy with rifampin should be considered. Three cases of prosthetic valve endocarditis with *K. schroeteri* were treated successfully with valve replacement and combination therapy of vancomycin, gentamicin and rifampin.

Rothia Mucilaginosa

Rothia mucilaginosa is a normal inhabitant of the human oral cavity and respiratory tract. It is an infrequent pathogen, mostly affecting severely immunocompromised patients.[70] Cases of bacteremia, endocarditis, catheter-associated bloodstream infection, central nervous system infections, endophthalmitis, spondylodiscitis, osteomyelitis, prosthetic joint infection, pneumonia, cholangitis and CAPD peritonitis have been described.

Obsolete names for *R. mucilaginosa* are *Staphylococcus salivarius*, *Micrococcus mucilaginosus* and *Stomatococcus mucilaginosus*.

DIAGNOSIS

Rothia mucilaginosa is a facultative anaerobe, oxidase-negative, catalase-variable Gram-positive coccus; in smears the bacterium appears in pairs or clusters. It grows well on most nonselective media and in standard blood culture systems. On sheep blood agar the bacterium forms clear to white, nonhemolytic, mucoid or sticky colonies that adhere to the agar surface. Further distinguishing characteristics include susceptibility to bacitracin, inability to grow on media containing 5% NaCl, and hydrolyzation of gelatin and esculin.

MANAGEMENT

In vitro, penicillin, ampicillin, second- and third-generation cephalosporins, imipenem, glycopeptides and rifampin have high activity against most *R. mucilaginosa* isolates; applying the non-species-specific breakpoints set by EUCAST (www.srga.org/eucastwt/MICTAB/index.html; no susceptibility cut-off points have been defined by the CLSI), the micro-organism may be considered susceptible to these agents. MICs for fluoroquinolones, aminoglycosides, co-trimoxazole, erythromycin, clindamycin and fosfomycin are generally higher,[69] suggesting that these drugs are less suitable as antibiotic therapy.

Clinical data are limited and mainly concern neutropenic patients. In case reports, vancomycin is mostly employed (sometimes intrathecally in case of meningitis); other drugs include piperacillin–tazobactam, meropenem and third-generation cephalosporins. Sometimes combination therapy is given, combining one of the former drugs with rifampin or an aminoglycoside. Treatment with penicillin and chloramphenicol has also been described.[70]

REFERENCES

References for this chapter can be found online at http://www.expertconsult.com

Chapter 166

Ellen M Mascini
Rob JL Willems

Streptococci, enterococci and other catalase-negative cocci

INTRODUCTION

The genus *Streptococcus* consists of round or slightly oval Gram-positive cocci with a diameter of <2 µm. The cocci are often in pairs or chains. The detection of cytochrome enzymes with the catalase test distinguishes members of the catalase-positive family of Micrococcaceae (see Chapter 165) from the members of the family of Streptococcaceae, which are catalase negative. Most of these micro-organisms are facultative anaerobes, but some need carbon dioxide for growth and others may be strictly anaerobic. The major representatives of the genus *Streptococcus* include *Streptococcus* and *Enterococcus*.[1] The further division of streptococci has been the topic of an extensive taxonomic re-evaluation and we still lack a complete picture of the genetic relationship between different subgenera and species. Some of the older classification systems are therefore still in use despite access to more accurate genetic taxonomic methods.

The traditional division of streptococci into α-streptococci, β-streptococci and γ-streptococci on the basis of the capacity of the bacterial colony to hemolyze erythrocytes in the blood agar medium is still considered the first step in the classification of streptococci. This allows separation of streptococci into 20 different serogroups (the Lancefield grouping system).[2] The clear zone produced by lysis of the erythrocytes around the colony is typical of the β-hemolytic streptococci, but the zone may vary considerably in size. Colonies of α-hemolytic streptococci are surrounded by a zone that is usually green, whereas no color changes (and no hemolysis) are seen around the colonies of γ-streptococci on blood agar plates. Optochin (ethylhydro-cupreinhydrochloride) inhibits the growth of most pneumococci, in contrast to other α-hemolytic streptococci, and is thus an easy method for distinguishing between these species.

Commercial kit systems in which group-specific antisera (usually containing A, B, C, D, F or G antibodies) are coupled to latex beads are now widely used for identification of β-hemolytic streptococci. Nonhemolytic streptococci do not possess Lancefield cell wall grouping antigens, but some strains may possess similar antigens that show cross-reaction with β-hemolytic streptococcal group-specific antisera.

The taxonomic classification of viridans streptococci has caused confusion for a long time but was substantially simplified by a scheme worked out by Facklam and Washington (Table 166.1).[3]

Five species or groups of species can be identified on the basis of various biochemical tests or fermentation capacities.[3]

The identification of these streptococci has long since been recognized as unsatisfactory, with the result that a significant proportion of these isolates remain misidentified or unidentified.[4] Therefore, a variety of molecular methods have been developed for correct identification of streptococci and related organisms. These methods include DNA–DNA and DNA–RNA hybridization, determination of tRNA intergenic length, arbitrarily primed polymerase chain reaction (PCR), restriction amplification of rRNA genes and sequencing of a large variety of targets,[5] such as 16S rRNA, *rnpB*, *sodA*, *tuf*, *groESL*, *rpoB* and the tRNA gene intergenic spacer. In general, sequencing will provide the highest discriminatory power but is limited by high costs and low speed. To overcome these disadvantages, pyrosequencing of the *rnpB* gene was recently proposed as an identification method of extreme high resolution, which is also relatively simple, fast and reproducible with lower costs than normal sequencing.[6] Oligonucleotide array-based identification has also been proposed as an alternative rapid and high-throughput system for identification of the genera *Abiotrophia*, *Enterococcus*, *Granulicatella* and *Streptococcus*.[7]

MOLECULAR BIOLOGY AND POPULATION STRUCTURE

Various methods are used to type streptococci and enterococci (Table 166.2). With the introduction of sequence-based typing methods such as multilocus sequence typing (MLST) it became possible to unambiguously assign names or codes to genotypes. This has improved worldwide tracking of clinically relevant (i.e. particularly virulent or resistant) circulating clones. MLST also allowed construction of web-based international databases of which three currently harbor MLST schemes for 42 bacterial species (http://www.mlst.net, http://pubmlst.org, http://mlst.ucc.ie), including schemes for *S. pyogenes*, *S. agalactiae*, *S. pneumoniae*, *E. faecalis* and *E. faecium*. In addition and also boosted by the rapid expansion in sequence capacity and reduction in sequencing costs, an increasing number of streptococcal genomes have been sequenced, providing data on strain variation and molecular correlates for disease specificity. At the moment whole genomes of 12 *S. pyogenes*, 6 *S. agalactiae*, 25 *S. pneumoniae*, 5 *E. faecalis* and 5 *E. faecium* strains are either completely or partially sequenced. Finally, numerous microarrays for the various *Streptococcus* and *Enterococcus* species have been developed, not only for comparative genomics but also for transcriptomics. A systems biology approach integrating comparative genome sequence, transcriptome, proteome, metabolome analysis and functional studies, including animal infection models and human patient studies, has given us insights in the population structure of streptococci and enterococci, revealed the extent of lateral gene transfer and identified particular clinically relevant genetic lineages. This is not only highly relevant for (molecular) epidemiologic research but also to link genetic differences to clinical behavior, to improve understanding of pathogenic behavior of particular clones and to identify novel targets for vaccines or immunotherapy.

Table 166.1 Biochemical identification of viridans group streptococci

Species or group	TEST					
	Mannitol	Sorbitol	Voges–Proskauer	Arginine	Esculin	Urease
S. mutans (includes *S. cricetus*, *S. downei*, *S. ferus*, *S. macace*, *S. rattus* (arginine positive), *S. sobrinus*)	1	1	1	2	1	2
S. salivarius (includes *S. intestinalis*, *S. vestibularis*)	2	2	1	2	1	6
S. sanguis (includes *S. gordonii*, groups H and W streptococci)	2	2 (1)	2	1	1	2
S. mitis (includes *S. mitior*, *S. oralis*, *S. sanguis* biotype II)	2	2	2	2	2	2
S. anginosus (includes *S. intermedius*, *S. constellatus*, *S. milleri*, DNA groups I and III)	2 (1)	2 (1)	1 (2)	1	1	2

1, positive reaction; 2, negative reaction; 6, some strains positive, other strains negative; 1 (2), usually positive but occasional exceptions; 2 (1), usually negative but occasional exceptions.
Data from Facklam & Washington.[3]

Table 166.2 Commonly used methods for typing streptococci

Serologic methods	M typing and T typing R typing OF typing (inhibition of the opacity reaction) group A streptococci Polysaccharide antigen typing combined with latex agglutination	
Molecular methods	DNA fingerprinting	• M genotyping • pulsed-field gel electrophoresis • amplified fragment length polymorphism
	Ribotyping	Multilocus sequence typing (MLST) *emm* gene sequencing
	DNA sequencing	
	PCR-based	Multiple locus variable number of tandem repeat analysis (MLVA)
Other methods	Multilocus enzyme electrophoresis Whole cell protein analysis	
Rarely used methods	Bacteriophage typing Bacteriocin typing	

Group A streptococci

The cell wall proteins of group A streptococci that are used in strain typing include T protein and M protein. M antigen, a potent virulence factor, is the basis for the further serologic and genetic identification of more than 100 types. Using specific antisera against these M proteins and sequence typing, more than 90% of fresh isolates of group A streptococci can be typed. During the last two decades invasive infections with M1 group A streptococci have increased. MLST, genome sequencing, single nucleotide polymorphism (SNP) analysis, transcriptomics and proteome analysis have revealed the molecular events involved in the emergence of this highly successful clone of group A streptococci. These molecular processes included the acquisition of prophages encoding particular virulence genes, accumulation of a very limited number of mutations that enhance expression of chromosomally located genes, reciprocal recombination of a chromosomal segment harboring two extracellular toxins and acquisition through lateral gene transfer of a M12-like 26 kbp region.[8] Full genome comparison of 12 group A *streptococcus* genomes, including (in addition to M1 and M3) M4, M5, M6, M12, M18 and M28 strains, indicated that, in agreement to what has been reported for M1 and M3 strains, a large portion of the *Streptococcus* metagenome resides on exogenous genetic elements. Interestingly, these elements not only include prophages, as reported previously for M1 and M3 strains, but also integrated conjugative elements (ICE), such as conjugative transposons and plasmids, encoding antibiotic-resistance genes, bacteriocins, putative virulence encoding cell-surface proteins tentatively involved in adhesion and extracellular matrix binding.[9] The identification of large numbers of ICE substantiates that lateral gene transfer contributes to metagenome diversification in group A streptococci. Genetic diversification by recombination appeared to be more prevalent and the dominant mechanism in *emm* patterns D and E, accounting for more than 80% of *emm* types.

Group B streptococci (GBS)

Comparison of the complete sequence of three *Streptococcus agalactiae* strains and draft genome sequences of five additional strains revealed that although the core genome accounts for 80% of any single genome, it represents only a fraction of the pangenome.[10] The seemingly ultimate large pool of genes from which GBS strains may acquire genes, resembling an open pangenome, has obvious consequences for pathogenesis-related questions and vaccine design. MLST of a global GBS collection showed recombination at the capsular locus and the existence of a highly invasive neonatal lineage of GBS.[11]

Streptococcus pneumoniae

Although multiple *S. pneumoniae* strains have been sequenced, the complete genome sequence of only four isolates have been reported.[12,13] Genome comparisons of completely sequenced *S. pneumoniae* strains revealed extensive variation in gene content and confirmed that lateral gene transfer and recombination is the most

important driving mechanism generating genetic diversity and mosaicism in the pneumococcal genome.[12] A remarkable feature of the *S. pneumoniae* genome is the presence of many insertion sequence (IS) elements and repeated sequences, which may have facilitated genome plasticity of this organism and the generation of pseudogenes. This extreme level of genetic diversity, most likely facilitated by genetic transformation, is also exemplified by the presence of over 90 distinct capsular serotypes. This, however, may not be true within all *S. pneumoniae* lineages since, despite its high potential of genetic variation, the genomes of strains R6 and D39 showed nearly 100% conserved gene synteny, suggesting that rearrangements and transpositions have not occurred frequently since R6 was separated from D36 several decades ago.[13] Frequent recombination, resulting in a panmictic rather than clonal population structure, was also inferred from MLST analysis of *S. pneumoniae*.[14]

Enterococcus

Extensive genetic variation driven by recombination has also been reported for *E. faecalis* and *E. faecium* when both enterococcal species MLST schemes were developed and applied to diverse strain collection from various ecologic sources and geographic locations.[15–17] In contrast to *S. pneumoniae*, horizontal gene transfer in enterococci is most likely the result of conjugation, involving conjugative plasmids and/or conjugative transposons. Enterococci have become especially notorious for their unprecedented abilities to acquire and disseminate antibiotic-resistance genes. As for group A streptococci and pneumococci, enterococcal intra- and interspecies genetic exchange facilitates rapid adaptation of enterococci to stringent and changing environmental conditions. This seems to be a repetitive theme for these low G+C Gram-positive organisms. The availability of the completely sequenced *Enterococcus* genome, *E. faecalis* V538,[18] underlined the enormous potential for genomic rearrangement, with more than 25% of the genome consisting of mobile or exogenously required genetic elements. Comparative genomic hybridization (CGH) of eight *E. faecalis* strains, representing different MLST-based genetic lineages, identified a core genome covering only 66% of the predicted open reading frames, which is more or less in line with the available genome information.[19] CGH, as well as MLST, also showed that virulence and antibiotic-resistance genes were distributed among multiple genetic lineages, but that in some lineage resistance, which could be designated as high-risk enterococcal clonal complexes (HiRECC), antibiotic-resistance and virulence genes converged, causing infections and outbreaks globally (Fig. 166.1).[19,20]

Surprisingly, no completely annotated *E. faecium* genome is yet available. Despite this, genotyping data, especially MLST, and CGH have provided ample information on the population structure of *E. faecium*. MLST identified a single genetic subpopulation responsible for 90% of the *E. faecium* hospital outbreaks and 70% of invasive infections occurring globally.[17] Based on MLST, this subpopulation was named CC17 after its presumed founder ST-17 (see Fig. 166.1).[17,20] Through CGH more than 100 genes that were more or less specific for CC17 were identified, including a putative pathogenicity island, genes encoding cell surface proteins and novel metabolic pathways, antibiotic-resistance genes and IS elements.[21]

EPIDEMIOLOGY

β-Hemolytic streptococci of groups A, C and G

β-Hemolytic streptococci of groups A, C and G are often found in the upper respiratory tract, especially in children aged between 5 and 15 years, although people of all ages may be infected. The frequency of streptococcal-induced upper respiratory tract infections and especially pharyngitis and tonsillitis is higher during the early autumn months and the spring. Spread among family members and in classrooms is a common finding. In residential schools and detention centers pharyngeal carriage rates approach 50%. Except in epidemics, skin carriage is not common. However, in certain geographic areas where streptococcal pyoderma is common, skin colonization rates may be as high as 40%. Colonization rates are higher in patients who have skin diseases, such as eczema and psoriasis, and wounds. Children who have streptococcal pharyngitis may excrete this organism in their feces or carry it in the perianal region, which may be the cause of widespread streptococcal infections in closed facilities.

Large numbers of pyogenic streptococci are shed in the immediate environment, where the bacteria may be cultivated from clothing as well as from sheets and mattresses belonging to the infected person. This is important, especially in the treatment of patients who have skin infections such as impetigo. Recurrent streptococcal throat or skin infection is a common finding within families or institutions. Generally, pets are not a common source of re-infection because group A streptococci are highly host specific. However, group C streptococcal infections can be acquired by close contact with infected horses and group G streptococci can be obtained from dogs, in which group G streptococci are the dominant cause of streptococcal pharyngitis.

In a given population only a limited number of M types are prevalent and the population gradually seems to acquire immunity to these. New types seem to enter the population when the immunity against these diminishes below a certain level.

Scarlet fever used to be a frightening disease, but during the past few decades it seems to have had a mild course in the Western world. However, since the mid-1980s a worldwide increase in the incidence of severe group A streptococcal disease has been reported, including rheumatic fever, cellulitis, necrotizing fasciitis and other invasive forms. These serious infections were reported as being associated with the reappearance of M1 and M3 strains and the production of pyrogenic exotoxins A and B, although subsequently other exotoxins have also been implicated.[22] The global burden of disease caused by group A streptococci is not known, but it is estimated that there are at least 517 000 deaths each year due to severe group A streptococcal disease, including rheumatic fever and invasive infections (Fig. 166.2).[23] Food-borne epidemics (especially carried by milk) were common before pasteurization and were usually caused by group A streptococci, but groups C and G streptococci were also implicated on occasion. Food-borne infection can cause epidemic outbreaks of streptococcal infections such as scarlet fever and pharyngitis.

Group B streptococci

Group B streptococci are less commonly found in the upper respiratory tract, but their presence in the vagina of women aged between 15 and 45 years is rather common. Their numbers fluctuate and are higher before the monthly menstrual period and in pregnancy. Isolation frequencies as high as 30–35% have been recorded in some surveys. During delivery, vaginal colonization causes transmission to the infant in 50–75% of cases. A small number of these infants will develop symptoms of disease (see Clinical manifestations, below).

Enterococci

Enterococci are the most prevalent aerobic cocci in the bowel. Resistance against glycopeptides has been emerging since 1990, especially in *E. faecium* isolated in intensive care units and nursing homes in the USA. This emergence can be attributed to epidemic spread of a single clonal lineage, CC17 (see above). CC17 is characterized by high-level ampicillin and quinolone resistance.[17,24] Ampicillin resistance preceded and may have facilitated the emergence of glycopeptide-resistant *E. faecium* in the USA. In Europe, prevalence of vancomycin-resistant enterococci (VRE) started to increase after the turn of the century, with prevalence rates

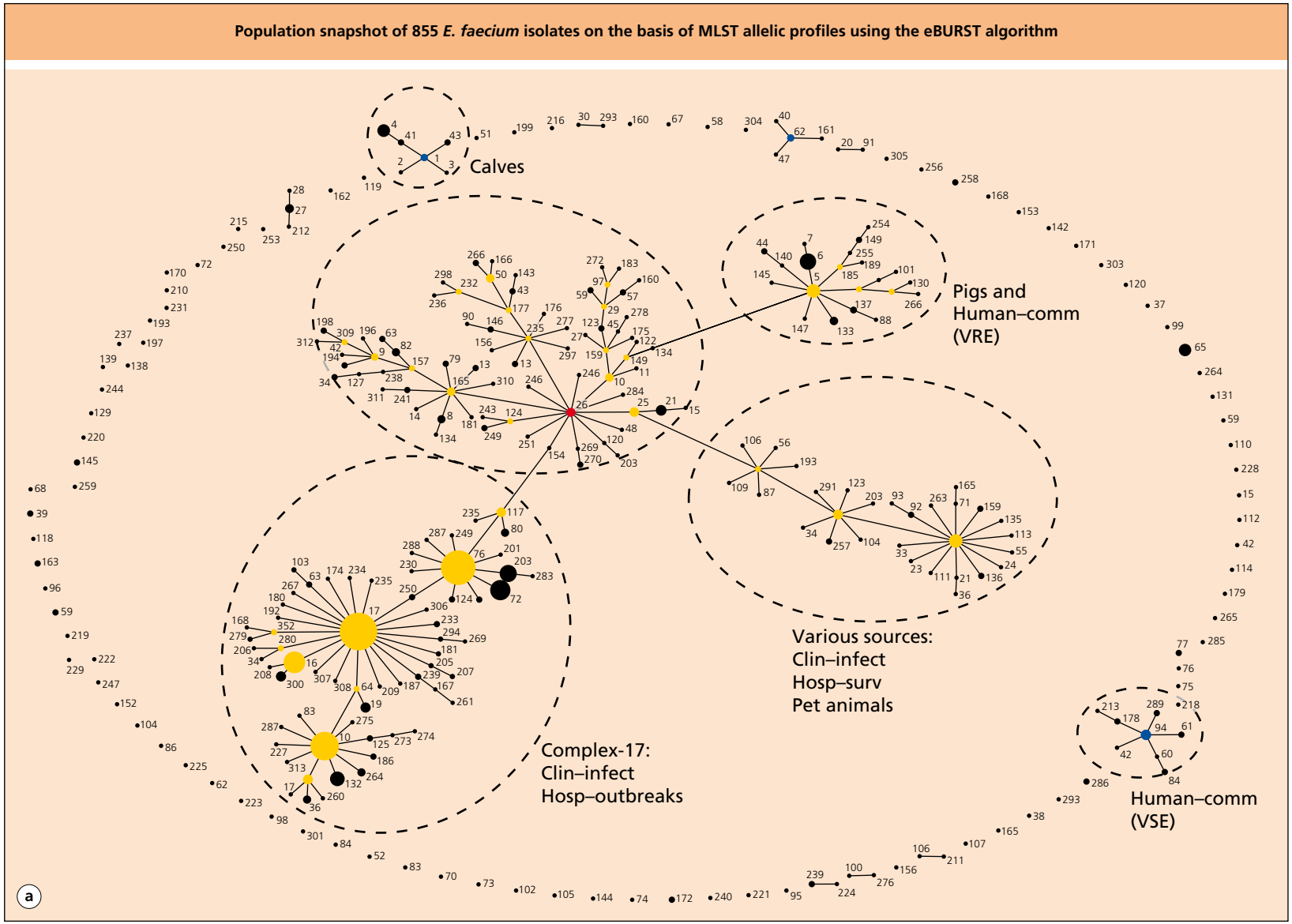


Fig. 166.1 (a) Population snapshot of 855 *E. faecium* isolates on the basis of MLST allelic profiles using the eBURST algorithm. CC17, the major subpopulation representing hospital outbreaks and clinical infections, is indicated, as well as the source of other major subgroups. Clin-infect, isolates from clinical sites (mainly blood) from hospitalized patients; Hosp–outbreaks, isolates from hospital outbreaks; Hosp–surv, feces isolated from hospitalized patients without an enterococcal infection and not associated with an enterococcal outbreak; Human–comm, feces isolated from human volunteers not connected to hospitals; VRE, vancomycin-resistant enterococci; VSE, vancomycin-susceptible enterococci. Copyright Elsevier 2006.

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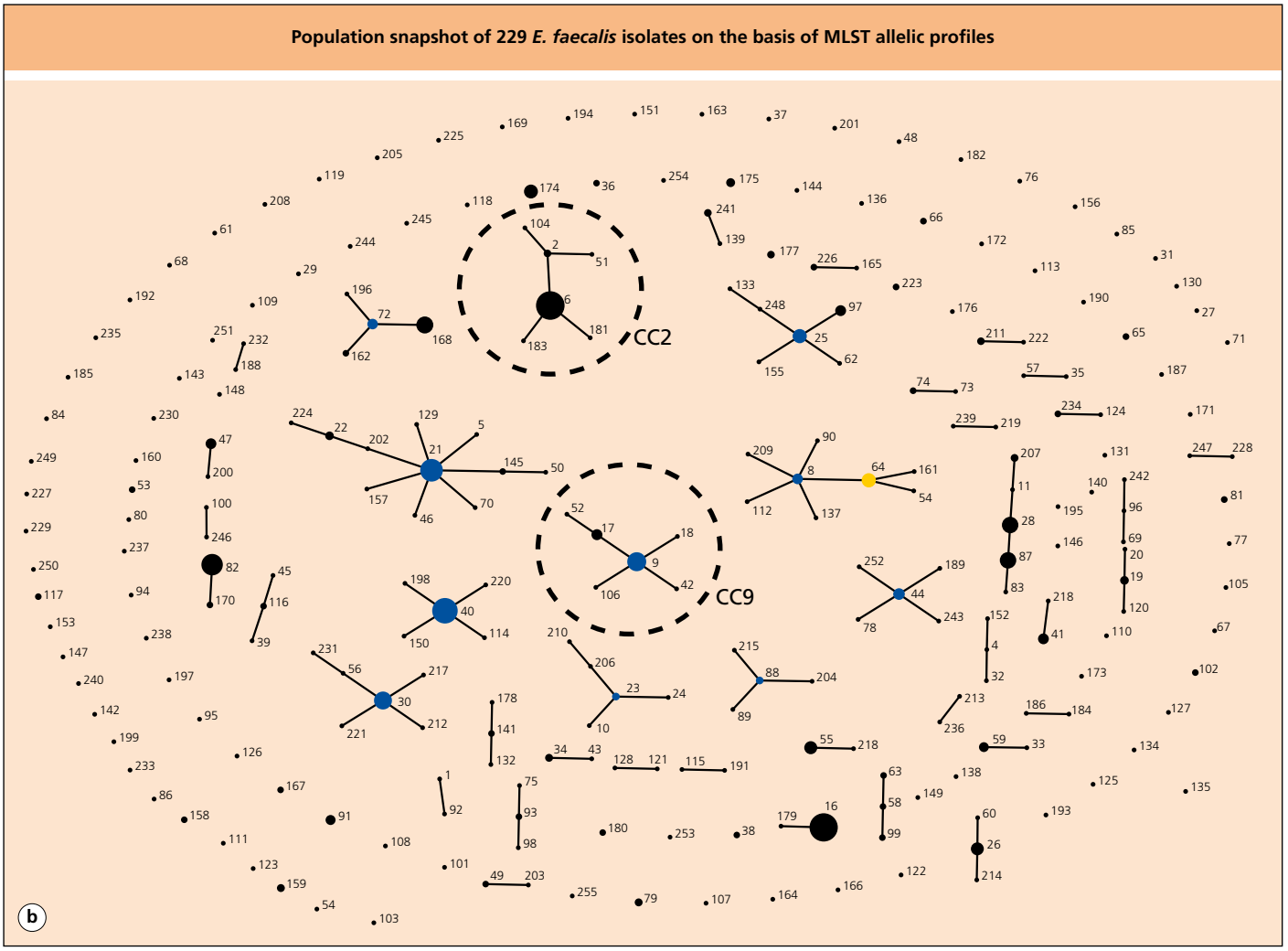


Fig. 166.1—cont'd (b) Population snapshot of 229 *E. faecalis* isolates on the basis of MLST allelic profiles. The high-risk enterococcal clonal complexes CC2 and CC9, exclusively containing hospital-related isolates, are indicated. These snapshots show all clonal complexes, singletons and patterns of evolutionary descent. The relative size of the circles indicates their prevalence. Numbers correspond to sequence types (STs) and lines connect single locus variants: STs that differ in only one of the seven housekeeping genes. Adapted from Leavis *et al.*[20] Copyright Elsevier 2006.

above 10% in countries such as Greece, Ireland, Israel, Italy, Portugal and the UK (Fig. 166.3). In other countries, such as the Netherlands, the prevalence of VRE has remained low but the number of ampicillin-resistant *E. faecium* has increased explosively during the past 10 years.[25]

Streptococcus pneumoniae

Streptococcus pneumoniae is an obligate parasite in humans, who may be colonized at a very early stage of life. The pneumococcus is a regular inhabitant of the nasopharynx in humans, especially among children, although adults are also frequently colonized. In certain populations carrier rates of 70% are found. In general, the rate of disease in a population depends on the frequency with which invasive serotypes are carried in the nasopharynx.[26] Nasopharyngeal carriage is often accompanied by the development of protection against infection by the same serotype. Carriage rates are inversely related to age and the levels of anticapsular antibody, and thus it is clear that the immune status of the host is an important determinant of the prevalence and longevity of carriage.[26] Of the 90 capsular types in the pneumococci, some seem to be more invasive than others (e.g. type 3 is among the most invasive strain types).[27] The capsular types 6a, 6b, 14, 19a, 19f and 23f are the dominant types found in pneumococcal infection during the first 2 years of life, whereas in adults these types and the types 1, 3, 4, 7f, 8, 9, 10a, 11a, 12f, 14, 15b, 17f, 18c, 20 and 22f seem to cause the greatest number of bacteremic infections recorded in the USA.

A viral infection may predispose to a pneumococcal infection; this seems to be the case in children, who very often get pneumococcal otitis media 1–2 weeks after a virus infection.

Certain penicillin-resistant pneumococcal clones may have a strong epidemic potential owing to their relative resistance to penicillin and other commonly used antibiotics, showing a facilitated spread in subpopulations regularly exposed to both the pneumococcus and to numerous antibiotics.[28] Since the 1980s, the prevalence of penicillin-nonsusceptible pneumococci has been constantly increasing worldwide with alarming high prevalences in countries bordering the Mediterranean (Fig. 166.4).

Viridans group streptococci

Viridans group streptococci are fairly constant in the upper respiratory tract and dominate the aerobic normal flora in this region in the same way that enterococci dominate the aerobic normal flora in the gut. Viridans group streptococci are part of the normal flora of human mucous membranes in the mouth and upper respiratory tract as well as in stool samples.

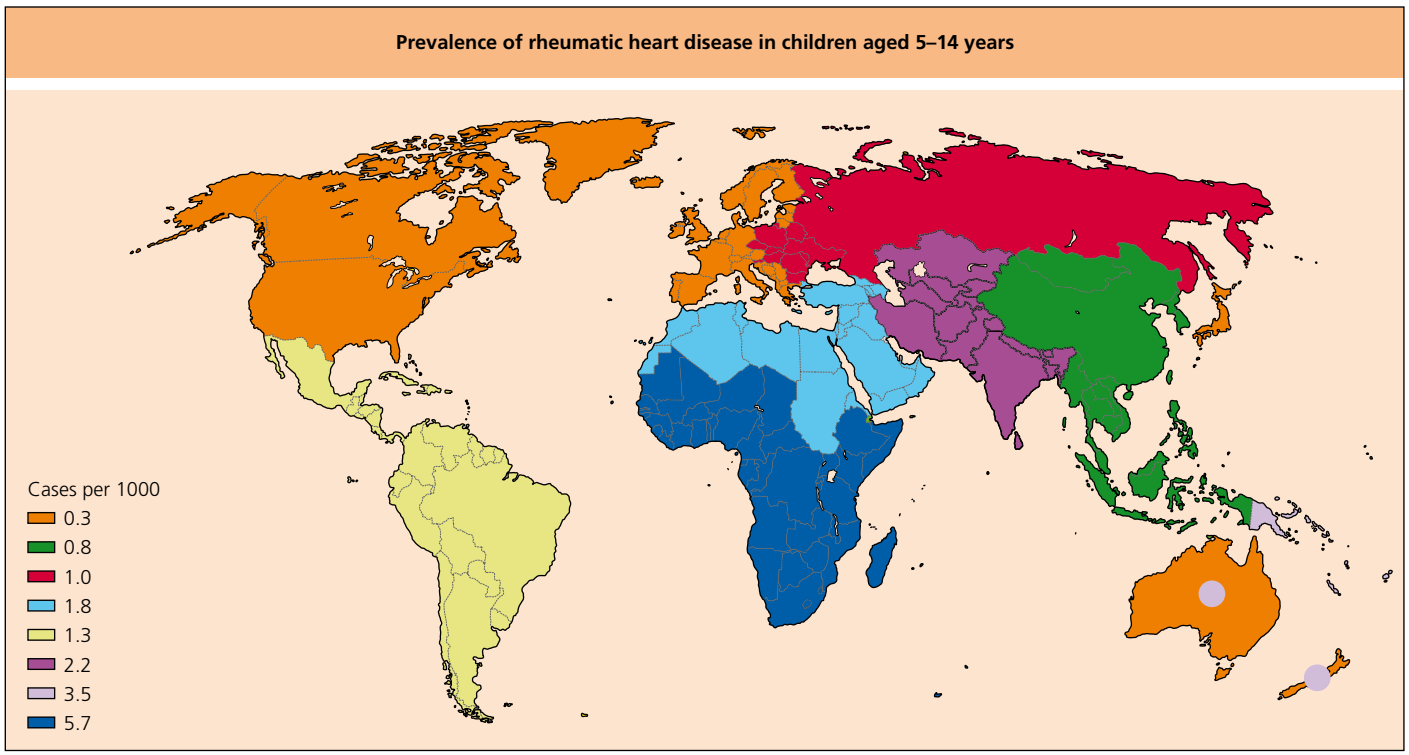


Fig. 166.2 Prevalence of rheumatic heart disease in children aged 5–14 years. The circles within Australia and New Zealand represent indigenous populations. Adapted from Carapetis *et al.*[23] Reprinted from The Lancet Infectious Diseases. Copyright Elsevier 2005.

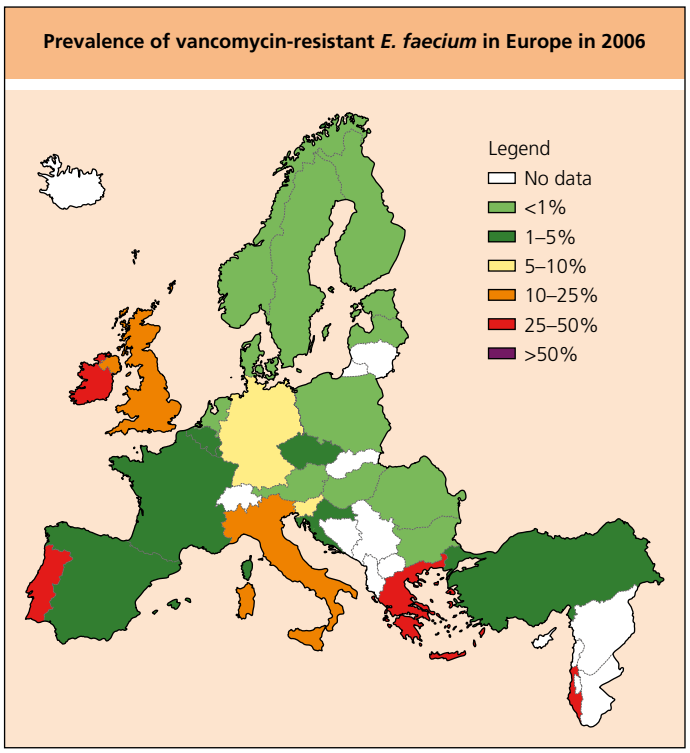


Fig. 166.3 Prevalence of vancomycin-resistant *E. faecium* in Europe in 2006 (see http://www.earss.rivm.nl).

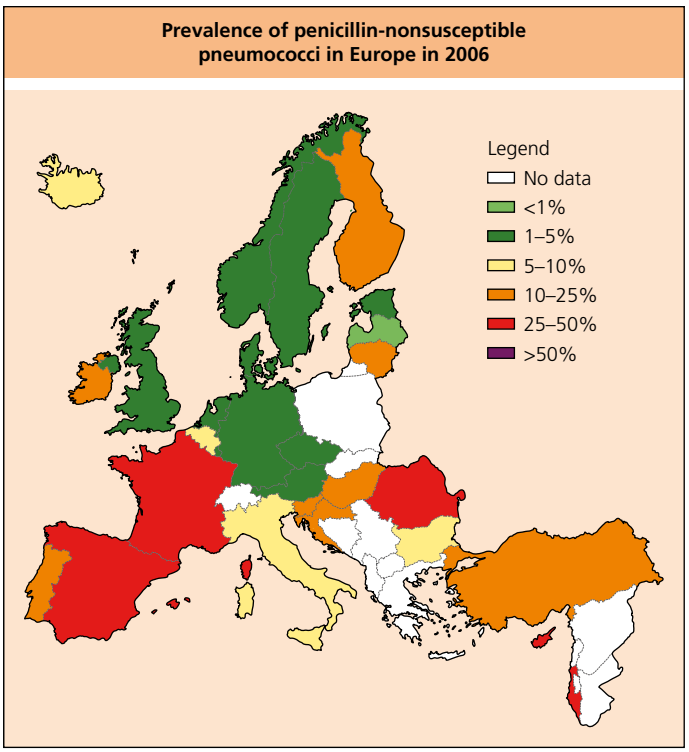


Fig. 166.4 Prevalence of penicillin-nonsusceptible pneumococci in Europe in 2006 (see http://www.earss.rivm.nl).

PATHOGENESIS

Group A streptococci (GAS)

The virulence factors of group A streptococci comprise both surface structures and proteins released from the cells during growth (Fig. 166.5).

Two-component regulatory systems

Two component regulatory systems in group A streptococci control co-ordinated expression of toxins, adhesins and other virulence-associated molecules that promote bacterial survival and pathogenesis in response to changing environmental conditions. Group A streptococci harbor 12 of these two-component systems and insertional activation followed by analysis in mouse infection models showed that these systems are involved in host–pathogen interactions (Fig. 166.6).[29,30] Co-ordinated regulation of virulence gene expression in response to different host environments is central to the success of group A streptococci. Mga is a virulence regulator that controls genes whose products are necessary for adherence, internalization and host immune evasion. Expression of the Mga regulon is influenced by conditions that signify favorable growth conditions, presumably allowing GAS to take advantage of promising new niches in the host.[31]

Cell wall antigens

The M protein is an α-helical structure similar to some muscle proteins such as myosin and tropomyosin and has also been shown to cross-react immunologically with these contractile proteins. It has been speculated whether this is of significance for the pathogenesis of rheumatic fever. A type-specific immunity is induced during the streptococcal infection and it has been shown in vaccination experiments that purified M protein can be used to induce immunity. Because these antibodies cross-react with human heart muscle proteins, there has been a reluctance to proceed with these experiments in humans. Lately it has been shown that M proteins may also function as superantigens and this has further emphasized that the proteins in the M-protein family may be important virulence factors.[32] M-negative streptococci are phagocytosed quickly in the serum milieu after being opsonized via the alternative complement pathway in the absence of antibodies against the M antigen, whereas M-positive streptococci resist phagocytosis in the absence of type-specific antibodies. The inability of M-positive streptococci to bind to phagocytosing cells efficiently indicates that C3b is not deposited on the surface of the streptococci or it is not accessible to the C3b receptor on the streptococci. The deposition of C3b is probably regulated by factor H, for which the M antigen has a high affinity.[32]

Several other M-like factors have been identified on the surface of group A streptococci. These have been shown to bind immunoglobulins such as IgG and IgA as well as several other serum proteins, including fibrinogen, plasmin, β_2 microglobulin and factor H. Binding of the M protein with the β_2 integrin adhesion molecule on the surface of neutrophils results in a massive inflammatory response which might be important in establishment of streptococcal toxic shock syndrome.[31] It has also been shown that some group A streptococcal strains have the ability to inhibit the activation of complement via the classic pathway by binding C4b to M protein-like structures. The way in which all these factors influence the virulence of the streptococci is not clear at present, but these factors can be seen as an expression of the complex interplay between the host and the micro-organism in group A streptococcal infections.[32]

One of the most the most exciting recent findings was the identification of pili in Gram-positive bacteria, including group A and group B streptococci, pneumococci and *E. faecalis*. In group A streptococci these pili are encoded by an 11 kb pathogenicity island and are involved in adherence and colonization of human tissue.[33]

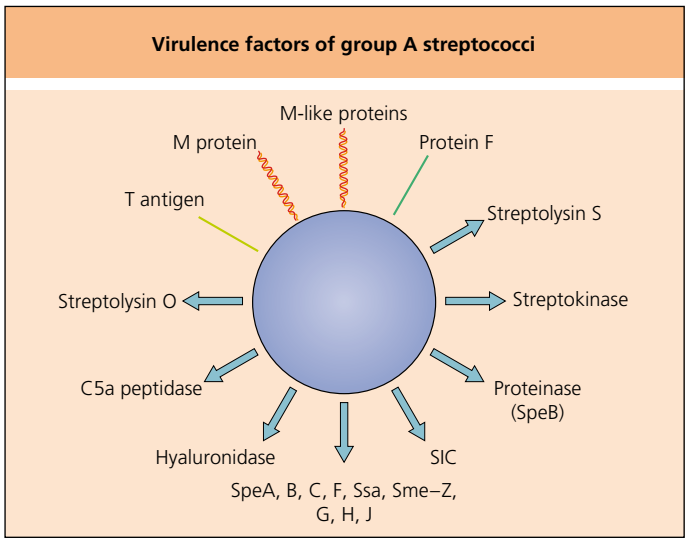


Fig. 166.5 Virulence factors of group A streptococci. SIC, streptococcal inhibitor of complement-mediated lysis; Spe, streptococcal pyrogenic exotoxins; Ssa, streptococcal superantigens.

Extracellular products, toxins and enzymes

Group A streptococci produce a great number of extracellular products (see Fig. 166.5). Streptolysin O is an oxygen-labile hemolysin that is biologically closely related to pneumolysin, tetanolysin and hemolysins from some other micro-organisms. Intravenous injections of this toxin in animals have demonstrated a cardiotoxic effect. It also inhibits chemotaxis, the mobility of neutrophils and phagocytosis by macrophages.

Streptokinase (fibrinolysin) is produced by groups A, C and G streptococci. Streptokinase binds to plasminogen in the blood, forming a complex that transforms plasminogen to plasmin, which has fibrinolytic activity. Purified streptokinase from group C streptococci is commonly used clinically to treat thrombosis in the coronary vessels and the deep veins of the leg. The plasmin formed by the plasminogen–streptokinase complex also activates complement via the alternative pathway.

The pyrogenic exotoxins (scarlatina toxins) have been proposed as being responsible for the scarlatina erythema. Numerous different toxins are produced; they are designated streptococcal pyrogenic exotoxin (Spe)A, SpeC, SpeF, SpeG, SpeH, SpeJ, SmeZ, mitogenic factor (MF) and streptococcal superantigen (Ssa). (SpeB is now known to be a constitutive cysteine protease and SpeE and MF have been shown to be identical.) Most group A strains produce more than one of these toxins. Several biologic effects have been attributed to the toxins, including pyrogenicity, a decrease in the blood–brain barrier permeability, cardiotoxic effect, T-cell activation and potentiation of the effects of endotoxin. The toxins may be responsible for the development of the shock syndrome that sometimes appears after streptococcal infections.[34] It has been suggested that this reaction, like many of the biologic effects, is induced by the release of cytokines because these toxins function as very active superantigens and thereby stimulate a high level of proliferation of T cells (Fig. 166.7). Patients who develop the toxic shock syndrome have been shown to have very low or undetectable levels of neutralizing antibodies against one or more of these toxins. Development of the streptococcal toxic shock syndrome might be hindered if toxin-neutralizing antibodies could be administered at a very early stage. Several of these extracellular products facilitate resistance of group A streptococci to killing by polymorphonuclear (PMN)-derived products.[35] In addition to evasion of neutrophil killing, group A streptococci are able to modulate PMN genes, resulting in apoptosis (Fig. 166.8).[29,35]

SpeB and Sic (streptococcal inhibitor of complement), in combination with polypeptides involved in carbohydrate transport and metabolism, like the maltodextrin-binding protein MalE, as well as regulation, MalR, have also been implicated in prolonged survival in saliva, thereby promoting enhanced colonization and survival in the upper respiratory tract.[29,31] Furthermore, SpeB is also implicated in the transition from local to systemic infection of group A streptococci.

Group A streptococci also produce three distinct DNases, two of which are encoded by bacteriophages, which are crucial in avoiding neutrophil killing, thus facilitating normal progression of both pharyngitis and invasive infections.[31]

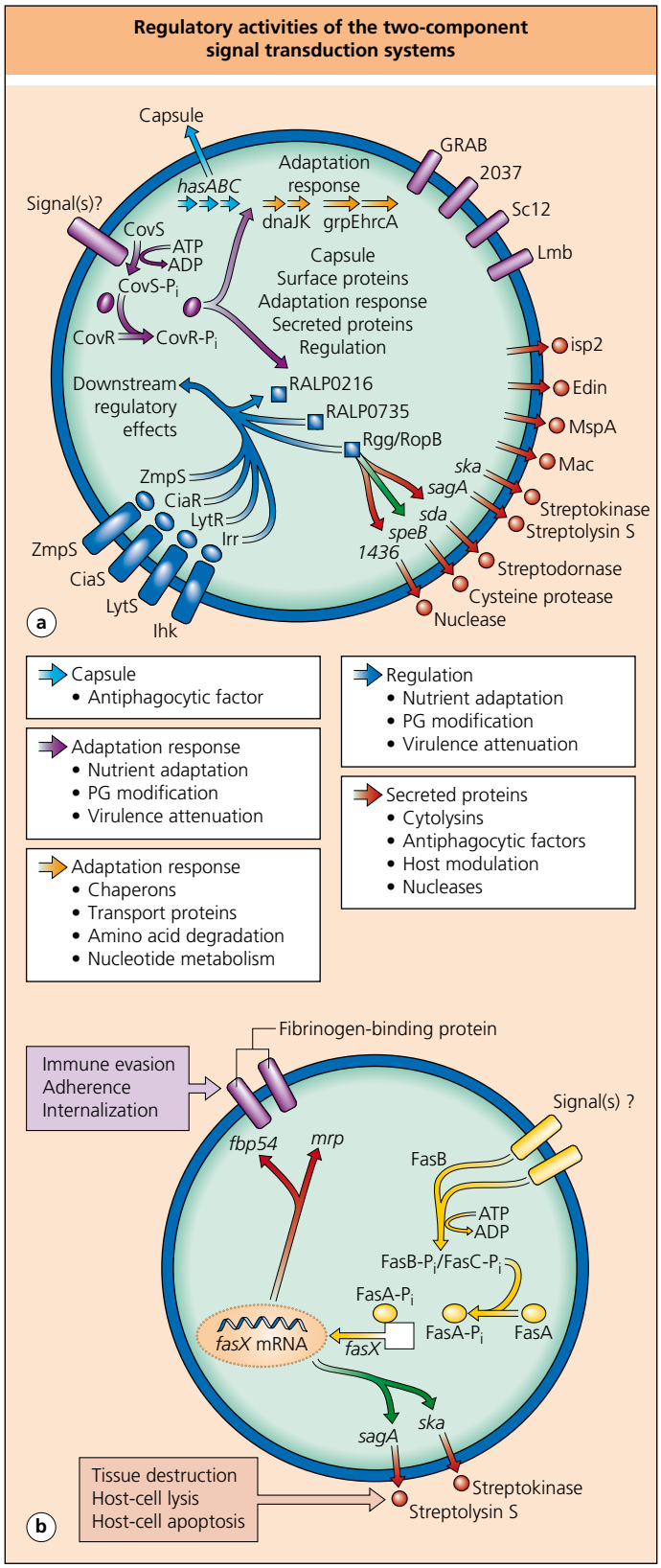


Fig. 166.6 Regulatory activities of the two-component signal transduction systems (TCSs) CsrRS/CovRS (a) and FasBCAX (b). (a) CsrRS/CovRS controls capsule expression, surface proteins, secreted proteins, other regulators (RofA-like protein (RALP) family and Rgg/RopB) and influences adaptive responses by transcriptional regulation of genes encoding chaperones, transport proteins and proteins associated with amino acid degradation and nucleotide metabolism. (b) Genes controlled by the FasBCAX system. This TCS appears to act on target genes through the action of a small putative regulatory RNA, FasX. TCSs are marked by rods and ovals (histidine kinases and response regulators, respectively), 'stand-alone' response regulators (RRs) by squares, secreted proteins by dots and surface proteins by rectangles. Red lines denote downregulation; green lines denote upregulation. Numbers denote SPy numbers assigned for serotype M1 group A streptococcus (GAS) strain SF370 ORFs. Adapted from Kreikemeyer *et al.*[30] Copyright Elsevier 2003.

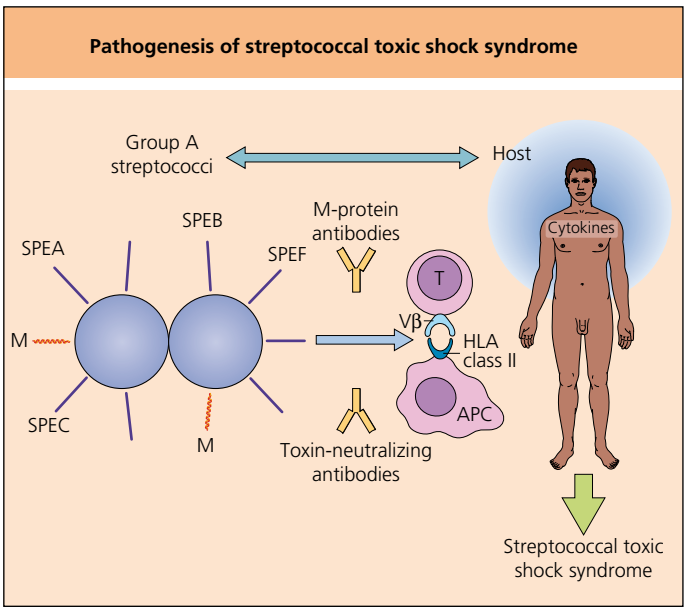


Fig. 166.7 Pathogenesis of streptococcal toxic shock syndrome. In this model, patients who have opsonizing antibodies against the M antigen are protected against infection. In the absence of these antibodies, they may develop a serious infection if they are also lacking neutralizing antibodies against the streptococcal pyrogenic exotoxins which, as superantigens, may induce a cytokine cascade. This process will be hindered by the presence of toxin-neutralizing antibodies. The affinity of the toxins to the class II major histocompatibility complex of antigen-presenting cells (APC) and T-cell receptors of specific Vβ (variable region of β chain of the T-cell receptor) bearing T cells determines the outcome in each individual patient.

Groups C and G streptococci

The species *S. equisimilis* (a group C streptococcal species) produces streptolysin O as well as streptokinase, and *S. zooepidemicus* and *S. equi* (also group C streptococci) form hemolysins that differ from streptolysin S and O. Group C streptococci often have a very strong antiphagocytic hyaluronic capsule similar to that of group B streptococci. Group C streptococci, like group G streptococci, have not been

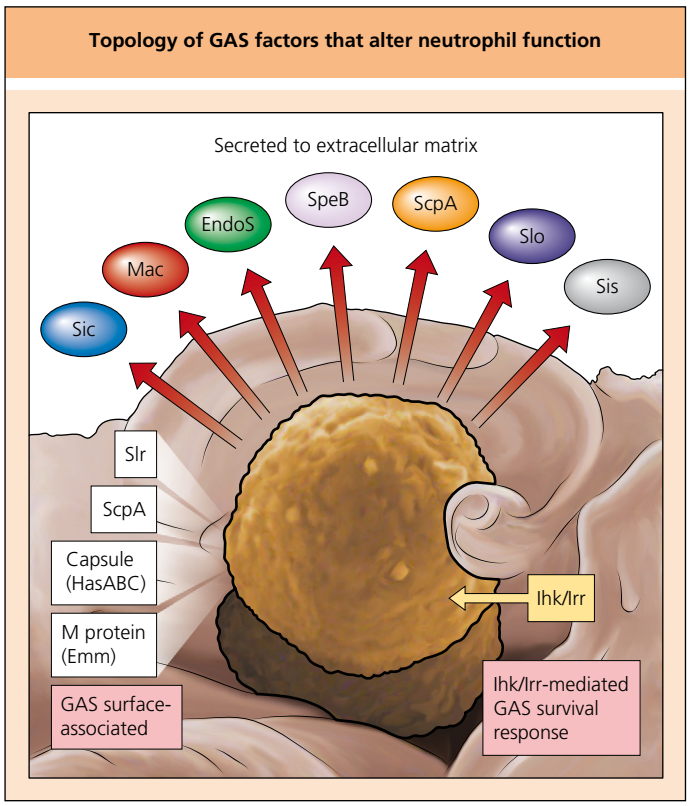


Fig. 166.8 Topology of group A streptococcus (GAS) factors that alter neutrophil function. GAS (orange) produces several proteins (ovals) that are secreted to the extracellular matrix. Most of these proteins inhibit polymorphonuclear (PMN) phagocytosis. GAS also generates surface-bound molecules (white squares) that block phagocytosis. It is not clear how much capsule is shed from the GAS cell surface and therefore not surface-associated. Ihk/Irr (yellow box) is a gene-regulatory system directly linked to inhibition of human PMN function. Ihk/Irr expression is triggered by interaction with human PMNs (gray) and/or associated PMN microbicidal components. Adapted from Voyich *et al*.[35] Copyright Elsevier 2004.

isolated from patients who have rheumatic fever, but both can cause bacteremia and sepsis. Like the group A streptococci, they have an antiphagocytic M protein on their surface and form several enzymes. They should be regarded as pathogens.

Group B streptococci

Vaginal colonization with group B streptococci is common among adult women and transmission to the infant during childbirth occurs in 50–75% of cases. A small proportion of these infants will be infected. This indicates that particular strains or a defect in the immunity of the host play a role in the establishment of infection. It has long been known that antibodies against the capsular polysaccharide are a crucial factor for protection against group B streptococcal infections.[36] These polysaccharide types are designated 1a, 1b and 2–10. *In-vitro* studies indicate that phagocytosis and killing by polymorphonuclear leukocytes require the presence of both antibodies and complement. The capsular polysaccharide types 1a, 1b, 2 and 3 are clinically the most important as they are responsible for infections during the early weeks after birth. Nonencapsulated mutants of group B streptococci belonging to type 3 have been demonstrated to lack virulence in an experimental neonatal rat model. The significance of the capsular polysaccharide for the virulence of group B streptococci was further strengthened by the finding that transplacentally acquired maternal antibodies to the group B streptococcal polysaccharides conferred protection against infection of the baby. Consequently, human experiments have been performed with polysaccharide–protein conjugate vaccines, which were capable of inducing opsonizing antibodies and killing group B streptococci.[37] The significance of sialic acid as part of the capsular polysaccharide in the inhibition of complement activation adds to the importance of the capsule in the pathogenesis of group B streptococcal disease.

Group B streptococci also produce other potential virulence factors, such as the CAMP factor, hemolysins, neuraminidase and surface proteins. Although these substances may contribute to pathogenesis, the exact role of some of these molecules awaits further studies. One class of surface proteins molecules comprises the α-like proteins or Alp family of proteins. Four members of the Alp protein family have been identified in group B streptococci: α, Rib, Alp2 and R28.[38] R28 was initially discovered in group A streptococci. Most, if not all, *S. agalactiae* strains express an Alp family protein and it has been suggested that these proteins may bind to human glycosaminoglycans, followed by human cell entry.[38] ScpB is a C5a peptidase that inactivates human C5a and inhibits recruitment of neutrophils. In addition, ScpB is able to bind fibronectin, which may promote adherence and/or invasion.[38] Of potential clinical importance is the fibrinogen-binding protein FbsA. It elicits a fibrinogen-dependent aggregation of platelets and may therefore may play an important role in *S. agalactiae*-induced endocarditis.[39]

As described above, pilus-like structures were also identified in group B streptococci.[40] These surface appendages confer adhesive properties, i.e. adhesion to human epithelial cells,[41] and contribute to adherence to and invasion of brain microvascular endothelial cells, which may facilitate penetration of group B streptococci through the blood–brain barrier.[42]

Enterococci

Although members of the genus *Enterococcus* cause many different infections in humans, little is known about the pathogenic mechanisms involved and the virulence factors carried by these microorganisms are less well understood than those of many other streptococci. It is clear, however, that infections caused by enterococci are often found in patients who have various types of immunodeficiency, in patients using mechanically compromising devices, such as intravascular lines and urinary catheters, and in seriously ill patients. The increasing importance of enterococci as nosocomial pathogens may in part be explained by their natural ability to acquire and exchange extrachromosomal elements encoding virulence traits or antibiotic resistance. Adhesion to extracellular matrix molecules and biofilm formation on indwelling medical devices leading to infections of artificial joints, implanted intravascular catheters, artificial hearts and artificial valves, stents and liquor shunt devices are often reported. A number of adhesins have been implicated in these processes including microbial surface components recognizing adhesive matrix molecules (MSCRAMMs) and the *E. faecalis* proteins Ace and Esp or Acm and Esp of *E. faecium*. The *bop* and *bee* loci, as well as two *sagA*-like genes, *salA* and *salB* of *E. faecalis*, appeared to be involved in biofilm formation.[43] Adherence to host tissue may also be mediated by enterococcal aggregation substance, fibronectin-binding protein and capsular polysaccharides in *E. faecalis*.[43,44] Furthermore, capsular polysaccharides mediate resistance to phagocytic killing of both *E. faecalis* and *E. faecium*.[44] Extracellular toxins such as cytolysin, gelatinase and a serine protease also contribute to *E. faecalis* pathogenesis.[44]

Pili were also discovered on the surface of *E. faecalis* and were demonstrated to be involved in biofilm formation and contributed to *E. faecalis* endocarditis in a rat endovascular infection model.[45]

Streptococcus pneumoniae

Pneumococci are a perfect example of the significance of the role that the mucous lining plays in the inhibition of potentially pathogenic micro-organisms. Thus, pneumococcal infections often occur

after a respiratory tract viral infection that has damaged the epithelium, thereby helping the pneumococci to establish themselves in the mucous membranes. Factors that disrupt the normal clearing process in the airways predispose patients to pneumococcal infections in the lungs. Defects in systemic host defenses (i.e. HIV infection, asplenia, immunoglobulin deficiency) may also contribute to pneumococcal infections in the respiratory tract, sometimes causing invasive infections such as bacteremia, meningitis and endocarditis. However, the above may suggest a relatively passive role of *S. pneumoniae* in the pathogenesis of pneumococcal infections, waiting for defects in host defenses to seize its opportunity. Actually, *S. pneumoniae* is equipped with an impressive armory of virulence determinants, favoring mucosal adherence and sustained colonization, migration across the extracellular matrix and dissemination into host tissue. Following dissemination, *S. pneumoniae* has strategies to evade host immunity.

The initial event in pneumococcal colonization is adherence to the mucosal surface. Macromolecular structures, including surface-exposed adhesins such as pneumococcal pili and LPxTG-containing cell-surface protein, are implicated in this process. Pneumococcal pili, extending beyond the polysaccharide capsule, are encoded by the *rlrA* pathogenicity islet, which is present in a subset of clinical isolates.[46] Pneumococcal pili have been shown to be involved in pneumococcal adherence to lung epithelial cells and virulence in a mouse infection model.[46] Whether pneumococcal pili are pivotal in human pneumococcal disease remains to be seen since they are present in only a minority of strains and are associated with only certain serotypes.[47] PspC (also known as CbpA and SpsA) binds to the polymeric immunoglobulin receptor (pIgR), facilitating adhesion to mucosal epithelial cells.[48] PspC belongs to a large family of choline-binding proteins (CBPs) with adhesive properties and roles in transcytosis and immune evasion (Fig. 166.9). Another member of the CBP family, cholin-binding protein E (CBPE), is an important receptor for plasminogen/plasmin, leading to plasmin activation and migration across the extracellular matrix (ECM).[49] Activation of host proteases like the plasminogen–plasmin system results in proteolytic cleavage of the ECM, thereby increasing bacterial invasion and dissemination in host tissue. Other adhesins include the fibronectin binder PavA (pneumococcal adherence and virulence factor A) and the pneumococcal surface antigen A (PsaA), which is a metal binding lipoprotein implicated in binding to nasopharyngeal cells through an interaction with E-cadherin.[48]

Expression of a capsule, which is structurally distinct for each of the known 90 serotypes, is important for survival in blood and is strongly associated with the ability of *S. pneumoniae* to cause invasive disease. Thus, laboratory strains that have lost the ability to produce a capsule are nonpathogenic. Expression of capsule polysaccharide (CPS) is essential for virulence due to its antiphagocytic capsular properties.[50] As the virulence capacity of pneumococci is neutralized by complement-dependent opsonizing antibodies, polyvalent polysaccharide vaccines have been developed to confer protection against pneumococcal invasive disease in children and adults. Protection is afforded by type-specific antibodies with opsonizing capacity.[27,50]

Viridans group streptococci

Viridans group streptococci can be divided into those that have a tendency to induce endocarditis and those that do not. The endocarditis-related species usually produce dextrans and this is true for *S. mutans*, *S. sanguis* and *S. mitior*, as well as *S. bovis* (recently renamed *S. gallolyticus*). A prerequisite for the endocarditis-inducing capacity seems to be an ability to adhere to endothelial cells in the endocardium and this, in turn, is related to dextran production. In contrast, *S. salivarius* produces levan as its exopolysaccharide and is usually not related to endocarditis. Dextran production is probably not the only virulence factor because these micro-organisms can cause several other types of infection.

Patients undergoing intensive cytotoxic treatment that results in neutropenia are predisposed to severe infections with viridans streptococci, the so-called viridans shock syndrome.[51] The mechanism is not well understood.

CLINICAL MANIFESTATIONS

Groups A, C and G streptococci

Group A streptococcus, or *Streptococcus pyogenes*, is considered the most pathogenic species of β-hemolytic streptococci. Group A streptococci are involved in a wide variety of clinical syndromes, which can be roughly divided into:

- uncomplicated superficial infections;
- severe invasive infections; and
- postinfectious autoimmune sequelae.

Depending on climate and portal of entry, the infections are preferentially related to the upper airways or to the skin.

Uncomplicated infections

Uncomplicated respiratory infections

Group A streptococci are the most prevalent cause of bacterial pharyngitis and tonsillitis, especially in school children aged between 5 and 15 years; however, people of all ages can be affected.

When the infecting strain produces one of the so-called pyrogenic (or erythrogenic) exotoxins, scarlet fever may be a complication of streptococcal pharyngitis. Group A streptococci are the main causative agents in scarlet fever, but streptococci of groups C and G are isolated from a small number of patients. As well as the signs observed in pharyngitis, scarlet fever is characterized by a diffuse erythematous rash over the trunk, the neck and face, and the limbs. The area around the lips is generally free of erythema (circumoral palor). The rash is a diffuse light erythema that blanches on pressure. A white coating on the tongue develops and then resolves, leaving red swollen papillae, the so-called 'strawberry tongue'. Patients usually recover within about 5–7 days (see also Chapter 24).

Uncomplicated skin infections

Streptococci can cause skin infections of various forms and the clinical features are determined by which skin layers are infected:

- infections immediately below the stratum corneum result in impetigo;
- infections in the epidermis can give rise to ecthyma; and
- infections in the dermis give rise to erysipelas and cellulitis.

Clinical descriptions of these conditions can be found in Chapter 9.

Invasive infections

Invasive group A streptococcal infections occasionally occur. Examples are sepsis (including puerperal sepsis), pneumonia, septic arthritis, meningitis and lymphangitis.[52]

Remarkably, the most severe infections, such as necrotizing fasciitis with or without the toxic shock-like syndrome (TSS), have been increasingly seen since the mid-1980s.[34] The skin serves as the main portal of entry, followed by the mucous membranes of the throat, but in many cases the portal of entry is unknown. Early clinical features of group A streptococcal infections, before the development of toxic shock, may be flu-like in nature, with fever, sore throat, vomiting and diarrhea. Sometimes there is a history of blunt trauma. Necrotizing fasciitis is often difficult to diagnose; although redness and bullae may be visible, edema is sometimes the only superficial sign. Discrepancy between the unimpressive clinical observations and the extremely severe pain indicated by the patient is then the only clue to the correct diagnosis. Subsequent surgery may reveal massive

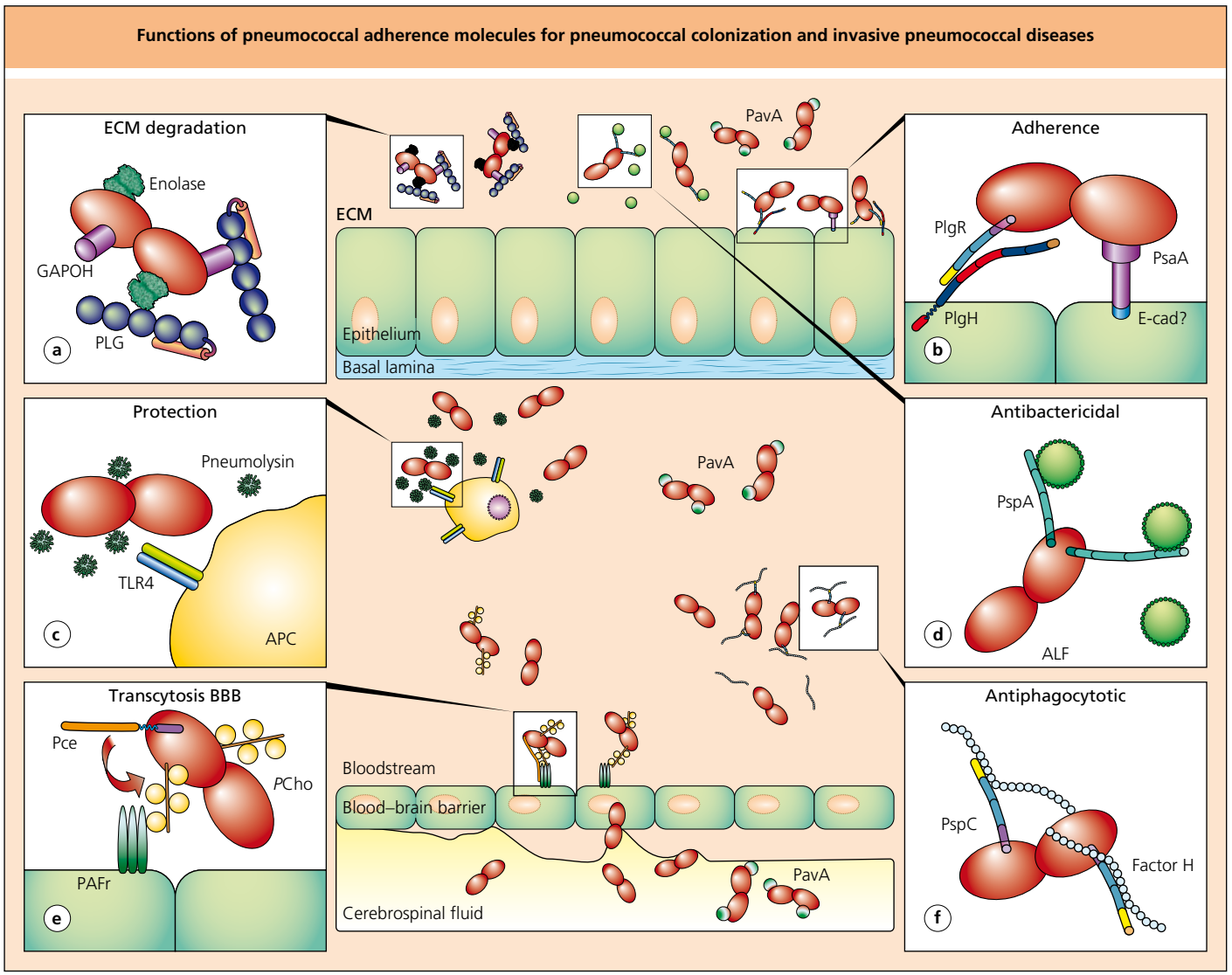


Fig. 166.9 Schematic representation highlighting functions of pneumococcal adherence molecules for pneumococcal colonization and invasive pneumococcal diseases. (a) The acquisition of plasmin(ogen) (PLG) by binding to surface-exposed GAPDH and enolase promotes degradation of the extracellular matrix (ECM). (b) The adhesins PspC and PsaA mediate adherence of pneumococci to epithelial cells. PspC binds to pIgR and PsaA probably interacts with E-cadherin (E-cad). (d) Binding of ALF by PspA on mucosal surfaces protects against the bactericidal effect of ALF. (f) Within the blood, binding of factor H by PspC/Hic protects against complement-mediated phagocytosis and (c) pneumolysin functions as the pathogen-associated molecular pattern for Toll-like receptor 4 (TLR4) on antigen-presenting cells (APC). It is suggested that the pneumolysin–TLR4 interaction provides protection against the innate immune response of the host. (e) On stimulated cells, the PAFr is targeted by the *P*Cho of pneumococci. This interaction is involved in transcytosis of pneumococci through the blood–brain barrier (BBB). The amount of *P*Cho is modulated by another choline-binding protein, the Pce protein. PavA, a fibronectin-binding protein of pneumococci, has also been shown to have a crucial impact on pneumococcal adherence and invasive diseases including septicemia and meningitis. Adapted from Hammerschmidt.[48] Copyright Elsevier 2006.

tissue necrosis along the fascial planes. In the course of hours or days, toxic shock with hypotension and multiple organ failure may develop. Clinically this disease resembles the staphylococcal toxic shock syndrome (Table 166.3). Despite adequate antibiotic treatment and intensive care, these infections are characterized by a high mortality rate of between 30% and 80%, with up to 50% of the survivors having to have limbs amputated and requiring major debridement of tissue.

Autoimmune sequelae

In some cases, streptococcal disease is followed by autoimmune sequelae, either acute rheumatic fever or acute glomerulonephritis.

Rheumatic fever

Rheumatic fever, a delayed, nonsuppurative sequela of a pharyngeal infection due to the development of cross-reacting antibodies, is seen predominantly during fall and winter in children aged between 5 and 15 years, who also show the highest incidence of streptococcal pharyngitis.[53] Clinical manifestations may vary from mild to highly aggressive. The disease is largely self-limiting. Most patients give a history of preceding pharyngitis. Acute rheumatic fever is a clinical syndrome; there is no specific diagnostic test. Guidelines for the diagnosis of initial attacks of acute rheumatic fever are described in the Jones criteria (Table 166.4). Typically, patients present 10–25 days after the preceding streptococcal infection with fever, tachycardia and nonsuppurative inflammatory changes of the joints, heart, skin and subcutaneous tissue. Migratory arthritis is the

Table 166.3 Definition for streptococcal toxic shock-like syndrome

I. Isolation of GAS from:
 A. a normally sterile site (e.g. blood, cerebrospinal, pleural or peritoneal fluid, tissue biopsy, surgical wound, etc.)
 B. a nonsterile site (e.g. throat, sputum, vagina, superficial skin lesion, etc.)

II. Clinical signs of severity
 A. Hypotension: systolic blood pressure ≤90 mmHg in adults or <5th percentile for age in children

 and

 B. ≥2 of the following signs
 1. Renal impairment: creatinine ≥177 μM for adults or ≥2 × the upper limit of normal for age. In patients with pre-existing renal disease, a ≥2-fold elevation over the baseline level.
 2. Coagulopathy: platelets ≤100 × 10^9/l or disseminated intravascular coagulation defined by prolonged clotting times, low fibrinogen level and the presence of fibrin degradation products.
 3. Liver involvement: alanine aminotransferase, aspartate aminotransferase or total bilirubin levels ≥2 × the upper limit of normal for age. In patients with pre-existing liver disease, a ≥2-fold elevation over the baseline level.
 4. Adult respiratory distress syndrome defined by acute onset of diffuse pulmonary infiltrates and hypoxemia in the absence of cardiac failure or evidence of diffuse capillary leak manifested by acute onset of generalized edema or pleural or peritoneal effusions with hypoalbuminemia.
 5. Generalized erythematous macular rash that may desquamate.
 6. Soft tissue necrosis, including necrotizing fasciitis or myositis or gangrene.

An illness fulfilling criteria IA and II (A and B) is defined as a *definite* case; an illness fulfilling criteria IB and II (A and B) is defined as a *probable* case if no other etiology for the disease is identified.

Table 166.4 Revised Jones criteria for the diagnosis of poststreptococcal rheumatic fever

Major manifestations	Minor manifestations	
Carditis	Clinical	Previous rheumatic fever or rheumatic heart disease
Polyarthritis		Arthralgia
Chorea		Fever
Erythema marginatum	Laboratory	Acute phase reactants
Subcutaneous nodules		Leukocytosis
Positive throat culture		Elevated erythrocyte sedimentation rate
Increased titer(s)		Elevated C-reactive protein
Recent scarlet fever		Prolonged P–R interval on electrocardiogram
	Supporting evidence of streptococcal infection	

The presence of two major criteria or of one major and two minor criteria is highly suggestive of rheumatic fever, if supported by evidence of preceding group A streptococcal infection.

most common manifestation and can be diagnosed in 70% of patients; carditis is found in about 50% of patients. There is an inverse relationship between the severity of joint involvement and the risk of developing carditis. The carditis is a pancarditis, which means that the pericardium, the myocardium and the endocardium can all be affected.

Repetitive group A streptococcal infections tend to cause relapses in these patients, resulting in chronic progressive damage to the cardiac valves. Carditis is the only manifestation of rheumatic fever that has the potential to cause long-term disability or death.

Strains of certain M types are strongly associated with rheumatic fever, especially strains that appear mucoid on blood agar plates. Other equally prevalent serotypes fail to initiate the disease or even to reactivate it in susceptible hosts. Rheumatic fever is associated with pharyngeal strains carrying M proteins of types 1, 3, 4, 5, 6, 12, 14, 18, 19 and 24.

In the developed world, the incidence of rheumatic fever declined throughout the 20th century, probably owing to improved living conditions, effective antibiotic treatment of upper respiratory tract infections and good health care. However, rheumatic fever is still a problem in developing countries, where it is still the most common cause of acquired heart disease.[23]

Acute glomerulonephritis

Acute glomerulonephritis is diagnosed on the basis of the clinical presentation in combination with evidence of a recent infection (in the previous 1–3 weeks) with *S. pyogenes*. This syndrome of acute inflammation of the glomeruli comprises oliguria, hematuria, proteinuria, hypertension and edema, but the presentation may be mild. The laboratory findings include proteinuria, hematuria and reduced renal function as evidenced by renal function tests. Decreased serum complement is an important parameter to support the diagnosis.

The prognosis in young adults is usually favorable, but sporadically acute disease is followed by chronic glomerulonephritis and subsequent renal failure. The incidence of glomerulonephritis is lower in Western Europe than in the USA, as is the incidence of pyoderma. The attack rate varies within wide ranges but seems to decrease substantially in the developed world and many cases can only be detected by slight proteinuria and confirmed by renal biopsies.

Glomerulonephritis is observed more in connection with skin isolates of M types M2, M49, M55, M57, M59, M60 and M61, and throat isolates of M1, M4, M12 and M25. Although the incidence of rheumatic fever has declined dramatically in the developed world, it is still a major concern in developing countries.

Chorea and other neurologic syndromes

Sydenham's chorea (St Vitus' dance) is characterized by rapid involuntary movements, sometimes associated with emotional lability and other neuropsychiatric features.[54] Although it can occur in close association with acute rheumatic fever, in which case diagnosis is relatively easy, it may present many years later as a seemingly distinct clinical syndrome, making the association with past streptococcal infection in these cases very difficult.

More recently, a syndrome with the rather unwieldy title of 'poststreptococcal autoimmune neuropsychiatric disorder associated with streptococci' (PANDAS) has been described to encompass a range of neurologic conditions such as tics and obsessive compulsive disorder.[55] The precise relationship with streptococcal infection is still being investigated.

Group B streptococci

Neonatal disease

Group B streptococci (*Streptococcus agalactiae*) are a leading cause of bacteremia and meningitis in neonates.[56] Most group B streptococcal infections are thought to be transmitted to the infant from the maternal genital tract during delivery.

Early-onset group B streptococcal infection (up to 5 days of age) is associated with a high mortality rate and occurs predominantly in immature neonates whose deliveries have been characterized by complications predisposing to infection (premature onset of labor, rupture of membranes for more than 24 hours before delivery, maternal fever or anogenital colonization of the mother).[56]

With rapid diagnosis, including screening of pregnant women, and better supportive care, mortality has decreased and is now between 15% and 40%. A substantial proportion of children with group B streptococcal sepsis develop meningitis and lumbar punctures should therefore be performed in all infants with suspected sepsis. Pulmonary involvement occurs in 40% of neonates with group B streptococcal sepsis, which cannot be distinguished from hyaline membrane disease on the basis of clinical or radiologic findings.[56]

Late-onset disease (occurring at 7 days of age or older) is infrequently associated with obstetric complications, but is acquired from exogenous sources (e.g. the mother or another infant). Typically, septic patients present with fever, poor feeding and irritability; 80% have meningitis. Death rates are much lower than in early-onset disease, but survivors of meningitis may have permanent neurologic sequelae, including central diabetes insipidus, thermal dysregulation, cortical blindness, deafness, mental retardation and generalized spasticity.[56]

As with group A streptococci, a correlation has been found between certain clinical conditions and the streptococcal type causing the infection. In early-onset neonatal infections, all four of the first serotypes are isolated at about the same frequency, whereas in late-onset disease, type 3 streptococci are the most prevalent.

Adult disease

In recent years, group B streptococci have been recognized with increasing frequency as a substantial cause of morbidity and mortality among adults.[57] Two groups of adults are at increased risk of group B streptococcal infection: pregnant women and patients with serious underlying disease (e.g. diabetes mellitus, malignancy).[56,57] The spectrum of group B streptococcal disease in adults includes (among others) bacteremia with or without sepsis, cellulitis and other soft tissue infections, pneumonia, arthritis, meningitis, osteomyelitis, endocarditis and urinary tract infection.

Enterococci

Initially thought of as merely harmless commensals because of their low intrinsic virulence compared with other organisms such as group A streptococci, enterococci have become the third most common nosocomial pathogen overall. This appears to have been caused by selection of these organisms by the widespread use of broad-spectrum antibiotics, such as the cephalosporins, which lack enterococcal activity, and acquisition of new mechanisms of antibiotic resistance.[58]

Enterococci are commonly associated with urinary tract infections, particularly in patients who have indwelling catheters, and are recovered from abdominal and pelvic wound infections and abscesses. However, most clinical studies suggest that patients who have normal host defenses are resistant to enterococcal infections.[58] Furthermore, there is a low frequency of enterococcal bacteremia originating from an intra-abdominal source when antimicrobial agents that do not cover enterococci are used.[58] Enterococcal bacteremia in surgical patients is almost always associated with the ongoing or previous use of antimicrobial agents that are not specific for enterococci.

The most frequently seen infections are infections of the cardiovascular system, with bacteremia related to an intravascular catheter being the most common,[58] although endocarditis also occurs. Other types of infection caused by enterococci include intra-abdominal and deep surgical wound infections; bone and joint infections, especially in prosthetic joints; infections of the central nervous system (CNS) associated with shunts; and urinary tract infections.

Classically, enterococcal endocarditis is a disease of older people, with a male predominance as a result of obstructive urinary tract disease; it can also affect those who have prosthetic valves.

It has been reported that 60% of enterococcal infections are nosocomial, with half of them occurring in intensive care units. Originally, *E. faecalis* was responsible for 80–90% of enterococcal infections and *E. faecium* for 10–20%.[58] This ratio, however, has changed during the last decade in favor of *E. faecium*.

Streptococcus pneumoniae

Streptococcus pneumoniae is an important agent of human disease at the extremities of age and in those who have underlying disease. Pneumococcal disease is most commonly associated with an antecedent viral respiratory infection such as influenza or with chronic conditions such as chronic obstructive pulmonary disease, diabetes mellitus, congestive heart failure, renal failure, smoking and alcoholism. Immunodeficiency such as that caused by splenic dysfunction or splenectomy is an additional risk factor for the development of severe pneumococcal disease because of decreased bacterial clearance and defective production of antibodies.

Streptococcus pneumoniae is the causative agent of the majority of cases of community-acquired pneumonia and otitis media, one of the three most common pathogens in bacterial meningitis and an important cause of sepsis and sinusitis.[26,59] Bacteremia occurs in 10% of patients who have pneumococcal pneumonia and in more than 80% of patients who have pneumococcal meningitis. Children with sickle cell disease and individuals with hypogammaglobulinemia or who have had a splenectomy (especially in the first year) are at greatly increased risk of fulminating pneumococcal sepsis, which has a high mortality.

Pneumococcal pneumonia is discussed in Chapter 27 and pneumococcal meningitis in Chapter 18.

Viridans group streptococci

Viridans group streptococci are usually found as normal inhabitants of the oral cavity, the upper respiratory tract and the bowel.[60] When these organisms gain access to the bloodstream, people who have damaged heart valves are at increased risk of endocarditis. Infection is usually endogenous and is preceded by disease, dental extraction or trauma to a mucosal surface. It is often associated with an immunocompromised condition.[61] However, blood cultures that are positive for α-hemolytic streptococci or nonhemolytic streptococci have no clinical relevance in many cases. If the same strain is isolated from more than one blood culture bottle or is isolated on repeated occasions, the assumption of clinical relevance is strengthened.

Viridans group streptococci are the most frequent cause of native valve endocarditis and are generally associated with a better clinical outcome than endocarditis caused by staphylococci, enterococci or fungi. A special population prone to develop infections with viridans streptococci is those with hematologic malignancies who are treated with cytotoxic drugs with high cytotoxicity for mucosal surfaces. The course of infection in these patients may vary from mild to highly severe, with shock, adult respiratory distress syndrome and death.

Streptococcus milleri is a cause of abscesses in association with previous surgery, trauma, diabetes and immunodeficiency. The digestive tract has been recognized as the most likely portal of entry.[62] These bacteria are an infrequent cause of endocarditis and pharyngitis.

Streptococcus gallolyticus (previously *S. bovis*) can be isolated from patients who have endocarditis and bacteremia. There is a strong association between bacteremia caused by this micro-organism and adenocarcinoma of the colon, as well as neonatal sepsis and meningitis.[63]

MANAGEMENT

Groups A, B, C and G streptococci

Penicillin is the traditional first-choice therapy for infections caused by groups A, B, C or G streptococci. *Streptococcus pyogenes* is still extremely sensitive to penicillin and erythromycin is a good alternative in case of penicillin allergy. However, an increase in the prevalence of erythromycin-resistant group A streptococcal strains has been reported in several areas. Resistance occurs in relation to an increased macrolide consumption and may reach 40%.[64]

Persistent oropharyngeal carriage after a complete course of penicillin therapy can occur in up to 30% of patients. β-Lactamase production by pharyngeal flora as well as penicillin tolerance of group A streptococci have been suggested as causes of failure of penicillin in the eradication of group A streptococci.

The object of therapy in patients who have acute rheumatic fever is to reduce inflammation, decrease fever and toxicity, and control cardiac failure. Depending on the clinical condition of the patient, treatment varies from analgesia to anti-inflammatory drugs (aspirin-like agents or, in severe cases, corticosteroids). Antibiotic treatment of streptococcal infections effectively prevents development of acute rheumatic fever and minimizes the possibility of transmission of rheumatogenic streptococcal strains.[53]

In life-threatening infections such as necrotizing fasciitis and streptococcal toxic shock, rapid diagnosis immediately followed by adequate treatment is essential. Surgery (debridement of necrotic tissue or even amputation) is the key event in the treatment of necrotizing fasciitis.[34] The combination of penicillin and clindamycin is the therapy of choice. Preliminary data suggest that the addition of intravenous immunoglobulin may be efficacious, although convincing clinical trial data are lacking.[34] Supportive therapy in the intensive care unit, including fluid replacement, inotropics, hemodialysis and resuscitation, is also required.

Group B streptococci

Group B streptococci remain susceptible to penicillin G. However, the minimum inhibitory concentration (MIC) of penicillin G for group B streptococci is considerably higher (average 0.04 μg/ml) than that observed for group A streptococci.[56] Susceptibility to ampicillin and cephalosporins is also uniform, whereas resistance to erythromycin and clindamycin occurs in 15–20% of isolates and tetracycline resistance is seen in 85–92% of recent isolates.[57] Resistance to aminoglycosides is uniform, but the combination of penicillin and gentamicin has synergistic activity and accelerates the killing of group B streptococci *in vitro*.

Because of the inoculum-like effect and the direct relation between delayed sterilization of the CNS and the occurrence of neurologic sequelae, initial therapy of suspected group B streptococcal meningitis should include increased dosages of penicillin to ensure efficacy.

Enterococci

Antimicrobial therapy for enterococcal infections is complicated because most antibiotics are not bactericidal at clinically relevant concentrations. Combination therapy with a cell wall agent plus an aminoglycoside improves the outcome of enterococcal endocarditis and probably of enterococcal meningitis, but may not improve the outcome in bacteremia.[65] High-level aminoglycoside resistance, vancomycin resistance and high-level ampicillin resistance are increasing in prevalence.[25,58,65]

Enterococcus faecium is typically high-level penicillin and ampicillin resistant and this type of resistance has been reported as a significant predictor of lack of cure.[58,65] Therapy of enterococcal infections caused by vancomycin-resistant *E. faecium* is very difficult because these organisms are usually resistant to alternative antibiotics currently available.[58] The newly developed streptogramins (quinupristin and dalfopristin), oxazolidinones (linezolid), daptomycin and tigecycline appeared promising against vancomycin-resistant enterococci.[66] Nitrofurantoin is active against many strains of vancomycin-resistant enterococci and should be useful for urinary tract infections.[65]

Streptococcus gallolyticus should be differentiated from the enterococci because it is usually susceptible to penicillin alone, and both endocarditis and meningitis due to *S. gallolyticus* have a better prognosis.[63]

Streptococcus pneumoniae

Penicillin used to be the first-choice therapy for pneumococcal disease, and in penicillin-sensitive strains it still is. For patients who have penicillin allergy, cephalosporins and erythromycin are valuable alternatives.

Resistance to penicillin is increasing in many parts of the world and is associated with a decreased affinity of the antibiotic for penicillin-binding proteins present in the bacterial cell wall. At least 30% of the pneumococcal strains in the USA show intermediate resistance to penicillin (MIC 0.1–2.0 μg/ml). Except for meningitis patients, these are readily treatable with increased doses of penicillin. Of more concern is the appearance of pneumococcal isolates that are regarded as highly resistant to penicillin (MIC ≥2.0 μg/ml). Penicillin resistance is associated with a higher mortality rate than is penicillin susceptibility in hospitalized patients with pneumococcal pneumonia. If these strains are circulating, it might be more reliable to treat severe pneumococcal infections with vancomycin. International surveillance studies demonstrate a high global prevalence of macrolide resistance up to 30% among pneumococcal isolates obtained from patients with community-acquired respiratory tract infections.[67] Low-level resistance to macrolides (usually <16 μg/ml) is due to the *mefA* gene encoding an efflux pump. High antibiotic concentrations might overcome the pump and exert an antibacterial effect. High-level resistance is due to the *ermB* gene that encodes methylation of the 23S rRNA. In this case increasing the dose will exert little effect.

Remarkably, the rate of resistance to erythromycin, tetracycline and trimethoprim–sulfamethoxazole is much greater in penicillin-resistant strains than in penicillin-sensitive strains.[28,59]

Viridans group streptococci

Viridans group streptococci have long been considered uniformly susceptible to β-lactams, macrolides, lincosamines, rifampin (rifampicin) and vancomycin. Occasionally, however, mostly under the pressure of long-term penicillin therapy, serious infections due to penicillin-resistant or penicillin-tolerant strains have been reported.[68] In endocarditis, high-dose penicillin in combination with an aminoglycoside is recommended. If MIC values are below 0.12 μg/ml, the duration of this combination therapy may be limited to 14 days; alternatively, penicillin monotherapy can be given for 4 weeks. In cases of penicillin allergy, vancomycin is a good alternative.

Surgical drainage remains central to the management of abscesses (caused by *S. milleri*) and is often augmented by antibiotics.[62] The antibiotic of choice for infections caused by *S. milleri* is penicillin, to which all but a few strains are very sensitive.[60,62] Suitable alternatives include erythromycin, clindamycin and cephalosporins.[62]

Leuconostoc and *Pediococcus* spp. are vancomycin resistant but are generally susceptible to clindamycin, carbapenems and gentamicin, and most are susceptible to penicillin as well.

PREVENTION

Several of the infections caused by streptococci are followed by complications of such a serious nature that preventive measures should be instituted. Preventive measures can include:

- improved hygiene;
- antibiotic prophylaxis; and
- vaccination.

Group A streptococci

Carefully controlled trials in patients who have rheumatic fever have demonstrated convincingly that a monthly intramuscular dose of benzathine penicillin produces a statistically significant reduction in the frequency of recurrent streptococcal infections and subsequent attacks of rheumatic fever. The recommended dose is benzathine penicillin intramuscularly 1.2 million units every 4 weeks or penicillin V orally 250 mg q12h. It is not clear how long prophylaxis should be continued, but presumably it should be for life.[53]

In contrast, a favorable effect of penicillin prophylaxis in cases of glomerulonephritis has never been demonstrated, and relapses of glomerulonephritis occur at such a low frequency that prophylaxis is not warranted. For patients who have streptococcal cellulitis or erysipelas with frequent recurrences, prophylaxis with penicillin is warranted. Close contacts of severe invasive manifestations of group A streptococcal disease should be considered for prophylaxis.[69]

No vaccine is at present available for protection against group A streptococcal infections, but intensive research is ongoing.

Group B streptococci

Group B streptococci colonize the vagina, vulva and rectum of about 25% of fertile women. This micro-organism therefore represents a threat of bacterial sepsis to the newborn baby, especially in those cases where obstetric complications or prematurity are likely. Although the incidence of serious infections, such as septicemia and meningitis, is only around 1%, antibiotic prophylaxis has been recommended for patients who have obstetric complications, especially if earlier pregnancies have been complicated by group B streptococcal infections. Studies have clearly demonstrated that intrapartum administration of penicillin or ampicillin reduces the transmission of group B streptococci from the mother to the infant, and at least 80% of early-onset group B streptococcal disease can be prevented in this way.[70] Reverse vaccinology strategies resulted in the identification of new protein antigens that in animal models were shown to be promising potential vaccine candidates.[71]

Streptococcus pneumoniae

In order to control the spread of (penicillin-resistant) pneumococci, restrictive antibiotic policies, isolation facilities and vaccination are indispensable. The currently available polysaccharide pneumococcal vaccines are composed of a mixture of 23 pneumococcal capsular polysaccharides; antibodies against these polysaccharides are protective.[72] However, the 23 vaccine serotypes do not cover more than 63% of the streptococcal types responsible for pneumococcal infections in other parts of the world. The response rate in the healthy adult population is variable.[26] Although there have been some conflicting data, pneumococcal vaccination does not appear to protect against nonbacteremic pneumonia in adults. The poor immunogenicity of the polysaccharide vaccine in children younger than 2 years and people at risk for pneumococcal disease is a major problem; an insufficient response is observed in asplenic or elderly people. An answer to immunogenicity would be to include a protein in the antigen preparation, thereby generating a T-cell-dependent memory response.[59] A 7-valent conjugate vaccine has been licensed in the USA. Surveillance after the introduction of PCV7 to the standard childhood immunization schedule has shown that the vaccine is effective in preventing invasive pneumococcal disease (IPD) and providing herd immunity. However, surveillance of pneumococcal isolates in children with IPD indicates that, since the introduction of PCV7 to the routine childhood immunization schedule, there has been an increase in the proportion of cases of IPD caused by nonvaccine serotypes (e.g. replacement serotypes).[73]

Prevention of endocarditis

Recommendations for the routine use of antibiotics for the prevention of (mainly streptococcal) endocarditis have recently been the subject of substantial review and discussion (Table 166.5).[74,75] For a more detailed discussion see Chapter 47.

REFERENCES

References for this chapter can be found online at http://www.expertconsult.com

Table 166.5 Endocarditis prophylaxis

Patients at the highest risk	Prophylaxis was recommended only in those settings associated with the highest risk of developing an adverse outcome if infective endocarditis (IE) were to occur. Patients with the following cardiac conditions were considered to meet this criterion: • prosthetic heart valves, including bioprosthetic and homograft valves • a prior history of IE • unrepaired cyanotic congenital heart disease, including palliative shunts and conduits • completely repaired congenital heart defects with prosthetic material or device, whether placed by surgery or by catheter intervention, during the first 6 months after the procedure • repaired congenital heart disease with residual defects at the site or adjacent to the site of the prosthetic device • cardiac 'valvulopathy' in a transplanted heart (valvulopathy is defined as documentation of substantial leaflet pathology and regurgitation). Similar limited criteria for prophylaxis were proposed in 2006 by the Working Party of the British Society for Antimicrobial Chemotherapy. 'Valvulopathy' in a transplanted heart was not included, but these guidelines also included complex left ventricular outflow abnormalities including aortic stenosis and bicuspid aortic valves, or acquired valvulopathy or mitral valve prolapse with echocardiographic evidence of substantial leaflet pathology and regurgitation.
No longer indicated	Common valvular lesions for which antimicrobial prophylaxis is no longer recommended in the 2007 American Heart Association guidelines include bicuspid aortic valve, acquired aortic or mitral valve disease (including mitral valve prolapse with regurgitation and those who have undergone prior valve repair) and hypertrophic cardiomyopathy with latent or resting obstruction.

Reproduced from Wilson *et al.*[75]

Chapter 167

Guy Prod'hom
Jacques Bille

Aerobic Gram-positive bacilli

INTRODUCTION

The last 20 years have led to the description of an explosion of new bacterial species, and aerobic Gram-positive bacilli are not an exception. Although most of the new species have been recovered only occasionally from human material, the key representative species have enjoyed renewed interest, for very different reasons.

Listeria monocytogenes, feared for its high associated morbidity and mortality, has extended its clinical presentations from the classic invasive food-borne pathogen to a sporadic or epidemic gastroenteritis. *Bacillus anthracis*, once an occasional agent of cutaneous anthrax in exposed persons, is now the most studied agent of bioterrorism. *Corynebacterium* spp. also enjoy renewed attention with their propensity to colonize and infect foreign materials. Finally, *Nocardia* spp. are still a rare but classic finding in the growing population of severely immunosuppressed patients.

Thus, this chapter will essentially focus on the few pathogenic species that play a major role in infectious diseases. Practical algorithms will be proposed to speed up their preliminary identification (Fig. 167.1). The major emphasis is on the laboratory diagnosis of these bacteria; their associated clinical presentations are discussed elsewhere in this book.

If most of the aerobic Gram-positive rods have an irregular shape, the most important (*Listeria*, *Bacillus*, *Erysipelothrix*) have a regular one. Almost all are nonpigmented, except *Oerskovia*. All are nonspore forming except *Bacillus* spp. A few exhibit filamentous growth: *Nocardia*, *Gordonia*, *Rhodococcus*, *Tsukamurella* and *Streptomyces*.

Commonly found aerobic Gram-positive rods can be preliminarily differentiated by a few simple tests:

- *Listeria monocytogenes* is catalase positive, motile and β-hemolytic on blood agar plates;
- *Bacillus* spp. display spores;
- *Erysipelothrix* is catalase negative and produces H_2S in triple sugar iron (TSI) slants; and
- *Corynebacterium* spp. are catalase positive, club-shaped, nonbranching rods.

Listeria monocytogenes

NATURE

The genus *Listeria* comprises six species: *L. monocytogenes*, *L. ivanovii*, *L. seeligeri*, *L. innocua*, *L. welshimeri* and *L. grayi*. Only *L. monocytogenes* is regularly pathogenic for humans; *L. ivanovii* very rarely causes clinical disease but it is pathogenic for animals. The genus *Listeria* belongs to the *Clostridium* subdivision of Gram-positive bacilli, where it forms a family with *Brochothrix* with a characteristic low G+C DNA content (<42%). The six *Listeria* species are separated into two lineages, one comprising *L. monocytogenes*, *L. innocua* and *L. ivanovii*, and the other *L. welshimeri*, *L. seeligeri* and *L. grayi*.[1]

Morphologically, *Listeria* colonies appear as nonspore forming, nonbranching, regular short (0.5–2 × 0.4–0.5 µm) Gram-positive rods occurring singly or in short chains. *Listeria* is motile at room temperature but not at 99°F (37°C). On blood agar plates, *Listeria* grows as smooth, gray, small colonies (1–2 mm in diameter after 1–2 days of incubation at 95°F/35°C). Its growth is optimal at 95–99°F (35–37°C) in an aerobic atmosphere, but occurs also at lower temperatures (including refrigerator temperature), in an anaerobic atmosphere, at low pH (≥4.5) and in a high salt concentration (up to 10% NaCl).

Listeria spp. produce catalase but not oxidase, and characteristically hydrolyze esculin. Indole reaction is negative and H_2S is not produced. Acid production from various sugars allows a differentiation within species. In addition, *L. monocytogenes* is β-hemolytic on blood agar plates, and can be differentiated from another β-hemolytic species, *L. ivanovii*, by the CAMP reaction (enhancement of β-hemolysis in the vicinity of *Staphylococcus aureus* streak culture).[2]

EPIDEMIOLOGY

Listeria spp. are widely distributed in the environment, and have been regularly recovered from soil, water, sewage, vegetables and animal feed.

Listeria monocytogenes has been isolated from a wide variety of food or food products such as milk products, raw or transformed meat, smoked fish, seafood and raw vegetables. It is a very robust organism, resisting many adverse conditions in the food processing chain or during storage.[3] *Listeria monocytogenes* colonizes or causes disease in many animals, mainly in bovines, ovines and caprines, but also in fish, crustaceans and birds. In humans, it can be carried asymptomatically and transiently in feces in up to 5% of the healthy population.

Listeria monocytogenes infections are almost all caused by the ingestion of contaminated food. They occur as sporadic cases or as outbreaks. Two major forms of clinical presentation can occur: the classic invasive disease causing bacteremia, meningitis or meningoencephalitis or infection of the offspring in pregnant women, and the newly recognized, less severe gastroenteritis form.[4] Host factors and level of inoculum are the main determinants of these two presentations.

The annual incidence of invasive listeriosis varies between 3 and 15 cases per 10^6 population in countries with a surveillance system.

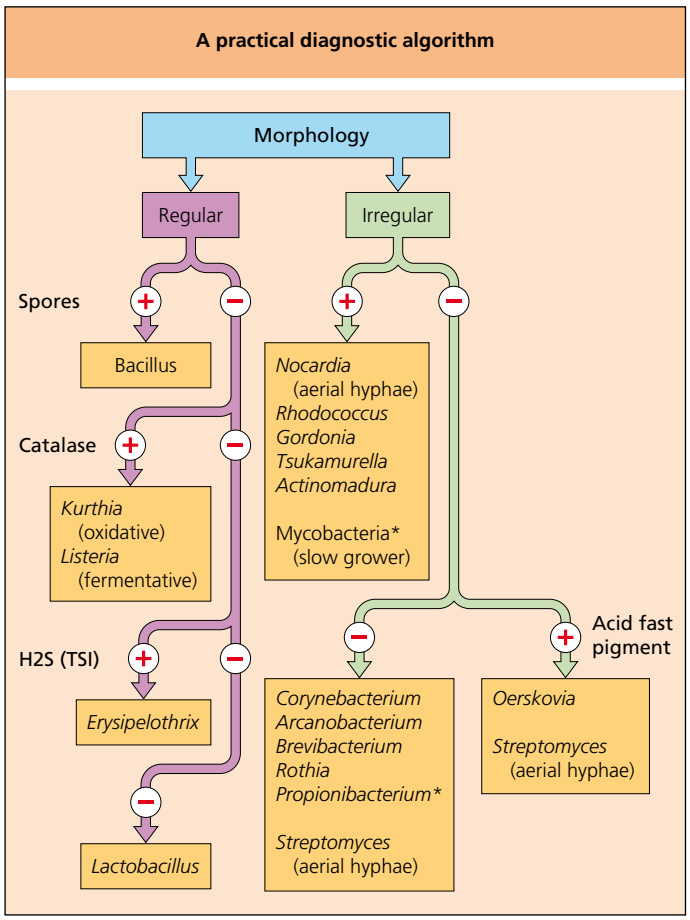


Fig. 167.1 A practical diagnostic algorithm. * Not dealt with in this review.

Although much less common than *Salmonella* or *Campylobacter*, foodborne listeriosis is much more severe, with an associated hospitalization rate of more than 95%, and a mortality rate of 20–40%. At particular risk of developing an invasive disease are the populations at the two extremes of age, as well as some immunocompromised patients (renal transplant patients and those with HIV infection with a low $CD4^+$ cell count). Other predisposing conditions are diabetes, alcoholism, liver or renal insufficiency and a high gastric pH. During outbreaks, immunocompetent persons exposed to a high inoculum can develop invasive disease. Outbreaks of invasive cases have been ascribed to different food vehicles. Among them, milk products (in particular ripened cheese), raw or undercooked meat and smoked fish have been implicated. When known, the level of contamination has been as high as 10^5 to 10^6 cfu/g of food. In these outbreaks the proportion of the maternofetal versus nonpregnant adult cases has varied greatly, as well as the distribution between bacteremia, meningitis and meningoencephalitis cases.

Not all *L. monocytogenes* strains are able to cause an invasive disease or an outbreak. Whereas *L. monocytogenes* strains can be classified into three serogroups and 13 serotypes, only three usually cause invasive disease (serotypes 4b, 1/2a and 1/2b). Most of the outbreaks described between 1980 and 1995 were caused by *L. monocytogenes* serotype 4b and within this serotype by restricted clones defined by phage or various molecular typing methods. Recently it has been shown that only the isolates harboring an intact internalin sequence are associated with invasive disease.[5]

Outbreaks of gastroenteritis seem to be associated with different strains and particularly with a very high level of contamination (>10^6 cfu/g of food). Major vehicles have been milk-based beverages, various salads or cold prepared dishes served during hot weather.[6]

Small nosocomial outbreaks have occasionally occurred in neonatal wards.

PATHOGENICITY

The mechanisms of pathogenicity of *L. monocytogenes* from its ingestion to its multiplication in selected target organs are incompletely understood. The inoculum necessary to trigger an invasive infection is not known, but it probably varies according to the magnitude of defects in host defenses. Resistant to the pH of gastric fluid, *L. monocytogenes* cells first attach to epithelial cells of the intestinal mucosa. The initial event involves the bacterial surface protein internalin A (InIA), a potent virulence factor which attaches to E-cadherin, a transmembrane glycoprotein adhesin molecule at the surface of intestinal epithelial cells. E-cadherin interacts with actin which polymerizes. A second protein, internalin B (InIB), contributes to *Listeria* entry by a different mechanism.[7]

After entry into the cell, *Listeria* cells are able to escape from the internalization vacuole thanks to listeriolysin O (LLO), a pore-forming toxin. In the cytosol, *Listeria* replicates freely and moves by the recruitment and polymerization of actin at one pole of the bacterium, the surface protein Ac+A. Moving to the opposite surface of the cell, *Listeria* protrudes into the neighboring cell and, by cell-to-cell spread, is able to disseminate in tissues. Recent advances have been made in the understanding of the very peculiar tropism of *Listeria* for specialized tissues such as placenta or the central nervous system (CNS). Both have an E-cadherin at the surface of some specialized cells (syncytiotrophoblasts, choroid plexus epithelium), allowing attachment of the bacteria.

Acquired immunity after infection is mediated by phagocytosis (by macrophages and monocytes), followed by binding of the bacterial peptides to major histocompatibility complex (MHC) class I molecules. This complex is then recognized by $CD8^+$ cytotoxic T cells and by T-helper cells. Cytokines – interferon gamma (IFNγ), macrophage colony-stimulating factor (M-CSF) and tumor necrosis factor (TNF) – are produced which enhance host resistance. In the premature infant and the neonate, macrophage activation is delayed and the number of natural killer (NK) cells reduced, increasing the susceptibility to *Listeria*. Both antibodies against somatic (O) and flagellar (H) antigens are produced after infection, but their determination is too complex to be used for diagnosis. More recently, the detection of antibodies to listeriolysin O in the blood has been claimed to be both sensitive and specific, but has not yet been used extensively.

Diagnostic Microbiology

Direct examination

Gram staining of clinical material should be attempted, particularly in normally sterile samples such as cerebrospinal fluid (CSF), amniotic fluid or placenta. Small Gram-positive bacilli or coccobacilli are suggestive in an appropriate specimen.

Listeria may sometimes be confused with enterococci, streptococci or corynebacteria.

The sensitivity of Gram stain direct examination of CSF to detect *Listeria* is rather low (30–40%) due to the usual low number of organisms.[8] It is much higher in meconium or ear swab of the infected neonate.

Culture

Listeria monocytogenes is easy to isolate from normally sterile clinical samples such as blood, CSF, placenta or amniotic fluid. These

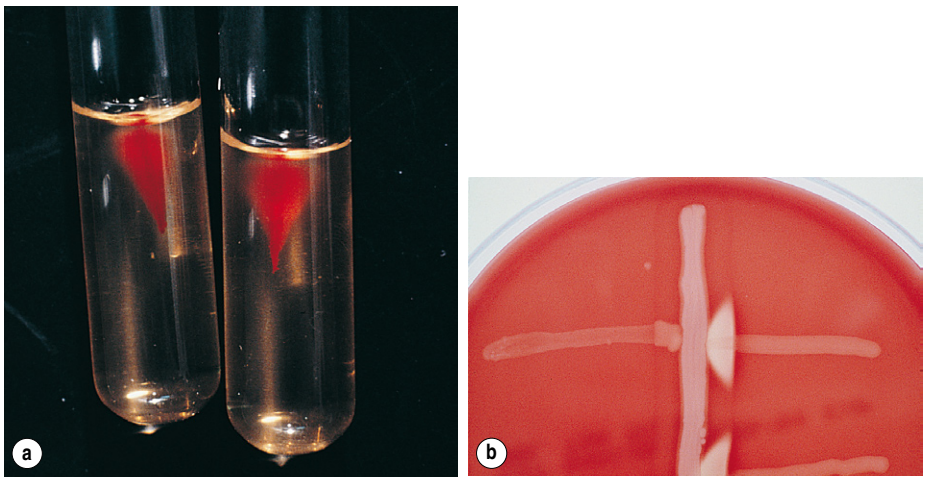


Fig. 167.2 Tests for the identification of *Listeria monocytogenes*. (a) Demonstration of motility of *L. monocytogenes* grown in semisolid agar at room temperature. Note that the migration of the organism from the central stab is more pronounced at the surface of the soft agar, forming the typical umbrella-shaped pattern in both tubes. (b) CAMP test. Enhanced hemolysis patterns of *L. monocytogenes* (left) and *Streptococcus agalactiae* (right) colonies are shown; these are growing adjacent to a streak of *Staphylococcus aureus* colonies in the center. Courtesy of Robert Bortolussi and Timothy Mailman.

specimens can be plated onto blood agar plates and blood inoculated in standard blood culture bottles.

Normally contaminated samples such as stool, genital swabs, gastric fluid and ear swab require the addition of selective solid or liquid media.

Food samples require special handling, with selective enrichment steps and indicator media.

On solid media, *L. monocytogenes* will grow in 18–24 hours as small grayish smooth colonies with a discrete β-hemolysis underneath the colonies.

Identification

Key characteristics to identify *L. monocytogenes* from colonies on agar media are the Gram stain morphology, tumbling motility in a wet mount or in semisolid agar slant (Fig. 167.2a), a positive catalase reaction and hydrolysis of esculin. A correct determination of hemolysis is essential to differentiate *L. monocytogenes* from other hemolytic or nonhemolytic *Listeria* spp. The CAMP test (for Christie, Atkins, Munch-Peterson) uses a strain of *S. aureus* producing a betalysin and a strain of *Rhodococcus equi* streaked in one direction on a sheep blood agar plate, and the *Listeria* spp. culture to test streaked at right angle (Fig. 167.2b).[1] *Staphylococcus aureus* betalysin enhances the hemolysis of *L. monocytogenes* whereas *R. equi* enhances the hemolysis of *L. ivanovii*.

Commercial kits allow the identification of *Listeria* to the genus level or to the species level (API-Listeria test, Vitek 2). A DNA probe assay performed on colonies provides rapid confirmation of *L. monocytogenes*.

Antimicrobial susceptibility testing is not mandatory for *L. monocytogenes*, the susceptibility pattern of which is rather predictable. Penicillin, aminoglycosides and trimethoprim–sulfamethoxazole are bactericidal. Cephalosporins are inactive *in vitro*, and should not be used when *Listeria* is among the suspected agents of CNS infection.

There is no currently commercially available serologic test to be recommended.

Several typing systems have been developed primarily for the investigation of food-borne outbreaks. Serotyping is still the reference phenotypic method whereas pulsed field gel electrophoresis is the most widely used for molecular typing. More recent methods (multilocus sequence typing, polymerase chain reaction (PCR)-based methods) are currently being evaluated.[9]

CLINICAL MANIFESTATIONS

Listeria monocytogenes causes invasive disease in two categories of populations: adults, often immunocompromised, and the pregnant woman and/or her offspring. In the nonpregnant adult population, *L. monocytogenes* causes two main types of disease: an invasive disease characterized by a bacteremic episode, with or without focal organ involvement (CNS, disseminated disease), and a febrile gastroenteritis.[4]

Invasive disease varies in presentation according to the age of the patient and the level of immunosuppression. Classically, one differentiates uncomplicated bacteremia without obvious organ involvement from bacteremia with CNS involvement. Several presentations of CNS disease can occur: meningitis, meningoencephalitis, rhombencephalitis and rarely brain abscesses.

Isolated bacteremia is the most frequent presentation of listeriosis, particularly among severely immunocompromised patients, representing about 40% of all cases of invasive diseases.

Associated clinical symptoms are rather nonspecific: fever, myalgias, nausea and diarrhea in a minority of patients. Focal infections following bacteremia are uncommon. The most frequently reported are liver abscess, cholecystitis, peritonitis, splenic abscess, joint infections and endophthalmitis. Endocarditis is rare and can occur both on native and prosthetic valves. *Listeria* endocarditis is a severe disease with a high rate of complications and a high mortality (>50%).

Central nervous system infections

Listeria display a particular tropism for CNS tissue, which has been recently explained at the molecular level. Following bacteremia, three main presentations may occur: meningitis, meningoencephalitis and brain abscess. A peculiar form of encephalitis involving the pons is classically described under the name of rhombencephalitis.

Meningitis due to *Listeria* ranks as number four or five, but as number one for mortality (≥20%). In neonates and patients older than 60 years, *Listeria* represents 20% of all bacterial etiologies of meningitis. In patients with lymphoma, solid-organ transplant or on steroid therapy, it ranks as number one. Almost all patients are either immunocompromised or older than 50 years, or both.

The clinical presentation is subacute to acute, nuchal rigidity is less common than with other etiologic agents, movement disorders can be seen in 15–20% of the cases, as well as seizures and fluctuation of mental status. The triad of fever, neck stiffness and alterations of mental status is present in less than 50% of cases.[8]

The diagnosis is based on blood cultures (positive in up to 75% of the cases) and CSF culture (40% of the cases have a low glucose level and a positive direct Gram stain). There is usually a moderate CSF pleocytosis; despite its name, listerial meningitis can be characterized by neutrophils rather than monocytes.

Listeria encephalitis, also named rhombencephalitis due to its similarity to the circling disease described in sheep, is more frequent in previously healthy adults than the meningitic form. It classically occurs in two phases, starting with an episode of fever, headaches, nausea and vomiting, followed 3–5 days later by the development of cranial nerve deficits, cerebellar signs or hemiparesis.[10,11] Nuchal rigidity is seen in about 50% of the cases and respiratory failure in 40%. Mortality is very high, in part due to the often delayed diagnosis and treatment. The microbiologic diagnosis of *Listeria* rhombencephalitis is sometimes particularly difficult because blood cultures are positive in only 60% of the cases and CSF culture in no more than 40%. MRI is more helpful than CT to document the lesions.

Brain abscess represents about 10% of CNS listerial infections, usually with a concomitant bacteremia, and in 25–40% of cases of concomitant meningitis. Typical locations are the thalamus, the pons and the medulla. Most occur as a single lesion. Associated mortality is high (40%), as well as the occurrence and severity of sequelae in surviving patients.[12]

In pregnant women, listeriosis presents classically in two phases. The first phase presents as an acute influenza-like illness with fever, chills, fatigue, myalgias and headaches, mostly in the second half of the pregnancy. When this episode is unnoticed and/or untreated, premature labor and delivery occur 2–14 days after the acute presentation. It is thus important to diagnose the first episode by blood culture. Early appropriate antibiotic treatment can prevent progression of the infection to the fetus. When the infection progresses (second phase), it can lead to death in utero or to the (premature) birth of an infected neonate. Neonatal mortality reaches 40–50%. The clinical manifestations of neonatal listeriosis differ greatly, as well as the mortality and the sensitivity of the diagnostic procedures according to the rapidity of disease onset at or after birth.[13]

Early-onset neonatal listeriosis is present at delivery or within the first 48 hours of life, and corresponds to an infection acquired in utero. The most common presentation is a pneumonia with or without sepsis (Table 167.1). The diagnosis is generally made by blood cultures or culture of meconium. Anemia and thrombocytopenia are common. The mortality of early-onset neonatal listeriosis is about 20%. Late-onset neonatal listeriosis develops generally after the first week of life and corresponds to the acquisition of this pathogen during birth or shortly thereafter from an infected mother or from the environment. The main presentation is meningitis.

Table 167.1 Neonatal listeriosis

	Early onset (1–2 days of life)	Late onset (>7 days of life)
Pneumonia	62%	
Meningitis	21%	94%
Anemia	62%	
Thrombocytopenia	35%	
Positive blood culture	73%	
Positive meconium stain and culture	69%	17%
Mortality (live-born)	20%	

Interestingly, the proportion of fetomaternal listeriosis has diminished considerably during the last two decades, most probably reflecting a higher awareness among pregnant women to at-risk food. On the whole, the incidence of invasive listeriosis has also decreased in many developed countries, reflecting the positive impact of various measures taken at many steps during food production and among consumers.

Listeria gastroenteritis is a rather recently described entity, undisputedly documented in several large outbreaks involving numerous previously healthy subjects exposed to a highly contaminated food product, as for example, chocolate milk, rice or corn salad. Major symptoms have been fever, vomiting, abdominal cramps and diarrhea, abating after 48 hours. Very few patients develop bacteremia.[6]

TREATMENT

Listeria monocytogenes is almost uniformly susceptible to penicillins, aminoglycosides and trimethoprim–sulfamethoxazole. Based on animal studies showing synergism, the current recommendations for a severe infection is the combination of intravenous ampicillin (2 g q4h) and intravenous gentamicin (1.5–2 mg/kg q8h) for 2–4 weeks. Fewer cases have been successfully treated with intravenous trimethoprim–sulfamethoxazole (20 mg/kg q24h trimethoprim part divided into two to four doses) for 2–3 weeks. Carbapenems are alternative drugs. Bacteremia without distant focal infection can be treated by intravenous amoxicillin for 10–14 days. In the neonate, the combination of ampicillin (150 mg/kg q24h divided into three to four doses) and gentamicin (2.5 mg/kg q8h) is recommended.

Listeria gastroenteritis is usually not treated by antibiotics due to its benign and rapidly favorable course, and low risk of associated invasive disease. Oral ampicillin or trimethoprim–sulfamethoxazole may be considered in patients at risk.[6] In pregnant women with positive blood cultures, intravenous ampicillin (2 g q6h) should be administered.

PREVENTION

The great majority of *Listeria* infections are food-borne, occurring more often sporadically (>90%) than as an outbreak.[14]

Several measures have been, or should be, taken at various levels in the food chain:

- pathogen elimination at production level of ready-to-eat at-risk food (especially meat or poultry); and
- in the consumer kitchen, wash raw vegetables, keep refrigerator temperature below 5 °C, keep raw food products separate, wash hands and kitchen instruments after use, and cook raw meat properly.

Individuals at risk, such as pregnant women and the immunocompromised, should not eat at-risk food products such as cold meat, refrigerated pates or meat spreads, unwashed salad, various soft cheese or smoked seafood.

Bacillus

NATURE

The genus *Bacillus* comprises more than 200 species of which two, *B. anthracis* and *B. cereus*, are especially important as agents of disease in humans. *Bacillus* spp. are ubiquitous Gram-positive bacilli. They

are characterized by their production of resistant endospores in the presence of oxygen. They have a wide range of G+C content (from 32 to 69). Morphologically, *Bacillus* spp. cells appear as spore-forming, large (3–5 × 1 µm), Gram-positive aerobic or facultative anaerobic mesophilic rods occurring singly or in pairs. Some *Bacillus* spp. are motile, others are not. On agar media, *Bacillus* spp. usually grow as large, irregular colonies easy to recognize. Most are saprophytes widely distributed in the environment.[15]

Bacillus anthracis

EPIDEMIOLOGY

Known since 1877 when Robert Koch first cultivated *B. anthracis*, this agent received increased attention only after its use as a bioterrorism agent in 2001.[16]

Bacillus anthracis is ubiquitous and can be recovered worldwide as a saprophytic organism from soil, water and dust. It belongs to the normal intestinal flora of many mammals including humans. As a sporulating bacterium, *B. anthracis* can survive for decades in soil before being ingested, usually by herbivore animals in which it can transform into a vegetative form or is eliminated in feces. In animals, *B. anthracis* causes severe gastrointestinal damage and high losses, particularly in nonimmunized herds in developing countries. Major outbreaks involving thousands of bovines have been reported in Africa, whereas the incidence of animal anthrax is very low in the Western world, particularly for inhalation anthrax. In humans the most frequent presentation by far is cutaneous anthrax (>95% of the reported cases), with very few cases of inhalation anthrax or gastrointestinal anthrax, which is the most frequent form in ruminants. Accidental anthrax can occur in laboratory workers or in relation to a bioterrorism event, leading to pulmonary anthrax. Naturally acquired human infections occur by accident, when *B. anthracis* spores or cells enter the body, generally through an abrasion. It is mainly restricted to persons in contact with an infected animal (farmers or veterinarians) or with animal products (skin handlers).

There are no documented cases of human-to-human transmission. The incubation time can vary widely, from a few days (1–7) to months when few spores have been inhaled. The lethal dose is probably small (2500–50 000 spores), as suggested by the tragic episode in Russia (Sverdlovsk) in 1979, when an explosion in an arms manufacturing plant caused at least 66 deaths, some of them up to 60 km from the epicenter.[17]

PATHOGENICITY

The virulence of *B. anthracis* is mediated by a complex interplay of three thermolabile proteins under the control of two plasmids. In addition, a large capsule made of poly-D-glutamic acid contributes to the virulence.[18]

The three components of the toxins are a protective antigen (PA, 83 kDa), an edema factor (EF, 89 kDa) and a lethal factor (LF, 90 kDa). Individually, they have no harmful effect. PA binds to two cell surface receptors – the tumor endothelium marker 8 (TEM8) and the capillary morphogenesis protein 2 (CMG2) – and is subsequently cleaved by a cell protease.[19] The cleaved particle (PA 63) binds to the two other components and enters by endocytosis. EF activity depends on calcium; LF interferes with the protein kinase signal transduction pathway. When assembled, PA–EF and PA–LF kill the macrophages by proteolysis and induce a release of cytokines, particularly interleukin (IL)-1 and TNF, interfering with the coagulation system and causing edema (PA–EF).

The large capsule observed mostly *in vivo* in clinical specimens is made of linear polymer of glutamic acid, and is antiphagocytic and very weakly immunogenic. These virulence factors are under the control of two large interconnected plasmids, pX01 (controlling for toxin production and carrying a pathogenicity island) and pX02 (controlling for capsule production). The toxins are mainly produced during the multiplication of the vegetative form in host tissues. External factors such as the temperature and the sodium bicarbonate concentration act on regulatory plasmid genes. In addition to the capsule, *B. anthracis* possess another unique cell-wall structure named the S layer, which is made of glycoproteins and is responsible for the shape of the bacteria.

DIAGNOSTIC MICROBIOLOGY

Direct examination

Gram staining of appropriate clinical material (skin lesion or ulcer biopsy, blood, CSF, sputum, pleural fluid) shows large, encapsulated, box-shaped, Gram-positive bacilli without spores. The sensitivity of direct examination in skin material from cutaneous anthrax is rather low (<30%). A direct fluorescent antibody (DFA) stain using an antibody against the capsular glutamic acid or a special stain (McFadyean methylene blue) can enhance detection of the capsule.

Direct detection in clinical samples can also be attempted by *B. anthracis*-specific molecular amplification or by antigen detection.[20]

Culture

Bacillus anthracis grows rapidly on aerobic standard agar media as large, flat, spreading, nonhemolytic, white to gray sticky colonies, with a typical morphology called 'medusa-head'. Cells are catalase-positive, nonmotile and produce endospores but no capsule on solid media.

Identification

Key characteristics to identify *B. anthracis* from appropriate clinical material are the Gram staining morphology, a positive catalase reaction, and the absence of motility and of frank hemolysis on blood agar. Confirmation of identification can be achieved with species-specific PCR and/or by lysis of colonies by a specific phage (γ phage).[21] Growth on agar medium enriched in sodium bicarbonate (at 0.8%) and incubated under 5% CO_2 will favor the formation of a large capsule. DFA assay and a rapid immunochromatographic test targeting the S layer to be used with colonies are also available.

Antibiotic susceptibility testing is generally not attempted because *B. anthracis* wild strains are usually susceptible to penicillins, tetracyclines, quinolones, rifampin (rifampicin), vancomycin and clindamycin, and resistant to the cephalosporins and to trimethoprim–sulfamethoxazole.

CLINICAL MANIFESTATIONS

There are three clinical presentations due to *B. anthracis* directly related to the mode of infection: a cutaneous form by direct transdermal inoculation of spores, a respiratory form following spore inhalation and a digestive form following ingestion of spores. By number of cases the most important is the cutaneous form and by severity the respiratory form. The digestive form, predominant in ruminants, is exceedingly rare in humans. (For further discussion, see Chapter 128.)

Bacillus cereus

NATURE

Bacillus cereus is the second *Bacillus* species of interest in human diseases. It is ubiquitous in nature, and can easily contaminate various raw or processed foods or damaged human skin.

Bacillus cereus is a large Gram-positive bacillus with four major properties, differentiating it from *B. anthracis*: motility, hemolysis, absence of capsule and resistance to penicillin.

EPIDEMIOLOGY

Bacillus cereus represents a significant cause of food poisoning (variable incidence, usually 1–3%). It is widely present in various raw or processed food products such as rice, vegetables, turkey meat and spices. Spores usually naturally contaminate the food environment.

PATHOGENICITY

Bacillus cereus carries two toxins, one responsible for a diarrheal syndrome (enterotoxin) and one for an emetic syndrome. The enterotoxin is a 38–57 kDa thermolabile protein, preformed or produced in the small intestine, acting on adenylcyclase. The emetic toxin is a 10 kDa peptide which is highly thermostable and resistant to proteolytic degradation. Its effect is classically observed when rice or pasta meals are consumed cold after cooking. In both cases, spores of *B. cereus* resist the heating process, and germinate and multiply during storage of the leftover food.[22]

DIAGNOSTIC MICROBIOLOGY

In clinical material – particularly in tissue – *B. cereus* appears as a large bacillus with a central endospore. *Bacillus cereus* can be cultured easily from blood or tissue biopsies, and appears on blood agar as large, flat, granular to ground-glass, β-hemolytic colonies of variable shape (circular to irregular) (Fig. 167.3, lanes 6C and 6D). They can be easily taken up with a loop, as opposed to *B. anthracis* colonies which are sticky. Its isolation from contaminated material such as feces, vomitus or food items requires selective media usually containing mannitol, egg yolk and antibiotics. *Bacillus cereus* is facultative anaerobe and gives a positive lecithinase reaction on egg yolk medium.[15]

CLINICAL MANIFESTATIONS AND MANAGEMENT

The diarrheal syndrome caused by the enterotoxin is characterized by an episode of profuse diarrhea with abdominal pain and cramps, occurring 8–16 hours after ingestion of the contaminated food. Fever and vomiting occur rarely.

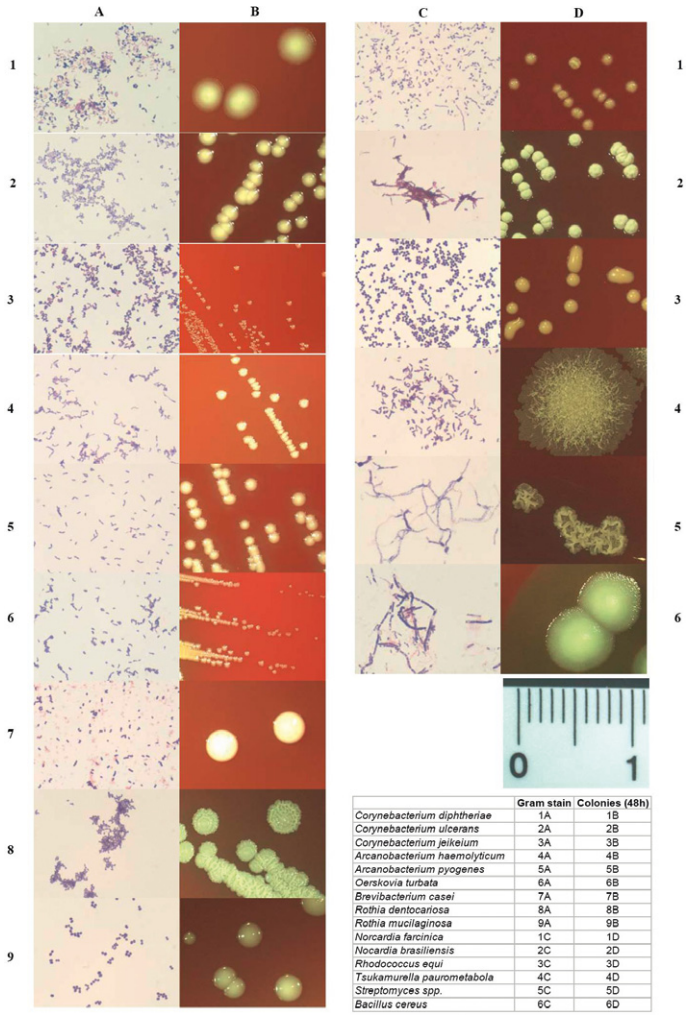
The other clinical syndrome is food poisoning. For an extensive clinical discussion, see Chapters 34 and 35.

As well as the classic gastrointestinal diseases, *B. cereus* also occasionally causes an array of focal or invasive diseases:

- in immunocompromised hosts, bacteremia, meningitis, endocarditis, brain abscess, pneumonia;
- in surgical patients, wound infections, after traffic accidents or burns;
- in neonates, infection of the umbilical cord; and
- in contact lens-wearing patients, keratitis or endophthalmitis.

It is important to consider the potential pathogenic role of *B. cereus* in these situations and not automatically disregard a positive culture for *Bacillus* considered as a simple contamination.

Bacillus cereus is resistant to penicillins and cephalosporins (due to the presence of a β-lactamase) but is usually susceptible to aminoglycosides, glycopeptides, clindamycin, ciprofloxacin and erythromycin. The recommended therapy for severe infection is the combination of vancomycin and an aminoglycoside, and clindamycin with gentamicin for ophthalmic infections. Ciprofloxacin has been used successfully to treat wound infections and bacteremia.



	Gram stain	Colonies (48h)
Corynebacterium diphtheriae	1A	1B
Corynebacterium ulcerans	2A	2B
Corynebacterium jeikeium	3A	3B
Arcanobacterium haemolyticum	4A	4B
Arcanobacterium pyogenes	5A	5B
Oerskovia turbata	6A	6B
Brevibacterium casei	7A	7B
Rothia dentocariosa	8A	8B
Rothia mucilaginosa	9A	9B
Norcardia farcinica	1C	1D
Nocardia brasiliensis	2C	2D
Rhodococcus equi	3C	3D
Tsukamurella paurometabola	4C	4D
Streptomyces spp.	5C	5D
Bacillus cereus	6C	6D

Fig. 167.3 Gram stain and colonies (48-hour incubation) of different Gram-positive bacilli.

Erysipelothrix

NATURE

Erysipelothrix rhusiopathiae is a small Gram-positive bacillus, widespread in nature, causing self-limited illnesses or systemic infections in various animals, swine in particular, and occasionally in humans. *Erysipelothrix rhusiopathiae* is a thin, elongated (0.2–0.4 × 0.8–2.5 µm) Gram-positive to gram-labile, aerobic to facultative anaerobic bacillus, nonmotile, nonsporulated and nonencapsulated, belonging to the clade of Gram-positive bacilli with a low G+C content (36–40%) (*Listeria*, *Lactobacillus*, *Kurthia*, *Brochothrix*).[23]

EPIDEMIOLOGY

Erysipelothrix rhusiopathiae is ubiquitous in nature, found on plants, in humid soil and used water, as well as in many animals as asymptomatic carriers. The major reservoir is the pig, where it is carried in the pharynx or digestive tract as a commensal. It can also cause a disease called swine erysipelas, presenting as skin infection, arthritis or septicemia.[24]

Humans usually develop a local infection through direct contact with an infected animal or animal product. At particular risk are butchers and fishermen handling crabs, but also farmers, abattoir workers and veterinarians. There is no documented human-to-human transmission.

PATHOGENICITY

Several components of *E. rhusiopathiae* are though to play a role in virulence: these include an antiphagocytic capsule, a surface protein (Spa A), the enzyme neuraminidase and the capacity to survive in macrophages. The exact mechanisms, however, are not firmly established.

DIAGNOSTIC MICROBIOLOGY

For localized erysipeloid lesion(s), the best clinical specimen is a deep tissue biopsy or liquid when present in vesicles. Swabs of the skin surface are to be discouraged. Blood cultures are taken for systemic disease.

The microscopy of a tissue biopsy is occasionally positive for thin Gram-positive bacilli. On blood agar, *E. rhusiopathiae* appears as punctiform colonies, α-hemolytic after 2 days, either as gray, translucent and regular S colonies (smooth variant) or as larger, opaque, irregular R colonies (rough variant). Growth is enhanced in 5–10% CO_2 at 91–99°F (33–37°C) and usually present in liquid media.

Preliminary identification relies on the pleomorphic Gram stain and cultural appearance of the colonies. *Erysipelothrix rhusiopathiae* is catalase-negative, nonmotile and non-β-hemolytic, but H_2S positive in 24–48 hours on TSI or Kligler slant agar.[2] There is an indirect immunofluorescence test, as well as a mouse inoculation test used in reference laboratories. There is no serologic test currently available.

Erysipelothrix rhusiopathiae isolates can be typed in two serogroups (A and B) and 23 serovars for epidemiologic purposes. *Erysipelothrix rhusiopathiae* is susceptible *in vitro* to penicillins, cephalosporins, clindamycin and erythromycin, more or less susceptible to tetracyclines and chloramphenicol, and resistant to vancomycin, aminoglycosides, sulfonamides and trimethoprim–sulfamethoxazole.[25]

CLINICAL MANIFESTATIONS AND MANAGEMENT

Erysipelothrix rhusiopathiae has three distinct clinical presentations, as follows.

- A cutaneous lesion called erysipeloid, localized to the upper extremities (generally the fingers) around an inoculation site, and characterized by a painful itching and burning erythematous, violaceous, well-demarcated lesion developing over 10 days after inoculation. Fever or arthralgias are uncommon, but localized lymphangitis (30%) and lymphadenopathy (30%) are frequent. In the absence of treatment, spontaneous healing generally occurs.
- A diffuse cutaneous disease with skin and soft tissue extension from the original inoculation site. This form is less frequent and is observed in some immunocompromised patients, in whom systemic manifestations (fever, arthralgia) are common.
- A disseminated disease, usually septicemia, often (up to 90%) complicated by an endocarditis on native valve. This form is usually a primary infection but can follow an erysipeloid lesion in about 40% of cases. The disease is often characterized by a long-lasting fever, with multiple cutaneous lesions on the trunk or the extremities. Classic underlying conditions are alcohol abuse and/or chronic liver disease, as well as corticosteroid use or chemotherapy. The associated mortality is rather high (38%).[26]

The treatment of uncomplicated erysipeloid skin lesions (with oral penicillin, 500 mg q6h, for 1 week or another active drug, such as another β-lactam, fluoroquinolones or clindamycin) will accelerate natural healing. For severe cutaneous disease or disseminated disease the recommended therapy is intravenous benzylpenicillin (2–4 million units q4h), ceftriaxone (2 g q24h) or ciprofloxacin (400 mg q12h) for 2–4 weeks.[24] As there are no human vaccines, the only form of disease prevention is to limit occupational exposure to the organism.

Corynebacterium diphtheriae and *C. ulcerans*

NATURE

Diphtheria is now a rare disease. This communicable infectious and vaccine-preventable disease may result in acute localized respiratory infection with typical 'croup' symptomatology due to the development of oropharyngeal adherent pseudomembranes. The mortality is due to airway obstruction or myocarditis caused by toxin production. *Corynebacterium diphtheriae* is a small, irregular, nonsporulated, Gram-positive bacillus with enlarged extremities and a typical 'club-shaped' morphology. A 'palisade' or 'V' arrangement or clusters with a so-called Chinese-letter appearance are observed in liquid media (Fig. 167.3, lanes 1A and 1B). *Corynebacterium* spp. belong to the broad class Actinobacteria. Phylogenetic studies show that *Corynebacterium* spp. are closely related to acid-fast bacilli such as *Mycobacterium*.

Corynebacterium ulcerans shares certain common features with *C. diphtheriae*. *Corynebacterium ulcerans* causes a rare oropharyngeal diphtheria-like illness, as well as extra pharyngeal infections due to the presence of a toxin similar to diphtheria toxin. Taxonomic studies show that *C. ulcerans* is closely related to *C. diphtheriae*.[27]

EPIDEMIOLOGY

Diphtheria remains endemic in numerous countries throughout the world – Eastern Europe, South East Asia, South America and the Indian subcontinent. In developed countries, diphtheria occurs sporadically and most cases are imported from areas of endemicity.[28]

During the 1990s, an important outbreak occurred in Russia and the newly independent states of the former Soviet Union, with more than 157 000 cases and 5000 deaths. Several factors may explain the re-emergence of epidemic diphtheria, notably the introduction of a toxigenic strain, insufficient coverage of vaccination of children and an increasing proportion of adults with waning vaccine-induced immunity. Contrary to the prevaccine era, where diphtheria was essentially a childhood disease, a shift in the age of patients was observed. The epidemic began in towns and in groups with close contacts (e.g. hospitals, military troops), before dissemination to socioeconomically disfavored groups (e.g. alcoholics).[29,30]

Achieving a high coverage (90–95%) of primary immunization is mandatory for diphtheria elimination and periodic booster doses for adults are necessary in regions at high risk of diphtheria. Control measures allow the control of epidemic diphtheria. In 1997, the World Health Organization (WHO) identified 10 countries with more than 10 cases for a total number of 15 839 cases reported, whereas in 2006 the number of reported cases decreased to 4000.

Humans represent the sole significant reservoir for *C. diphtheriae*. Acquisition occurs essentially through direct transmission (or via airborne droplet) with contaminated respiratory secretions or skin lesions.

In temperate climates, respiratory infections due to toxigenic strains predominate, while in the tropics, cutaneous diphtheria is more commonly caused by nontoxigenic strains. A peak incidence of respiratory infections is observed during the cold months.

In diphtheria endemic areas with high prevalence of cutaneous diphtheria, respiratory diphtheria is rare due to the high rate of natural immunity attained through exposure to cutaneous diphtheria. In most cases, transmission of *C. diphtheriae* to susceptible individuals results in transient pharyngeal carriage rather than in disease. Cutaneous diphtheria appears to be more contagious than the respiratory form of diphtheria and persists longer than *C. diphtheriae* infections of the tonsils or nose in a carrier state.

Corynebacterium ulcerans is a commensal of wild and domestic animals. Human *C. ulcerans* infection is considered a zoonosis, since human transmission occurs via manipulation of infected dairy animals or consumption of contaminated milk; however, these risk factors are absent in half of the cases. Global epidemiologic data on *C. ulcerans* infection are missing but *C. ulcerans* strains represent 58% of toxigenic *Corynebacterium* strains submitted to WHO reference laboratories.[31]

PATHOGENICITY

Corynebacterium diphtheriae contains pili or fimbriae involved in initial adhesion of the bacteria to host-cell receptors; the pili also promote the aggregation of other bacteria to ensure colonization. These structures contain subunits of pilin proteins, a backbone protein component (SpaA; Spa for sortase-mediated pilus assembly) and two ancillary proteins (SpaB, SpaC). The pili are covalently linked to the peptidoglycan structure of the bacterial cell wall. Minor pilins, SpaB and SpaC, are required for pharyngeal cell adhesion, the specific receptor being currently unknown.[32] This type of pilus is encoded on a pathogenicity island, probably acquired by recent horizontal transfer. *Corynebacterium diphtheriae* is responsible for a localized predominantly pharyngeal infection with the appearance of a so-called 'pseudomembrane' made of fibrin, neutrophilic inflammation and abundant colonies of *C. diphtheriae*. The major virulence factor is a 58 kDa exotoxin responsible for the systemic complications of diphtheria, notably for myocardial and neurologic toxicity.[33] This exotoxin is very effective, since one molecule introduced into a cell can kill it.

The exotoxin gene (*tox*) is present on a family of corynephages hosted by some *C. diphtheriae* strains. The expression of toxin is reliant on an iron-dependent regulatory element (*dtxR*). The presence of iron activates *dtxR* and blocks the transcription of *tox*. Under iron-limiting conditions, generally observed on the mucosal surface, *dtxR* is inactivated and diphtheria toxin is produced. The toxin is excreted through the bacterial cell and diffuses both locally and via the circulation to all organs, the myocardium and the peripheral nerves being the most affected. Diphtheria toxin has a complex activity, resulting in cytolysis through cell-protein inhibition.

Three distinct domains of diphtheria toxin have been described:

- the carboxyl-terminal R-domain (fragment B) which is responsible for binding to the heparin-binding epidermal-like growth factor, the specific receptor for the toxin;
- internalization into endocytic vesicles which is mediated by the central part of the toxin; and
- the amino-terminal C-domain (fragment A) which is delivered in the cytosol and is responsible for the inhibition of protein synthesis by inactivating the eukaryotic elongation factor 2 (eEF-2) through adenosine diphosphate (ADP) ribosylation of diphthamide residue[34] (Fig. 167.4).

Schematic representation of *C. diphtheriae* pathogenesis

Entry site	• Oropharynx • (skin wound)	• Adhesion to pili • Toxin production	
Toxin dissemination	• Oropharynx • Blood		
Target of toxin	• Oropharynx • Myocardium • Peripheral nerves	• Fixation to heparin-binding EGF-like growth factor • Endocytosis • Disruption of toxin part A • Translocation into cytoplasm • Inhibition of eEF2 after ADP-ribosylation of diphthamide residue	

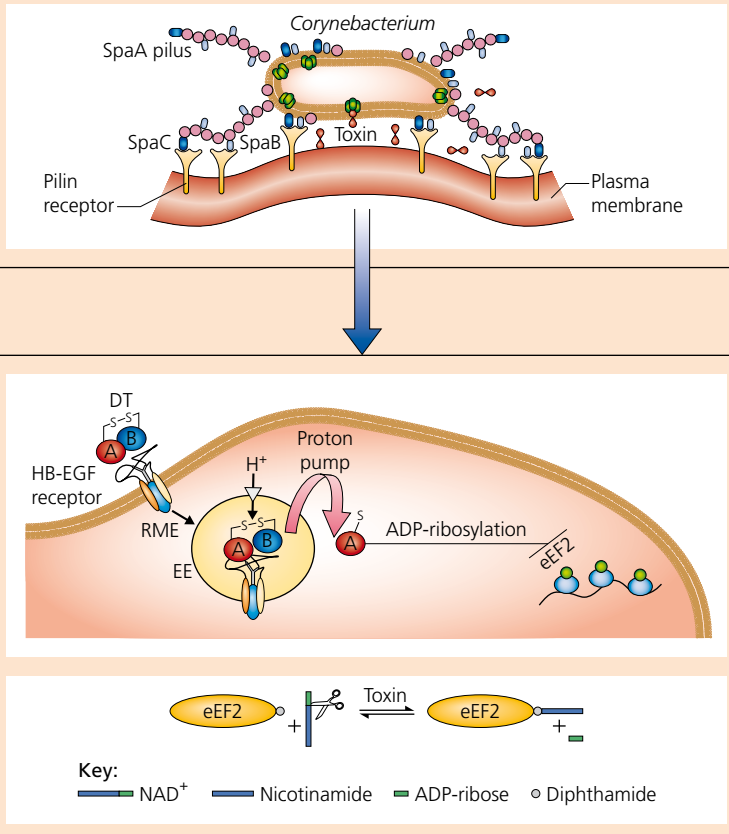


Fig. 167.4 Schematic representation of *C. diphtheriae* pathogenesis. Adapted from Mandlik et al.[32] and Yates et al.[34]

Histology of heart tissues shows necrosis associated with active inflammation in the interstitial space. Cardiac conduction tissue may also be affected. Diphtheria toxin may affect peripheral nerves, causing a demyelination localized around the nodes of Ranvier. In severe cases, axonal degeneration can occur.[35]

Corynebacterium ulcerans also carries a corynephage coding for the diphtheria toxin, and the *C. ulcerans tox* gene has 95% homology with *C. diphtheriae tox* gene. Most differences are located in the B fragment of the toxin in the translocation (T) region and in the receptor-binding (R) domain.[36]

DIAGNOSTIC MICROBIOLOGY

Due to the declining incidence of diphtheria the policy for screening throat swabs varies in different countries. In endemic regions, routine screening for *C. diphtheriae* in throat swabs is recommended since milder infections or atypical infections may be misdiagnosed. When diphtheria is suspected, clinicians should inform the laboratory.

Cotton or polyester-tipped swabs are appropriate for sampling. When membranes are removed, they should be sent to the laboratory for culture and a swab from areas under the membranes should be obtained. Standard Amies transport media ensure adequate maintenance of viability during transport to the laboratory. When molecular testing is available, Dacron polyester swabs are preferred for sampling. Suspected cases should have specimens taken from both the nose and the throat. For wound infections, swabs or aspirates of inflamed lesions are recommended.

For culture, selective media such as cysteine–tellurite blood agar or Tinsdale medium and nonselective sheep blood agar are recommended.[37] When selective media are not available, multiple colonies from sheep blood agar are picked and biochemically identified to rule out *C. diphtheriae*. Commercial biochemical tests accurately identify *C. diphtheriae* strains. *Corynebacterium diphtheriae* appear as small 0.5 mm up to 2 mm large colonies with a gray to white opaque surface (see Fig. 167.3, lanes 1A and 1B). Some strains (biotype intermedius) appear dysgonic on standard media, their colonies being enlarged on lipid-enriched media (lipophilism). Based on colony morphology and biochemical reactions, *C. diphtheriae* can be divided into four biotypes: *gravis*, *mitis*, *belfanti* and *intermedius*. However, biotyping is of limited use and is generally carried out only in reference laboratories.

Corynebacterium ulcerans grows on standard sheep blood agar media, but the use of selective media such as cysteine–tellurite blood agar or Tinsdale medium facilitates the isolation of pathogenic corynebacteria.

After 24 hours, 1 mm colonies are observed with a gray-white appearance (see Fig. 167.3, lanes 2A and 2B) and slight hemolysis on sheep blood agar. Presence of urease and a positive reverse CAMP test (inhibition reaction) differentiate *C. ulcerans* from *C. diphtheriae*.[38]

The detection of toxin is the most important test; molecular tests based on the amplification of the *tox* gene constitute an alternative to the classic Elek immunoprecipitation test. Recently, the direct detection of the *tox* gene from clinical specimens has been described; however, the amplification test cannot differentiate the *C. diphtheriae tox* gene from other toxin-producing corynebacteria such as *C. ulcerans* and *C. pseudotuberculosis*.[39]

Some toxigenic *C. ulcerans* strains show atypical results in real-time PCR for toxin detection. Specific amplification of the *C. ulcerans tox* gene have been described based on the polymorphic region of the *tox* gene.[40]

CLINICAL MANIFESTATIONS

Respiratory tract disease

Corynebacterium diphtheriae is responsible for localized infection of the upper respiratory tract. The oropharynx and rhinopharynx are most frequently affected. Symptoms generally occur 2–5 days after transmission, the most common symptoms being a moderate fever and sore throat followed by weakness, painful swallowing and headaches. Classical adherent and gray pseudomembranes are not always present and their extent can vary from small patches on infected tonsils to an extensive involvement of the posterior oropharynx and larynx. A serous or serosanguinous discharge can be observed in nasal diphtheria which is often milder and chronic. Signs of toxicity may be present with edema of the neck, hoarseness and stridor and a marked local lymphadenopathy. In adults, atypical presentations are observed with oral lesions.[35]

Respiratory infections due to *C. ulcerans* are similar to infection due to *C. diphtheriae*.

Cutaneous disease

Cutaneous diphtheria appears usually as a chronic and nonspecific disease. In fact, *C. diphtheriae* may colonize any kind of primary cutaneous lesion. Superinfection with *C. diphtheriae* begins with vesicles or pustules evolving as skin ulcers, with edematous surrounding tissues. Dark pseudomembranes may be observed during the first 1–2 week(s). Their localization is predominantly on the legs and hands. Co-infection with other pathogens such as *S. aureus* and *Streptococcus pyogenes* is common. Systemic toxic manifestations of cutaneous diphtheria are rarely seen. Chronic cutaneous infections with gray membranes due to toxigenic *C. ulcerans* have been described.[41]

Infections due to *C. diphtheriae* and *C. ulcerans* are undoubtedly underreported since cutaneous manifestations may be mild and corynebacteria are often considered as contaminants.

Myocardial disease

Myocardial toxicity occurs in 10–20% of patients with respiratory diphtheria and is associated with a mortality rate of 40%. Its frequency depends on the extension of the oropharyngeal infection and on the delay until antitoxin therapy is started. Cardiac manifestations appear during the second week of the disease with a dilated cardiomyopathy and depressed left ventricular function. ECG shows anomalies of conduction and dysrhythmia may be observed with atrioventricular block, bundle branch block and hemiblock. Markers of poor prognosis and carditis in children are a high myoglobin level and an increased lactate dehydrogenase (LDH) isoenzyme level, with an LDH1/LDH2 ratio >1.[42]

Neurologic disease

Neuronal toxicity appears generally in severe respiratory diphtheria. Distinct types of damage are observed due to the regional effect of the toxin, with paralysis of the soft palate, of the posterior pharyngeal wall with dysphonia, dysphagia and defects of ocular accommodation; a delayed complication consisting of a peripheral neuritis affecting the limbs and/or the diaphragm can also occur up to 3 months later. Recovery is slow and only 20% of patients with diphtherial peripheral neurotoxicity were completely healthy after 1 year.[43] The incidence of severe paralysis seems reduced when prompt antitoxin therapy is initiated within the first 2–3 days of infection.

Systemic disease

Deep seated infections due to *C. diphtheriae* (e.g. endocarditis, septic arthritis, osteomyelitis and splenic abscesses) have also been documented. Risk factors such as intravenous drug use, homelessness and alcoholism have been identified. The portal of entry is likely to be skin colonization or skin infections with *C. diphtheriae*. Strains belonging to different biotypes (*gravis*, *mitis*, *belfanti*) have been isolated and are generally nontoxigenic.[44] These strains may represent a potential reservoir for the emergence of toxigenic *C. diphtheriae* strains since they possess the functional regulation machinery represented by the 'diphtheria toxin repressor (*dtxR*) genes' and thus could become toxigenic by acquiring the *tox* gene.[45]

MANAGEMENT

The management of respiratory tract diphtheria includes specific and nonspecific measures. Specific measures include the administration of diphtheria antitoxin and antibiotic treatment for *C. diphtheriae* eradication, whereas nonspecific measures are the surveillance and support of respiratory and cardiac functions. Neutralization of circulating toxin through diphtheria antitoxin represents the mainstay of diphtheria therapy. Since diphtheria antitoxin is prepared with horse hyperimmune serum, the sensitivity of the patient to horse serum should be tested before administration. According to the extent and duration of the illness, a variable dose of antitoxin is administered (Table 167.2).[46] Penicillin or erythromycin constitutes the standard antibiotic treatment. *In-vitro* resistance to penicillin has not yet been described but *in-vitro* penicillin tolerance has been documented and could explain some eradication failures.[47] Erythromycin is generally highly active *in vitro* but inducible resistance has been described.[48]

Antibiotics are used to reduce the carrier state and are combined with antitoxin to treat the disease. Erythromycin 40–50 mg/kg/day intravenously for 7–14 days is the treatment of choice, but oral penicillin (benzylpenicillin G 50 000 IU/kg/day for 5 days followed by penicillin V 50 mg/kg/day for 5 days) has been shown to be superior in a randomized trial.[48]

Testing for *C. diphtheriae* eradication is recommended at the end of treatment. Continue treatment for 10 days if culture remains positive.[46]

Close contacts should receive a single dose of penicillin or a 7-day course of erythromycin.[46]

PREVENTION

The following measures to control diphtheria have been proposed by international experts:

- primary prevention of disease based on a childhood immunization program;
- secondary prevention through rapid investigation of close contacts of index cases to prevent emergence of secondary cases, with investigation of carriage rate, clinical surveillance, penicillin prophylaxis and administration of a booster dose of diphtheria toxoid-containing vaccine; and
- tertiary prevention based on early diagnosis and proper management to prevent complications of suspected cases.[49]

Table 167.2 Dosage of antitoxin recommended for various types of diphtheria

Type of diphtheria	Dosage (units)	Route
Nasal	10 000–20 000	Intramuscular
Tonsillar	15 000–25 000	Intramuscular or intravenous
Pharyngeal or laryngeal	20 000–40 000	Intramuscular or intravenous
Combined types or delayed diagnosis	40 000–60 000	Intravenous
Severe diphtheria: extensive membrane and/or severe edema (bull-neck diphtheria)	40 000–100 000	Intravenous or part intravenous and part intramuscular

From Bonnet & Begg.[46]

Primary prevention with diphtheria toxoid obtained from formaldehyde-treated toxin is highly effective, providing high immunization rates. Recent epidemics have shown that more than 90% of children should be vaccinated to interrupt efficiently the transmission of epidemic clones.[50] The primary vaccination is based on a series of three doses started at 6 weeks of age and given at intervals of 4 weeks. Three doses induce the formation of protective toxin-neutralizing antibodies in 95.5% of children. This series is completed by at least one booster dose in the preschool period. The waning of adult immunity should be prevented with booster doses of diphtheria toxoid every 10 years throughout life. Recently, a new vaccine formulation called Tdap (for tetanus toxoid, reduced diphtheria toxoid and acellular pertussis vaccine) has been recommended for adults aged 19–64 years.[51] In endemic regions, booster doses are less necessary since natural boosting of immunity may occur through *C. diphtheriae* colonization.

Coryneforms

NATURE

The genus *Corynebacterium* contains more than 70 species, half of them being considered as pathogenic for humans in case reports or small series of cases. Taxonomic studies have shown that the genus is quite homogeneous except for a distinct subgroup formed by *Corynebacterium diphtheriae*, *C. kutscheri*, *C. pseudotuberculosis*, *C. vitarumen* and *C. ulcerans*.[27] Some *Corynebacterium* spp. have been reported as opportunist pathogens with some frequency, including *C. amycolatum*, *C. jeikeium* (formerly group JK) and *C. urealyticum*.

The so-called coryneforms represent a heterogeneous group of asporogenous, irregularly shaped, nonacid-fast, generally Gram-positive rods. This group contains the following genera representative species considered as human pathogens: *Arcanobacterium*, *Brevibacterium*, *Oerskovia* and *Rothia* (Tables 167.3, 167.4).

EPIDEMIOLOGY

For most *Corynebacterium* spp. and coryneforms, the reservoir and ecology are unknown. *Corynebacterium* spp. may be isolated from normal skin and mucous membranes, whereas *C. jeikeium* has also been isolated from inanimate hospital environments. *Arcanobacterium* may colonize mucous membranes from humans and farm animals. *Brevibacterium* can be isolated from dairy products (e.g. cheese) and *Oerskovia* from the inanimate environment. *Rothia* are members of the oropharyngeal microflora. Possible human-to-human or animal-to-human transmission may occur with *Arcanobacterium*.

PATHOGENICITY

Except for *Arcanobacterium*, most coryneforms and *Corynebacterium* spp. are opportunistic human pathogens. The major risk factors are immunosuppressive diseases such as malignancy, organ transplantation, iatrogenic manipulation (e.g. urinary tract instrumentation for *C. urealyticum*), breaks in the skin barrier (e.g. medical devices for *C. jeikeium*, *Brevibacterium* spp., *Oerskovia* spp., *Rothia* spp.) and therapy with broad-spectrum antibiotics (*C. jeikeium*, *C. urealyticum*, *C. amycolatum*).

DIAGNOSTIC MICROBIOLOGY

Several *Corynebacterium* spp. and coryneforms colonize the mucocutaneous epithelium and are part of the normal microflora. Their isolation from clinical specimens is difficult to interpret. Criteria have been proposed to help clinical microbiologists and clinicians to distinguish colonization from a possible role in infection (Table 167.5).

Table 167.3 Taxonomy of medically relevant Actinomycetales

Suborders	Families	Genera	Frequency	Practical classification
Actinomycineae	Actinomycetaceae	*Actinobaculum*, *Actinomyces*, *Arcanobacterium**, *Mobiluncus*	+	Ac
Propionibacterineae	Propionibacteriaceae	*Propionibacterium*	+++	An
Micrococcineae	Micrococcaceae	*Arthrobacter*, *Micrococcus*, *Rothia**	+	Co
	Cellulomonadaceae	*Cellulomonas*, *Oerskovia**, *Tropheryma*†	+	Co
	Dermatophilaceae	*Dermatophilus*	+	Aa
	Brevibacteriaceae	*Brevibacterium**	+	Co
	Dermabacteraceae	*Dermabacter*	+	Co
	Microbacteriaceae	*Microbacterium*	+	Co
Corynebacterineae	Corynebacteriaceae	*Corynebacterium**, *Turicella*	+++	Co
	Mycobacteriaceae	*Mycobacterium*†	++	My
	Nocardiaceae	*Nocardia**, *Rhodococcus**	+	Aa
	Gordoniaceae	*Gordonia**	+	Aa
	Tsukamurellaceae	*Tsukamurella**	+	Aa
	Dietziaceae	*Dietzia*	+	Aa
Streptomycineae	Streptomycetaceae	*Streptomyces**	+	Aa
Streptosporangineae	Nocardiopsaceae	*Nocardiopsis*	+	Aa
	Thermomonosporaceae	*Actinomadura**	+	Aa

Aa, aerobic actinomycetes; Ac, actinomycetes; An, anaerobic Gram-positive rods; Co, coryneforms; My, mycobacteria.
*Genera dealt with in this overview; †genera discussed in separate chapters.

With few exceptions, *Corynebacterium* spp. and coryneforms grow in 24–48 hours in aerobic conditions in standard liquid media (blood culture) or on solid media (blood-containing media). *Corynebacterium jeikeium* (see Fig. 167.3, lanes 3A and 3B) and *C. urealyticum* grow better in lipid-enriched media such as those containing Tween. Some morphologic or physiologic characteristics of colonies may help to differentiate them; for example:

- *C. amycolatum* produces dry colonies;
- β-hemolysis is observed around *Arcanobacterium* spp. colonies;
- *Brevibacterium* produces a cheese-like smell;
- *Oerskovia* colonies are pigmented in yellow to orange;
- white colonies with a 'spoked-wheel' aspect are observed in *Rothia dentocariosa*; and
- *R. mucilaginosa* produces mucoid colonies which adhere on agar (see Fig. 167.3, lanes 4–9A and 4–9B).

Antibiotic susceptibility testing is obtained generally by using E-test technology which allows the determination of the minimal inhibitory concentration on blood-containing agar. However, standardized interpretation criteria are lacking for these bacteria.

Corynebacterium jeikeium, *C. urealyticum* and *C. amycolatum* exhibit high levels of resistance to several antibiotics (including resistance to penicillins, cephalosporins, macrolides and aminoglycosides) but are uniformly susceptible to glycopeptides. Resistance to glycopeptides attributed to the presence of the *vanA* gene has been described in *Oerskovia turbata* and *Arcanobacterium haemolyticum* strains.[52]

CLINICAL MANIFESTATIONS AND MANAGEMENT

Corynebacterium spp. and coryneform bacteria have a low index of pathogenicity and are considered as opportunistic. Clinical syndromes that have been reported include bacteremia, endocarditis, infections of prosthetic joints, infections of the urinary tract and pneumonia in immunocompromised hosts.

For detailed discussions, see specific chapters and Table 167.4.

Aerobic Actinomycetes

NATURE

The term 'aerobic actinomycetes' helps clinical microbiologists to define noncoryneform Gram-positive rods which grow better under aerobic conditions. Table 167.6 shows the different genera belonging to this heterogeneous group. Only the most frequently encountered species in human pathology will be discussed. Important pathogens such as *Mycobacterium* and *Tropheryma* are covered elsewhere (see Chapters 30, 36 and 174).

EPIDEMIOLOGY

The prevalence of disease due to aerobic actinomycetes is not precisely established but remains rare. For nocardiosis, which represents the most frequently encountered disease in this group of micro-organisms, a frequency of about 2 per million cases annually has been proposed.[53,54] During the period prior to highly active antiretroviral therapy (HAART), the incidence of nocardiosis in AIDS patients with fewer than 100 CD4 cells/microliter was estimated to vary from 0.3% to 1.8% according to geographic differences, and predominated in rural environments.[55] In organ transplant recipients, nocardiosis was diagnosed in 0.6% of the patients in a recent case-control study. Independent risk factors were high-dose steroids, history of cytomegalovirus disease and high levels of calcineurin inhibitors.[56]

Rhodococcus equi, the second most frequent aerobic actinomycete, occurs in patients with the same predisposing factors. Rarely, infection may be observed in immunocompetent patients with a male-to-female ratio of 3:1 and a history of exposure to livestock.[57]

Table 167.4 Medically relevant *Corynebacterium* spp. and coryneform bacteria (all acid-fast stain negative)

Genera	Gram stain	Predominant species	Natural habitat	Site of infection	Risk factors, transmission	Diagnostic comments
Corynebacterium	Club-shaped morphology, arrangement in liquid media: V forms, in palisades or in clusters with so-called Chinese letter appearance	*C. diphtheriae*	Human	Upper respiratory tract, wound	No or insufficient vaccination, human-to-human transmission (close contact)	Four biotypes: *gravis*, *mitis*, *belfanti* and *intermedius C. diphtheriae* biotype intermedius: lipophilism (growth enhanced in presence of Tween 80)
		C. ulcerans	Cattle, domestic cats and dogs	Upper respiratory tract, wound	Contact with animals, rural setting, ingestion of unpasteurized dairy products	Small β-hemolytic colonies; reverse CAMP test with *S. aureus* positive; positive urease test
		C. jeikeium	Human skin (axillary, inguinal and perineal areas), mucous membranes (urinary tract), inanimate hospital environment	Bacteremia, endocarditis, foreign body infections, wound infections	Immunosuppression, medical devices, prolonged hospital stay, therapy with broad-spectrum antibiotics	Lipophilism; multiresistance to antibiotics
		C. amycolatum	Human skin	Bacteremia, endocarditis, foreign body infections, wound infections	Immunosuppression, underlying heart disease (endocarditis)	–
		C. urealyticum	Urinary tract, skin (axilla and groin of hospitalized patients)	Bacteremia, urinary tract infections, alkali-encrusted cystitis (struvite stones), wound infections	Immunosuppression (kidney transplant), urologic procedures	Lipophilism; strong positive urease test; multiresistance to antibiotics
Arcanobacterium	Gram-positive rod (to gram-variable, with branching bacilli)	*A. haemolyticum*	Oropharyngeal microflora	Pharyngitis (20% with morbilliform rash), soft tissue infections, sepsis (immunosuppression), endocarditis	Possible human-to-human transmission (pharyngitis in teenagers and young adults), immunosuppression	Small β-hemolytic colonies; reverse CAMP test with *S. aureus* positive
		A. pyogenes	Normal flora of farm ruminants and swine	Abdominal abscesses, soft tissue abscesses, bacteremia, endocarditis	Contacts with animals, rural setting	Small β-hemolytic colonies
Brevibacterium	Small Gram-positive rods (coccoid forms – old colonies)	*B. casei*	Dairy products/skin flora	Bacteremia, endocarditis	Immunosuppression, medical devices (intravascular catheter)	Nonhemolytic colonies; typical cheese-like smell
Oerskovia	Branching bacilli, coccoid forms (breaking up of mycelia)	*O. turbata*	Inanimate environment (soil)	Bacteremia, endocarditis	Immunosuppression, medical devices (intravascular catheter)	Yellow-orange pigmented colonies
Rothia	Gram-positive rods	*R. dentocariosa*	Oro-pharyngeal microflora	Bacteremia, endocarditis, meningitis	Immunosuppression, medical devices, underlying heart disease (endocarditis)	First isolation from carious lesions; nonhemolytic white colonies with a 'spoked-wheel' aspect
	Coccoid forms, encapsulated	*R. mucilaginosa*	Oro-pharyngeal microflora	Bacteremia, endocarditis, meningitis, lower respiratory tract infections	Immunosuppression, indwelling foreign material	Nonhemolytic white to gray mucoid colonies; adherence of older colonies to the agar surface

Table 167.5 Clinical conditions where precise identification of coryneform bacteria is recommended

- When isolated from an upper respiratory tract specimen, notably throat and nose swabs (*C. diphtheriae*)
- When isolated from a wound or skin specimen from a person having recently traveled abroad (*C. diphtheriae*)
- When isolated from normally sterile body fluids or if coccobacilli have been seen on Gram stain, notably when multiple specimens are positive for the same coryneforms
- When two or more blood cultures are positive for coryneforms
- When coryneform bacteria are seen on direct Gram stain associated with a strong leukocyte reaction, especially if other organisms recovered from the same specimen have a low pathogenicity
- When isolated from a urine specimen, if the organisms are the sole agent in counts of 10^4 cfu/ml or greater or if they are the predominant agent in counts of 10^5 cfu/ml or greater

Adapted from Efstratiou *et al.*[37] and Funke & Bernard.[38]

The reservoir of all aerobic actinomycetes is the soil environment where micro-organisms are implicated in decaying plant matter. *Rhodococcus equi* is found in high proportion in dung from horses and foals. Aerobic actinomycetes are thought to be acquired essentially by inhalation from the soil. Colonization or infections may also be acquired through a traumatic lesion contaminated with soil, wound infections or catheter colonization.

PATHOGENICITY

Few studies have investigated the virulence mechanisms of aerobic actinomycetes. The complete genome sequence of *Nocardia farcinica* has revealed the presence of potent virulence determinants such as mammalian cell entry (Mce) proteins with homology with *Mycobacterium tuberculosis* Mce proteins,[58] allowing mycobacteria to multiply in a mammalian host. Similar Mce genes have also been identified in *R. equi*[59] and *Streptomyces coelicolor*.[60]

Nocardia farcinica and *R. equi* also possess adherence factors with proteins belonging to fibronectin-binding protein families presenting a homology with antigen 85 of *M. tuberculosis*.[58,59]

Murine models have shown the importance of cell-mediated immunity in nocardiosis.[61] Studies with human monocytes show a resistance of *Nocardia* spp. to killing mechanisms of phagocytes by inhibition of phagosome–lysosome fusion and by bacterial detoxification enzymes (superoxide dismutase, catalase).[61] *In-vitro* studies have also demonstrated the induction of apoptosis related to caspase activation during contact of cells with *N. asteroides*.[62] This may play a role in the progression of nocardiosis.

Abscess formation with polymorphonuclear cell infiltration and later cell necrosis represents the hallmark of histologic lesions of both *Nocardia* and *Rhodococcus*. Malakoplakia is a granulomatous inflammation in which large macrophages have basophilic inclusions known as Michaelis–Gutmann bodies. This entity is observed in *R. equi* infection in HIV-infected patients.[63] The origin of Michaelis–Gutmann bodies is attributed to an abnormal degradation of bacteria by phagocytes.

DIAGNOSTIC MICROBIOLOGY

Microscopy and culture on appropriate media are critical for the precise clinical diagnosis of these infections, and contact with the laboratory is mandatory. Samples from the respiratory tract represent the majority of specimens, others being blood, cutaneous tissue, subcutaneous tissue biopsy and abscess collection from a large variety of organs. Multiple specimen examination is recommended, particularly from the respiratory tract.

Macroscopic examination of skin and soft tissue specimens is important to detect the presence of granules or concretions. These granules will be used selectively for microscopic examination since they represent masses of filaments embedded in necrotic tissue. Gram staining is preferred to modified acid-fast stain for visualization of this group of micro-organisms. However, determination of acid fastness is used as confirmatory staining. According to the species, the microscopic appearances may vary from coccoid and diphtheroid bacteria seen for *Rhodococcus* spp. and *Gordonia* spp. to branched and filamentous Gram-positive bacilli for *Nocardia* spp. and *Streptomyces* spp. (see Table 167.6 and Fig. 167.3, lanes 1–5C and 1–5D).

Routine media are generally used for recovery of aerobic actinomycetes; for specimens containing surface or mixed flora, selective media with antibiotic cocktails such as buffered charcoal yeast extract (BCYE) agar is recommended, particularly for *Nocardia* and for respiratory tract specimens. Media appropriate for mycobacteria such as Löwenstein may also be used. The incubation period for bacterial growth is prolonged, up to 2–3 weeks. Conventional blood cultures may be used for the detection of bacteremia due to *Rhodococcus* or *Gordonia*, but prolonged incubation is mandatory. In a review of *Nocardia* bacteremia, the detection time ranged from 2 to 14 days with a median of 4 days.[64]

A minimum of 48–72 hours is necessary before colonies became visible on routine media. Phenotypic aspects such as pigmentation or aerial hyphae appear later (see Table 167.6). Aerial hyphae give a powdery aspect to most *Nocardia* and *Streptomyces* isolates; sparse aerial hyphae can be observed with *Actinomadura* isolates or rarely for *Rhodococcus* strains. Mucoid colonies are typically seen for *R. equi* strains and *Actinomadura*. Only a small fraction of bacteria appear acid fast, this property being enhanced in lipid-rich media such as Löwenstein.

Biochemical identification to species level is difficult since most commercial kits are not appropriate for aerobic actinomycete differentiation. Molecular techniques, notably sequencing of 16S rRNA, have now replaced biochemical identification.[65] Analysis of heat shock protein genes by PCR paired with restriction fragment length polymorphisms has also been described.[66]

The distinction of pattern of resistance to antimicrobial agents has been used to subtype *N. asteroides* complex into six different groups.[67] Species most frequently isolated in human specimens are *N. asteroides* sensu stricto type VI, *N. brasiliensis*, *N. farcinica* and *N. nova*, the proportion of each species varying according to the type of specimen and the geographic location.

Antibiotic susceptibility testing with broth microdilution is the recommended method.[68] The breakpoints for *Nocardia* and other aerobic actinomycetes are based on pharmacokinetic/pharmacodynamic (PK/PD) data. For clinical laboratories not performing these tests, collaboration with reference laboratories is important since these tests remain problematic due to the slow growth and the degradation of the antimicrobial agent.

CLINICAL MANIFESTATIONS AND MANAGEMENT

Pulmonary infections and disseminated infections

Nocardiosis

Pulmonary infections and disseminated infections represent the most frequent form of nocardiosis. The patients are generally severely immunocompromised due to advanced HIV infections with low (<100)

Table 167.6 Medically relevant aerobic Actinomycetes

Genera	Gram stain	Acid-fast stain	Predominant species	Natural habitat	Site of infection	Risk factors, transmission	Diagnostic comments
Nocardia	Branched filamentous Gram-positive bacilli, short rods (breaking up of mycelia)	Positive	*N. asteroides, N. brasiliensis, N. farcinica*	Inanimate environment (soil)	Soft tissue infection, nonmycetomic (mycetoma), respiratory and disseminated infection	Penetrating wound (occupational disease, male predominance, tropical and subtropical regions), immunosuppression (inhalation), underlying chronic lung disease	Presence of white to yellow intralesional grains (mycetoma); colonies white to red, superficially chalky appearance (powdery aerial hyphae); slow growth with aerial mycelium (3–7 days)
Rhodococcus	Coccoid forms to coccobacilli (may be dismissed as diphtheroids)	Weakly positive	*R. equi*	Inanimate environment (coprophilic soil – dung of horses)	Pulmonary (lung abscesses) and bacteremic infections, rare cutaneous infections (traumatic wounds)	Immunosuppression (AIDS, organ transplant recipients), contact with animals	Pink mucoid colonies (variant: yellow pigmentation, rough appearance)
Gordonia	Thin Gram-positive coccobacilli (may be dismissed as diphtheroids)	Weakly positive	*G. terrae*	Inanimate environment (soil)	Bacteremia, endocarditis	Immunosuppression, indwelling foreign material, wound infections	Dry, wrinkled beige to orange colonies
Tsukamurella	Gram-positive rods	Positive	*T. paurometabola*	Inanimate environment (soil)	Bacteremia, endocarditis	Immunosuppression, indwelling foreign material	Psychrophilic micro-organism (grows best at cooler temperatures); slow growth
Actinomadura	Branched filamentous Gram-positive bacilli with short chains of spores	Negative	*A. madurae*, *A. pelletieri*	Inanimate environment (soil)	Soft tissue infection (mycetoma), rare nonmycetomic disseminated infection, pneumonia (immunosuppression)	Penetrating wound (occupational disease, tropical and subtropical regions), inhalation (immunosuppression)	Colonies white to red, usually mucoid, molar tooth appearance after a few days of growth; aerial hyphae if present are sparse and appear later during growth incubation
Streptomyces	Filamentous Gram-positive bacilli	Negative	*Streptomyces somaliensis*	Inanimate environment (soil)	Soft tissue infection (mycetoma), perianal soft tissue infection	Penetrating wound (occupational disease, tropical and subtropical regions)	Presence of yellow to brown intralesional grains (mycetoma); smooth to rugous colonies, generally with powdery aerial hyphae

CD4 counts or are receiving chemotherapy with cytotoxic agents or immunosuppressive drugs. Pulmonary nocardiosis is also observed in patients with impaired local defenses due to chronic lung disease such as chronic obstructive pulmonary disease, bronchiectasis and chronic sarcoidosis.

Clinical presentation is subacute or chronic with cough, dyspnea, productive sputum, hemoptysis and systemic symptoms such as fever, weight loss and sweats. Lung infiltrates with and without abscess formation represent a frequent radiologic finding. Other findings are the presence of nodules, reticulonodular infiltrates, interstitial infiltrates and pleural effusions. Nocardial lesions are poorly localized and may extend to adjacent tissues with pleural effusion, pericarditis or mediastinitis. Misdiagnosis as malignancy or tuberculosis is frequent. In a recent observational study performed on adult patients diagnosed with pulmonary nocardiosis during a 13-year period, disseminated nocardiosis was observed in 30% of patients.[69] Any organ may be involved; however, a predilection for brain and cutaneous dissemination is frequently mentioned. In a review of 1050 cases in the literature, 44% of patients with systemic nocardiosis had cerebral infections, with pulmonary infection being the primary site.[61] Brain abscess may be an unapparent infection; in other cases seizures, headache or focal deficits predominate.

Pulmonary and disseminated nocardiosis treatment is based on antibiotic therapy. Standard treatment is co-trimoxazole for 3–6 months. Combination therapy with imipenem or a third-generation cephalosporin with or without amikacin is recommended for severely ill patients or for those with cerebral lesions.[70] There is, however, considerable debate over the extent to which *in-vitro* antibiotic susceptibility testing is helpful in guiding the choice of therapy.

Clinical management of nocardial brain abscess has been reviewed.[71] If the nocardial infection is not documented elsewhere, aspiration or biopsy is recommended. When the nocardial infection is already documented, medical management is feasible for a brain abscess smaller than 2 cm. Surgical intervention and aspiration are needed if the clinical condition deteriorates, if the lesion does not shrink within 1 month or if the lesion is larger than 2.5 cm.

Mortality of pulmonary nocardiosis remains high in spite of adequate therapy. In one recent series, the mortality from pulmonary nocardiosis was 39%, 64% in case of disseminated nocardiosis and 100% in case of cerebral abscess.[69] In another series of organ recipients, 89% of patients were cured including six of seven cases with disseminated disease.[56] The mortality rates may vary from series to series according to diagnostic procedure, optimization of treatment and the severity of the patient's immunosuppression.

Rhodococcus equi

The lung represents the primary site of infection due to *R. equi*. Inhalation is the portal of entry and severe immunosuppression is the major predisposing factor, in particular advanced HIV infection. In a large retrospective study in HIV patients, the lung was involved in more than 90% of cases.[72] The clinical presentation develops insidiously over days to weeks, with fever, nonproductive cough, dyspnea and pleuritic pain predominating. Lung infiltrates or nodules are observed on chest radiographs and pleural effusions may occur. Cavitation is seen in 63% of immunocompetent patients and in 41–77% of HIV-immunosuppressed patients.[57,73] Bacteremia is detected in more than 50% of cases and dissemination to the CNS, bone and soft tissue may occur.

Treatment of *R. equi* lung infection is based on antibiotic therapy. Surgical resection is considered for isolated lung abscesses and failure of medical treatment. *Rhodococcus equi* is usually susceptible *in vitro* to erythromycin, rifampin, fluoroquinolones, aminoglycosides, glycopeptides and imipenem. Several successful antibiotic regimens exist. A few principles guide the treatment:

- a combination regimen is necessary to avoid the appearance of resistance;
- prolonged therapy (2–6 months) is recommended to avoid frequently observed relapses;
- initial intravenous therapy with bactericidal agents such as imipenem and vancomycin is followed by oral therapy (e.g. macrolide plus quinolone);
- use antibiotics able to kill intramacrophagic micro-organisms; and
- initiate HAART in HIV patients to reduce mortality.[72]

Rhodococcus equi causes a serious disease with a high mortality rate. The outcome depends on predisposing factors. In the pre-HAART period, mortality was estimated to be greater than 50%. In an extensive series of *R. equi* infections in patients with HIV, HAART was the unique independent factor associated with reduced mortality.[72] In transplant recipients and immunocompetent patients, mortality is about 20%.[57,74]

Cutaneous and soft tissue infection

Nocardia spp., *Actinomadura*, *Streptomyces*

Cutaneous nocardiosis can be divided into two main groups: primary lesions observed generally in immunocompetent patients and secondary lesions observed following dissemination in immunosuppressed patients representing metastatic lesions due to a bacteremic episode. In primary cutaneous nocardiosis, infection results from inoculation of the micro-organism during skin trauma (outdoor activities) or following iatrogenic trauma such as surgery. Rarely, in immunosuppressed patients, primary cutaneous lesions may evolve in a disseminated disease.[75]

Clinical presentation of primary cutaneous lesions is as cellulitis or abscess, or in chronic state as mycetoma. *Nocardia braziliensis* is isolated in the majority of cases.[67] Mycetoma predominates in men living in rural and subtropical or tropical areas. Lesions are usually on the feet, legs, shoulders and back. Mycetoma is a general term for a chronic granulomatous infection of the subcutaneous tissue due to either aerobic actinomycetes (actinomycetoma) or filamentous fungi (eumycetoma) (see Chapter 179). In actinomycetoma the following etiologic agents predominate: *Nocardia*, *Streptomyces* and *Actinomadura*. Mycetoma begins as nodules which fistulate to the skin. Exudates contain granules corresponding to masses of bacteria embedded in necrotic tissue. The lesion is chronic, and pain or systemic manifestations are generally absent. In late-stage infection, deep soft tissue and bone are implicated. Superinfection with pyogenic bacteria is frequent. Identification of the etiologic agent is necessary to determine the appropriate antibiotic regimen. Co-trimoxazole prescribed for several months is the treatment of choice.[76] For treatment failure, amoxicillin–clavulanate is recommended.[77]

Bacteremia and catheter-related Infections

Nocardia

A positive blood culture for *Nocardia* is a rare event. In a large review, the incidence was estimated at 0.003%.[64] Secondary bacteremia associated with pulmonary infection and cutaneous infection predominates. In this series, less than 20% of *Nocardia* positive blood culture was attributed to contamination. Catheter-related nocardial infections are also rare and catheter removal is recommended.

Rhodococcus equi

Bacteremia due to *R. equi* represents a secondary bacteremia during disseminated or lung infection. In HIV co-infected patients, up to 50% with *R. equi* lung disease have bacteremia.[72]

Gordonia

Gordonia is a rarely isolated human pathogen and only case reports or small series have been published. Several of these infections were bacteremias associated with an intravenous catheter.[78] The propensity of *Gordonia* spp. to biodegrade rubber may contribute

to the pathogenesis of these infections.[79] Infections generally resolved with catheter removal and an antibiotic regimen containing vancomycin.[80] *In vitro*, *Gordonia* strains are susceptible to imipenem and ciprofloxacin, frequently susceptible to gentamicin, vancomycin, azithromycin, ceftriaxone and ceftazidime (80–90% of isolates) and variably susceptible to doxycycline, trimethoprim–sulfamethoxazole, erythromycin, penicillin, ampicillin and rifampin (30–70% of isolates).[80]

Tsukamurella

Tsukamurella have also been isolated in relation to central venous catheter-related infection.[81] In a review published in 2004, fewer than 20 cases of *Tsukamurella* infections were identified, the majority being catheter related, generally in immunosuppressed patients.[82]

Management is based on medical device removal associated with an antibiotic regimen, generally with a β-lactam and an aminoglycoside.

REFERENCES


References for this chapter can be found online at http://www.expertconsult.com

Chapter 168

Jos van Putten
Tone Tønjum

Neisseria

INTRODUCTION

The genus *Neisseria* was named after Albert Neisser who observed gonococci (*Neisseria gonorrhoeae*) in leukocytes in urethral exudates from patients with gonorrhea in 1879. Eight years later Weichselbaum isolated the meningococcus (*Neisseria meningitidis*) from six of eight cases of primary sporadic community-acquired meningitis. The gonococcus and the meningococcus are exclusively human pathogens and the most studied species of the Neisseriaceae family.

Neisseria gonorrhoeae causes gonorrhea, a sexually transmitted disease. Gonorrhea was named by Galen in AD 130 after the Greek words *gonor* (seed) and *rhoia* (flow), suggesting that the disease was related to the flow of semen. In the 13th century Maimonides recognized that the urethral discharge of male gonorrhea patients was not semen, but a sexually transmitted disease. Gonorrhea was not clearly distinguished from syphilis until the 19th century. In 1885 Bumm proved gonococci as the cause of gonorrhea by inoculating human volunteers. Effective therapy became available in the 1930s when sulfonamides became available and in 1943 when penicillin was produced. Sulfonamide resistance occurred early and rapidly and the prevalence of strains resistant to penicillin and other antibiotics has risen steadily worldwide. Vaccine development is hampered by the multitude of gonococcal immune evasion strategies.

Neisseria meningitidis is a cause of endemic and epidemic meningitis and/or septicemia worldwide. Epidemic meningococcal meningitis was first described by Vieusseaux in 1805 in Geneva. Throughout the 19th century periodic epidemics occurred, involving mainly young children and adolescents including military recruits. In 1896 Kiefer described the nasopharyngeal meningococcal carrier state among healthy people. The outcome of meningococcal disease improved drastically by treatment with antibiotics. Selected meningococcal conjugate polysaccharides vaccines are now successfully used worldwide. A universal vaccine that protects against *N. meningitidis* serogroup B, which causes most cases of disease in temperate countries, is still under development. The absence of a serogroup B vaccine limits the effective control of meningococcal disease.

MICROBIOLOGY

Taxonomy

In the 2005 edition of *Bergey's Manual of Systemic Bacteriology* the family of the Neisseriaceae consists of the genus *Neisseria* as well as the heterogeneous genera (in order of decreasing relatedness to genus *Neisseria*) *Kingella*, *Eikenella*, *Alysiella*, *Simonsiella*, *Microvirgula*, *Laribacter*, *Vogesella*, *Vitreoscilla*, *Chromobacterium*, *Aquaspirillum*, *Prolinoborus*, *Formivibrio* and *Iodobacter*.[1] The taxonomy of the family has been extensively revised over the past decades, mainly based on 16S rRNA sequence analysis, even though this methodology does not reflect all the complex levels of relationships between these heterogeneous entities.

The members of the amended family of Neisseriaceae typically are Gram-negative coccal or coccoid micro-organisms. Some species are rod shaped (*N. weaveri* and *N. elongata*) or exhibit a multicellular micromorphology (*Simonsiella* spp. and *Alysiella* spp.). Several species possess capsules and are fimbriated (piliated). Endospores are not found and flagella are absent. Members of the genera *Simonsiella* and *Alysiella* show gliding motility. Some *Neisseria* spp., including *N. gonorrhoeae* and *N. meningitidis*, may show surface-bound twitching motility. All species are aerobic, but *Kingella* and *Eikenella* spp. also grow under anaerobic conditions. The G+C content of DNA ranges from 41 to 58 mol%.

Among the Neisseriaceae, *N. gonorrhoeae* and *N. meningitidis* are genetically very closely related human pathogens (Fig. 168.1). *Neisseria lactamica* and *N. cinerea* are related to *N. gonorrhoeae*, but frequently colonize the nasopharynx of children and adults. The saccharolytic Neisseriaceae (*N. polysaccharea*e, *N. subflava*, *N. sicca* and *N. mucosa*) are more distantly related to the other Neisseriaceae and colonize humans less frequently. The species of animal origin are even more distantly related to the human pathogens. The *Kingella* spp. and *Eikenella corrodens* are commensals that are occasionally isolated from human infections. These species are discussed in Chapter 172.

Growth characteristics

The genus *Neisseria* is composed of 17 species that may be isolated from humans and six species that colonize various animals (Table 168.1).[1] *Neisseria* spp. grow best aerobically in an atmosphere containing 5–10% carbon dioxide at a temperature of 89.6–98.6°F (32–37°C) and a pH of 7–7.5. The micro-organisms typically appear in pairs (diplococci) and occasionally in tetrads or clusters. *Neisseria elongata* and *N. weaveri* are medium-to-large plump rods that sometimes occur in pairs or short chains. Diplococci have flattened opposing sides, imparting the characteristic kidney or coffee-bean appearance seen in stained smears. Cell size ranges from 0.6 to 1.5 mm depending upon the species source of the isolate and the age of the culture.

Neisseria spp. are fastidious. Blood agar and chocolate medium (blood heated at 176–194°F/80–90°C) are suitable growth media. Bacterial colonies usually appear after 24–48 hours of growth. Colonies of *N. gonorrhoeae* are 0.5–1 mm in size. Colonies of *N. meningitidis* are usually larger (1–2 mm) and flatter. Colonies of the nonpathogenic *Neisseria* spp. are similar in size, appearance and consistency, except for the saccharolytic *Neisseria* spp. (*N. subflava*, *N. sicca* and *N. mucosa*) that are larger (1–3 mm), more convex and smooth (*N. mucosa*). Colonies of *N. subflava* and *N. sicca* are opaque and have varying consistency. *Neisseria sicca* adhere to the agar surface and become wrinkled

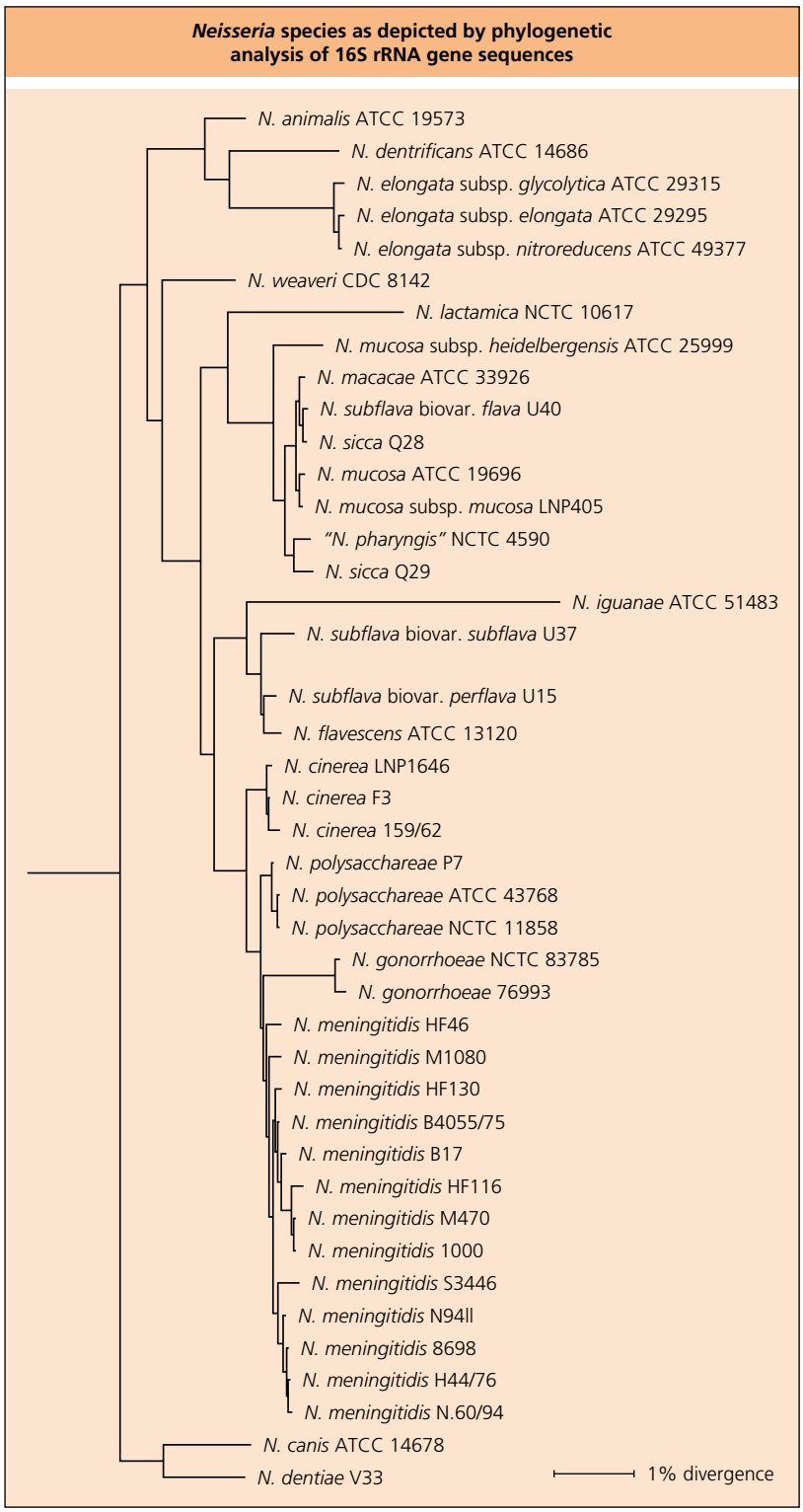


Fig. 168.1 *Neisseria* species as depicted by phylogenetic analysis of 16S rRNA gene sequences. Redrawn from Tønjum.[1]

with prolonged incubation. Some nonpathogenic *Neisseria* spp. form a yellow pigment (*N. flavescens*) or a greenish-yellow pigment (*N. mucosa*, *N. subflava*).

Neisseria spp. are oxidase positive and catalase positive, except *N. elongata*, which is catalase negative. All species produce acid from a few carbohydrates by oxidation. The ability to produce polysaccharide from sucrose, to produce catalase and deoxyribonuclease, to reduce nitrate and nitrite, and to oxidize the tributyrin fatty acid are also used to identify *Neisseria* spp.

Genome dynamics

The size of the *Neisseria* chromosome is approximately 2.2–2.3 Mb. The average G+C content is 48–56 mol%. *Neisseria meningitidis* and *N. gonorrhoeae* share about 95% of their gene content.[2] The vast majority of genes are also present in nonpathogenic *N. lactamica*, but gene regulation may be different in pathogenic and commensal strains.[3] Due to genetic instability, the *Neisseria* spp. have hyperdynamic genomes.[4] The genome plasticity contributes to the pathogenic

Table 168.1 Amended classification of micro-organisms within the family Neisseriaceae

Neisseria			***Kingella***	***Eikenella, Alysiella***	***Simonsiella***	**Recent genera**
Humans		Animals	Humans	Humans	Humans and animals	*Microvirgula* *Laribacter*
Urogenital tract	Oropharynx	Oropharynx	Oropharynx and urogenital tract	Oropharynx	Oral cavity	*Vogesella* *Vitreoscilla*
N. gonorrhoeae	*N. gonorrhoeae* *N. meningitidis* *N. lactamica* *N. sicca* *N. subflava* *N. flavescens* *N. mucosa* *N. cinerea* *N. polysaccharea* *N. elongata*	Dogs: *N. weaveri* Guinea-pigs: *N. animalis*, *N. denitrificans* Cows: *N. dentiae* Iguanas: *N. igaunae* Dolphins: *N. mucosa* Monkeys: *N. macacae*	*K. kingae* *K. denitrificans*	*E. corrodens* *A. filiformis*	*S. crassa*	*Chromobacterium* *Aquaspirillum* *Prolinoborus* *Formivibrio* *Iodobacter*

potential of *Neisseria* spp. and the development of hypervirulent lineages. The most important sources of neisserial genome instability (Fig. 168.2) are as follows.

- *Phase variation*, i.e. variable protein expression due to slipped-strand mispairing of nucleotide runs found within or close to the promoter region (affect transcription) or within open reading frames (affect translation).
- *Recombination*, i.e. the genetic exchange or rearrangement of DNA from external or internal sources. This may, for example, lead to the generation of millions of variants of pilin subunits.
- *Horizontal gene transfer*, i.e. the introduction of genes predominantly via natural transformation (the natural uptake of DNA) and integration into the chromosome by RecA-mediated homologous recombination. *Neisseria* spp. are naturally competent for transformation throughout their growth cycle.[6]
- *Hypermutation*, i.e. increased global mutation rates often associated with DNA repair deficiencies, replication infidelity or overexpression of DNA translesion polymerases. High mutation rates in specific loci (more than 100 phase-variable candidate genes in the pathogenic *Neisseria* spp.) result in rapid generation of variants.

The pathogenic *Neisseria* spp. share several genomic regions, including up to nine prophage and eight genetic islands, that are absent from *N. lactamica*.[7] There are no classic pathogenicity islands as present in many other bacterial species. *Neisseria meningitidis*-specific DNA sequences include the *cps* locus encoding the polysaccharide capsule, genes that encode the RTX family of toxins and an orthologue of the filamentous hemagglutinin of *Bordetella pertussis*. The genomes of disease and carriage isolates show no consistent differences. Certain hypervirulent lineages contain the filamentous prophage Nf1.[8] About 80% of the gonococcal clinical isolates and *N. meningitidis* strains of serogroups W135, H and Z contain the 'gonococcal genetic island' (GGI, 57 kb).[7] This is an often chromosomally integrated conjugative plasmid that encodes a type IV secretion system involved in DNA secretion. The genome of *Neisseria* spp. has a variable number of non-coding repeat arrays and insertion (IS) elements among which IS*1655* appears unique to *N. meningitidis*.

Most isolates of *N. gonorrhoeae* but not *N. meningitidis* carry plasmids.[9] Nearly all gonococcal strains carry a 4.2 kb cryptic plasmid of unknown function and many strains carry plasmids encoding β-lactamase causing resistance to penicillin. The conjugative plasmid TetM confers tetracycline resistance.

Genome-based phylogenetic reconstruction indicates that pathogenic *N. meningitidis* has relatively recently (several hundreds of years ago) emerged from a common unencapsulated ancestor by acquisition of capsule genes, probably from members of the family Pasteurellaceae.[10]

Structure and virulence factors

Neisseria spp., like other Gram-negative bacteria, have a cell wall that consists of two membranes separated by a thin peptidoglycan layer (Fig. 168.3). The inner cytoplasmic membrane consists of proteins embedded in a phospholipid bilayer that is impermeable for hydrophilic compounds. The outer membrane is an asymmetric bilayer composed of phospholipids in the inner leaflet and lipo-oligosaccharide (LOS) in the outer leaflet. The LOS renders the outer membrane relatively resistant to detergents and is semipermeable due to the presence of protein channels, called porins. Other surface-exposed outer membrane proteins and extracellular appendages such as capsular structures and type IV pili particularly contribute to neisserial survival and virulence.[12,13] The neisserial outer membrane continuously sheds vesicles (blebs) that contain DNA, protein and high levels of LOS.

Capsules

Neisseria meningitidis produces a polysaccharide capsule (see Fig. 168.3). On the basis of structural differences in capsule, meningococci are divided into at least 13 serogroups (A, B, C, D, 29E, H, I, K, L, W135, X, Y and Z). Serogroups A, B, C, Y and W135 cause more than 90% of meningococcal disease. Capsular types are normally stable but strains can acquire variant capsule gene alleles.[14] Serogroup B can thus switch to C or vice versa. The serogroup A capsule contains *N*-acetyl-mannosamine-1-phosphate. The capsules of the serogroups B, C, Y and W135 consist of polymers of *N*-acetylneuraminic (sialic) acid. The B polysaccharide resembles structures present in human neural tissues, limiting its immunogenicity and vaccine potential. The carbohydrates can be variably *O*-acetylated. The capsule polymers are anchored in the outer membrane through a 1,2-dipalmitoyl glycerol moiety. Capsule biosynthesis can vary and is subject to regulation. Isolates from healthy carriers are frequently unencapsulated due to reversible changes in capsule gene expression. A substantial proportion of meningococcal carrier isolates are incapable of capsule production due to deletions in or a lack of capsule genes. Isolates from the bloodstream or cerebrospinal fluid (CSF) are invariably encapsulated.

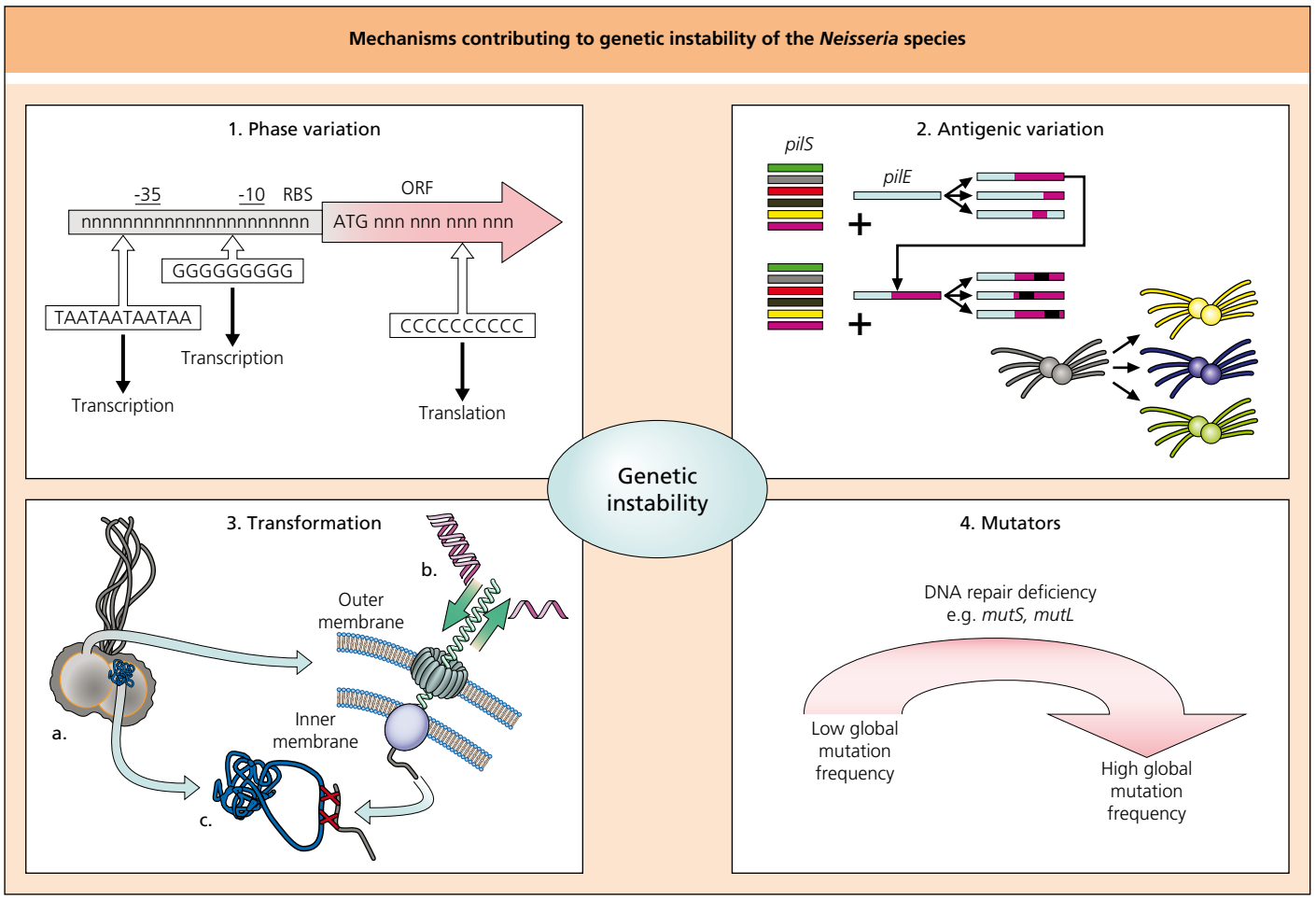


Fig. 168.2 Mechanisms contributing to genetic instability of the *Neisseria* species. Redrawn from Davidsen *et al.*[5]

Neisseria gonorrhoeae does not produce a polysaccharide capsule. However, both meningococci and gonococci are covered with a loosely adherent capsular-like structure containing high-molecular weight polyphosphate. This layer provides protection against environmental stress.

Pili

Pili are hair-like fibers consisting of thousands of protein subunits (pilin, 16–20 kDa).[15] Pathogenic *Neisseria* spp. have long pili (up to 4300 nm in length) termed type IV pili that protrude from the bacterial surface (see Fig. 168.3). Nonpathogenic *Neisseria* spp. may express both long and short pili (175–210 nm in length). Type IV pili confer bacterial cell-to-cell interactions and twitching motility – a form of locomotion that is powered by extension and retraction of the pilus filament. Pili are essential for adhesion to epithelial and endothelial cells and impart tissue tropism.[12,13] Expression of type IV pili is also required for efficient transformation of DNA.[6]

The main structural constituent of the type IV pilus fiber is the pilin subunit (PilE). During infection pilins undergo rapid phase shifts and antigenic variation. The single *pilE* expression locus is changed by unidirectional donation of coding sequences from multiple silent partial *pilS* genes in a process similar to gene conversion (see Fig. 168.2), producing an extensive repertoire of antigenic variants.[15] The frequency of antigenic pili variation may be as high as 10^3. Pilin can be post-translationally modified with phosphorylcholine, phosphoethanolamine and variable acetylated O-linked glycans.[16,17]

Neisseria gonorrhoeae produces one type of pili (class I). *Neisseria meningitidis* can express class I or class II pili which are antigenically and structurally distinct. Class II pili are encoded by a different *pilE* gene that has no silent cassette counterparts.

Surface proteins

The repertoire of the meningococcal and gonococcal surface proteins is very similar.[12,13] Trimeric protein channels (porins) confer transport of low-molecular nutrients across the outer membrane. The principal gonococcal porin is PorB (formerly protein I, 32–36 kDa). Gonococci express either of two PorB isotypes, termed PorB-IA and PorB-IB. The proteins are stably expressed but display interstrain variation due to amino acid differences in surface-exposed regions. The antigenic heterogeneity is the basis for PorB-based serologic typing. PorB-IA strains are resistant to normal human serum and can cause disseminated gonococcal infection. The gonococcal PorB porin is essential for bacterial survival.

Neisseria meningitidis can express two types of porin simultaneously: PorA and PorB. PorA (class 1 protein, 44–47 kDa) is variably expressed and PorA-negative variants can be isolated from patients. PorA antigenic differences serve as the basis for serologic subtyping of meningococci. Meningococcal PorB is equivalent to the gonococcal PorB protein and is present as either PorB-IA (class 2 protein, 40–42 kDa) or PorB-IB (class 3 protein, 37–39 kDa).[12,13] Serotyping of meningococci is based on antigenic differences in PorB. Expression of at least one of the porin types is required for meningococcal survival.

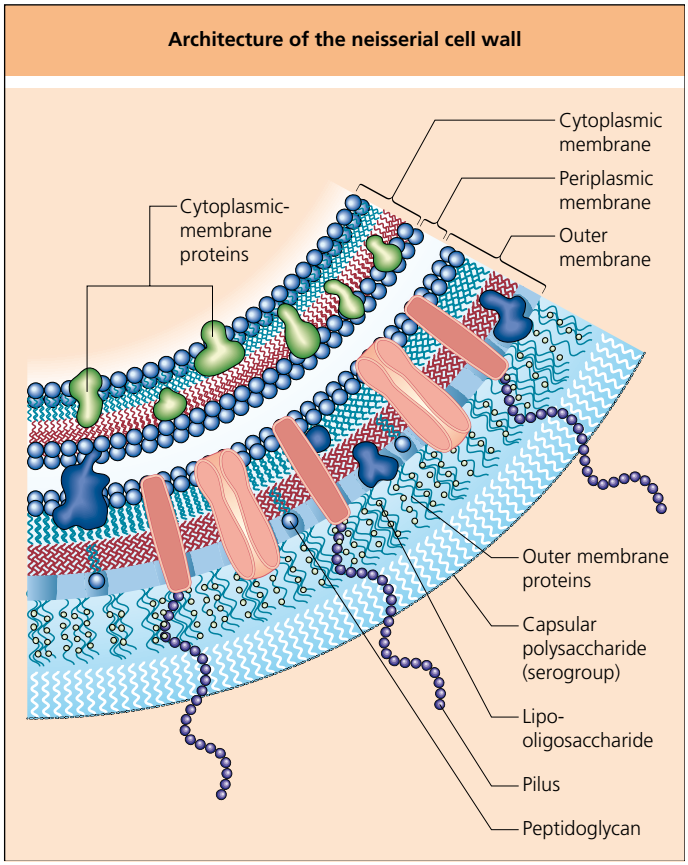


Fig. 168.3 Architecture of the neisserial cell wall. Redrawn from Stephens *et al.*[11]

The neisserial RmpM protein (formerly protein III or class 4 protein) is complexed to and likely stabilizes outer membrane protein complexes including porins. The protein is stably expressed by gonococci and meningococci. Its C-terminal periplasmic region resembles *Escherichia coli* OmpA domains and interacts with peptidoglycan. Rmp-specific antibodies may interfere with the bactericidal activity of antibodies directed to other surface antigens and increase the risk of infection.[18]

Both pathogenic *Neisseria* spp. can express different opacity (Opa) proteins (formerly protein II or class 5 proteins, 20–28 kDa).[12,13] Colonies of gonococci expressing Opa proteins often have a more opaque appearance. In meningococci colonial opacity is most evident at 88°F (31°C) when capsule production is low. Opa proteins are structurally very similar but display considerable intra- and interstrain variation in their surface-exposed regions. Antigenic variation occurs during natural infection due to selective immunologic pressure. Intrastrain variation results from intragenomic recombination and horizontal gene transfer. Opa proteins are variably expressed. The high-frequency phase variation is due to translational frame shifting caused by the presence of pentameric nucleotide repeats in each of the *opa* genes (see Fig. 168.2). The expression of each Opa protein can thus independently be switched on and off, enabling expressing of multiple proteins simultaneously.[13] The gonococcus contains up to 11 different *opa* genes. The meningococcal genome contains three to four *opa* genes. The proteins have an important role in neisserial adherence and invasion of eukaryotic cells. Their phase and antigenic variation limits their vaccine potential. Many meningococcal strains (~70%) express the Opc protein. This protein also confers colonial opacity and has functional similarity with Opa proteins. The Opc protein is absent in gonococci.

In addition to the aforementioned major outer membrane proteins, both pathogenic *Neisseria* spp. express more than 80 other outer membrane proteins, among which the pilus-related secretin complex PilQ is naturally overexpressed. Protein expression may vary, depending on the bacterial growth conditions. Iron-regulated proteins are essential for survival of gonococci and meningococci *in vivo*. The transferrin-binding proteins (Tbp-1 and Tbp-2) and the lactoferrin-binding proteins (Lbp) are scavenger proteins that mediate internalization of iron into the bacterium. Conserved surface-exposed proteins such as OMP85, NspA, NadA, GNA1870 (factor H binding protein) and GNA2132 (hypothetical lipoprotein) are candidate vaccine antigens.

Lipo-oligosaccharide

Approximately half of the neisserial surface comprises lipid-anchored oligosaccharides (LOS) (see Fig. 168.3). The LOS lacks the repeating carbohydrate units (O chain) of enterobacterial lipopolysaccharide (LPS). Neisserial LOS is composed of hexa-acylated lipid A, two KDO molecules, and one or more carbohydrate chains of 8–12 saccharide units, the core oligosaccharide. Lipid A anchors LOS in the outer membrane and is one of the most potent bacterial endotoxins.

The core oligosaccharide of neisserial LOS is divided into an inner and an outer core region. The composition of the inner core is heterogeneous due to variable substitutions (phosphoethanolamine, glycine, glucose, *O*-acetyl groups).[19] This variation in glycoforms is partially regulated by environmental cues. The outer core is highly variable and undergoes high-frequency phase and antigenic variation due to frequent nucleotide mismatching during replication of LOS biosynthesis genes and horizontal gene transfer.[20] A single strain can simultaneously express up to six related LOS structures. The terminal structure of neisserial LOS is the target for sialylation by bacterial sialyltransferase. Gonococci utilize host sialic acid (CMP-NeuNAc) to modify their LOS. Meningococci produce their own CMP-NeuNAc. The terminal LOS of the pathogenic *Neisseria* spp. often shares epitopes with host glycolipids.[20] This molecular mimicry is exploited by the pathogens to interact with host cell lectin receptors and limits the vaccine potential of the LOS.

Peptidoglycan

Neisserial peptidoglycan consists of long chains of repeated disaccharide units cross linked via peptide bridges.[21] Covalent linkages of peptidoglycan to lipoproteins have not been found, unlike in *E. coli*. Peptidoglycan metabolism is a dynamic process involving co-ordinated activity of lytic and synthetic enzymes. The peptidoglycan is synthesized by up to four penicillin-binding proteins (PBPs). *O*-acetylation of peptidoglycan protects against autolysis by endogenous lytic transglycosyates and host lysozymes. Released peptidoglycan fragments activate the innate immune response and contribute to the inflammatory response.

Secreted factors

Meningococcal and gonococcal genome analyses predict the presence of autotransporter-, two-partner-, type I and type II secretion mechanisms.[22] The pathogenic *Neisseria* spp. secrete immunoglobulin A1 (IgA1) protease. This serine protease directs its own transport across the outer membrane and secretion into the environment. The enzyme cleaves in the hinge of IgA1 separating the Fab and Fc regions, making IgA ineffective. IgA protease also cleaves other proteins such as endosomal Lamp1 important for intracellular vesicle trafficking. The function of the other secreted proteins including the filamentous hemagglutinin (FHA)-like protein TpsA and FrpA/C is largely unknown. A subset (~80%) of gonococcal strains secrete DNA via a type IV secretion system.[23] The genes encoding this system are located on the gonococcal genetic island (GGI) that is acquired by horizontal gene transfer. *Neisseria* spp. lack a type III secretion mechanism which present in many other pathogens.

PATHOGENESIS

Gonorrhea

Neisseria gonorrhoeae infects the human urogenital epithelia. Other frequently infected anatomic niches are the rectum, oropharynx and conjunctiva.[24] Certain gonococcal strains may cause disseminated infection and/or arthritis.[25–27] All *N. gonorrhoeae* strains are considered to be pathogenic. The infective dose for the male urethra is as low as 250 gonococci; for the uterine cervix this ranges from 10^2 to more than 10^7 gonococci.

In males infection usually manifests after development of inflammation caused by local induction of cytokines and influx of polymorphonuclear cells (PMNs). Examination of male biopsies and exudates shows gonococci attached to and within the epithelial cells, development of (sub)mucosal microabscesses and exudation of pus with gonococci inside PMNs. Several weeks after the start of the infection gonococci can no longer be observed histologically or recovered by culture. Gonococcal infection in women is often asymptomatic (50–80%). The basis for this gender difference in host response may be niche-related differences in host receptors and/or innate host defense.[28]

At the cellular level, a large repertoire of often phase-variable adhesins and invasion-promoting surface factors enables gonococcal infection of different niches and cell tropism (Table 168.2).[12,13] The first step in gonococcal infection is the type IV pilus-mediated attachment to mucosal cells (Fig. 168.4). Once attached, PilT-mediated pilus retraction brings the bacteria into intimate contact with the cell surface and stimulates mechanosensitive host cell signaling pathways.[29] Shortly after initial attachment Opa proteins interact with CEACAM and/or heparan sulfate proteoglycan (HSPG) receptors.[29] Different Opa proteins bind to distinct receptors types. *In vitro* Opa-receptor interactions result in efficient internalization of the gonococci by host cells[12,13] (Fig. 168.4). PorB-IA expressing strains also efficiently invade cells in the absence of Opa proteins.[30] Distinct LOS variants may invade through binding of lectin receptors.[28] Sialylated LOS variants are impaired in invasion but more resistant to killing by complement, indicating the importance of the presence of variant phenotypes in the bacterial population. Gonococci induce recruitment and show intimate association with polymorphonuclear leukocytes (Fig. 168.4). Type IV pili and distinct Opa protein confer non-opsonophagocytosis by this cell type.[12,13] Part of the ingested gonococci resist killing by phagocytes.

In women gonococcal infection can ascend to the upper genital tract and selectively adhere to nonciliated cells of the fallopian tubes. Ciliated cells are lost through direct or indirect (tumor necrosis factor-mediated) cytotoxic effects of released peptidoglycan fragments and LOS. Gonococci do not produce exotoxins. Tissue invasion and the generated inflammatory response may manifest as pelvic inflammatory disease and lead to infertility.

The presence of *N. gonorrhoeae* is sensed by the host innate immune system. Neisserial LOS is recognized by the Toll-like receptor (TLR)4–MD2 complex. Porins interact with the TLR2 receptor complex. Peptidoglycan fragments activate the cytoplasmic Nod-1 receptors. These interactions induce the secretion of cytokines, chemokines and antimicrobial peptides.

Gonococcal infection elicits a strong humoral immune response, even though distinct Opa proteins that bind CEACAM1 suppress $CD4^+$ T-lymphocyte function.[31] Dominant antigens are PorB, Opa proteins, RmpM, LOS and iron-regulated proteins. Nevertheless, the antibodies provide limited protection due to bacterial surface variation and an array of bacterial immune evasion mechanisms (see Table 168.2). The extensive phenotype variation and immune escape mechanisms are major obstacles in the generation of a broadly protective gonococcal vaccine.

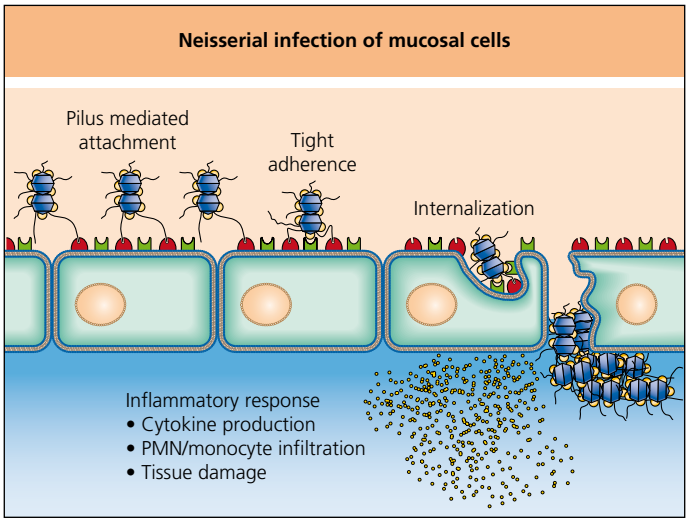


Fig. 168.4 Neisserial infection of mucosal cells.

Table 168.2 Neisserial virulence factors

Virulence factor	Function
Lipopolysaccharide (LPS/LOS)	Lipo-oligosaccharide (LOS) has endotoxin activity and is released as bacterial outer membrane vesicles (blebs) or through cellular lysis. LOS is responsible for toxic damage to the human tissue, development of septic shock and disseminated intravascular coagulation (DIC) through interactions with Toll-like receptors (TLR4) and cytokine induction
Polysaccharide capsule (*N. meningitidis* only)	Polysaccharide surface component which works as a protective shell and blocks the insertion of the membrane attack complex of the complement system and protects the bacteria from phagocytosis. The capsule is the main component enabling bacterial survival in blood and resisting bactericidal antibodies. The serogroup B capsule can also mimic human antigens
Type IV pili	Major adhesins that mediate initial attachment to nonciliated human cells. Also required for efficient transformation of DNA
Outer membrane proteins (OMP)	Dominant antigens. Porin protein promotes intracellular survival. Opacity proteins mediate firm attachment to eukaryotic cells. RmpM protein may protect other antigens from bactericidal interactions with antibodies. Frequent antigenic variation makes it difficult for the host immune system to recognize the porin and opacity protein antigens
Iron-binding proteins	Transferrin-, lactoferrin- and hemoglobin-binding proteins. Pathogenic *Neisseria* spp. are dependent on a constant iron supply for growth
IgA1 protease	Destroys mucosal IgA which is a part of the local immune system
β-Lactamase	An enzyme that hydrolyzes the β-lactam ring of penicillin. Important for antibiotic resistance development in *Neisseria* spp.

Meningococcal disease

Infection due to *N. meningitidis* commonly results from asymptomatic oronasopharyngeal carriage.[32] At any time about 10% of the general population is colonized with meningococci. The carrier state may persist for many months. In children under 4 years of age the carriage rate is less than 1%, but this progressively increases with age to peak at about 20–25% in late teenage and early adult life. During carriage, nongroupable meningococci are most often isolated as the expression of capsule polysaccharide is absent or low. Meningococcal carriage may not be confined to the mucosal surface. Immunohistochemistry has demonstrated meningococci within the tonsilar tissues in 45% of patients undergoing tonsillectomy compared to a carriage rate of 10% determined by nasopharyngeal swabbing.[33]

Meningococcal carriage induces bactericidal antibodies within 1–2 weeks after colonization. Antibodies may last for several months after carriage. *Neisseria lactamica* carriage elicits bactericidal antibodies that cross-react with various meningococcal serogroups and serotypes. As carriage of *N. lactamica* is approximately 4% by 3 months of age and peaks at 21% by 18–24 months of age, this is much higher than carriage of *N. meningitidis* at this age.[32] *Neisseria lactamica* may contribute to protection against meningococcal disease. Development of invasive meningococcal disease correlates with the absence of bactericidal antibodies.[34]

Meningococcal colonization of the nasopharynx largely resembles the events following gonococcal infection (see above and Fig. 168.4). Type IV pili facilitate initial adherence, and opacity-associated proteins (Opa and Opc) and PorB trigger uptake of the bacteria into the cells largely via similar types of receptor (CEACAM, HSPG). Particular sets of Opa protein variants are found in hyperinvasive meningococcal lineages. Opa and Opc proteins that bind heparan sulfate and thus are able to recruit heparin-binding proteins such as vitronectin and fibronection enter cells via integrin receptors.[35] This is accompanied by a downregulation of pili and capsule, enabling optimal contact between bacterial adhesins and the host mucosa. Transferrin (TbpA, TbpB) and hemoglobin (Hbp) binding proteins recruit the iron sources required for growth.[36] Ciliated mucosal cells may be damaged by released peptidoglycan fragments and LOS. Meningococcal (and gonococcal) infection of the oropharynx is usually entirely asymptomatic, possibly because of the relatively high threshold for activation of an inflammatory response compared to more sterile anatomic niches such as the urethra.

The major difference in pathogenesis between gonococci and meningococci is the ability of distinct meningococcal lineages to survive in the bloodstream and to access the CSF (Fig. 168.5). The few phylogenetic groups of *N. meningitidis* that cause meningococcal disease often carry a filamentous prophage in their genome that is secreted from the bacteria via the type IV pilin secretin.[8] The prophage may promote the development of new epidemic clones. The mechanism of meningococcal penetration and passage of the mucosa is only partially understood.[12] Meningococci survive and multiply during epithelial cell traversal. The IgA1 protease and PorB may promote survival inside epithelial cells. Meningococci isolated from the bloodstream invariably produce polysaccharide capsule. The capsule protects the bacterium from phagocytosis and complement-mediated lysis by preventing insertion of the terminal complement attack complex. Invasive meningococci express sialylated LOS which influences binding of C4b, while the proteins PorA and GNA1870 recruit the negative regulators of complement activation C4BP and factor H.[37] Individuals with inherited deficiencies in the late complement components (C6–C9) have a high risk of developing meningococcal disease. Intriguingly, they acquire the infection at a much later age, have frequent recurrences, and the fatality rate is much lower than for normocomplementemic individuals.[37]

In the blood *N. meningitidis* replicates to high levels and sheds outer membrane vesicles (blebs). The blebs may subvert the complement system and high levels of circulating LOS overactivate the innate immune system. Circulating levels of proinflammatory mediators – tumor necrosis factor (TNF), interleukin (IL)-1 and interferon gamma (IFN-γ) – strongly correlate with development of lethal septic shock.[11]

Meningococci enter the CSF likely by the hematogenous route via the veins in the subarachnoidal space (the blood–CSF barrier) and the choroid plexi rather than through the brain parenchyma (blood–brain barrier). Encapsulated *N. meningitidis* invade the CSF probably via the transcellular route.[38] The absence of non-opsonophagocytosis in CSF enables uncontrolled bacterial growth and inflammation of the leptomeninges and subarachnoid space. In the CSF *N. meningitidis* produce polysaccharide capsule and pili and stimulate proinflammatory cytokine (IL-6, IL-8, MCP-1) and chemokine (RANTES, granulocyte–macrophage colony-stimulating factor) production in meningeal cells.[39] Attracted PMNs aggravate the inflammatory response and release cytotoxic mediators.

Immunity to invasive meningococcal disease depends upon the presence of bactericidal IgG antibodies directed against capsule (except serogroup B), PorA, PorB, Opa, LOS, iron-regulated proteins and minor surface proteins. Carriership and nonpathogenic *Neisseria* spp. (e.g. *N. lactamica*) in the nasopharynx elicit cross-reactive antibodies

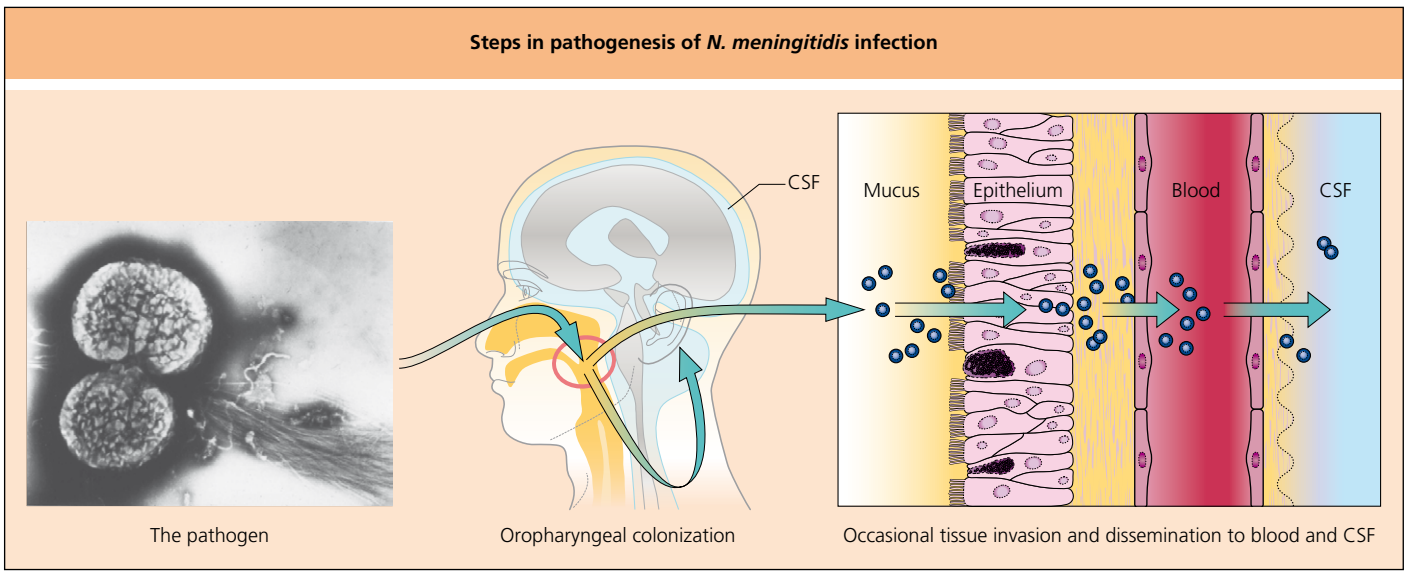


Fig. 168.5 Steps in pathogenesis of *N. meningitidis* infection. Redrawn from Davidsen *et al.*[5]

that contribute to development of immunity against *N. meningitidis*. *In vivo* phase and antigen variation of meningococcal surface antigens indicate selective immunologic pressure during natural infection.

EPIDEMIOLOGY

Gonorrhea

Gonorrhea is a common sexually transmitted disease worldwide. *Neisseria gonorrhoeae* infection is the second most common notifiable disease in the USA, with 358 366 cases reported in 2006.[40] Incidences in Europe and in the developing world are 10–30 and 4000–10 000 per 100 000 population, respectively.[41] The actual disease burden is probably higher due to underdiagnosis and underreporting. The highest attack rates occur in 15–25-year-old men and women. In regions where collected statistics include sexual orientation, rates of gonococcal infection more than tripled from 1995 to 2003 among homosexual men.

Gonococci are exclusive human pathogens. They cannot spontaneously infect animals. The risk of acquiring a urethral infection for men is approximately 20% after a single vaginal exposure to an infected woman, rising to 60–80% after four or more exposures. The transmission rate from male to female is approximately 50% per contact, rising to 90% after three exposures. Gonococci can be transmitted by orogenital contact or rectal intercourse. Perinatal transmission may also occur. Although gonococci can survive for brief periods outside the human reservoir, extracorporeal transmission is extremely rare.

The major reservoir for continued spread of gonorrhea is the asymptomatic patient. Among infected women, 30–50% are asymptomatic or show no symptoms associated with a sexually transmitted disease.[42] Among infected men, only 5–10% are asymptomatic. Asymptomatic women and men remain infectious for months. Maintenance and transmission of gonorrhea are also related to a social subset of 'core transmitters' who have unprotected intercourse with multiple new partners and either are asymptomatic or choose to ignore symptoms.[43] The average incubation period for developing gonorrhea is 2–7 days but varies between 1 and 14 days.

Meningococcal disease

Meningococcal disease is a global major health problem (Fig. 168.6).[11,44] Disease patterns differ among populations and infecting strains and can be endemic, hyperendemic, epidemic and pandemic. In 2007, mortality from meningococcal disease worldwide was estimated at 170 000.[41] The case fatality rate is 5–10% in industrialized countries and 10–20% of survivors develop permanent sequelae. Transmission of meningococci occurs by respiratory droplets, requiring close contact. Invasive disease particularly occurs when bactericidal antibodies against the invading strain are lacking and in complement factor-deficient individuals.[34,44] Concurrent viral or mycoplasmal respiratory tract infections facilitate systemic invasion.

The major virulence factor of disease-associated meningococci is the polysaccharide capsule. Most infections are caused by strains belonging to serogroups A, B, C, Y and W135[11,44] (see Fig. 168.6). In Western Europe and the Americas, meningococcal disease is endemic and caused mainly by serogroups B or C with incidences of 1–3/100 000. Periodically, local hyperendemic outbreaks occur when new lineages spread through the population. A serogroup B infection has spread worldwide, culminating in outbreaks in Australia and New Zealand in 2001–2006.[11,44] In China, the Middle East and parts of Africa, serogroups A and C predominate. Large epidemics are attributed predominantly to serogroup A strains. In the African 'meningitis belt', major periodic epidemics of serogroup A disease occur every 5–12 years, with attack rates of 500/100 000 population or higher.[45] The emergence and global importance of serogroups W135, X and Y has been recognized only in the last 10 years. Serogroup W135 was identified in 2002–2003 as a major threat and the main pathogen during outbreaks in Africa.[41]

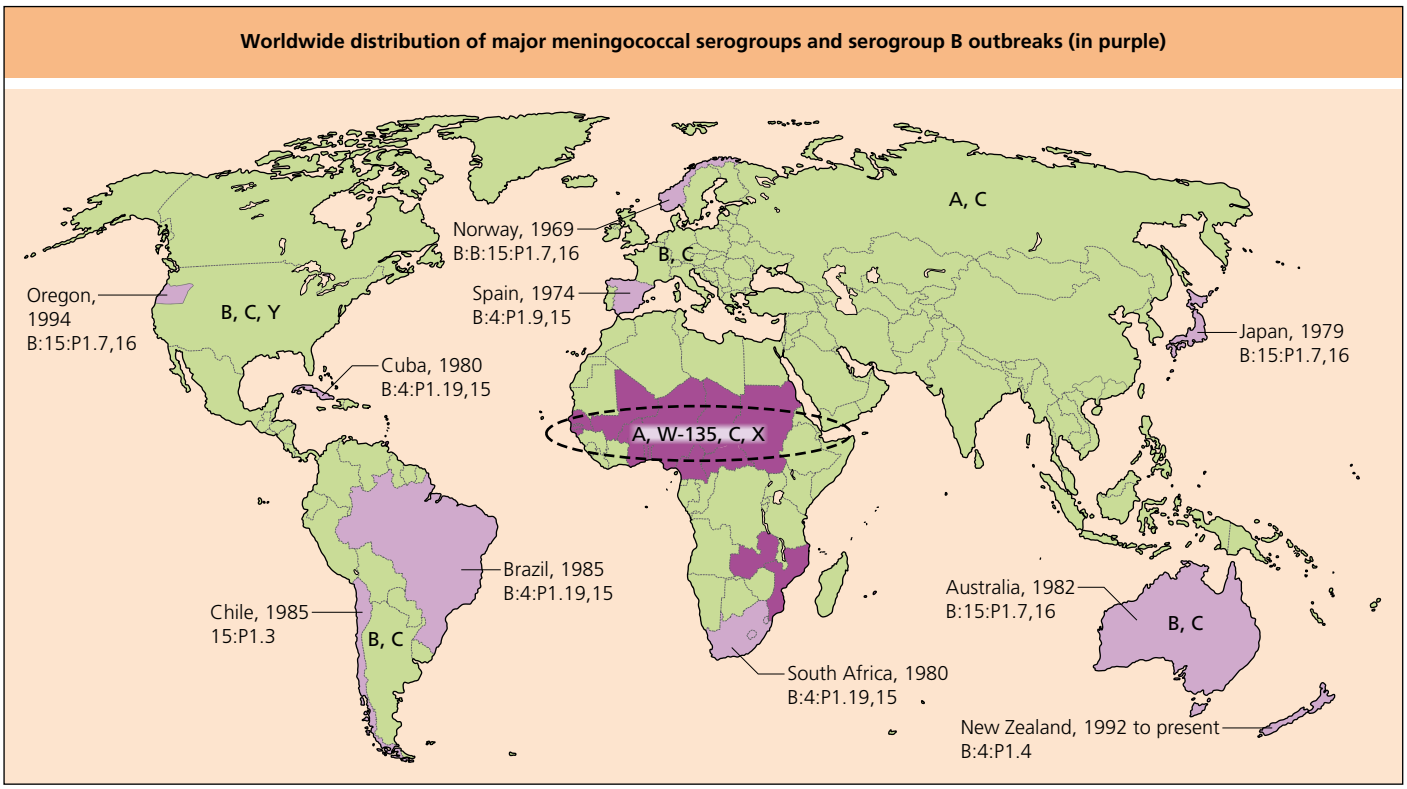


Fig. 168.6 Worldwide distribution of major meningococcal serogroups and serogroup B outbreaks (in purple). The meningitis belt (dotted line) of sub-Saharan Africa is shown. Redrawn from Stephens *et al.*[11]

An unprecedented incidence of serogroup X meningitis was observed in Niger in 2006.[46] Occasionally, particularly virulent strains arise that cause pandemic outbreaks across continents.[11] In the USA, Israel and Sweden, disease due to serogroup Y strains has increased.[47]

The occurrence of meningococcal disease varies with climate, age and social behavior. Serogroup A and C disease increases during the dry season in Africa. The number of serogroup B and C cases peaks during the winter months in the developed countries.[11,44] In the meningitis belt, young school-aged children represent the peak age group. In developed countries, children aged between 1 and 4 years account for the majority of cases with a second peak among teenagers.[11,44] Smoking young adults who socialize frequently at discos and parties may be particularly at risk. The attack rate among family members of a clinical case is 1000-fold higher than in the general population. The acquisition of meningococcal disease requires a combination of a pathogenic organism, a susceptible host, and possibly coincidental mucosal damage by other infections or low humidity. Hereditary factors such as late complement deficiencies and receptor polymorphisms may be additional predisposing factors.[11,37]

Molecular typing of *N. gonorrhoeae* and *N. meningitidis*

The classic methods of monitoring transmission of *N. gonorrhoeae* are auxotyping and serotyping.[48] Auxotyping is based on the different requirements of gonococcal strains for amino acids (proline, arginine) and nucleotides (hypoxanthine, uracil). *Neisseria gonorrhoeae* can be subdivided in 35 auxotypes. Gonococcal serotyping is based on a panel of monoclonal antibodies directed against variant epitopes on PorB-IA and PorB-IB. At least 55 serovars have been identified. These methods have, however, been replaced by DNA-based analysis such as multilocus sequence typing (MLST).[49,50]

Phenotypic classification of *N. meningitidis* is based on antigenic differences of the major surface antigens which provides information about the serogroup (capsule, e.g. B), serotype (PorB porin, e.g. 15), serosubtype (PorA porin, e.g. P1.7) and LOS immunotype (e.g. L3) of a particular strain. This results in the classification: B, 15, P1.7, L3. Multiple epitopes may be recognized depending upon the presence of phase or antigen variants in the bacterial population. Antigen-based typing is currently only relevant for vaccine efficacy studies.

For larger studies on *N. meningitidis* genome evolution and surveillance, multilocus enzyme electrophoresis (ET) typing, DNA restriction analysis, randomly amplified polymorphic DNA (RAPD), restriction fragment length polymorphism (RFLP) and ribotyping have been employed.[32] The current strain typing method, MLST, shows that epidemics are often caused by specific complexes of related hypervirulent lineages.[32,51] Targeted and complete genome sequencing is the next generation of typing methods that is being applied.[52]

CLINICAL FEATURES

Gonococcal infection

Neisseria gonorrhoeae usually causes an infection of the urethra (urethritis) and cervix (cervicitis).[53] Ascending gonococcal infection in infected women can result in pelvic inflammatory disease (PID). Dissemination to more distant sites occurs in about 0.5–3% of gonococcal infection.[24,25]

Urogenital gonococcal infection

In men, acute anterior urethritis is the most common manifestation of gonorrhea. Symptoms are a purulent urethral discharge and dysuria. Acute epididymitis is the most common local complication. In women, the endocervix is the primary site of infection. The infection is characterized by (muco)purulent discharge and intermenstrual bleeding, but is often asymptomatic. Urethral infection is present in 70–90% of women who have gonococcal cervicitis. Infection of the Bartholin's glands leads to abscess formation in about 35% of the patients.

Gonococcal infection in women may ascend to cause endometritis or acute salpingitis in 10–20% of the cases. Symptoms of the clinical syndrome of PID include lower abdominal pain, abnormal cervical discharge and bleeding, fever and peripheral leukocytosis. The onset of gonococcal salpingitis occurs early rather than late in infection and often during or shortly after menstruation. PID causes obstruction of the fallopian tubes and infertility in 10–20% of patients. In pregnant women gonorrhea is associated with an increased risk of spontaneous abortion, premature labor, early rupture of fetal membranes and perinatal infant mortality. PID is uncommon after the first trimester.[54,55]

Gonorrhea in children

Historically gonococcal infection in children included only ophthalmia neonatorum (acute gonococcal conjunctivitis).[56] However, children can acquire gonococcal infection by sexual contact with an infected person.[49,50] Such infection indicates sexual abuse. In girls the infection often produces a vulvovaginitis. Complications affecting the internal genital organs are rare.

Localized gonococcal infection outside the urogenital tract

Proctitis

Anorectal gonorrhea is present in up to 5% of women who have gonorrhea, although gonococci can be cultured from the anorectal region of 40% of women with gonorrhea. A similar proportion of homosexual men have positive rectal cultures for gonococci. Proctitis manifests as anal pruritus, tenesmus, purulent discharge or rectal bleeding.

Pharyngeal gonorrhea

Pharyngeal infection occurs in 10–20% of women who have gonorrhea, in 10–25% of homosexual men who have the infection and in 3–7% of heterosexual men. The infection is due to orogenital sexual exposure. Most cases are asymptomatic and resolve spontaneously.

Acute conjunctivitis

Ocular gonococcal infection is seen in neonates. Ophthalmia neonatorum is acquired during passage through an infected birth canal. In adults ocular infection usually results from autoinoculation of the conjunctiva in a person who has the infection. Gonococcal conjunctivitis is usually severe, with an overt purulent exudate and corneal ulceration.

Disseminated gonococcal infection

Dermatitis–arthritis–tenosynovitis syndrome

This infection is characterized by fever, hemorrhagic painful skin lesions, primarily on the hands or feet, tenosynovitis, polyarthralgias and frank arthritis.[27]

Monoarticular septic arthritis

In 30–40% of patients who have disseminated gonococcal infection, gonococci are localized in one joint and cause a purulent arthritis. The elbows, wrists, fingers and knee or ankle joints are commonly involved.[26]

Perihepatitis

Acute perihepatitis (Fitz-Hugh–Curtis syndrome) occurs by direct extension of gonococci from the fallopian tube, lymphangitic spread or bacteremic dissemination to the liver capsule.

Endocarditis and meningitis

These complications are reported in 1–2% of patients who have disseminated gonococcal infection.[53]

Meningococcal infection

The clinical spectrum of meningococcal disease includes meningoencephalitis, meningococcemia without meningitis and bacteremia without septic complications.[44,47] On admission, 60% of cases have had symptoms for less than 24 hours and 12–20% for less than 2 days. These symptoms may occur discretely or may blend into one another during clinical disease progression. The disease usually begins abruptly with headache, meningeal signs including stiffness of the neck (Fig. 168.7a) and fever. However, classic signs of meningitis (e.g. confusion, headache, fever and nuchal rigidity) are seen in only about one-half of the patients. Very young children often have only nonspecific signs such as fever, abdominal pain and vomiting. Other signs may be reduced consciousness and photophobia. Mortality approaches 100% in untreated cases, but is around 10% when appropriate antibiotic therapy is instituted. The incidence of neurologic sequelae is low, with hearing deficits, epilepsy and arthritis most commonly noted. These sequelae are most probably underreported.

Meningitis

The most frequent form of meningococcal infection is acute pyogenic meningitis due to inflammation of the meninges. Meningitis may occur with or without meningococcemia. Among patients who have meningococcal disease, 75% have meningitis; 40% of them also have bacteremia.

Meningococcemia

Meningococcemia may be transient, occult or result in severe sepsis. Bacteremia without meningitis occurs in 7–10% of patients. Meningococcemia can manifest as pink maculopapular petechial eruptions (Fig. 168.7b).[11] Rapidly progressive infections may result in purpuric/petechial or ecchymotic skin lesions that are hemorrhagic and necrotic (Fig. 168.7c,d). However, skin lesions may be atypical, evanescent or even entirely absent in patients who have blood culture-positive meningococcal sepsis. Fulminant shock may dominate the clinical picture of acute meningococcal sepsis.[11,58] Sepsis may progress to disseminated intravascular coagulation (DIC) characterized by increasing petechiae or purpura fulminans, resulting in extensive areas of tissue destruction secondary to coagulopathy, rapid onset of hypotension and adrenal hemorrhage (Waterhouse–Friderichsen syndrome). Gangrenous cases in the extremities may occur due to peripheral vasoconstriction and death may supervene as a result of DIC.

Other meningococcal infections

Persistent meningococcal bacteremia is associated with low-grade fever, rash and arthritis (arthritis–dermatitis syndrome). Meningococci are implicated as the etiologic agent in approximately 5–14% of patients who have community-acquired pneumonia. Pharyngitis is associated with recent contact with individuals who are colonized by meningococci and is often a prior symptom and sign of serious meningococcal disease. Due to hematogenous spread meningococci may cause



Fig. 168.7 Typical symptoms of meningococcal infection. Stiffness of the neck may indicate meningitis. Fine erythematous macules, maculopapular petechial eruptions and purpuric/petechial or ecchymotic skin lesions that are hemorrhagic and necrotic may accompany meningococcal sepsis. From Brandtzaeg *et al.*[57]

endocarditis, pericarditis, arthritis, endophthalmitis, osteomyelitis, conjunctivitis and peritonitis. Arthritis is particularly common with an incidence of 11% in adults and 5% in children.

LABORATORY DIAGNOSIS

Gonococcal infection

Diagnosis of gonococcal infection is made at two levels: presumptive and confirmed. Antimicrobial treatment must be started based on the results of the presumptive tests, but additional tests must be performed to yield a confirmatory diagnosis.

Collection of specimens for diagnosis depends on the clinical manifestations and the sites exposed. When appropriate, cultures from blood and biopsies from skin lesions and joint fluid aspirates are started. Male urethral exudates and female cervical swabs should be taken from all cases of suspected gonococcal infection for direct examination and culture. Neutrophils containing Gram-negative cocci in the Gram-stained smear are presumptive evidence of gonococcal infection (Fig. 168.8). Gram stain is highly sensitive and specific for diagnosing genital gonorrhea in men. Gram-stained smears from endocervix specimens of symptomatic women have a sensitivity of only 40–60% relative to culture, but have a high predictive value. In asymptomatic women, Gram stain has a low predictive value and is not useful.

Direct detection (i.e. without culture) of gonococci may also involve rapid and sensitive diagnostic nucleic acid amplification tests (NAATs) that simultaneously detect *N. gonorrhoeae* and *Chlamydia trachomatis*.[42] NAATs require only a freshly voided urine sample. Limitations are cost, risk of carryover contamination, inhibition and inability to provide antibiotic resistance data.[59] Frequent horizontal genetic exchange leading to commensal *Neisseria* spp. acquiring *N. gonorrhoeae* genes may give false-positive results. Furthermore, some *N. gonorrhoeae* subtypes may lack specific sequences targeted by a particular NAAT due to sequence variation in the dynamic gonococcal populations, leading to false-negative results.

Maximal recovery of gonococci requires immediate plating of the collected specimen.[1] If this is not possible, swabs can be transported in commercially available charcoal-containing transport media. Commonly used culture media are Thayer–Martin and Martin–Lewis. These media contain lysed or heated blood (chocolate agar) supplemented with growth factors and a variety of antimicrobials to suppress the growth of other bacteria and yeast. Specimens taken from sites that are normally sterile are cultured on antibiotic-free media to enable growth of occasional strains that are susceptible to the antibiotics added to the growth media. Growth is performed in an atmosphere with 5–10% carbon dioxide at 95–98.6°F (35–37°C) for 24–48 hours.

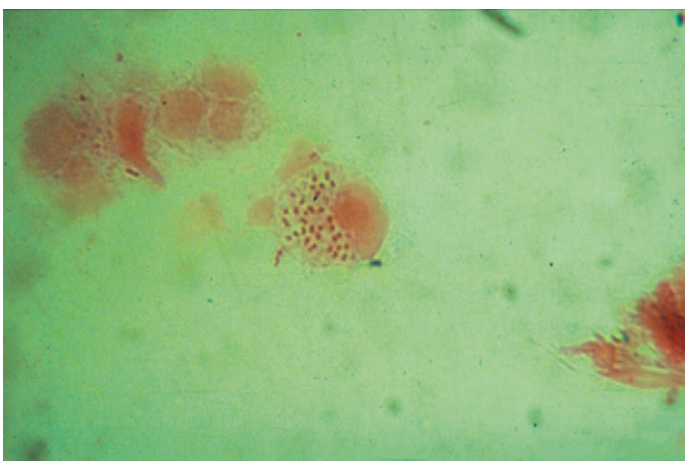


Fig. 168.8 Gram stain of a urethral discharge from a male who has gonorrhea. Note the intracellular Gram-negative diplococci with neutrophils.

Isolates on primary culture media are predominantly of the P^+ (piliated) colony type, being small, glistening and raised. After subculture, P^- (nonpiliated) colonies appear that are larger, smoother and flatter than P^+ colonies. Smears are prepared from suspicious colonies and examined with Gram stain. Gram-negative diplococci with a positive oxidase and catalase test may be *N. gonorrhoeae*.

For confirmatory identification of gonococci, other types of *Neisseria* spp. as well *Moraxella* and *Kingella* spp. have to be considered.[1] Confirmatory identification includes carbohydrate utilization tests, monoclonal antibody fluorescence, chromogenic detection of specific enzyme activities and DNA-based culture confirmation tests. Gonococci oxidize glucose (but not maltose, sucrose or lactose). Serologic tests do not have sufficient sensitivity and specificity for clinical use.

Meningococcal infection

Cerebrospinal fluid, blood, skin biopsies, nasopharyngeal swabs and aspirates are relevant specimens for the diagnosis of meningococcal disease. Additionally, synovial fluid, sputum and conjunctival swabs may be cultured if clinically indicated. Because meningococci, like gonococci, are susceptible to desiccation and temperature extremes, collected specimens should be cultured as soon as possible.

For presumptive diagnosis specimens are examined by Gram stain. Gram-stained smears are made directly from CSF if the CSF is cloudy or after centrifugation if the CSF is clear. The majority of the smears will show Gram-negative diplococci inside and outside PMNs when the CSF bacterial count is $>10^5$ ml. Smears from CSF containing $<10^3$ ml of bacteria will be positive in only 25%; on average 60–90% of culture-positive CSF specimens are positive in the Gram stain. Gram-stained smears from petechial skin lesions due to meningococcemia may detect meningococci in more than 70% of cases.

Direct detection of meningococcal capsular polysaccharides in CSF is performed by latex agglutination and coagulation tests with polyclonal antibodies for serogroups A, B, C, Y and W135.[60] These methods can detect 0.02–0.05 mg of antigen per ml. Their sensitivity is only about 50% compared to 82–90% for direct detection of meningococci in CSF and blood by NAATs/PCR.[61] The latter tests are also useful in confirming the diagnosis in patients who had antibiotic treatment prior to collection of CSF and whose CSF Gram stain, antigen test and culture are negative.

The proportion of PMNs in CSF from patients who have meningitis ranges from 49% to 98% (mean of 86%). Other CSF abnormalities include low glucose and an elevated protein concentration. In patients partially treated with antibiotics, the CSF leukocyte count, glucose and protein concentration, and the antigen tests remain abnormal for several days, whereas bacteria might not be evident on smear or by CSF culture. Blood cultures are positive in only 50% of patients with meningococcal disease. In those who have received antibiotics prior to the collection of blood for culture, blood cultures are sterile. A nasopharyngeal swab from young children will provide valuable information in cases of suspected meningococcal disease.

For isolation of *N. meningitidis* the clinical specimen should be inoculated on selective and nonselective growth media (Table 168.3).[1] Appropriate nonselective media are 5% sheep blood agar (in contrast to gonococci, meningococci grow well on this medium) and chocolate agar. Selective media used to culture nasopharyngeal specimens are the same as those mentioned for gonococci. Most blood-containing media support the growth of meningococci. Meningococci are grown on agar media in a 5–10% carbon dioxide-enriched atmosphere with rather high humidity at 95–98.6°F (35–37°C). After 18–24 hours, flat, gray-brown, translucent, smooth, 1–3 mm in diameter colonies of *N. meningitidis* are present which can be analyzed by Gram stain.[1] The finding of oxidase- and catalase-positive Gram-negative diplococci is sufficient to support a tentative diagnosis of meningococcal disease. For confirmatory identification, other species of *Neisseria*, *Kingella* and *Moraxella* have to be taken into account (Table 168.4). Differentiation characteristics are the production of acid from glucose

Table 168.3 Specimens and culture media for the isolation of *N. gonorrhoeae* and *N. meningitidis*

Species	Disease	Specimen/site	Media for cultivation
N. gonorrhoeae	Cervicitis	Endocervix, urethra (Bartholin's glands, rectum, pharynx)	Selective
	Pelvic inflammatory disease (PID)	Endocervix, endometrium, fallopian tubes	Selective, nonselective
	Disseminated infection (DGI)	Endocervix, urethra, skin lesions Joint fluid Blood	Selective, nonselective Nonselective, selective Blood culture medium
	Ophthalmia	Conjunctiva	Nonselective, selective
N. meningitidis	Meningitis/septicemia	Cerebrospinal fluid Blood Nasopharynx Skin lesions	Nonselective, blood culture medium Blood culture medium Selective, nonselective Nonselective

Table 168.4 Characteristics of the most common human *Neisseria* spp.

Species	Colony morphology on chocolate agar	ACID FROM		Lactose	Sucrose	Fructose	Reduction of nitrate
		Glucose	Maltose				
N. gonorrhoeae	Gray-brown, translucent, smooth (0.5–1 mm diameter)	+	–	–	–	–	–
N. meningitidis	Gray-brown, translucent, smooth (1–3 mm diameter)	+	+	–	–	–	–
N. lactamica	Gray-brown, translucent, smooth (1–2 mm diameter)	+	+	+	–	–	–

and maltose. Gonococci acidify only glucose and *N. lactamica* produces acid from glucose, maltose and lactose, although a number of commensal *Neisseria* spp. may be misidentified as *N. meningitidis* on the basis of carbohydrate oxidation. Enzyme tests may further differentiate these species.[1] Isolation of *N. meningitidis* can also be finally confirmed by NAATs/PCR, DNA sequence or MALDI/TOF analysis.

Infections caused by other Neisseriaceae

The so-called nonpathogenic *Neisseria* spp. colonize the human nasopharynx and oropharynx. They comprise eight species (*N. lactamica*, *N. cinerea*, *N. polysaccharea*, *N. sicca*, *N. subflava*, *N. flavescens*, *N. mucosa* and *N. elongata*). These species do not grow on the enriched antibiotic-containing media used for the isolation of gonococci and meningococci.[1]

Strains of *N. lactamica*, *N. subflava*, *N. cinerea* and *N. polysaccharea* can be isolated from the selective media and differentiated by their patterns of acid production (see Table 168.4).[1] Without additional tests, including chromogenic enzyme substrate tests and serologic tests, the eight species may be misidentified as meningococci or gonococci.

Neisseria lactamica produces acid from lactose and can thus be differentiated from meningococci. This and other nonpathogenic neisserial species are only occasionally associated with disease, although *N. lactamica* has been isolated from cases of meningitis or sepsis in both adults and children (Table 168.5).

Neisseria animalis, *N. denitrificans*, *N. canis*, *N. dentiae*, *N. macacae* and *N. iguanae* are present in the oropharynx of guinea pigs, cats, cows, monkeys and iguanas, respectively, and have not been associated with human disease.[1]

In general, the nonpathogenic *Neisseria* spp. are susceptible to penicillin, ampicillin and tetracyclines. Only *N. mucosa* is penicillin resistant and sensitive to chloramphenicol. Some strains of *N. lactamica* have an altered penicillin-binding protein 2 as found in relatively penicillin-resistant *N. meningitidis*. Rare strains of *N. sicca*, *N. flavescens* and *N. subflava* are penicillin resistant because of production of β-lactamase. Such strains are a potential source of β-lactamase genes that are transferable to meningococci and gonococci.[1]

THERAPY AND MANAGEMENT

Gonorrhea

Without treatment male urethral infection subsides after 2–3 months. Repeated infection, if untreated, leads to stricture of the urethra. Such sequelae are now rare, because the signs of urethritis bring most men to diagnosis and treatment. In 10–20% of women, gonorrhea results in ascending genital infection and PID, manifested by endometritis, salpingitis, tubo-ovarian abscess and pelvic peritonitis. Infertility due to fallopian tube obstruction is the most common and serious sequela of PID and occurs in 15–20% of women after a single episode and in 50–80% of women after three or more episodes.[62] In addition, disseminated gonococcal infections may occur. In the pre-antibiotic era, *N. gonorrhoeae* was a frequent cause of endocarditis and meningitis. Patients with gonorrhea may have concurrent sexually transmitted disease (STD) infections. *Chlamydia trachomatis* can be recovered from 15–30% of men who have gonococcal urethritis and from 35–50% of women who have endocervical gonorrhea,[63]

After the emergence of sulfonamide-resistant strains, penicillin was introduced as treatment for gonorrhea. Since 1976, gonococcal isolates with a decreased sensitivity for penicillin (minimum inhibitory concentration (MIC) >0.1 mg/1) have been recovered. This chromosomally encoded penicillin insensitivity results from mutations at the *pen* A, *pen* B, *env* and *mtr* genes, which can be spread by

Table 168.5 Nonpathogenic neisserial species rarely isolated from clinical disease in humans

Neisserial species	Clinical disease observed when isolated
N. lactamica	Meningitis or sepsis in adults and children
N. cinerea, *N. polysaccharea*, *N. sicca*, *N. subflava*	Native and prosthetic endocarditis, often in patients with heart abnormalities or intravenous drug use
N. sicca	Native and prosthetic valve endocarditis Meningitis cases Rarely pneumonia and osteomyelitis
N. subflava	Rarely endocarditis, meningitis and sepsis
N. flavescens	Once in an outbreak of meningitis Occasionally in sepsis resembling chronic meningococcemia
N. mucosa	Occasional endocarditis, meningitis, ocular infections, cellulitis, pneumonia and empyema.
N. cinerea	Conjunctivitis in newborns (ophthalmia neonatorum) Proctitis and lymphadenitis Meningitis in patients with facial trauma Pneumonia in immunodeficient patients
N. elongata	Endocarditis or sepsis Wound infections, osteomyelitis after oral surgery
N. weaveri	Human wounds due to dog bites

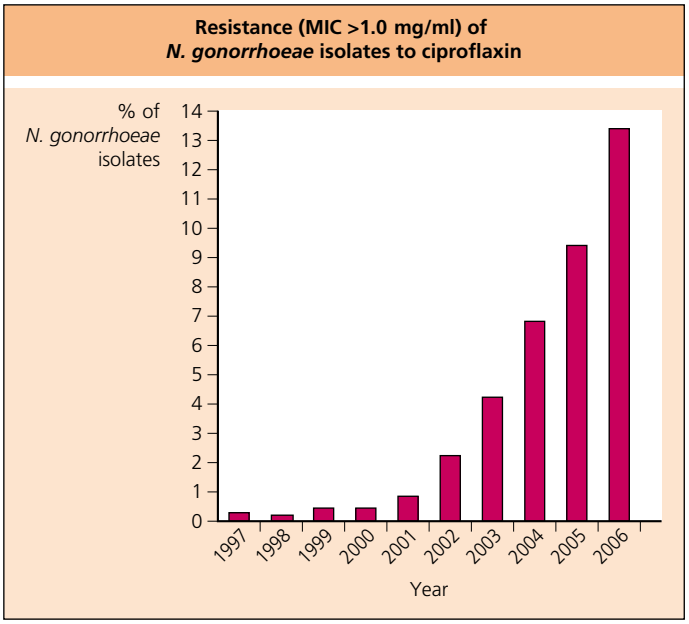


Fig. 168.9 Resistance (MIC >1.0 mg/ml) of *N. gonorrhoeae* isolates to ciprofloxacin, United States, 1997–June 2006. Redrawn from Centers for Disease Control and Prevention.[40]

natural transformation. Mutations in the *pen* genes alter penicillin-binding proteins, reducing their affinity to penicillin. The *mtr* locus encodes an efflux pump. Mutations of *mtr* and *env* alter the permeability of the gonococcal membrane. These strains represent 10–16% of all gonococci in the Western world. In 1976, strains of gonococci that were totally resistant to penicillin due to β-lactamase production were also recovered. These isolates contain a plasmid (Pc^r) that carries the genes for a TEM-1 type β-lactamase. Even though the prevalence of penicillinase-producing *N. gonorrhoeae* (PPNG) remains stable at about 5–10% in Western Europe and the USA, it is no longer common practice to treat gonococcal infections with penicillin. One reason is that therapy failure has implications for the infected patient as well as for public health.[64] Antibiotic regimens for gonorrhea require high patient compliance and an efficacy of 100%. Fluoroquinolones (i.e. ciprofloxacin, ofloxacin or levofloxacin) are also no longer recommended for treatment of gonococcal infections[40] due to the sharp increases in antibiotic resistance[65] (Fig. 168.9). The high-level quinolone resistance (MIC >0.1–16 mg/l) is chromosomally mediated, mainly due to mutations in *gyrA* (the gene encoding DNA gyrase) and *parC* (topoisomerase IV). Tetracycline is not recommended as primary treatment of uncomplicated gonococcal infections due to the high-level of tetracycline resistance caused by a conjugative plasmid into which the *tetM* transposon has inserted.

Present recommendations for treatment of uncomplicated gonorrhea are based on regimens that provide high efficacy, that have potential effect against concurrent STDs (particularly *C. trachomatis*) and that can be administered as a single dose to obtain maximal patient compliance.[40] Typical regimens are ceftriaxone (one intramuscular dose) or a single oral dose of cefixime. Each of the therapies also includes a single oral dose of azithromycin (or doxycycline for 1 week) for the treatment of co-infecting *C. trachomatis*. One oral dose of azithromycin is most often active against both gonococci and *C. trachomatis*. Doxycycline is contraindicated during pregnancy. Pregnant women who have gonococcal infection are treated with ceftriaxone followed by 7–10 days of erythromycin.[40]

Treatment of PID has been performed with a large number of antibiotic combinations. The important role of both gonococci and *C. trachomatis* in producing PID has been elucidated and, although the role of various anaerobes in PID has not yet been ascertained, many studies have shown that anaerobic cover is essential for treatment of PID patients. The recommended regimens now include intravenous cefotetan or cefoxitin plus doxycycline and/or metronidazole.[40] The recommended regimen for treatment of outpatients is a 2-week combination of oral ofloxacin and clindamycin or metronidazole. Amoxicillin–clavulanic acid together with doxycycline also covers the major pathogens responsible for PID. Azithromycin in combination with amoxicillin–clavulanic acid may also give excellent activity against *N. gonorrhoeae*, anaerobes and *C. trachomatis*.

Complicated gonococcal infection in males (acute epididymitis) is treated with a single dose of ceftriaxone plus doxycycline for 10 days. Treatment of disseminated gonorrhea always starts with ceftriaxone. Children who have gonococcal infection are treated with ceftriaxone. The latest USA recommended treatment regimens are available at http://www.cdc.gov/std/treatment.

Meningococcal disease

The mainstay of treatment for patients who have meningococcal disease is benzylpenicillin or a third-generation cephalosporin (e.g. ceftriaxone).[11] For most cases of uncomplicated meningococcal meningitis, 7-day treatment is adequate. When the etiology is not known at admission, ceftriaxone or cefotaxime is used for the first 24–48 hours to cover the possibility of other bacterial pathogens.[11] β-Lactamase-producing strains have occasionally been recovered. These strains harbor the same plasmid as many PPNG. In addition, there are

N. meningitidis strains that are not β-lactamase positive but have decreased sensitivity to penicillin due to reduced affinity of penicillin to penicillin-binding proteins 2 and 3, resulting from an altered *penA* gene. Although the frequency of relatively penicillin-resistant meningococci is low, continued surveillance is necessary. Cefotaxime or ceftriaxone is used when relatively penicillin-resistant strains are isolated. Treatment of meningococcal septic shock with plasmapheresis or inhibitors of inflammatory mediators is still in the experimental phase.

PREVENTION

Gonococcal infection

Condoms provide a high degree of protection from acquisition of gonorrhea, as well as other STDs. Other preventative measures are early diagnosis and treatment, partner notification and screening, and case finding. A new approach is the use of topical microbicides for intravaginal or intrarectal use.

Attempts to develop a gonococcal vaccine have been hampered by the high degree of antigenic variability in pili, outer membrane proteins and LOS during the natural course of infection. There are currently no suitable animal models for testing gonococcal vaccine efficacy. Transfected animals expressing human proteins involved in *Neisseria* infection such as transferrin receptors, CR3, CD46, CEACAMs and Toll-like receptors may aid vaccine development.

Meningococcal disease

Prevention of meningococcal disease is based on chemoprophylaxis and vaccination.[47]

Chemoprophylaxis

The aim of chemoprophylaxis is to reduce secondary cases of meningococcal disease and to arrest outbreaks. The risk of a secondary case among close contacts in the household setting is 150–1000 times higher than that in the general population. Children are at greatest risk, but secondary disease can occur at all ages. Risk is maximal in the week following recognition of the index case but extends for several weeks.

Many antibiotics used for therapy do not effectively eradicate or prevent carriage of meningococci because of inadequate levels in oropharyngeal secretions. Rifampin (rifampicin), ceftriaxone, azithromycin and the quinolones are effective against meningococci in the naso- and oropharynx.[11] However, rifampin resistance can develop rapidly and quinolone resistance in meningococci has recently been reported. Ceftriaxone as a single intramuscular dose is 97% effective in household contacts 1–2 weeks after infection. The advantage of ceftriaxone is that it can be used in pregnancy and in small children.

Chemoprophylaxis is recommended only for close household contacts of cases and other intimate contacts. Patients who have meningococcal disease should receive chemoprophylaxis before discharge from the hospital, because parenteral antibiotic treatment of meningococcal disease (except ceftriaxone) is unreliable in eliminating meningococci from the nasopharynx.

Polysaccharide vaccines

Polysaccharide vaccines reduce the incidence of infection among military recruits, reduce the progress of epidemics of serogroup A disease and protect susceptible complement factor-deficient individuals.[11] Capsule polysaccharide vaccines are available for the pathogenic meningococcal serogroups A, C, Y and W135. These vaccines are safe, with mild local adverse events, and have good efficacy (>85%) in older children and adults. However, due to lack of a T-helper response, the vaccines are poorly immunogenic below 2 years of age, fail to induce immunologic memory and provide protection for only 3–5 years. Polysaccharide vaccines are used by travelers visiting countries with a high incidence of meningococcal disease. A polysaccharide vaccine against serogroup B meningococci is not available due to carbohydrate mimicry and poor immunogenicity.

The immunogenicity of polysaccharide vaccines is greatly improved by chemical conjugation to a protein carrier. The resulting polysaccharide conjugate vaccines are safe, immunogenic in young infants and induce long-term memory. Polysaccharide conjugate vaccines against serogroups A, C, W135 and Y are now available. These vaccines are so far safe and immunogenic, are anticipated to provide long-term protection (as they induce a T-cell-dependent response) and are effective in young children.[66] Introduction of the C conjugate meningococcal vaccines in 2000 markedly reduced the incidence of serogroup C disease in the UK with estimated vaccine efficacies of 88% in young children and 95% in young adolescents. Immunization also decreased nasopharyngeal carriage by 66% and transmission of the pathogen (herd immunity).[67] However, widespread use of monovalent serogroup conjugate vaccines may become ineffective when the capsule types switch due to genetic exchange or strains arise that show reduced capsule expression.

Outer membrane protein vaccines

Complement-mediated killing of encapsulated strains is also achieved with cross-reactive antibodies directed against outer membrane components. Developed outer membrane vesicle (OMV) vaccines with a low LOS composition show efficacies of 50–80% in clinical trials, but do not protect young children and are in general too strain specific, i.e. the vaccines can be used against clonal disease outbreaks, but not for prevention of sporadic disease caused by diverse strains. Multivalent vaccine strains based on common variants of PorA (a major inducer and target of bactericidal antibodies) may provide protection against multiple subtypes of *N. meningitidis*. Recently, novel conserved candidate vaccine antigens have been identified using a 'reverse vaccinology' approach.[68] Currently, the lipoproteins GNA1870 and GNA2132, the conserved surface proteins OMP85, NspA and NadA, as well as PorA, pilin and LOS conjugates, are being evaluated for their vaccine potential.

REFERENCES


References for this chapter can be found online at http://www.expertconsult.com

Chapter 169

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Enterobacteriaceae

INTRODUCTION

As a whole, the family Enterobacteriaceae can be considered ubiquitous as members are found in various ecologic sources such as soil, water, vegetation and animals.[1–3] Some species are important pathogens of plants,[4] while many species are part of the normal flora of many animals including humans. Because many Enterobacteriaceae species are believed to be natural inhabitants of the gut, the terms 'enteric bacteria' or 'enterics' are sometimes used to refer to the taxonomic family Enterobacteriaceae. However, this practice is not advised, since these terms can cover all species of the gut (including Enterobacteriaceae) and some Enterobacteriaceae members (e.g. the epiphytes or plant pathogens) do not have the gut as a natural habitat.

Some Enterobacteriaceae species are more frequently associated with infection in animals and humans.[2] Some of the most important pathogens in human history, such as the agent of plague *Yersinia pestis*, belong to Enterobacteriaceae, and members of the family represent a huge public health concern (e.g. *Salmonella enterica* serotype Typhi, *Shigella*, *Escherichia coli*). Two main types of infectious disease are associated with Enterobacteriaceae: intestinal diseases and extraintestinal diseases. The transmission route of intestinal infection is classically orofecal with exogenous ingestion of specific pathogens, either by person-to-person transmission or, more often, by environmental (water and food) contamination. An endogenous pathway of infection is also possible (e.g. bacterial translocation from the gut to blood), as is more often observed in immunocompromised hosts or persons with underlying conditions such as cirrhosis or chemotherapy.

This chapter focuses on aspects of taxonomy, pathogenesis, clinical diagnosis and management of human Enterobacteriaceae infections. Other aspects have been covered elsewhere.[1–3,5]

TAXONOMY, PHYLOGENY AND CLONAL RELATIONSHIPS

In the prokaryotic taxonomy, Enterobacteriaceae represent the only family within the Order Enterobacteriales, itself one of 15 Orders within class Gammaproteobacteria, which belongs to phylum Proteobacteria (sometimes still referred as 'purple bacteria and their relatives'). Based on 16S rRNA gene sequences, the closest phylogenetic relatives of Enterobacteriaceae appear to be the Pasteurellaceae, the Vibrionaceae, and members of the Orders Aeromonadales and Alteromonadales (Fig. 169.1).

The family Enterobacteriaceae currently includes (as of mid-2009) more than 210 species and 50 genera, and these numbers will doubtless continue to increase rapidly. Release 7.7 of the Taxonomic Outline of Bacteria and Archaea includes 43 Enterobacteriaceae genera (http://www.taxonomicoutline.org). A history of taxonomic changes and synonyms of species is maintained at http://www.bacterio.cict.fr. Recent taxonomic changes for taxa of medical relevance include:

- the proposal of *Escherichia albertii* for *eae*-positive diarrhea-causing strains that are closely related to *Shigella boydii* serotype 13 and were previously attributed to *Hafnia alvei*;

The phylogenetic relationships determined based on 16S rRNA gene sequences for some members of class Gammaproteobacteria

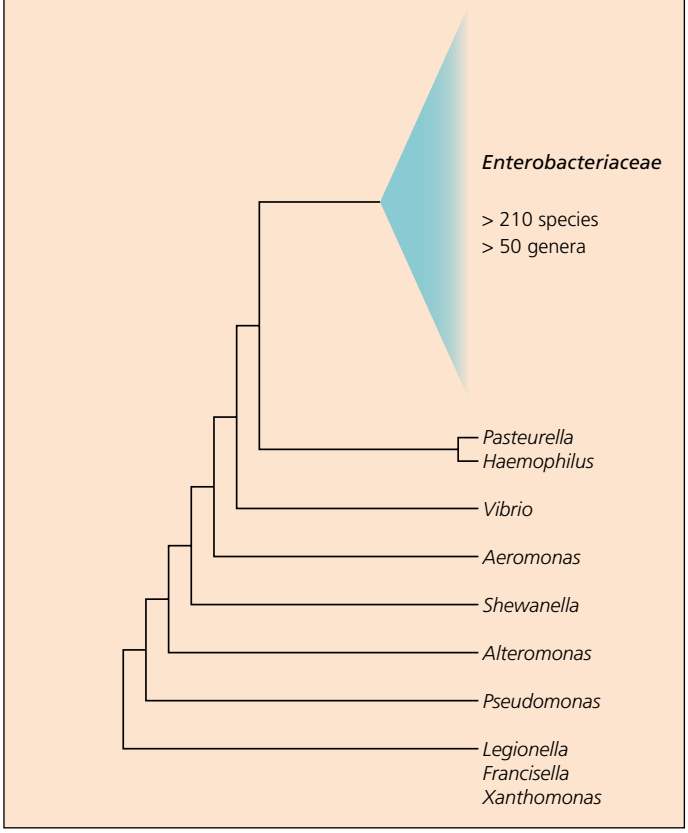


Fig. 169.1 A schematic representation of the phylogenetic relationships determined based on 16S rRNA gene sequences for some members of class Gammaproteobacteria. Based on currently available data, the bacterial groups that are most closely related to Enterobacteriaceae are families Pasteurellaceae and Vibrionaceae, Order Aeromonadales, genus *Shewanella* and Order Alteromonadales. Data from the Ribosomal Database Project, http://rdp.cme.msu.edu.

- the demonstration that *Plesiomonas shigelloides*, which is associated with water-borne diarrhea, belongs to Enterobacteriaceae even if it is oxidase positive;
- the creation of genus *Cronobacter* that includes several former *Enterobacter* species, including *C. sakazakii*;
- the realization that most '*E. cloacae*' nosocomial isolates actually belong to *E. hormaechei*; and
- the transfer of *Calymmatobacterium granulomatis*, the agent of granuloma inguinale (donovanosis), to genus *Klebsiella*, as it is closely related to *K. pneumoniae*.

Further, one should note that since its proposal, the validity of genus *Raoultella* has been questioned by several authors.[3] The recent review by Janda[3] provides detailed information on some of the newest Enterobacteriaceae taxa, including a review of the evidence for their medical significance.

A precise phylogeny of Enterobacteriaceae genera and species would be useful for taxonomic purposes and would allow understanding of the evolution of characteristics such as biochemical capabilities, host range, ecology and virulence. Unfortunately, the 16S rRNA molecule is poorly informative within this family,[6] and sequencing of other genes will be needed to establish a robust phylogeny of Enterobacteriaceae species.

All *Salmonella* strains are currently classified into two species: *S. enterica* and *S. bongori*. The type strain of a proposed third species, *Salmonella subterranea*, does not appear to belong to the genus *Salmonella* based on gene sequences, and the taxonomic position of this species within *Salmonella* should be reconsidered. *Salmonella enterica* itself is subdivided into six subspecies: *enterica, arizonae, diarizonae, houtenae, indica* and *salamae*. *Salmonella enterica* subsp. *enterica* is by far the most important from a medical standpoint, representing 99% of clinical infections. For details of *Salmonella* nomenclature, including serovar naming, see Figure 169.2.

The genus *Escherichia* currently includes the species *E. coli, E. fergusonii, E. albertii, E. hermanii, E. vulneris* and *E. blattae*. Clinically, *E. coli* is by far the most important species. Sequence comparisons indicate that *E. fergusonii* and *E. albertii* are closely related to *E. coli*, while the three remaining species may be evolutionarily more distant.

The clonal diversity of *E. coli* strains was initially disclosed based on multilocus enzyme electrophoresis (MLEE), but is currently best described by large-scale multilocus sequence typing (MLST) approaches.[7,8] Genome-wide sequence data of multiple strains are now being produced.[9] As shown earlier, based on DNA–DNA hybridization[10] and MLEE studies,[11] the genomic species that includes *E. coli* strains also includes *Shigella* strains, with the exception of *Shigella boydii* serotype 13 (Fig. 169.3). Hence, *Shigella* species were distinguished historically based on clinical and biochemical characteristics, but *Shigella* strains are phylogenetically more closely related to some *E. coli* strains than are some *E. coli* strains between themselves. In addition, the three taxonomic *Shigella* species *S. flexneri, S. dysenteriae* and *S. boydii* do not correspond to three phylogenetic clusters within the *E. coli* species.[12] Rather, the major *Shigella* clusters may comprise strains from two or more *Shigella* 'species'. In contrast, both *S. sonnei* and *S. dysenteriae* group 1 form unique and genetically homogeneous clusters.

Other pathotypes of *E. coli*, such as enteropathogenic *E. coli* (EPEC), enterotoxigenic *E. coli* (ETEC) or enterohemorrhagic *E. coli* (EHEC), also show multiple independent origins and parallel evolution (see Fig. 169.3).[8,12,14] There are currently several MLST schemes in use and efforts are ongoing to harmonize the set of genes used to identify *E. coli* strains. In the near future, nearly complete genome sequences of multiple *E. coli*/*Shigella* strains will be available (see, e.g., http://xbase.bham.ac.uk).

DIAGNOSTIC MICROBIOLOGY

All Enterobacteriaceae are Gram-negative, nonspore-forming bacilli. They are either motile by peritrichous flagella (except *Tatumella ptyseos*) or nonmotile. With a few exceptions (e.g. *Klebsiella granulomatis*), they grow rapidly on ordinary laboratory media, under either aerobic or anaerobic conditions. Growth is generally optimal at 99°F (37°C). Some specific properties are common: all species utilize glucose fermentatively (often with gas production), are oxidase negative (with the exception of *Plesiomonas shigelloides*) and catalase positive, and reduce

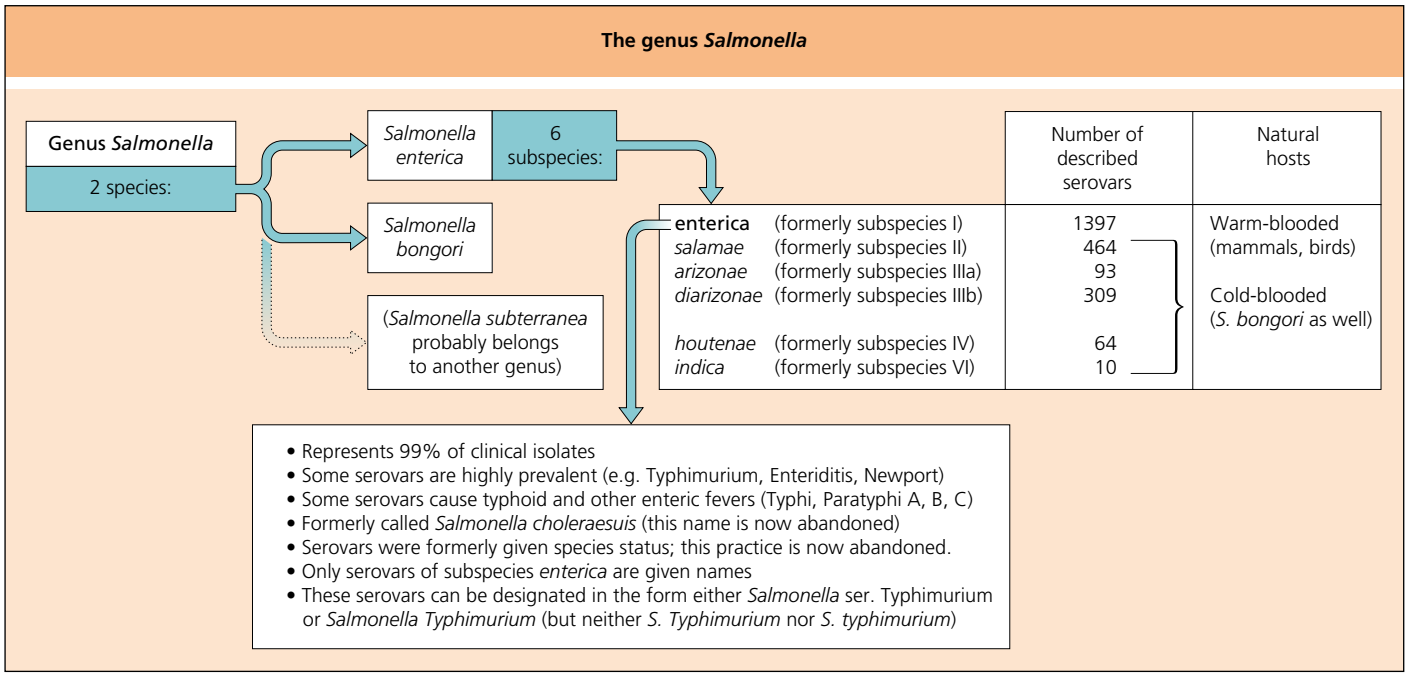


Fig. 169.2 The genus *Salmonella* includes two species, *S. bongori* and *S. enterica* (formerly *S. choleraesuis*), with the latter including six subspecies. Nomenclature has evolved from a system where all serovars (the combination of O antigen and the two phases of the H antigen) were considered as species (e.g. *Salmonella typhi*) into the current system, which gives names to serovars of subspecies *S. enterica* subsp. *enterica* (e.g. *S. enterica* subsp. *enterica* serotype Enteritidis), but designates the serovars of other subspecies and of *S. bongori* simply by their antigenic formula. More details can be found at http://www.bacterio.cict.fr/s/salmonella.html.

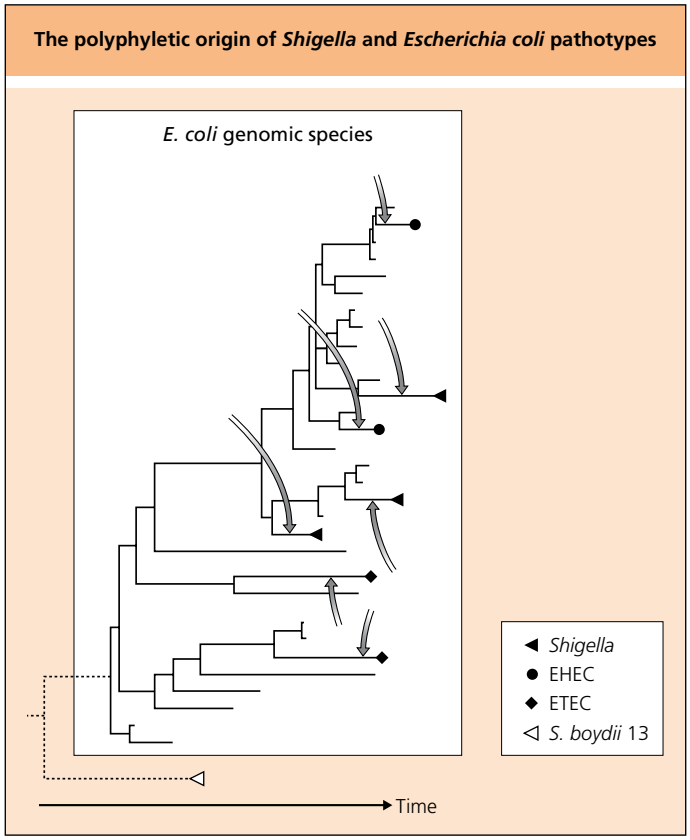


Fig. 169.3 Schematic representation of the polyphyletic origin of *Shigella* and *Escherichia coli* pathotypes, which are each found on several branches that are nested inside the diversity of *E. coli*. *Shigella* (black triangles), enterotoxigenic *E. coli* (ETEC; black diamonds) and enterohemorrhagic *E. coli* (EHEC; black circles) pathotypes have several independent origins from ancestral strains that have incorporated virulence genes by horizontal transfer (grey arrows). Among the *Shigella*, only *Shigella boydii* serotype 13 (open triangle) represents a lineage distinct from the phylogenetic cluster corresponding to the *E. coli* species.

nitrates to nitrites (excepted *Photorhabdus* and *Xenorhabdus*). Some useful features for identification of Enterobacteriaceae species that are common in clinical samples are given in Table 169.1; more details on identification methods can be found in other reference texts.[5]

In classic nonselective media, colonies are circular and convex, with a smooth surface and a diameter of 1–3 mm after 24 hours. Selective media are used to recover Enterobacteriaceae from fecal samples or other specimens containing complex flora. These media enhance the growth of particular Enterobacteriaceae species, while inhibiting non-desired species. Classically, these media (e.g. MacConkey lactose- or sorbitol-containing media) contain substrate(s) selectively used by one or a few species and facilitate distinction between colonies. More precise distinction of genera and species is obtained by differences in biochemical activity (enzyme profiles, carbon source utilization, pH-based reactions). Several miniaturized tests are available commercially (e.g. Api 20E or Biotype-100 strips from BioMerieux). Typically, isolates are identified by comparison of their biochemical profile with a reference database that contains the percentages of positive results for each substrate/species pair. More automated identification systems based on the same approach are classically used in larger microbiology laboratories.[15] One limitation of this approach is that isolates that are biochemically atypical or correspond to rare or novel species (thus not incorporated in the reference databases) cannot be identified or can even be misidentified. Typically, Enterobacteriaceae species that are common pathogens are easily identified but identification of environmental species can be problematic. Automated methods can also test antimicrobial susceptibility, which is important in regard to the increase of resistant strains isolated in human infections.

In some species, serotyping remains the predominant means by which routine identification is performed beyond the species level, given the high epidemiologic and medical relevance of serotype characterization. This is the case, for example, for *Salmonella* strains. In fact, biochemical profiling is not sufficient for diagnosis of the serotypes causing typhoid or typhoid-like fevers (Typhi, Paratyphi A, B and C). Serotyping of Enterobacteriaceae is based on antigenic variation of the O (somatic, corresponding to the polysaccharide side chain of the lipopolysaccharide), H (flagellar) and K (capsular) surface antigens. In some species, most or all strains do not have a capsule (*E. coli*, *Salmonella*), whereas the capsule is the predominant and most discriminatory antigen of *Klebsiella*. Serotyping is typically performed

Table 169.1 Major properties used to identify the most common Enterobacteriaceae implicated in human infections

	BIOCHEMICAL TESTS					
Species	**TDA**	**VP**	**ONPG**	**Indole production**	**Citrate (Simmons)**	**Remarks**
Escherichia coli	(–)	(–)	(+)	(+)	(–)	Utilization of inositol (–)
Citrobacter spp.	(–)	(–)	(+)	Variable	(+)	Urease variable, H_2S (–) except *C. freundii*
Shigella spp.	(–)	(–)	(–) except *S. sonnei*	(+)*	(–)	Utilization of xylose (–)
Salmonella enterica	(–)	(–)	(–) except *S. arizonae*	(–)	(+) except Typhi and Paratyphi A	H_2S (+)
Yersinia spp.	(–)	(–)	Variable	Variable	(–)	H_2S (–), urease (+) except *Y. pestis*
Enterobacter spp.	(–)	(+)	(+)	(–)	(+)	LDC (–) except *E. aerogenes* and *E. gergoviae*, ODC (+)
Klebsiella spp.	(–)	(+)†,‡	(+)†	(–) except *K. oxytoca*	(+)†	ODC (–) except *K. ornithinolytica*
Serratia spp.	(–)	(+)	(+)	(–)	(+)	Gelatin hydrolysis (+) except *S. fonticola*

(Continued)

Table 169.1 Major properties used to identify the most common Enterobacteriaceae implicated in human infections—cont'd

	BIOCHEMICAL TESTS					
Species	**TDA**	**VP**	**ONPG**	**Indole production**	**Citrate (Simmons)**	**Remarks**
Proteus spp.	(+)	(–)	(–)	(–) except *P. vulgaris*	Variable	H_2S (+), urease (+)
Morganella morganii	(+)	(–)	(–)	(+)	(–)	H_2S (–), urease (+)
Providencia spp.	(+)	(–)	(–)	(+)	(+)	H_2S (–)

*30–70% positive reaction for *S. dysenteriae* or *S. flexneri*, and negative for *S. Sonnei*
†Except *K. pneumoniae* subsp. *rhinoscleromatis*.
‡Except *K. pneumoniae* subsp. *ozaenae*.
(–), 70–100% negative reaction; (+), 70–100% positive reaction; variable, different reactions between different species or <70% positive/negative reaction for strains of the species.
H_2S, hydrogen sulfide production; LDC, lysine decarboxylase; ODC, ornithine decarboxylase; ONPG, O-nitrophenyl-β-D-galactopyranoside; TDA, phenylalanine deaminase; VP, Voges–Proskauer.

by slide agglutination using sets of O, H and K antisera, and the resulting combination of antigens defines the serotype (also called serovar). The most familiar serotyping scheme is the Kauffman–White scheme used for *Salmonella*, which distinguishes more than 2540 serotypes.[16] In *E. coli*, serotyping is important for identification of particular pathovars such as the EHEC strains or for identification of *E. coli* strains that possess the K1 capsular type and are implicated in neonatal infections. Serotyping is also used for *Shigella* identification. In *Klebsiella*, K typing can confirm identification of *K. pneumoniae* subspecies *rhinoscleromatis* and *ozaenae*, as the former has capsular type K3 (although rare *K. pneumoniae* subsp. *pneumoniae* are K3) and the latter is most often K4 (and sometimes K5).

Finally, knowledge of specific virulence factors and the pathogenesis of infection allowed the development of molecular diagnostic methods. For example, virulence factors of pathogenic strains include specific adhesins which confer the ability to colonize the intestinal mucosal surface, and specific factors which interfere with the normal physiology of epithelial cells. These genotypic methods are very important in particular situations, for example to make the distinction between diarrheagenic *E. coli* and commensal strains, or to detect toxin-producing *E. coli* strains causing the hemolytic–uremic syndrome. Advances in genomic characterization of virulence factors will continue to allow the development of new diagnostic methods based for example on nucleic acid-based probes, polymerase chain reaction (PCR) methods or DNA arrays.[17]

GENERAL PATHOPHYSIOLOGIC CONSIDERATIONS

Enterobacteriaceae are associated with both intestinal and extraintestinal infections, among which, ordered by frequency, are urinary tract infection, bacteremia, pneumonia, abdominal or pelvic infection, surgical site infections, meningitis and various abscesses including wound infections (Fig. 169.4). Despite the fact that many Enterobacteriaceae are part of the normal facultative flora of the gastrointestinal tract of mammals, this family represents a major contributor to human infections, ranking among the most frequent groups of pathogens in many clinical sources.

The balance between host defenses and virulence factors of Enterobacteriaceae members is a key factor that determines commensalism or disease (Fig. 169.5). Commensal bacteria themselves represent an important barrier against infection, as colonization of the gut by harmless commensals protects the host from intruding pathogens. Recognition by the host of the commensal flora occurs through the tolerance phenomenon.[18,19] The tolerogenic process that maintains homeostasis is based on various specific interactions between microbes and the digestive mucosa. The physical barrier of the mucus together with antibacterial molecules creates a distance that maintains commensal bacteria separate from the epithelial surface. Specific features of commensal species, such as inhibition of nuclear factor kappa B (NF-κB) by *Lactobacillus casei*, or particular characteristics of epithelial cells, such as the reduced expression of Toll-like receptor 4 (TLR4) at the gut surface epithelium, reduce the effects of bacterial stimuli, thus avoiding inflammation that would be detrimental for the host.[20] Enteric bacteria modulate inflammation leading to host responses that are compatible with their survival and restrain other intestinal resident flora members.[19,21] The specific digestive Enterobacteriaceae pathogens *E. coli*, *S. enterica*, *Shigella* spp. and *Yersinia*, as well as examples of mechanisms by which they breach the intestinal barrier, are described in more detail below.

Extraintestinal infections by Enterobacteriaceae occur through microbial colonization of normally sterile sites. Each anatomic site presents specific molecular structures and defenses against infection, and bacteria must therefore express specific colonization factors to adhere to these structures and specific virulence factors to counter these defenses.

Many pathogenicity factors have been described in Enterobacteriaceae. These factors are often clustered in chromosomal regions called pathogenicity islands (PAIs), which were first described in uropathogenic and diarrheagenic *E. coli*.[22,23] Most pathogenic Enterobacteriaceae are characterized by specific sets of PAIs (Table 169.2) whereas these PAIs are absent in nonpathogenic strains. PAIs are acquired by horizontal transfer and are typically associated with tRNA genes, flanked by repeated sequences, and may differ from the core genome in guanine and cytosine (G+C) content and in codon usage.

CLINICAL MANIFESTATIONS

Intestinal infections

The most important enteric pathogens are *S. enterica*, some strains of *E. coli*, *Shigella* and *Y. enterocolitica*. Although other Enterobacteriaceae are occasionally implicated in gastrointestinal infections, clinical significance is sometimes controversial (e.g. for *Plesiomonas shigelloides* or diarrhea-associated *K. pneumoniae*). Indeed, Enterobacteriaceae isolated from stool specimens during acute diarrhea could reflect the drastic change in stool flora, rather than being the cause of the symptoms.

Escherichia coli

Six distinct pathotypes of diarrheagenic *E. coli* are classically distinguished: enterotoxigenic, enteropathogenic, enterohemorrhagic, enteroinvasive, enteroaggregative and diffusely adherent *E. coli*. Identification

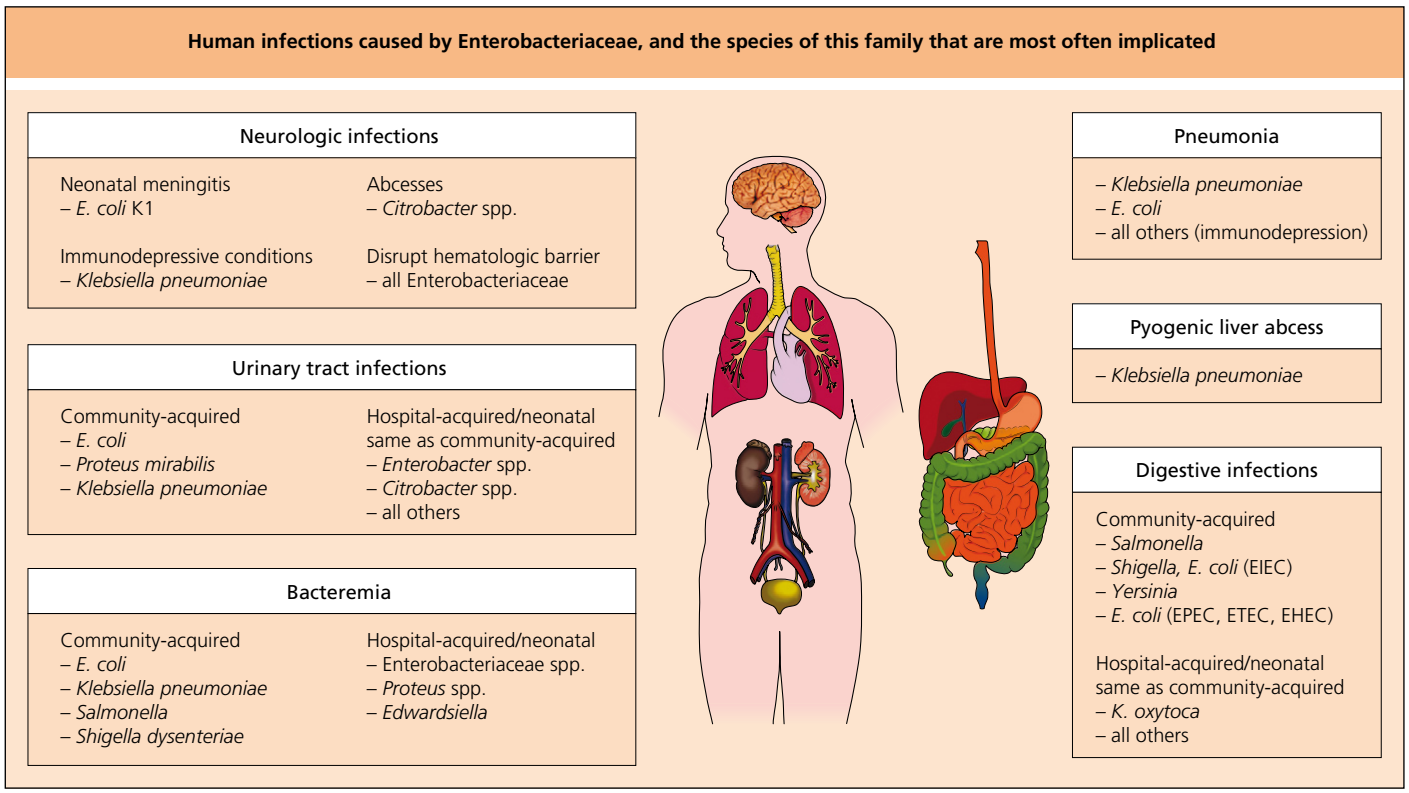


Fig. 169.4 Human infections caused by Enterobacteriaceae and the species of this family that are most often implicated.

of diarrheagenic *E. coli* strains requires their distinction from commensal *E. coli* strains, which is rendered possible by their specific sets of virulence factors, sometimes in combination with serotyping. Table 169.3 summarizes clinical, epidemiologic and biologic features of the distinct pathotypes.

Enterotoxigenic Escherichia coli *(ETEC)*

First described in Calcutta in 1956, ETEC is a major etiologic agent of diarrhea in infants younger than 2 years of age in developing countries, with an estimated 1.5 million deaths per year.[24] The illness caused by ETEC is abrupt with a short incubation period. Diarrhea is watery, similar to *Vibrio cholerae* infection, and fever is often absent. Two clinical syndromes are distinguished: weaning diarrhea, mostly observed among children in the developing world, and travelers' diarrhea. Immunologic protective response could explain the decrease in incidence after 5 years of age. Co-infection with ETEC and other enteric pathogens is common, rising to 40% of cases in endemic areas. ETEC is endemic all year round in numerous countries from Africa, Asia and Latin America. However, the infection is more common in warm and wet months. ETEC may be the cause of 20–40% of travelers' diarrhea and represents the most frequent cause of diarrhea for North American and European travelers visiting developing countries.[24]

Two classes of enterotoxins contribute to the pathogenesis of ETEC:

- the LT-I and LT-II heat-labile oligomeric toxins, which are closely related to cholera enterotoxin; and
- STa and STb heat-stable monomeric toxins.

A large number of ETEC fimbrial antigens have been characterized constituting multiple Colonization FActors (CFAs; more than 22 CFAs are known). The initial nomenclature that designated them as 'colonization factor' (CFA/I to IV) was replaced in the mid-1990s by a uniform designation composed of the initials 'CS' for 'coli surface antigen' and number (e.g. CS2), except for CFA/I.[24]

Unfortunately, ETEC is still difficult to recognize in minimally equipped laboratories. Serotyping was rapidly abandoned because ETEC isolates may belong to a large number of serotypes that change over time. Several immunoassays (including radioimmunoassay and enzyme linked immunosorbent assays) were widely used in ETEC diagnosis until the use of PCR to detect LT- and ST-producing *E. coli*, either in isolated colonies or directly from stools. DNA probes and PCR methods were also developed to detect ETEC colonization factors.

Enteropathogenic Escherichia coli *(EPEC)*

EPEC infections cause diarrhea in infants younger than 2 years except for rare cases in adults attributed to ingestion of large inocula. Outbreaks are common in developed countries, where EPEC is a major cause of infant diarrhea, with high mortality rates. Clinical manifestations include profuse watery diarrhea, vomiting and fever, all of which contribute to severe dehydration.

EPEC pathogenesis consists of bacterium-to-bacterium localized adherence pattern (LA phenotype), signal transduction and intimate adherence of the bacteria to the enterocyte membrane with disruption of the apical cytoskeleton.[25] Infection by EPEC induces attaching and effacing (A/E) lesions characterized by effacement of microvilli and intimate adherence to the epithelial cell membrane, with formation of pedestal-like structures. Two specific genetic elements are implicated: the EPEC adherence factor (EAF) plasmid encoding a cluster of 14 genes coding for the expression and assembly of the bundle-forming pilus (BFP) and a 35 kb chromosomal pathogenicity island called the locus of enterocyte effacement (LEE).[25] This locus encodes a type III secretion system (T3SS) and multiple EPEC-secreted protein (Esp) effectors. One of these, the translocated intimin receptor (Tir), acts as a receptor for intimin (Eae), a 97 kDa bacterial outer membrane protein. Adherence of EPECs to epithelial cells induces increases in the intracellular calcium levels, host cell protein phosphorylation with activation of kinases that leads to changes in intestinal water and electrolyte secretions. The formation of A/E lesions reduces the

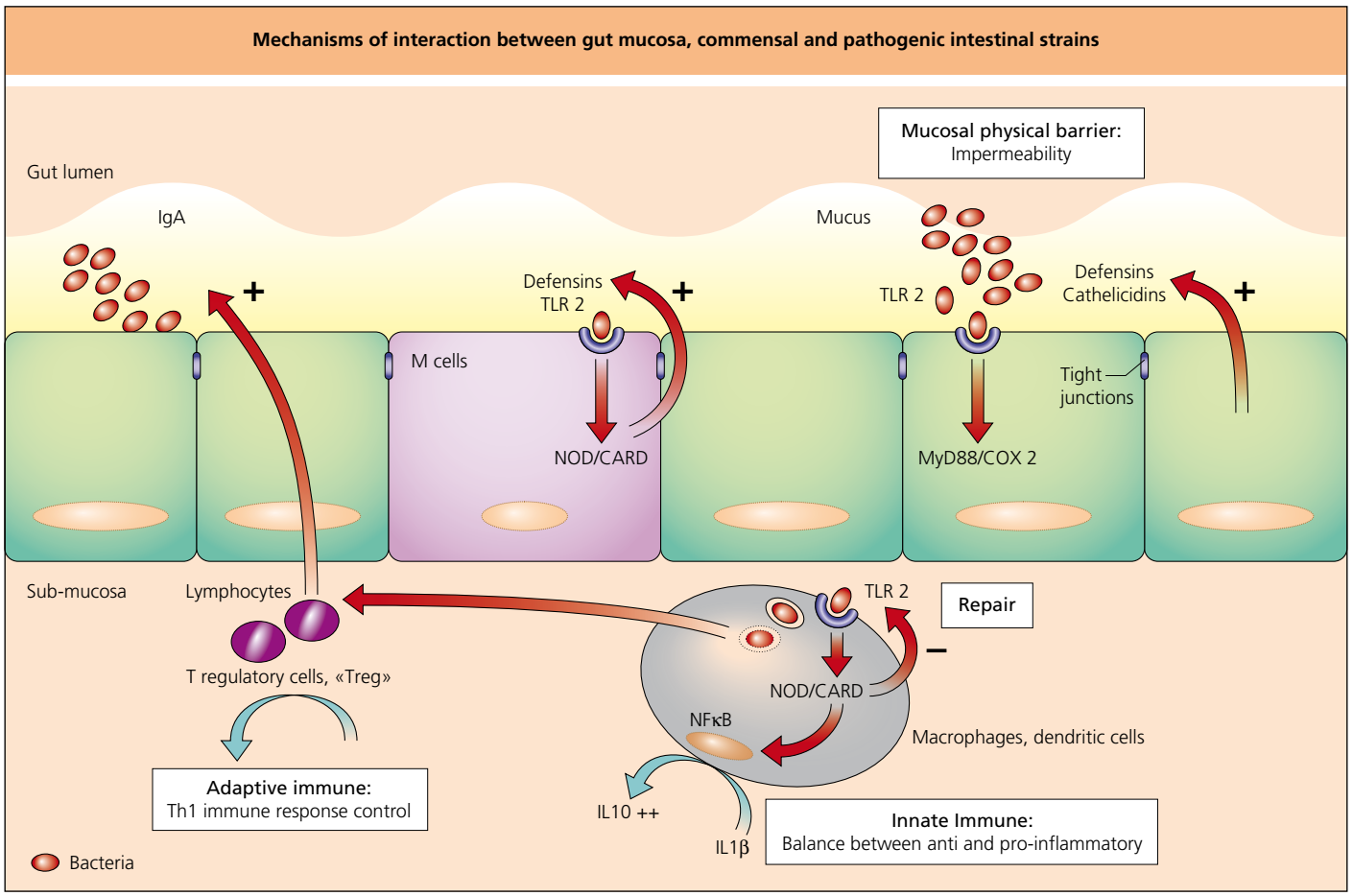


Fig. 169.5 Interplay between intestinal bacteria and the host. At the proximity of the intestinal mucosa, physical barriers exclude bacteria by the impermeability of the epithelium with tight junctions, and by production of factors excreted by epithelial cells themselves such as defensins or cathelicidins. The mucus is composed of mucin glycoproteins that interplay with bacteria, which are trapped and maintained at a distance from the epithelium. When the intestinal mucosa is exposed to bacteria (pathogens or commensal in hosts with underlying conditions), an effective immune activation is essentially based on three major regulated events. (1) Activation of TLR2/MyD88-dependent signaling is essential for effective intestinal repair in response to epithelial damage in the presence of commensal bacteria. (2) Innate immune mechanisms with activation of macrophages, dendritic cells or neutrophils are central in the control of local inflammation (NOD2/CARD15 pathway). A central role of the NOD/CARD system implicated in the controlled production of NF-κB is demonstrated. (3) Induction of regulatory T cells by commensal bacteria, with low induction of Th1 differentiation, is observed and explains at least in part the associated tolerance.

Table 169.2 Classification of virulence factors described in Enterobacteriaceae species

	DESCRIPTION		
Type of virulence factor (VF)	**Bacterial source**	**VF-specific nomenclature**	**Major function**
Colonization factors, adhesins	*Escherichia coli*	P-fimbriae: type 1 common pili; sfa fimbriae: CFA/I to IV in ETEC; bundle-forming pilus (BFP) and Tir in EPEC; AAF/I and AAF/II in EAEC; Afa adhesins in DAEC	Adherence to target epithelial cells via specific receptors
	Shigella	–	
	Salmonella	Curli	
	Yersinia	Invasin	
	Proteus	MR/P fimbriae; PMF (*P. mirabilis* fimbriae), ATF (ambient temperature fimbriae), NAF (nonagglutinating fimbriae)	
	Klebsiella	Type 1 common fimbriae, type 3 fimbriae, type 6 fimbriae	

Table 169.2 Classification of virulence factors described in Enterobacteriaceae species—cont'd

	DESCRIPTION		
Type of virulence factor (VF)	**Bacterial source**	**VF-specific nomenclature**	**Major function**
Component of secretion system	*Escherichia coli*	T3SS *sep/esp* locus in EPEC and EHEC	Molecular needle that permits export of secreted bacterial proteins across bacterial membranes and injection directly into target eukaryotic cells
	Salmonella	T3SS *inv/spa* locus	
	Shigella	T3SS *mxi/spa* locus (also described in EIEC)	
	Yersinia	T3SS *ysc* locus	
Flagella	Many members of Enterobacteriaceae except *Shigella*	–	Mobility, proinflammatory activity
Capsular polysaccharide	*Escherichia coli*	K1 antigen	Resistance to antimicrobial complement activity and prevention of antibacterial serum resistance
	Salmonella serotype Typhi	Vi antigen	
	Klebsiella	K capsular antigens: K1, K2, K4 and K57 emergent capsule	
	Yersinia	YadA, AIL (attachment invasion locus)	
Iron capture system	*Escherichia coli*	Aerobactin, chu protein, yersiniabactin in EAEC	Iron acquisition
	Shigella	*foc, fet, iuc* and *iut* loci	
	Yersinia	Yersiniabactin	
Toxins, cytotoxic effectors	*Shigella spp.*	Pic serine protease	Mucolytic activity; modification of 28S ribosomal subunit leading to apoptosis of target cells
	Shigella dysenteriae	Shiga-toxin (Stx) (ADP ribosyltransferase toxins)	
	Enterohemorrhagic *Escherichia coli*	Shiga-like toxin (Stx)	
	Enterotoxigenic *Escherichia coli*	Thermolabile and thermostable enterotoxins (LT, ST)	Increase of intracellular cAMP causes modification of apical ion channel activity in target cells
	Enteroaggregative *Escherichia coli*	EAST-1, antiaggregative toxin, plasmid-encoded toxin, Pic protease	Unknown
	Uropathogenic *Escherichia coli*	Hemolysin alpha (RTX toxins), CNF1	Proinflammatory activity; modification of Rho GTPase (for CNF1)
T3SS effectors			
Effectors targeting or mimicking Rho GTPase family members	*Salmonella*	SopE2, SopE, SptP	Modification of cytoskeletal actin and macropinocytosis
	Shigella	IpaA, IpaC, IpgB1, IpgB2	
	Yersinia	YopT, YopE, YpkA/YopO	
Effectors targeting innate immune signaling pathways	*Salmonella*	SipB (SspB), AvrA, SspH1, SpvC	Apoptosis in macrophages and dendritic cells; inhibition of NF-κB signaling and IL-8 production
	Shigella	OspG, OspF, IpaB	
	Yersinia	YopJ	
Effectors targeting actin polymerization directly	Enteropathogenic *Escherichia coli*	Tir	Actin nucleation and pedestal formation
	Salmonella	SipA (SspA), SipC (SspC)	Induction of actin polymerization
	Shigella	IcsA (VirG), IcsP, VirA	Actin nucleation
	Yersinia	YopH	Antiphagocytic activity targeting a major focal adhesin
Effectors promoting intracellular survival	*Salmonella*	SseF, SseG, SseJ, SopD2, SifA, PipB2, SspH2, SptP	Contribution of Sif formation and microtubule formation
	Shigella	IcsB	Host cell survival and prevention of autophagic recognition of IcsA/VirG

cAMP, cyclic adenosine monophosphate; DAEC, diffusely adherent *E. coli*; EAEC, enteroaggregative *E. coli*; EHEC, enterohemorrhagic *E. coli*; EIEC, enteroinvasive *E. coli*; EPEC, enteropathogenic *E. coli*; ETEC, enterotoxigenic *E. coli*; IL, interleukin; NF-κB, nuclear factor kappa B.

Table 169.3 *Escherichia coli* and intestinal infections

Pathotype of *E. coli*	Clinical manifestations	Histologic effects	Specific virulence factors	Diagnostic methods	References
Enterotoxigenic *E. coli* (ETEC)	Watery diarrhea; travelers' diarrhea; endemic cholera-like illness in children; developing countries	LT-B subunit binding GM1 and LT-A subunit increase cyclic AMP production, leading to chloride and neutral sodium chloride secretion	LT (enterotoxin, heat labile) and ST (enterotoxin, heat stable); various colonization factors (CFs) including CS1 at CS7, CFA/I and CFA/II	Immunologic detection of LT (ELISA, passive latex agglutination); specific cytopathic effects on CHO cells; detection of LT or ST gene (*stx*) (PCR)	Qadri *et al.*[24] Nataro & Kaper[25]
Enteropathogenic *E. coli* (EPEC)	Watery diarrhea with vomiting and fever; children <2 years; developing countries	A/E adhesion; actin-rich pedestal	Outer membrane intimin adhesin (*eae* gene); LEE pathogen (T3SS, *esc* genes, Esp effectors); EAF plasmid (bundle-forming pilus); intimin receptor Hp90 or Tir; EAST-1 (*astA* gene)	A/E adhesion effect on Hep-2 cytotoxic assay; detection of *eae* gene and/or EAF probe DNA (typical strain) (PCR or hybridization); O:H serotyping (O55, O86, O111, O119, etc.)	Nataro & Kaper[25] Franckel & Phillips[26]
Enterohemorrhagic *E. coli* (EHEC)	Late bloody diarrhea, hemolytic–uremic syndrome triad (thrombocytopenia, hemolytic anemia, renal failure); children and young adults; industrialized countries	A/E adhesion; actin-rich pedestal (identical to EPEC)	Outer membrane intimin adhesin (*eae* gene); LEE pathogen (T3SS, *esc* genes, Esp effectors); pO157 plasmid with notably enterohemolysin gene (in O157:H7 strains); *stx* genes ++ (specific virulence); EAST-1 (*astA* gene)	Detection of *stx* (PCR or hybridization), accessory detection of *eae* gene; O:H serotyping (O157:H7 ++, O26, O103, O111, etc.)	Nataro & Kaper[25]
Enteroinvasive *E. coli* (EIEC)	Classic watery diarrhea; minority dysentery syndrome	*Shigella*-like invasion of epithelium	Large virulence plasmid (220 kb, 100 genes); TTSA type secretion (*mxi/spa* locus); Ipa, Ipg and Osp effectors; Vir regulators; IcsA/VirG	Guinea pig keratoconjunctivity or Sereny test; DNA probe hybridization (17 kb pMR17-*Eco*RI fragment or 2.5 kb *Hin*dIII fragment pInv-ial); detection of *ial* gene (PCR)	Nataro & Kaper[25] Parsot[27]
Enteroaggregative *E. coli* (EAEC)	Watery diarrhea; travelers' diarrhea; children and adults; HIV patients; developing and industrialized countries	Adherence to epithelial cells with typical 'stacked-brick' pattern; mucoid biofilm formation; toxic effects	Large plasmid (typical strains); EAST-1; transcriptional activator gene; *she* patho-island; HPI *Yersinia* island; 18.30 kDa outer membrane adhesin; AAF/I, AAF/II, AFA/III factors	Adherence with specific pattern in HEp-2 cell assays; detection of CVD432 (PCR)	Jenkins *et al.*[28] Huang *et al.*[29] Weintraub[30]
Diffusely adherent *E. coli* (DAEC)	Watery diarrhea persistent in young children; developing and industrialized countries	–	Afa, dra operon; others?	DA pattern in HEp-2 adherence assays; detection of *daaC* gene (PCR or hybridization)	Le Bouguenec & Servin[31]

A/E adhesion, 'attaching and effacing' adhesion; ELISA, enzyme linked immunosorbent assay; PCR, polymerase chain reaction.

absorptive capacity of the intestinal epithelium and could also explain diarrhea. Moreover, the two EPEC effectors EspF and Map (*m*itochondrial-*a*ssociated *p*rotein) act to disrupt tight junction integrity and to induce apoptotic cell death, respectively, disrupting intestinal barrier function.[32]

Diagnosis of EPEC has long been determined by serotyping, as EPEC corresponds to only a few serotypes. In fact, the hallmark of EPEC infection is the ability to induce the A/E histopathologic effect, which can be reproduced in cell culture. A diagnostic method that relies on the fluorescent actin staining (FAS) test is very specific when associated with negative Shiga toxin production (see EHEC section). However, this phenotypic test is not easy to perform. Genotypic tests were developed for the detection of the three major characteristics of EPEC: the *eae* gene responsible for the A/E phenotype, an EAF probe (not constant, with atypical strains) and absence of *stx* genes coding for Shiga toxins.

Enterohemorrhagic Escherichia coli *(EHEC)*

First reported in 1983, EHEC strains are responsible for gastrointestinal illnesses including severe abdominal pain and grossly bloody diarrhea that can be accompanied by the hemolytic–uremic syndrome (HUS) several days after the onset of diarrhea (sometimes 10–15 days later). HUS is specifically associated with thrombocytopenia and hemolytic anemia, as well as acute renal failure which often requires dialysis treatment. EHEC constitutes the most common etiologic agent of infectious HUS in children and appears as an emergent diarrheic agent in industrialized countries.[33] Contamination by food, particularly of bovine origin, caused the first outbreaks reported in the USA, but water-borne and person-to-person transmission were also reported.[34]

Also named STEC for *stx*-producing *E. coli*, EHEC infection is recognized based on the association of the LEE pathogenicity island (containing an *eae* gene as in EPEC), which confers an A/E histopathology effect, and the production of a cytotoxin of the Stx family including a prototype Stx1, identical to Stx1 of *S. dysenteriae* type 1 and Stx2 variants.[34] The presence of Stx toxins diffusing in the circulation appears to be a determinant in the development of HUS, leading to vascular damage in the colon and kidneys, specifically by induction of apoptosis.[35] Other toxins have been described in EHEC, such as EAST-1 (also present in the enteroaggregative *E. coli* (EAEC) pathotype) and hemolysin.

Bacteriologic diagnosis is not easy and depends on early investigation, as HUS is often observed several days after the onset of diarrhea, when patients no longer have STEC in their stools. The recommended diagnostic method consists of detecting DNA encoding *stx* and *eae* factors directly in the fecal flora. Initially restricted to the O157:H7 serotype, which does not ferment D-sorbitol (as opposed to other *E. coli* strains), EHEC were detected by sorbitol nonfermenting colonies in specific agar. At present, the increase in incidence of *stx*-producing *E. coli* of other serotypes (e.g. O26, O103, O111 and O145), which do not have specific biochemical characteristics, complicates the detection of STEC in stools.[25]

Enteroinvasive Escherichia coli *(EIEC)*

First described in 1971 as being capable of causing diarrhea in volunteers, EIEC is closely similar to *Shigella* biochemically as well as for its genetic and pathogenic characteristics (see Phylogeny section). EIEC infections are mostly described in developing countries, appearing in the form of outbreaks. Although person-to-person transmission can occur, as also described for shigellosis, diarrhea is usually food- or water-borne.

The major virulence factor is a 220 kb plasmid, named the virulence plasmid (VP). The pathogenic mechanism of EIEC is virtually identical to that of *Shigella* (see below). Although EIEC can be responsible for a dysenteric syndrome with blood, mucus and leukocytes in stools, this clinical presentation is less frequent than upon infection with *Shigella*. Watery diarrhea is common and indistinguishable from ETEC infections.

For bacteriologic diagnosis, specific polynucleotide probes have been defined based on virulence factor sequences, which are detected by a multiplex PCR system or hybridization.[17,36]

Enteroaggregative Escherichia coli *(EAEC)*

This pathotype was first described in 1991 as 'enteroadherent *E. coli*' for non-EPEC adherent strains associated with diarrhea.[37] Nataro and colleagues[25] later recognized two phenotypes, diffuse (see below) and aggregative adherence (AA), characterized by a stacked-brick formation of bacterial cells attached to HEp-2 cells, consisting of the first description of EAEC. EAEC is an important emerging agent in developing countries, mostly in children younger than 2 years of age.[30] This pathogen is also an important cause of diarrhea in HIV-infected patients and travelers. The typical illness is a watery, mucoid, secretory diarrhea with low-grade fever. Vomiting is classically absent. Although the exact mechanism of pathogenesis is not fully understood, a model in three stages was proposed:[25]

- adherence to intestinal mucosa by aggregative fimbriae (AAF/I and AAF/II);
- stimulation of mucus production and mucoid biofilm production; and
- elaboration of EAEC cytotoxins responsible for damage to intestinal cells.

A 108 kDa plasmid encodes a toxin that elicits destructive lesions in the rat ileal loop and could constitute a putative enteroaggregative virulence factor. Another EAEC heat-stable enterotoxin encoded by the *astA* gene is a 4.1 kDa homologue of the ST protein (EAST-1); however, although this may play a role, it is detected in only 40% of cases of EAEC. The diagnosis of EAEC diarrhea is difficult and is performed in only a few laboratories. The gold standard method for the identification of EAEC is the HEp-2 adherence assay with the characteristic aggregative or 'stacked-brick' adherence pattern. EAEC strains are highly heterogeneous and many strains are auto-agglutinable, which excludes serotyping as a bacteriologic diagnostic method. DNA probe CVD432, corresponding to an anti-aggregative protein transporter gene *aat* encoded on a plasmid, was proposed as a good candidate (89% sensitive and 99% specific compared to the HEp-2 adherence test). More recently, multiplex PCR was proposed to identify EAEC.[28] However, as EAEC strains could lack some virulence markers, the HEp-2 adherence assay remains the preferred diagnostic test.

Diffusely adherent Escherichia coli *(DAEC)*

Diffusely adherent *E. coli* constitutes a heterogeneous group of isolates which are associated with watery diarrhea in children older than 1 year of age. All DAEC strains are defined by a diffuse adherence (DA) pattern to HEp-2 or HeLa culture cells. This adherence phenotype is due to the production of adhesins encoded by a family of operons related to *afa/dra/daa* genes.[31,38] These adhesive structures are both afimbrial (such as Afa) or fimbrial (such as F1845 encoded by daaC or Dr) and have been demonstrated to recognize the Cromer blood group antigen Dr(a) on the human decay-accelerating factor. Afa/Dr DAEC was reported initially in extraintestinal *E. coli* infection by a uropathogenic strain[39] and does not appear specific to strains associated with diarrheal diseases (DAEC). Moreover, these structures were also described among commensal *E. coli* from normal digestive flora. Although the interaction between Afa/Dr adhesins and receptors leads to cell signaling resulting in actin modification, the implication of Afa/Dr DAEC strains in diarrhea remains controversial.[38]

Shigella

Symptoms of shigellosis are mild watery diarrhea or more severe inflammatory bacillary dysentery, characterized by fever, abdominal cramps, and blood and mucus in stools. A large proportion of all diarrheal episodes worldwide are attributed to *Shigella*, with 1.1 million fatal cases annually; approximately 99% of cases occur in developing countries.[40]

The four *Shigella* species, a genus first described by K. Shiga, are now known to be part of the genomic species *E. coli*, with the exception of *S. boydii* serotype 13, which is closely related to *E. albertii*.[41] The genus *Shigella* includes *S. dysenteriae, S. flexneri, S. sonnei* and *S. boydii*, each being subdivided into several serotypes based on O antigen variation. *Shigella dysenteriae* is responsible for the most severe form and the majority of fatal shigellosis cases; *S. flexneri* and *S. sonnei* are found predominantly in developing and industrialized countries, respectively.[42]

Shigella are facultative intracellular pathogens derived from *E. coli* by parallel acquisition of virulence factors, including chromosomal PAIs and the pINV virulence plasmid (see above, Taxonomy and EIEC sections). Evolution of *Shigella* has also involved substantial loss of gene functions, such as flagella and fimbriae. Various PAIs have been described, including sequences encoding several iron acquisition systems (e.g. *iuc* or *fec* locus) or specific toxins (enterotoxin ShET1 encoded by setA1 and set1B). The prominent virulence determinant of *Shigella* is the large virulence plasmid (pINV) which encodes approximately 100 genes including the T3SS machinery, regulators VirB and MxiE, and several protein effectors and chaperones that stabilize the T3SS substrates. The *mxi/spa* locus encodes the components of the T3SS machinery, deriving from flagellar architecture consisting of a needle protruding from the basal body to the bacterial surface.[43] This essential machinery is required for the translocation of approximately 25 effector proteins from the bacterial cytoplasm into epithelial cells, leading to bacterial invasion and intracellular survival[42] (Fig. 169.6).

Shigella's effectors modulate the immune response, favoring bacterial invasion and intracellular survival.[40,42] Severe tissue damage during *Shigella* infection results from an adaptive inflammatory response with caspase-1 dependent macrophage killing. Tissue destruction and secretion by *Shigella* of enterotoxins 1 and 2 (shET1, shET2) which induce fluid secretion into the intestine explain diarrhea. In addition, *S. dysenteriae* serotype 1 secretes the Shiga toxin, responsible not only for vascular lesions in the colon but also in the central nervous system (CNS) and kidney, thus being associated with life-threatening complications.

Salmonella

The genus *Salmonella* includes the two species *S. bongori* and *S. enterica*, the latter being subdivided into six subspecies (see Fig. 169.2). *Salmonella enterica* subsp. *enterica* is responsible for 99.9% of human infections. To date, more than 2540 serovars of *S. enterica* have been described, with 1400 of these within the subspecies *enterica*. Two major forms of salmonellosis are distinguished: enterocolitis (gastroenteritis) and typhoid (enteric) fever. *Salmonella enterica* serovars Typhi and Paratyphi A, B and C and Sendai are responsible for typhoid and typhoid-like fevers and are only known to infect humans. Typhoid fever is characterized by a systemic infection where bacteria colonize the liver, spleen and bone marrow, associated with discrete gastrointestinal symptoms. This systemic illness leads to an estimated 20 million cases and 200 000 deaths worldwide each year. The population structure of the genetically monomorphic serotype Typhi has recently been disclosed,[44] indicating that most genotypic variants that diversified from the ancestor of Typhi approximately 50 000 years ago still survive in the present human population. This observation is best explained by the superposition of two epidemiologic patterns, with outbreaks of acute infection leading to a proportion (approximately 5%) of asymptomatic carriers that allow the survival and transmission of most Typhi genotypes over long periods of time.

Gastroenteritis can be caused by a large number of serovars including Typhimurium and Enteritidis, the most frequent ones in many countries. Infection is localized to the intestine, without systemic

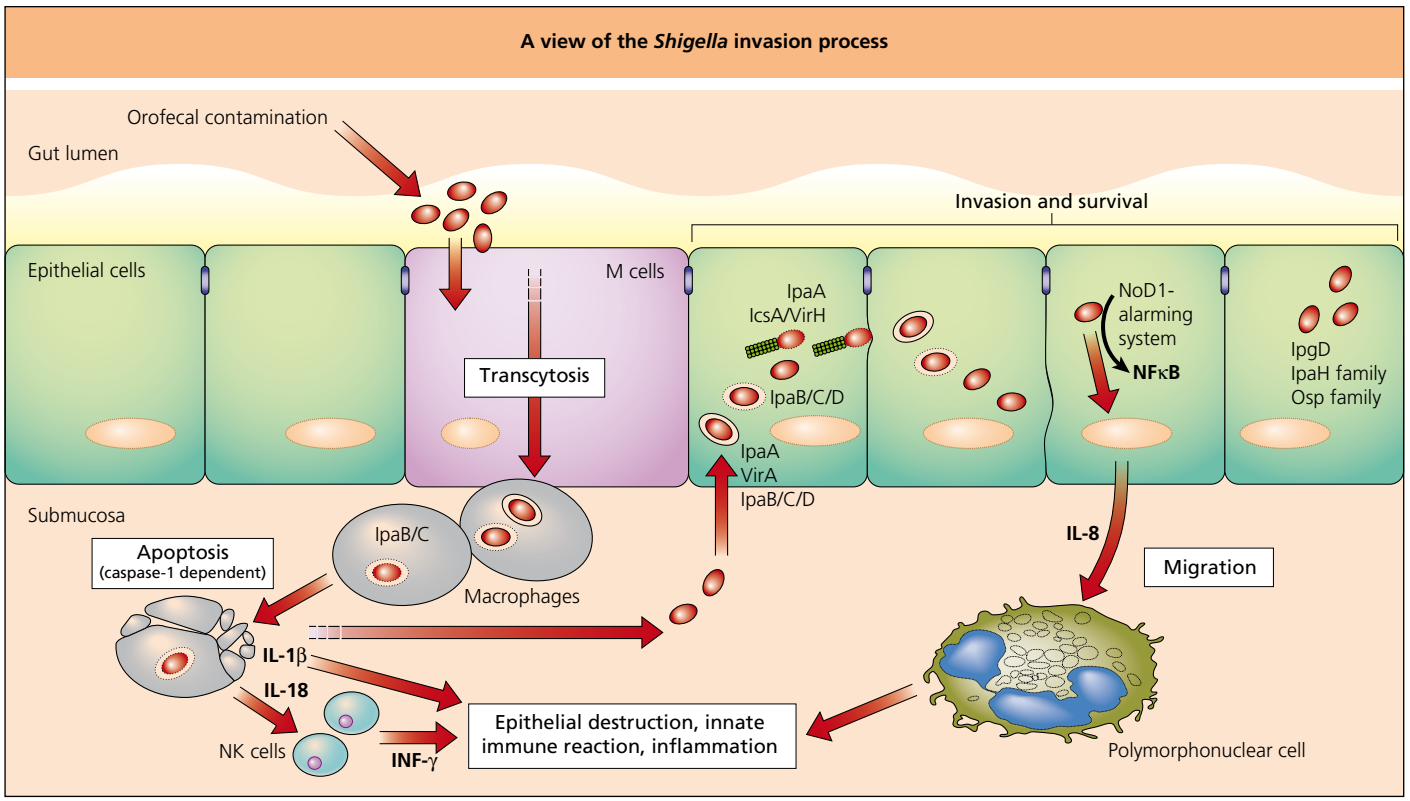


Fig. 169.6 A view of the *Shigella* invasion process. *Shigella* transmitted by the fecal–oral route reach the large intestine, transcytose across the epithelial layer through M cells, and encounter resident macrophages. *Shigella flexneri* is capable of survival in macrophages, inducing apoptosis and release of proinflammatory cytokines such as IL-1β or IL-8. IL-8 induces a massive infiltration of neutrophils, which destroy the integrity of the epithelial layer. Once released from macrophages, bacteria invade the epithelial cells from the basolateral side, escape to phagosomal vesicles, and move in the intracellular cytoplasm by polymerization of actin, ultimately invading neighboring cells. Adapted from Schroeder & Hilbi.[40]

diffusion in most cases. Some serovars cause disease in both humans and animals and have large host ranges, whereas others are more host restricted.

Typically acquired by contaminated foods or water, *Salmonella* colonize the intestine and can translocate across the intestinal epithelium by multiple mechanisms including invasion of M cells, via solitary intestinal lymphoid tissue[45] or a subset of dendritic cells,[46] or by disruption of tight junctions.[47] Translocation of *Salmonella* allows interaction with intestinal epithelial cells through their basolateral surface and leads to the production of proinflammatory cytokines and chemokines. Interaction between TLR5 and the major subunit of bacterial flagella, encoded by *fliC*, has been highlighted.[48] Although downregulation of *fliC* expression in *Salmonella* helps to explain how bacteria could evade the host immune defense in mesenteric lymph nodes, TRL5-dependent detection of flagellin seems to facilitate dissemination to these lymphoid tissues.[46,48]

Similar to *Shigella*, *Salmonella* injects effectors directly into the cytoplasm of epithelial cells. Manipulation of the host cytoskeleton leads to macropinocytosis and active invasion of enterocytes.[49] Specific targets of *Salmonella* effectors are a Ras subfamily of GTPases, the Rho GTPases. These proteins are essential targets for bacterial virulence factors. The active form is associated with GTP interacting with effectors at the eukaryotic membrane cell, while the inactive form associated with GDP is sequestered in the cytosol by GDP dissociation inhibitor (GDI)-Rho protein. The switch between activation and inactivation of Rho GTPases constitutes a specific balance implicated in the regulation of the organization of the actin cytoskeleton and in various essential physiologic events. Different *Salmonella* effectors targeting Rho GTPases alter the function and lead to internalization of the bacteria in nonphagocytic cells. After internalization, *Salmonella* are enclosed in a spacious phagosome, the SCV (for *Salmonella* containing vacuole), derived from membrane ruffles.

Two major *Salmonella* pathogenicity islands (SPIs), SPI-1 and SPI-2, are required for invasion of the epithelial cells in the early steps of infection and for intracellular survival and systemic infection, respectively. Other virulence factors and several additional SPIs have been described. For example, SPI-7 is responsible for the production of the Vi polysaccharide capsule, which is important for survival within the macrophage and is known as a major virulence factor of serovar Typhi.[50]

Yersinia

Three taxonomic species of the genus *Yersinia* – *Y. enterocolitica*, *Y. pseudotuberculosis* and *Y. pestis* – cause human infections. *Yersinia pestis*, the agent of plague, has species status in the nomenclature for obvious clinical and historic reasons, even if *Y. pestis* actually corresponds to a recently emerged pathogenic clone of *Y. pseudotuberculosis*.[51] As plague is discussed in Chapter 120, and a recent review is available,[52] it will not be developed further in this chapter.

Yersinia enterocolitica is the most common species causing enteric yersiniosis, a specific enteric pathology including acute gastroenteritis (with fever, abdominal pains and diarrhea), mesenteric adenitis (pseudo-chirurgical appendicular symptoms), bacteremia and postinfectious immunologic complications (arthritis and erythema nodosum). The most important source of infection is believed to be contaminated porcine products. Most cases of yersiniosis occur sporadically, predominantly affecting young children (<10 years old) in industrialized countries. This infection could represent the third most frequently reported food-borne gastroenteritis in Europe.[53] A recent epidemiologic study found more than 5000 cases of gastroenteritis implicating *Yersinia* spp., representing 5% of the total number of intestinal infections observed between 1997 and 2004.[53]

Pathogenic *Yersinia* invade Peyer's patches and disseminate to lymphoid tissues, including liver and spleen, where they suppress and reorient the immune system. The bacteria contain a plasmid that encodes a T3SS, which is essential for virulence expression of *Yersinia*.[54] The virulence plasmid also encodes many effectors (named Yop for '*Yersinia* outer proteins'), which translocate via the T3SS directly into eukaryotic cells and prevent proinflammatory warning signals by hijacking the host's intracellular machinery.[55] YopH constitutes a potent tyrosine phosphatase targeting a focal adhesion protein that is essential for phagocytosis.[55] Other effectors modulate the activities of Rho GTP binding proteins. YopE is a GTPase activating protein, YopT a cysteine protease and YpkA/YopO a multifunctional protein with both serine–threonine kinase activity and GDI activity. The latter protein prevents phagocytosis by macrophages and modifies cytoskeletal dynamics.[55–57] YopP/YopJ is able to interfere with inflammatory pathways, inhibiting the phosphorylation and degradation of IκB, the inhibitor of NF-κB.[58]

Extraintestinal infections

Urinary tract infections

Urinary tract infections (UTIs) are among the most common bacterial infections in humans and account for significant morbidity and high medical costs (see Chapters 53, 55 and 56).

The uroepithelium is a specialized stratified transitional epithelium composed of three cell layers, which prevent the entry of pathogens. The apical membrane of umbrella cells exhibits extended horizontal compact structures called 'plaques' and 'hinges'. These structures cover the totality of the bladder uroepithelium and are required for its impermeability.[59,60] Nevertheless, both plaques and hinges are highly detergent-insoluble lipid domains, thus assimilated to lipid rafts, and it has been reported that uropathogenic *E. coli* (UPEC) may penetrate the bladder uroepithelium through lipid rafts.[61] Moreover, plaques and hinges contain integral membrane proteins called uroplakins (UPIa, UPIb, UPII and UPIIIa/b) and UPIa is the receptor for one important adhesive appendage of UPEC, the type 1 pili.[60]

UPEC differs from commensal *E. coli* by the presence of specific virulence factors such as adhesins, toxins, capsule and iron uptake systems, which are often encoded on PAIs.[22] Adhesins include the fimbrial adhesins such as the type 1 pili, P-fimbriae and S-fimbriae, and the afimbrial adhesins such as AfaE. Other specific virulence factors include various toxins. α-Hemolysin (Hlyα) is the most common toxin encountered in extraintestinal *E. coli* infections and is associated with more than half of *E. coli* strains responsible for pyelonephritis. Hlyα seems to interfere with inflammatory and apoptotic signaling pathways via pore formation in the membrane of target epithelial cells, which modifies the intracellular calcium concentration.[62,63] A co-regulated Hlyα toxin, CNF1, is found in one-third of all UPEC strains[64,65] and in some K1 *E. coli* strains.[66] These specific UPEC virulence factors act together to bypass the various host defense mechanisms of the urinary tract.

Other common Enterobacteriaceae associated with UTIs are *Klebsiella pneumoniae* and *Proteus mirabilis*.[67,68] These species infect specific groups of patients such as those with diabetes mellitus or neuropathic bladders and urinary catheters. A common pathogen in both community and hospitalized UTIs, *K. pneumoniae* possesses a number of virulence determinants, including adhesins, urease activity, capsule and iron-scavenging systems.[69–71] *Proteus mirabilis* is an important agent of UTI, particularly in hospitalized patients and notably those who are catheterized. A hallmark of infection with this species is stone formation, resulting from active urease which hydrolyzes urea to ammonia, raising urinary pH and subsequently leading to precipitation of magnesium ammonium phosphate and calcium phosphate. Another specific characteristic of *Proteus* infections is the ability to colonize the surfaces of catheters, linked to the capacity of swarmer cells to differentiate between and to express large numbers of fimbriae.[67,72]

Respiratory tract infections

Enterobacteriaceae may cause community-acquired pneumonia in the elderly and are implicated in inhalational pneumonia.[73] In particular, *K. pneumoniae* and *E. cloacae* are among the most frequent bacteria

responsible for ventilation-associated pneumonia, after *Staphylococcus aureus* and *Pseudomonas aeruginosa*. *Klebsiella pneumoniae* was originally described as the agent of Friedländer's pneumonia, a severe lobar pneumonia, and remains an important cause of hospital- and community-acquired pneumonia, even though the incidence of community-acquired pneumonia has decreased in North America and Europe[74] (see 'Other infections and rare or emerging Enterobacteriaceae pathogens' below, and Chapter 28).

Meningitis and neurologic infections

Two clinically distinct situations can be described for neurologic infections by Enterobacteriaceae: primary infection associated with bacteremia, especially observed in neonates, and direct inoculation after traumatic or surgical disruption of the CNS (e.g. during neuroperitoneal dialysis).

The *E. coli* pathotype neonatal meningitis *E. coli* (NMEC) is the most common cause of Gram-negative neonatal meningitis, the incidence of which seems to be increasing. Eighty percent of strains express the K1 capsular type and belong to a limited number of serogroups (O1, O45, O18, O7, O16, O83).[34,75] Levels of bacteremia correlate with the development of meningitis.[34] As well as its classic antiphagocytic function, the K1 capsular polysaccharide also shows structural similarities with human tissue components, leading to an immune tolerance and resistance to antibacterial serum activity, thus possibly contributing to the high virulence by high resistance to antibacterial serum activity.[76] Some other virulence factors leading to invasion of the blood–brain barrier have been described for the NMEC pathotype, such as the outer membrane protein A, Ibe membrane proteins and the cytotoxic necrotizing factor.[77]

Citrobacter koseri is another classic bacterium described in neonatal neurologic infections, in particular as a cause of devastating neonatal meningitis characterized by the formation of multiple brain abscesses. The ability of *C. koseri* to survive phagolysosome fusion and replicate within macrophages, which could be dependent on *fliP* expression, may contribute to the establishment of a chronic CNS infection.[78]

One possible clinical complication of *K. pneumoniae* liver abscess is meningitis (see 'Other infections and rare or emerging Enterobacteriaceae pathogens', below).

Bacteremia

Bacteremia is a major complication of infection by Enterobacteriaceae as it can lead to severe sepsis with acute organ failure and septic shock (see Chapter 44).

The primary source of bacteremia is urinary tract infections. Other classic sources of blood infections include the digestive tract, implicating both specific intestinal pathogens and opportunistic enteric bacteria, which can translocate from the intestinal lumen to blood in hosts with underlying conditions (e.g. digestive solid tumor, cholecystitis or immunosuppressive treatment). *Salmonella* serovars that cause typhoid fever and, more rarely, nontyphoidal *Salmonella* can cause sepsis.[79]

Other infections and rare or emerging Enterobacteriaceae pathogens

Many if not most Enterobacteriaceae can cause infection, even if rarely, and those species that are considered medically most important are described in Table 169.4. *Klebsiella* spp., principally *K. pneumoniae* and to a lesser extent *K. oxytoca*, are responsible for frequent hospital-acquired infections and can occur in the form of outbreaks, in particular in immunocompromised persons and neonatal intensive care units (ICUs).[70] *Klebsiella pneumoniae* ranks second in frequency to *E. coli* among nosocomial Gram-negative bacteremia isolates.

However, *K. pneumoniae* cannot be viewed only as an opportunist pathogen, as it causes a number of distinctive infections in previously healthy persons in the community.[69] An update on the virulence and clinical characteristics of *Klebsiella* infections has recently been published.[74] *Klebsiella pneumoniae* owes its name to Friedländer's pneumonia, a severe form of lobar pneumonia that used to devastate chronic alcoholics. In addition, two chronic and somewhat neglected diseases of the upper respiratory airways, rhinoscleroma and ozena, are also associated with strains related to *K. pneumoniae*, which were given subspecific status based on clinical criteria: *K. pneumoniae* subsp. *rhinoscleromatis* and *K. pneumoniae* subsp. *ozaenae*, respectively. Note that the etiologic role of *K. pneumoniae* subsp. *ozaenae* in ozena is still not well established. Finally, renewed interest in *K. pneumoniae* is now emerging due to the increasing prevalence of pyogenic liver abscesses, especially – but not exclusively – in Asian countries. Pyogenic liver abscesses are generally community acquired and often without underlying hepatobiliary diseases. Moreover, *K. pneumoniae* liver abscesses can be associated with metastatic complications including meningitis and endophthalmitis. Most reports implicate hypermucoviscous strains of the capsular serotype K1.[90] A recent population study by MLST[91] has defined a number of virulent clones of *K. pneumoniae*, one of which is associated with pyogenic liver abscess and two of which correspond to *K. pneumoniae* subsp. *rhinoscleromatis* and *K. pneumoniae* subsp. *ozaenae*, respectively. These two subspecies should therefore be regarded as clones of *K. pneumoniae* that have evolved particular characteristics.[91]

Klebsiella planticola and *K. terrigena* are often misidentified as *K. pneumoniae* or *K. oxytoca*; as a result, knowledge of their clinical importance remains poor (note that the validity of genus *Raoultella*, which was proposed for the two former species, has been questioned). Cytotoxin-producing *K. oxytoca* were recently shown to be a cause of antibiotic-associated hemorrhagic colitis.[92]

Klebsiella (*Calymmatobacterium*) *granulomatis* is the causative agent of donovanosis (granuloma inguinale), a chronic genital ulceration (see Chapter 60). This species was shown to have a very close phylogenetic relationship to *Klebsiella* members and it has been proposed that it should be reclassified as *Klebsiella granulomatis*.[83,93] Specific diagnosis is possible by detection of typical intracellular Donovan bodies in biopsy samples and amplification of DNA sequences of the *phoE* gene *in situ*.[93] In fact, comparison of *phoE* sequences of *K. granulomatis* and *K. pneumoniae* suggests that the former may represent particular strains of *K. pneumoniae*.[91]

Serious infections by *Cronobacter* (formerly *Enterobacter*) *sakazakii* with fatal outcome have been described, in particular in children. This species has been associated with small outbreaks or sporadic cases of sepsis, meningitis and necrotizing enterocolitis reported in infants younger than 1 month, especially in premature babies and neonatal ICUs. High morbidity and mortality were associated with neurologic forms of this infection. Powdered infant formulae were incriminated as a source of *C. sakazakii* infections.[94]

Other species such as *Enterobacter cloacae* (itself a complex of several species), *Citrobacter freundii*, *Citrobacter koseri*, *Proteus mirabilis*, *Hafnia alvei*, *Morganella morganii*, *Serratia marcescens* and others can cause disease in humans. These are generally considered as opportunistic pathogens, as they ordinarily occur as commensals in the gastrointestinal tract. The clinical significance of most unusual Enterobacteriaceae species remains unclear, given limited data, and specific virulence factors remain unknown in most.

Rare pathogenic species are more difficult to identify with classic methods than more frequent pathogens. Molecular methods, such as the sequencing of 16S rRNA or *rpoB* genes, are especially helpful in these cases and may also lead to the discovery of novel species. For example, some strains that were misidentified as *Hafnia alvei* expressing the *eae* gene have now been reclassified as *Escherichia albertii*, leading to reconsideration of the implication of *H. alvei* in gastrointestinal disease.[95]

PREVENTION, MANAGEMENT AND CONTROL OF INFECTIONS

Symptomatic treatment

Rapid oral rehydration using intravenous fluids is the first recommended therapy in digestive Enterobacteriaceae infections, specifically during EPEC infection affecting very young children or ETEC-related

Table 169.4 Unusual, emerging or recently reclassified Enterobacteriaceae species causing human infections

Species	Human infections	Properties	Risk factors	Recent references
Escherichia albertii	Diarrhea	*Eae*-positive strains, previously misidentified as *Hafnia alvei*; closely related to *Shigella boydii* serotype 13	–	Hyma *et al.*[41]
Enterobacter (*Cronobacter*) *sakazakii*	Bacteremia, meningitis and cerebrolysis, necrotizing enterocolitis	Described in 1980, phenotypically close to *E. cloacae* but sorbitol negative, specific yellow pigment, α-glucosidase activity detected in specific agar medium	Neonatality, low birth weight	Drudy *et al.*[80] Bowen & Braden[81]
Klebsiella pneumoniae subsp. *rhinoscleromatis*	Rhinoscleroma, a chronic granulomatous disease of upper airways	Related to *K. pneumoniae* but ONPG negative; no acid production with lactose; urease, LDC and citrate negative; K3 capsular type	Nutritional deficiency, tropical and subtropical areas	Botelho-Nevers *et al.*[82] Brisse *et al.*[91]
Klebsiella pneumoniae subsp. *ozaenae*	Ozena, a chronic rhinitis with atrophic nasal mucosa	Closely related to *K. pneumoniae* but always urease negative; K4 or rarely K5	Developing countries	Botelho-Nevers *et al.*[82] Brisse *et al.*[91]
Klebsiella granulomatis	Donovanosis (granuloma inguinale)	Previously named *Calymmatobacterium granulomatis*; uncultured in defined medium	Southern Africa or Asia, Papua New Guinea, northern and central Australia	Carter *et al.*[83]
Enterobacter hormaechei	Intra-abdominal abscesses, urinary tract infections, bacteremia	Often misidentified as *E. cloacae*; a frequent cause of nosocomial infections	–	Hoffmann *et al.*[84]
Edwarsiella tarda	Gastrointestinal disease (controversial), bacteremia, wound infections with abscesses following trauma in aquatic environment	Described in 1965, resides in intestine of cold-blooded animals and water. LDC, ODC, H_2S and indole reaction positive; absence of fermentation of most sugars except glucose and maltose	Immunocompromised	Slaven *et al.*[85]
Hafnia alvei	Bacteremia, respiratory tract infections associated with abscesses, gastroenteritis (controversial)	Voges–Proskauer reaction positive, indole production negative, absence of fermentation of lactose, motility at 22°C positive	Immunocompromised	Janda[3]
Kluyvera ascorbata	Urinary tract infections, soft tissue infections, intra-abdominal abscesses	Described in 1936, including four species (*ascorbata, cryocrescens, cochleae, georgiana*) closely related to *E. coli* but esculin negative, citrate and malonate positive, growth in KCN	Neutropenia, underlying malignancy	Cheruvattath *et al.*[86]
Leclercia adecarboxylata	Bacteremia, wound infections, pneumonia	Described in 1962, closely related to *E. coli* but lysine decarboxylase negative, malonate positive; acid production from arabitol and cellobiose but not from adonitol and sorbitol	Immunocompromised status, neutropenia, cirrhosis	Temesgen *et al.*[87]
Pantoea agglomerans	Bacteremia associated with venous catheter, bone or joint infections, soft tissue infections with abscesses	Includes strains originally named *Enterobacter agglomerans* or *Erwinia herbicola*; presents a characteristic LDC-, ADH- and ODC-negative profile	Blood products, intravenous fluids; wounds from thorns or knives	Cruz *et al.*[88]
Plesiomonas shigelloides	Water-borne gastrointestinal infections	Oxidase positive	Consumption of seafood or untreated water; biliary tract diseases or acute cholangitis	Salerno *et al.*[89]

ADH, antidiuretic hormone; H_2S, hydrogen sulfide production; LDC, lysine decarboxylase; ODC, ornithine decarboxylase.

diarrhea and shigellosis.[24] Symptomatic treatment of EHEC infections is critical for management of HUS, which requires dialysis in more than half of cases[33] (see Chapters 34 and 35 for a discussion on the management of these conditions).

Antimicrobial resistance and emergence of multidrug resistance

Resistance mechanisms to antimicrobial agents can be intrinsic and acquired. The latter mechanisms result from mutational events (especially for quinolone resistance) or, more often, acquisition of various mobile genetic elements such as transposons and plasmids.

Recent changes have been witnessed regarding the epidemiology of resistance of Enterobacteriaceae. In particular, strains producing extended-spectrum β-lactamases (ESBLs) and expressing multidrug resistance to different antibiotic families are spreading rapidly. ESBLs can hydrolyze a wide range of β-lactam substrates including cephalosporins. First observed in the early 1980s in Germany and France, these enzymes evolved by mutation of common penicillinases and were initially widespread in *Klebsiella*,[96] especially for β-lactamases of the SHV family since this species harbors a chromosomal gene that is the progenitor of the plasmid-harbored SHV ESBLs.[97] During the 1990s, most outbreaks were reported in ICUs, involving unrelated strains of the same species and dissemination to different species. During this period, most ESBL-positive Enterobacteriaceae strains belonged to *K. pneumoniae*, *P. mirabilis*, *E. coli* and *E. aerogenes*, and were especially isolated in hospital infections. A significant increase in fecal carriage of ESBL-producing Enterobacteriaceae was also noted.[98] Risk factors for infection by ESBL-producing Enterobacteriaceae included, as is common for other nosocomial infections, increased length of hospital stay, admission to the ICU or pre-existing co-morbidities. A more specific factor for ESBL-producing Enterobacteriaceae infection is prior use of third-generation cephalosporins.[96] Note that family Enterobacteriaceae is the evolutionary source of several important β-lactamases which have diversified in the chromosome of Enterobacteriaceae members before being mobilized and evolving into ESBL enzymes. This is the case, for example, for CTX-M in *Kluyvera*,[13] SHV and LEN in *K. pneumoniae*,[97] or TEM in a yet-undiscovered ancestral Enterobacteriaceae species (as deduced from its close sequence relatedness to SHV).

The past few years have witnessed the emergence of ESBLs of the CTX-M family, first described in Japan in 1986. CTX-M enzymes hydrolyze cefotaxime in preference to ceftazidime and are prevalent among community-acquired infections. In *E. coli*, one predominant CTX-M15 clone represented two-thirds of all EBSLs isolated in 2004 in the UK.[99,100] CTX-M is also a common ESBL identified in Enterobacteriaceae isolated in fecal flora.

Carbapenems (the most widely used is imipenem, followed by meropenem and ertapenem) constitute classic antibiotic molecules used in infections by ESBL-producing strains. Three distinct mechanisms have been described for carbapenem resistance:

- outer membrane protein mutation causing decreased permeability;
- production of carbapenemases (four classes encoded either on chromosomes or plasmids are known; classes A and B are mostly identified in Enterobacteriaceae); and
- altered affinity of the penicillin-binding proteins for carbapenems.[101]

Some carbapenemases such as IMI-1, the first acquired carbapenemase identified in *Serratia marcescens* (isolated in Japan in 1991), or KPC-1, reported from a *K. pneumoniae* isolate from the USA in 2001, are β-lactamase enzymes that hydrolyze most β-lactams, conferring a high level of resistance even to carbapenems and thus compromising treatment. Their dissemination has significantly increased over the last decade among various Enterobacteriaceae, as the corresponding genes are often located on transferable plasmids.[102]

Vaccination and prevention

Prevention of digestive infections depends on adequate treatment of drinking water and food contamination control, which remains very difficult in many places in the world.

Vaccination constitutes a major perspective and challenge for the control of frequent Enterobacteriaceae infections specifically against digestive illnesses which affect several million people in developed countries.[24,103–105] In addition, the emergence and rapid dissemination of multidrug resistance and the challenges faced by the development of novel agents should encourage vaccine development. Multiple vaccination programs are currently being developed, such as:

- the rCTB-CF ETEC vaccine consisting of a combination of recombinant cholera toxin B subunit and formalin-inactive ETEC bacteria;[24,106]
- a clinical trial to obtain *Shigella* protective vaccines (http://clinicaltrials.gov/ct2/home); and
- enterohemorrhagic *E. coli* vaccines to prevent human disease as well as carriage of STEC in animals.[33]

REFERENCES

References for this chapter can be found online at http://www.expertconsult.com

Chapter 170

Hilmar Wisplinghoff
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Pseudomonas spp., *Acinetobacter* spp. and miscellaneous Gram-negative bacilli

INTRODUCTION

Strictly aerobic Gram-negative bacilli have become increasingly important as human pathogens over the past 20 years.[1,2] Molecular methods for identification have led not only to a number of changes in taxonomy, but also contributed important insights into the clinical significance and epidemiology of these organisms. While *Pseudomonas aeruginosa*[1] remains the clinically most prevalent species among the nonfermenting Gram-negative bacilli, other species, mainly *Acinetobacter baumannii*, have emerged as important nosocomial pathogens contributing significantly to morbidity and mortality.[2] *Stenotrophomonas maltophilia* and *Burkholderia cepacia* are commonly isolated from patients in the setting of the intensive care unit (ICU) and patients with cystic fibrosis (CF).[3] Species of *Acidovorax*, *Alcaligenes*, *Brevundimonas*, *Comamonas*, *Chryseobacterium* (*Flavobacterium*), *Pandoraea* and *Ralstonia* groups have increasingly been recognized as potential pathogens since the late 1980s.[3]

EPIDEMIOLOGY

Most of the nonfermenting Gram-negative bacilli can survive or even replicate under adverse environmental conditions. *Pseudomonas* spp. and other nonfermenting aerobic Gram-negative bacilli, in particular *Acinetobacter* spp., may survive for extended time periods in dry, cold or warm environments, and have been isolated from a variety of surfaces as well as from medical products and foods such as dairy products, poultry and frozen foods. Most species are saprophytic organisms and can also be recovered from water, soil, plants, vegetables, insects and various other sources due to their ability to use a wide variety of substrates as sole carbon and energy sources and to grow in environments providing only limited nutrients.[1–3] Even though several of these species have been isolated from clinical specimens, the clinical significance of many of them remains to be determined. However, combining intrinsic resistance to antimicrobial agents, resistance to the protective conditions of the skin (such as dryness, low pH, the resident normal flora and toxic lipids) and the mucous membranes (such as the presence of mucus, lactoferrin and lactoperoxidase), and high resistance to environmental conditions, these organisms may pose an important problem in the susceptible host and in health-care facilities.

PATHOGENIC ROLE

Aerobic nonfermenting Gram-negative bacilli can be isolated as part of the transient physiologic flora. Although most species are considered low-virulent, saprophytic organisms, others such as *P. aeruginosa* and *A. baumannii* are recognized as important human pathogens worldwide and can be responsible for severe nosocomial, health-care-associated and (less frequently) community-acquired infections contributing significantly to morbidity and mortality.[1,2]

ANTIBIOTIC THERAPY

Most nonfermenting aerobic Gram-negative bacilli have a high intrinsic resistance which makes them frequently multiresistant to the major classes of antimicrobial agents, often leaving few therapeutic options.[1–3] Antimicrobial therapy should be guided by *in-vitro* susceptibility testing results; when possible, minimal inhibitory concentrations (MICs) of the respective antimicrobials should be determined, in particular in cases of severe infection. Various mechanisms of both intrinsic and acquired resistance have been identified, their type and frequency varying widely among different species.

This chapter deals with *P. aeruginosa*, *A. baumannii* and other potentially pathogenic nonfermenting aerobic Gram-negative bacilli.

Pseudomonas aeruginosa and Other *Pseudomonas* spp.

NATURE AND TAXONOMY

The taxonomic classification of *Pseudomonas* spp. has undergone significant changes since the proposition of the genus in 1894 and at times more than 800 different species have been included in the genus *Pseudomonas*. Historically, phenotypic characterization and subsequent classification were attempted on the basis of utilization of various organic compounds as sole energy sources.[4] Based on rRNA–DNA hybridization, five different groups have been identified that were previously included in *Pseudomonas* spp.:[1]

- I: *Pseudomonas* spp.;
- II: *Burkholderia* spp. and *Ralstonia* spp.;
- III: *Comamonas* spp.;
- IV *Brevundimonas* spp.; and
- V: *Xanthomonas* spp. and *Stenotrophomonas* spp.

One example of these nomenclature changes is *Stenotrophomonas maltophilia* which was formerly designated *Pseudomonas maltophilia* in 1961 and then *Xanthomonas maltophilia* in 1983 (Table 170.1).

The taxonomic status of *Pseudomonas* spp. other than *P. aeruginosa* has evolved significantly (see Table 170.1). Some species, such as *P. fluorescens* and *P. putida*, are heterogeneous and several biovars have been recognized: five biovars in *P. fluorescens* (I–V) and two biovars in *P. putida* (A and B), which differ in a few metabolic properties.[1]

Table 170.1 Current nomenclature of nonfermenting Gram-negative bacilli*

Genus	Current name	Previous name(s)
Achromobacter	*Achromobacter denitrificans*	*Alcaligenes xylosoxidans* subsp. *denitrificans*, *Alcaligenes denitrificans* CDC group VD-3
	Achromobacter piechaudii	*Alcaligenes piechaudii*
	Achromobacter ruhlandii	*Alcaligenes ruhlandii*
	Achromobacter xylosoxidans	*Alcaligenes xylosoxidans* subsp. *xylosoxidans*, *Alcaligenes denitrificans* subsp. *xylosoxidans*, *Achromobacter xylosoxidans* CDC groups IIIa, IIIb
Acidovorax	*Acidovorax delafieldii*	*Pseudomonas delafieldii*
	Acidovorax facilis	*Pseudomonas facilis*
	Acidovorax temperans	*Pseudomonas temperans*
Acinetobacter	*Acinetobacter baumannii*	*Acinetobacter anitratus*, *A. calcoaceticus*, *Diplococcus mucosus*, *Micrococcus calcoaceticus*, *Alcaligenes haemolysans*, *Mima polymorpha*, *Herellea vaginicola*, *Bacterium anitratum*, *Moraxella lwoffi* var. *glucidolytica*, *Neisseria winogradskyi*, *Achromobacter anitratus*, *Achromobacter mucosus*
	Acinetobacter baylyi	
	Acinetobacter bouvetii	
	Acinetobacter calcoaceticus	*Acinetobacter anitratus*, *Acinetobacter calcoaceticus* subsp. *anitratus*
	Acinetobacter gerneri	
	Acinetobacter grimontii	
	Acinetobacter haemolyticus	*Acinetobacter anitratus*
	Acinetobacter johnsonii	
	Acinetobacter junii	
	Acinetobacter lwoffii	*Acinetobacter anitratus*, *Moraxella lwoffi*, *Acinetobacter calcoaceticus* subsp. *lwoffi*
	Acinetobacter parvus	
	Acinetobacter radioresistens	
	Acinetobacter schindleri	
	Acinetobacter tandoii	
	Acinetobacter tjernbergiae	
	Acinetobacter towneri	
	Acinetobacter ursingii	
	Acinetobacter venetianus	
Agrobacterium	*Agrobacterium tumefaciens*	
Alcaligenes	*Alcaligenes faecalis*	*Alcaligenes odorans*, *Pseudomonas odorans*
Bergeyella	*Bergeyella zoohelcum*	*Weeksella zoohelcum*
Brevundimonas	*Brevundimonas aurantiaca*	*Caulobacter henricii* subsp. *aurantiacus*
	Brevundimonas diminuta	*Pseudomonas diminuta*
	Brevundimonas intermedia	*Caulobacter intermedius*
	Brevundimonas subvibrioides	*Caulobacter subvibrioides*
	Brevundimonas variabilis	*Caulobacter variabilis*
	Brevundimonas vesicularis	*Pseudomonas vesicularis*
Burkholderia	*Burkholderia ambifaria*	
	Burkholderia anthina	
	Burkholderia arboris sp. nov.	
	Burkholderia cenocepacia	
	Burkholderia cepacia	*Pseudomonas cepacia*, *Pseudomonas multivorans*, *Pseudomonas kingae* CDC group EO-1
	Burkholderia diffusa sp. nov.	
	Burkholderia gladioli	*Pseudomonas gladioli*, *Pseudomonas marginata*
	Burkholderia latens sp. nov.	
	Burkholderia mallei	*Pseudomonas mallei*, *Actinobacillus mallei*
	Burkholderia metallica sp. nov.	
	Burkholderia multivorans	
	Burkholderia pseudomallei	*Pseudomonas pseudomallei*
	Burkholderia pyrocinia	
	Burkholderia seminalis sp. nov.	
	Burkholderia stabilis	
	Burkholderia vietnamensis	
Chryseobacterium	*Chryseobacterium gleum*	*Flavobacterium gleum* CDC group IIb
	Chryseobacterium indologenes	*Flavobacterium indologenes* CDC group IIb
	Chryseomonas luteola	*Pseudomonas luteola* CDC group Ve-1

(Continued)

Table 170.1 Current nomenclature of nonfermenting Gram-negative bacilli*—cont'd

Genus	Current name	Previous name(s)
Comamonas	*Comamonas aquatica* *Comamonas kerstersii* *Comamonas terrigena* *Comamonas testosteroni*	 *Pseudomonas terrigena* *Pseudomonas testosteroni* CDC group EF-19
Cupriavidus	*Cupriavidus necator*	*Wautersia eutropha*, *Ralstonia eutropha*, *Pseudomonas/Alcaligenes eutrophus*
Delftia	*Delftia acidovorans*	*Comamonas acidovorans*, *Pseudomonas acidovorans*
Elizabethkingia	*Elizabethkingia meningoseptica*	*Chryseobacterium meningosepticum*, *Flavobacterium meningosepticum* CDC group IIa, *Flavobacterium meningosepticum*
Myroides	*Myroides odoratus*	*Chryseobacterium odoratum*, *Flavobacterium odoratum* CDC group M-4f
Ochrobactrum	*Ochrobactrum anthropi* *Ochrobactrum intermedium*	*Achromobacter* Vd *Ochrobactrum* sp. nov.
Oligella	*Oligella ureolytica* *Oligella urethralis*	CDC group IVe *Moraxella urethralis*, CDC group M-4
Pandoraea	*Pandoraea* spp.	*Burkholderia* spp.
Pedobacter	*Pedobacter heparinum* *Pedobacter piscium*	*Sphingobacterium heparinum* *Sphingobacterium piscium*
Pseudomonas	*Pseudomonas aeruginosa* *Pseudomonas alcaligenes* *Pseudomonas chlororaphis* *Pseudomonas delafieldii* *Pseudomonas fluorescens* *Pseudomonas kingii* *Pseudomonas mendocina* *Pseudomonas oryzihabitans* *Pseudomonas pertucinogena* *Pseudomonas pseudoalcaligenes* *Pseudomonas putida* *Pseudomonas* sp. group 1 *Pseudomonas stutzeri* *Pseudomonas stutzeri*-like *Pseudomonas*-like group 2	 *Pseudomonas aureofaciens* CDC group Vb-2 *Bordetella pertussis* rough phase IV *Pseudomonas alcaligenes* biotype B *Pseudomonas denitrificans* CDC group Vb-1 CDC group Vb-3 CDC group IV-d
Ralstonia	*Ralstonia pickettii*	*Burkholderia pickettii*, *Pseudomonas pickettii*, CDC groups Va-1, Va-2, *Pseudomonas thomasii*
Rhizobium	*Rhizobium radiobacter* *Rhizobium rhizogenes* *Rhizobium rubi* *Rhizobium vitis*	*Agrobacterium radiobacter*, *Agrobacterium tumefaciens*, *Agrobacterium radiobacter* CDC group Vd-3 *Agrobacterium rhizogenes* *Agrobacterium rubi* *Agrobacterium vitis*
Shewanella	*Shewanella algae* *Shewanella hanedai* *Shewanella putrefaciens*	 *Alteromonas hanedai* *Pseudomonas/Alteromonas putrefaciens*, CDC group Ib, *Achromobacter putrefaciens*
Sphingobacterium	*Sphingobacterium antarcticum* *Sphingobacterium faecium* *Sphingobacterium mizutaii* *Sphingobacterium multivorum* *Sphingobacterium spiritivorum* *Sphingobacterium thalpophilum* *Sphingobacterium yabuuchiae*	 *Flavobacterium mizutaii* *Flavobacterium multivorum* CDC group IIk-2 *Flavobacterium spiritivorum*, *Sphingobacterium versatilis* CDC group IIk-3 *Flavobacterium thalpophilum* *Flavobacterium yabuuchiae*
Sphingomonas	*Sphingomonas paucimobilis*	*Pseudomonas paucimobilis* CDC group IIk-11
Stenotrophomonas	*Stenotrophomonas maltophilia*	*Xanthomonas maltophilia*, *Pseudomonas maltophilia*
Weeksella	*Weeksella virosa*	*Flavobacterium genitale* CDC group II-f

*The list is limited to those potentially involved in human infections.[1,6,7]

EPIDEMIOLOGY

Currently, there are 160 *Pseudomonas* spp.,[1] most of which are ubiquitous organisms and are widely distributed in nature.[5] To date, a total of 12 *Pseudomonas* spp., including *P. aeruginosa*, *P. fluorescens*, *P. luteola*, *P. putida* and *P. stutzeri*,[6] have been implicated in human infections. The increasing importance of *Pseudomonas* spp. can be attributed in part to the increasing usage of broad-spectrum antimicrobial agents and the resulting selective pressure in favor of these inherently resistant Gram-negative organisms. Also, the increasing number of immunocompromised and intensive care patients as well as the increase in invasive procedures may have contributed to the emergence of mainly saprophytic organisms as important nosocomial pathogens. *P. aeruginosa* remains the most prevalent aerobic nonfermenting Gram-negative organism identified as the causative pathogen in a large variety of nosocomial infections.

Recent studies have shown the ability of individual *P. aeruginosa* strains to acquire or discard genomic segments, giving rise to strains with customized genomic repertoires that can survive in a wide range of environmental habitats that can serve as reservoirs for the infecting organisms.[7] Studies have identified a variety of environmental reservoirs in hospitals and nursing homes. For example, *P. aeruginosa* has been isolated from surgical instruments, disinfectants (quaternary ammonium compounds) and contact lens cleaning solutions, as well as in ventilatory equipment in ICUs and surgical equipment such as an ultrasonic aspirator used in neurosurgical procedures.[5,8] Surveys using molecular typing methods (exotoxin A typing) also linked *P. aeruginosa* isolates from sinks, wash basins and toilets to those isolated from the hands of staff and the urinary tracts of paraplegic patients, and transmission over a 6-month period in an ICU for newborns has been related to a source implicating air valves in the ventilator tubes.[9] *Pseudomonas* spp. are not part of the normal flora and colonization of healthy individuals with *Pseudomonas* spp., particularly *P. aeruginosa*, is rare.[10] While *P. aeruginosa* has been isolated from many different sites, including the respiratory tract, genitourinary tract and skin, recovery of these bacteria, especially mucoid variants, should prompt the search for infections.[1] In contrast, intestinal colonization reaches 18% in hospitalized patients and figures up to 73% have been reported in patients recovering from gastrointestinal surgery. In neutropenic patients intestinal colonization has been identified as the source of subsequent *P. aeruginosa* bloodstream infection.

Several *Pseudomonas* spp. other than *P. aeruginosa*, particularly *P. fluorescens*, *P. stutzeri* and *Sphingomonas paucimobilis* (*P. paucimobilis*), are more frequently isolated from clinical specimens than other *P.* non-*aeruginosa* and have been implicated in a variety of infections in adult and pediatric patients. There have also been individual reports of common source outbreaks due to these organisms.

EPIDEMIOLOGIC MARKERS

A variety of typing methods using both phenotypic and molecular techniques have been assessed for epidemiologic and clinical purposes. A selection of conventional and molecular methods is summarized in Table 170.2.

Conventional phenotypic methods

Conventional methods such as biochemical profiles, antibiotic susceptibility patterns, bacteriophage and bacteriocin susceptibilities, as well as outer membrane protein profiles and multilocus enzyme electrophoresis, are based on unstable properties of the organisms and phenotypic characters may change during the course of an outbreak or during a prolonged period of occurrence of endemic cases with apparently identical pathogens. To date, molecular methods have largely replaced conventional typing methods. Various different methodologies are used.

Restriction fragment length polymorphism (RFLP)

DNA probes specific for genes coding for exotoxin A (*exoA*), alginate (*algD*), elastase (*lasB*) or pilin (*PAK*), can be used for differentiating isolates from different patients, provided that at least two DNA probes are used (5% of *P. aeruginosa* strains are deficient in the *exoA* gene).

Pulsed-field gel electrophoresis (PFGE)

Advantages of this method include a very high discriminatory power, making it uniquely suitable for outbreak investigation. On the downside, PFGE is a labor-intensive method and time to result ranges between 48 and 96 hours.

Random amplified polymorphic DNA (RAPD) polymerase chain reaction (PCR)

RAPD-PCR has been shown to be a useful tool for typing of *P. aeruginosa*. RAPD-PCR has only a moderate discriminatory power that is lower than that of PFGE, but can be improved by combining RAPD with an RFLP analysis of the fragments. Although allowing for a quick estimate in a defined epidemiologic outbreak setting, this method is not suitable for large-scale epidemiologic studies.

Multilocus sequence typing (MLST)

MLST is a sequence-based, highly discriminative typing method that was developed in the late 1990s and has been applied to a variety of bacterial pathogens including *Neisseria meningitidis*, *Streptococcus pneumoniae*, *Staphylococcus aureus*, *S. epidermidis*, *A. baumannii*[11] and *P. aeruginosa*.[12] For MLST of *P. aeruginosa*, sequences of the conserved regions of the housekeeping genes *acsA*, *aroE*, *guaA*, *mutL*, *noD*, *ppsA* and *trpE* are used.[13] Compared to PFGE, MLST showed a higher sensitivity for the detection of genetic relatedness in recent studies, while the discriminator power of PFGE was higher than that of MLST in *P. aeruginosa*.[12]

DIAGNOSTIC MICROBIOLOGY

Bacteriology of *Pseudomonas* spp.

Pseudomonas spp. are thin, rod-shaped, nonspore-forming Gram-negative bacilli with a guanine/cytosine content of 57–68 mol%. *Pseudomonas* spp. are motile due to one (e.g. *P. aeruginosa*, *P. stutzeri*, *P. oryzihabitans*; Fig. 170.1) or more polar flagella (e.g. *P. fluorescens*, *P. putida*, *P. luteola*).[1,5] *Pseudomonas* spp. including *P. aeruginosa* can be recovered from clinical and environmental specimens using standard collection, transport and storage techniques, and can be cultured using standard broth or solid media including all nonselective (e.g. Columbia or tryptic-soy agar) as well as a number of selective media including MacConkey agar. While selective media containing inhibitors such as acetamide, nitrofurantoin or cetrimide may be helpful for the isolation of *Pseudomonas* spp., inhibition of some strains of *P. aeruginosa* (isolated from specimens from CF patients) by cetrimide and nalidixic acid has been reported.

Pseudomonas spp. grow at temperatures between 82°F and 108°F (28–42°C) and visible growth is usually achieved within 24–48 hours. Colonies of *P. aeruginosa* on solid media are usually flat (Fig. 170.2), but a variety of culture variants including small colony variants (SCV)[14] and mucoid forms have been described. Mucoid forms are particularly observed in patients suffering from CF and can pose problems for identification and susceptibility testing. Pyoverdin (fluorescein, yellow) is produced by all fluorescent *Pseudomonas* spp.; *P. aeruginosa* may also produce pyocyanin (blue), pyorubin (red) or pyomelanin (black). The distinct green color of *P. aeruginosa* colonies usually results from a combination of pyoverdin and pyocyanin. *P. aeruginosa* can be separated from the other fluorescent *Pseudomonas* spp. (e.g. *P. fluorescens*, *P. putida*) by its ability to grow at 108°F (42°C).

Table 170.2 Epidemiologic markers for *Acinetobacter baumanii* and *Pseudomonas aeruginosa* typing

Epidemiologic markers		Species	Principles and characteristics	Advantages	Drawbacks
Phenotypic	Biotyping	*A. baumannii*	Utilization of substrates, production of enzymes, biotyping schemes for identification, commonly carbon-source utilization test using levulinate, citraconate, L-phenylalanine, phenylacetate, 4-hydroxybenzoate and L-tartrate	Rapid, easy to perform, inexpensive, also useful for identification to species level	Unstable, variability of metabolic characters, limited discriminatory power
		P. aeruginosa	Utilization of substrates, production of enzymes, biotyping schemes for identification	Rapid, easy to perform, API 20NE panel or automated identification systems (Vitek, MicroScan, Phoenix), inexpensive	Unstable, variability of metabolic characters, poorly discriminating
	Resistance phenotype	*A. baumannii*, *P. aeruginosa*	Antimicrobial susceptibility pattern always obtained, multiple resistance markers	Rapid, easy to perform, standardized (national/international guidelines), automated systems (Vitek, MicroScan, Phoenix) can be used, early and often useful during outbreak	Unstable profiles, plasmid acquisition or loss during an outbreak, derepression of inducible enzymes, mutations, poorly discriminating, unreliable
	Serotyping	*A. baumannii*	O-antigenic polysaccharide of the lipopolysaccharide, 34 O-antisera	No advantages for *A. baumannii*	Not all strains typeable, low discriminatory power
		P. aeruginosa	Based on somatic O-specific antigen (LPS), polyclonal/monoclonal antibodies, 20 serotypes, 17 antisera (IATS)	Rapid, early results, easy to perform, inexpensive	50–70% of CF strains nontypeable, polyagglutination of some CF strains, reproducibility of anti-LPS monoclonal antibodies is 75%, only available in specialized laboratories
	Phage typing	*A. baumannii*	Two systems (21 and 14 phages, respectively)	Limited requirements, inexpensive	Lack of reproducibility, low discriminatory power, approx. 20% of strains nontypeable, only available in specialized laboratories
		P. aeruginosa	Colindale set of 21 phages, cell surface receptors (OM, LPS, slime)	Limited requirements, inexpensive	Lack of reproducibility, low discrimination, insensitivity of CF- and LPS-defective strains, only available in specialized laboratories
	Pyocin typing	*P. aeruginosa*	R, F, S pyocins, specific lytic activity, 105 types, 26 subtypes	Limited requirements, inexpensive	Poor discrimination, complexity of the system, time-consuming technique, only available in specialized laboratories
Genotypic	Plasmidotyping	*A. baumannii*	Plasmids present in many *A. baumannii* strains	No advantage for *A. baumannii* typing	Plasmids are easily transferable and may be gained or lost, cumbersome technique
		P. aeruginosa	Relatively rare plasmid in *P. aeruginosa*, plasmids of 1.2–60 MDa in 15% of strains	No advantage for *P. aeruginosa* typing	Low frequency, acquisition or loss during epidemics
	Genomic DNA, total DNA	*P. aeruginosa*	Polymorphism of DNA, REA endonucleases (*Eco*R1, *Hin*dII, *Sma*I), conventional agarose gel electrophoresis	Good discriminatory power	Large number of fragments making resolution of bands difficult to interpret

	DNA RFLP	*P. aeruginosa*	Detection of genes coding for exotoxin A (*exoA*), elastase (*lasB*), alginate (*algD*), two probes necessary	Good discriminatory power, good correlation with ribotyping	Laborious techniques, small numbers of isolates can be compared
	Ribotyping (ribosomal DNA)	*A. baumannii*	*Hin*dIII, *Hin*cII, *Eco*R1, *Cla*I	Results comparable to AFLP, automated systems available	Limited discriminatory power, labor intensive, PFGE and others provide more accurate results for *A. baumannii*
		P. aeruginosa	Three genes coding for rRNA, probes for 16S and 23S RNA, restriction enzymes (*Eco*R1, *Cla*I, *Sal*I)	Universal, excellent reproducibility, stable ribotype patterns within outbreaks	Laborious techniques, sensitivity and specificity not established for *P. aeruginosa*
	Pulsotyping (PFGE)	*A. baumannii*	*Apa*I and/or *Sma*I	Gold standard for *A. baumannii*, highest discriminatory power, interlaboratory reproducibility possible with standardized protocols	Labor intensive
		P. aeruginosa	DNA fingerprinting, restriction enzymes DraI, SpeI	The most specific discriminatory technique	Interpretation somewhat delicate, heavy workload
	RAPD-PCR	*A. baumannii*	Amplification of random DNA sequences	Fast, easy and low cost, relatively high discriminatory power	Inter- and intralaboratory reproducibility difficult to achieve, not suited for large-scale epidemiologic studies, discriminatory power inferior to PFGE
		P. aeruginosa	Amplification of random DNA sequences	Fast, easy and low cost, relatively high discriminatory power	Inter- and intralaboratory reproducibility difficult to achieve
	Repetitive extragenic palindromic (REP)-PCR	*A. baumannii*	PCR-based typing method, amplification of highly-conserved REP sequences using specific primers	Simple, rapid	Inter- and intralaboratory reproducibility remains to be determined, expensive
	AFLP	*A. baumannii*	PCR-based typing method, amplification of restriction fragments (*Hin*dIII and TaqI) using specific primers	Can be used for typing and species identification, relatively robust method, high discriminatory power	*Acinetobacter* only, requires a high level of standardization and extensive experience, cumbersome and expensive, restricted to reference laboratories, not suited for routine epidemiologic analyses, data are not readily transportable
	MLST	*A. baumannii*, *Acinetobacter* genomic species 13TU, *P. aeruginosa*	Amplification and sequencing of several specific loci in different housekeeping genes: *A. baumannii* and *Acinetobacter* genomic species 13TU: *gltA*, *gyrB*, *gdhB*, *recA*, *cpn60*, *gpi*, *rpoD*; *P. aeruginosa*: *acsA*, *aroE*, *guaA*, *mutL*, *nuoD*, *ppsA*, *trpE*	Highly portable, highly reproducible	Time-consuming, very expensive
	PCR/ESI-MS)	*A. baumannii*	Amplification of specific sequences of six housekeeping genes (*efp*, *trpE*, *adk*, *mutY*, *fumC*, *ppa*), detection by ESI-MS	Results similar to MLST, easy, high throughput, fastest method to date	Novel method, not suited for routine laboratories, expensive equipment, detailed evaluation warranted

AFLP, amplified fragment length polymorphism; CF, cystic fibrosis; ESI-MS, electrospray ionization mass spectrometry; IATS, International Antigenic Typing Scheme; LPS, lipopolysaccharide; MLST, multilocus sequence typing; OM, outer membrane; PCR, polymerase chain reaction; PFGE, pulsed-field gel electrophoresis; RAPD, random amplified polymorphic DNA; REA, restriction endonuclease analysis; RFLP, restriction fragment length polymorphism.

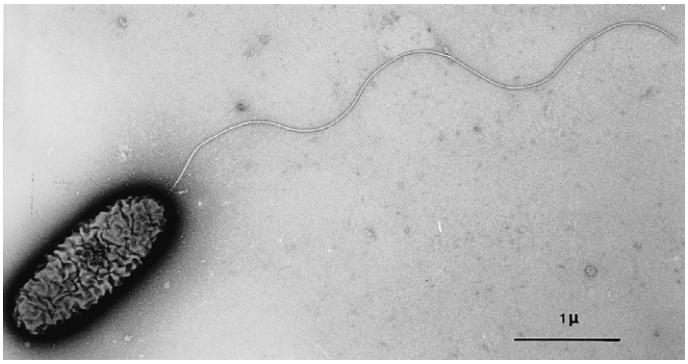


Fig. 170.1 *Pseudomonas aeruginosa* monotrichous polar flagellum seen on electron microscopy. Courtesy of Professor A Marty.

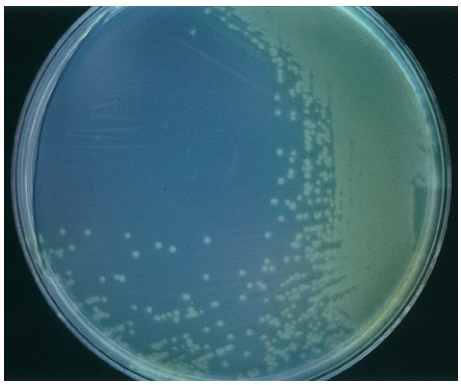


Fig. 170.2 *Pseudomonas aeruginosa* colonies on agar medium. Courtesy of Professor E Bingen.

Characteristics differentiating *Pseudomonas* spp. are shown in Table 170.2. This simplified scheme for identification of these bacteria is mainly based on morphologic features (flagella), pigment production and the main metabolic characteristics. Automated systems can usually identify *P. aeruginosa* from clinical specimens; however, additional testing is often required for identification of *Pseudomonas* spp. including mucoid or other forms of *P. aeruginosa* from CF patients.

Other methods to identify *Pseudomonas* spp. include conventional and real-time PCR, probes directed at or sequencing of species-specific 16S rRNA,[15] fluorescence in situ hybridization (FISH) and matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF) mass spectrometry.[16]

PATHOGENICITY AND PATHOGENESIS

Pseudomonas aeruginosa expresses a variety of virulence factors. While host-related factors also play an important role in the course of *P. aeruginosa* infection, some specific bacterial factors have been characterized in recent years (Table 170.3).

Host-related factors

As with other opportunistic infections, host-related factors such as anatomic and physiologic barriers play an important role in the pathogenesis of *P. aeruginosa* infections. Several of the more severe and more frequent *P. aeruginosa* infections such as burn wound infections, as well as colonization of the upper respiratory or gastrointestinal tract, largely depend on the impairment of the natural barriers provided by intact skin or mucous membranes. Animal studies showed that *P. aeruginosa* can cause lethal infections originating from as little as 10 colony forming units (cfu) inoculated into burned skin, while similar infections of the intact skin require five to six logs more organisms.

As in skin infections, disruption of the integrity of the corneal mucosa has been identified as the major factor in severe *P. aeruginosa* infections of the eye. Mucosal surfaces provide protection against *P. aeruginosa* colonization and infection by a variety of mechanisms such as mucus-containing antimicrobial factors including lysozyme, lactoferrin and defensins, the presence of physiologic flora, and mucociliary clearance. However, if impaired by underlying diseases such as CF or therapeutic interventions such as antimicrobial or anti-cancer chemotherapy, *P. aeruginosa* has a high propensity for colonization and infection of the respective site.

A variety of soluble factors involved in the pathogenesis of *P. aeruginosa* infections, such as complement, collectins, cytokines, chemokines and immunoglobulins, as well as cellular mediators, have been characterized. A detailed summary of their function can be found elsewhere.[1,17]

Bacterial factors

Virtually all major classes of bacterial virulence systems (see Table 170.3) have been reported in *P. aeruginosa* such as endo- and exotoxins, pili, flagella, proteases, lipases, iron-binding proteins and exopolysaccharides. Adherence of *P. aeruginosa* to host tissue is in part promoted by pili (encoded by the *pilA* gene, regulated by *rpoN*, *pilS* and *pilR*), flagella (*fliC* (flagellin) and *fliD* (protein caps)) and other appendage-like structures that have recently been identified in multidrug-resistant strains of *P. aeruginosa*. In addition, lipopolysaccharide may bind to the cystic fibrosis transmembrane conductance regulator (CFTR). Defective expression (ΔF508 allele) of the CFTR has been identified as one of the factors contributing to the hypersusceptibility of CF patients to chronic *P. aeruginosa* infection. Recent studies have identified numerous CFTR-dependent factors that are recruited to the epithelial plasma membrane in response to infection and that are needed for bacterial clearance. Several factors mediating resistance to host defenses have been identified in *P. aeruginosa*, including lipopolysaccharide O (LPS O) preventing lysis by complement and a variety of proteases (e.g. protease IV, elastolytic proteases and alkaline protease) that are involved in the evasion of innate immunity, degradation of cells and host proteins, and syndecan 1.

Manifestation of infection largely depends on the expression of a variety of toxins or other substances that damage host cells such as ferripyochelin that has been shown to destroy endothelial cells alone or in combination with hydrogen peroxide. Toxins that may be produced by *P. aeruginosa* include exotoxin A (an inhibitor of protein biosynthesis by means of inactivating elongation factor 2), leukocidin, plcHR (a hemolytic phospholipase C), pyocyanin and rhamnolipids. Most of the toxins and hydrolytic enzymes that are involved in virulence are regulated by a complex system, many components of which are influenced by environmental factors. In *P. aeruginosa* three major quorum-sensing systems have been identified to date, termed las, rhl and PQS. These interacting quorum-sensing systems seem to play an important role in the regulation of biofilm formation and expression of virulence factors. Degradation of acyl-homoserine lactones, the signaling molecules of the quorum-sensing system, has even been discussed as a treatment option. In addition, the GacS/GacA two-component system positively controls the transcription of two sRNAs (RsmY, RsmZ) that are crucial for the expression of virulence genes and which regulate the response to oxidative stress in *P. fluorescens*. The spread of *P. aeruginosa* and invasion of the bloodstream requires a smooth LPS substituted with O side chains and is likely enhanced by bacterial factors stimulating the release of tumor necrosis factor (TNF) and quorum-sensing molecules.

Mucoid *Pseudomonas aeruginosa*

In the early 1960s the importance of mucoid variants of *P. aeruginosa* was recognized. Mucoid strains are morphologic and functional variants of *P. aeruginosa* characterized by the ability to produce copious

Table 170.3 *Acinetobacter baumanii* and *Pseudomonas aeruginosa* virulence-associated factors

Category	Pathogen	Classes of virulence factors (examples)	Effects in humans
Enzyme	*A. baumannii*	Antibiotic-inactivating enzymes (β-lactamases) Proteases Protein S	Inactivation of antimicrobial agents Tissue damage Interference with phagocytosis
	P. aeruginosa	Adhesins (exoenzyme S) Antibiotic-inactivating enzymes (β-lactamases) Elastolytic activity (two enzymes Las A, Las B) Glycolipid-rhamnolipid (heat-stable) Hemolysins (phospholipase C) Neuraminidase Cytoplasmic lectins, *P. aeruginosa* lectin I (PA I), PA II Proteases (alkaline and neutral metalloproteinase)	Binding specificity for glycolipids (glycosphingolipid) Inactivation of antimicrobial agents Break down elastin of blood vessels, hemorrhages Disruption of phospholipids of cell membranes, hydrolysis of lung surfactant and ciliostatic action Enhances pilin-mediated adherence PA I specific for D-galactose, PA II specific for D-mannose Tissue damage (active on elastin, collagen, fibrin), digestion of protecting host defense proteins
Structural	*A. baumannii*	Aerobactin Fimbriae Iron-repressible outer membrane receptor proteins Iron-uptake components Lipopolysaccharide Lipopolysaccharide (hydrophobic sugars in the O side chain) Outer membrane proteins Pili Polysaccharide capsule	Increased virulence (mechanism to be determined) Adhesion to epithelial cells Increased virulence (mechanism to be determined) Survival in the bloodstream Proinflammatory response Adhesion to host cells Interference with cell permeability Adhesion to epithelial cells Interference with phagocytosis, survival in dry environment
	P. aeruginosa	Lipopolysaccharide Mucopolysaccharide capsule (alginate) Pili (fimbriae) Siderophores (pyochelin and pyoverdin)	Cascade of inflammatory events Adhesion to epithelial cells, barrier to antibiotics, increased viscosity of bronchial secretions (cystic fibrosis) Adhesion to epithelial cells Help growth in iron-limited condition, generation of toxic oxygen-free radicals
Toxin	*A. baumannii*	Lipid A Outer membrane protein A (Omp 38)	Toxicity, pyrogenicity Cytotoxicity, apoptosis, cell death
	P. aeruginosa	Cytotoxins (leucocidin) Endotoxins Exotoxin A Phenazine pigment (pyocyanin)	Cytopathic effects on leukocytes and alteration of phospholipids of cell membrane Septic shock Tissue damage, inhibition of phagocytes, inhibition of protein synthesis Ciliary disruption

amounts of alginate, an acetylated polymer of D-mannuronic and L-glucuronic acids regulated by ClpXP proteases, AlgB, Alg8 and Alg44[18] (Figs 170.3, 170.4). Alginate has been implicated in the pathogenesis of respiratory tract infections due to *P. aeruginosa* in CF patients as well as in chronic oropharyngeal colonization.

In vivo, in areas with impaired local defenses, such as in the airways of CF patients, the organism grows in microcolonies surrounded by a thick polysaccharide matrix. In autopsied lungs of CF patients there are microcolonies adherent to the walls of larger airways and in the alveoli. Mucoid *P. aeruginosa* is present in abundance in foci of active inflammation in small bronchioles but not in destroyed parenchymal areas. This observation is consistent with the simultaneous role of bacterial growth in the active inflammatory process and of toxins produced by *P. aeruginosa* diffusing away from microcolonies. Several studies have addressed the role of antibodies against alginate and their failure to confer protection against infection in CF patients, showing that the biofilm-like growth may interfere with the opsonizing capabilities of the alginate antibodies. An exopolysaccharide–alginate conjugate vaccine has been discussed as a therapeutic approach.

CLINICAL MANIFESTATIONS

Bacteremia/bloodstream infection

Assessment of the incidence of *P. aeruginosa* bloodstream infection (BSI) is difficult. While in older series *P. aeruginosa* accounted for 20–35% of all BSI isolates, this figure has been considerably lower in recent series, where 4.4% were due to *P. aeruginosa*. In another recent study analyzing 24 179 nosocomial BSIs from 52 hospitals in the USA over a 7-year period, *P. aeruginosa* accounted for 3.8% of all BSI isolates from non-ICU wards and 4.7% from ICUs, making it the

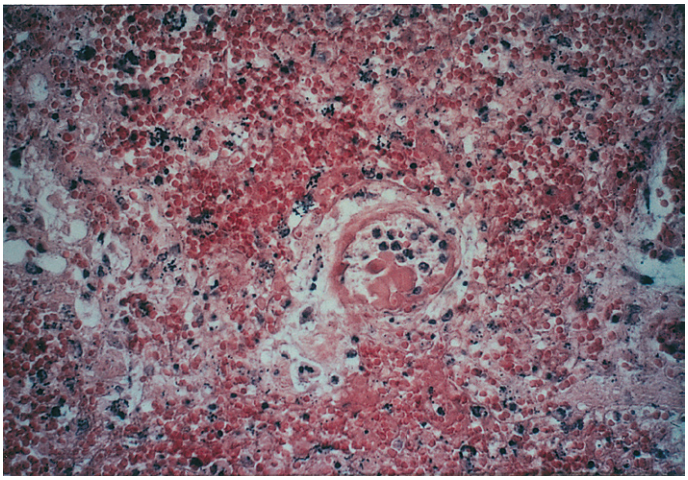


Fig. 170.3 Anatomic pathology of *Pseudomonas aeruginosa* pneumonia showing acute inflammatory exudate, necrosis of alveolar membranes and fibrinous thrombosis in a venula. Hematoxylin–eosin stain. Courtesy of Professor Groussard.

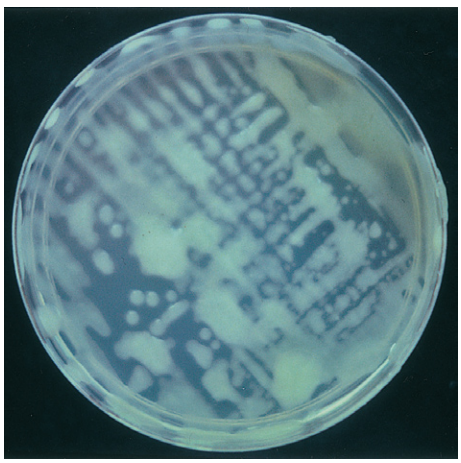


Fig. 170.4 Mucoid colonies of a strain of *Pseudomonas aeruginosa* isolated from a patient with cystic fibrosis. Courtesy of Professor E Bingen.

Table 170.4 Predominant sites and incidences of nosocomial infections due to *Acinetobacter baumanii*, *Burkholderia cepacia*, *Pseudomonas aeruginosa* and *Stenotrophomonas maltophilia*

Organism	Clinical presentation	Incidence (% of patients)
Pseudomonas aeruginosa	Bloodstream infection Pneumonia (VAP) Urinary tract infection Wound infection Cystic fibrosis respiratory infection Burn wound infection	3–5 21–24 11–15 8–10 70–90 1–10
Stenotrophomonas maltophilia	Bloodstream infection Pneumonia (VAP) Urinary tract infection Wound infection Cystic fibrosis respiratory infection Burn wound infection	<1 to 6 2 3–9 8–17 – –
Burkholderia cepacia	Bloodstream infection Pneumonia (VAP) Urinary tract infection Wound infection Cystic fibrosis respiratory infection Burn wound infection	<1 <1 4 – 80 –
*Acinetobacter baumannii**	Bloodstream infection Pneumonia (VAP) Urinary tract infection Wound infection Cystic fibrosis respiratory infection Burn wound infection	<1 to 14 5–10 10–30 2–32 – –

–, no data available; VAP, ventilator-associated pneumonia.
*Includes *Acinetobacter* genomic species 2, 3 and 13TU.

seventh and fifth most commonly isolated pathogen, respectively. In this series *P. aeruginosa* BSI had an incidence of 2.1 per 10 000 hospital admissions.[19] Historically, crude mortality rates of 50–70% have been reported in patients with *P. aeruginosa* BSI, while more recent studies found crude mortality rates of 28% in non-ICU and 48% in ICU patients, respectively,[19] as well as an attributable mortality ranging from 15% to 44%.

Secondary *P. aeruginosa* BSI most often originates from the respiratory tract (Table 170.4) while other sources such as the urinary tract or burn wounds are less common. Colonization of the gastrointestinal tract, which may develop in the presence of a variety of risk factors such as hospitalization in an ICU, the presence of neutropenia or treatment with cytotoxic chemotherapy, has also been discussed as a source of *P. aeruginosa* BSI, particularly in neutropenic patients receiving chemotherapy. Approximately 50% of these patients develop intestinal carriage, compared to only 5–15% of the general population, and translocation of *P. aeruginosa* has been implicated as the potential mechanism for invasion and metastatic infection. In addition, contaminated medical equipment used for endoscopic retrograde cholangiopancreatography (ERCP), placement of left ventricular assist devices and other invasive procedures have been reported as sources of *P. aeruginosa* BSI.[20]

The clinical presentation of *P. aeruginosa* BSI may range from benign transient bacteremia to fulminant septic shock with high mortality. The usual presentation does not differ from sepsis due to other Gram-negative pathogens. In neutropenic patients, typical skin lesions (e.g. ecthyma gangrenosum; see Chapter 12) may present and point to *P. aeruginosa* as the causative pathogen. Even though these lesions may on rare occasions present in non-neutropenic patients or be associated with other pathogens such as *Aspergillus* spp. or *Mucor* spp., the presence of ecthyma gangrenosum in a septic patient should prompt antimicrobial chemotherapy active against *P. aeruginosa*.[1]

Endocarditis

Pseudomonas aeruginosa is the second most common cause of endocarditis due to non-HACEK Gram-negative bacilli (after *Escherichia coli*), accounting for 22% (n=11) of all cases caused by non-HACEK Gram-negative bacilli in a recent observational study from the International Collaboration on Endocarditis-Prospective Cohort Study (ICE-PCS) database, including 2761 cases of definite endocarditis from 61 hospitals in 28 countries.[21] In contrast to older studies that reported endocarditis due to *P. aeruginosa* predominantly in intravenous drug users,[20] the ICE-PCS study found that 57% of patients with non-HACEK Gram-negative endocarditis had a health-care-associated infection, whereas intravenous drug use was observed in only 4% of patients. Implanted endovascular

devices and prosthetic heart valves were frequent risk factors.[21] The in-hospital mortality of patients with endocarditis due to *P. aeruginosa* reached 21% in recent studies despite high rates of cardiac surgery (55%).[21]

Lower respiratory tract infections (see also Chapter 28)

Pseudomonas aeruginosa is a leading cause of nosocomial infections and is responsible for 10% of all hospital-acquired infections. It is the most common Gram-negative pathogen causing ventilator-associated pneumonia (VAP) and is consistently associated with a measurable attributable mortality.[22,23]

Since *P. aeruginosa* is also a frequent colonizer of endotracheal tubes and the nasopharynx of patients with mechanical ventilation receiving antimicrobial chemotherapy, the significance of a culture from the upper respiratory tract yielding *P. aeruginosa* in the absence of clinical and/or radiologic signs of pneumonia is less clear. The delay in eradication of *P. aeruginosa* in patients with VAP may be explained by an increased apoptosis in neutrophils.[22]

Typical pathologic features include necrosis of alveolar septa (see Fig. 170.3) and arterial walls, with areas of focal hemorrhage and, in intact areas, infiltration with macrophages, mononuclear cells and polymorphonuclear leukocytes. A different lung pathology has been described in bacteremic pneumonia, which is rarely seen and mainly affects patients with severe underlying conditions. Risk factors are listed in Table 170.5.

The clinical course is characterized by a rapid progression with a diffuse and often bilateral infiltration, usually in combination with pleural effusion. On cross-section, the lesions are nodular, hemorrhagic with necrotic foci or umbilicated nodules surrounded by dark hemorrhage. Intra-alveolar hemorrhage with patchy alveolar septal necrosis is seen on microscopy. The lesions contain many bacteria but lack infiltration with macrophages, mononuclear cells and polymorphonuclear leukocytes. Similar to fulminant pneumonia caused by Panton–Valentine leukocidin (PVL)-positive *S. aureus*, pulmonary edema and necrotizing bronchopneumonia are associated with a poor prognosis. This form of pneumonia is generally complicated by bloodstream invasion that often spreads metastatically to distant sites of infection. The case fatality rate in patients with fulminant bacteremic pneumonia due to *P. aeruginosa* is extremely high (up to 80–100% of cases).

Historically, community-acquired pneumonia due to *P. aeruginosa* was mainly seen in immunocompromised patients, such as HIV/AIDS patients with low CD4 counts or patients suffering from CF, but it has also been reported in previously healthy patients with no underlying immunosuppression.

Inappropriate antibiotic therapy, mainly due to lack of coverage against *P. aeruginosa*, has been identified as a significant risk factor for adverse outcome.[24]

Skin and soft tissue infections

Commonly seen skin and soft tissue infections in otherwise healthy patients are dermatitis or superinfections of predisposing skin lesions. Folliculitis due to *P. aeruginosa* has been repeatedly described and outbreaks have been linked to swimming pools, whirlpools, hot tubs and, recently, contamination of an industrial closed loop water recycling system in a cardboard factory.[25]

Burn wound sepsis

Pseudomonas aeruginosa is the most common cause of burn wound sepsis, which is the predominant form of skin and soft tissue infection complicating severe thermal injury. The mortality rate is high (50–78%) despite improvements in management and antibiotic therapy.[20] The incidence of *P. aeruginosa* BSI, however, has declined considerably from 10% of burn patients in older reports to about 1% in more recent studies.[26]

Colonization of the burned skin surface by *P. aeruginosa* may result from the patient's own flora or from environmental sources. The bacteria penetrate into the subcutaneous tissues via hair follicles and breaks in the burned skin, and may enter the bloodstream with the

Table 170.5 Sources, means of transmission and risk factors for nosocomial infections due to aerobic Gram-negative bacilli

Organisms and reference	Settings*	Sources/means of transmission	Risk factor/comments
Achromobacter (Alcaligenes) xylosoxidans	ICU, hemodialysis units	Contaminated chlorhexidine solution, dialysis fluid, aerosols, respirators	Aqueous source, hemodialysis, severe underlying disease
Acinetobacter baumannii	ICU	Contaminated ventilators, intravascular catheters	Severely ill patients, cross-contamination, outbreaks
Burkholderia cepacia	ICU	Airborne transmission, contaminated skin preparations, ventilator, thermometer, antiseptic solutions	Cystic fibrosis patients, hand carriage, immunocompromised patients
Elizabethkingia (Chryseobacterium) meningosepticum	NICU	Contaminated water, ice, disinfectants, humidifiers	Wounds, mechanical ventilation
Pseudomonas aeruginosa	ICU, burns units	Contaminated equipment, solutions, antiseptics, endogenous	Cross-contamination, exposure to broad-spectrum antibiotics, severely ill patients, outbreaks in burn patients
Pseudomonas putida, *Pseudomonas fluorescens*	ICU	Contaminated blood and blood by-products, antiseptics	Large (burn) wounds, intravascular devices
Stenotrophomonas maltophilia	ICU, hematology/oncology units	Contaminated devices, disinfectants, catheters	Dialysis fluids, exploratory procedures, neutropenia, respiratory devices, tracheostomized patients, backflow from nonsterile tubes

*Most frequently reported occurrence. ICU, intensive care unit; NICU, neonatal intensive care unit.

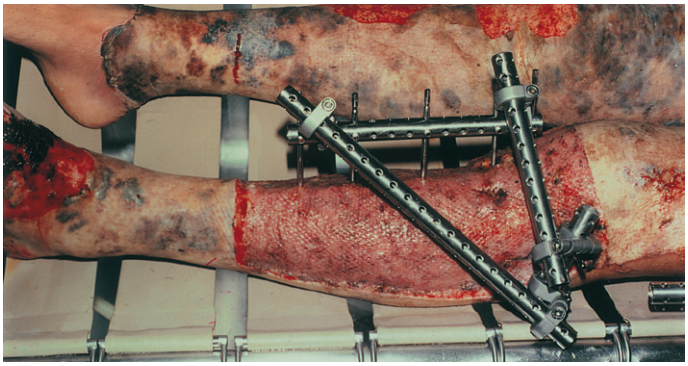


Fig. 170.5 Burned leg superinfected with *Pseudomonas aeruginosa*. Courtesy of Professor H Carsin.

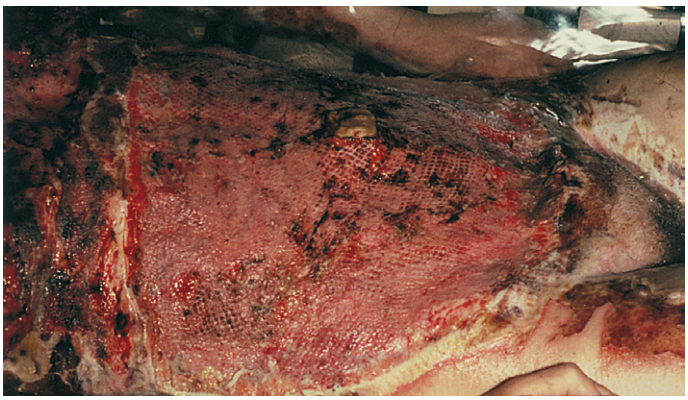


Fig. 170.6 Burned abdominal wall superinfected with *Pseudomonas aeruginosa*. Courtesy of Professor H Carsin.

help of the proteolytic enzymes they produce. Other virulence factors (see Table 170.3) contribute significantly to the severity of the burn infection, which can be the source of bloodstream invasion. Recently Gr-1$^+$/CD11b$^+$ cells have been identified as an accelerator of sepsis stemming from *P. aeruginosa* wound infection in thermally injured mice. Sepsis requires specific management in burn centers (Figs 170.5, 170.6). Recent studies comparing BSI in burn patients due to *P. aeruginosa*, *A. baumannii*, *Klebsiella pneumoniae* and *S. aureus* showed that *K. pneumoniae* had the greatest impact on mortality relative to all other pathogens.

Ecthyma gangrenosum

This focal skin lesion constitutes a rare manifestation of *P. aeruginosa* BSI. The lesion is characterized by an erythema surrounded by hemorrhage, necrosis of skin tissues and bacterial invasion. These extensive and destructive lesions of the skin are particularly seen in neutropenic patients, but have also been reported in non-neutropenic pediatric and adult patients.

Eye infections

Pseudomonas aeruginosa is the most common Gram-negative pathogen causing infections of the eye, despite not forming part of the normal ocular flora. Infections most commonly originate from an exogenous source and are related to (superficial) injuries. Ocular infections vary from mild (conjunctivitis) to extremely severe (orbital cellulitis).

Keratitis

Keratitis is the most frequent eye infection. Keratitis due to *P. aeruginosa* has only been reported secondary to an injury of the corneal surface. Recent studies support this observation, stating that *P. aeruginosa* does not cause keratitis in animals with an intact cornea. The predominant predisposing factors in *P. aeruginosa* keratitis are wearing of contact lenses, congenital abnormalities, burns or trauma, altered host defenses (in particular patients with HIV infection) and prematurity. Contact lens-associated keratitis due to *P. aeruginosa* has mainly been observed in association with extended-wear contact lenses, inappropriate disinfecting regimens and poor hygiene. The bacteria may adhere to the lens, resulting in the development of a thick coat of mucopolysaccharides forming a biofilm similar to that seen on other prosthetic devices. Viral keratitis (herpes simplex) may also be associated with secondary bacterial infection.

Exocellular products of *P. aeruginosa* and strong adhesion to the exposed basement membrane of the epithelium increase the corneal damage; exotoxins, proteases and phospholipases degrade the corneal stroma, resulting in extensive loss of collagen fibers from the stroma. Keratitis due to *P. aeruginosa* may progress rapidly, leading to endophthalmitis and loss of eyesight.

Endophthalmitis

Endophthalmitis most often results from an endogenous origin, occurring by hematogenous spread from other infected sites. It may also occur after intraocular inoculation of *P. aeruginosa* by trauma, burns or ocular surgery, or following keratitis. Endophthalmitis may present as an acute fulminant disease with severe pain, chemosis and decreased acuity, and can progress rapidly to panophthalmitis. The prognosis is poor without appropriate local and systemic management.

Urinary tract infections

Urinary tract infections due to *P. aeruginosa* are usually health-care associated. Common risk factors include the presence of a foreign body (e.g. long-term catheter or stent), surgery or any cause of obstruction of the urinary flow or persistent infection (e.g. chronic prostatitis). While *P. aeruginosa* urinary tract infections usually affect patients with prolonged hospitalization, patients receiving antimicrobial therapy over a prolonged period of time and/or patients with the above-mentioned risk factors, infections in otherwise healthy children with no known risk factors have also been reported.

P. aeruginosa urinary tract infections have no specific clinical presentation but tend to evolve with frequent recurrences, treatment failures and chronic evolution. A characteristic picture of ulcerative or necrotic lesions and multiple renal abscesses is seen in patients who have metastatic bacteremia with urinary tract invasion.[20]

Ear infections

Pseudomonas aeruginosa is frequently isolated from the external auditory canal, particularly in infants. Infections may range from benign transient colonization to severe infections with a prolonged clinical course that may be associated with permanent neurologic sequelae and adverse outcome. *P. aeruginosa* has been identified as the cause of a mild superficial infection of the external ear canal (e.g. swimmer's ear). Although this benign infection usually resolves without sequelae, it may proceed to invasion of the epithelium between cartilage and bone in the lateral portion of the auditory canal, penetrating soft tissue, cartilage and bone.

Malignant (necrotizing) otitis externa

Malignant otitis externa or necrotizing otitis externa is a severe invasive ear infection, clinically characterized by decreased hearing, otalgia, otorrhea, early facial nerve paralysis and a swollen erythematous external auditory canal. Adjacent soft tissue is often involved and the infection may progress to the middle ear, mastoid, temporal bone and cranial nerves. Clinical presentation may include visible extension

with cellulitis and bone erosions, and purulent discharge from the inner ear if the tympanic membrane is perforated.[20] *P. aeruginosa* may be isolated from superficial swabs of the external auditory canal and from surgical specimens. Most cases occur in elderly people with diabetes mellitus, but cases have also been reported from patients suffering from AIDS, elderly patients without immunosuppression or infants with severe underlying diseases.

Management of this severe and extensive ear infection requires prolonged antibiotic therapy, surgical debridement and drainage. The fatality rate is high (about 15–20%). Relapses are frequent and malignant external otitis requires prolonged follow-up.

Miscellaneous

Central nervous system infections

Infections of the central nervous system (CNS) are rarely due to *P. aeruginosa*. Meningitis, epidural or subdural empyema and brain abscesses have been reported in adults and children, usually as a result of either direct inoculation (head trauma, surgery) or a contiguous infection (sinus, mastoid), or following bloodstream infection.

Bone and joint infections

Bone and joint infections are infrequently caused by *P. aeruginosa*. As in CNS infections, direct inoculation (trauma, surgery), contiguous infection from surrounding tissue or resulting from hematogenous seeding following BSI are the most common routes of infection. Recent studies in war casualties found that *P. aeruginosa* was more frequently seen in primary osteomyelitis, whereas Gram-positive pathogens were more likely to be isolated from recurrent episodes.

Infections predominantly occur in patients with predisposing factors such as diabetes mellitus, intravenous drug use and chronic debilitation. More frequently reported associations include:

- vertebral osteomyelitis and arthritis involving the sternoclavicular or sternochondral joints in intravenous drug users;
- vertebral osteomyelitis in elderly patients following genitourinary instrumentation or surgery; and
- osteochondritis of the foot in children following puncture wounds or in patients with diabetic foot ulcers.

While *P. aeruginosa* seems to have a particular affinity for cartilaginous joints of the axial skeleton, infections at a distant site such as pneumonia rarely spread to the vertebral column or the axial skeleton. Following pelvic surgery or femoral catheterization, osteomyelitis of the pubic symphysis has also been reported as well as rare cases of pelvic osteomyelitis in children.

The clinical presentation may be discrete, and pain, swelling, fever and other systemic signs are variable.[20] The diagnosis of these infections may be difficult as blood cultures are frequently negative and imaging studies may also be normal in the earlier stages.

PATHOGENICITY AND CLINICAL MANIFESTATIONS OF *PSEUDOMONAS* SPP. OTHER THAN *P. AERUGINOSA*

Some of the non-*aeruginosa Pseudomonas* spp. have been isolated from human clinical specimens (blood, urine, stools) and occasional cases of opportunistic infection as a result of transfusions, contamination of indwelling catheters, antiseptics or dialysis fluid, and various other mechanisms of transmission have been reported. For example, *P. fluorescens*, a psychrophilic organism, may grow at 39°F (4°C), which favors its presence in blood products and infusates such as heparin. Outbreaks of bacteremia, respiratory infections in adult and pediatric CF and non-CF patients, wound infections and rare cases of community-acquired pneumonia have been reported. These *Pseudomonas* spp., particularly *P. fluorescens*, *P. stutzeri* and *Sphingomonas paucimobilis* (*P. paucimobilis*), have also been implicated in rare cases of brain abscess, arthritis, endophthalmitis and keratitis. Most cases occur in patients with severely impaired host defenses such as immunocompromised patients in the ICU setting (Table 170.6).[20]

ANTIMICROBIAL RESISTANCE AND THERAPY

Although *P. aeruginosa* is most frequently involved in nosocomial infections, it is not the most resistant organism in the group of aerobic nonfermenting Gram-negative bacilli. Although these organisms are intrinsically resistant to many antimicrobials, several antimicrobial agents from different classes remain potentially active against *P. aeruginosa* and other *Pseudomonas* spp. (Tables 170.7, 170.8). However, the percentage of multi- or pandrug-resistant clinical

Table 170.6 Epidemiology and pathogenicity of *Acinetobacter* spp. and *Pseudomonas* spp.

Species	Habitat and epidemiology	Clinical significance
Acinetobacter non-*baumannii*, i.e. *A. johnsonii*, *A. junii*, *A. lwoffii*, *A. radioresistens*, *A. ursingii*	Ubiquitous, soil, water, sewage water, hospital environment, antiseptics, injectable solutions, more susceptible to antibiotics than other species	Outbreaks of pseudobacteremia involved in catheter-related bloodstream infection, isolation from wounds, urine, blood, cerebrospinal fluid
A. baumannii (Acinetobacter genomic species 2, 3, 13TU)	Hospital environment, colonized patients, health-care personnel	Opportunistic pathogen, severe infections mostly in immunocompromised patients
Pseudomonas alcaligenes, *P. pseudoalcaligenes*	Environment, water, plants, hospital environment, rare opportunistic pathogens	Occasional bacteremia (contaminated blood products, solutions)
P. putida, *P. fluorescens*, *P. aeruginosa*	Soil, water, plants, hospital sinks, floor, food spoilage (eggs, meat, fish, milk), opportunistic pathogens	Rarely isolated from clinical specimens, rare cases of colonization in patients with cystic fibrosis, bacteremia, urinary tract infection, wounds
P. stutzeri	Ubiquitous, soil, water, sewage water, hospital environment, antiseptics, injectable solutions, relatively more frequent than other non-*aeruginosa Pseudomonas* spp., opportunist, more susceptible to antibiotics than other species	Outbreaks of pseudobacteremia, isolation from pus, urine, blood, cerebrospinal fluid, contamination of bone marrow transplant
P. aeruginosa, *P. fluorescens*	Hospital environment, patients, health-care personnel	Opportunistic pathogen, severe infections mostly in immunocompromised patients

Table 170.7 Resistance mechanisms in *Acinetobacter baumannii* and *Pseudomonas aeruginosa*

Antibiotic class	Antibiotics	Organism	Mechanisms	Genetic basis (examples)
Aminoglycosides	Aminoglycosides	*A. baumannii*	Aminoglycoside-modifying enzymes	aph(3′)-VIa and aac(6′)-I
	Aminoglycosides	*A. baumannii*	Ribosomal (16s rRNA) methylation	
	Aminoglycosides	*A. baumannii*	Efflux	AdeABC, AdeM
	Aminoglycosides	*P. aeruginosa*	Enzymatic inactivation	aph(3′)-IIb
	Aminoglycosides	*P. aeruginosa*	Reduced permeability	PprA, PprB
Fosfomycin	Fosfomycin	*P. aeruginosa*	Altered transport system	Chromosomal mutation (GlpT), plasmid-mediated (fosA, fosB)
Quinolones	Quinolones	*A. baumannii*	Modification of target binding site	*gyrA*, *parC*
	Quinolones	*A. baumannii*	Efflux	AdeABC, AdeM
	Quinolones	*P. aeruginosa*	Altered DNA gyrase target	*gyrA*, *parC*
	Quinolones	*P. aeruginosa*	Efflux	mexCD-oprJ, mexAB-oprM
Rifampin (rifampicin)	Rifampin	*P. aeruginosa*	Altered DNA polymerase target	arr-2
Tetracyclines and glycylcyclines	Tetracyclines and glycylcyclines	*A. baumannii*	Tetracycline-specific efflux	Tet(A), tet(B)
	Tetracyclines and glycylcyclines	*A. baumannii*	Ribosomal protection	tet(M)
	Tetracyclines and glycylcyclines	*A. baumannii*	Multidrug efflux	AdeABC
β-Lactams	Carbapenems	*A. baumannii*	Multidrug efflux	AdeABC
	Carbapenems	*A. baumannii*	Carbapenemases	OXA, IMP
	Carbapenems	*P. aeruginosa*	Altered protein porin D2	Chromosomal, orpD
	Carbapenems	*P. aeruginosa*	Imipenemase	VIM, IMP
	Cephalosporins	*P. aeruginosa*	Reduced permeability	Chromosomal
	Cephalosporins	*P. aeruginosa*	β-Lactamase inactivation (83%)	AmpC, PSE-1, PSE-3, PSE-4, OXA-10 (=PSE-2), IMP-1, TEM-1, TEM-2, OXA-1
	Monobactams	*P. aeruginosa*	β-Lactamase inactivation	AmpC, PSE-1, PSE-3, PSE-4, OXA-10 (=PSE-2), IMP-1, TEM-1, TEM-2, OXA-1
	Penicillins	*A. baumannii*	Altered penicillin binding proteins (PBPs)	Chromosomal (altered PBP-4)
	Penicillins	*P. aeruginosa*	Altered PBP targets	Chromosomal (altered PBP-4)
	β-Lactams	*A. baumannii*	β-Lactamases	TEM, SHV, ADCs, VEB, PER, CTX-M, OXA, IMP, VIM, SIM
	β-Lactams	*A. baumannii*	Outer membrane proteins	CarO (29-kDa), 22-, 33-, 36-, 43-, 44-, 47-kDa, HMP-AB, OmpW
	β-Lactams	*A. baumannii*	Efflux	AdeABC

Table 170.8 Antibiotic susceptibility of *Acinetobacter baumannii* and *Pseudomonas aeruginosa*

		A. BAUMANNII		*P. AERUGINOSA*	
Antibiotics		**MIC_{50} (mg/l)**	**MIC_{90} (mg/l)**	**MIC_{50} (mg/l)**	**MIC_{90} (mg/l)**
β-Lactams	Ampicillin	64	>256	64	>256
	Ampicillin–sulbactam	1	16	32	256
	Amoxicillin	32	256	32	>256
	Amoxicillin–clavulanic acid	2	256	32	256
	Mezlocillin	16	512	16	128
	Piperacillin	8	512	8	512
	Piperacillin–tazobactam	4	128	4	128
	Ticarcillin	64	512	32	512
	Ticarcillin–clavulanic acid	32	256	16	512
	Cefazolin	256	512	128	512
	Cefuroxime	32	128	32	256
	Ceftriaxone	8	128	8	128
	Cefotaxime	16	128	16	64
	Ceftazidime	4	128	4	32
	Cefepime	4	16	4	16
	Imipenem	0.06	0.25	1	2
	Meropenem	0.25	1	0.5	2
	Aztreonam	16	64	4	16
Aminoglycosides	Amikacin	2	8	0.5	2
	Gentamicin	1	32	0.5	8
	Tobramycin	0.5	8	0.125	4
Quinolones	Ofloxacin	0.5	16	2	4
	Ciprofloxacin	0.5	64	0.06	0.25
	Levofloxacin	0.25	8	1	32
	Moxifloxacin	0.12	16	4	32
Miscellaneous	Trimethoprim–sulfamethoxazole	2	256	128	≥128

From Zhanel *et al*.[27]

P. aeruginosa strains has increased considerably in the past decade and often leaves few therapeutic options. Antimicrobial agents with potential antipseudomonal activity include semisynthetic penicillins such as carboxypenicillins (ticarcillin), ureidopenicillins (piperacillin), some third- (group IIIb) and fourth-generation cephalosporins (ceftazidime, cefpirome, cefepime), carbapenems (imipenem, meropenem), monobactams (aztreonam), aminoglycosides (gentamicin, tobramycin, amikacin), fluoroquinolones (ciprofloxacin, levofloxacin) and polymyxins (polymyxin B, polymyxin E). Novel agents that display *in-vitro* activity against *P. aeruginosa* belong to well-known classes and include doripenem, ceftobiprole and ceftaroline. There is an urgent need for antimicrobials active against multidrug-resistant Gram-negative bacilli, and new antimicrobial classes active against *Pseudomonas* spp. need to be developed. A summary of antipseudomonal treatment options in various indications is given in Table 170.9.

P. aeruginosa displays a wide array of resistance determinants, most of which are chromosomally located. The incidence of plasmids in *P. aeruginosa* is relatively low. Mechanisms of resistance include altered outer membrane permeability (altered protein porins or lack of protein porin OprD), production of chromosomally and plasmid-mediated β-lactamases, aminoglycoside-inactivating enzymes and an efflux mechanism actively pumping out different antibiotic classes from the bacterial cell.

β-Lactamases, as well as IMP, GES and VIM metallocarbapenemases, may be augmented by a number of efflux systems and decreased OprD expression which together confer multidrug resistance to β-lactam antibiotics. In clinical settings these are often encountered in combination with other mechanisms conferring resistance to fluoroquinolones and aminoglycosides, thus considerably limiting the remaining therapeutic options.

Recent multicenter studies report rates of multidrug-resistant (i.e. resistance to three or more antimicrobial classes) *P. aeruginosa* ranging from 3% to 50%, with considerable variation between countries.

The therapy of serious *Pseudomonas* infections should be based on the results of adequately performed antimicrobial susceptibility testing, including determination of minimal inhibitory concentrations (MICs). Of note, in mucoid strains of *P. aeruginosa* determination of MICs and susceptibility testing using commercial systems may not be accurate, and the addition of agar dilution or gradient diffusion techniques (such as E-test) may be warranted. Data shown in Table 170.8 indicate current susceptibility rates, but due to considerable geographic variation empiric therapy should be based on local susceptibility data.

Pseudomonas spp. other than *P. aeruginosa* are generally more susceptible to currently used antimicrobial agents including trimethoprim–sulfamethoxazole. Based on *in-vitro* susceptibilities, carbapenems and fluoroquinolones remain the drugs of choice, while aminoglycosides have considerably less activity, especially in *P. putida* and *P. fluorescens* which are also resistant to trimethoprim–sulfamethoxazole. While *P. stutzeri* is usually susceptible to all antipseudomonal agents, higher rates of resistance to aztreonam and β-lactam antibiotics have been reported in *P. putida*, *P. fluorescens* and *P. oryzihabitans*.

Treatment of infections due to *P. aeruginosa* with a single agent is possible using third-generation cephalosporins such as ceftazidime or cefpirome, carbapenems such as imipenem or meropenem, or fluoroquinolones such as ciprofloxacin if the strain is susceptible.[28] A meta-analysis of controlled clinical trials has also shown that monotherapy (β-lactam) provided similar survival compared to combination therapy (β-lactam and aminoglycoside) in patients with cancer and neutropenic fever.[29] However, especially with fluoroquinolones and carbapenems, resistance may develop quickly during therapy and close microbiologic

Table 170.9 Empiric antibiotic therapy for *Acinetobacter baumannii* and *Pseudomonas aeruginosa* infections

Choices	Organism	Drugs	Indications
Monotherapy	*P. aeruginosa*	Antipseudomonal penicillins: ticarcillin, piperacillin, azlocillin; Cephalosporins: ceftazidime, cefoperazone, cefpirome; Carbapenems: imipenem, meropenem; Fluoroquinolones: ciprofloxacin	Limited to nongranulocytopenic patients, non-life-threatening infections
	A. baumannii	Carbapenems: imipenem, meropenem; Fluoroquinolones: ciprofloxacin, levofloxacin; Polymyxins: colistin (in carbapenem-resistant strains); Glycylcycline: tigecyclin (in multidrug-resistant strains)	Due to high and increasing resistance therapy must be based on susceptibility testing results
Conventional combinations	*P. aeruginosa*	Azetreonam, ticarcillin or ceftazidime plus tobramycin, imipenem plus amikacin, ciprofloxacin plus ceftazidime, ciprofloxacin plus fosfomycin	Severe *P. aeruginosa* infections – pneumonia, bacteremia, burns (plus topical), malignant external otitis media (plus surgery), central nervous system infection (plus intrathecal), cystic fibrosis (plus topical, colistin or tobramycin inhalative)
	A. baumannii	Combination of two *in vivo* active compounds such as imipenem plus ciprofloxacin or amikacin, combination of any monotherapy drug with sulbactam or polymyxin	Critically ill patients with severe infections; data on sulbactam and polymyxin therapy are from *in vitro* and animal models and from rare case studies in humans
Alternatives	*P. aeruginosa*	Antipseudomonal penicillin plus fluoroquinolone, aztreonam plus aminoglycoside, aminoglycoside plus fluoroquinolone	
	A. baumannii	Various combinations of β-lactams and amikacin, imipenem plus rifampin (rifampicin)	Salvage therapy, combinations of antibacterial agents that are resistant by *in-vitro* testing should be used with great caution or avoided

monitoring is advised including repeated determination of susceptibility. In addition to systemic antimicrobial therapy, other interventions such as surgical debridement or local antimicrobial therapy have been shown to be useful in specific infections due to *P. aeruginosa*.

Central nervous system infections due to *P. aeruginosa* require bactericidal antibiotics that reach high concentrations in cerebrospinal fluid (CSF). Patients with meningitis require a combination of ceftazidime, which is highly concentrated in CSF when the meninges are inflamed, and an aminoglycoside. If there is obstruction of the subarachnoid space the aminoglycoside may be instilled directly into the ventricular system. *P. aeruginosa* brain abscesses may require surgical intervention in addition to prolonged antibiotic therapy.[20]

Malignant external otitis, which may present as an extremely severe infection, usually requires a combination of local surgical debridement and drainage together with antimicrobial therapy consisting of a (local) aminoglycoside or quinolone preparation and a parenteral antipseudomonal β-lactam.

In burn wound sepsis frequent emergence of resistance due to high bacterial counts and limited access of antibiotic to burn sites has been reported. Local procedures with topical agents and surgical debridement of necrotic tissue are always applied in addition to systemic antibiotics.[20]

Strategies for the treatment of pneumonia due to *P. aeruginosa* have emphasized the importance of early initiation of appropriate empiric therapy because the majority of fatal cases occur within the first 48–72 hours. For treatment of severe infections such as pneumonia or bacteremia, combination therapy is generally recommended (antipseudomonal β-lactam and an aminoglycoside), but there is a poor correlation between clinical response and the *in-vitro* synergistic effects of antibiotics.[20] In CF patients, eradication of *Pseudomonas* spp. as well as other pathogens such as *Burkholderia cepacia* or *Stenotrophomonas maltophilia* from the airways occurs temporarily only, whatever strategy is used. Proper management of acute exacerbations of *Pseudomonas* lung infection in these patients usually requires combination therapy and higher dosing. Fluoroquinolones have been used successfully both in adult and pediatric CF patients, and a combination of ciprofloxacin with fosfomycin has demonstrated *in-vitro* synergy. Repeated courses of aggressive antibiotic therapy every 3 months in combination with other measures such as mucolytics, antiproteases, topical (inhalative) antibiotic therapy and physiotherapy have increased the long-term survival of patients with CF. Current treatment options have been extensively reviewed elsewhere.[30] Administration of antibiotics directly into the tracheobronchial tree has been shown to increase local antibiotic concentrations. Inhalation therapy utilizing aminoglycosides and colistin is generally well tolerated, although some reduction of the maximum expired volume per second has occasionally been observed. Currently, tobramycin and polymyxin B are being used successfully in prevention and therapy of respiratory tract infections due to *P. aeruginosa* as well as *B. cepacia* in patients with CF.

PREVENTION

Prevention plays a major role in controlling *Pseudomonas* infections. Preventative measures can be based on the identification of sources and interruption of ways of transmission (see Table 170.2) and a variety of guidelines have been established in the USA and in European countries, implementing isolation policies, administrative and regulatory measures and hospital epidemiology surveillance. Attempts to reduce the risk of colonization and subsequent infection in high-risk patients have included:

- elimination of endogenous nosocomial *P. aeruginosa* and reduction of oropharyngeal, intestinal and skin colonization in ICU patients;
- prevention of cross-contamination and monitoring of various sources of *P. aeruginosa*; and
- prevention of contamination in burns patients, in surgical wounds and in the oropharyngeal area in ventilated patients.

Active vaccination has been discussed for high-risk patients and a variety of antigenic determinants are being evaluated as potential vaccine targets including outer membrane proteins, flagella and pili. In addition, vaccination with live attenuated strains has been shown to be efficacious in animal models. Current approaches to vaccines and immunotherapy against *P. aeruginosa* have recently been reviewed in detail.[31]

Acinetobacter Species

NATURE AND TAXONOMY

The genus *Acinetobacter* has undergone significant taxonomic modification over the last 30 years. To date, the genus *Acinetobacter* consists of 32 different species, 17 of which have been assigned names (see Table 170.1). While most *Acinetobacter* spp. are considered to be of minor clinical importance, *A. baumannii* and unnamed *Acinetobacter* genomic species 3 and 13TU have emerged as important clinical pathogens, contributing significantly to morbidity and mortality. Due to their phenotypic similarity, these three species have been grouped together with the environmental organism *A. calcoaceticus* in the so-called *A. calcoaceticus–A. baumannii* (Acb) complex. Overall, *A. baumannii* has emerged as one of the most significant nosocomial pathogens over the past decades, especially in patients with impaired host defenses in the ICU and has been implicated in a variety of infections including BSI, pneumonia and meningitis.

In severe nosocomial infections, mortality rates as high as 64% have been reported. Similar to methicillin-resistant *Staph. aureus* (MRSA), major epidemiologic features of these organisms include their propensity for clonal spread and their involvement in hospital outbreaks, as well as the ability to express or acquire resistance determinants, making it one of the most resistant organisms known to date.

EPIDEMIOLOGY

Acinetobacter spp. are widely distributed in nature and can be isolated from virtually 100% of soil and freshwater samples when appropriate enrichment and culture techniques are used. Nevertheless, current studies indicate that not all *Acinetobacter* spp. are found naturally in the environment (some such as *A. schindleri* and *A. ursingii* have until now only been recovered from human specimens) and that the clinically important species in particular (e.g. *A. baumannii*) are in fact not ubiquitous.[3] A variety of *Acinetobacter* spp. recovered from the axilla, groin and toe webs, the oral cavity, the respiratory tract and normal intestine have also been identified as part of the commensal human flora. In contrast, some *Acinetobacter* spp., such as *A. baylyi*, *A. bouvetii*, *A. grimontii*, *A. tandoii*, *A. tjernbergiae* and *A. towneri*, that were isolated from activated sludge have as yet never been observed in human specimens. Studies investigating the colonization of human skin and mucous membranes found *Acinetobacter* spp. in up to 44% of nonhospitalized individuals and in up to 75% of patients hospitalized on a regular ward. The most frequently isolated species were *A. lwoffii* (58–61%), *A. johnsonii* (20%), *Acinetobacter* genomic species 15BJ (12%), *A. junii* (10%), *A. radioresistens* (8%) and *Acinetobacter* genomic species 3 (5%), whereas *A. baumannii* was found only rarely on human skin (0.5–3%) and in human feces (0.8%), and was not found at all in *Acinetobacter* genomic species 13TU.[32] Recent studies investigating potential skin contamination in healthy US soldiers did not report any *Acinetobacter* spp., but the lack of enrichment techniques and long transport time may have contributed to this finding. In addition, seasonal and geographic variations in skin colonization with *Acinetobacter* spp. have been reported from different geographic locations where *Acinetobacter* genomic species 3 (36%), *Acinetobacter* genomic species 13TU (15%), *Acinetobacter* genomic species 15TU (6%) and *A. baumannii* (4%) were the most frequently recovered species, whereas *A. lwoffii*, *A. johnsonii* and *A. junii* were only rarely found.[33,34]

Molecular epidemiology

Only after 1986, when the taxonomy of the genus *Acinetobacter* was revised and molecular methods provided the necessary tools to identify *Acinetobacter* at the species level, did detailed studies of the epidemiology of the different members of this genus became possible. Commonly used methods include ribotyping, pulsed-field gel electrophoresis, random amplified polymorphic DNA analysis, amplified fragment length polymorphism (AFLP) and, most recently, multilocus sequence typing (MLST)[11]Bartual, 2005 and polymerase chain reaction/electrospray ionization mass spectrometry (PCR/ESI-MS).[35]

Acinetobacter outbreaks published between 1977 and 2004 (http://www.outbreak-database.com) have recently been extensively reviewed in two studies.[36,37] Recent studies increasingly report outbreaks involving multidrug-resistant *A. baumannii*. The persistence of endemic strains of *A. baumannii* over an extended period of time and the spread of single clones within a medical center have been documented in several institutions worldwide, with some studies linking individual outbreaks to a common source such as computer keyboards, blood pressure cuffs, enteral nutrition or dust in the interior of a mechanical ventilator and inside continuous venovenous hemofiltration (CVVH) dialysis machines, while others failed to identify a common source despite extensive environmental surveillance.

Several large studies failed to detect major interinstitutional spread of *A. baumannii* while other recent studies from New York, London and Johannesburg reported involvement of several different medical centers within a city, or even of health-care facilities in several cities within one country. Following reports of the so-called European clones I, II and III,[38] some authors suggested that a few epidemic strains may be involved in outbreaks across countries; however, in contrast to the aforementioned multisite outbreaks, no epidemiologic link in time or space could be established.

DIAGNOSTIC MICROBIOLOGY

Acinetobacter spp. are nonfermenting, nonmotile, oxidase-negative, aerobic Gram-negative coccobacilli usually found in diploid formation or in chains of variable length (Fig. 170.7) and may be mistaken for Gram-negative (or even Gram-positive) cocci. Strictly aerobic, *Acinetobacter* spp. grow well on most routinely used solid media at temperatures of 68–111°F (20–44°C). *Acinetobacter* spp. isolated from human specimens grow readily at 99°F (37°C). In contrast to the species of the *Acinetobacter calcoaceticus–baumannii* complex, several other *Acinetobacter* spp. do not grow on MacConkey agar or may show hemolysis on sheep blood agar (*A. haemolyticus*, *Acinetobacter* genomic species 6, 13BJ, 14BK, 15BJ, 16 and 17).

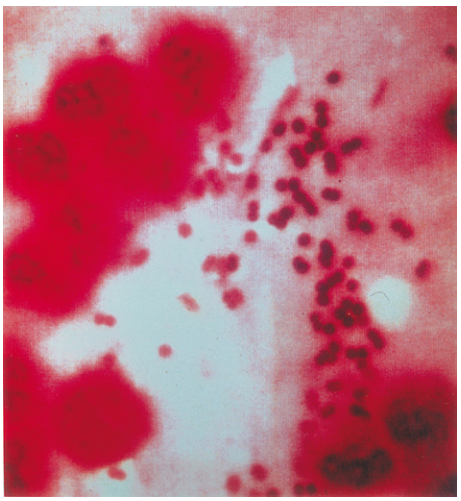


Fig. 170.7 Morphology of *Acinetobacter baumannii* on Gram stain. Preparation from a lung infection in mice. Courtesy of Dr ML Joly-Guillou.

While presumptive identification at the genus level is possible using the above-mentioned criteria, unambiguous identification of *Acinetobacter* spp. to the genus level is possible by transformation of the naturally transformable tryptophan auxotroph *A. baylyi* ADP1 by crude DNA of any *Acinetobacter* to wild-type phenotype.[39] There are several methods that can be used for the identification of *Acinetobacter* spp., unfortunately none of which is readily available in the routine laboratory. Phenotypic identification of 11 of the 12 initially described genomic species (but not of the novel species) is possible using a scheme proposed by Bouvet and Grimont.[40] Molecular methods include amplified 16S ribosomal DNA restriction analysis (ARDRA), high-resolution fingerprint analysis by AFLP, ribotyping, tRNA spacer fingerprinting, restriction analysis of the 16S–23S rRNA intergenic spacer sequences, sequence analysis of the 16S–23S rRNA gene spacer region, sequencing of the *rpoB* (RNA polymerase β-subunit) gene and its flanking spacers, PCR/ESI-MS and MALDI-TOF mass spectrometry,[41] as well as methods to identify *A. baumannii* by detection of the $bla_{\text{OXA-51}}$-like carbapenemase gene or using differences in the *gyrB* genes to differentiate between *A. baumannii* and *Acinetobacter* genomic species 13TU by means of a conventional PCR amplifying a specific region of the *gyrB* gene. While most of these methods are not suitable for the routine laboratory, some of the novel methods such as MALDI-TOF mass spectrometry may be an interesting option in the future, but require further evaluation.

Species identification using currently available commercial systems such as API 20NE, Vitek 2, Phoenix and MicroScan WalkAway remains problematic, especially since *A. baumannii*, *Acinetobacter* genomic species 3 and *Acinetobacter* genomic species 13TU are uniformly identified as *A. baumannii* by the most widely used identification systems.

PATHOGENICITY AND PATHOGENESIS

Several factors may be responsible for the virulence of *Acinetobacter* spp., including the presence of a polysaccharide capsule formed of L-rhamnose, L-glucose, D-glucuronic acid and D-mannose that makes the surface of strains more hydrophilic, factors facilitating adhesion to human epithelial cells in the presence of fimbriae and/or mediated by the capsular polysaccharide; enzymes such as butyrate esterase, caprylate esterase and leucine arylamidase potentially involved in damaging tissue lipids; and the LPS component of the cell wall and the presence of lipid A, which are likely to participate in the pathogenicity of *Acinetobacter* spp.

Recent studies using whole genome sequencing identified a large number of antibiotic drug resistance determinants as well as several pathogenicity islands,[42] a significant number of which likely originated in other bacterial species including *Pseudomonas* spp., *Salmonella* spp. and *E. coli*.[42] Relevant genes included those encoding the cell envelope, pilus biogenesis, iron uptake and metabolism, as well as sensor kinases that are important for the regulation of virulence determinants in other Gram-negative organisms such as *P. aeruginosa*. Several studies in *A. baumannii* have described siderophore-mediated iron acquisition systems, biofilm formation, adherence and outer membrane protein function and a specific lipopolysaccharide.

Important elements of the host response may include Toll-like receptor signaling and the stimulation of proinflammatory cytokines; the LPS of *Acinetobacter* seems to be equally potent to *E. coli* LPS at similar concentrations. Humoral immune responses include antibodies targeted toward iron-repressible outer membrane proteins and the O-polysaccharide component of LPS that have bactericidal and opsonizing *in-vitro* activity. While several potential host response mechanisms have been described in recent studies, the role of the host responses in the pathogenesis of *A. baumannii* infections remains to be determined.

CLINICAL MANIFESTATIONS

Acinetobacter spp. (mainly *A. baumannii*) have been implicated as the causative pathogen in nearly all types of nosocomial infection, including BSI, pneumonia, urinary tract infection, wound infection and meningitis. Crude mortality in patients with *A. baumannii* BSI ranges from 32% to 52%[43] but mortality rates as high as 64% have been reported in patients with meningitis due to *A. baumannii*.

Pneumonia

Acinetobacter baumannii accounts for 5–10% of cases of ICU-acquired pneumonia in the USA and is usually observed in patients with a prolonged ICU stay. The proportion of nosocomial pneumonia due to *A. baumannii* in institutions where this organism is endemic may be much higher. Predisposing factors include endotracheal intubation, surgery, prior antibiotic therapy and underlying pulmonary disease. The clinical presentation may include multilobular involvement, pleural effusion and formation of a bronchopulmonary fistula. Mortality rates of up to 70% have been reported, but as with other severe *A. baumannii* infections this may reflect the patients' underlying condition rather than the virulence of the organism. Community-acquired pneumonia due to *A. baumannii* has mainly been reported from tropical regions of Australia and Asia, and affects patients with impaired host defenses such as those with diabetes, renal failure, chronic alcohol abuse or underlying pulmonary disease. Similar to infections with community-acquired MRSA, it is characterized by a fulminant clinical course, secondary BSI and a high mortality of 40–60%.

Bloodstream infection

In a recent series analyzing almost 25 000 cases of nosocomial BSI, *A. baumannii* was the tenth most common pathogen, accounting for 1.3% of all monomicrobial BSIs (0.6 BSIs per 10 000 admissions).[19] *Acinetobacter baumannii* BSI tends to occur late during hospitalization, a mean of 26 days after hospital admission.[19] Sources include intravascular catheters, pneumonia, urinary tract infection and wound infection. Crude mortality in patients with *A. baumannii* BSI ranged from 16.3% to 43.4% and was exceeded only by the crude mortality in patients with BSI due to *P. aeruginosa* and *Candida* spp. Even though a significantly higher mortality was seen in *A. baumannii* BSI compared to BSIs due to other Gram-negative pathogens and in patients with colonization or infection due to multidrug-resistant *A. baumannii*, none of the studies formally adjusted for severity of illness or comorbidities. *Acinetobacter* spp. other than *A. baumannii* – in particular *A. johnsonii*, *A. lwoffii* and *Acinetobacter* genomic species 3, as well as *A. haemolyticus*, *A. junii*, *A. radioresistens* and *Acinetobacter* genomic species 10, *A. ursingii* and *A. schindleri* – have been mainly associated with catheter-related BSIs.

Wound infection

Acinetobacter baumannii has been implicated in 2.1% of ICU-acquired skin/soft tissue infections, but has recently been isolated from up to 32.5% of wound infections in combat casualties sustained in Iraq or Afghanistan. Colonization and infection of burn wounds with *A. baumannii* has also been reported. Even though colonization is one of the major risk factors for BSI in these patients, the impact of *A. baumannii* infection on the outcome of burn patients or combat casualties remains to be determined.

Miscellaneous

Acinetobacter baumannii is responsible for 1.6% of ICU-acquired urinary tract infections. Most cases of pyelonephritis and other UTIs have been

associated with a urinary catheter or nephrolithiasis. Nosocomial, post-neurosurgical *A. baumannii* meningitis has increasingly been reported. Risk factors include neurosurgery and external ventricular drainage, and crude mortality rates up to 70% have been reported. Other infections include endocarditis (commonly associated with prosthetic valves), endophthalmitis or keratitis, as well as osteomyelitis and arthritis.

MANAGEMENT AND RESISTANCE

Antibiotic resistance

Prior to the 1970s it was possible to treat *Acinetobacter* infections with a range of antibiotics, including aminoglycosides, β-lactams and tetracyclines. However, since 1980 successive surveys have shown increasing resistance in clinical isolates of *Acinetobacter* spp. High proportions of strains are now resistant to most commonly used antibacterial drugs including aminopenicillins, ureidopenicillins, cephalosporins of the first (cephalothin) and second generation (cefamandole), cephamycins such as cefoxitin, chloramphenicol and tetracyclines, and resistance to all known antibiotics (i.e. pandrug resistance) has now emerged in *A. baumannii*. Therapy of serious *A. baumannii* infections should be based on the results of adequately performed antimicrobial susceptibility testing, and empiric therapy should consider recent institutional level susceptibility data.[2] To date, carbapenems remain the agents of choice for serious *A. baumannii* infections, but increasing resistance has been reported from several countries including Portugal, Spain, France and the USA. In the case of multi- or pandrug-resistant strains, combination therapy or the use of agents such as rolistin may be considered. A detailed review of the currently available antimicrobial agents and their potential use in the therapy of *A. baumannii* infections has recently been published.[19] Newer compounds with anti-Gram-negative activity such as tigecycline (with reported MIC_{90}s between 2 and 32 μg/ml)[44] or doripenem will most likely not have better activity against *A. baumannii* than the currently existing antimicrobials.

Burkholderia Species

NATURE AND TAXONOMY

Burkholderia spp. (as well as *Ralstonia* spp.) were transferred from rRNA group II of the former genus *Pseudomonas*. The genus *Burkholderia* currently consists of more than 35 species, most of which have been assigned species names. Some of the novel *Burkholderia* spp. have recently been reclassified as *Pandoraea* spp. All *Burkholderia* spp. are genetically related on the basis of DNA–DNA homologies, 16S rRNA sequences and phenotypic characters. They are ubiquitous organisms, being widespread in water, soil and plants, and are present in the human environment.

The *B. cepacia* complex currently harbors nine genovars:

- I: *B. cepacia*;
- II: *B. multivorans*, *B. gladioli*;
- III: genovar III, *B. cenocepacia*;
- IV: *B. stabilis*;
- V: *B. vietnamensis*;
- VI: genovar VI;
- VII: *B. ambifaria*;
- VIII: B. *anthina*; and
- IX: *B. pyrrocinia*

and represents the most frequently isolated clinical pathogen among the *Burkholderia* spp., followed by *B. mallei* and *B. pseudomallei*. It has recently been proposed that five other species be added to the novel species within the *B. cepacia* complex: *Burkholderia latens* sp. nov., *Burkholderia diffusa* sp. nov., *Burkholderia arboris* sp. nov., *Burkholderia seminalis* sp. nov. and *Burkholderia metallica* sp. nov.[16]

EPIDEMIOLOGY

Similar to *P. aeruginosa* or *Stenotrophomonas maltophilia*, *Burkholderia* spp. can be isolated from a variety of environmental sources. *Burkholderia cepacia* is an environmental organism that has no specific nutritional requirements and may survive for months in water, sinks, antiseptic solutions (chlorhexidine, quaternary ammoniums, povidone–iodine), pharmaceutical products, dialysis fluid and various injectable solutions. It may also survive on environmental surfaces[5] and has been found in nebulizers, ventilatory equipment and many other medical and dental devices (see Table 170.5).

Person-to-person transmission has been reported for strains of the *B. cepacia* complex, but so far there are no reports of person-to-person transmission of *B. mallei* and *B. gladioli*. Overall, about 3% of pediatric and 7% of adult CF patients are colonized or infected with strains of the *B. cepacia* complex, with a considerable geographic variation of species. *Ralstonia pickettii* (*B. pickettii*) and *B. gladioli* are ubiquitous organisms that can be found in water and soil and may play a role as nosocomial pathogens. Rare outbreaks of infection have been described and emergence of multidrug resistance is a potential problem.[5] *Burkholderia mallei* and *B. pseudomallei* are predominantly found in Asia, Africa and South America. *Burkholderia pseudomallei*, the causative agent of melioidosis, can be isolated from environmental samples such as soil and water.

Transmission to humans usually occurs by percutaneous inoculation, ingestion or inhalation from the environment; however, person-to-person transmission, zoonotic disease and laboratory-acquired infections have been reported. While cases of human melioidosis have been detected worldwide, the highest incidence has been reported from north-east Thailand and the Northern Territory of Australia where *B. pseudomallei* accounts for up to 36% of cases of fatal community-acquired pneumonia and is the most common cause of this infection. *Burkholderia mallei* is the causative agent of glanders, a disease primarily affecting horses and donkeys. In contrast to *B. pseudomallei*, *B. mallei* does not survive in the environment, although laboratory infections have been reported.[45]

DIAGNOSTIC MICROBIOLOGY

Identification tests for *Burkholderia* spp. are summarized in Table 170.10. While commercial test systems such as API 20NE, Phoenix, MicroScan and Vitek 2 can identify bacteria of the *B. cepacia* complex, misidentification is frequent and differentiation of species within the complex, as well as differentiation from other nonfermenting Gram-negative rods such as *Achromobacter* spp. or *Ralstonia* spp., may require additional testing. Of note, *B. gladioli* is often misidentified as *B. cepacia* complex by commercial test systems. *Burkholderia pseudomallei* may be falsely identified as *B. cepacia*, *B. mallei*, *P. stutzeri* or other *Pseudomonas* spp. *Burkholderia pseudomallei* and *B. mallei* cannot be distinguished by morphology or serologic tests, and molecular methods such as 16S rRNA gene sequencing may be required for species identification.

PATHOGENICITY AND PATHOGENESIS

Although only weakly virulent with a limited invasive capacity in the normal host, *B. cepacia* has become an important nosocomial pathogen. *Burkholderia cepacia* has been isolated from a variety of nosocomial infections including BSI, urinary tract infection, arthritis, peritonitis, endophthalmitis and pneumonia,[20] most commonly in patients with impaired host defenses such as CF patients or patients in the ICU. The predominant site of infection is the respiratory tract (Table 170.11).

Table 170.10 Diagnostic microbiology of *Burkholderia* species

Characteristics	*Burkholderia cepacia*	*Burkholderia mallei*	*Burkholderia pseudomallei*
Call length (μm)	1.6–3.2	1.4–4.0	1.5
Flagella number	>1	0	>1
Pigment (diffusible)			
Oxidase			
Poly-β-hydroxybutyrate accummulation			
Gelatin	V		
Lipase (Tween 80 hydrolysis)		V	
Denitrification			
Arginine dihydrolase			
Growth at 105.8°F	V		
Guanine + cytosine content in DNA (%)	67.4	69.0	69.5

Main characteristics differentiating species.[1,11] Red indicates a positive result, white a negative result. V, variable.

Table 170.11 Infections due to *Acinetobacter baumanii* and *Pseudomonas aeruginosa*

Infection		Associated factors
Respiratory tract infections	*A. baumannii* pneumonia, nosocomial, 60% mortality with BSI	Mechanical ventilation Endotracheal or tracheostomy tube Neurologic disease
	P. aeruginosa pneumonia (30–60% mortality rate)	Nasogastric tube Prolonged stay in intensive care unit Broad-spectrum antibiotics
	P. aeruginosa pneumonia, nosocomial, with BSI (80–100% mortality rate)	Neutropenia Underlying malignant neoplasm Cytotoxic chemotherapy Chronic bronchiectasis (terminal state) Diabetes mellitus Severe immunodepression Severe burns
	A. baumannii pneumonia (community acquired, 60% mortality in bacteremic pneumonia)	Chronic obstructive pulmonary disease Smoking Alcoholism
	P. aeruginosa respiratory tract infection in people with cystic fibrosis (ultimately fatal unless a pulmonary transplant is carried out)	Presence of the lethal genetic disease cystic fibrosis Chronic colonization with *P. aeruginosa* Progressive lung deterioration Altered immune response to *P. aeruginosa*
Bloodstream infection (BSI)	*A. baumannii*	Prolonged stay in intensive care unit, mechanical ventilation, underlying immunosuppression, intravenous devices Leukemia, lymphoma Colonization with *A. baumannii* Various endoscopic instrumentation procedures
	P. aeruginosa	Prolonged stay in intensive care unit, broad-spectrum antibiotics, invasive procedures, underlying immunosuppression, intravenous devices Leukemia, lymphoma Mechanical ventilation, Intravenous drug use Trauma Prematurity Ulceration of the gastrointestinal tract Solid organ or bone marrow transplant Various endoscopic instrumentation procedures

Table 170.11 Infections due to *Acinetobacter baumanii* and *Pseudomonas aeruginosa*—cont'd

Infection		Associated factors
Skin and soft tissue infections	*P. aeruginosa*	Burn wound sepsis Wound infection Ecthyma gangrenosum Invasive procedures, surgery Dermatitis, pyoderma
Urinary tract infections	*A. baumannii*	Invasive procedures, urinary catheters
	P. aeruginosa	Acute (rare) Chronic (obstruction)
Endocarditis	*P. aeruginosa*	Intravenous drug use Prosthetic heart valves
Miscellaneous	*A. baumannii*	Meningitis (secondary)
	P. aeruginosa	Brain abscesses, meningitis (secondary, following neurosurgical procedures) Bone and joint infections (chronic osteomyelitis) Ear infections (otitis externa, malignant external otitis) Eye infections (keratitis, endophthalmitis, contact lens keratitis)

Virulence factors of *B. cepacia* include exoproducts (proteases, lipases, exopolysaccharides) that act in addition to the LPS forming part of O-antigen and which are responsible for severe pneumonia and sepsis in CF patients. Cellular virulence factors have recently been reviewed in detail.[46]

Attachment to epithelial cells is mediated by pili, followed by penetration, biofilm formation and invasion, which is in part aided by flagella and LPS. In addition, intracellular survival of clinical *B. cepacia* has been demonstrated. As in *Pseudomonas* spp., quorum-sensing system(s) may be responsible for the regulation of virulence factors in *B. cepacia*.

In *B. pseudomallei*, host-related factors such as diabetes, chronic renal disease or alcoholism seem to play a major role in the acquisition and clinical course of disease. Production of LPS and the ability of *B. pseudomallei* to survive intracellularly have been identified as important factors in the pathogenesis of melioidosis. The latter is conferred by the production of a capsular polysaccharide and the ability to form small colony variants or mucoid variants.

CLINICAL MANIFESTATIONS

While several *Burkholderia* spp. such as *B. gladioli* or *B. thailandiensis* have been isolated only occasionally from clinical specimens, the *B. cepacia* complex (including *B. cepacia*, *B. multivorans*, *B. cenocepacia*), *B. mallei* and *B. pseudomallei* are classified as human pathogens. *Burkholderia gladioli* has been reported to cause disease in patients with CF or chronic granulomatous disease and other immunocompromising conditions.

Respiratory tract infection and pneumonia are the most frequent manifestations of infection due to bacteria of the *B. cepacia* complex, most commonly *B. cepacia*. In patients with CF, increased mortality and a higher rate of fatal complications following lung transplantation has been associated with *B. cepacia* colonization. While chronic colonization of the respiratory tract with little or no clinical symptoms is frequent, cases of fulminant necrotizing pneumonia and sepsis with a rapidly fatal outcome have been reported. In addition, *B. cepacia* has been associated with catheter-related BSI, ventilator-associated pneumonia, skin and soft tissue infections following burns, surgery or invasive diagnostic procedures.

Although *Burkholderia mallei* is normally found in infections of horses and *B. pseudomallei* is present in soil and water, these organisms can also cause glanders and melioidosis in humans. Clinical manifestations of melioidosis (*B. pseudomallei*) in humans include pneumonia, genitourinary manifestations, osteomyelitis and skin or soft tissue abscesses, commonly associated with BSI and sepsis (see Chapter 119). Clinical presentation of BSI may range from transient bacteremia to fulminant septic shock with mortality rates of up to 87%.

MANAGEMENT AND RESISTANCE

In-vitro efficacy against *B. cepacia* has been demonstrated for ureidopenicillins, third-generation cephalosporins, carbapenems, fluoroquinolones, trimethoprim–sulfamethoxazole, chloramphenicol and minocycline.[47] Among the novel antimicrobials, tigecycline has shown less activity compared to minocycline. However, resistance to several classes of antimicrobial agents is often observed, especially in patients receiving multiple courses of antibiotics over a prolonged period of time, such as CF patients. Therapy of *B. cepacia* infections should be based on the results of antimicrobial susceptibility testing. In cases of multi- or pandrug-resistant strains, combination therapy is recommended and the use of agents such as polymyxins may be considered. In addition, inhalation therapy in combination with intravenously administered antimicrobials can control pulmonary exacerbation by *B. cepacia* infection.[48] Management of *B. mallei* and *B. pseudomallei* infections are discussed elsewhere in this book (see Chapter 119).

Stenotrophomonas maltophilia

NATURE AND TAXONOMY

Stenotrophomonas maltophilia is an opportunistic pathogen that was transferred from the genus *Pseudomonas* via the *Xanthomonas* group to the newly defined genus *Stenotrophomonas*. *Stenotrophomonas maltophilia* is a ubiquitous environmental bacterium that has also emerged as an important nosocomial pathogen contributing substantially to morbidity and mortality of immunocompromised patients, particularly in the ICU setting.[49]

EPIDEMIOLOGY

Stenotrophomonas maltophilia can be readily isolated from soil, plants, water and raw milk, and can also be recovered from the hospital environment where it has been isolated from ventilatory equipment and from respirometers, as well as from dialysis fluids and antiseptic solutions (see Table 170.5). There is a high incidence of infections due to *S. maltophilia* in immunocompromised patients such as those with solid malignancies, leukemia or lymphoma.[49] In addition, *S. maltophilia* is increasingly implicated in pulmonary infections in CF patients (see Table 170.4). Recent surveys report a point prevalence of 11% of CF patients with transient colonization with *S. maltophilia*, thus affecting approximately 30% of these patients during a 6-month period, even though the importance of *S. maltophilia* in patients with CF remains to be determined.

DIAGNOSTIC MICROBIOLOGY

Stenotrophomonas maltophilia grows readily on most routinely used media. These bacteria are characterized by the presence of a single or a small number of polar flagella (motile bacteria), frequently pigmented colonies (yellow or yellowish orange) and a negative oxidase reaction. *Stenotrophomonas maltophilia* acidifies sugars (except for rhamnose and mannitol) and is generally proteolytic.

PATHOGENICITY AND PATHOGENESIS

Stenotrophomonas maltophilia produces proteolytic enzymes and other pathogenic extracellular enzymes such as DNAse, RNAse, elastase, lipase, hyaluronidase, mucinase and hemolysin, which may contribute to the severity of *S. maltophilia* infection in immunocompromised patients.

CLINICAL MANIFESTATIONS

Stenotrophomonas maltophilia, particularly in immunocompromised patients, has been implicated as the causative pathogen in respiratory tract infections, endocarditis, bacteremia, meningitis and urinary tract infections. In addition, it is also known to cause severe cutaneous infections (ecthyma gangrenosum similar to that due to *P. aeruginosa*), cellulitis and abscesses, and has been identified as one of the most common causes of infection in wounds resulting from agricultural machinery.

MANAGEMENT AND RESISTANCE

Stenotrophomonas maltophilia displays intrinsic resistance to most classes of antimicrobial agents as a result of chromosomally mediated mechanisms, poor permeability of the outer membrane and naturally produced inactivating enzymes. Mechanisms of resistance include production of several β-lactamases, rendering it susceptible only to latamoxef and combinations of ticarcillin plus clavulanic acid or piperacillin plus tazobactam, as well as carbapenemase production conferring resistance to carbapenems.[50] Few strains are susceptible to gentamicin, neomycin and kanamycin, and susceptibility to doxycycline is reported in less than 50% of strains. Resistance to tetracyclines is associated with alteration of outer membrane proteins.

Currently, trimethoprim–sulfamethoxazole remains the drug of choice for treatment of infections due to *S. maltophilia* although *in-vitro* studies indicate that ticarcillin–clavulanic acid, minocycline, some of the new fluoroquinolones and tigecycline may be useful alternative agents in case of resistance or allergy.[50] Due to increasing rates of resistance, therapy should always be guided by susceptibility testing. Of note, susceptibility testing results may not correctly predict clinical treatment response. In addition, novel fluoroquinolones such as moxifloxacin have displayed reasonable *in-vitro* activity. Severe infections may require combination therapy including ticarcillin plus clavulanic acid or piperacillin plus tazobactam, quinolones, and trimethoprim–sulfamethoxazole.

Miscellaneous Aerobic Nonfermenting Gram-Negative Bacteria

Many other nonfermenting Gram-negative bacilli have been identified from clinical specimens. Some are increasingly involved in human infection.[3,5,51] These genera and species have undergone many taxonomic changes; some have been identified recently and the wide use of analysis of ribosomal 16S RNA gene sequences has allowed a clearer taxonomic position to be established for most of these organisms. The following section includes a short description of the pathogenic role of nonfermenting Gram-negative bacteria involved in human infections and of the management of these infections. For easy reading, the generic groups are described in alphabetical order.

ACHROMOBACTER SPP.

See *Alcaligenes* spp. and *Ochrobacterium* spp.

AGROBACTERIUM SPP.

See *Rhizobium* spp.

ALCALIGENES SPP. (INCLUDING *ACHROMOBACTER* SPP.)

The genera *Alcaligenes*, *Achromobacter* and *Ochrobacterium* have undergone confusing taxonomic changes in the past decade (see Table 170.1). Currently *Alcaligenes faecalis* remains the only *Alcaligenes* spp. of clinical importance, after several other *Alcaligenes* spp. have been transferred to the genus *Achromobacter*, including *A. denitrificans*, *A. xylosoxidans*, *A. ruhlandii* and *A. piechaudii*.

Epidemiology

The natural habitat of *Alcaligenes* spp. is the same as that of *Pseudomonas* spp. In the hospital environment, *A. faecalis* and *A. xylosoxidans* have been isolated from various environmental sources such as respirators, hemodialysis systems, intravenous solutions and disinfectants.[1]

Diagnostic microbiology

Achromobacter spp. and *Alcaligines* spp. are Gram-negative nonfermenting rods, strictly aerobic and motile, with one to eight peritrichous flagella, usually described as degenerated. They are oxidase-positive, catalase-positive and indole-negative. In contrast to *A. xylosoxidans*, the species *A. faecalis*, *A. piechaudii* and *A. denitrificans* are not saccharolytic. Not all *Alcaligenes* spp. or *Achromobacter* spp. possess specific physiologic or biochemical characteristics allowing a biochemical identification to species level.

Pathogenesis and clinical manifestations

Alcaligenes spp. and *Achromobacter* spp. have been isolated from various clinical sources such as blood, feces, sputum, urine, cerebrospinal fluid, wounds, burns and swabs taken from throat, eyes and ear discharges. *Alcaligenes* strains do not seem to possess any specific virulence determinants. They are infrequent causes of hospital-acquired infections in patients who often have severe underlying disease. Rare cases of peritonitis, pneumonia, bacteremia, meningitis and urinary tract infections have been reported in the literature.[52] In many instances the organism is considered to be a colonizer.[52] Nosocomial outbreaks of infection are usually associated with an aqueous source of contamination. *Alcaligenes* spp. are predominantly isolated from respiratory tract specimens and recovery of these organisms from the sputum of CF patients has been associated with an exacerbation of pulmonary symptoms.

Alcaligenes xylosoxidans has been implicated in BSI (mostly catheter related), pneumonia, endocarditis, meningitis, osteomyelitis, peritonitis and urinary tract infection, often in patients with underlying malignancy, HIV and CF.

Management

As with other nonfermenting Gram-negative bacteria, available susceptibility data for *Alcaligenes* spp. and *Achromobacter* spp. are based on a limited number of isolates, and antimicrobial therapy should be guided by appropriate susceptibility testing. *Alcaligenes faecalis* is generally resistant to aminoglycosides, chloramphenicol and tetracyclines and usually susceptible to trimethoprim–sulfamethoxazole and β-lactam antibiotics such as ureidopenicillins, ticarcillin–clavulanic acid, cephalosporins and carbapenems. *Alcaligenes xylosoxidans* has been shown to be usually susceptible to ureidopenicillins, imipenem and polymyxins and variably resistant to fluoroquinolones. In contrast to *A. faecalis*, *Achromobacter* spp. are often resistant to cephalosporins. There have been several reports of resistance to broad-spectrum penicillins in *A. xylosoxidans* due to constitutive β-lactamase production; three different types of cephalosporinase and the presence of other β-lactamases have been demonstrated.

BERGEYELLA SPP.

See *Weeksella* spp.

CHROMOBACTERIUM SPP.

The genus *Chromobacterium* contains only one species, *C. violaceum*. On agar plates these bacteria usually form violet colonies (violacein). The bacterium has some fermentative activities on sugars and is proteolytic. Nonpigmented strains may be misidentified as *Aeromonas* spp.

Found in soil and water in tropical climates, *C. violaceum* has been implicated in skin, soft tissue and bloodstream infections, mainly in patients with neutrophil dysfunction.[53] In immunocompromised patients, bacteremia may be associated with a high mortality.[53] Empiric treatment can be attempted using imipenem, fluoroquinolones, gentamicin, tetracycline or trimethoprim–sulfamethoxazole.

CHRYSEOBACTERIUM SPP., *ELIZABETHKINGIA* SPP., *FLAVOBACTERIUM* SPP. AND *MYROIDES* SPP.

The clinically important *Chryseobacterium* spp. (*C. meningosepticum* and *C. indologenes*) have been reclassified from the genus *Flavobacterium* (see Table 170.1), while other *Flavobacterium* spp. such as *F. multivorum* and *F. spiritivorum* have been moved to the genus *Sphingobacterium*. In addition, *F. odoratum* has been reclassified as *Myroides odoratus* and *M. odoratimimus*.

Chryseobacterium meningosepticum has recently been reclassified as *Elizabethkingia meningoseptica*.[54] *Chryseobacterium* spp. and *Elizabethkingia* spp. are ubiquitous organisms that can be found not only in soil and water but have also been recovered from foods and from the hospital environment. Epidemiologic studies have traced the bacterial source to contaminated water, ice machines and humidifiers. Phenotypic markers used for the delineation of outbreaks of *E. meningoseptica* infections were serology based on the O-antigenic type; nine O-serovars have been identified (A–H and K).

Microbiology

Chryseobacterium spp. usually grow between 41 °F and 86 °F (5–30 °C), but strains isolated from human specimens including *E. meningoseptica* readily grow at 99 °F (37 °C). On nutrient agar they produce colonies 1–2 mm in diameter, which are frequently pigmented light yellow or yellowish orange (nondiffusible pigment, Fig. 170.8). The metabolism is strictly aerobic and sugars are metabolized by the oxidative pathway, except for *Myroides odoratus* (*C. odoratum*) and *Sphingobacterium multivorum* (*F. multivorum*) which do not acidify glucose. Indole-positive species (i.e. *E. meningoseptica*, *C. gleum*) are usually strongly proteolytic; esculin, citrate and urease tests are variably positive.

Clinical manifestations

Elizabethkingia meningoseptica and *C. indologenes* have been isolated from patients with sepsis, osteomyelitis, meningitis and endocarditis.[55] Meningitis due to *E. meningoseptica* has often been observed in neonates but has been reported infrequently in immunocompromised adult patients. Bloodstream infections due to *E. meningoseptica* and *C. indologenes* have been associated with intravascular catheters or contaminated infusates and often present as benign transient bacteremia. In otherwise healthy individuals *E. meningiseptica* has also been implicated in cellulitis, arthritis and community-acquired pneumonia.[55]

Management

Elizabethkingia meningoseptica and *Chryseobacterium* spp. are intrinsically resistant to many commonly used antimicrobial agents including aminoglycosides, third-generation cephalosporins, penicillins (mezlocillin, piperacillin, ticarcillin), aztreonam, imipenem and tetracycline.



Fig. 170.8 Colonies of *Flavobacterium–Chryseobacterium* group grown on Mueller–Hinton agar. Courtesy of Professor H Monteil.

However, most of these species, including *E. meningoseptica*, are generally susceptible to agents that are usually active against Gram-positive bacteria such as rifampin (rifampicin), clindamycin, erythromycin and vancomycin. Cases of neonatal sepsis have been treated with clindamycin combined with piperacillin. Recent studies have reported the highest *in-vitro* activities in minocycline, rifampin, trimethoprim–sulfamethoxazole and levofloxacin. *Chryseobacterium indologenes* is uniformly resistant to aztreonam, third-generation cephalosporins, aminoglycosides, erythromycin, clindamycin, vancomycin and teicoplanin. Therapy should be guided by antimicrobial susceptibility testing using MICs, since disk-diffusion results are unreliable in predicting susceptibility of *Chryseobacterium* spp. Interpretation of MICs, however, is complicated by the lack of Clinical and Laboratory Standards Institute (CLSI) clinical breakpoints.[51]

COMAMONAS SPP., *DELFTIA* SPP. AND *ACIDOVORAX* SPP

Previously designated as *Pseudomonas* rRNA homology group III, the family Comamonadaceae now includes the genera *Comamonas*, *Delftia* and *Acidovorax*. The genus *Comamonas* consists of four named species – *C. aquatica*, *C. kerstersii*, *C. terrigena* and *C. testosteroni* – that have been isolated from human specimens, as well as several other species that so far have been recovered from environmental samples only. The genus *Delftia* consists of *D. acidovorans*, formerly designated *Comamonas acidovorans*. Three clinically relevant species – *Acidovorax facilis*, *A. delafieldii* and *A. temperans* – currently belong to the genus *Acidovorax* in addition to several environmental species that have recently been identified or reclassified from the genus *Pseudomonas*.

Members of these genera are aerobic, Gram-negative, oxidase-positive rods that are commonly found in soil, water and on plants, but are seldom implicated in human infections. Rare cases of catheter-related bacteremia (*C. testosteroni*, *D. acidovorans*, *Acidovorax* spp.), meningitis (*C. testosteroni*), endocarditis (*C. testosteroni*, *D. acidovorans*), conjunctivitis (*C. testosteroni*) and otitis media (*D. acidovorans*) have been reported.[56]

OCHROBACTRUM SPP.

Derived from the genus *Achromobacter*, two species have been recognized as clinical pathogens: *Ochrobactrum anthropi* and *O. intermedium*. These nonfastidious bacteria grow readily on most conventional media and can be identified to genus level by classic biochemical tests; however, no biochemical reaction can separate the two species.

Ochrobactrum spp. are environmental organisms and are considered opportunistic pathogens of low virulence in humans. *Ochrobactrum anthropi* has been associated with catheter-related bloodstream infections, and individual cases of meningitis, endocarditis and other infections have been published. In contrast, *O. intermedium* has only been implicated in one case of pyogenic liver infection; however, due to the biochemical indistinguishability, these data should be interpreted with caution.

Ochrobactrum spp. are usually resistant to most β-lactam antibiotics except carbapenems. Aminoglycosides (except tobramycin in *O. intermedium*), fluoroquinolones, tetracycline and trimethoprim–sulfamethoxazole are usually active. In addition, *O. intermedium* is resistant to polymyxins.[51]

OLIGELLA SPP.

This genus was created in 1987 and includes *Oligella urethralis* (derived from *Moraxella urethralis* and CDC group M-4) and *Oligella ureolytica* (derived from CDC group IVe). These small rods, often occurring in pairs, grow slowly on blood agar and exhibit only limited metabolic activity. They are oxidase- and catalase-positive. *O. ureolytica* is motile, while *O. urethralis* is not. Both species have been implicated in bacteremia, arthritis and genitourinary infections including urosepsis, although the causative role could not be established in all cases.[57]

Both species are usually susceptible to most antimicrobial agents, with *O. ureolytica* being the more resistant species. Therapy should be guided by the results of antimicrobial susceptibility testing.

RALSTONIA SPP.

Ralstonia spp. were reclassified from *Pseudomonas* rRNA group II and originally consisted of *R. pickettii* (formerly *Burkholderia pickettii* or *Pseudomonas pickettii*) and *R. eutropha* (formerly *Alcaligenes eutrophus*). More recently, *R. eutropha* has been reclassified as *Cupriavidus necator*, after intermittently being named *Wautersia eutropha*.[58] Both are emerging pathogens isolated from infections in immunocompromised patients or from respiratory tract infections in CF patients.[59]

RHIZOBIUM SPP.

The genus *Rhizobium* currently consists of four species – *R. radiobacter*, *R. rhizogenes*, *R. rubi* and *R. vitis* – all of which were transferred from the genus *Agrobacterium* (see Table 170.1). *Rhizobium* spp. are phytopathogenic organisms, present in water, soil and environmental plants; they are strictly aerobic coccobacilli, motile with peritrichous flagella (one to six). They grow easily on conventional media, produce oxidase and catalase, and can be identified by most commercially available systems.

Thus far, only *R. radiobacter* has been implicated in infections in humans, mostly device related. Individual cases of endocarditis, catheter-related BSI, peritonitis and urinary tract infections have been published.[60]

Rhizobium spp. are generally susceptible to cephalosporins (second- and third-generation), ticarcillin, imipenem, tetracyclines, colistin, trimethoprim–sulfamethoxazole and fluoroquinolones. In device-related infections, removal of the device may be necessary.[60]

SHEWANELLA SPP.

Shewanella putrefaciens, formerly designated CDC group Ib, *Alteromonas*, *Pseudomonas* and *Achromobacter putrefaciens*, currently belongs to the genus *Shewanella*, which also includes *Shewanella algae*. *Shewanella* spp. grow in media used for Enterobacteriaceae and produce H_2S, which may result in misidentification as *Salmonella* spp. or *Proteus* spp., even though *Shewanella* spp. are nonfermenting. *Shewanella putrefaciens* is present in the environment and has occasionally been isolated from meningitis, otitis media, keratitis, intra-abdominal infections and bacteremia, most cases occurring in immunocompromised patients.[61] In contrast, *Shewanella algae* accounts for the majority of clinical isolates and has been associated with a broad range of diseases, including BSI, peritonitis, osteomyelitis, skin and soft tissue infections, and otitis media.[61]

Shewanella spp. are generally resistant to penicillins but susceptible to third-generation cephalosporins, imipenem, ciprofloxacin, aminoglycosides, trimethoprim–sulfamethoxazole and tetracyclines.[61]

SPHINGOBACTERIUM SPP.

Two species of the genus *Sphingobacterium*, *S. multivorum* and *S. spiritivorum*, are derived from several *Flavobacterium* spp. and CDC groups IIk-2 and -3 (see Table 170.1). In addition, the genus also harbors *S. antarcticum*, *S. faecium*, *S. thalpophilum* and *Sphingobacterium* genospecies 1 and 2. *Sphingobacterium mizutaii* has been transferred to the genus *Flavobacterium*. Other species formerly included in the genus,

S. heparinum and *S. piscium*, have been reclassified as *Pedobacter* spp., none of which has been implicated in clinical manifestations in humans. *Sphingobacterium* spp. are characterized by colonies that develop a yellow pigment after a few days at room temperature. *Sphingobacterium multivorum*, *S. spiritivorum* and *S. thalpophilum* have been isolated from a variety of infections including bloodstream infections, peritonitis, wound infections, urinary tract infections and abscesses.[62]

Sphingobacterium spp. *in vitro* are usually resistant to aminoglycosides and polymyxin B and susceptible to fluoroquinolones and trimethoprim–sulfamethoxazole.

WEEKSELLA SPP. AND *BERGEYELLA* SPP.

Weeksella virosa (see Table 170.1) and *Bergeyella zoohelcum* have both been implicated in infections in humans. While *W. virosa* has been recovered from urine and vaginal swabs, *B. zoohelcum* has been implicated in wound infections following animal bites but individual cases of BSI and meningitis have also been reported.[63] Both grow as pigmented colonies (brown or yellow) and can be distinguished by urease (positive in *B. zoohelcum*) and susceptibility to polymyxin B (*B. zoohelcum* is resistant). Both organisms are usually susceptible to most antimicrobial agents, but susceptibility testing is nevertheless warranted in all cases.

REFERENCES

References for this chapter can be found online at http://www.expertconsult.com

Chapter 171

Francis Mégraud
Philippe Lehours
Steven FT Thijsen

Curved and spiral bacilli

INTRODUCTION

The curved and spiral bacilli are a heterogeneous group of bacteria that share little morphology.

Helicobacter pylori was previously called *Campylobacter pylori*[1] and in the first part of the 20th century *Campylobacter* was called *Vibrio*. *Campylobacter*, *Helicobacter* and *Vibrio* spp. all produce infections of the gastrointestinal tract. Moreover, they are all Gram-negative organisms and have a similar, curved shape. Using nucleic acid sequence determination of 16S rRNA, the genera *Campylobacter* and *Helicobacter* have been classified (together with *Arcobacter*, *Sulfurospirillum* and *Wolinella*) as members of the superfamily VI of Gram-negative bacilli now called Epsilonproteobacteria (Table 171.1).[2] Only Epsilonproteobacteria that are involved in human infections are discussed in this chapter.

Treponema, *Borrelia* and *Leptospira* are all members of Spirochaetales. These spirochetes are thin, helical, Gram-negative bacteria.

Campylobacter spp., *Helicobacter pylori* and *Vibrio cholerae*

CAMPYLOBACTER SPP.

Nature

Campylobacter spp. are microaerophilic, Gram-negative, curved rods which obtain their energy by using fatty acids and amino acids rather than carbohydrates, and are adapted to life in mucus of the digestive tract. With the genera *Arcobacter* and *Sulfurospirillum*, they form the family Campylobacteraceae. At least 16 species and six subspecies have been differentiated.[2] Not all, however, cause disease in humans.[3] *Campylobacter jejuni* and *Campylobacter coli* are responsible for enteric infections and are the most common Campylobacters found in humans. *Campylobacter fetus* is the third most frequently isolated, but is mostly involved in systemic diseases. The other species (e.g. *Campylobacter lari*, *Campylobacter upsaliensis*) occur only anecdotally. They also lead to enteric infections. Clinical aspects of *Campylobacter* diarrhea are discussed in more detail in Chapter 35.

Epidemiology

Campylobacter spp. infections can be considered as zoonoses, because the primary reservoir for *Campylobacter* spp. is animals. These bacteria are essentially present in the digestive tract of animals, especially birds, where they do not cause disease. Only *C. fetus* spp. *venerealis* causes septic abortion in cattle. Humans can become infected by ingesting contaminated food or water or through contact with infected animals, including pets. The majority of *Campylobacter* spp. infections are sporadic, although outbreaks mostly of limited size do occur. Large outbreaks involving water as a vehicle have also been described. *Campylobacter* enteritis is more common than *Salmonella* and *Shigella* enteritis and is a major cause of travelers' diarrhea. Infections can be caused by the ingestion of undercooked, contaminated poultry or contaminated milk as well as by cross-contamination of foods which will

Table 171.1 Epsilonproteobacteria of potential clinical interest[2]

FAMILY CAMPYLOBACTERACEAE		FAMILY HELICOBACTERACEAE
Genus Campylobacter	**Genus Arcobacter**	**Genus Helicobacter**
Campylobacter jejuni	*Arcobacter butzleri*	*Helicobacter pylori*
Campylobacter coli	*Arcobacter cryaerophilus*	*Helicobacter acinonychis*
Campylobacter concisus	*Arcobacter nitrofigilis*	*Helicobacter aurati*
Campylobacter curvus	*Arcobacter skirrowii*	*Helicobacter bilis*
Campylobacter fetus		*Helicobacter bizzozeronii*
Campylobacter gracilis		*Helicobacter canadensis*
Campylobacter helveticus		*Helicobacter canis*
Campylobacter hominis		*Helicobacter chrolecystus*
Campylobacter hyointestinalis		*Helicobacter cinaedi*
Campylobacter lanienae		*Helicobacter felis*
Campylobacter lari		*Helicobacter fennelliae*
Campylobacter mucosalis		*Helicobacter ganmani*
Campylobacter rectus		*Helicobacter hepaticus*
Campylobacter showae		*Helicobacter mesocricetorum*
Campylobacter sputorum		*Helicobacter muridarum*
Campylobacter upsaliensis		*Helicobacter mustelae*
Related species: *Bacteroides ureolyticus*		*Helicobacter nemestrinae*
		Helicobacter pametensis
		Helicobacter pullorum
		Helicobacter rodentium
		Helicobacter salomonis
		Helicobacter trogontum
		Helicobacter typhonius
		Candidatus H. bovis
		Candidatus H. suis
		Related species: *'H. winghamensis'* *'H. muricola'* CLO-3 *'Gastrospillum hominis'*

be consumed raw.[4] This is an important consideration since the simple preventive measure of cleaning cutting boards, cutlery and hands with soap and hot water may not be often applied in kitchens.[5] Eating undercooked beef was also identified as a risk factor in a case control study in France.[6] As for other enteric infections, taking acid suppressing drugs, especially proton pump inhibitors, is a risk factor for *Campylobacter* infection.[7]

It has been estimated that there are 1.4 million cases annually in the USA.[8] Transmission of the disease from human to human has also been described but seldom occurs. In temperate countries there is a peak incidence in summer and early autumn, although infections occur throughout the year.[9] The highest incidence is found in young children, with a second peak in young adulthood. Infants can also be infected but the risk factors appear to be different.[10] The incidence in developing countries, with less hygienic living conditions, is even higher than in developed countries and direct transmission from poultry to humans seems to occur.

Pathogenesis

Campylobacter bacilli are acid sensitive. Because of the relative barrier imposed by the gastric acid environment, infection is more likely to occur when large numbers of bacteria are ingested. Histologic examination of gut biopsies obtained from patients who have *Campylobacter* enteritis reveals inflammation and edema of the mucosa, with infiltration of neutrophils in the lamina propria. *Campylobacter* colonizes the small bowel but lesions are mainly restricted to the ileum and colon.

In vitro co-culture of epithelial cells with *C. jejuni* has shown that these bacteria can adhere and penetrate into the cells. *C. jejuni* can adhere to epithelial cells via a number of different adhesins: a surface exposed lipoprotein (JlpA) which binds to Hsp90α,[11] a fibronectin-binding protein (CadF),[12] an ABC binding protein (Peb1)[13] and a putative autotransporter (CapA).[14] *C. jejuni* is able to circumvent the induction of innate immunity, Toll-like receptors 5 and 9 not being efficiently stimulated. *C. jejuni* is also able to synthesize proteins which may play a role in internalization and cytoskeletal rearrangement.[15] Cia proteins (for *Campylobacter* invasion antigens) are secreted through the flagella filament upon contact with a eukaryotic cell and appear to contribute to the invasion.[16] *C. jejuni*'s translocation may occur by a transcellular as well as a paracellular route. The disruption of tight junctions of epithelial cells leads to proinflammatory cytokine response[17] and allows the bacterium to move to the basolateral side and reinvade epithelial cells or be taken up by macrophages.

During this process the transcription factors nuclear factor kappa B (NF-κB) and MAP kinases are activated,[18] contributing to both inflammatory diarrhea and clearance of the infection. In addition, *C. jejuni* produces a cytolethal distending toxin (CDT) acting on the cell cycle and leading to apoptosis.[19] Nevertheless, despite the numerous pathogenicity studies performed, the role of CDT in diarrheal disease caused by *C. jejuni* remains unclear.

The molecular mimicry of human ganglioside with the lipo-oligosaccharide (LOS) molecules present in strains of *C. jejuni* expressing the O:19 antigen has been implicated in the association of *C. jejuni* infection with the Guillain–Barré syndrome.[20]

Immune persons in endemic areas, where infections are frequent, can become asymptomatic carriers. Infection can have a protracted course in the case of immune deficiencies, such as in patients suffering from hypogammaglobulinemia. In AIDS patients, opportunistic infections with *C. jejuni* and atypical *Campylobacter* spp. also suggest a role for cellular immunity.

The presence of *Campylobacter*-specific secretory IgA and serum IgA in breast milk correlates with protection against diarrhea. IgG also plays an important role against disease.[21]

An interesting mechanism to avoid elimination by the immune system has been developed by *C. fetus*. Almost all *C. fetus* strains express a surface protein that abrogates complement C3b binding. This prevents opsonization, thereby conferring resistance to killing by phagocytes and adding to the pathogenicity of the species.[22]

In summary, pathogenesis remains poorly understood, in part because of the lack of an animal model that mimics human diarrheal disease, but also because of the genetic variability within the organism resulting from genomic reorganization and phase variation that has made it difficult to identify genomic correlates of virulence.[23]

Prevention

Preventive measures for *Campylobacter* spp. infections include adequate heating of contaminated food and reinforcement of hygiene in the kitchen in order to avoid cross-contamination as well as adequate disinfection of drinking water supplies. Eradication of the animal reservoir is extremely difficult and costly but adequate measures taken in poultry farms and abattoirs can at least decrease the level of contamination.

Development of vaccines is an alternative but no vaccine against campylobacteriosis is currently available.[24] However, several approaches are being followed – for example, the development of:

- live attenuated vaccines;
- vaccines based on heat-killed/formalin-killed bacteria with or without mucosal adjuvants;
- subunit vaccines delivered together with adjuvants; and
- live attenuated *Salmonella* strains expressing *Campylobacter* proteins.[21]

While the link between *Campylobacter* infection and Guillain–Barré syndrome may stimulate the development of a vaccine, whole-cell vaccines cannot be used until we know more about the determinants of this disease.

Diagnostic microbiology

The curved motile rods can be observed in a fecal sample using Gram staining or darkfield microscopy. Culturing *Campylobacter* spp. necessitates special conditions, sometimes difficult to implement.[25] Cultures are commonly performed with an atmosphere comprising 5% oxygen, 10% carbon dioxide and 85% nitrogen. Some species, such as *Campylobacter rectus* and *Campylobacter hyointestinalis*, also require hydrogen in the atmosphere for growth. Selective culture media contain antibiotics such as cefoperazone to suppress the growth of normal Gram-negative intestinal bacteria, vancomycin to suppress the growth of Gram-positive bacteria, and an antifungal compound, as well as blood or charcoal, to neutralize inhibiting factors such as oxygen-free radicals. Commonly used media are Skirrow's, Butzler's, Karmali's agar and cefoperazone charcoal deoxycholate agar. An important disadvantage of selective media is that some *Campylobacter* spp. such as *C. hyointestinalis*, *C. fetus* and *C. upsaliensis*, which are sensitive to antibiotics used in these selective media, can be missed. To circumvent this problem, membrane filtration of feces can be performed to eliminate contaminants followed by culturing on nonselective media. However, this filtration technique is less sensitive than direct plating.

Although the most important species (i.e. *C. jejuni* and *C. coli*) grow at 107.6°F (42°C), some species (e.g. *C. fetus*) grow best at 98.6°F (37°C) and will be missed when cultures are only incubated at 107.6°F (42°C). Typically, *C. jejuni* and *C. coli* colonies with a gray color and growing flat and confluently are visible after 2–3 days of culture. Definite identification of suspect colonies is performed using standard biochemical tests showing positive oxidase and catalase tests.

Campylobacter jejuni is the only *Campylobacter* spp. that is capable of hydrolyzing hippurate, which is essential for its differentiation from other Campylobacters, especially *C. coli*; however, some *C. jejuni* strains may appear hippurate negative. Growth at 77°F (25°C) is essential for diagnosing *C. fetus*. Given the high level of resistance of Campylobacters to quinolones, the nalidixic acid susceptibility test is no longer a key test in *Campylobacter* identification. Molecular identification – polymerase chain reaction (PCR), sequencing – is now being performed more frequently in this group of bacteria. A comparison of seven PCRs, mostly multiplex PCRs, to differentiate well-characterized strains showed a perfect sensitivity and specificity for *C. coli* but not for *C. jejuni*.[26] Real-time PCR has also been used successfully.[27]

Campylobacter spp. can also be detected using PCR directly on feces. The sensitivity is much higher than detection by culture.[28] However, the specificity cannot be proven given the lack of a reference method. Typing of isolates is important for epidemiologic studies. More than 60 serotypes of *C. jejuni* and *C. coli* have been identified with the Penner O typing system but molecular typing methods are now commonly used, including polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) of the *fla* gene, macrorestriction of the genomes, amplified fragment length polymorphism and multilocus sequence typing.[29] Serology can also be helpful in diagnosing a *Campylobacter* spp. infection because serum IgG and IgM levels start to rise in response to infection 5 days after infection and reach a peak 2–4 weeks later. This is a key investigation in the diagnosis of *C. jejuni*-associated Guillain–Barré syndrome.

Clinical features

Most *Campylobacter* spp. infections manifest as acute enteritis.[30] The ensuing diarrhea can vary from modest to voluminous stools that may be watery or bloody. The infection can also run a subclinical course, especially in hyperexposed populations. Disease will develop 1–3 days after ingestion of the bacilli and symptoms usually disappear after a week. Stool samples typically remain positive for *Campylobacter* spp. for several weeks. In most cases, *Campylobacter* enteritis is a self-limiting disease and it tends to be more severe in patients at the extremes of life. Fever, malaise and abdominal pain may precede diarrhea or may be the most predominant signs. Infection with *Campylobacter* spp. gives rise to inflammation of the gut mucosa. The accompanying pain and fever may also lead to symptoms resembling inflammatory bowel disease. When pain is the major feature of the infection, differentiation from appendicitis may be difficult. *Campylobacter jejuni* can grow in bile and can occasionally cause acute cholecystitis and pancreatitis.[31]

Only a few patients who have a *C. jejuni* infection develop systemic disease. Bacteremia can occur, but generally in patients who have an underlying disease.[32]

A postinfectious reactive arthritis may occur[33] which is very similar to the complication seen after enteritis caused by *Salmonella* spp., *Shigella* spp. or *Yersinia* spp. and appears to be associated with the presence of the HLA-B27 antigen. It also appears that *C. jejuni* is the major cause of postinfectious irritable bowel syndrome.[34]

C. jejuni has also been associated with a rare form of mucosa-associated lymphoid tissue (MALT) lymphoma called immunoproliferative small intestinal disease (IPSID).[35]

Another important complication of *Campylobacter* enteritis is the Guillain–Barré syndrome.[20] A variant, Miller Fisher syndrome, can also occur. *Campylobacter jejuni* enteritis is the infection most frequently observed before Guillain–Barré syndrome and occurs in 20–40% of cases. The risk of developing Guillain–Barré syndrome after *Campylobacter* spp. infection is estimated at 1 per 2000 infections. Major neurologic sequelae exist in 20% of the cases.

Campylobacter coli infections are very similar to infections with *C. jejuni*, but they tend to follow a less severe course.

Infections with *C. fetus* tend to disseminate from the intestine, especially in patients who have conditions that cause impaired immunity, such as chronic alcoholism, diabetes mellitus, malignancies and HIV infection, and in the elderly. Systemic *C. fetus* infections can lead to endocarditis, thrombophlebitis, mycotic aneurysm, meningitis, bone and joint infections and septic abortion.

Campylobacter upsaliensis, *C. lari* and *C. hyointestinalis* can also cause enteritis. *Campylobacter consisus*, *C. gracilis*, *C. curvus*, *C. mucosalis*, *C. rectus*, *C. showae*, *C. sputorum* and *Bacteroides ureolyticus* can be associated with periodontal infections.

HELICOBACTER PYLORI

Helicobacter pylori was cultured for the first time in 1982, from the stomach which was previously thought to be sterile.[36] Currently this bacterium is considered to be the most important bacterium responsible for chronic infections. It is also the first bacterium known to be involved in a cancer in humans. Clinical aspects of *Helicobacter* infection are discussed in more detail in Chapter 33.

Nature

Helicobacter spp. are spiral-shaped, flagellate Gram-negative bacilli. They are microaerophilic and use amino acids and fatty acids rather than carbohydrates to obtain their energy. At present, about 23 species of *Helicobacter* and two candidate species have been identified, only eight of which cause disease in humans (see Table 171.1).[2] *Helicobacter pylori* is the third bacterium for which the entire genome has been sequenced[37] and the first for which the genome of two strains has been sequenced.[38]

Diagnostic microbiology

Invasive methods for diagnosing *H. pylori* infection depend on endoscopy to obtain biopsies. These biopsies can then be processed for histologic examination and stained with hematoxylin–eosin, Giemsa stain or silver stain. *H. pylori* is usually abundant and its typical morphology and the presence of polymorphs make diagnosis easy. However, previous treatment with proton pump inhibitors may render histologic diagnosis more difficult. The use of immunoperoxidase staining can be considered when atypical bacilli are detected. The examination of a smear can be performed in the endoscopic ward using either darkfield examination of a wet smear or Gram staining.

Because *H. pylori* is a fragile organism, transport conditions are extremely important for culture. Biopsies can be transported in saline for 3–4 hours, but (commercially) available transport medium must be used if preservation for up to 24 hours is necessary. The biopsies must be maintained at a low temperature (39°F/4°C) before being ground with a homogenizer and plated on media enriched with 5–10% blood and supplemented with antibiotics to inhibit growth of contaminant bacteria. The plates must then be incubated for up to 12 days in a microaerobic atmosphere. Growth usually occurs within 3–4 days. *Helicobacter pylori* colonies are easily identified by their morphology and their urease, catalase and oxidase activities. These tests are sufficient when the bacteria are grown from the stomach; molecular tests must be added when they are grown from other specimens.

The urease test is specific for *H. pylori*. A color change is observed if *H. pylori* urease is present when the biopsy is introduced into a medium containing urea and a pH indicator. Tests using semisolid agars show an optimal sensitivity after 24 hours. In contrast, strip tests show a high sensitivity after just 2 hours.

Polymerase chain reaction for diagnosing *H. pylori* infection does not require specific transport conditions and can be performed with a urease test kit sent by mail. Several genes can be targeted: urease, 16S rRNA, 23S rRNA, 29 kDa antigen, *cag*A gene or *vac*A gene. A real-time PCR has been developed which allows detection of both *H. pylori* and its resistance to macrolides. Primers target the 23S rRNA gene and the dissociation curve of the amplicons indicates the mutation present.[39]

Less invasive methods for obtaining material are aspiration of gastric juice or a capsulated string. Diagnosis can also be made using noninvasive tests. The urea breath test measures urease production by *H. pylori*. Samples of breath air are collected by having the patient blow into a tube before and 30 minutes after ingestion of ^{13}C-labeled urea. The tubes can be maintained for months and sent by mail to a laboratory that has a mass spectrometer in order to measure the ^{13}C:^{12}C ratio.

Another method is based on the detection of *H. pylori* antigens in stools using enzyme-linked immunosorbent assay (ELISA). Polyclonal antibodies were first used as a reagent. A second generation of tests employs monoclonal antibodies and gives excellent results.

Antibodies are mainly detected by ELISA. There are numerous kits commercially available with different sensitivities and specificities. Immunoblot methods can also be used. Detection of specific antibodies has also been proposed but shows a lower sensitivity than in blood.

Rapid one-step immunoassays have been developed for detection either of *H. pylori* antigens in stools or *H. pylori*-specific antibodies in blood or urine, but they do not reach a sufficient level of sensitivity.

Since none of the tests is perfect in terms of sensitivity, a combination of tests is recommended.[40] All tests have a comparable sensitivity except for the smear examination, the agar-based urease and rapid serology tests which are inferior. The urea breath test is ideal for follow-up 4–6 weeks after eradication therapy. Serology cannot be used for this purpose because the antibody titer may be high for months after the disappearance of *H. pylori*.[41]

VIBRIO CHOLERAE

Nature

Vibrio cholerae is a Gram-negative, comma-shaped rod belonging to the family Vibrionaceae. Its natural habitat consists of fresh-water and salt-water environments. Based on differences in the composition of the major cell wall antigen (O), 139 serotypes have been differentiated. *V. cholerae*, which belongs to either serogroup O1 or serogroup O139, has been associated with epidemic cholera. *V. cholerae* serogroup O1 can be subdivided into El Tor and classic biotypes as well as the Ogawa, Inaba and Hikojima serotypes. Other serogroups of *V. cholerae*, in addition to nontoxigenic *V. cholerae* O1 or O139, do not cause epidemic cholera; they may, however, cause individual cases of diarrhea.

Epidemiology

Cholera has raged in seven pandemics since 1817. It is possible that an eighth is superimposed on the seventh. The fifth and sixth pandemics were caused by the classic biotype and originated in the Indian subcontinent. The seventh and current one was caused by the El Tor strain and began in 1961 in Indonesia. It has gradually affected most of Asia and Africa and is found incidentally in parts of Europe. In 1991, this pandemic reached South America and has since spread throughout Latin America (Fig. 171.1).[42] Persons who have blood group O are at higher risk of El Tor cholera than those with blood group A, B or AB.[43] This is particularly important for Latin America, where 73% of the population carries the blood type O. An episode of classic cholera protects nearly entirely against recurrent cholera of either biotype, but an episode of El Tor cholera does not protect against future attacks.

In 1992, a novel *V. cholerae* variant O139 (synonym Bengal), which has the same origin as the El Tor strain, emerged in southern Asia. This 1992 epidemic was the first one caused by a serogroup other than O1, and it occurred in populations assumed to be largely immune to *V. cholerae* O1. The impact of a *V. cholerae* O139 infection on the risk of a subsequent *V. cholerae* infection, either O1 or non-O1, has not yet been determined. The Bengal strain has the potential to spread pandemically. It has now affected areas throughout the Indian subcontinent, neighboring states and other parts of Asia. Cases occurring as far away as the USA and Western Europe have also been described.

The distribution and subdistribution of 632 reported outbreaks have been reviewed from the Program for Monitoring Emerging Diseases (ProMED) from 1995 to 2005.[44] Of the reported outbreaks, 66% occurred in sub-Saharan Africa followed by South East Asia. In West and Southern Africa, the most commonly cited risk factor was heavy rainfall and flooding. The lack of infrastructure and economic development, and especially poor sanitation and the lack of clean water have made many parts of Africa susceptible to cholera.[45] The largest identified risk factor for cholera outbreaks is a contaminated water source. In more developed regions, the risk factor is more likely to be related to seafood or vegetables that have not been properly washed or cooked.

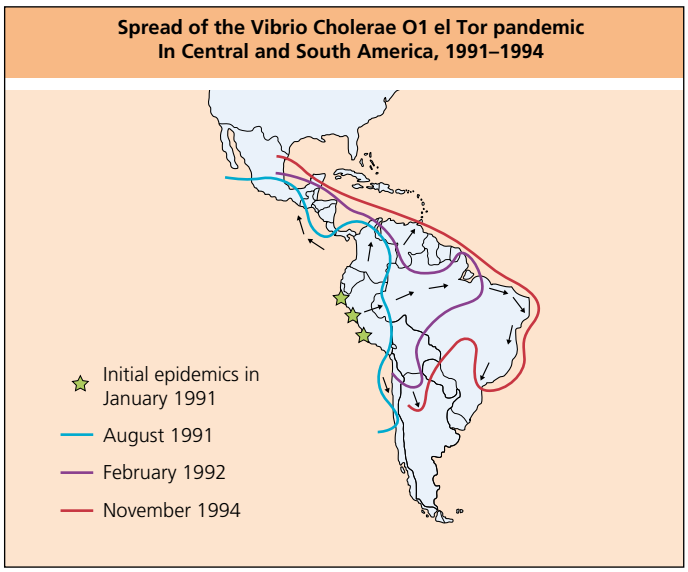


Fig. 171.1 Spread of the *Vibrio cholerae* O1 El Tor pandemic in Central and South America, 1991–94. Adapted from Tauxe *et al*.[43]

Vibrio cholerae, including serogroups O1 and O139, exist as natural inhabitants of aquatic ecosystems and are therefore facultative human pathogens. Within the marine environment they attach to zooplankton and are able to form biofilm, facilitating environmental persistence. *V. cholerae* O1 and O139 are endemic in freshwater zooplankton[46] and indeed an important role of the environment was supported by the correlation between cholera incidence and elevated sea surface temperature. Following events related to El Niño, it was shown that the detection of *V. cholerae* followed ambient temperature increases and coincided with or preceded annual outbreaks of cholera in the summer.[47] The extreme weather conditions of higher temperature, increased rainfall and consequent flooding may explain the global increase in the number of reported outbreaks from 1997 to 1999.[48] Finally, it should be noted that underreporting of cholera is a notorious problem, and the estimation of between 3 and 5 million cases and 120 000–200 000 deaths per year due to cholera could represent a small proportion, perhaps only 10–20% of all cases.[49,50]

Pathogenesis

The infectious dose is high due to the acid sensitivity of the bacteria. Persons who have impaired gastric acidity or who take acid-suppressing medication have an increased risk of infection. In addition, *H. pylori* gastritis is also associated with an increased risk of cholera.[51] The surviving bacteria adhere to and colonize the small intestine epithelial cells, producing the cholera toxin and causing acute watery diarrhea. In the intestine *V. cholerae* is faced with growth inhibitory substances (e.g. bile salts) and defense factors (e.g. complement and defensins), against which it has developed survival strategies. The powerful enterotoxin then released is a 68 kDa protein consisting of an active (A) subunit and five binding (B) subunits (Fig. 171.2). The cholera toxin is released from *V. cholerae* via an extracellular protein secretion machinery which excretes more than 90% of the toxin.[53]

The genes encoding the cholera toxin are part of a single-stranded DNA filamentous bacteriophage CTXΦ.[54] More precisely, the cholera toxin is composed of two types of subunit, a single toxic-active A subunit (CTA) and a circular B subunit heptamer (CTB) responsible for toxin binding to cells. CTA is synthesized as a single polypeptide chain, which is post-translationally modified via the action of a *V. cholerae* protease which generates two fragments, CTA1 and CTA2. CTA1 possesses an ADP-ribosylating activity whereas CTA2 inserts CTA into CTB.[55] The CTB subunits attach to the GM1 ganglioside receptor in

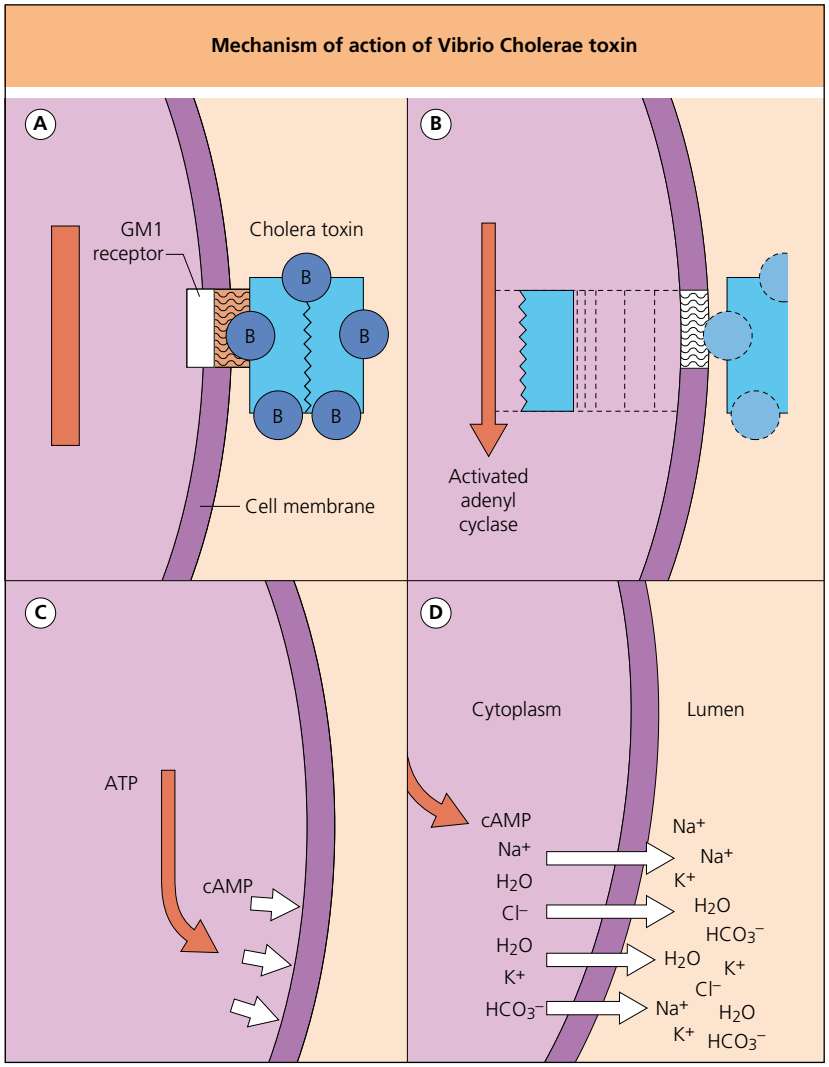


Fig. 171.2 Mechanism of action of *Vibrio cholerae* toxin. The toxin binds to the GM1 ganglioside receptor on the intestinal mucosal cell membrane via the binding (B) subunits (a). The active portion of the A subunit enters the cell and activates adenyl cyclase (b), which results in the accumulation of cyclic adenosine monophosphate (cAMP), derived from adenosine triphosphate (ATP), along the cell membrane (c). The cAMP causes active secretion of sodium (Na^+), chloride (Cl^-), potassium (K^+), bicarbonate (HCO_3^-) and water (H_2O) out of the cell into the lumen of the intestine (d). Adapted from Murray *et al*.[52]

the lining of the mucosal cells; the cholera toxin is then endocytosed by the cell either through caveoli-coated vesicles or clathrin-coated vesicles and travels to the endoplasmic endothelium reticulum, probably in association with the actin cytoskeleton via lipid drafts.[56,57] The A subunit triggers a cascade of reactions involving cyclic adenosine monophosphate, prostaglandins, 5-hydroxytryptamine and calmodulin.[58] This results in an increased level of intracellular cAMP leading to an increase in intestinal chloride secretion and a decrease in sodium chloride absorption. The outcome is a passive watery excretion that leads to diarrhea. The volume typically exceeds 1 liter per hour in adults and 10 ml/kg per hour in children. Since cholera results from a locally acting enterotoxin, it is not accompanied by systemic manifestations caused by a cytokine-induced acute-phase reaction.

The complete genome sequences of the two chromosomes of *V. cholerae* O1 El Tor, now available, helped to unveil the mechanisms of cholera pathogenesis. The bacteria contain two chromosomes, one of which is approximately 3 Mb and codes for virulence factors such as the cholera toxin.[59] A toxin co-regulated pilus (TCP) was identified as the most important colonization factor. The bacterium first colonizes the intestinal surface utilizing TCP and then may receive a signal that induces full cholera toxin and TCP expression and the onset of disease. The genes required for TCP synthesis (*tcp* operon) are located on a pathogenicity island (PI) named Vibrio PI (VPI). VPI contains a G+C content lower than the rest of the genome (35.6% vs 47.7%) suggesting an external origin.[60–62] VPI also encodes a regulator (ToxT) which has been demonstrated to coordinate the expression of TCP and cholera toxin. ToxT is under the control of several regulatory systems that are known to respond to various environmental conditions (osmolarity, pH and nutriment composition) and to bacterial cell density.[63–65]

Motility, surface antigens (capsule, lipopolysaccharide, outer membrane proteins) and hemagglutination protease are additional important virulence factors. Finally, the role of extracellular capsular polysaccharides in intestinal adherence, virulence and biofilm formation has also been characterized.

In *V. cholerae*, quorum-sensing regulation is considered as the key mechanism coordinating virulence gene expression with that of cell population density. *V. cholerae* contains two well-characterized quorum-sensing systems, CqsA/S and LuxS/PQ.[66] It has been proposed that biofilm formation increases acid resistance of *V. cholerae* and is critical for entry into the host as it passes the stomach milieu. Once in the intestinal environment, quorum-sensing signals within the biofilm repress virulence factor gene expression, such as genes encoding for the cholera toxin and TCP.[67,68] The bacterium then detaches from the biofilm and colonizes the intestines where quorum-sensing signals are low and cholera toxin and TCP are expressed.

Prevention

Cholera is difficult to eradicate from water and is likely to remain a serious threat to public health for some time. Measures to prevent cholera include separating sewage and drinking water systems, disinfection of drinking water and food, hygiene measures (e.g. hand washing with soap), active case finding and effective case management with

the use of oral rehydration. Breast-feeding provides important protection to infants, not because of transmission of maternal antibodies but because of the lower exposure to contaminated food and water. During epidemics, the bodies of persons who died of cholera should be disinfected and buried rapidly, and the consumption of food at gatherings, including funerals, should be discouraged.

The currently licensed, parenteral, killed cholera vaccine is no longer recommended by the World Health Organization (WHO) because of its limited protective efficacy. To induce mucosal immunity, oral vaccines, both inactivated (whole cell/cholera toxin B-subunit or WC-BS) and live (CVD103-HgR), have been developed.[69] WC-BS is currently recommended by the WHO (Dukoral; licensed by SBL Vaccine, Sweden) and consists of four batches of heat- or formalin-killed whole-cell *V. cholerae* O1, representing both serotypes (Inaba and Ogawa) and both biotypes (classic and El Tor), supplemented with purified recombinant cholera toxin B-subunit.

Mass vaccination campaigns of populations considered to be at high risk of cholera outbreaks have been performed in several countries. When the WC-BS vaccine was studied in Bangladesh, a short-term protection of 6 months was found in 85% of adults and children over 6 years of age; the protection was as good after two doses as after three. However, the duration of protection dramatically decreased after 1 year. Similar protection rates were obtained in other studies in Peru (86% protection efficacy), Mozambique (60.5% protection efficacy), Uganda and Darfour after two or three doses.[70–72] Vaccines that include the B-subunit of the toxin provide cross-protection against heat-labile enterotoxigenic *Escherichia coli*, a result of the close relationship between the two toxins.[73,74] A variant of the Dukoral killed whole cell vaccine containing no recombinant CTB-subunit has been produced and tested in Vietnam. It is administered in two doses 1 week apart and an efficacy of 66% against *V. cholerae* El Tor was obtained after 8 months in all age groups tested.[75]

CVD103-HgR consists of a live attenuated, genetically modified *V. cholerae* O1 Inaba strain which has been engineered to produce CTB but not the A subunit of CT, and produces higher resistance to the homologous classic strain than to the El Tor strain. Attenuation of El Tor has led to several new candidate vaccines that have proven safe and highly effective against El Tor cholera.[76] The vaccine (Orochol; licensed by Berna Biotech, Switzerland) is given orally along with buffer to neutralize stomach acidity. The vaccine is available in two formulations, either a low-dose formulation for nonendemic countries or a 10-fold higher dose formulation for cholera-endemic developing countries. The vaccine is currently licensed in several industrialized countries. The safety and immunogenicity of a single dose of Orochol has been demonstrated.[77–79]

Because immunity against the O1 type is not protective against O139, *V. cholerae* O139 type vaccines have been developed. New attenuated strains of serogroup O139 for use as oral vaccines (CVD112 and Bengal 15), as well as new candidate vaccines, have been developed.[80–82] A bivalent O1 and O139 whole-cell oral vaccine without CTB was also recently developed in Vietnam and shown to be safe and immunogenic in both adults and children, generating 90% anti-O1 and 68% anti-O139 vibriocidal responses after administration of a two-dose regimen. It elicits a robust immune response and can be produced at a low cost.[83,84] Similar results were obtained during a randomized placebo-controlled trial in adults and children in a cholera-endemic area in Kolkata, India.[85]

Diagnostic microbiology

For rapid diagnosis, darkfield examination of a fresh, unstained stool specimen is highly sensitive and specific; a characteristic finding is the 'shooting star' phenomenon caused by the motility of the single polar flagellum of the organism. The diagnosis of cholera is confirmed when *V. cholerae* is identified in a stool culture on thiosulfate-citrate-bile salts-sucrose (TCBS) agar directly or after enrichment in alkaline peptone water, taking advantage of *V. cholerae*'s tolerance to alkaline conditions. Sucrose-positive, smooth, yellow colonies grown on TCBS agar are submitted to an oxidase test and the identification of *V. cholerae* is completed by biochemical and serologic tests.

Once identified at the species level, additional tests include serotyping for O antigens and the three antigen variants recognized, as well as biotyping to differentiate classic and El Tor biotypes. A rapid, colorimetric immunodiagnostic method is available for the detection of *V. cholerae* O1. The immunochromatographic strip test may be the simplest, most rapid and most sensitive and specific test for the detection of *V. cholerae*. Two rapid immunodiagnostic test kits based on the use of monoclonal antibodies have been developed for the direct detection of *V. cholerae* O139: a co-agglutination test and Bengal DFA (a direct fluorescent-antibody test). In epidemic settings in developing countries, a bacteriologic diagnosis is not indicated in all suspected cases because the management of dehydrating diarrhea is guided by the extent of fluid loss rather than by the nature of the infecting organism. In contrast, the diagnosis should be confirmed in individual cases of suspected cholera in the developed world.

Such classical methods can be time-consuming. Thus molecular methods, when available, are more suitable for *Vibrio* spp. detection directly from environmental water samples, since they can detect dead bacteria and viable but nonculturable (VNC) bacteria. Several molecular techniques based on DNA amplification have been developed and could be useful in the detection of *V. cholerae* O1 directly from environmental water in cholera endemic areas and in complementing the identification of toxigenic strains isolated by culture.[86–88]

Clinical features

Cholera is an acute diarrheal disease in which the fluid loss is responsible for all other clinical manifestations of the disease. Most cases are so mild that they may escape detection, but a small percentage are so serious that death can occur within a few hours of the onset of symptoms. *V. cholerae* needs to be considered among the possible diarrheal causes in children living in endemic areas.[50,89] In Latin America, well-established case management has kept fatality rates low (at about 1%). In contrast, cholera in Africa has been sporadic and as a result less well recognized. The fatality rates there also tend to be higher (about 10%).[90] Children are more likely to have only subclinical infection or mild diarrhea, whereas adults tend to develop more severe disease and require hospitalization.

Cholera is in principle a self-limiting disease if dehydration is sufficiently corrected. Cholera gravis is a voluminous painless watery diarrhea that can lead to dehydration and even death within a few hours. The case fatality rate for untreated cholera gravis is 50%. Symptoms include nausea, vomiting (especially early in the illness) and muscle cramps, followed by signs of hypovolemic shock. With adequate treatment, mortality is reduced to 1% or less in all age groups. After an incubation period of between 18 hours and 5 days, symptoms are generally abrupt and include watery diarrhea and vomiting. The most distinctive feature of cholera is the painless purging rice-water stools with a fishy odor. The vomitus is generally a clear, watery, alkaline fluid. The rate of diarrhea may reach 500–1000 ml/h in severe cases, leading to severe dehydration. Most patients have no urine output until the dehydration is corrected. Because *V. cholerae* does not invade the epithelial lining of the intestine, there is little inflammatory response and hence the stool contains few if any leukocytes and patients are afebrile. Metabolic abnormalities may also occur, including hypokalemia as the result of potassium loss, acidosis from both bicarbonate loss and increased lactate production resulting from anaerobic glycolysis, and hypoglycemia due to deficient gluconeogenesis resulting in seizures and other neurologic abnormalities. Hyperventilation (Kussmaul breathing) may occur as a result of the metabolic acidosis. Shock from cholera can precipitate abortion in pregnant women, although this is less likely to occur if rehydration is prompt.

Complications include acute renal failure, low blood glucose concentrations and electrolyte imbalance, especially hypokalemia, but these are generally from improper treatment. Severe muscle cramps of

arms and legs are common. They are probably due to the electrolyte imbalance, although the exact explanation is not known. They subside within a few hours of treatment.[50]

Management

The management of cholera patients consists of two components:

- rehydration, which is critical; and
- antimicrobial therapy, which is optional and intended to shorten the duration of the illness and prevent spread of the disease.

Effective fluid replacement can be expected to reduce mortality to less than 1% of the severely affected individuals. If fluids are given promptly, nearly all deaths are avoided. However, as effective treatment is not always available in remote areas where cholera occurs, cholera deaths are still common. Mild and moderately dehydrated patients can be treated using oral rehydration solution (ORS), which has three basic components: sugars, salts and water. Physiologically, the sugar acts as the vehicle whereby salts can be absorbed by the mucosal cell; water follows passively.[91] Patients on ORS should remain on ORS until the diarrhea stops. Severely dehydrated patients should be treated intravenously. When intravenous solutions and equipment are not available, severely dehydrated patients may be treated through a nasogastric tube.

Antibiotic treatment serves to shorten the illness and save rehydration fluids. Incomplete courses have contributed to antibiotic resistance. Options are guided by local sensitivity patterns. These are tetracycline (250 mg q12h for 7–10 days) or amoxicillin (250 mg q6h for 5 days). Tetracyclines and quinolones are not recommended during pregnancy. During epidemics, prophylactic antibiotics should be used for the immediate family only and should be limited to single-dose therapy. Reports from several cholera-endemic countries of strains resistant to antibiotics, including tetracycline, ampicillin, kanamycin, streptomycin, sulphonamides and fluoroquinolones, have emerged with a trend of spatial and temporal fluctuations. On a molecular basis, conjugative plasmids, transposons, integrons, mutations and proton-motive-force-dependent efflux have been associated with this antibioresistance.[92,93]

Apart from fluid replacement, novel and potentially useful strategies to reduce diarrhea in cholera have been developed. First, the inhibition of toxin binding is a particularly attractive approach because binding inhibitors can be administered prophylactically in high-risk areas or as therapy after the infection as begun, as well as administrated orally.[94] Second, enkephalin inhibitors such as racecadotril can be effective in *V. cholerae* and *E. coli*-related pediatric diarrhea.[95] Third, small-molecule thiazolidinone inhibitors of CFTR Cl^- conductance have been shown to be effective in preventing Cl^- and fluid secretion in human intestinal cells and rodents.[96] In conclusion, all of these new agents could be promising compounds for cholera treatment, but further studies must be carried out to prove their antidiarrheal efficacy *in vivo*.

Other pathogenic *Vibrio* spp.

Vibrio parahaemolyticus is a halophilic, or salt-requiring, *Vibrio* sp. that has been related to food poisoning and ingestion of raw or inadequately cooked seafood. The epidemiology results from the ubiquitous presence of the organism in coastal waters. The median incubation time is estimated at 23 hours (range, 5–92 hours); secondary cases are rare. Preventive measures include the use of boiled water for cooking food. It is not known whether clinical disease confers immunity. There is no effective vaccine available. Stool culture on selective TCBS agar demonstrates distinct opaque green colonies; final identification is made using standard biochemical tests. The clinical picture ranges from mild, watery diarrhea with low-grade fever to frank dysentery. Specific treatment is not required in most cases because the illness is self-limiting and no benefit has been established from antimicrobial agents.

Other less common halophilic *Vibrio* spp. include *Vibrio vulnificus*, *Vibrio alginolyticus*, *Vibrio fluvialis*, *Vibrio hollisae* and *Vibrio damselae*. Molecular methods, especially PCR, can be used for identification or detection of these species, including in the environment. In contrast to the *Vibrio* spp. discussed so far, *V. vulnificus* and *V. alginolyticus* are associated more frequently with soft tissue wound infection and sepsis than with diarrhea.[97] *V. vulnificus* may be considered an emerging pathogen and its virulence is the strongest among the noncholera *Vibrio* spp. Pathogenicity is generally reserved for the immunocompromised host and is related to disease states that exhibit high serum iron levels, including liver disease. Prevention involves avoidance of contaminated salt water. Immunocompromised patients should be warned against the ingestion of raw oysters and shellfish. At higher latitudes, severe *V. vulnificus* infections have been reported in association with very hot weather conditions and sea water temperatures above 68°F (20°C).

A *Vibrio* polysaccharide conjugate vaccine may be useful in the management of *V. vulnificus* infections. Antibodies that react with the capsular polysaccharide of the organism are detectable in infected patients and in persons without known exposure to the organism, suggesting that cross-reacting antibodies are present in the general population.

V. vulnificus infections are the cause of two main clinical syndromes:

- primary sepsis secondary to ingestion of raw oysters; and
- localized infection from wound exposure to salt water in which the organism lives.

Both syndromes demonstrate characteristic skin lesions of the trunk and extremities. They are characterized by hemorrhagic bullae which progress to necrotic ulceration. Necrotizing fasciitis of the foot associated with *V. vulnificus* can cause death within 48 h and has an overall mortality rate of 50%, even with appropriate antibiotic and surgical treatment.[98] Besides these two syndromes, *V. vulnificus* may also cause acute diarrhea in those on antacid therapy. Early suspicion is critical, because *V. vulnificus* is not always susceptible to aminoglycosides, and tetracycline is the first-choice treatment.

V. alginolyticus may cause bacteremia and death in immunocompromised hosts. Among immunocompetent hosts, it may cause cellulitis and otitis media in swimmers and fishermen.

Vibrio mimicus, a nonhalophilic *Vibrio*, produces a clinical spectrum that is indistinguishable from that of *V. parahaemolyticus*. The epidemiology of *V. mimicus* reflects a global distribution; outbreaks have been associated with heavy contamination of water sources. Prevention involves adequate purification of water sources and proper cooking practices. The diagnosis is confirmed by identifying the agent on TCBS agar and subsequent specific antiserum testing. Treatment is limited to fluid and electrolyte replacement.

V. alginolyticus represents a diagnostic obstacle, because it is often outnumbered by other *Vibrio* spp. in environmental and seafood samples. As *V. parahaemolyticus* is the species responsible for most seafood-borne outbreaks worldwide,[99] the differentiation of this species from other *Vibrio* spp. is of great importance. For this purpose Bauer *et al.* have recently developed a novel multiplex PCR for the identification of *V. parahaemolyticus*, *V. cholerae* and *V. vulnificus* which could be very useful for clinical laboratories.[100]

Treponema spp., *Borrelia* spp. and *Leptospira* spp.

The order of the Spirochaetales is divided into the families Spirochaetaceae and Leptospiraceae. These families include, among others, the genera *Treponema*, *Borrelia* and *Leptospira* (Table 171.2). Syphilis and endemic treponematoses are discussed in Chapters 57 and 104, respectively. Infection caused by *Borrelia burgdorferi* (Lyme disease) is covered in Chapter 43. The remainder of this chapter deals with *B. recurrentis*, leptospirosis and *Spirillum* infections.

Table 171.2 Order Spirochaetales

Spirochaetaceae		Etiologic agent	Human disease
Family Spirochaetaceae	Genus *Cristispira*		None
	Genus *Serpulina*		None
	Genus *Spirochaeta*		None
	Genus *Treponema*	*Treponema pallidum* subsp. *pallidum*	Syphilis
		Treponema pallidum subsp. *endemicum*	Bejel
		Treponema pallidum subsp. *pertenue*	Yaws
		Treponema carateum	Pinta
	Genus *Borrelia*	*Borrelia recurrentis*	Epidemic relapsing fever
		Many Borrelia spp.	Endemic relapsing fever
		Borrelia burgdorferi, Borrelia garinii, Borrelia afzelii	Lyme borreliosis
Family Leptospiraceae	Genus *Leptonema*		None
	Genus *Leptospira*	*Leptospira interrogans*	Leptospirosis

The genera *Treponema*, *Borrelia* and *Leptospira* contain pathogens associated with human infections. The genera *Cristispira*, *Serpulina*, *Spirochaeta* and *Leptonema* are not known to cause disease in humans.

BORRELIA RECURRENTIS

Nature and epidemiology

The louse-borne epidemic relapsing fever, caused by *Borrelia recurrentis*, is transmitted by the human body louse and is associated with unhygienic living circumstances. The tick-borne endemic relapsing fever is caused by different *Borrelia* spp. and is transmitted by the *Ornithodorus* tick.

Pathogenesis and prevention

When lice are crushed, *B. recurrentis* is released and penetrates the skin or mucous membranes. Organisms can be detected in the blood when the patient has a fever. They sequester in internal organs in the afebrile periods. Mutational changes in the antigenic properties of the organism and consequent antibody production account for the relapses, which end when sufficient borrelicidal antibody has been developed.

Preventive measures are similar to those recommended for Lyme disease and involve the elimination or avoidance of the spirochete vector. A vaccine is not yet available for public health purposes.

Diagnostic microbiology

Diagnosis is based on the clinical presentation and detection of the bacteria in the blood. Spirochetes are found in the majority of wet or stained blood smears. They have also been detected in the CSF of patients who have signs of central nervous system involvement. Up to 10% show positive serologic tests for syphilis.

An early clinical diagnosis of louse-borne relapsing fever is not difficult during epidemics. In contrast, the initial differential diagnosis in an isolated case includes many different diseases such as malaria, typhoid fever, leptospirosis and dengue fever.[101]

Clinical features

Epidemic louse-borne relapsing fever and endemic tick-borne relapsing fever have very similar clinical manifestations; heterogeneity reflects differences in, for example, spirochete strains and the condition of the patient. Louse-borne relapsing fever and tick-borne relapsing fever both have a very acute onset of high fever with chills and muscle and joint aches. Headache and lethargy may be accompanied by neurologic findings, including meningitis and seizures leading to coma. Photophobia and iridocyclitis may develop, causing permanent damage to the sight. Coughing may result from bronchitis or pneumonia. A 2-day truncal rash may occur at the end of the first febrile period. Abdominal tenderness with enlargement of liver and spleen may be associated with jaundice, lymphadenopathy and hemorrhagic diathesis.

The first bout of fever usually lasts 3–6 days, sometimes with fatal complications including myocarditis, cerebral hemorrhage and hepatic failure. The fever may suddenly reappear after a 7–10-day interval. Louse-borne disease typically has a longer incubation period and a more elongated cycle of febrile and afebrile periods, and is associated with a single relapse. Tick-borne disease manifests with multiple relapses that taper off in duration and intensity.

Management

First-choice antibiotic is doxycycline 100 mg q12h for 5–10 days. One 500 mg dose of erythromycin is the second-choice option. Antibiotic treatment may be accompanied by a Jarisch–Herxheimer reaction that cannot be prevented by prior corticosteroid therapy[102] and that reflects clearance of the spirochetemia. It may be advisable to give the first dose of antibiotics at a lower dose (e.g. erythromycin 250 mg) to limit the severity of the Jarisch–Herxheimer reaction (see Chapter 125 for further management guidelines).

LEPTOSPIRA INTERROGANS SPP.

Nature

Leptospira interrogans spp. are thin, motile, coiled bacilli. The serotypes of the *Leptospira interrogans* sp. group include *icterohaemorrhagiae*, *canicola*, *pomona*, *autumnalis*, *grippotyphosa*, *hebdomadis*, *ballum* and *australis*.[103] Each of these has a different natural habitat. For example, *L. interrogans* serotype *icterohaemorrhagiae* is most commonly found in rats, *L. interrogans* serotype *canicola* in dogs and *L. interrogans* serotype *pomona* in pigs.

Epidemiology

Leptospirosis is a zoonosis that occurs worldwide (see Chapter 124). In the USA, rats are the most common vector for human infection, followed by dogs, livestock, rodents, wild mammals and cats. Humans are dead-end hosts because person-to-person transmission is very rare. The peak incidence is during the summer and early autumn; young adult men often become infected, for example, after recreational exposure to contaminated water. Leptospirosis has long been considered an occupational disease affecting farmers, veterinary surgeons and abattoir workers through indirect contact with infected animals via urine-contaminated water or soil.[104]

Pathogenesis

Leptospira spp. penetrate the intact mucous membranes or abraded skin, enter the bloodstream and spread to all parts of the body, including the cerebrospinal fluid (CSF) and eyes. Liver damage is subcellular and may lead to jaundice. Renal failure may result from a direct toxic effect of the leptospires on the tubules. Meningeal irritation may occur when the leptospires enter the CSF. They are abundant during the early stage of the meningeal disease and disappear when serum antibodies develop. A chronic or recurrent uveitis may result from leptospires persisting in the corpus vitrium of the eye. Myalgia, particularly of the calves, may be an early sign, and pathologic changes such as polymorphonuclear infiltration of the muscle occur late.[105]

Prevention

Effective control of leptospirosis is difficult because leptospires can establish a symbiotic relationship with many hosts, even if they have been vaccinated, and persist in the renal tubules (with excretion in the urine) without causing illness or pathologic changes in the kidneys. In addition, wild animals represent a reservoir from which domestic animals are continually infected. Widespread vaccination of livestock and pets in the USA has reduced the incidence of infection in some species, although adequately vaccinated dogs can still infect humans.[106] Immunization of rice fieldworkers in China with polyvalent vaccines has been practiced. In France a vaccine against serovar *icterohaemorrhagiae* is available for human use.[107] Prohibition of recreational activities in contaminated waters and measures to reduce the number of rats have been successful in reducing the incidence of disease. Prevention can be considered using 200 mg doxycycline once a week for heavily exposed individuals such as soldiers who train in the jungle.[108]

Diagnostic microbiology

Isolating the organism from patient material or the detection of seroconversion confirms the clinical suspicion of leptospirosis. Leptospires can be isolated from blood or CSF during the first 10 days of illness. During the second week, they appear in the urine and can be detected in biopsies from various organs. Tween 80-albumin is viewed as the best available medium.[109] Cultures should be maintained in the dark for up to 6 weeks at 82–86°F (28–30°C). Leptospires grow in semisolid media in a ring 0.5–1 cm below the surface, appearing 6–14 days after inoculation. They remain viable in heparinized blood for more than 1 week and can be mailed to a reference laboratory for identification.

The laboratory technique most commonly used to diagnose leptospirosis is a two-step serologic procedure, although antibiotic treatment may interfere with antibody development and account for false-negative results.

Macroscopic slide agglutination is used for screening purposes, followed by a microscopic agglutination test (MAT) with live antigens for determining antibody titers and identifying serotypes. Both tests use pools of antigens representative of most serogroups. Nevertheless, cross-reactions often occur and serologic tests cannot accurately identify the serotype responsible for infection. Currently, the sensitivity and specificity necessary for reliable clinical diagnosis is best achieved using a battery of 23 antigens. The presumptive diagnosis may be based on a microscopic agglutination titer of 1:100 or greater or on a positive slide agglutination test. Agglutinins appear after 1 week of illness and decline after the third or fourth week. For areas in the world where laboratory resources are limited a dipstick assay using a broadly reactive leptospiral antigen on a solid support for the detection of IgM antibodies was demonstrated to have satisfactory specificity and sensitivity.[110]

Other techniques to identify leptospires include darkfield examination and silver staining. Polymerase chain reaction has proven valuable as a rapid, sensitive and specific means of diagnosing leptospiral infection, especially during the first 10 days of illness when the clinical expression of the disease may be confusing.[111]

Clinical manifestations

Leptospirosis may evolve subclinically or clinically. It has an incubation period of approximately 7–12 days; clinical illness typically follows a biphasic course. During the initial phase of 4–7 days, patients present with sudden onset of fever, severe general malaise, muscular pain (especially in the calves) and conjunctival congestion; leptospires can be isolated from most tissues. Two days without fever follow. During the second phase of up to 30 days, leptospires are still detectable in the urine, kidney and vitreous body. Circulating antibodies emerge and meningeal inflammation, uveitis and rash develop.[112]

Icteric leptospirosis, or Weil's disease, occurs in 10% of the clinically ill patients; 90% remain anicteric. Icteric leptospirosis can be caused by any type of leptospire including *L. interrogans* subtype *icterohaemorrhagiae*. Prominent features are renal and liver malfunction, hemorrhage and impaired consciousness; the mortality rate is 5%.[113] The combination of a direct serum bilirubin level of less than 20 mg/100 ml (342 mmol/l), a marked elevation in serum creatine phosphokinase and an elevation in transaminase (SGOT and SGPT) of less than 200 units is suggestive of the diagnosis.[114] Hepatocellular necrosis rarely occurs and no residual hepatic dysfunction ensues. Hepatomegaly is found in 25% of cases. The severity of the illness probably reflects the degree of the vasculitis.

Anicteric leptospirosis is a more common and less severe form of leptospirosis. It has a sudden onset and is accompanied by remittent fever and chills, persistent headache, severe myalgia, malaise, prostration and abdominal pain with nausea and vomiting lasting up to 7 days. During the following so-called immune stage, the fever virtually subsides. Delirium and meningism, however, may develop. Several organ systems can be affected, including the lungs, which may show pulmonary infiltrates, and the eyes.[115] A rash may develop as well. Distinct erythematous pretibial lesions can be found in the Fort Bragg fever syndrome, which is caused by *L. interrogans* serotype *autumnalis*.

MANAGEMENT

Severely ill patients should be treated with intravenous penicillin or amoxicillin for 7 days (Table 171.3).[116,117] Less severely ill patients should receive oral doxycycline for 7 days.[118] Severe disease is not limited to adults but may also affect children, who benefit from antibiotics even late during the illness.[119] Careful monitoring and supportive therapy are important in order to prevent possible complications, including renal failure, hypotension and major hemorrhage.

Table 171.3 Treatment options for leptospirosis

	ANTIBIOTIC	
Bacteria	**First choice**	**Second choice**
Leptospira icterohaemorrhagiae, *copenhagi* (Weil's disease) *Leptospira hardjo* (milker's disease) *Leptospira grippotyphosa*	Penicillin G 1 MU q4–6h iv for 7 days Alternative: Amoxicillin 500 mg q6h for 7 days	Doxycycline 100 mg q12h po for 7 days

Adapted from Speelman *et al.*[117]

SPIRILLUM MINUS

Rat-bite fever can be caused by either *Streptobacillus moniliformis* or *Spirillum minus*. The first description of the spirillum dates back to 1888 when Carter reported a spiral organism which he called *Spirillum minor* in the blood of wild rats. Later the organism was named *Spirocheta morsus muris* and in 1924 the organism was renamed *Spirillum minus*.[120]

Nature

The literature on *Spirillum minus* is very puzzling since largely conflicting properties of the organism have been reported. The basic problem lies in the fact that the description of the organism is purely morphologic.[121] No biochemical properties or nucleotide sequence data are available. *Spirillum minus* is a Gram-negative spiral micro-organism. It measures 0.2–0.5 mm in width and 3–5 mm in length. It possesses two to three windings and is actively motile by means of bipolar tufts of flagella.

Epidemiology and pathogenesis

Spirillum minus can be detected in the oropharynx of rats and small rodents. Infections occur throughout the world. Data on incidence are lacking so the widely held belief that infections with *S. minus* are more often seen in the Pacific region cannot be substantiated. Transmission from human to human has not been documented.

Prevention

No studies have been reported on the use of prophylactic antibiotics after a rat bite.

Diagnostic microbiology

The spiral bacteria can be made visible using darkfield microscopy or Giemsa staining of blood smears, smears from eschars or lymph node aspirates. *Bergey's Manual of Determinative Microbiology* states that the organism cannot be cultured on artificial media but can be detected in the blood of mice or guinea-pigs 3 weeks after intraperitoneal injection of clinical samples.[121] A practical problem concerning this approach is the fact that these animals are the natural reservoir of these spirilla. Animal inoculation studies should include control animals and pre-inoculaton evaluation for spirilla.[122] Most reports in the literature do not describe if such controls were performed.

Diagnosis of *S. minus* infection in the literature is based on clinical presentation,[123] direct visualization of the organism[124] or animal inoculation.[122] These spirilla could not be cultured on artificial media. Other reports indicate an easily cultivable *S. minus* as an etiologic agent for endocarditis[125,126] and recurrent fever.[127] These spirilla could be cultured on simple media such as blood agar and an antibiogram could be established. Further characterization of the spirilla is warranted to determine if we are dealing with one or multiple species or even genera.[128] No specific serologic test can be used; however, the VDRL is positive in 50% of cases.

Clinical features

Spirillum minus rat-bite fever is also called sodoku: 'so' means rat and 'doku' means poison in Japanese.[120] The initial lesion of the rat bite usually heals promptly. One to 36 days after the exposure the patient becomes ill and develops fever. At the site of the bite, the wound reactivates and an eschar develops. Regional lymphadenitis is present in the majority of cases. A violaceous maculopapular rash on palms and soles, very similar to the one seen in syphilis, is a common feature. If untreated, relapsing fever will develop. As compared to the rat-bite fever caused by *Streptobacillus moniliformis*, sodoku has a longer incubation period and arthritis is not a frequent event.

Management

Penicillin is the treatment of choice although no clear-cut guidelines can be presented about dose and duration. A Jarisch–Herxheimer reaction can occur after initiation of antibiotic therapy. Tetracycline can be used for patients with an allergy for penicillin.

REFERENCES

References for this chapter can be found online at http://www.expertconsult.com

Chapter 172

Mary PE Slack

Gram-negative coccobacilli

INTRODUCTION

The Gram-negative coccobacilli that are important human pathogens including *Bordetella*, *Brucella*, *Francisella*, *Haemophilus*, *Legionella*, *Pasteurella* and *Yersinia* spp. *Actinobacillus*, *Aggregatibacter*, *Cardiobacterium*, *Eikenella*, *Kingella* and *Moraxella* spp. occasionally cause human disease. All of these genera except *Yersinia* are fastidious, requiring special nutrients and growth factors for isolation. Like other members of the Enterobacteriaceae, *Yersinia* spp. are not exacting in their growth requirements.

The genera *Brucella*, *Francisella*, *Pasteurella* and *Yersinia* cause zoonotic infections in humans. *Legionella* infections are acquired through exposure to environmental contamination. *Haemophilus* and most *Bordetella* infections arise through person-to-person transmission. Rarely *Bordetella bronchiseptica*, an animal pathogen, produces opportunistic human infections.

Bordetella Species

NATURE

Bordetella spp. are minute Gram-negative coccobacilli. They often demonstrate bipolar staining. They may be nonmotile or motile by means of peritrichous flagella. *Bordetella pertussis* and *Bordetella parapertussis* are nonmotile. *Bordetella* spp. are strictly aerobic (except for *B. petrii*) and the optimum temperature for growth is 95–98.6°F (35–37°C). No acid is produced from carbohydrates. There are eight species in the genus (a further putative species, *Bordetella ansorpii*, is awaiting confirmation of its validity); these differ in their fastidiousness and growth requirements. *Bordetella pertussis* is the most exacting and requires special media for isolation; *Bordetella parapertussis* is slightly less exacting; *Bordetella bronchiseptica* grows on ordinary laboratory media. Multilocus enzyme electrophoresis, insertion sequence polymorphisms and sequencing data derived from 16S rDNA sequence analysis and microarray-based comparative genome hybridization have confirmed the close genetic relatedness of all known *Bordetella* species.[1] *Bordetella pertussis* and *B. parapertussis* are probably human adapted subspecies of *B. bronchiseptica*. The genomes of *B. pertussis*, *B. parapertussis*, *B. bronchiseptica*[2] and *B. avium*[3] have all been sequenced.

Bordetella pertussis and *B. parapertussis* are human pathogens of the respiratory tract causing pertussis or whooping cough. *B. bronchiseptica* and *B. avium* are primary respiratory tract pathogens of birds and mammals. *B. bronchiseptica*, *B. hinzii*, *B. holmesii*, *B. trematum*, *B. petrii*, *B. ansorpii* and *B. avium* may infrequently cause infection in humans, particularly in the immunocompromised host.

EPIDEMIOLOGY

Pertussis is highly contagious, being transmitted via aerosolized droplets of respiratory secretions, and in the prevaccine era nearly all children became infected between the ages of 1 and 5 years. Attack rates range from 50% for school contacts to 80–90% for close family contacts. Patients disseminate organisms for weeks or months and are highly infectious in the nonspecific catarrhal and early paroxysmal stages of the infection. It is therefore easy for the infection to spread to susceptible individuals before the possibility of whooping cough is considered. There is little evidence of asymptomatic carriage.

Whooping cough is still a major disease worldwide and an important cause of death in malnourished children. It is estimated that there are over 51 000 000 cases each year worldwide and 300 000 deaths.[4] In most populations the disease is endemic, with epidemics occurring every 4 years in late winter and spring. There is no animal reservoir.

With the use of whole-cell pertussis vaccine the incidence of pertussis in children aged 1–5 years declines sharply but there is an increase in the incidence of the disease in children younger than 1 year. Unfortunately, these young infants are those at greatest risk of morbidity and mortality from pertussis. Females are more likely to be infected than males. Immunized contacts may become transiently colonized. Adolescents and adults with waning vaccine-induced immunity are increasingly suffering from pertussis, which often goes undiagnosed because the infection may be atypical, and these older patients act as a source of transmission to young children. It has been suggested that up to 20% of adults with a prolonged cough may be suffering from pertussis.[4] There are three serotypes of *B. pertussis* pathogenic for humans, which contain agglutinins 1,2; 1,2,3; and 1,3. Strains may switch serotype both *in vitro* and *in vivo*. For this reason, a genotypic method of classification, based on techniques such as restriction fragment length polymorphism (RFLP) or pulsed-field gel electrophoresis (PFGE) is preferable.

PATHOGENICITY

Bordetella pertussis, *B. parapertussis* and *B. bronchiseptica* all possess a virulence control system regulated by the BvgAS operon.[1] BvgAS is a two-component phospho-relay system that responds to environmental conditions by switching between three phenotypic phases. The Bvg^+ phase occurs with respiratory tract colonization and is associated with the expression of a number of virulence factors. The Bvg^i phase is associated with respiratory transmission and Bvg^- is an avirulent phase that enables *B. bronchiseptica* to survive in the environment.[1] Some Bvg-regulated genes are expressed in all three subspecies. Others, although they are present in all three subspecies, are differently expressed. Importantly the *ptx-ptl* operon which encodes pertussis toxin (PT) is present in *B. pertussis*, *B. parapertussis* and *B. bronchiseptica* but only expressed in the Bvg^+ phase of *B. pertussis*.

Table 172.1 Virulence factors of *Bordetella pertussis**

Toxin	Synonyms	Composition	Actions	Comments
Filamentous hemagglutinin	FHA	Protein	Adhesin	Highly immunogenic component of acellular pertussis vaccines
Pertactin	PRN	Outer membrane protein	Adhesin	Enhances protective immunity
Pertussis toxin	PT	Protein	Attachment to ciliated respiratory epithelium Activation of cAMP, HSF, LPF, IAP Impairs leukocyte function Haemolytic	Adjuvant component of acellular pertussis vaccines
Adenylate cyclase toxin	ACT	Protein	Activation of cAMP Antiphagocytic Anti-inflammatory Haemolytic	Calmodulin-activated RTX toxin
Dermonecrotic toxin	DNT	Polypeptide	Vascular smooth muscle contraction Dermal necrosis	
Tracheal cytotoxin	TCT	Glycopeptide	Ciliostasis Inhibits DNA synthesis	
Lipopolysaccharide	LPS	Lipopolysaccharide	Endotoxin	
Fimbriae	FIM	Protein	Facilitates persistent tracheal colonization	Component of some acellular pertussis vaccines

cAMP, cyclic adenosine monophosphate; HSF, histamine sensitizing factor; IAP, islet-activating protein; LPF, lymphocytosis promoting factor; RTX, repeats in toxin.
*With the exception of pertussis toxin, similar toxins are expressed in *B. parapertussis* and *B. bronchiseptica*.

Bordetella pertussis produces a number of adhesins and toxins, which are important in pathogenesis[1] (Table 172.1).

Filamentous hemagglutinin (FHA), pertactin (PRN) and fimbriae (FIM) are adhesins that play a role in colonizing the ciliated respiratory tract epithelium. Pertussis toxin (PT) is a four-polypeptide toxin with many biologic activities: a histamine-sensitizing factor, a lymphocytosis-promoting factor, an enhancer of insulin secretion and a potent adjuvant. It is composed of two subunits, A and B. Subunit B mediates toxin binding and subunit A carries out the toxin's biologic activities. Pertussis toxin activates adenyl cyclase, resulting in increased cyclic adenosine monophosphate (cAMP) in the host cell. Adenylate cyclase toxin (ACT) is an extracytoplasmic protein that can enter leukocytes. Once inside the cells, ACT is activated by calmodulin to catalyze the production of cAMP from adenosine triphosphate (ATP). The increased cAMP interferes with leukocyte function. Dermonecrotic toxin (DNT) is a heat-labile toxin that can cause vascular smooth muscle contraction resulting in focal ischemic necrosis. Tracheal cytotoxin (TCT) is a peptide derived from bacterial peptidoglycan. It causes ciliostasis, inhibits DNA synthesis and kills ciliated epithelial cells, which are then sloughed off. The endotoxin lipo-oligosaccharide (LOS) of *B. pertussis* probably also plays a part in pathogenicity.[1]

Following inhalation, *B. pertussis* adheres to ciliated epithelium in the trachea and bronchi. Adhesion is mediated by filamentous hemagglutinin, pertactin and possibly pertussis toxin. The bacilli then begin to multiply, producing pertussis toxin, which disrupts cell function; tracheal cytotoxin, which inhibits ciliary motion; and adenylate cyclase toxin, which interferes with phagocytosis. The dermonecrotic toxin causes local necrosis. The organisms remain localized on the respiratory epithelium. They do not invade.

PREVENTION

Prevention of pertussis depends on immunization. Whole-cell pertussis vaccines consist of killed suspensions of whole bacterial cells adsorbed with the adjuvant aluminum hydroxide. To be effective the vaccine must contain all three agglutinogens, 1, 2 and 3. Pertussis vaccine was introduced into the UK during the 1950s and resulted in a steady decline in the size of pertussis epidemics until the mid-1970s.

Many concerns have been voiced about the safety of whole-cell vaccines and fears of possible neurologic sequelae. In the UK these fears resulted in a dramatic fall in the rate of vaccine uptake and three large epidemics of pertussis occurred in the late 1970s (Fig. 172.1). Since that time, parental confidence in the vaccine has gradually been restored and the number of cases has again declined. There is no strong evidence that whole-cell pertussis vaccine does produce long-term adverse effects but it may trigger the appearance of pre-existing neurologic problems. A severe adverse reaction to a previous dose of vaccine is a contraindication for further doses. Vaccination should be delayed in children who are unwell.

A number of acellular pertussis vaccines, containing up to five *B. pertussis* antigens, including pertussis toxin, filamentous hemagglutinin, pertactin and fimbrial antigens FIM2 and FIM3, have been developed and have replaced whole-cell pertussis vaccine in the routine infant immunization program in many developed countries, including the UK.[5] They are generally less reactogenic than whole-cell preparations and may be used for booster immunization in older children and adults.

As the incidence of pertussis in older children and adults is increasing there may be a case for using acellular pertussis vaccine to boost waning vaccine-induced immunity in older age groups. Whole cell pertussis vaccine should be avoided for adolescents and adults because of significant degrees of reactogenicity.

DIAGNOSTIC MICROBIOLOGY

With careful sampling and culture techniques *B. pertussis* can be recovered for up to 3 months after the onset of the illness. Nasopharyngeal aspirates, pernasal swabs or nasopharyngeal swabs are the specimens of choice. Dacron TM or rayon pernasal swabs should be used to increase recovery of *B. pertussis* as this organism has a greater affinity for Dacron than for cotton wool. Samples collected using Dacron swabs

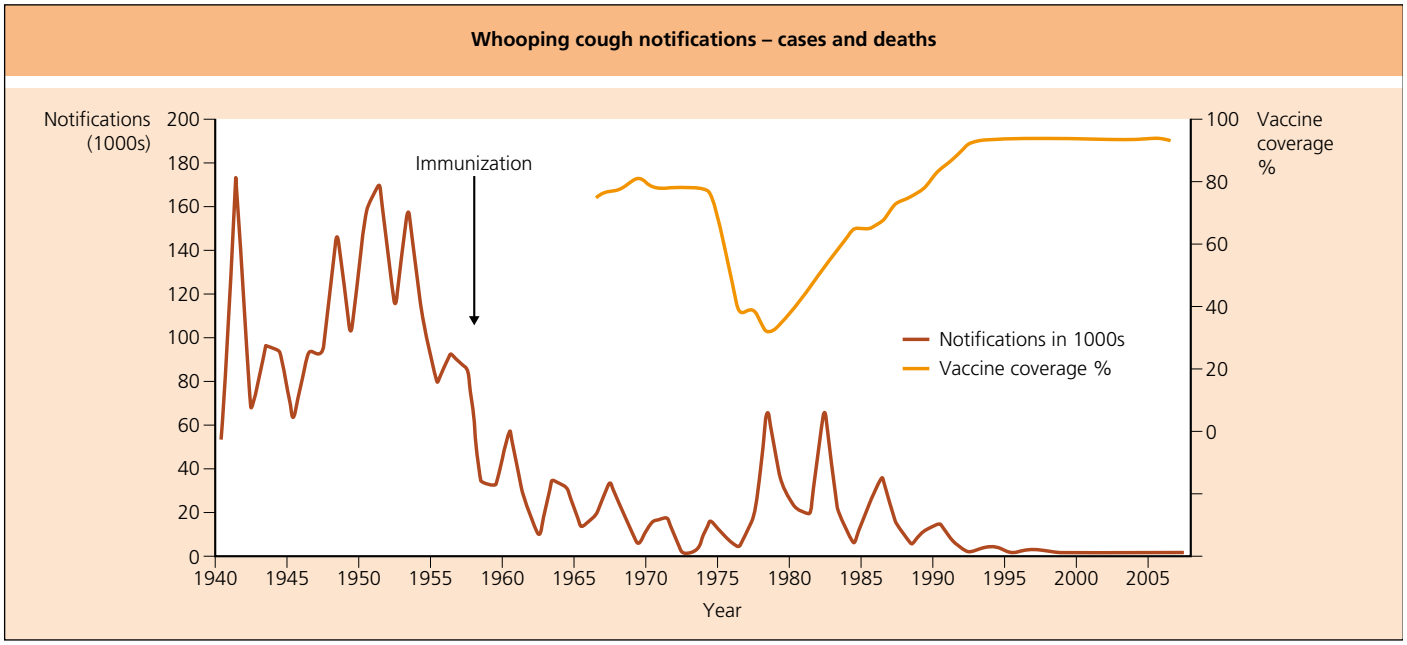


Fig. 172.1 Whooping cough notifications – cases and deaths. Figures are for England and Wales 1940–2007. Data supplied by the Health Protection Agency Centre for Infections.

are preferable for polymerase chain reaction (PCR) testing. These samples are far superior to 'cough plates'. Material collected for bacterial culture should be plated immediately on a suitable medium. If this is not possible it should be placed in a charcoal transport medium.[1] The traditional Bordet–Gengou agar, a potato infusion agar containing 10% glycerol and 20% sheep blood, has the disadvantage of a short shelf life. Better growth is obtained on charcoal blood agar, which can be made selective by the addition of cephalexin.

Cultures should be incubated in a moist aerobic atmosphere at 98.6°F (37°F) for at least 7 days. After 3–5 days of incubation, typical 'bisected pearl' colonies appear (Fig. 172.2). Suspect colonies should be checked by Gram staining and identified using slide agglutination with type-specific antisera. *B. pertussis* and *B. parapertussis* can be differentiated by a series of simple tests; *B. bronchiseptica* can be identified using a commercial galley test kit such as API 20NE.

Bordetella pertussis has three major surface agglutinogens (1, 2 and 3) which form the basis of a serotyping scheme. There are three serotypes responsible for infections: 1,2; 1,3 and 1,2,3. The serotype of an isolate can be determined by slide agglutination using cross-absorbed antisera. Type 1,3 is the most common serotype, accounting for 90% of isolates.



Fig. 172.2 Colonies of *Bordetella pertussis* on charcoal blood agar.

Direct immunofluorescence may be applied to respiratory secretions to give a rapid diagnosis, but this method lacks sensitivity and specificity[1] and has been largely superseded by PCR-based detection. Real-time PCR methods are more sensitive than culture, especially for specimens collected more than 3–4 weeks into the illness and following antibiotic treatment. A number of different primers have been used, including *IS481* which detects *B. pertussis* (and *B. holmesii*); *IS1001*, which detects *B. parapertussis* and *B. holmesii*; and *ptxA* which is specific for *B. pertussis*.[6]

Serologic diagnosis of *B. pertussis* is based on ELISA assay of IgG and IgA antibodies to pertussis toxin. A rise in titer of IgG or IgA (twofold or greater) to pertussis toxin in acute and convalescent phase samples taken at least 2 weeks apart is considered significant. In adolescents and adults a single high titer of antipertussis toxin IgG has a good predictive value of acute pertussis infection, with reported sensitivity of 76% and specificity of 99%.[5] Serology is unreliable in young infants.

CLINICAL MANIFESTATIONS

Bordetella pertussis is the cause of pertussis, or whooping cough. In a typical case, following an incubation period of 7–14 days, the patient develops red eyes, a runny nose, mild cough and sneezing ('catarrhal stage'), clinically indistinguishable from many viral upper respiratory tract infections. After about 1 week the cough becomes more severe and the patient experiences paroxysmal bouts of a severe, hacking cough. A paroxysm consists of repeated coughing followed by an inspiratory gasp as the patient finally inspires. This is the characteristic 'whoop'. Paroxysms may be triggered by a variety of stimuli, including cold air and loud noises. A child may become cyanosed during a paroxysm and can suffer fatal hypoxia. Often the child vomits at the end of a paroxysm and is left exhausted. The 'paroxysmal stage' may last for 1–4 weeks. This is followed by a lengthy 'convalescent stage' as the paroxysms decline and the patient slowly recovers. Many cases, but not all, develop a lymphocytosis.

Common complications include pneumonia (which may be primary or secondary to a supervening infection with another pathogen) and otitis media. The child may suffer cerebral hypoxia during a paroxysm which can lead to encephalopathy or fitting. Other possible complications include intraventricular hemorrhage, subconjunctival hemorrhages, umbilical or inguinal herniae, fractured ribs, ruptured diaphragm and rectal prolapse. Pertussis is most severe in very young

infants and in this age group the presentation may be atypical with no characteristic paroxysmal whooping. Similarly, partially immunized children and adults may have an atypical form of pertussis.[1]

Bordetella parapertussis is a cause of acute bronchitis or pertussis-like infections in children.[1] *Bordetella bronchiseptica* may rarely cause pneumonia, meningitis or whooping cough in highly immunocompromised patients.[1]

MANAGEMENT

Antimicrobial therapy for pertussis, when administered early in the course, can decrease transmission to susceptible contacts and possibly ameliorate symptoms. Macrolides are the antibiotics of choice and appear to reduce the severity and duration of the disease. Trimethoprim–sulfamethoxazole (co-trimoxazole) or fluoroquinolones are alternatives for older patients who are unable to tolerate erythromycin. Corticosteroids may be indicated in infants who have life-threatening disease. Secondary bacterial infections should be treated with appropriate antibiotics.

Brucella Species

NATURE

Brucella spp. are small, nonmotile, nonsporing, noncapsulate Gram-negative coccobacilli. They are aerobic, but some strains require 5–10% carbon dioxide for primary isolation. Growth on culture media is improved by the addition of serum or blood. Growth *in vitro* is slow and primary isolation may require up to 4 weeks' incubation. *Brucella* spp. are catalase-positive and usually oxidase-positive, but urease activity and hydrogen sulfide production are variable. *Brucella* is a monospecific genus, *Brucella melitensis*, with multiple biovars. In practice it is more useful to refer to separate species, which have differing host specificities. There are six species of *Brucella* (and two provisional species, *B. pinnipediae* and *B. cetaceae*, awaiting full validation), four of which can infect humans (Table 172.2). *B. melitensis* is the most virulent and is responsible for the majority of human infections. Both *Brucella abortus* and *Brucella suis* cause considerable morbidity in countries where brucellosis persists in domestic animals. The genomes of *B. melitensis*,[7] *B. abortus*[8] and *B. suis*[9] have been sequenced.

EPIDEMIOLOGY

Brucella spp. can infect a wide variety of domestic and wild animals. Brucellosis is a true zoonosis because humans acquire the infection directly or indirectly from infected animals. About 500 000 cases of human brucellosis are reported annually worldwide but this almost certainly is a gross underestimate of the true incidence.

Brucellosis is prevalent in Mediterranean countries, the Middle East, the Indian subcontinent, Mongolia and Central and South America.[10] Some countries have eradicated brucellosis, including the UK, much of northern Europe, Australia, New Zealand and Canada.

In their natural hosts, *Brucella* spp. cause chronic infections that are mild or asymptomatic. The bacteria localize in the reproductive tissues of ruminants, which are rich in mesoerythritol. Erythritol stimulates the multiplication of *Brucella* spp. This accounts for the main symptoms of brucellosis in animals – sterility or abortion. Erythritol is not present in human tissue. *Brucella* organisms are shed in large numbers in the products of conception, in urine and in milk, and when the animals are slaughtered. Humans are infected either by direct contact with infected animals or animal products or indirectly by ingesting infected milk or dairy produce.

In endemic areas the majority of cases occur in dairymen, herdsmen, abattoir workers, butchers and veterinary surgeons. Children may become infected in rural areas of developing countries if they live in close proximity with domestic animals. The general public generally acquires the infection through the ingestion of unpasteurized milk and milk products such as fresh soft cheese. Case-to-case transmission in humans is very rare. Self-inoculation with live *Brucella* vaccine is a recognized risk among veterinary surgeons. Laboratory workers are at risk of acquiring brucellosis through the inhalation of aerosols.[11] This may occur when staff handle as yet unidentified Gram-negative coccobacilli without adequate safety precautions. Brucellosis is considered a potential biologic weapon via airborne transmission.

Brucella melitensis is responsible for the majority of human infections and is primarily food-borne. The primary hosts for *B. melitensis* are sheep and goats. *Brucella abortus* and *B. suis* infections are generally sporadic and occur in people who work with cattle or pigs, respectively. *Brucella canis* infections are the least common in humans and are generally laboratory acquired.[12]

PATHOGENESIS

Brucella spp. are facultative intracellular parasites, surviving and multiplying within cells of the reticuloendothelial system. The organisms enter the body by inhalation, ingestion or after penetration of intact skin, abrasions or the conjunctival mucosa. The lipopolysaccharide (endotoxin) of smooth strains (S-LPS) is a major virulence determinant. S-LPS is antiphagocytic, facilitates cell entry, inhibits the fusion of *Brucella*-containing vacuoles (BCV) with lysosomes and inhibits host cell apoptosis. A two-component regulatory system, BvrR/BvrS is important for invasion and intracellular survival, and acts by regulating the expression of outer membrane proteins, Omp 3a (Omp25) and Omp 3b (Omp22).[13] The altered expression of surface proteins permits Brucellae to bind to and penetrate host cells.

Table 172.2 *Brucella* species pathogenic to humans

Species	Primary host	Humans as secondary host
Brucella abortus (biovars 1–6, 9)	Cattle, camels, yaks, buffalo	++
Brucella melitensis (biovars 1–3)	Goats, sheep, camels	++++
Brucella suis		
biovars 1–3	Pigs	+
biovar 2	Hares	+
biovar 4	Reindeer	+
biovar 5	Small rodents	+
Brucella canis	Dog, fox, coyote	+
Brucella pinnipediae, *B. cetaceae*	Minke whales, dolphins, porpoises, seals	+

The type IV secretion system (VirB) of *Brucella*, encoded by *virB* operon, is also important for adherence, cell entry and bacterial survival and replication within macrophages.[13]

After ingestion by phagocytic cells, the organisms proliferate in the local lymph nodes. The infection spreads hematogenously to tissues rich in elements of the reticuloendothelial system, including the liver, bone marrow, lymph nodes and spleen. Organisms may also localize in other tissues, including joints, central nervous system, heart and kidneys.

Endotoxin and hypersensitivity to *Brucella* antigens may cause some of the symptoms of brucellosis, including fever and weight loss. Antibodies against the *Brucella* LPS appear within a few days of the onset of the acute phase of the disease and are important in preventing re-infection. However, cell-mediated immunity, particularly the production of activated mononuclear phagocytes, is more important in promoting recovery.

PREVENTION

The ideal method of prevention of human brucellosis is to eliminate the disease from domestic livestock. This has been achieved in several countries. There are effective vaccines for cattle (S19) and goats (Rev-1).

Where eradication has not been achieved, people who are occupationally exposed should minimize the risk of infection by wearing protective clothing, gloves, goggles and masks. These measures are particularly important when handling animals that have recently given birth or aborted.

In the past, live, attenuated animal vaccines have been given to workers at high risk of contracting brucellosis. However, these vaccines may produce infection in humans and are far from ideal. Candidate vaccines, including an LPS–protein conjugate vaccine and a live attenuated strain are under development.[14] *Brucella* spp. are killed by heating at 140°F (60°C) for 10 minutes. Boiling or pasteurizing milk will therefore eliminate the risk of transmission via dairy products.

DIAGNOSTIC MICROBIOLOGY

Clinicians should inform laboratory staff when brucellosis is suspected so that they can handle samples and cultures in a containment level 3 laboratory and in a biologic safety cabinet to avoid laboratory-acquired infections. A definitive diagnosis depends on the isolation of *Brucella* spp. from cultures of blood, bone marrow or tissue taken early in the disease. Bone marrow cultures are reported to give a slightly higher yield than peripheral blood cultures but, since modern blood culture methods can produce positive results in up to 80% of cases, bone marrow cultures are not generally required or advocated. Biphasic Castaneda bottles were traditionally recommended to reduce the risk of contamination during subculturing. Cultures should be incubated at 98.6°F (37°F) for at least 6 weeks before they are discarded. Modern semiautomated blood culture techniques (e.g. Bactec®, Bact-Alert®) are suitable for the isolation of *Brucella* spp. The lysis centrifugation method also gives good results. These commercial systems may give positive results in a matter of days rather than weeks, depending on the initial inoculum of organisms in the sample. In developing countries where automated blood culture systems are not available, blood clot culture is more sensitive than whole blood culture.

Positive cultures should be subcultured onto serum dextrose agar or a similar medium. Selective culture media are not necessary for human blood cultures but may be required for contaminated material. *Brucella* spp. produce small, smooth, translucent colonies after 48 hours of incubation at 98.6°F (37°C) in air plus 5–10% carbon dioxide. Commercial galley-test identification systems may misidentify *Brucella* spp. as *Moraxella phenylpyruvica*,[11] and isolates should be sent to a reference laboratory for further tests to confirm the identity and to determine the biovar of the strain. This classification is based on the production of hydrogen sulfide, urease activity, the tolerance of the organism to the bacteriostatic dyes basic fuchsin and thionin and agglutination with monospecific antisera. Tbilisi (Tb) phage typing is also used to define *Brucella* spp. A real-time multiplex PCR assay has been described which can also be used to confirm the identity and species of *Brucella* isolates.[15] A number of PCR assays for diagnosing brucellosis in clinical samples have been described and can provide a rapid diagnosis of acute brucellosis and also an early detection of relapses. Antigen detection is another potential diagnostic tool.

Many serologic tests have been described for brucellosis. Interpretation of results is often difficult since both IgG and IgM antibodies can persist for a long time. A sample of serum should be collected as early as possible in the course of the disease. The serum agglutination test (SAT) using *B. abortus* strain 119 is widely used. False-negative results can occur if serum samples are not diluted beyond 1:320, owing to a prozone phenomenon. False-positive reactions may occur due to cross-reactivity with strains of *Escherichia coli*, *Vibrio cholerae*, *Yersinia enterocolitica* and *Francisella tularensis*. The SAT measures both IgM and IgG. To measure the quantity of specific IgG, serum is first treated with 2-mercaptoethanol to inactivate IgM. IgG and IgA antibodies seem to be the best indicators of acute infection. A rise in antibody titer is observed in more than 97% of patients who have acute brucellosis and positive blood cultures. An initial IgM antibody response is followed after 1–2 weeks by a rise in IgG. During recovery the IgG antibody titers slowly decline over a period of months but IgM antibodies may persist at a low level in the serum for several years. Sustained IgG antibody titers or a second rise in IgG antibody levels are seen in chronic infection or relapse. The SAT test cannot be used to diagnose *B. canis* infection.

Commercial ELISA tests based on antibodies to S-LPS are highly sensitive but appear to be less specific than the SAT. Rapid point-of-care tests, including fluorescent polarization immunoassay (FPA) and immunochromatographic *Brucella* IgM/IgG lateral flow assay (LFA) are highly sensitive and specific and have been used successfully to diagnose brucellosis in endemic areas.

CLINICAL MANIFESTATIONS (SEE ALSO CHAPTER 123)

Brucellosis is a systemic infection that can affect any tissue of the body. The protean, vague clinical symptoms are generally not specific for brucellosis. The possibility of brucellosis should be considered in any fever of unknown origin, especially in patients who have occupational exposure or have recently traveled in endemic areas.

MANAGEMENT

Before the introduction of antibiotics, brucellosis was a chronic, relapsing illness. A number of antimicrobial agents are active against *Brucella* spp. *in vitro* but they are not always clinically effective. *Brucella* spp. are sequestered intracellularly, so agents that achieve adequate intracellular concentrations should be used. It is also necessary to give a prolonged course of treatment to reduce the rate of relapsing infection. The World Health Organization (WHO) recommendations for treating brucellosis are either an oral regimen of doxycycline plus rifampin (rifampicin) daily for at least 6 weeks or oral doxycycline for 6 weeks supplemented by parenteral streptomycin daily for the first 2–3 weeks.[12] Gentamicin may be a suitable alternative to streptomycin (see also Chapter 123).

Francisella Species

NATURE

Francisella spp. are very small, faintly staining, pleomorphic Gram-negative, nonmotile and nonspore-forming coccobacilli. They are strictly aerobic, oxidase-negative and weakly catalase-positive. The organisms are surrounded by a thin, lipid-rich capsule. They attack carbohydrates slowly, producing acid but no gas. *Francisella tularensis* requires cysteine or cystine for growth and grows slowly at 98.6°F (37°C) on suitably enriched media.

Francisella tularensis causes tularemia in animals and humans. Within the species *F. tularensis* there are four subspecies, which differ in their virulence and their epidemiology (Table 172.3). *F. tularensis* subsp. *tularensis* (type A) is the most virulent and is found primarily in North America. There are two distinct genetic clades of *F. tularensis* subsp. *tularensis*; clade A1 is found in the central United States and has a high case-fatality rate; clade A2 occurs in the western USA and has a lower case-fatality rate. *Francisella tularensis* subsp. *holarctica* (type B) is more widely distributed than *F. tularensis* subsp. *tularensis* and is less virulent. The genomes of strains of both clades of *F. tularensis* subsp. *tularensis* have been sequenced.[16]

EPIDEMIOLOGY

The majority of human infections with *F. tularensis* occur in the northern hemisphere between latitudes 30° and 71°. It is well recognized in North America, Scandinavia and Russia, but has not been described in the UK.[17] The organism is found in many species of wild and domestic animals, birds, fish and blood-sucking arthropods. *Francisella tularensis* may persist in water, possibly in association with free-living amebae.

In the USA the most important reservoirs of *F. tularensis* are rabbits, hares, muskrats and ticks. In Scandinavia and Russia, rodents (such as voles and mice) and mosquitoes are important reservoirs. The modes of transmission to humans include tick or mosquito bites, contact with infected animal tissues, inhalation of an infectious aerosol or ingestion of contaminated meat or water. There is an increased risk of tularemia in occupations that bring people into contact with infected material.

There are approximately 200 cases each year reported in the USA, with over 60% occurring in the southern and southern-central states of Missouri, Oklahoma, Arkansas, Texas and Kansas. Most infections occur in males. Infections usually occur in summer following insect bites or winter when animals are hunted and trapped.

PATHOGENICITY

The infectious dose in humans depends on the subspecies and the route of entry. Inhalation of fewer than 10 organisms of *F. tularensis* subsp. *tularensis* can result in infection and up to 30% of untreated infections can be lethal.[17] The infecting dose rises to 10^8 when the organisms are ingested. Laboratory workers are at high risk through handling infected laboratory animals or cultures of the organism. *Francisella tularensis* is a hazard group 3 pathogen. *Francisella tularensis* subsp. *tularensis* (type A) is more virulent to animals and humans than the other subspecies. The low infecting dose, aerosol transmission, high virulence and high mortality of consequent infection with *F. tularensis* subsp. *tularensis* mean that this organism is a potential bioweapon.

It does not produce an exotoxin. Virulence determinants of *F. tularensis* include the lipopolysaccharide (LPS), capsule and type IV pili. The *Francisella* pathogenicity island (FPI) contains 19 genes that are necessary for intracellular growth and virulence. Genes within the FPI are regulated by the Mg1A protein.[18] The application of new molecular methods should further elucidate the molecular basis of *F. tularensis* pathogenicity, which is still poorly understood.

After entry into the body, the organisms spread to the regional lymph nodes, from where they may disseminate via the lymphatic system or bloodstream to involve multiple organs. There is probably a transient bacteremia at this early stage. *Francisella tularensis* is a facultative intracellular parasite and can multiply and survive for prolonged periods within macrophages, hepatocytes and endothelial cells. Intracellular survival is associated with a failure of phagosome–lysosome fusion and the bacteria replicate in the cell cytosol. The bacilli are protected from humoral antibodies, which are directed against carbohydrate antigens. Recovery from tularemia depends on cell-mediated immunity, which is directed against the protein antigens of the organism.

PREVENTION

Prevention of tularemia is best achieved by avoiding exposure to the organism. Gloves, masks and goggles should be worn when skinning or eviscerating animals. Animals that look sick should be left intact. Game meat should be thoroughly cooked and fresh water that is possibly contaminated should not be drunk. Ticks should be promptly removed and chemical insect repellants may be used.

A live attenuated vaccine (LVS) was used to immunize laboratory staff working with *F. tularensis* in the USA until recently. It provided good protection against an aerosol challenge of 10 organisms of a virulent strain

Table 172.3 *Francisella tularensis* subspecies and biovars

Subspecies	Geographical distribution	Virulence in humans	Virulence in rabbits	Main animal hosts
Tularensis (type A)				
Clade 1*	Central United States	+++	+++	Rabbits, hares, ground squirrels
Clade 2†	Western United States			
Holartica (type B)				
Biovar I	North America, Europe, Siberia, Far East, Kazakhstan	++	+	Rodents, beavers, muskrats
Biovar II	Eurasia			
Biovar japonica	Japan			
Mediasiatica	Central Asia	+	+	Rodents, rabbits
Novocida‡	North America	+	?	Muskrats

*Clade 1 is associated with distribution of Lone Star tick and American dog tick; it has a high case-fatality rate.
†Clade 2 is associated with distribution of Rocky Mountain wood tick and deer fly; it has a low case-fatality rate.
‡Low virulence and usually infections only occur in immunocompromised hosts.

of *F. tularensis* subsp *tularensis* but the degree of protection declined as the infecting dose increased. Problems with LVS, including reversion to virulence and variable immunogenicity, have led to its withdrawal. New novel epitope-based and subunit tularemia vaccines are being developed.[19]

DIAGNOSTIC MICROBIOLOGY

Isolation of *F. tularensis* from clinical material is potentially hazardous and it is imperative that the laboratory is notified if tularemia is suspected so that appropriate containment precautions can be taken. *Francisella tularensis* is a category 3 pathogen and should only be knowingly handled at containment level 3 in a biologic safety cabinet by trained and experienced staff.

The detection of *F. tularensis* in a Gram-stained smear of aspirates or other samples is rarely successful. PCR methods have been described that can be applied to clinical samples and to identify the subspecies of an isolate.

The organism requires cysteine or cystine for growth. Specialized media such as cysteine–glucose–blood agar have been devised, but *F. tularensis* will grow on some media that are routinely used for other organisms. Chocolate blood agar is often supplemented with cysteine (e.g. IsoVitaleX) and will then support the growth of *F. tularensis*. Similarly, *F. tularensis* will grow on buffered charcoal yeast extract agar (BCYE), which is normally used to isolate *Legionella* spp.

Cultures should be incubated at 98.6°F (37°F) with additional carbon dioxide in the atmosphere of incubation. *Francisella tularensis* grows slowly, taking 2–4 days to produce visible colonies, and cultures should be incubated for 3 weeks before being discarded as negative. The addition of penicillin to the medium will suppress the overgrowth of contaminants.

Colonies of *F. tularensis* are blue-gray, round, smooth and somewhat mucoid. They are β-hemolytic on blood-containing media. The identity of the organism can be confirmed by slide agglutination or fluorescent antibody staining. Alternatively, PCR targeting the *fopA* or *tul4* gene may be used.[20] Biochemical tests are of little value. Some commercial identification tests may misidentify *F. tularensis* as *Aggregatibacter actinomycetemcomitans*. Further subspeciation should only be undertaken in a reference laboratory.

Real-time PCR tests for the diagnosis of tularemia have been described. Many cases of tularemia are diagnosed serologically. An agglutination test using killed *F. tularensis* antigen or an ELISA is generally used. A fourfold rise in antibody titer or a single titer of more than 1:160 is considered diagnostic. IgG and IgM antibodies can persist for months or years and it may be difficult to distinguish past from current infection. Infections with *Brucella* spp. can give rise to antibodies that cross-react with *F. tularensis*. A commercial latex agglutination test is also available.

CLINICAL MANIFESTATIONS (SEE ALSO CHAPTER 121)

The clinical manifestations of *F. tularensis* infection depend on the portal of entry, the virulence of the infecting organism and the immune status of the individual host.

MANAGEMENT

Streptomycin remains the drug of first choice for treating all forms of tularemia. Gentamicin is an acceptable alternative. Ciprofloxacin and other quinolones have been used successfully to treat tularemia. Tetracycline and chloramphenicol have been used to treat infections but both are bacteriostatic for *F. tularensis* and there is a high rate of relapse when these agents are used. *Francisella tularensis* produces a β-lactamase, and β-lactamase-stable β-lactams, such as cefotaxime, ceftriaxone and imipenem, may be of use, although they have not been fully evaluated. For postexposure prophylaxis oral doxycycline or ciprofloxacin for 14 days is recommended.[21]

Haemophilus Species

NATURE

Haemophilus spp. are small, pleomorphic, nonmotile, nonsporing Gram-negative rods or coccobacilli. They are aerobic and facultatively anaerobic. Growth is often enhanced by the addition of 5–10% carbon dioxide to the incubation atmosphere. The oxidase and catalase reactions vary among the species. *Haemophilus* spp. require one or both of two accessory growth factors (X and V). X factor can be provided by hemin, protoporphyrin IX or other iron-containing porphyrins. X-dependent *Haemophilus* spp. cannot synthesize protoporphyrin from δ-aminolevulinic acid, a process involving several enzyme-mediated steps, some or all of which may be defective. V factor is nicotinamide adenine dinucleotide (NAD) or NAD phosphate or certain unidentified precursors of these compounds. It is essential for oxidation–reduction processes.

The differential requirements for X and V factors are important criteria for defining *Haemophilus* spp., which are obligate parasites of humans and animals. The species of *Haemophilus* associated with infections in humans are shown in Table 172.4. *Haemophilus influenzae* is the major human pathogen in the group. Some strains of *H. influenzae* have a polysaccharide capsule, of which there are six distinct antigenic types, designated a–f. The most important is type b, which has a capsule consisting of polyribosyl ribitol phosphate (PRP). *Haemophilus influenzae* type b (Hib) strains are associated with the majority of invasive infections.

Haemophilus influenzae biogroup *aegyptius* (formerly *Haemophilus aegyptius*) is a cause of epidemic conjunctivitis and Brazilian purpuric fever.[22] *Haemophilus ducreyi* is the causative agent of chancroid. *Haemophilus aphrophilus*, *H. paraphrophilus* and *H. parainfluenzae* are occasionally implicated in infective endocarditis and in brain and liver abscesses. *Haemophilus pittmaniae*[23] is part of the normal oropharyngeal flora that has been isolated from various sites of infection, including blood and bile. *Haemophilus haemolyticus* is a pharyngeal commensal that has classically been differentiated from *H. influenzae* by the demonstration of β-hemolysis on blood agar. However a significant number of *H. haemolyticus* strains are nonhemolytic and may be erroneously identified as *H. influenzae*.[24] Neither hemolytic nor nonhemolytic strains of *H. haemolyticus* have ever been implicated as the cause of infection.

The two species *H. aphrophilus* and *H. paraphrophilus* have recently been combined as a single species and reassigned to the genus *Aggregatibacter* as *Aggregatibacter aphrophilus* sp. nov. comb.[25] Similarly, *Haemophilus segnis* has been reassigned as *Aggregatibacter segnis*. The name *H. quentini* has been proposed for a group of *Haemophilus* strains isolated from neonatal and female genital tract infections which were previously termed a cryptic genospecies of *Haemophilus* but this name has not to date been validly published.

The genome of *Haemophilus influenzae* strain Rd was the first bacterial genome to be fully sequenced.[26]

EPIDEMIOLOGY

Haemophilus influenzae is an obligate parasite of human mucous membranes; it is not found in any other animal species. It colonizes the throat and nasopharynx, and to a lesser extent the conjunctivae and genital tract. The respiratory tract is colonized by *Haemophilus* spp. – mainly *H. parainfluenzae* and noncapsulate strains of *H. influenzae* (ncHi) – in 25–84% of healthy people. The carriage rates for Hib are much lower (3–5%).

Table 172.4 Species of *Haemophilus* associated with infection of humans

Species	REQUIREMENT FOR			Hemolysis	ACID FROM			
	X factor	V factor	CO_2		Glucose	Sucrose	Lactose	Mannose
H. influenzae	+	+	–	–	+	–	–	–
H. influenzae biogroup *aegyptius*	+	+	–	–	+	–	–	–
H. haemolyticus	+	+	–	+‡	+	–	–	–
H. parainfluenzae	–	+	–	–	+	+	–	+
H. pittmaniae	–	+	–	+	+	+	–	+
H. parahaemolyticus	–	+	–	+	+	+	–	–
*H. aphrophilus**	h	–	+	–	+	+	+	+
*H. paraphrophilus**	–	+	+	–	+	+	+	+
H. segnis†	–	+	–	–	w	w	–	–
H. ducreyi	+	–	–	–	–	–	–	–

h, requires hemin for primary isolation; w, weak reaction.
**H. aphrophilus* and *H. paraphrophilus* have been reassigned to the genus *Aggregatibacter* as *Aggregatibacter aphrophilus* sp. nov. comb.
†*H. segnis* has been reassigned to the genus *Aggregatibacter* as *Aggregatibacter segnis*.
‡*H. haemolyticus* strains can be nonhemolytic.

Table 172.5 Spectrum of infections caused by *Haemophilus influenzae*

Infection	Age group affected	Strains
1. Invasive infections: Meningitis Epiglottitis Pneumonia Septic arthritis Osteomyelitis Cellulitis Bacteremia	90% children <4 years old* 10% older children and adults	90% type b (Hib) 10% ncHi 1% types e and f
2. Neonatal and maternal sepsis	Neonates Parturient mothers	>90% ncHi
3. Noninvasive respiratory infections: Otitis media Sinusitis Conjunctivitis Acute exacerbations of chronic bronchitis	Children and adults	>90% ncHi2

ncHi, noncapsulated *Haemophilus influenzae*.
*Figures reflect prevaccine incidence and age distribution.

Haemophilus influenzae is associated with two types of infection, invasive infections and noninvasive infections which have distinctive epidemiologic profiles (Table 172.5).

Haemophilus influenzae type b meningitis principally affects children aged 2 months to 2 years. Epiglottitis occurs in slightly older children, with the peak incidence being in children aged 2–3 years. Risk factors for invasive disease are mainly socioeconomic and include overcrowding, attendance at day-care centers, chronic illness and lack of access to good health care facilities.

Invasive Hib infections are distributed worldwide but the incidence varies from country to country with very high incidence rates being found in certain racial groups, for example Apache and Navajo Native Americans, Alaskan Inuit and Australian Aborigines (Table 172.6). In these racial groups the peak incidence of infection occurs at a younger age. By contrast, a very low incidence rate has been reported in Hong Kong Chinese. There is seasonal variation, with most cases occurring in the winter months.

The introduction of vaccines effective against Hib in infants has dramatically reduced the incidence of invasive disease in children in countries using this vaccine, and consequently the epidemiology is changing.

The noninvasive infections of the respiratory tract associated with *H. influenzae* include otitis media, sinusitis, acute exacerbations of chronic bronchitis and conjunctivitis. The majority of these infections are caused by noncapsulate strains and many occur in adults. There is often an underlying physiologic or anatomic abnormality. These infections have a worldwide distribution and all of them are more common in the winter months.

Haemophilus influenzae biogroup *aegyptius* is associated with an acute pediatric condition of seasonal conjunctivitis and septicemia (Brazilian purpuric fever).

Table 172.6 Annual incidence of *Haemophilus influenzae* type b meningitis

Country	Incidence*
England	25
Finland	26
USA (Texas, North Carolina, Maryland, California)	19–69
Australia (New South Wales, Victoria)	20–25
Hong Kong Chinese	2.7
Gambia	60
Alaskan Inuit	282
Apache Native Americans	254
Australian Aborigines	454

*Figures for incidence per 100 000 children under the age of 5 years and are from various countries and ethnic groups prior to the introduction of routine immunization with Hib conjugate vaccine.

PATHOGENICITY

Haemophilus influenzae is transmitted by aerosols of respiratory secretions or by direct contact with contaminated material. The primary event is colonization of the nasopharynx. Prior infection with respiratory viruses (e.g. influenza) promotes colonization and subsequent infection by *Haemophilus* spp. Noninvasive infections of the respiratory tract result from contiguous spread of organisms colonizing the respiratory tract. Acute sinusitis, otitis media and acute exacerbations of chronic bronchitis usually follow a viral infection. This predisposes to secondary infection with potentially pathogenic components of the local resident bacterial flora by mechanisms including obstruction to the outflow of respiratory secretions, depression of local immunity and suppression of mucociliary clearance. The majority of these infections are caused by noncapsulate strains.

There are a number of microbial factors that promote colonization, including fimbriae and other adhesins, lipo-oligosaccharide (LOS) and IgA1 protease.[27] LOS consists of two covalently linked moieties, lipid A and core oligosaccharide. LOS is a ciliotoxin and is important for colonization, persistence and survival of the bacteria in the human host. The lipid A moiety mediates the endotoxic effects that are associated with severe infection.

IgA1 protease inactivates secretory IgA, facilitating bacterial access to the mucosal surface. Fimbriae and the autotransporter proteins, Hap, HMW1/HMW2 and Hia/Hsf are associated with binding to respiratory tract epithelium. Hap is found in both noncapsulate and capsulate strains and promotes the entry of bacteria into epithelial cells. HMW1/HMW2 proteins are expressed by the majority of noncapsulate *H. influenzae* but are not found in capsulate strains. Hia is found in nearly all noncapsulate strains. Its analogue, Hif, is expressed in almost all capsulate strains. Possession of the outer membrane protein P2 is another important virulence factor for noncapsulate *H. influenzae*. Lipoprotein D (LPD) is a highly conserved 42 kDa surface lipoprotein found in all *H. influenzae*, which impairs respiratory ciliary function.

Invasive infections, notably meningitis and epiglottitis, are mainly caused by Hib and result from invasion of the bloodstream. The pathogenesis of these infections has been elegantly elucidated using an infant rat model.[28] The capsule of type b *H. influenzae* is the single most important virulence determinant for invasion because it protects the organism from phagocytosis and complement-mediated lysis. The rarity of infections in the first 2 months of life correlates with the presence of maternal antibodies to PRP and the occurrence of infection in early infancy with the absence of antibodies having such specificity. As the mean level of PRP antibodies in the population rises, so Hib infections become less common. It is unclear whether natural antibody production is stimulated by exposure to Hib or to some other organism (e.g. *Escherichia coli* K100) that possesses cross-reacting antigens.

Brazilian purpuric fever is caused by a single clone of *H. influenzae* biogroup *aegyptius*.[29] The LOS phenotype is a critical determinant of virulence for *H. influenzae* biogroup *aegyptius*.

PREVENTION

Vaccination

Haemophilus influenzae type b conjugate vaccines have now been developed in which the PRP is covalently linked to a protein carrier. Several different protein carriers have been used, including tetanus toxoid, diphtheria toxoid, a non-toxic mutant diphtheria and an outer membrane protein complex from *Neisseria meningitidis* group B. These vaccines produce a lasting anamnestic response, which is not age related. They can be given effectively to infants as young as 2 months of age and are also effective in high-risk groups of patients who have a poor response to polysaccharide vaccines. The Hib vaccine can be given concurrently with diphtheria–tetanus–pertussis immunization. In the UK Hib vaccine is administered at 2, 3 and 4 months as a component of a pentavalent vaccine (diphtheria, tetanus, acellular pertussis, Hib and inactivated polio vaccine), with a booster dose (Hib combined with meningococcus C vaccine) being given at 12 months of age. Countries where Hib vaccine is routinely offered have witnessed a dramatic decline in the occurrence of Hib infections (Fig. 172.3; see also Chapter 3).

Immunization of infants with conjugate Hib vaccine results in a reduction in the rate of nasopharyngeal colonization by Hib and a marked herd immunity effect.

Haemophilus influenzae type b vaccine is recommended after splenectomy, or for patients who have functional asplenia, together with pneumococcal vaccine and meningococcal vaccine.

There is currently considerable interest in developing vaccines for nontypeable *H. influenzae*. A 10-valent pneumococcal conjugate vaccine, using *H. influenzae*-derived protein D as a carrier protein for the pneumococcal polysaccharide, showed protection against both pneumococcal and noncapsulate *H. influenzae* otitis media.[30] Several candidate antigens are being investigated (Table 172.7).[31]

Chemoprophylaxis

Oral rifampin for 4 days is recommended for eradicating carriage of *H. influenzae* type b and has been used to prevent secondary infection in household and nursery contacts. Its efficacy is unproven. Widespread use of the vaccine may soon render chemoprophylaxis unnecessary.

DIAGNOSTIC MICROBIOLOGY

Gram-stained smears of cerebrospinal fluid, pus, sputum or aspirates from joints, middle ear or sinuses may provide a rapid diagnosis. *Haemophilus influenzae* tends to stain poorly and dilute carbol fuchsin is a better counterstain than neutral red or safranin.

Rapid tests for Hib capsular antigens, such as latex agglutination or a PCR-based method, can be applied to clinical material. False-positive latex agglutination results have been reported in cerebrospinal fluid samples collected from children who had recently received Hib vaccine.

Specimens should be plated on appropriate culture media as soon as possible. Chocolate agar is the most commonly used medium for culturing *Haemophilus* spp. It can be used without further

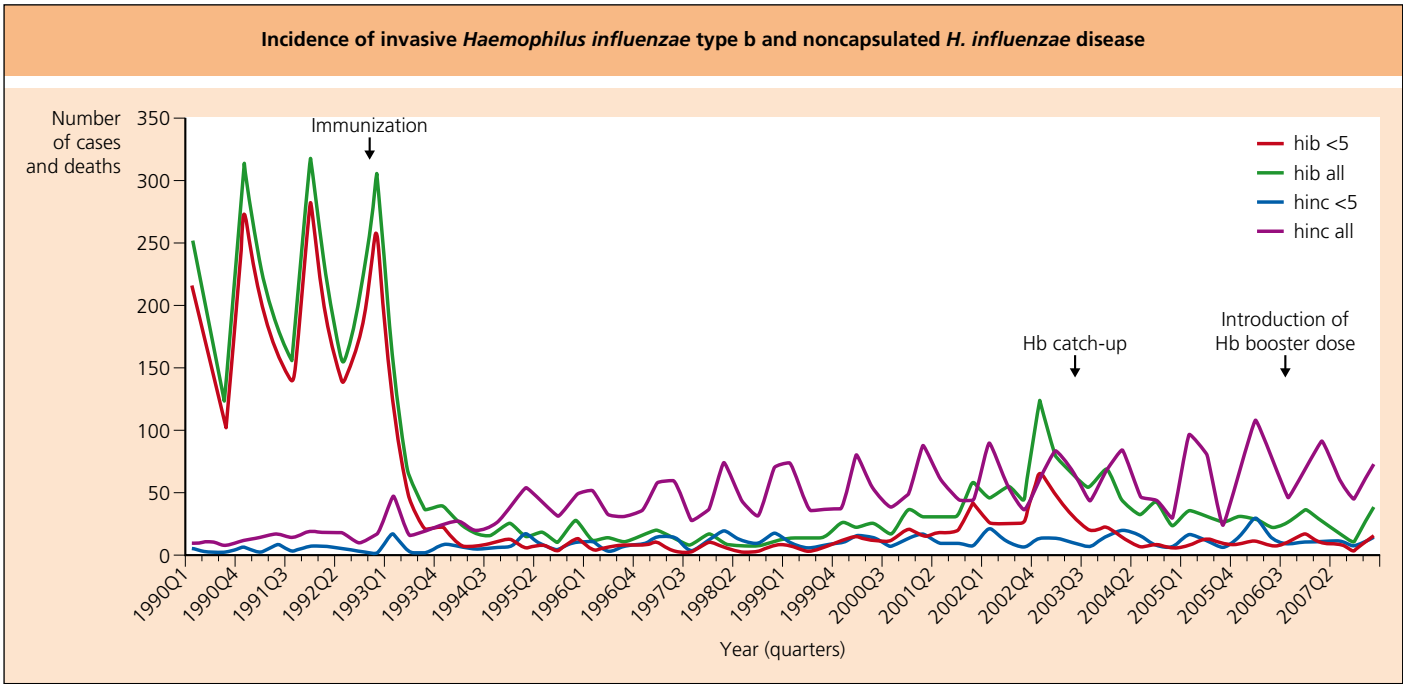


Fig. 172.3 Incidence of invasive *Haemophilus influenzae* type b and noncapsulated *H. influenzae* disease. Figures are for the period from October 1990 to June 2008 in England and Wales. Data supplied by the Health Protection Agency Centre for Infections.

Table 172.7 Candidate vaccine antigens of noncapsulate *Haemophilus influenzae* (ncHi)

Antigen	Protective in animal model	Comments
Lipo-oligosaccharide (LOS)	+	Common epitopes among ncHi strains
P6	+	Highly conserved lipoprotein
OMP26	+	Highly conserved
OMP P5	+	Highly conserved
Lipoprotein D (LPD)	+	Recombinant deacylated form (protein D) used as carrier protein for pneumococcal conjugate vaccine
OMP P2		>50% of total OMP of ncHi Heterogeneous and conserved regions
HtrA	±	
HMW1/HMW2	+	Found in >75% ncHi strains
P4 lipoprotein	+	Highly conserved

supplementation for specimens from normally sterile sites. The addition of bacitracin to chocolate agar will assist the recovery of *H. influenzae* from respiratory samples because it will suppress the growth of other respiratory organisms. Good growth can also be obtained on certain transparent media that contain blood extracts (e.g. Levinthal's agar or Fildes peptic digest agar).

The cultures should be incubated at 98.6°F (37°C) in an aerobic atmosphere enriched with 5–10% carbon dioxide. After 24 hours of incubation, colonies of noncapsulate *H. influenzae* are small, circular, smooth and pale gray. Capsulated strains produce somewhat larger, mucoid colonies, which have a characteristic seminal odor. On clear media, such as Levinthal's agar, colonies of capsulated strains exhibit iridescence when viewed obliquely with transmitted light. Type b capsulated strains can also be detected by the presence of precipitin haloes on transparent media containing hyperimmune Hib antiserum.

Confirmation of the identity of colonies depends on demonstrating a requirement for X factor, V factor, or both. This is usually performed as a disk test. The culture is plated onto a nutrient agar that is deficient in both X and V factors, and paper disks containing X factor alone, V factor alone, and both X and V factors are placed on the surface of the agar. After overnight incubation, growth is observed around the disks supplying the required growth factors (Fig. 172.4). An alternative method of demonstrating X-factor requirement is the porphyrin test.[32]

Haemophilus influenzae strains should be typed to determine their capsular serotype. This is normally carried out by slide agglutination using type-specific antisera. This method is prone to misinterpretation, with problems of autoagglutination, cross-reacting antisera and observer error. The definitive method of typing *H. influenzae* is capsular genotyping using a PCR-based method.[33]

CLINICAL MANIFESTATIONS

Haemophilus influenzae is associated with two distinct types of infection: invasive and noninvasive infections.

Invasive infections

Haemophilus influenzae type b (Hib) meningitis is the commonest and most serious manifestation of systemic infection with *H. influenzae*. Prior to the use of Hib conjugate vaccine, Hib meningitis principally occurred in young children between the ages of 2 months and 2 years. In countries that have introduced routine infant immunization with Hib conjugate vaccine the incidence

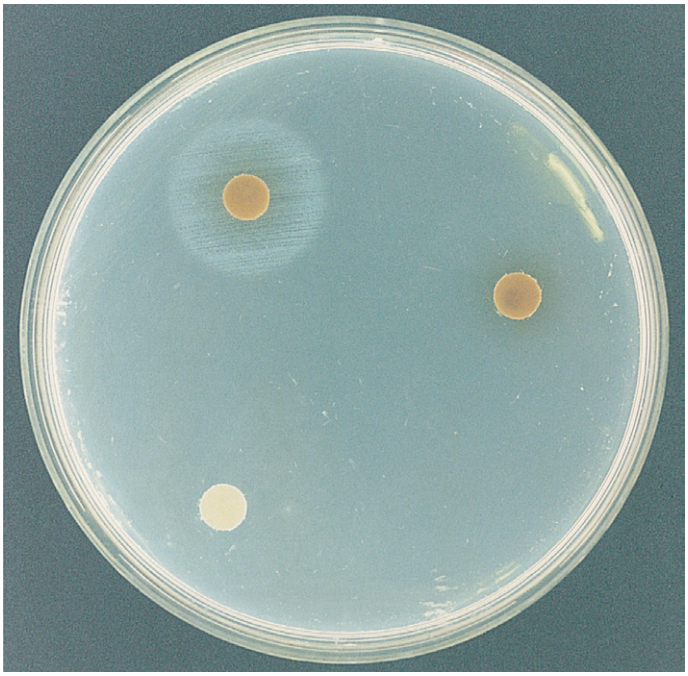


Fig. 172.4 Growth factor requirement of *Haemophilus influenzae*. Strain of *H. influenzae* sown on Columbia agar plate. Filter paper disks containing X factor, V factor, and both X and V factors have been placed on the surface of inoculated plate, but colonies of *H. influenzae* grow only around the disk with both X and V factors.

of invasive *H. influenzae* infections has dramatically declined. *Haemophilus* meningitis can also occur in neonates, in whom the causative organism is almost invariably noncapsulate and the presentation can resemble group B streptococcal sepsis. Meningitis caused by either type b or noncapsulated strains can occur in older children and adults.

Epiglottitis is a life-threatening infection that is almost always caused by Hib. It occurs in children, aged 2–5 years, with a peak incidence in those aged 2–3 years. Examination of the larynx is potentially hazardous because it may provoke respiratory obstruction. The diagnosis should be based on the clinical findings confirmed by blood culture results (see also Chapter 24).

Other less common invasive *H. influenzae* infections include pneumonia, periorbital cellulitis, osteomyelitis, septic arthritis and a bacteremia with no obvious focus of infection. *Haemophilus influenzae* pneumonia is a major cause of mortality in young children in the developing world.

Noninvasive infections

A variety of local infections, principally in the respiratory tract, may be seen. These include otitis media, sinusitis, conjunctivitis and acute exacerbations of chronic bronchitis (see Chapter 26). Most of these infections are caused by noncapsulated strains of *H. influenzae*. *Haemophilus influenzae* is an important cause of purulent conjunctivitis. This may be sporadic but can occur in outbreaks. Severe conjunctivitis associated with severe sepsis and a high mortality was first described in 1984 in Brazilian children aged 1–4 years. The causative organism is a clone of *H. influenzae* biogroup *aegyptius*, which is indistinguishable in routine laboratory tests from noncapsulate *H. influenzae*.

Rarely *H. influenzae* may be associated with other infections, including endocarditis, urinary tract infections, cholecystitis, salpingitis and epididymo-orchitis. Many of these unusual infections are associated with pre-existing damage, including the presence of urinary stones or gallstones.

Haemophilus aphrophilus, *H. paraphrophilus* (now reclassified as *Aggregatibacter aphrophilus*) and *H. parainfluenzae* may be associated with infective endocarditis, meningitis and cerebral abscesses.[34] These species form part of the HACEK group (see below).

Haemophilus segnis (*Aggregatibacter segnis*) is occasionally associated with acute appendicitis.

MANAGEMENT

Cefotaxime, ceftriaxone and related third-generation cephalosporins are the antibiotics of choice for treating *Haemophilus* meningitis. They are bactericidal for *H. influenzae*, achieve high concentrations in the meninges and cerebral tissues, and have proved highly effective in clinical practice.

Chloramphenicol was formerly the treatment of choice. Resistance may be encountered but in most parts of the world this remains uncommon. Ampicillin is also effective but resistance due to a β-lactamase is now encountered in an unacceptably high percentage of strains. For example, 13% of Hib strains in the UK are β-lactamase-producing. In view of this, ampicillin should no longer be used as a single agent in *Haemophilus* meningitis in countries where ampicillin resistance is prevalent.

For the treatment of less serious respiratory infections, oral antibiotics including amoxicillin, amoxicillin–clavulanate, tetracycline, erythromycin or newer macrolides such as clarithromycin or azithromycin are all effective. β-Lactamase-mediated amoxicillin resistance may again be a problem. In the UK, 19% of noncapsulate isolates are β-lactamase producers. Occasional strains are resistant to ampicillin by an intrinsic mechanism.

Haemophilus ducreyi

EPIDEMIOLOGY

Haemophilus ducreyi (see Chapter 60) is the causative agent of the sexually transmitted disease chancroid, or soft sore. Chancroid is endemic in tropical areas, particularly central and southern Africa, South East Asia, India and the Caribbean where it is responsible for 25–50% of genital ulcers.[35] It occurs sporadically in other parts of the world and recently there have been major outbreaks in Canada and the USA. It is more common in nonwhite, uncircumcised males and in low socioeconomic groups living in poor hygienic conditions. Infection may be asymptomatic or inapparent in females. Genital ulcers, including chancroid, are associated with increased transmission of HIV. Co-infection with *Treponema pallidum* or herpes simplex virus (HSV) is common. The incidence of *H. ducreyi* has been decreasing over recent years, whilst the incidence of HSV type 2 genital ulcers has increased. The reason for this declining incidence is unclear.

PATHOGENICITY

Haemophilus ducreyi has not been isolated from nonhuman sources and is sexually transmitted. The organisms gain access via a break in the epithelium and establish infection in a focal area of mucosa or skin in the genital tract. An inoculum of 10^4 organisms is required to initiate infection. The pathogenesis of chancroid is poorly understood. Hemolysin is important in epithelial cell invasion and ulcer formation. Superoxide dismutase enzymes contribute to survival of the bacteria within the host. Potential virulence factors include fimbriae, LPS, cytotoxins and outer membrane proteins (OMP).[35]

PREVENTION

The use of condoms dramatically reduces the transmission of *H. ducreyi*. All sexual partners should be identified by contact tracing and treated. Education is vitally important. *Haemophilus ducreyi* hemolysin is a possible candidate for vaccine development.

DIAGNOSTIC MICROBIOLOGY

Gram-stained examination of material from ulcers or buboes reveals pleomorphic coccobacilli or short rods. The organisms are more commonly extracellular but may be intracellular. Characteristically they resemble 'shoals of fish'. However, ulcer material is generally contaminated with other bacteria and direct Gram staining identifies only about 10% of culture-positive cases and microscopy is not recommended as a diagnostic test.[36] Specimens collected from the base and margins of ulcers using a saline-moistened swab or by aspirating a bubo should be plated directly onto culture plates which should be incubated in a humid atmosphere with 5% carbon dioxide at 91.4°F (33°C) for 2–3 days.[36]

Haemophilus ducreyi is a fastidious organism that grows slowly on chocolate agar. In general a suitable medium contains a growth supplement such as IsoVitaleX and is made selective by the addition of vancomycin. The sensitivity of culture is low but can be increased somewhat by using more than one medium.

The appearance of colonies of *H. ducreyi* varies with the medium used. Typically, after 24 hours' incubation the colonies are pinpoint, yellow-gray and translucent or semiopaque. Cultures often appear mixed, with a variety of colonial forms. The colonies are very cohesive and can be pushed across the agar surface with an inoculating loop.

Haemophilus ducreyi requires X factor but not V factor for growth. It can be identified using a number of rapid test systems, including API-ZYM and RapIDNH.

PCR is the most sensitive diagnostic technique and has been shown to be 95% sensitive compared to culture.[36] Multiplex PCR tests that also amplify *Treponema pallidum* and HSV have been developed.[37]

CLINICAL MANIFESTATIONS

These are described in Chapter 60.

MANAGEMENT

The susceptibility of *H. ducreyi* to antimicrobial agents varies from one geographic area to another. Many strains are β-lactamase producers and resistance to tetracycline and trimethoprim–sulfamethoxazole is common. Azithromycin and ceftriaxone exhibit excellent *in-vitro* activity against *H. ducreyi* and have the advantage that they can be used as single-dose therapy.[38] Ciprofloxacin and erythromycin are alternative agents, but strains with intermediate resistance to both of these agents have been reported.

Buboes should be drained to prevent sinus formation.

HACEK Group

NATURE

The HACEK group of organisms includes *H. parainfluenzae*, *H. aphrophilus*, *H. paraphophilus*, *Actinobacillus actinomycetemcomitans*, *Cardiobacterium hominis*, *Eikenella corrodens* and *Kingella kingae*. *Haemophilus aphrophilus* and *Haemophilus paraphrophilus* have now been reassigned to the genus *Aggregatibacter* and combined as a single species, *Aggregatibacter aphrophilus* sp. nov. comb. Similarly, *Actinobacillus actinomycetemcomitans* has been renamed *Aggregatibacter actinomycetemcomitans* sp. nov.[23] It was previously thought that the genus *Cardiobacterium* consisted of a single species, *C. hominis*. However a second species, *C. valvarum*, has now been described[39] which has also been associated with infective endocarditis. All of the HACEK group are small, fastidious, pleomorphic Gram-negative bacilli that form part of the normal commensal flora of the oropharynx and are infrequent causes of human infections. They are slow growing, their growth being enhanced by the use of enriched culture media and an increased carbon dioxide tension.

Cardiobacterium hominis and *Eikenella corrodens* usually require hemin (X factor) and carbon dioxide on primary isolation and may be misidentified as *Haemophilus* spp.; these requirements are lost on subculture. The colonies of *E. corrodens* have a characteristic 'bleach-like' odor and often 'pit' the surface of the agar. Colonies of *K. kingae* produce slight β-hemolysis on blood agar.

CLINICAL MANIFESTATIONS

Collectively, the HACEK group of organisms causes 3% of all cases of infective endocarditis[40] and is responsible for a variety of other infections (see Chapter 47).

Aggregatibacter actinomycetemcomitans was originally thought to be a secondary pathogen in actinomycotic infections caused by *Actinomyces israelii*. It is now recognized as a cause of periodontal infections, brain and other soft tissue abscesses, osteomyelitis and infective endocarditis.[41]

Cardiobacterium hominis and *C. valvarum* have been implicated in cases of natural and prosthetic valve endocarditis[42] but rarely cause other types of infection.

Eikenella corrodens tends to cause indolent infections, often in association with other organisms. Infections associated with *E. corrodens* include periodontitis, human bite wounds, sinusitis, meningitis, brain abscess, pulmonary infections, pelvic infections, endocarditis, osteomyelitis and septic arthritis.[43] Clenched fist injuries are the most serious human bite wounds and are sustained when the knuckles of the assailant's hand strike the teeth of the adversary.

Kingella spp. (*K. kingae* and *K. denitrificans*) are associated with endocarditis and osteoarticular infections. Since the introduction of routine Hib immunization in the USA, and consequent decline in Hib septic arthritis, *K. kingae* has emerged as a major cause of septic arthritis in children less than 2 years of age.[44] *K. kingae* expresses an RTX toxin which is cytotoxic to respiratory epithelium, synovial cells and macrophage-like cells.[45]

Haemophilus parainfluenzae and *Aggregatibacter aphrophilus* (formerly *H. aphrophilus* and *H. paraphophilus*) may cause endocarditis. *Haemophilus parainfluenzae* has been reported as a rare cause of the spectrum of infections associated with *H. influenzae*: meningitis, epiglottitis, septic arthritis, osteomyelitis, pneumonia and bacteremia. *Aggregatibacter aphrophilus* has been implicated in cases of brain abscesses, septic arthritis, osteomyelitis, epiglottitis and meningitis.

PREVENTION

Approximately 60% of cases of endocarditis caused by HACEK organisms are associated with poor oral hygiene, bad dentition or recent dental work.[40] Prevention of endocarditis should therefore be based on good dental and oral hygiene in patients at risk of endocarditis.

MANAGEMENT

The treatment of endocarditis caused by HACEK organisms should be based on antimicrobial susceptibility tests, including tests for β-lactamase

activity. Since many of these organisms are slow growing, such tests can be problematic. Currently, third-generation cephalosporins, including cefotaxime or ceftriaxone, are the drugs of choice for treating endocarditis caused by the HACEK organisms.

Eikenella corrodens is generally susceptible to penicillin, but consideration should be given to the possibility of β-lactamase-producing aerobic and anaerobic organisms when treating mixed infections, such as human bites.

Legionella Species

NATURE

Legionella spp. are slender, aerobic, noncapsulated, non-spore-forming pleomorphic Gram-negative coccobacilli. In clinical material they often appear as coccobacilli but they tend to form elongated filamentous forms on some culture media. They are motile with a single polar flagellum. *Legionella* spp. stain poorly with Gram stain. Basic fuchsin is a better counterstain for these organisms. They do not attack carbohydrates, and nitrate is not reduced. The cell wall contains branched-chain fatty acids with high concentrations of ubiquinones, which give characteristic profiles by gas–liquid chromatographic analysis. The genome sequences of several *L. pneumophila* strains (Philadelphia, Paris, Lens and Corby) have been established.[46]

Legionella spp. are nutritionally fastidious and will not grow on ordinary culture media. A suitable medium for their isolation is buffered charcoal yeast extract (BCYE) supplemented with L-cysteine, α-ketoglutarate and ferric ions. The pH of the medium should be adjusted to pH 6.[9] *Legionella* can grow in the temperature range 68–107.6°F (20–42°C). There are more than 50 species of *Legionella* and human infections have been documented for 19 of these species (Table 172.8). Infections due to species other than *L. pneumophila* are rare and usually occur in immunocompromised hosts. The exception is *Legionella longbeachae* serogroup 1 which is a major cause of community-acquired pneumonia in Australia.[47] More than 90% of human infections are caused by *Legionella pneumophila* serogroup 1.

Some *Legionella* grow very poorly on BCYE but can be isolated by co-cultivation with amebae. Originally called *Legionella*-like amebal pathogens (LLAPs), they have been renamed as *L. lytica*, *L. drozanskii*, *L. falloni* and *L. rowbothamii*.[48]

Table 172.8 *Legionella* species associated with disease

Species	Number of serogroups
Legionella pneumophila	15
Legionella anisa	1
Legionella bozemanii	2
Legionella cincinnatensis	1
Legionella dumoffii	1
Legionella feeleii	2
Legionella gormanii	1
Legionella hackeliae	2
Legionella jordanis	1
Legionella longbeachae	2
Legionella lansingensis	1
*Legionella lytica**	1
Legionella maceachernii	1
Legionella micdadei	1
Legionella oakridgensis	1
Legionella parisiensis	1
Legionella sainthelensi	2
Legionella tucsonensis	1
Legionella wadsworthii	1

*Formerly called *Legionella*-like amebal pathogens (LLAP).

EPIDEMIOLOGY

Legionella spp. are distributed worldwide. They are found in fresh water streams, rivers, ponds and lakes and in moist soil and mud. The organisms are able to survive in moist environments for long periods of time and can withstand temperatures of 32–154°F (0–68°C) and a pH range of 5.0–8.5. They can survive chlorination and can proliferate in air-conditioning cooling towers, hot water systems, shower heads, taps, whirlpool spas and respiratory ventilators. The organisms are located in biofilms on the surfaces of these systems, where they are far less susceptible to the effects of biocides and chlorine. The growth of *Legionella* spp. is aided by coexisting bacteria and algae, which provide nutrients, and protozoa, in which the *Legionella* spp. can live and multiply.

The exact incidence of *Legionella* infections is difficult to determine. There is no doubt that exposure to *Legionella* spp. is a fairly frequent event and serologic surveys suggest that asymptomatic infection and seroconversion are common. Large-scale surveys of pneumonia suggest that *Legionella* spp. cause 2–5% of community-acquired pneumonia and up to 30% of nosocomial pneumonia.

Cases may be sporadic or occur as part of an outbreak. The original description of Legionnaires' disease was of an outbreak of a febrile respiratory illness in delegates at an American Legion convention in Philadelphia in 1976. A total of 221 people developed pneumonia and 34 died.[49] Retrospective studies have revealed that the first proven case occurred in 1947 and the first known epidemic was in 1965 in Washington, DC.

Sporadic cases are reported throughout the year, but most cases of epidemic infection occur in the summer and autumn, presumably because warmer weather encourages proliferation of the bacteria in water. The disease tends to occur in the middle-aged and elderly, especially in people who have impaired respiratory and cardiac function, who are heavy smokers or who are immunocompromised. Cases have been documented in children. Person-to-person spread has not been demonstrated. Surgery is a major predisposition in nosocomial infection, with transplant patients at the greatest risk.

Legionella is a good example of an organism that has been present in the environment for a long time but has been brought into close contact with humans as a result of technical developments. Organisms are disseminated via contaminated water droplets from nebulizers and humidifiers and in aerosols from cooling towers, whirlpools and evaporative condensers. Infection arises from inhalation of contaminated aerosols, direct instillation during surgery or possibly by ingestion of contaminated water. Hospital equipment that has been rinsed in tap water prior to use may be a source of infection.

There are more than 50 species and 64 subgroups in the genus *Legionella*. *L. pneumophila* contains 15 different serogroups. *L. pneumophila* serogroup 1 can be subdivided into subtypes, on the basis of monoclonal antibody typing or genotyping. Identifying the subtype of a strain is of importance in defining outbreaks where an environmental isolate can be matched to isolates from human cases of legionellosis. Several different methods have been used to genotype strains of *L. pneumophila*, including pulsed-field gel electrophoresis (PFGE), restriction fragment length polymorphism (RFLP) analysis, multilocus sequence typing (MLST), amplified fragment length polymorphism (AFLP) and sequence-based typing (SBT).

PATHOGENICITY

Legionella spp. residing in water are transmitted to humans via aerosols. Droplet nuclei of less than 5 µm will reach the alveoli, where the organisms are ingested by alveolar macrophages. *Legionella* is a facultative intracellular parasite and multiplies freely within the macrophages. The bacteria bind to the alveolar macrophages via complement receptors and are engulfed in phagosomes. Phagolysosome fusion is inhibited by an unknown mechanism that prevents intracellular killing. The bacteria multiply within the phagosomes. When the supply of nutrients is depleted the bacteria respond by expressing virulence proteins which lead to lysis of the host cell and release of bacteria, which are then taken up by other macrophages and the infection cycle is repeated.[50] This process produces chemotactic substances that attract polymorphonuclear leukocytes and monocytes. The inflammatory response results in a destructive pneumonia.

There are two phases in the life cycle of *Legionella pneumophila* – an intracellular replicative phase (RP) and an infectious nonreplicating transmissive phase (TP) which promotes transmission to a new host. Bacilli in the RP are avirulent, not flagellated and sodium resistant, whereas those in the TP are virulent, flagellated and motile. Complex regulatory systems govern the differentiation between these two phases.[51] In the TP the bacteria express genes concerned with transmission and host cell attack including substrates of the dot/Icm secretion system type IV pilus and more than 90 proteins; RP bacteria express components of aerobic metabolism. Switch is linked not only to expression of virulence trait but also to motility.

The virulence factors of *L. pneumophila* have not been fully determined. A 24 kDa protein, macrophage infectivity potentiator (mip), appears to be required for virulence. The major outer membrane protein may be important in the uptake of *Legionella* spp. by macrophages. A variety of extracellular enzymes are produced by *Legionella* spp., including proteases, esterases and hemolysins, but these have not been shown to be important virulence factors. The virulence of *L. pneumophila* may be enhanced by multiplication in amebae.

PREVENTION

Prevention of legionellosis depends upon identification of the environmental source and reduction of *Legionella* colonization. The most commonly used method of decontamination is periodic superheating and flushing of water supplies. This method is particularly useful for urgent disinfection during an outbreak. Hyperchlorination is no longer recommended because chlorine decomposes in hot water and *Legionella* spp. are relatively chlorine tolerant. Biocides are relatively ineffective. The most effective method appears to be copper–silver ionization units, which generate metallic ions that disrupt and kill bacterial cells.[52] Despite these costly measures it is often impossible to eliminate *Legionella* spp. from water supplies.

New water systems should be designed to minimize the risk of heavy colonization with *Legionella* spp., avoiding 'dead spaces', stagnation, materials that support the growth of *Legionella* spp. and the build-up of sediment. Regular monitoring for *Legionella* spp. is also advisable, particularly in high-risk areas.

Several putative vaccines have been developed that induce cell-mediated immunity in guinea pigs. These may be an option in the future for highly susceptible patients.

DIAGNOSTIC MICROBIOLOGY

Definitive diagnosis of legionellosis depends on culturing *Legionella* organisms, detecting *Legionella* antigens or DNA in body fluids or demonstrating a serologic response to the infection.

Legionella spp. can be cultured from sputum, endotracheal aspirates, bronchoalveolar lavages, lung biopsies and pleural fluid. Sputum samples should not be diluted in saline, because this can prove inhibitory to some *Legionella* spp., but in distilled water. Gram-stained smears of the sample will reveal poorly staining Gram-negative rods or coccobacilli, which are suggestive of legionellosis. Direct immunofluorescence of the smear using a monoclonal antibody to a major outer membrane protein common to all the serogroups of *L. pneumophila* can give a rapid provisional diagnosis but this test is not as sensitive as culture and will only detect *L. pneumophila*.

Cultures should therefore also be carried out. The sample should be cultured on a specific medium such as BCYE. *Legionella* spp. do not grow on blood agar. It is possible to render BCYE semiselective by the inclusion of antibiotics, but this may prove inhibitory to some strains of *Legionella* spp. It is therefore prudent to culture samples on both BCYE and BCYE supplemented with antibiotics. Contaminated material, such as sputum, can be heated at 122°F (50°C) for 30 minutes to suppress the growth of other less heat-stable organisms prior to inoculating the culture plates. Cultures should be incubated at 98.6°F (37°C) in 2–5% carbon dioxide for up to 14 days. *Legionella* spp. are slow growing, and on primary isolation it may take 3–5 days for colonies to appear. Cultures should not be regarded as negative until they have been cultured for at least 10 days. Colonies of *Legionella* spp. have a ground-glass appearance, which may be white, gray, pale blue or purple-tinged (Fig. 172.5). Often the colonies will fluoresce under ultraviolet light.

Colonies that grow on BCYE but not blood agar, that are catalase-positive, oxidase-negative and have a characteristic Gram-stain morphology should be regarded as presumptive *Legionella* spp. *Legionella pneumophila* hydrolyzes hippurate, starch and gelatin but in general biochemical tests are not of great help in identifying *Legionella* spp. Serologic typing using direct or indirect fluorescent antibody tests with polyclonal or monoclonal antibodies will confirm the identity of clinical isolates. Sequence analysis of the *mip* gene can be used to differentiate most *Legionella* spp.[53] Other schemes based on the 5S rRNA gene and partial 16S rRNA gene sequencing have also been described, but are less discriminatory than *mip* gene sequencing. Real-time PCR based on the *mip* gene can also be used to provide a rapid diagnosis.

Legionella lytica (and other LLAPs) can be isolated on BCYE if the cultures are incubated at 86°F (30°C).

Urine samples can be examined for *Legionella* soluble antigen using either an enzyme immunoassay or a radioimmunoassay kit. These tests are specific for *L. pneumophila* serogroup 1 (which causes 70–80% of human infections) and can give a rapid diagnosis. However, they will not detect infections caused by other *Legionella* spp. Rapid immunochromatographic tests for urinary antigen are also commercially available, including the Binax NOW urinary antigen test. The antigen persists in the urine for 1–2 weeks but some patients continue to excrete antigen for months.

Serologic tests can be used to make a diagnosis of legionellosis. The most commonly used technique is an indirect fluorescent antibody test (IFAT), but other techniques, such as ELISA or a rapid microagglu-

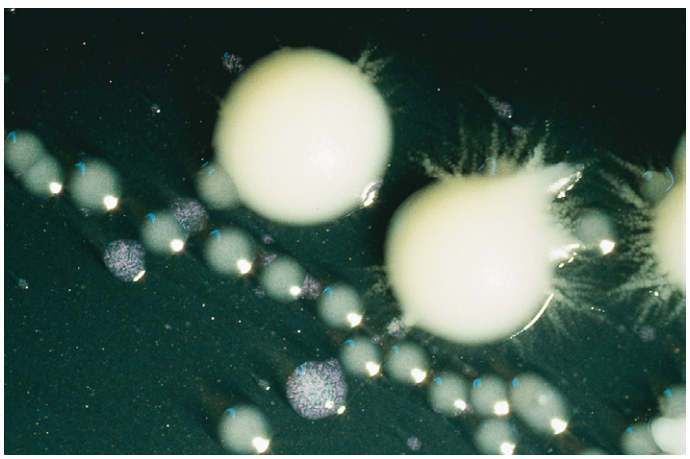


Fig. 172.5 Colonies of *Legionella pneumophila* on BCYE agar, showing the typical ground-glass appearance. With permission from Harrison TG, Taylor AG (eds). A laboratory manual for *Legionella*. Chichester: Wiley; 1998.

tination test, are also available. A fourfold or greater rise in antibody titer in a patient who has had clinical pneumonia indicates *Legionella* infection. It may take weeks or even months for the antibody titer to rise, and antibodies can persist for years, so serology is less useful than culture or antigen detection in the diagnosis of acute cases. Serology is much more useful in epidemiologic studies. The only validated serologic test is for *L. pneumophila* serogroup 1. Cross-reacting antibodies may be found in patients who have *Campylobacter* spp. infection.

CLINICAL MANIFESTATIONS

Asymptomatic *Legionella* infections are relatively common, as demonstrated by seroepidemiologic surveys. Symptomatic *Legionella* infection can present in two distinct ways: a severe pneumonia (Legionnaires' disease) and an acute influenza-like illness known as Pontiac fever. Clinical aspects are discussed in detail in Chapter 27.

MANAGEMENT

Macrolides or quinolones are the treatments of choice. In the patient who is acutely ill with Legionnaires' disease, a macrolide (e.g. azithromycin or clarithromycin) or a quinolone (e.g. levofloxacin or moxifloxacin) should be administered. The co-administration of rifampin does not appear to give any additional benefit.

Cases of Pontiac fever resolve spontaneously without the need for antibiotic therapy.

Moraxella Species

NATURE

Moraxella spp. are Gram-negative diplococci that morphologically and phenotypically resemble *Neisseria* spp. They are strictly aerobic, oxidase-positive, catalase-positive, DNAse-positive and asaccharolytic. *Moraxella catarrhalis* is now recognized as an important cause of upper and lower respiratory tract infections in children and adults.

Moraxella lacunata is associated with conjunctivitis. *Moraxella nonliquefaciens* is a commensal in the upper respiratory tract and may be a secondary invader in respiratory infections. *Moraxella osloensis* is a common commensal in the genital tract and may be misidentified as *Neisseria gonorrhoeae*. Unlike *N. gonorrhoeae*, *M. osloensis* grows well on blood agar. *M. osloensis* has been reported in cases of septic arthritis, osteomyelitis and bacteremia. These three organisms will not be further discussed.

EPIDEMIOLOGY

Moraxella catarrhalis is exclusively found in humans. The nasopharyngeal carriage rate of *M. catarrhalis* is 1–5% in healthy adults, but up to 75% of infants and young children are colonized with this organism.[54] This marked disparity may reflect the age-dependent development of secretory IgA antibodies. Nasopharyngeal carriage rates are significantly higher in the autumn and winter months. It also appears that the carriage rate is higher in adults who have chronic lung disease than in healthy adults.

PATHOGENICITY

M. catarrhalis is a mucosal pathogen, causing infections of the upper respiratory tract in young children and the lower respiratory tract in chronic obstructive airways disease (COPD). A number of potential virulence factors have been identified among the outer membrane proteins of *Moraxella catarrhalis*. These include the adhesins UspA1/A2, MID/Hag, CD and McaP, and the nutrient uptake proteins LbpA, LpbB, TbpA, TbpB and CopB. UspA1 and UspA2 are thought to have a role in infections in COPD patients since they bind to fibronectin which is produced in abundance in the chronically damaged airways. Lipo-oligosaccharide (LOS) is another highly conserved surface component that has been implicated as a possible virulence factor.[31]

PREVENTION

Currently there is considerable interest in the development of a vaccine to prevent *M. catarrhalis* infections. UspA1, UspA2 proteins and MID/Hag are the most immunogenic of these proteins but they exhibit considerable heterogeneity between strains. The functional adhesive domains of these proteins are more conserved and it may be possible to combine these with more conserved proteins[55] (Table 172.9).

DIAGNOSTIC MICROBIOLOGY

Moraxella catarrhalis grows well on blood agar and chocolate agar, producing small, nonhemolytic, grayish-white colonies that slide across the agar surface, like a hockey puck, when pushed with a bacteriologic loop. It may be difficult to distinguish *M. catarrhalis* from commensal *Neisseria* spp. in respiratory tract specimens, and the use of selective media may be necessary. Gram-stained films reveal Gram-negative diplococci but *M. catarrhalis* often resists destaining and thus may appear to be Gram-positive (Fig. 172.6). *M. catarrhalis* is DNAse positive, reduces nitrate and hydrolyses tributyrin, tests that differentiate it from the majority of *Neisseria* spp.

PCR tests can be applied to clinical material without the need for bacterial culture. A multiplex PCR for the detection of *Haemophilus influenzae*, *Moraxella catarrhalis* and *Streptococcus pneumoniae* has been successfully used to identify the bacterial etiology of middle ear infections.[56]

CLINICAL MANIFESTATIONS (SEE ALSO CHAPTER 25)

Moraxella catarrhalis is an important cause of otitis media and sinusitis in children, being the third most common cause of these conditions after *S. pneumoniae* and *H. influenzae*.[54]

In adults *M. catarrhalis* is associated with acute exacerbations of chronic bronchitis, being the third most common isolate after *H. influenzae* and *S. pneumoniae*. *Moraxella catarrhalis* may cause pneumonia, especially in the elderly and is associated with a poor prognosis. There have been several reports of nosocomial outbreaks of

Table 172.9 Candidate vaccine antigens of *Moraxella catarrhalis*

Antigen	Action	Comments
UspA1	Adhesin	Highly immunogenic Contains both conserved and variable regions
UspA2	Induces serum resistance	Highly immunogenic Contains both conserved and variable regions
MID/Hag	IgD binding Adhesin Hemagglutinin	Highly immunogenic Contains both conserved and variable regions
OMP CD	Porin	Highly conserved
McaP	Adhesin	Highly conserved

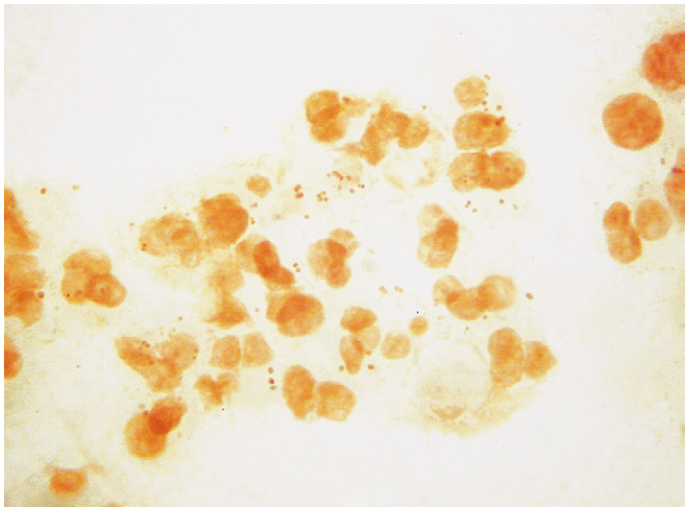


Fig. 172.6 Gram-stained smear of sputum containing *Moraxella catarrhalis*. Sample from a patient suffering an acute exacerbation of chronic bronchitis showing Gram-negative diplococci and leukocytes (*Moraxella catarrhalis*).

lower respiratory tract infections due to *M. catarrhalis* in respiratory units. *Moraxella catarrhalis* may occasionally cause invasive infections, including bacteremia, meningitis and endocarditis.

MANAGEMENT

Before 1977 no β-lactamase-positive isolates of *M. catarrhalis* had been reported. By 1980, 75% of *M. catarrhalis* isolates in the USA were β-lactamase-positive and now virtually all strains of *M. catarrhalis* are β-lactamase-positive. Among isolates collected in a European study from 1997 to 1999, more than 90% of *M. catarrhalis* carried BRO-1 β-lactamase.[57] There are two *M. catarrhalis* β-lactamases, BRO-1 and BRO-2, which are unique to this genus. There is some evidence that, in mixed infections, *M. catarrhalis* β-lactamases can protect other respiratory pathogens such as *S. pneumoniae* and *H. influenzae* from the action of β-lactam antibiotics. The β-lactamases of *M. catarrhalis* are chromosomal and inducible; therefore ampicillin therapy should be avoided.

Many infections due to *M. catarrhalis* can be treated with oral antibiotics, including amoxicillin–clavulanate, trimethoprim–sulfamethoxazole, tetracyclines (contraindicated in children aged under 7 years), cefaclor, clarithromycin, azithromycin and ciprofloxacin.

Pasteurella Species

NATURE

Pasteurella spp. are very small, nonmotile, nonspore-forming Gram-negative bacteria that are coccoid, oval or rod-shaped. They often exhibit bipolar staining. They are aerobic and facultatively anaerobic. Most species are catalase-positive and oxidase-positive. They attack carbohydrates readily, forming acid but no gas. Nitrates are reduced to nitrite. *Pasteurella* spp. will grow on ordinary laboratory media at 98.6°F (37°C). There are 15–20 species included in the genus *Pasteurella*, but it is probable that not all of these are true members of the genus. The type species is *Pasteurella multocida*. *Pasteurella multocida* is subdivided into four subspecies – *multocida*, *septica*, *gallicida* and *tigris*.

EPIDEMIOLOGY

Pasteurella spp. are distributed worldwide. They are commensals or parasitic organisms in the upper respiratory tract and gastrointestinal tracts of many domestic and wild animals and birds.

Pasteurella multocida is found in the oropharynx of 50–90% of domestic cats, 50–70% of dogs and 50% of pigs. It is the cause of hemorrhagic septicemia and bronchopneumonia in cattle and water buffalo, pneumonia in pigs and sheep, swine atrophic rhinitis, snuffles in rabbits and fowl, and fowl cholera in chickens, ducks and turkeys. It is generally carried asymptomatically by cats and dogs but may infect bites inflicted on these animals by feline or canine assailants. Humans become infected through contact with infected animals.

Pasteurella multocida can remain viable in soil and water for up to 4 weeks and may survive in animal carcasses for up to 3 months. Most human infections are caused by *P. multocida* subsp. *multocida* and *P. multocida* subsp. *septica*, though other species are sometimes implicated (Table 172.10).

PATHOGENICITY

Pasteurella multocida is transmitted to humans by contact with infected animals. The most common source of infection is bites, scratches, or licks from cats or dogs (see Chapter 68). Respiratory tract infections in those handling animals may result from airborne transmission. In a few patients it is not possible to document an animal source of the infection.

Virulence in *P. multocida* is associated with the degree of encapsulation. Strains with large capsules are more resistant to phagocytosis *in vivo*. There are five serogroups based on capsular antigens – A, B, D, E and F – and 16 serotypes (1–16) based on lipopolysaccharide (LPS) antigens. Capsular types A and D produce an exotoxin (PMT), encoded by the *toxA* gene. The role of this toxin in human infections is not known. *Pasteurella multocida* lipopolysaccharide is an important virulence factor. Fimbriae are probably important adhesins. Other putative virulence factors include neuraminidases, iron-sequestering proteins, surface adhesins and metabolic enzymes.[58]

PREVENTION

The only way of effectively preventing *Pasteurella* infections is to avoid contact with domestic or wild animals. Animal bites should be cleaned promptly and devitalized tissue should be debrided. Prophylactic antibiotics may be indicated after cat and dog bites, particularly if the patient is immunocompromised or diabetic or the wound affects the hands or face.

DIAGNOSTIC MICROBIOLOGY

A clinical history of animal bites will, in most instances, alert the laboratory to the possibility of *Pasteurella* spp. Samples should be cultured on blood agar plates at 98.6°F (37°C) for 24 hours. *Pasteurella multocida*

Table 172.10 Species of *Pasteurella* most commonly associated with infections in humans

- *Pasteurella multocida subsp. multocida*
- *Pasteurella multocida subsp. septica*
- *Pasteurella canis*
- *Pasteurella dagmatis*
- *Pasteurella stomatis*

grows as small, gray, nonhemolytic colonies, with a strong odor of indole. Gram-stained films reveal small Gram-negative coccobacilli. The organisms often show bipolar staining in methylene blue preparations. The identity of the organism can be confirmed by a series of biochemical and serologic tests and serology.

CLINICAL MANIFESTATIONS

Pasteurella infections in humans usually present as destructive soft tissue infections of acute onset following a bite or scratch from a domestic or wild animal. Open wounds may be infected by animal licks. Some *Pasteurella* infections present as respiratory infections, which may be chronic. In other cases the patient presents with a bacteremic infection. Approximately one-third of patients who have bacteremia have underlying cirrhosis.

MANAGEMENT

Animal bites should be carefully cleaned, irrigated and debrided. Penicillin is the treatment of choice for *Pasteurella* infections. Other agents with good activity include ampicillin, amoxicillin–clavulanate, cefuroxime and ciprofloxacin. Many infected animal bites have a polymicrobial etiology and it is prudent to use a regimen that provides good cover against both anaerobes and streptococci, such as amoxicillin–clavulanate. A strain of *P. multocida* that produced ROB-1 β-lactamase has been reported.

Yersinia Species

NATURE

Yersinia spp. are members of the Enterobacteriaceae. They are short, pleomorphic Gram-negative rods or coccobacilli, which often exhibit bipolar staining. *Yersinia pestis* is nonmotile. Other species are nonmotile at 98.6°F (37°C) but motile at temperatures less than 86°F (30°C) by means of peritrichous flagella. They are aerobic and facultatively anaerobic, oxidase-negative and catalase-positive. They are nonlactose fermenters. *Yersinia pestis* is urease-negative, and *Yersinia enterocolitica* and *Yersinia pseudotuberculosis* are both urease-positive. *Yersinia* spp. grow on simple laboratory media and are tolerant of bile salts. The optimal temperature for growth is 82–86°F (27.8–30°C). They do not form spores or capsules, but *Y. pestis* produces a capsule-like envelope. They share antigens with other members of the Enterobacteriaceae.

Multilocus sequence typing suggests that *Y. pestis* is a clone derived from *Y. pseudotuberculosis*.[59] The genome of five strains of *Y. pestis* and one strain of *Y. pseudotuberculosis* have been sequenced.[60]

The genus *Yersinia* contains three important human pathogens associated with zoonotic infections – *Y. pestis* causes plague, and *Y. pseudotuberculosis* and *Y. enterocolitica* give rise to infections known as yersiniosis.

Because these two diseases are very different they are described separately.

PLAGUE

The clinical features, pathogenesis, epidemiology and treatment of plague are discussed in detail in Chapter 120.

Diagnostic microbiology

Plague should be suspected in febrile patients exposed to flea bites or rodents in endemic parts of the world. It is important to notify the laboratory if plague is suspected so that appropriate precautions can be taken. There is a considerable risk of laboratory-acquired infection. Material suspected of containing *Y. pestis* must be handled in a containment level 3 laboratory in a category I or III exhaust protective cabinet by experienced, trained personnel. Smears taken from bubo aspirates, blood, sputum or cerebrospinal fluid should be examined using Gram stain and methylene blue. Gram stain will reveal Gram-negative pleomorphic coccobacilli with rounded ends. Wright–Giemsa or Wayson staining will demonstrate bipolar staining. *Yersinia pestis* appear light blue with dark blue polar bodies. Smears can also be examined by direct immunofluorescence.

Material can be cultured on blood agar or MacConkey agar or in broth cultures. Cultures should be incubated at 80°F (26.7°C). Tiny, translucent, nonhemolytic colonies appear on blood agar after 24 hours. After further incubation the colonies enlarge and become opaque. *Yersinia pestis* grows rather poorly on MacConkey agar and the colonies tend to autolyse after 2–3 days. In fluid culture *Y. pestis* tends to form chains.

The identity of presumptive colonies of *Y. pestis* can be confirmed using cultural and biochemical tests. Typically, *Y. pestis* is oxidase-negative, catalase-positive, urea-negative, indole-negative and lactose-negative. However strains may be misidentified as *Y. pseudotuberculosis* or other members of the Enterobacteriaceae. Alternatively, definitive identification can be made by detection of F1 antigen by immunofluorescence of the organisms. A bacteriophage that lyses *Y. pestis* but not *Y. pseudotuberculosis* can be used as a rapid identification in reference laboratories. A real-time PCR assay for *Y. pestis pla* gene has been developed.[61] A serologic diagnosis can be made using a passive hemagglutination test using tanned sheep red cells to which F1 antigen has been adsorbed, or a complement fixation test. A fourfold or greater rise in titer or a single titer over 1:16 in convalescent serum indicates plague infection. For large-scale seroepidemiologic surveys an ELISA test for antibodies to F1 antigen can be used.

YERSINIOSIS

Yersiniosis encompasses a wide spectrum of infections caused by *Y. enterocolitica* and *Y. pseudotuberculosis*. The clinical features range from gastroenteritis, enterocolitis or mesenteric adenitis to sepsis.

Epidemiology

Yersinia enterocolitica is harbored in the gastrointestinal tract of a wide range of mammals, including rodents, cattle, sheep, pigs, cats and dogs. Infected animals tend to become chronic carriers and excrete large numbers of bacilli, which can contaminate water and dairy products. Humans are infected by eating inadequately cooked meat (especially pork) or other food contaminated with *Yersinia* spp. Infection may also arise following contact with an infected domestic animal. In the USA, outbreaks associated with dairy products, chocolate and milk have been reported. Rarely, water is the source of the infection. The ability of *Y. enterocolitica* to grow at 39°F (4°C) means that refrigerated meat and meat products can become a potent source of infection. Infections are more common in children than in adults. Most cases occur in the autumn and winter. *Yersinia enterocolitica* sepsis has been reported following the transfusion of blood that has been stored for more than 3 weeks at 39°F (4°C).

Yersinia enterocolitica is a well-recognized cause of abdominal pain and diarrhea in Scandinavia and northern Europe. It is less commonly seen in the USA. Cases have been reported from other parts of the world. In Europe, infections are generally sporadic and the predominant serotypes are 03 and 09. In the USA, sporadic infections are less common but food-borne outbreaks caused by serotypes 03 and 09 have been described.[62]

Yersinia pseudotuberculosis infection is less commonly seen. The organism is harbored in the gastrointestinal tract of rodents, farm animals and birds. Human infections have been reported from all parts of

the world but, as with *Y. enterocolitica*, it is more common in northern Europe than elsewhere. Infection is generally seen in children and affects males more frequently than females. The majority of infections occur in the winter.

Pathogenicity

Yersinia enterocolitica generally gains entry to the body via the gastrointestinal tract. The infecting dose is 10^8–10^9 organisms/ml. Within the ileum the organisms invade the mucosa via specialized follicle-associated epithelial cells (M cells) that are involved in antigen uptake. Adherence and invasion of epithelial cells requires two chromosomal genes, *inv* and *ail*.[63] Following invasion the bacteria enter Peyer's patches and macrophages, where they can survive intracellularly and multiply. This results in a severe gastroenteritis. The bacteria may also spread to the mesenteric lymph nodes causing mesenteric adenitis, which may mimic acute appendicitis.

The organisms may disseminate through the body, producing sepsis and splenic and hepatic abscesses. A reactive polyarthritis can occur, particularly in HLA-B27-positive patients.

Yersinia pseudotuberculosis usually gives rise to mesenteric adenitis. Both species produce V-W and YOPs antigens, which are essential for virulence, and an outer membrane protein, invasin, which is involved in adherence to and invasion of cells (Table 172.11). A clone of *Y. pseudotuberculosis* has been identified as the cause of a toxic-shock syndrome associated with a desquamating, erythematous rash called Far East scarlet-like fever.

Prevention

Prevention of yersiniosis depends on good animal husbandry and careful slaughtering techniques to minimize contamination of meat with orofecal material. Meat should not be consumed raw and should not be stored at 39°F (4°C) for prolonged periods before consumption. There is no effective vaccine.

Diagnostic microbiology

Material for culture will include stool specimens, blood, lymph nodes and food samples. Specimens should be cultured on blood agar and MacConkey agar at 80°F (26.7°C) for 24 hours. Heavily contaminated

Table 172.11 Virulence factors of *Yersinia* species

Virulence factor	Gene	Location	*Y. pestis*	*Y. enterocolitica*	*Y. pseudotuberculosis*	Action
Pigment	*hms*	Chromosomal	+	+	+	Promotes biofilm formation and blocks proventriculus in flea vector for *Y. pestis*
Phospholipase D	*ymt*	pFra plasmid	+	–	–	Promotes survival of *Y. pestis* in flea vector
F1 antigen	*caf1*	pFra plasmid	+	–	–	Fibrillar capsule inhibits macrophage activity
Plasminogen activator	*pla*	pPst plasmid	+	–	–	Activates mammalian plasminogen and anticomplementary Promotes dissemination from subcutaneous inoculation of *Y. pestis*
Lipopolysaccharide	*rfb*	Chromosomal	+	+	+	Endotoxin
Yersiniabactin	*irp2*	Chromosomal	+	+	+	Siderpophore-essential for full virulence of *Y. pestis* by subcutaneous inoculation
V antigen, low calcium response plasmid, *Yersinia* outer proteins (YOPs)	*yopH*, *yopM*, *yopE*, *ypkA/yopO*, *yopJ/yopP*, *yadA*, *lcrV*	pLCR/pYV plasmid	+	+*	+	YOPs production essential for virulence of *Y. pestis* YOPs deregulate signaling pathways in host Type III secretion system, adhesin
pH 6 antigen	*psaA*	Chromosomal	+	+	+	Fibrillar surface protein Binds host lipoprotein Antiphagocytic
Invasin	*inv*	Chromosomal	–	+	+	Invasion of epithelial cells
Ail protein	*ail*	Chromosomal	–	+	+	Host cell attachment and serum resistance

* Yop T exclusive to *Y. enterocolitica*.

samples such as feces can be subjected to a cold enrichment technique whereby the sample is inoculated into phosphate-buffered saline and incubated at 39°F (4°C) for 3 weeks or more. The broth is subcultured at weekly intervals on to MacConkey agar or a special selective medium, CIN (cefsulodin, irgasan, novobiocin) agar. A chromogenic medium for detecting *Y. enterocolitica* has been described.[64] *Yersinia pseudotuberculosis* is rarely isolated from feces.

Presumptive colonies of *Yersinia* spp. are identified using a range of physical and biochemical tests. The serotype can be determined using slide agglutination tests.

A serologic diagnosis depends on demonstrating a significant rise of serotype-specific antibodies.

Clinical manifestations

A variety of clinical presentations have been documented in yersiniosis. The commonest form is acute gastroenteritis due to *Y. enterocolitica*. This is often indistinguishable from *Salmonella* or *Campylobacter* gastroenteritis. The commonest presentation of *Y. pseudotuberculosis* infection is mesenteric adenitis, which can mimic acute appendicitis.

Management

Yersinia enteritis and mesenteric adenitis do not generally require antimicrobial chemotherapy. Sepsis, extraintestinal foci of infection and enteritis in immunocompromised patients should be treated with antimicrobials. *Yersinia enterocolitica* is generally resistant to penicillin, ampicillin and first-generation cephalosporins. Aminoglycosides, quinolones, tetracyclines and third-generation cephalosporins have all been used successfully to treat *Y. enterocolitica* infections. *Yersinia pseudotuberculosis* is usually sensitive to benzylpenicillin and ampicillin.

REFERENCES

References for this chapter can be found online at http://www.expertconsult.com

Chapter 173

Itzhak Brook

Anaerobic bacteria

INTRODUCTION

Infections caused by anaerobic bacteria are common, and may be serious and life-threatening. Anaerobes are the predominant components of the bacterial flora of normal human skin and mucous membranes,[1] and are therefore a common cause of bacterial infections of endogenous origin. Because of their fastidious nature, these organisms are difficult to isolate from infectious sites and are often overlooked. Delay in appropriate therapy against these organisms often leads to clinical failures. Their isolation requires appropriate methods of collection, transportation and cultivation of specimens.[2–4] Treatment of anaerobic bacterial infection is complicated by the slow growth of these organisms, which makes diagnosis in the laboratory only possible after several days, by the often polymicrobial nature of the infection and by the growing resistance of anaerobic bacteria to antimicrobial agents.

NATURE

Anaerobic bacteria do not grow on solid media in the presence of room air (10% carbon dioxide and 18% oxygen), whereas facultative anaerobic bacteria grow both in the presence and in the absence of air. Microaerophilic bacteria grow poorly or not at all aerobically, but grow better under 10% carbon dioxide or anaerobically. Anaerobes can be divided into strict anaerobes that are unable to grow in the presence of more than 0.5% oxygen and moderate anaerobes that are capable of growing at between 2% and 8% oxygen.[4] Anaerobes generally do not possess catalase, but some clinical isolates produce superoxide dismutase that can protect them from oxygen.

The clinically important anaerobic bacteria in decreasing frequency are:

- six genera of Gram-negative rods (*Bacteroides, Prevotella, Porphyromonas, Fusobacterium, Bilophila* and *Sutterella* spp.);
- Gram-positive cocci (primarily *Peptostreptococcus* spp.);
- Gram-positive spore-forming (*Clostridium* spp.) and nonspore-forming bacilli (*Actinomyces, Propionibacterium, Eubacterium, Lactobacillus* and *Bifidobacterium* spp.); and
- Gram-negative cocci (mainly *Veillonella* spp.) (Table 173.1).[4–6]

The frequency of recovery of anaerobic bacterial strains differs in various infectious sites (Table 173.2). Mixed infections caused by numerous aerobic and anaerobic organisms are observed commonly in clinical situations (Fig. 173.1).[2,3]

Approximately 95% of the anaerobes isolated from clinical infections are members of these genera. The remaining isolates belong to species not yet described, but these usually can be assigned to the appropriate genus on the basis of morphologic characteristics and fermentation products. The frequency of recovery of anaerobic strains differs in various infectious sites.

The taxonomy of anaerobic bacteria has changed in recent years because of improved characterization methods using genetic studies.[4,6] The ability to differentiate between similar strains enables better characterization of type of infection and predicted antimicrobial susceptibility.[4]

EPIDEMIOLOGY

Most infections are caused by anaerobes that belong to the normal flora. Anaerobic infection generally occurs when members of the normal flora contaminate a previously sterile body site or gain access to the body from an external source of normal flora such as a bite. They also occur when the local defenses are decreased in capacity. Some anaerobic infections are caused by contamination of wounds by soil containing anaerobes. Such is the case with some *Clostridium* infections. *Clostridium difficile* enterocolitis can lead to epidemics in the hospital. In geriatric wards and nurseries in particular these bacteria are transmitted by hands from one patient to another. *Clostridium* botulinum causes food poisoning due to the presence of the toxin produced by *C. botulinum* in food.[7]

Gram-positive spore-forming bacilli

Anaerobic spore-forming bacilli belong to the genus *Clostridium*. Morphologically, the clostridia are highly pleomorphic, ranging from short, thick bacilli to long filamentous forms, and are either ramrod straight or slightly curved. The clostridia found most frequently in clinical infections are *Clostridium perfringens* (Fig. 173.2), *Clostridium septicum, Clostridium ramosum, Clostridium novyi, Clostridium sordellii, Clostridium histolyticum, Clostridium fallax, Clostridium bifermentans* and *Clostridium innocuum*.

Clostridium perfringens, the most commonly recovered *Clostridium* isolate, is an inhabitant of soil and of intestines of humans and animals, and is the most frequently encountered histotoxic *Clostridium* species. This micro-organism, which elaborates a number of necrotizing extracellular toxins,[8] is easily isolated and identified in the clinical laboratory. It can be characterized in direct smears of a purulent exudate by the presence of stout gram-variable rods of varying length, frequently surrounded by a capsule. *Clostridium perfringens* can cause a devastating illness with a high mortality rate. Bacteremia caused by *Clostridium* spp. is associated with extensive tissue necrosis, hemolytic anemia and renal failure.

Clostridium septicum has often been found to be associated with malignancy. The intestinal tract is thought to be the source of the organism, and most of the isolates are recovered from blood and subcutaneous tissue.[9] *Clostridium sordellii* infections have been reported in women undergoing therapeutic abortion, during natural childbirth, and in injection drug users.[10]

Table 173.1 Predominant anaerobic bacteria

Gram-positive cocci	*Peptostreptococcus* spp.: *P. magnus, P. asaccharolyticus, P. prevotii, P. intermedius, P. anaerobius, P. micros* Microaerophilic streptococci (not true anaerobes)
Gram-positive nonspore-forming bacilli	*Propionibacterium* spp.: *P. acnes, P. propionicum, P. granulosum* *Eubacterium tentum* *Bifidobacterium* spp.: *B. eriksonii, B. dentium* *Actinomyces* spp.: *A. israelii, A. naestundii, A. viscosus, A. odontolyticus, A. meyerii*
Gram-positive spore-forming bacilli	*Clostridium* spp.: *C. perfringens, C. ramosum, C. septicum, C. novyi, C. histolytica, C. sporogenes, C. difficile, C. bifermentans, C. butyricum, C. innocuum, C. sordellii, C. botulinum, C. tetani*
Gram-negative bacilli	*Bacteroides fragilis* group: *B. fragilis, B. thetaiotaomicron, B. distasonis, B. vulgatus, B. ovatus, B. uniformis* Other *Bacteroides* spp.: *B. gracilis, B. ureolyticus* *Bilophila wadsworthia, Sutterella* spp. Pigmented *Prevotella* spp.: *P. melaninogenica, P. intermedia, P. denticola, P. loescheii, P. corporis, P. nigrescens* Other *Prevotella* spp.: *P. oris, P. buccae, P. oralis* group (*P. oralis, P. buccalis, P. veroralis*), *P. bivia, P. disiens* *Porphyromonas* spp.: *P. asaccharolytica, P. gingivalis, P. endodontalis* *Fusobacterium* spp.: *F. nucleatum, F. necrophorum, F. gonidiaformans, F. naviforme, F. mortiferum, F. varium*

Although *C. botulinum* is usually associated with food poisoning, wound infections caused by this organism are being recognized with increasing frequency. Proteolytic strains of types A and B have been reported from food poisoning and wound infections. Infant botulism occurs with types A, B and F.[7] Disease caused by *C. botulinum* is usually an intoxication produced by ingestion of contaminated food (uncooked meat, poorly processed fish, improperly canned vegetables) containing a highly potent neurotoxin.[8] The polypeptide neurotoxin is relatively heat labile, and food containing this toxin may be rendered innocuous by exposure to 212 °F (100 °C) for 10 minutes. Infection of a wound with *C. botulinum* occurs rarely and can produce botulism.

Clostridium difficile has been incriminated as the causative agent of antibiotic-associated and spontaneous diarrhea and colitis.[11] *Clostridium tetani* is found in soil and is rarely isolated from human feces. Infections caused by this bacillus are a result of contamination of wounds with soil containing *C. tetani* spores.[8] The spores will germinate in devitalized tissue and produce the neurotoxin that is responsible for the clinical findings.

Laboratory diagnosis of *C. difficile*

Most laboratories use direct stool toxin assays (as opposed to organism detection assays) given their relatively rapid turnaround time. This practice has made investigation of the changing epidemiology of *C. difficile* more challenging, since isolation of a pathogen from culture is needed for epidemiologic surveillance.[12]

Direct stool toxin assays

Cytotoxicity assay Cytotoxicity assay (also known as tissue culture assay) is the gold standard for the diagnosis of *C. difficile*.[13] The test is performed by adding a prepared stool sample (diluted, buffered and filtered) to a monolayer of cultured cells. If *C. difficile* toxins (A and/or B) are present, they exert a cytopathic effect characterized by rounding of fibroblasts in tissue culture (see Fig. 173.1).

Because only a few toxin molecules per cell are sufficient to cause rounding, the cytotoxicity assay can detect as little as 10 pg of toxin B, making it the most sensitive *C. difficile* test (94–100%).[14,15] In addition, the sample is preincubated with neutralizing antibody against the toxins, allowing for high specificity (99%). However, most clinical laboratories do not perform this assay routinely given its relatively high cost and long turnaround time (48 hours).

Enzyme immunoassay Enzyme immunoassay (EIA) allows direct detection of *C. difficile* toxin.[16] Commercially available reagents can detect toxin A alone or both toxins A and B. EIA for both toxins is

Table 173.2 Anaerobic bacteria most frequently encountered in specific infection sites

Organism		Infection site
Gram-positive cocci	*Peptostreptococcus* spp. Microaerophilic streptococci (not obligate anaerobes)	Respiratory tract, intra-abdominal and soft-tissue infections Sinusitis, brain abscesses
Gram-positive bacilli	Nonspore-forming: *Actinomyces* spp.	Intracranial abscesses, chronic mastoiditis, aspiration pneumonia, head and neck infections
	Propionibacterium acnes	Shunt infections (cardiac, intracranial), infections associated with foreign body
	Bifidobacterium spp.	Chronic otitis media, cervical lymphadenitis, abdominal infections
	Spore-forming: *Clostridium perfringens*	Soft-tissue infection, sepsis, food poisoning
	Clostridium septicum	Sepsis, neutropenic enterocolitis
	Clostridium difficile	Colitis, antibiotic-associated diarrheal disease
	Clostridium botulinum	Botulism
	Clostridium tetani	Tetanus
	Clostridium ramosum	Soft-tissue infections
Gram-negative bacilli	*Bacteroides fragilis* group	Intra-abdominal and female genital tract infections, sepsis, neonatal infections
	Pigmented *Prevotella* and *Porphyromonas* spp.	Orofacial infections, aspiration pneumonia, periodontitis
	Prevotella oralis	Orofacial infections
	Prevotella oris-buccae	Orofacial infections, intra-abdominal infections
	Prevotella bivia, Prevotella disiens	Female genital tract infections
	Fusobacterium nucleatum	Orofacial and respiratory tract infections, brain abscesses, bacteremia
	Fusobacterium necrophorum	Aspiration pneumonia, bacteremia

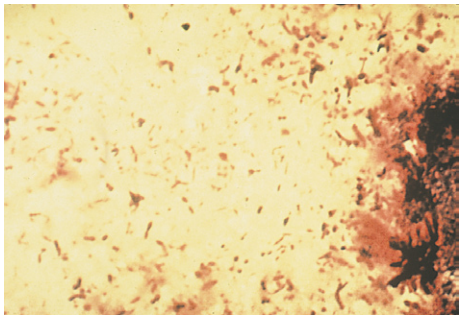


Fig. 173.1 Gram stain of a perirectal abscess caused by polymicrobial aerobic and anaerobic flora.

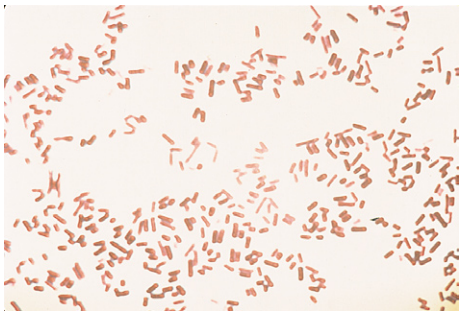


Fig. 173.2 Gram stain of *Clostridium perfringens*.

preferred because clinical cases may involve strains with a mutation in toxin A (rendering the EIA for toxin A ineffective) or strains that produce only toxin B.[17–20] In one study of 276 stool samples positive for *C. difficile* by EIA, for example, 19% were positive for toxin A alone, 48% for toxin B alone, and 33% for both toxins.[21]

EIA is the preferred diagnostic assay in most clinical laboratories because the technique is relatively simple and results are available within 24 hours. Despite good specificity (up to 99%), EIA is reported to have only moderate and variable sensitivity (60–95%).[12] There is a relatively high false-negative rate since 100–1000 pg of toxin must be present for the test to be positive.[22] Up to three serial EIA tests may increase the diagnostic yield by as much as 10% if the initial test is negative, although this incurs excessive cost without substantially enhancing diagnosis.[23] Supplementing negative EIA stool tests with tissue culture assay may also be useful.

Organism detection assays

An inherent problem with detection of the organism rather than the toxin is that up to 30% of hospitalized patients are colonized without disease.[3] Some laboratories use one of these methods to screen stool samples, with subsequent cytotoxin testing for positive samples.[24]

Anaerobic culture

Anaerobic culture is used less frequently for diagnosis of *C. difficile*. Although the test is extremely sensitive, anaerobic culture requires about 3 days. In addition, the assay cannot distinguish toxin-producing strains from nontoxin-producing strains.[15] To address this limitation, some laboratories perform an EIA on anaerobic culture isolates to determine whether they are toxin-producing strains; this approach appears to be more sensitive than assays that use stool specimens directly[25] (see 'Direct stool toxin assays' above).

Anaerobic culture is not a routinely used diagnostic tool in most clinical laboratories since it is relatively slow and labor intensive. However, it remains an important tool for epidemiologic surveillance.[12]

Polymerase chain reaction

Real-time polymerase chain reaction (PCR) using primers targeting a region of the toxin B gene could be a useful tool for diagnosis of *C. difficile* infection.[26] In a study evaluating 618 stool samples comparing PCR and EIA techniques with anaerobic culture (the 'gold standard'), sensitivity and specificity were better for PCR than EIA (67% vs 94% and 92% vs 97%, respectively).

Common antigen testing

Common antigen testing detects glutamate dehydrogenase (GDH) antigen, an essential enzyme produced constitutively by all *C. difficile* isolates. Some laboratories use this method to screen stool samples, with subsequent cytotoxin testing for positive samples.[24,27]

Endoscopy

Endoscopy can be a useful adjunctive tool for the diagnosis of *C. difficile* in the following settings:

- high clinical suspicion for *C. difficile* with negative laboratory assay(s);
- prompt *C. difficile* diagnosis needed before laboratory results can be obtained;
- failure of *C. difficile* infection to respond to antibiotic therapy; and
- atypical presentation with ileus or minimal diarrhea.[10,28]

Endoscopy is not warranted in patients with classic clinical findings and a positive stool toxin assay. In the setting of fulminant colitis, care should be taken to introduce minimal amounts of air given the risk of perforation. Gross and histologic findings obtained by endoscopic evaluation are discussed in detail above.

Gram-positive, nonspore-forming bacilli

Anaerobic, Gram-positive, nonspore-forming rods comprise part of the microflora of the gingival crevices, the gastrointestinal tract, the vagina and the skin. Several distinct genera are recognized: *Actinomyces, Arcanobacterium, Atopobium, Bifidobacterium, Eubacterium, Lactobacillus, Mobiluncus, Propionibacterium* and *Pseudoramibacter*. The most frequently recovered species are *Propionibacterium, Eubacterium, Bifidobacterium* and *Lactobacillus*.

The *Actinomyces, Arcanobacterium* and *Bifidobacterium* spp. are Gram-positive, pleomorphic, anaerobic to microaerophilic bacilli.

Actinomyces israelii, Actinomyces naeslundii and *Propionibacterium propionicum* are normal inhabitants of the human mouth and throat and are the most frequent cause of actinomycosis. The organisms have been recovered from intracranial abscesses, chronic mastoiditis, aspiration pneumonia and peritonitis.[3,5] The lesions of actinomycosis occur most commonly in the tissues of the face, neck, lungs, pleura and female and male genital and urinary tracts. Bone, pericardial and anorectal lesions are less common, but virtually any tissue may be invaded; even disseminated infection with bacteremia has been described.[29]

Most organisms of the genus *Eubacterium* and anaerobic lactobacilli seem to be nonpathogenic. They are almost invariably isolated as part of mixed flora in the oral, vaginal and gastrointestinal areas. These organisms have been recovered most commonly in infections associated with predisposing or underlying conditions that include malignancy, previous surgery, immunodeficiency, diabetes mellitus, the presence of a foreign body, dental extraction and broad-spectrum antibiotic therapy.[30]

Propionibacterium spp. ordinarily are not pathogens, but can be found in association with implanted cardiac prostheses or central nervous system (CNS) shunts or as a cause of endocarditis on previously damaged valves. They have been recovered from parotid and dental infections, brain abscesses, conjunctivitis associated with contact lens, peritonitis and foreign body and pulmonary infections. The most common species, *Propionibacterium acnes*, may be isolated from blood cultures but is associated only rarely with bacteremia or endocarditis. Because these organisms are part of the normal skin flora, they are common laboratory contaminants or may grow in blood cultures from skin contamination if the skin surface has been improperly decontaminated before the blood sample is drawn. *Propionibacterium acnes* can cause bacteremia, especially in association with shunt and infections,[31,32] and is believed to play a role in the pathogenesis of acne vulgaris.[33]

Gram-negative bacilli

Bacteroides fragilis group are the most frequently recovered species of Bacteroidaceae in clinical specimens (Fig. 173.3). *Bacteroides fragilis* group are resistant to penicillins, mostly through the production of β-lactamase. They include several members – the most commonly isolated ones are *B. fragilis* (the most commonly recovered member), *Bacteroides thetaiotaomicron, Bacteroides distasonis, Bacteroides ovatus* and *Bacteroides vulgatus*. These organisms are part of the normal gastrointestinal flora[1] and predominate in intra-abdominal infections and other infections that originate from the gut flora (i.e. perirectal abscesses, decubitus ulcers).[3,5] *Bilophila wadsworthia* and *Centipeda periodontii* are new genera and species found in abdominal and endodontal infection, respectively.[34]

Pigmented *Prevotella* spp. (*Prevotella melaninogenica, Prevotella intermedia*), *Porphyromonas* spp. (*Porphyromonas asaccharolytica*) and non-pigmented *Prevotella* spp. (*Prevotella oralis, Prevotella oris*) are part of the normal oral and vaginal flora and are the predominant Gram-negative anaerobic species isolated from respiratory infections and their complications, aspiration pneumonia, lung abscess, chronic otitis media, chronic sinusitis, abscesses around the oral cavity, human bites, paronychia, brain abscesses and osteomyelitis.[35] *Prevotella bivia* and *Prevotella disiens* are important isolates from obstetric and gynecologic infections.

Fusobacterium species

Fusobacterium spp. are moderately long and thin organisms with tapered ends, and have typical fusiform morphology (Fig. 173.4). The species of *Fusobacterium* seen most often in clinical infections are *Fusobacterium nucleatum, Fusobacterium necrophorum, Fusobacterium mortiferum* and *Fusobacterium varium*. *Fusobacterium nucleatum* is the predominant *Fusobacterium* sp. from clinical specimens, and is often associated with oral, pulmonary and intracranial infections.[36] *Fusobacterium* spp. are frequently isolated from abscesses, obstetric and gynecologic infections, blood and wounds.

A growing resistance of Gram-negative anaerobic bacilli to penicillins has been noted in the past decade.[37] These include the pigmented *Prevotella* and *Porphyromonas, Prev. oralis, Prev. disiens, Prev. bivia* and *Fusobacterium* spp. The main mechanism of resistance is through the production of the enzyme β-lactamase. Testing for antimicrobial susceptibility and the ability to produce β-lactamase of all Gram-negative anaerobic bacilli can assist in the selection of appropriate antimicrobials.

The recovery rates of the different anaerobic Gram-negative bacilli in infected sites are similar to their distributions in the normal flora.[3,5] The *B. fragilis* group were more often isolates in sites proximal to the gastrointestinal tract (abdomen, bile), pigmented *Prevotella* spp. were more prevalent in infections proximal to the oral cavity (bones, sinuses, chest), and *Prev. bivia* and *Prev. disiens* were more often isolates in obstetric and gynecologic infections (see Table 173.2). Knowledge of this common mode of distribution allows for logical choice of antimicrobials that are adequate for the therapy of infections in these sites.

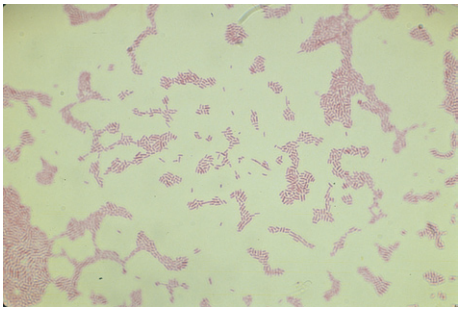


Fig. 173.3 Gram stain of *Bacteroides fragilis*. Courtesy of Mike Cox.

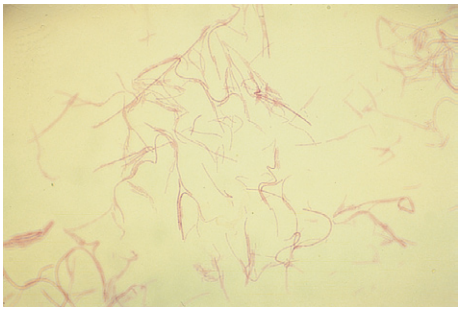


Fig. 173.4 Gram stain of *Fusobacterium nucleatum*. Courtesy of Mike Cox.

Gram-positive cocci

The species most commonly isolated are *Peptostreptococcus magnus, Peptostreptococcus asaccharolyticus, Peptostreptococcus anaerobius, Peptostreptococcus prevotii* and *Peptostreptococcus micros*. Additional anaerobic cocci include *Coprococcus, Peptococcus, Ruminococcus sarcina* and *Staphylococcus saccharolyticus*. These organisms are part of the normal flora of the mouth, upper respiratory tract, intestinal tract, vagina and skin.

These organisms can be isolated in all types of anaerobic infection. They also predominate in all types of respiratory infection (including chronic sinusitis, mastoiditis, acute and chronic otitis media, aspiration pneumonia and lung abscess), and necrotizing, subcutaneous and soft-tissue infections.[38] They are generally recovered mixed with other aerobic or anaerobic organisms, but in many cases they are the only pathogens recovered. This may be of particular significance in cases of bacteremia. Microaerophilic streptococci are not true anaerobes as they can also become aerotolerant after subculture; however, they grow better anaerobically and are often grouped under anaerobes in many studies. These organisms include the *Streptococcus anginosus* group (previously called *Streptococcus milleri* group, which includes *Streptococcus constellatus* and *Streptococcus intermedius*) and *Gemella morbillorum* (previously called *Streptococcus morbillorum*). Microaerophilic streptococci are of particular importance in chronic sinusitis and brain abscesses. They are also recovered from obstetric and gynecologic infections and abscesses.[39]

Gram-negative cocci

There are three genera described as anaerobic Gram-negative cocci: *Veillonella, Acidaminococcus* and *Megasphaera* spp. There are two described species of *Veillonella* and only one each of the other two genera. *Veillonella* is the most frequently involved of the three genera and *Veillonella* spp. are part of the normal flora of the mouth, vagina and the small intestine of some persons. Although they are rarely isolated from clinical infections, these organisms have been recovered occasionally from almost every type of anaerobic infection.[40]

PATHOGENICITY

Anaerobes as part of the normal flora (Table 173.3)

The human body mucosal and epithelial surfaces are colonized with aerobic and anaerobic micro-organisms.[1] Differences in the environment, such as oxygen tension, pH and variations in the ability of bacteria to adhere to these surfaces, account for changing patterns of colonization. Microflora also vary in different sites within the body system, as in the oral cavity; for example, the micro-organisms present in the buccal folds vary in their concentration and types of strain from those isolated from the tongue or gingival sulci. However, the organisms that prevail in one body system tend to belong to certain major

bacterial species, and their presence in that system is predictable. The relative and total counts of organisms can be affected by various factors, such as age, diet, anatomic variations, illness, hospitalization and antimicrobial therapy. However, these sets of bacterial flora, with predictable pattern, remain stable through life, despite their subjection to perturbing factors. Anaerobes outnumber aerobic bacteria on all mucosal surfaces, and certain organisms predominate in the different sites (see Table 173.3).

Knowledge of the composition of the flora at certain sites is useful for predicting which organisms may be involved in an infection adjacent to that site and can assist in the selection of a logical antimicrobial therapy, even before the exact microbial etiology of the infection is known. Recognition of the normal flora can also help the clinical microbiology laboratory to identify appropriate culture media that will be selective in inhibiting organisms that may interfere with the growth of certain anaerobic bacteria.

The anaerobic microflora of the skin is largely made up of the genus *Propionibacterium*[34] (mostly *Propionibacterium acnes*) and, to a lesser extent, *Peptostreptococcus* spp. The perineum and lower extremity may harbor members of the colonic and vaginal flora.

The microflora of the upper airways, including oral cavity, nasopharynx and oropharynx, is complex and contains many types of obligate anaerobes. The distribution of bacteria within the mouth seems to be a function of their ability to adhere to the oral surfaces. The differences in numbers of the anaerobic microflora probably occur because of considerable variations in the oxygen concentration in parts of the oral cavity. The ratio of anaerobic bacteria to aerobic bacteria in saliva is approximately 10:1. The total count of anaerobic bacteria in the saliva and elsewhere in the oral cavity reach 10^7–10^8 bacteria/ml. The predominant anaerobic bacteria in the upper airways include *Fusobacterium* spp. (especially *F. nucleatum*), pigmented *Prevotella* and *Porphyromonas* spp., *Prev. oralis* and *Peptostreptococcus* spp. These organisms also predominate in oropharyngeal, otolaryngologic and pulmonary infections.

The gastrointestinal flora varies in bacterial concentration at different levels. The stomach acidity reduces the number of organisms swallowed from the oropharynx. The stomach, duodenum, jejunum and proximal ileum normally contain relatively few bacteria. However, the flora becomes more complex, and the number of different bacterial species increase in the distal portion of the gastrointestinal tract. Interruption in intestinal motility may result in an increase in the number of anaerobic and aerobic bacteria. The bacterial counts in the small intestine are relatively low, with total counts of 10^2–10^5 organisms/ml. The organisms that predominate up to the ileocecal valve are Gram-positive facultative, whereas below that structure *Bacteroides, Peptostreptococcus* and *Clostridium* spp. and coliform bacteria are the major isolates.[2] The mean number of bacteria in the colon exceeds 10^{11} bacteria/g fecal material. Approximately 99.9% of these bacteria are anaerobic (ratio of aerobes to anaerobes is 1:1000–10 000). In the colon 300–400 different species or types of bacteria can be found, many of which are not yet identified.

The female genital flora is composed of mixed aerobic and anaerobic flora. However, the concentration and type of bacteria is less stable than that of the gastrointestinal flora and can be influenced by antimicrobial therapy, pregnancy and gynecologic surgery. A concentration of 10^8 organisms/ml is found during the reproductive years. Changes occur in the number of organisms at the various stages of the menstrual cycle.[41] The predominant aerobic organisms are aerobic lactobacilli, and the predominant anaerobic bacteria are anaerobic *Lactobacillus, Peptostreptococcus, Prevotella* and *Bacteroides* and *Clostridium* spp. Other anaerobic isolates include *Porphyromonas, Fusobacterium, Bilophilia, Bifidobacterium, Actinomyces, Eubacterium* and *Propionibacterium* spp. Enterobacteriaceae can be found in postmenopausal flora. Bacterial vaginosis is associated with an increase in the number of anaerobic flora and a decrease in the concentration of lactobacilli.[42]

Most infections caused by anaerobic bacteria originate from the endogenous mucosal membrane and skin flora. An exception is *C. difficile*, the major cause of antibiotic-associated colitis. Anaerobes belonging to the indigenous flora of the oral cavity can be recovered from various infections adjacent to that area, such as cervical lymphadenitis, subcutaneous abscesses and burns in proximity to the oral cavity, human and animal bites, paronychia, tonsillar and retropharyngeal abscesses, chronic sinusitis, chronic otitis media, periodontal abscess, thyroiditis, aspiration pneumonia and bacteremia associated with one of the above infections.[3,5] The predominant anaerobes recovered in these infections are *Prevotella, Porphyromonas, Fusobacterium* and *Peptostreptococcus* spp., which are all part of the normal flora of the mucous surfaces of the oropharynx (Fig. 173.5).

A similar correlation exists in infections associated with the gastrointestinal tract. Such infections include peritonitis after rupture of a viscus, liver and spleen abscess, abscess and wounds near the anus, intra-abdominal abscess, and bacteremia associated with any of these

Table 173.3 Normal flora

Site	NO. OF ORGANISMS/g FECAL MATERIAL			Predominant anaerobic bacteria
	Aerobes	Anaerobes	Anaerobe/aerobe ratio	
Skin	–	–	–	*Propionibacterium acnes* *Peptostreptococcus* spp.
Mouth/upper respiratory tract	10^8–10^9	10^9–10^{11}	Nasal washings 3–5:1 Saliva 1:1 Tooth surface 1:1 Gingival crevice 1000:1	Pigmented *Prevotella* and *Porphyromonas* spp. *Fusobacterium* spp. *Peptostreptococcus* spp. *Actinomyces* spp.
Gastrointestinal tract: Upper Lower	 10^2–10^5 10^5–10^9	 10^3–10^7 10^{10}–10^{12}	 Stomach 1:1 Small bowel 1:1 Ileum 1:1 Colon 1000:1	 *Bacteroides fragilis* group *Clostridium* spp. *Peptostreptococcus* spp. *Bifidobacterium* spp. *Eubacterium* spp.
Female genital tract	10^8	10^9	Endocervix 1–5:1 Vagina 1–5:1	*Peptostreptococcus* spp. *Prevotella bivia* *Prevotella disiens*

For predominant anaerobic bacteria in the gastrointestinal tract, all organisms can be found in both the upper and lower gastrointestinal tracts.

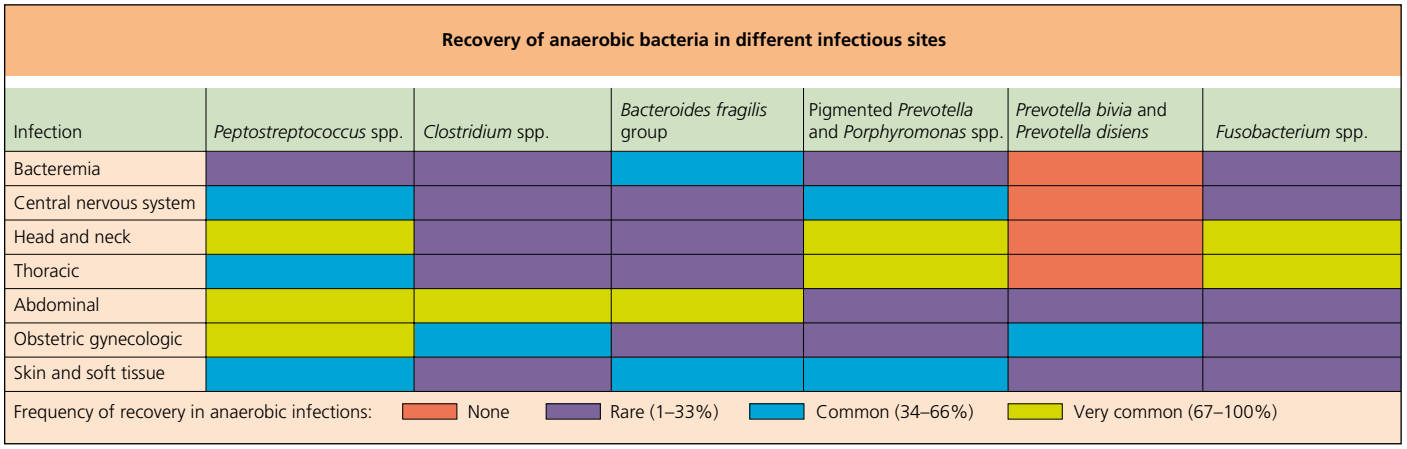


Fig. 173.5 Recovery of anaerobic bacteria in different infectious sites.

infections.[3,5] The anaerobes that predominate in these infections are *B. fragilis* group, *Clostridium* spp. (including *C. perfringens*) and *Peptostreptococcus* spp.

Another site where a correlation exists between the normal flora and the anaerobic bacteria recovered from infected sites is the genitourinary tract. The infections involved are amnionitis, septic abortion and other pelvic inflammations.[3,5] The anaerobes usually recovered from these sites are species of anaerobic Gram-negative bacteria and *Peptostreptococcus* spp. Organisms belonging to the vaginal–cervical flora are also important pathogens of neonatal infections. They can be acquired by the newborn before delivery in the presence of amnionitis or during passage through the birth canal.

Conditions predisposing to anaerobic infection

The clinical situations that predispose to anaerobic infections include exposure of the sterile body sites to a high inoculum of indigenous mucous membrane flora. Poor blood supply and tissue necrosis lower the oxidation and reduction potential and favor the growth of anaerobic bacteria. Any condition that lowers the blood supply to an affected area of the body can predispose to anaerobic infection. Therefore, trauma, foreign body, malignancy, surgery, edema, shock, colitis and vascular disease may predispose to anaerobic infection. Other predisposing conditions include diabetes mellitus, splenectomy, immunosuppression, hypogammaglobinemia, neutropenia, leukemia, collagen vascular disease and cytotoxic drugs. Previous infection with aerobic or facultative organisms may make the local tissue conditions more favorable for the growth of anaerobic bacteria. The human defense mechanisms may also be impaired by anaerobic conditions and anaerobic bacteria. The noted effects include impairments in phagocytosis and intracellular killing (often caused by encapsulated anaerobes[43] and by succinic acid produced by *Bacteroides* spp.), inhibition of chemotaxis (by *Fusobacterium, Prevotella* and *Porphyromonas* spp.), degradation of serum proteins by proteases (by *Bacteroides* spp.) and production of leukotoxins (by *Fusobacterium* spp.).[44]

Suppuration, abscess formation, thrombophlebitis and gangrenous destruction of tissue associated with gas formation are the hallmarks of anaerobic infection (Table 173.4). Anaerobes are especially common in chronic infections, and they are commonly seen after failure of therapy with antimicrobials that are effective against them, such as aminoglycosides, trimethoprim–sulfamethoxazole (co-trimoxazole) and earlier quinolones.

Certain infections are very likely to involve anaerobes as important pathogens, and their presence should always be assumed. Such infections include brain abscess, oral or dental infections, human or animal bites, aspiration pneumonia and lung abscesses, peritonitis after perforation of viscus, amnionitis, endometritis, septic abortions, tubo-ovarian abscess, abscesses in and around the oral and rectal areas, pus-forming necrotizing infections of soft tissue or muscle and postsurgical infections following procedures on the oral or gastrointestinal tract or female pelvic area.[5] Certain solid malignant tumors, such as colon, uterine and bronchogenic carcinomas, and necrotic tumors of the head and neck, have the tendency to become infected with anaerobic bacteria.[45] The anoxic conditions in the tumor and exposure to the endogenous adjacent mucous flora may predispose to these infections.

Table 173.4 Clinical signs of anaerobic infection

Infection adjacent to a mucosal surface
Foul-smelling discharge
Necrotic gangrenous tissue and abscess formation
Free gas in tissue
Bacteremia or endocarditis with no growth on aerobic blood cultures
Infection related to the use of antibiotics effective against aerobes only
Infection related to tumors or other destructive processes
Infected thrombophlebitis
Infection after bites
Black discoloration of exudates containing *Bacteroides melaninogenicus*, which may fluoresce under ultraviolet light
'Sulfur granules' in discharges caused by actinomycosis
Clinical presentation of gas gangrene
Clinical condition predisposing to anaerobic infection (after maternal amnionitis, perforation of bowel, etc.)

Virulence factors

Anaerobic bacteria contain a number of components that have been defined as virulence factors in facultative bacteria including toxins, polysaccharide capsules, and lipopolysaccharides. Most of these also have been shown to be virulence factors in anaerobic species. The capacity to survive and grow under anaerobic conditions has also led to the identification of other properties of anaerobes which may play a role in virulence.

Toxins

The most clearly identified virulence factors for anaerobic bacteria are the exotoxins produced by clostridial species including botulinum toxins, tetanus toxin, *C. difficile* toxin A and B, and five toxins produced by *C. perfringens* (as well as many other clostridial species); these are among the most virulent bacterial toxins in mouse lethality assays. Alpha toxin is the major toxin produced by *C. perfringens*, but studies have shown a complex interaction between alpha and theta toxin in the production of experimental gas gangrene.[1,2] Both toxins appear to be involved in upregulation of intercellular adhesion molecule (ICAM)-1 and platelet aggregating factor (PAF), which contributes to vascular leukostasis and absence of a polymorphonuclear leukocyte (PMN) response to the infection.[3]

Clinical expression of these histotoxic clostridial syndromes depends upon the site of toxin production and the physiologic effects of the toxin (see 'Epidemiology, microbiology, and pathophysiology of *Clostridium difficile* infection', 'Clostridial myonecrosis' and 'Botulism').

An enterotoxin has also been identified in *Bacteroides fragilis*, which is a metalloprotease.[4] Strains producing the toxin have been recovered from patients with diarrhea and from healthy controls but seem to be more common in those with diarrhea.[5]

Capsular polysaccharides

In the same way that *Clostridium* spp. serve as the prototypes for toxins produced by anaerobes, *B. fragilis* has been studied as a unique virulent anaerobe. The organism constitutes 0.5% of normal colonic flora and yet is the most common anaerobe isolated from intra-abdominal infections and is the most frequent anaerobic bacteremic isolate.[6] This explains why investigators have chosen to examine *B. fragilis* virulence factors in more detail.

Based upon the rat model of intra-abdominal sepsis, it became apparent that *B. fragilis* alone among the anaerobes studied was capable of provoking abscesses without a synergistic facultative organism and that heat-killed *B. fragilis* retained this capacity.[7] Following extraction and purification of the polysaccharide capsule of a prototype strain, this complex sugar was also able to provoke abscesses in the model system. Further study of the capsular polysaccharide has led to an appreciation that it is actually a complex of polysaccharides with zwitterionic properties (containing alternating oppositely charged sugars).[8,9] Adherence of the capsular polysaccharide complex to mesothelial cells *in vitro* stimulates ICAM-1 and tumor necrosis factor (TNF); pretreatment of mice with antibodies to ICAM-1 or TNF results in failure of the animals to develop intraperitoneal abscesses in a mouse model of intra-abdominal sepsis.[10]

Lipopolysaccharides

Anaerobic Gram-negative bacteria, like all Gram-negative bacteria, contain lipopolysaccharide (LPS) that can be extracted from the envelope, but the biologic activity of this endotoxin (mouse lethality assays, the chick embryo death test, and the Shwartzman reaction) is 100–1000 times less than that of LPS from Enterobacteriaceae.[11] The LPS of *B. fragilis* contains a lipid A moiety (the endotoxin portion of LPS), but there are structural and chemical composition differences which render this LPS less potent than the LPS of *Escherichia coli*.[11]

Other anaerobic Gram-negative bacteria, such as fusobacteria, are thought to contain endotoxin with substantial biologic activity. One study of extracted LPS from a strain of *Fusobacterium nucleatum* found biologic activity equal to the LPS of Enterobacteriaceae and correlated this with inhibition of gingival fibroblasts.[29] Thus, LPS of fusobacteria may play a pathogenic role in periodontal disease and presumably accounts for the severity of illness with Lemierre disease[30,31] (see 'Anaerobic bacterial infections').

Volatile fatty acids

Another virulence factor of anaerobes is the production of short chain volatile fatty acids. Production of these fatty acids is a characteristic feature of the metabolic system of anaerobes that is used to identify these microbes in the microbiology laboratory and may be responsible for the characteristic putrid drainage. These volatile fatty acids have been shown to inhibit phagocytic killing of bacteria.[32,33]

Ability to tolerate oxygen

A number of anaerobic bacteria, including *B. fragilis*, can tolerate exposure to oxygen. These organisms contain varying concentrations of superoxide dismutase, an enzyme present in aerobic bacteria which protects against the toxic effects of oxygen.[34] The ability to survive exposure to oxygen is a different type of factor which facilitates the survival and thus pathogenicity of the organism.

Formation of and host defense to abscesses

As noted above, an abscess is a unique biologic phenomenon, representing at one and the same time a pathologic process and host defense to contain infection. Within the fibrous capsule of a well-formed abscess, bacteria and PMN coexist; these same neutrophils *in vitro* will kill the bacteria.

Anaerobes contribute to the severity of infection through their synergy with their aerobic counterparts and with each other.[22] Anaerobic bacteria generally take longer than aerobic bacteria to become virulent. This is because some of the major virulence factors of certain anaerobic bacteria (i.e. the production of a capsule) are expressed only after the infection has become chronic.[43]

Anaerobic bacteria possess several virulence factors that assist them to adhere to and invade epithelial surfaces. These factors include the presence of surface structures (such as capsule polysaccharide or lipopolysaccharide), production of superoxide dismutase and catalase, immunoglobulin proteases, coagulation promoting, spreading factors (such as hyaluronidase, collagenase and fibrinolysin), adherence factors and the production of toxins.[44] Other factors that enhance the virulence of anaerobes include mucosal damage, oxidation–reduction potential drop and the presence of hemoglobin or blood in an infected site.

An indirect pathogenic role of some anaerobes is their ability to produce the enzyme β-lactamase. Several anaerobic bacteria can produce β-lactamase (Table 173.5). β-Lactamase-producing bacteria (BLPB) can protect not only themselves but also other penicillin-susceptible organisms from the activity of penicillins. This can occur when the enzyme β-lactamase is secreted into the infected tissue or abscess fluid in sufficient quantities to degrade the β-lactam ring of penicillin before it can kill the susceptible bacteria.[46]

In-vitro and *in-vivo* studies have demonstrated protection of penicillin-susceptible bacteria from penicillin by aerobic and anaerobic BLPB (i.e. protection of group A streptococci by *Staphylococcus aureus* or *Bacteroides* spp.).[46] The predominant anaerobic BLPB are pigmented *Prevotella* and *Porphyromonas, Bacteroides* and *Fusobacterium* spp.

Table 173.5 Principal β-lactamase-producing anaerobes

Fusobacterium spp.: *F. nucleatum, F mortiferum, F. varium*
Pigmented *Prevotella* and *Porphyromonas* spp., *Prevotella oralis* group
Other *Prevotella* spp.: *P. oris, P. buccae, P. bivia, P. disiens*
Bacteroides fragilis group, *Bacteroides splanchnicus*
Bilophila wadsworthia
Clostridium spp.: *C. ramosum, C. clostridioforme* and *C. butyricum*

PREVENTION

Proper therapy of acute infections can prevent the occurrence of chronic infections where anaerobes predominate. In settings where anaerobic infections are expected to occur, such as intra-abdominal and wound infections after surgery, proper antimicrobial prophylaxis reduces the chance of such infections.

Prevention and early therapy of conditions that may lead to anaerobic infection can reduce their rate. Preventing oral flora aspiration by improving the neurologic status of the patient, repeated suctioning of oral secretions, improving oral hygiene and maintaining lower stomach pH can reduce the risk of aspiration pneumonia and its complications. Skin and soft-tissue infections can be prevented by irrigation and debridement of wounds and necrotic tissue, drainage of pus and improvement of blood supply.

Prophylactic therapy before surgery is generally administered when the operative field is expected to be contaminated by the normal flora of the mucous membrane at the operated site. Cefazolin is effective in surgical prophylaxis in sites distant from the oral or rectal areas, when anaerobic cover is not required. Cefoxitin, cefotetan or ertapenem are used in procedures that involve the oral, rectal or vulvovaginal surfaces because their spectrum includes the anaerobic flora likely to be encountered.

COLLECTION, TRANSPORT AND DIAGNOSTIC MICROBIOLOGY

Collection of specimens for anaerobic bacteria

The proper management of anaerobic infection depends on appropriate documentation of the bacteria causing the infection. Without such an approach, the patient may be exposed to inappropriate, costly and undesirable antimicrobial agents with adverse side-effects. Some laboratories may fail to recover certain or all of the anaerobes present in a specimen. This situation can occur particularly when the specimen is not promptly placed under anaerobic conditions for transport to the laboratory. If care is not taken to avoid specimen contamination with normal flora, anaerobes may not be recovered.

Appropriate cultures for anaerobes are especially important in polymicrobial infections.[4] Techniques or media that are inadequate for isolation of anaerobes because of a lack of an anaerobic environment, or because of an overgrowth of aerobic organisms, can be misleading. This may cause the clinician to direct therapy toward only the isolated aerobic organism(s).

The most acceptable documentation of an anaerobe is through recovery of anaerobic organisms from the infected site. Three essential elements require the physician's cooperation with the microbiology laboratory for appropriate documentation of anaerobic infection: collection of appropriate specimens, their expeditious transportation and careful laboratory processing.

Specimens must be obtained free of contamination so that saprophytic organisms or normal flora are excluded and culture results can be interpreted correctly. Because indigenous anaerobes are often present on the surfaces of skin and mucous membranes in large numbers, even minimal contamination of a specimen with normal flora can give misleading results. On this basis, specimens can be designated according to their acceptability for anaerobic culture to the acceptable or the unacceptable category. Materials that are appropriate for anaerobic cultures should be obtained using a technique that bypasses the normal flora (Table 173.6). Unacceptable or inappropriate specimens can yield normal flora also and therefore have no diagnostic value.

Acceptable specimens (Table 173.7) include blood specimens, aspirates of body fluids (pleural, pericardial, cerebrospinal, peritoneal and joint fluids), urine collected by percutaneous suprapubic bladder aspiration, abscess contents, deep aspirates of wounds and specimens collected by special techniques, such as transtracheal aspirates or direct lung puncture. Specimens of the lower respiratory tract are difficult to obtain without contamination with indigenous flora. Double-lumen catheter bronchial brushing and bronchoalveolar lavage, cultured quantitatively, can be useful. Direct needle aspiration is probably the best method of obtaining a culture, and the use of swabs is much less desirable. Specimens obtained from sites that are normally sterile may be collected after thorough skin decontamination, as is the case for the collection of blood, or spinal, joint or peritoneal fluids.

Table 173.6 Methods for collection of specimens for anaerobic bacteria

Infection site	Methods
Abscess or body cavity	Aspiration by syringe and needle Incised abscesses: syringe or swab (less desirable); specimen obtained during surgery after cleansing the skin
Tissue or bone	Surgical specimen using tissue biopsy or curette
Sinuses or mucus surface abscesses	Aspiration after decontamination or surgical specimen
Ear	Aspiration after decontamination of ear canal and membrane; in perforation, cleanse ear canal and aspirate through perforation
Pulmonary	Transtracheal aspiration, lung puncture, bronchoscopic aspirate (using double lumen catheter and quantitative culture)
Pleural	Thoracentesis
Urinary tract	Suprapubic bladder aspiration
Female genital tract	Culdocentesis after decontamination, surgical specimen Transabdominal needle aspirate of uterus Intrauterine brush (using double lumen catheter and quantitative culture)

Table 173.7 Appropriate and inappropriate specimens for anaerobic culture

Appropriate	Inappropriate
Feces or rectal swabs	All normally sterile body fluids other than urine (e.g. blood, pleural and joint fluids)
Throat or nasopharyngeal swabs	Urine obtained by suprapubic bladder aspiration
Sputum or bronchoscopic specimens	Percutaneous transtracheal aspiration or direct lung puncture
Routine or catheterized urine	Culdocentesis fluid obtained after decontamination of the vagina
Vaginal or cervical swabs	Material obtained from closed abscesses
Material from superficial wound or abscesses not collected properly to exclude surface contaminations	Material obtained from sinus tracts or draining wounds
Material from abdominal wounds obviously contaminated with feces (e.g. an open fistula)	

Transportation of specimens

Prompt delivery of specimens to the laboratory to allow for microbiologic processing is essential. Various transport devices are available that

generate an oxygen-free environment. These systems generally contain an oxygen-free environment provided by mixture of carbon dioxide, hydrogen and nitrogen, plus an indicator that illustrates aerobic conditions. The specimens should be placed into an anaerobic transporter as soon as possible after collection. Aspirates of liquid specimen or tissue are always preferred to swabs, although systems for the collection of all culture forms are commercially available.

Liquid specimens may be inoculated into a commercially available anaerobic transport vial. A plastic or glass syringe without a needle may also be used for transport. After the specimen is collected and all air bubbles are expelled, the syringe is capped. Because air gradually diffuses into the plastic syringe, no more than 30 minutes should elapse before the specimen is processed. This inexpensive transport device for liquid specimens is especially useful in the hospital where it can be rapidly transported to the microbiology laboratory.

Swabs may be placed in the sterilized tubes containing carbon dioxide or prereduced, anaerobically sterile Carey and Blair semisolid media. Tissue specimens or swabs can be transported in an anaerobic jar or in a Petri dish inside a sealed plastic bag that can be rendered anaerobic by use of a catalyzer.

Laboratory diagnosis

For the laboratory diagnosis of *Clostridium difficile*, see above.

Certain findings are suggestive of anaerobic infection (Table 173.8). However, laboratory diagnosis of anaerobic infections begins with the examination of a Gram-stained smear of the specimen (see Fig. 173.1). The appearance of the Gram-stained organisms will give important preliminary information regarding the types of organism present, suggest appropriate initial therapy and serve as a quality control on the final culture analysis. The laboratory should be able to recover all of the morphologic types in the approximate ratio in which they are seen.

The techniques for cultivation of anaerobes should provide optimal anaerobic conditions throughout processing. Detailed procedures of these methods can be found in microbiology manuals.[4] Briefly, these methods could be the prereduced tube method, the anaerobic glove-box technique (Fig. 173.6), which provides an anaerobic environment throughout processing, or the anaerobic jar or bag systems, which are more simplified.

As a minimum requirement for the recovery of anaerobes, specimens should be inoculated onto enriched blood agar medium (containing vitamin K1 and hemin) and a selective medium (for *Bacteroides* spp.), such as laked sheep blood agar with kanamycin and vancomycin, should be used. The use of a selective medium along with a nonselective one increases the recovery rate and can shorten the time to identification of organisms.

Although prereduced vitamin K1-enriched thioglycolate broth is generally used as a back-up culture, this medium alone should never be used as a substitute for a solid medium. The major limitation of liquid media is the possibility of overgrowth of slow-growing strict anaerobes by rapid-growing aerobic and facultative organisms. Selective media can supplement nonselective media in increasing the bacterial yield and facilitating recovery.

Table 173.8 Bacteriologic findings suggestive of anaerobic infection

Organisms seen on Gram stain that cannot be grown in aerobic cultures
Typical morphology for anaerobes on Gram stain
Anaerobic growth on proper media containing antibiotic-suppressing aerobes
Growth in anaerobic zone of fluid or agar media
Gas, foul-smelling odor in specimen or bacterial culture
Characteristic colonies on anaerobic plates
Colonies of pigmented *Prevotella* or *Porphyromonus* spp. may fluoresce red under ultraviolet light, and older colonies produce a typical dark pigment

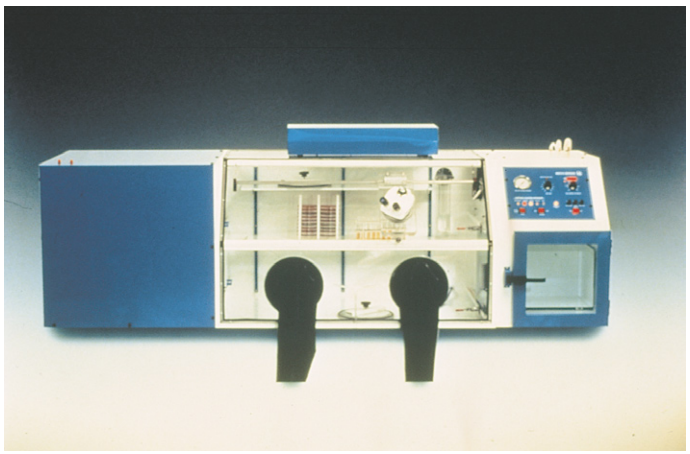


Fig. 173.6 Anaerobic glove-box used in the microbiology laboratory for processing of specimens and identifying anaerobic bacteria. Courtesy of Mike Cox.

Cultures should be placed immediately under anaerobic conditions and incubated for 48 hours or longer. Plates should then be examined for approximate number and types of colonies present. Each colony type should be isolated, tested for aerotolerance and identified.

An additional period of 36–48 hours is generally required to identify completely the anaerobic bacteria to a species or genus level using biochemical tests. Kits containing these biochemical tests are commercially available. These are good with fast-growing anaerobes (i.e. *B. fragilis* and *C. perfringens*). Rapid enzymatic tests have recently been introduced. This panel test enables identification of the anaerobes after only 4 hours of aerobic incubation and seems to be as good as the biochemical tests. Other rapid tests that have potential use and can also be used directly on clinical isolates are direct fluorescent microscopy and direct gas–liquid chromatography. Direct fluorescent microscopy can identify *Actinomyces* spp. and *Propionibacterium propionicus*. This method is inaccurate in identification of *B. fragilis* group, pigmented *Prevotella* and *Porphyromonas* spp. Gas–liquid chromatography of metabolites can be employed to assist in the identification of anaerobes. Direct cellular fatty acid analysis using gas–liquid chromatography with capillary column can also be useful. Nucleic acid probes and PCR methods are also being developed for rapid identification of anaerobic bacteria.

Most clinical microbiology laboratories are able to identify the major anaerobic bacteria. Peptostreptococci are generally not speciated because they are usually susceptible to commonly used antimicrobials. *Clostridium* spp. can be identified by the presence of spores and their ability to survive 30 minutes exposure to ethanol or heating to 176°F (80°C) for 10 minutes. Speciation requires biochemical testing. Nonspore-forming Gram-positive bacilli can be speciated by gas–liquid chromatography and biochemical tests. *Propionibacterium* spp., which are often a contaminant, can be separated from other nonspore-forming Gram-positive bacilli by using a catalyze test and indole reaction. *Bacteroides fragilis* group organisms grow on 20% bile and are generally catalase positive. Pigmented *Prevotella* and *Porphyromonas* spp. produce black or brown pigment within a week when growing on rabbit blood agar medium. *Fusobacterium* spp. have a distinct morphology on Gram stain and, in contrast to *Bacteroides* spp., are susceptible to kanamycin.

Identification of an anaerobe to a species level is often cumbersome, expensive and time-consuming, taking up to 72 hours. Identification is most helpful in selecting an antibiotic against a species that has predictable antibiotic susceptibility. The level of speciation adequate for identifying an anaerobe is often controversial.

Occasionally, species identification of an organism will provide the diagnosis, as with *C. difficile* in patients who have colitis or *C. botulinum* in infants with botulism. However, because most anaerobes are endogenous, there are rarely epidemiologic reasons to perform complete

identification. Identifying *B. fragilis* group, which often cause bacteremia and septic complications, has significant prognostic value.

Antimicrobial susceptibility testing

The antimicrobial susceptibility of anaerobes has become less predictable. Resistance to several antimicrobials, especially by anaerobic Gram-negative bacilli and *Fusobacterium* spp., has increased over the past decade.[38] It is important, therefore, to perform susceptibility testing for anaerobes recovered from sterile body sites, those with particular epidemiologic or prognostic significance (e.g. *C. difficile*), or those that are clinically important and have variable susceptibilities.

Screening of anaerobic Gram-negative bacilli isolates (particularly *Prevotella, Bacteroides* and *Fusobacterium* spp.) for β-lactamase activity may be helpful. This can provide information regarding their penicillin susceptibility. However, occasional bacterial strains may resist β-lactam antibiotics through other mechanisms.

Routine susceptibility testing of all anaerobic isolates is extremely time-consuming and in many cases unnecessary. Susceptibility testing should be limited to anaerobes isolated from blood cultures, bone, CNS and serious infections, as well as to those isolated in pure culture from properly collected specimens. Antibiotics tested should include penicillin, a broad-spectrum penicillin, a penicillin plus a β-lactamase inhibitor, clindamycin, a second-generation cephalosporin (e.g. cefoxitin), an extended-spectrum fluoroquinolone, tigecycline, metronidazole and a carbapenem (e.g. imipenem).

The method recommended by the Clinical and Laboratory Standards Institute (CLSI), formerly the National Committee for Clinical Laboratory Standards (NCCLS), includes agar dilution testing, microbroth and macrobroth dilution.[47] Newer methods include the E-test and the spiral gradient end-point system.

CLINICAL MANIFESTATIONS

Anaerobic bacteria have been recovered in infections at all anatomic locations. However, their frequency of isolation and the types of bacterial isolate depend on the microbial flora at their source or at the adjacent mucocutaneous sites.

Central nervous system infections

Anaerobic bacteria can cause a variety of intracranial infections. These organisms induce brain abscess, subdural empyema and infrequently cause epidural abscess and meningitis. The main source of brain abscess is an adjacent, generally chronic infection in the ears, mastoids, sinuses, oropharynx, teeth or lungs.[48] Ear or mastoid infection tends to spread to the temporal lobe or cerebellum, whereas facial sinusitis often causes abscess of the frontal lobe. Hematogenous spread often occurs after dental, oropharyngeal or pulmonary infection. Rarely bacteremia of another origin or endocarditis can lead to such infection.

Meningitis caused by anaerobes is rare and can follow respiratory infection or develop as a complication of a cerebrospinal fluid shunt. Shunt infections are generally caused by skin flora such as *Prop. acnes*,[11] or in instances of ventriculoperitoneal shunts that perforate the gut, by anaerobes of enteric origin (i.e. *B. fragilis*).[49] *Clostridium perfringens* has been reported as a cause of brain abscesses and meningitis after head injuries or after intracranial surgery.[2]

The anaerobic bacteria generally recovered from brain abscesses that complicate respiratory and dental infections include *Prevotella, Porphyromonas, Bacteroides, Fusobacterium* and *Peptostreptococcus* spp. Microaerophilic and other streptococci are also often isolated. Actinomyces are less frequently encountered.

At the stage of encephalitis, antimicrobial therapy accompanied by measures to control the increase in intracranial pressure can prevent abscess formation. Once an abscess has formed, surgical excision or drainage may be needed, combined with a long course of antibiotics (4–8 weeks). Some neurosurgeons advocate complete evacuation of the abscess, whereas others advocate repeated aspirations.[50] In patients who have multiple abscesses or in those who have abscesses in essential brain areas, repeated aspirations are preferable. High-dose antibiotics for an extended period may represent an alternative approach in this group of patients and have often replaced surgical drainage.[50]

Because of the difficulty involved in the penetration of various antimicrobial agents through the blood–brain barrier, the choice of antibiotics is restricted. The antimicrobials advocated for these infections are metronidazole, penicillins, meropenem and chloramphenicol. However, the choice may vary according to the specific isolates and their susceptibilities. A significant improvement in the mortality rate has been associated with the introduction of computed tomography (CT) and use of metronidazole therapy.

Head and neck and upper respiratory tract infections

Anaerobic bacteria can be recovered from a variety of head and neck and upper respiratory tract infections and predominate more in their chronic forms (Table 173.9). These include chronic otitis media, sinusitis and mastoiditis, tonsillar, peritonsillar and retropharyngeal abscesses, all deep neck space infections, parotitis, sialadenitis, thyroiditis, odontogenic infections, and postsurgical and nonsurgical head and neck wounds and abscesses. The predominant organisms are of oropharyngeal flora origin and include *Prevotella, Porphyromonas, Bacteroides, Fusobacterium* and *Peptostreptococcus* spp.

Most dental infections involve anaerobes. These include endodontal pulpitis and periodontal (gingivitis and periodontitis) infections, periapical and dental abscesses, and perimandibular space infection.[28,51] Pulpitis may progress to an abscess and eventually involve the mandible and other neck spaces. In addition to the organisms mentioned above, microaerophilic streptococci and *Streptococcus salivarius* can also be involved.

Vincent's angina (or trench mouth) is a distinct form of ulcerative gingivitis; the causative organisms include *Fusobacterium* spp. and anaerobic spirochetes.[2]

Deep neck infections after oral, dental and pharyngeal infections are usually polymicrobial. These include mediastinitis after perforation of the esophagus, extension of retropharyngeal abscess or cellulitis, and dental abscess.[52]

Otitis media

Anaerobes were isolated in 5–15% of patients who had acute otitis media[52a] and 42% of culture-positive aspirates of patients who had serous otitis media.[52b] The predominant isolates in acute otitis media were *Peptostreptococcus* spp. and *Peptostreptococcus acnes*. These organisms, as well as Gram-negative anaerobic bacilli, were recovered in serous otitis media.

Several studies reported the recovery of anaerobes in about 50% of the patients who have chronic suppurative otitis media.[3,5,52c,d] The infection is often polymicrobial and the predominant anaerobes were Gram-negative bacilli and peptostreptococci, and the common aerobes were *Pseudomonas aeruginosa* and *Staph. aureus*. Many of these organisms can produce β-lactamase and might have contributed to the high failure rate of β-lactam antibiotics in the therapy of this infection. Anaerobes were isolated from 23 out of 24 (96%) specimens of chronic mastoiditis[52e] and from most patients who have intracranial abscesses that complicate chronic suppurative otitis media.[3,5,48]

Anaerobes were recovered from infected cholesteatomas.[52f,g] The production of organic acids by anaerobic bacteria may promote the process of bone destruction in cholesteatoma.[52g] Because infected cholesteatoma contains bacteria similar to those recovered from chronically infected ears, it may serve as a nidus for chronic infection.

Table 173.9 Aerobic and anaerobic bacteria isolated in head and neck and upper respiratory tract infections

Type of infection	Aerobic and facultative organisms	Anaerobic organism
Otitis media and mastoiditis: acute	*Streptococcus pneumoniae* *Haemophilus influenzae** *Moraxella catarrhalis**	*Peptostreptococcus* spp.
chronic	*Staphylococcus aureus** *Escherichia coli** *Klebsiella pneumoniae** *Pseudomonas aeruginosa**	Pigmented *Prevotella* and *Porphyromonas* spp.* *Bacteroides* spp.* *Fusobacterium* spp.* *Peptostreptococcus* spp.
Peritonsillar and retropharyngeal abscess	*Streptococcus pyogenes* *Staphylococcus aureus**	*Fusobacterium* spp.* Pigmented *Prevotella* and *Porphyromonas* spp.*
Recurrent tonsillitis	*Streptococcus pyogenes* *Haemophilus influenzae** *Staphylococcus aureus**	*Fusobacterium* spp.*
Suppurative thyroiditis	Streptococcus pyogenes Staphylococcus aureus*	Pigmented *Prevotella* and *Porphyromonas* spp.* *Peptostreptococcus* spp.
Sinusitis: acute	*Haemophilus influenzae** *Streptococcus pneumoniae* *Moraxella catarrhalis**	*Peptostreptococcus* spp.
chronic	*Staphylococcus aureus** *Streptococcus pneumoniae* *Haemophilus influenzae**	Pigmented *Prevotella* and *Porphyromonas* spp.* *Fusobacterium* spp.* *Bacteroides fragilis* group*
Cervical lymphadenitis	*Staphylococcus aureus** *Mycobacterium* spp.	Pigmented *Prevotella* and *Porphyromonas* spp.* *Peptostreptococcus* spp.
Postoperative infection disrupting oral mucosa	*Staphylococcus* spp.* Enterobacteriaceae* *Streptococcus pyogenes*	*Fusobacterium* spp.* *Bacteroides* spp.* Pigmented *Prevotella* and *Porphyromonas* spp.* *Peptostreptococcus* spp.
Deep neck abscesses and parotitis	*Streptococcus* spp. *Staphylococcus* spp.*	*Bacteroides* spp.* *Fusobacterium* spp.* *Peptostreptococcus* spp.*
Odontogenic complications	*Streptococcus* spp. *Staphylococcus* spp.*	Pigmented *Prevotella* and *Porphyromonas* spp.* *Peptostreptococcus* spp.
Oropharyngeal: Vincent's angina and necrotizing	*Streptococcus* spp. *Staphylococcus* spp.*	*Fusobacterium necrophorum**

* Organisms that have the potential of producing β-lactamase.

Sinusitis

Sinus disease may develop when allergy, viral infection, or anatomic obstruction occurs, preventing normal drainage. In the first stages of infection, the most common pathogens are similar to those recovered in acute otitis media: *Streptococcus pneumoniae, Haemophilus influenzae* and *Moraxella catarrhalis*. Anaerobic organisms become involved as the infection becomes chronic and the levels of tissue oxygen decline.[52h] The transition in the bacterial flora from aerobic to anaerobic flora has been demonstrated.[52i] An elevated serum antibody level to *Prevotella* and *Fusbacterium* spp. was demonstrated in patients with chronic sinusitis.[52j] Although anaerobes are generally isolated from only about 7% of patients who have acute sinusitis (generally in maxillary sinusitis secondary to periodontal infection), they can be isolated from up to 67% of patients who have chronic infection.[3,5,52k] An average of three anaerobes per aspirate were recovered in patients who had chronic sinusitis.[52l]

Sinus infection may spread via anastomosing veins or contiguously to the CNS. Complications include orbital cellulitis,[52m] meningitis, cavernous sinus thrombosis, and epidural, subdural and brain abscesses.[3,5]

Parotitis

Viral parotitis can be caused by paramyxovirus (mumps), Epstein–Barr virus, coxsackievirus, HIV, and influenza A and parainfluenza virus. Acute suppurative parotitis is generally caused by *Staph. aureus*, streptococci and, rarely, aerobic Gram-negative bacteria. Anaerobes, mostly *Peptostreptococcus*, *Bacteroides*, and pigmented *Prevotella* and *Porphyromonas* spp., have also been recognized as an important cause of this infection.[52n] Empiric antibiotic therapy should be directed against both aerobic and anaerobic bacteria. Surgical drainage may be indicated when pus has formed.

Cervical lymphadenitis

The anterior cervical, the submandibular or the posterior cervical nodes are the most prevalent sites of infection. The most common causes in children are viruses. The organisms causing acute unilateral infection associated with facial trauma or impetigo are *Staph. aureus* and *Streptococcus pyogenes*. *Bartonella henselae* and mycobacterial infections are important in chronic infections. Anaerobes (mostly *Fusobacterium* and *Peptostreptococcus* spp.) were isolated in about 25%

of the infections, often in pure culture.[27] Their recovery was associated with a primary dental, periodontal or tonsillar infection.

Infection after head and neck surgery

Infections after head and neck surgery are caused by the exposure of the surgical site to the oropharyngeal flora, by the decreased blood supply and by the presence of necrotic tissue. They are common after surgery for malignant tumors. The wounds are generally infected by polymicrobial aerobic and anaerobic flora; the average number of isolates varies from one to nine (average six).[53] The most common isolates are peptostreptococci, *Staph. aureus*, anaerobic Gram-negative bacilli (i.e. *Bacteroides* spp.), *Fusobacterium* spp. and Enterobacteriaceae. The presence of this flora warrants the use of antimicrobial agents that are effective against these organisms in the prophylaxis and therapy of this infection.[54]

Tonsillitis

Indirect evidence is mounting that anaerobes are involved in both acute and chronic tonsillitis. The evidence is mainly derived from studies of complications of anaerobic tonsillitis (i.e. bacteremia, abscesses) where anaerobes play a major role. The organisms associated with the infection are *Fusobacterium* spp., peptostreptococci and Gram-negative anaerobic bacilli. Polymicrobial flora predominate in peritonsillar and retropharyngeal abscesses, where the number of isolates is between one and 12 (average five anaerobes and two aerobes).[3,5,55] Anaerobes have also been isolated from the cores of tonsils of children with recurrent group A β-hemolytic streptococci (GABHS) infection[23,46,56] and non-GABHS tonsillitis.[56] These organisms can be isolated from 25% of suppurative cervical lymph nodes and are mostly associated with the presence of dental or tonsillar infections.[27] Anaerobic organisms were associated with thrombophlebitis of the internal jugular veins, which often causes postanginal sepsis.[3,5]

The pathogenic role of anaerobes in the acute inflammatory process in the tonsils is also supported by several clinical observations:

- their recovery in tonsillar or retropharyngeal abscesses, often without any aerobic bacteria;[55]
- their isolation from tonsils in Vincent's angina;[5]
- the recovery of encapsulated pigmented *Prevotella* and *Porphyromonas* spp. in acutely inflamed tonsils;
- the isolation of anaerobes from the core of recurrently inflamed non-GABHS tonsils;[56] and
- the response to antibiotics in patients who have non-GABHS tonsillitis.[57,58]

Furthermore, immune response against *Prev. intermedia* can be detected in patients who have non-GABHS tonsillitis[59] and against *Prev. intermedia* and *F. nucleatum* in patients who recovered from peritonsillar cellulitis or abscesses[60] and infectious mononucleosis.[61]

Metronidazole therapy alleviated the symptoms of tonsillar hypertrophy and shortened the duration of fever in patients who had infectious mononucleosis.[57] Because metronidazole has no antiviral or aerobic antibacterial efficacy, suppression of the oral anaerobic flora may contribute to diminishing the inflammation induced by the Epstein–Barr virus. This is supported by the increased recovery of *Prev. intermedia* and *F. nucleatum* during the acute phases of mononucleosis.[62]

Recurrent pharyngotonsillitis and failure to eradicate the GABHS with penicillin can be a serious clinical problem. One explanation for penicillin failure is that repeated administrations result in selection of BLPB.[46] β-Lactamase-producing strains of pigmented *Prevotella* and *Porphyromonas* spp., *B. fragilis, Fusobacterium* spp., *Haemophilus influenzae* and *Staph. aureus* were isolated from the tonsils of more than 75% of children with recurrent GABHS tonsillitis[23,46,63,64] and from 40% of children with non-GABHS tonsillitis.[56] Similar organisms were recovered from patients who had adenoiditis and adenoid hypertrophy.[65]

The recovery of these bacteria in more than three-quarters of the patients who had recurrent GABHS tonsillitis,[46,63,64] the ability to measure β-lactamase activity in the core of these tonsils[66] and the response of patients to antimicrobials effective against BLPB (i.e. clindamycin or amoxicillin plus clavulanic acid)[46,67,68] support the role of these aerobic as well as anaerobic organisms in the inability of penicillin to eradicate GABHS tonsillitis.

Pleuropulmonary infections

Aspiration of oropharyngeal secretions or gastric contents, and severe periodontal or gingival disease are the most prevalent risk factors for developing anaerobic pleuropulmonary secretion. The infection can progress from pneumonitis into necrotizing pneumonia and pulmonary abscess, with or without empyema.[69] The lesions tend to develop in the dependent pulmonary segments, in either of the superior segments of the lower lobes and in the posterior segments of the upper lobes. The infection is generally polymicrobial where the causative organisms of community-acquired infection (in 60–80% of cases) are aerobic and anaerobic members of oropharyngeal flora. The anaerobes isolated are *Prevotella, Porphyromonas, Fusobacterium* and *Peptostreptococcus* spp., and the aerobes are β-hemolytic streptococci and microaerophilic streptococci (Table 173.10).[70] Anaerobes can also be recovered in about one-third of patients who have nosocomial-acquired aspiration pneumonia and pneumonia associated with tracheostomy with and without mechanical ventilation,[71] where they are generally recovered mixed with Enterobacteriaceae, *Pseudomonas* spp. and *Staph. aureus*. Specimens for culture should be obtained in a manner that will avoid their contamination by the oral flora. They can be obtained using bronchoalveolar lavage, bronchoscopy via bronchial brush protected in a double-lumen plugged catheter (using quantitative cultures in the last two methods), percutaneous transtracheal aspiration, lung biopsy and thoracentesis (of empyema fluid). Management of these infections includes drainage of the pleural space in the presence of empyema, and antimicrobials effective against the anaerobic and aerobic bacteria (see Chapter 29).

Intra-abdominal infections

Secondary peritonitis and intra-abdominal abscesses generally occur because of the entry of enteric micro-organisms into the peritoneal cavity through a defect in the wall of the intestine or other viscus as a result of obstruction, infarction or direct trauma. Perforated appendicitis, inflammatory bowel disease with perforation and gastrointestinal surgery are often associated with polymicrobial infections caused by aerobic and anaerobic bacteria, where the number of isolates can average 12 (two-thirds are generally anaerobes). Diverticulitis and its complications is also associated with such an infection.[72]

The initial infection that follows perforation is peritonitis. This is a synergistic infection in which more than one organism is involved. Characteristically, the more types of bacteria that can be isolated, the graver the morbidity. The specific micro-organisms involved in peritonitis are generally those of the gastrointestinal tract flora where anaerobes outnumber aerobes in the ratio 1:1000–10 000.[1] Of the more than 400 bacterial species that constitute the gut flora, only the virulent ones survive in the peritoneum to cause the infection. The more distal the perforation in the gastrointestinal tract, the more numerous are the types and number of organisms that spill into the peritoneal cavity. An average of 11.6 organisms per specimen (8.5 anaerobes and 3.1 nonanaerobes) was recovered in a study of 71 patients with gangrenous and perforated appendicitis.[73] The predominant aerobic and facultatives are *E. coli* and *Streptococcus* spp. (including *Enterococcus* spp.), and the most frequently encountered anaerobes are the *B. fragilis* group, and *Peptostreptococcus, Clostridium, Fusobacterium* and *Eubacterium* spp. (see Table 173.10).[3,5]

Intra-abdominal infections are characteristically biphasic: in the initial stages a generalized peritonitis occurs, which is primarily associated with *E. coli* sepsis, and in the later stages, in which the infection is contained and intra-abdominal abscesses emerge, *B. fragilis* can be recovered.[74]

The clinical manifestations of secondary peritonitis are a reflection of the underlying disease process. Fever, diffuse abdominal pain, nausea and vomiting are characteristic. Physical examination reveals signs of peritoneal inflammation, including rebound tenderness, abdominal wall rigidity and decrease in bowel sounds. These early findings may be followed by signs and symptoms of shock.

Table 173.10 Aerobic and anaerobic bacteria isolated in various types of infection

Type of infection	Aerobic and facultative organisms	Anaerobic organism
Pleuropulmonary	*Staphylococcus aureus** viridans streptococci *Pseudomonas aeruginosa** Enterobacteriaceae*	Pigmented *Prevotella* spp. (*P. denticola, P. melaninogenica, P. intermedia, P. nigrescens, P. loescheii*) Nonpigmented *Prevotella* spp. *(P. oris, P. buccae, P. oralis)* *Fusobacterium nucleatum* *Peptostreptococcus* spp. *(P. micros, P. anaerobius, P. magnus)* *Bacteroides fragilis* group Nonspore-forming Gram-positive rods (*Actinomyces, Eubacterium, Lactobacillus* spp.)
Intra-abdominal	*Escherichia coli* *Enterococcus* spp. *Pseudomonas aeruginosa**	*Bacteroides fragilis* group *Bilophila wadsworthia* *Peptostreptococcus* spp. (especially *P. micros*) *Clostridium* spp.
Female genital tract	*Streptococcus* (groups A, B, others) *Escherichia coli* *Klebsiella pneumoniae* *Neisseria gonorrhoeae* (in sexually active patients) *Chlamydia* spp. (in sexually active patients) *Mycoplasma hominis* (in postpartum patients)	*Peptostreptococcus* spp. *Prevotella* spp. (especially *P. bivia, P. disiens*) *Bacteroides fragilis* group *Clostridium* spp. (especially *C. perfringens*) *Actinomyces, Eubacterium* spp. (in intrauterine contraceptive device-associated infections)
Skin and soft tissue	*Staphylococcus aureus* *Streptococcus* (*Strep. milleri group*, groups A and B, viridans group) *Enterococcus* spp.‡ Enterobacteriaceae‡ *Pseudomonas aeruginosa**	*Peptostreptococcus* spp. (*P. magnus, P. micros, P. asaccharolyticus*) Pigmented *Prevotella* spp.† *Actinomyces* spp. Fusobacterium nucleatum† *Bacteroides fragilis* group‡ *Clostridium* spp.‡

* Recovered in hospital-acquired infection.
† After exposure to oral flora.
‡ After exposure to colonic flora.

The manifestations of shock from a ruptured viscus merge with those of peritonitis and may be followed by toxemia, restlessness and irritability, a higher temperature, an increase in pulse rate, chills and convulsions. Biliary tract infection is caused by *E. coli*, *Klebsiella* and *Enterococcus* spp. Anaerobes (mostly *B. fragilis* group and rarely *C. perfringens*) can be recovered in complicated infections associated with carcinoma, recurrent infection, obstruction, bile tract surgery or manipulation.[75]

Laboratory studies reveal an elevated blood leukocyte count in excess of 12 000/mm^3 with a predominance of polymorphonuclear forms. Radiographs of the abdomen may reveal free air in the peritoneal cavity, evidence of ileus or obstruction and obliteration of the psoas shadow. Diagnostic ultrasound, gallium and CT scanning[76] may be useful in detecting appendiceal or other intra-abdominal abscesses. Postoperative wound infections can occur after appendectomy.

Appropriate management of mixed aerobic and anaerobic intra-abdominal infections requires the administration of antimicrobials that are effective against both aerobic and anaerobic components of the infection[2,5] as well as surgical correction and drainage of pus.[77] Single and easily accessible abscesses can be drained percutaneously, thus avoiding a surgical procedure. The outcome of the infection depends on a variety of factors that include the general condition of the patient (as measured by the Apache score[78]), the site of perforation, the bacteriology of the infection and the antimicrobial chosen for therapy. The principle of using antimicrobial coverage effective against both the aerobic and anaerobic offenders has become the cornerstone of practice and its efficacy has been confirmed by numerous studies.[77]

The choice of therapy should cover Enterobacteriaceae and anaerobes (mainly *B. fragilis* group), and can be achieved by combination or single-agent therapy. Single-agent therapy provides the advantage of avoiding the ototoxicity and nephrotoxicity of aminoglycosides and may be less expensive. However, a single agent may not be effective against hospital-acquired resistant bacterial strains, and the use of a single agent is devoid of antibacterial synergy, which may be important in immunocompromised hosts. However, for otherwise healthy persons, when therapy is initiated without a long delay, single agents provide adequate therapy. An anti-Enterobacteriaceae agent, such as an aminoglycoside, a quinolone or a third-generation cephalosporin, plus an anti-anaerobic agent, such as clindamycin, metronidazole or cefoxitin, is used as combination therapy. Single-agent therapy includes a carbapenem (e.g. imipenem, meropenem, ertapenem), tigecycline, or a penicillin plus a β-lactamase inhibitor (i.e. ticarcillin–clavulanate).[77] The need to add therapy directed at *Enterococcus* spp. is controversial and some authorities advocate adding such a drug (i.e. amoxicillin or vancomycin). However, these organisms are isolated in only 10–20% of cases and rarely in pure culture (see also Chapter 37).

Antimicrobial prophylaxis before colonic surgery includes either an oral preparation such as erythromycin plus neomycin or a parenteral antimicrobial such as cefoxitin. Use of prophylaxis has reduced the rate of postsurgical wound infection (see also Chapter 132).[79]

Female genital tract infection

Female genital tract infections involving anaerobes are polymicrobial and include the following: bacterial vaginosis; soft-tissue perineal, vulvar and Bartholin gland abscesses; endometritis; pyometra; salpingitis; tubo-ovarian abscesses; adnexal abscess; pelvic inflammatory disease, which may include pelvic cellulitis and abscess; amnionitis; septic pelvic thrombophlebitis; intrauterine contraceptive device-associated infection; septic abortion; and postsurgical obstetric and gynecologic infections.[3,5,80,81] Obtaining proper cultures is often difficult, and avoiding their contamination by the normal genital flora can be achieved by use of culdocentesis, laparoscopy or quantitative endometrial cultures of transcervical samples obtained with a telescoping catheter.

The predominant anaerobes include *Prev. bivia, Prev. disiens* and *Peptostreptococcus, Porphyromonas* and *Clostridium* spp. *Bacteroides*

fragilis group is less often isolated in these infections than in intra-abdominal infection.[81] *Actinomyces* spp. and *Eubacterium nodatum* are commonly isolated in infections associated with intrauterine devices. *Mobiluncus* spp. may be involved with bacterial vaginosis.[3,5,80] The aerobic organisms also isolated mixed with these anaerobes include Enterobacteriaceae, *Streptococcus* spp. (including groups A and B), *Neisseria gonnorhoeae* and *Chlamydia* spp. (in sexually active females) and *Mycoplasma hominis* (see Table 173.10).

Clinical findings associated with the presence of anaerobes include gas in the tissues, abscess formation and foul-smelling discharge. Management of polymicrobial pelvic infection includes the use of antimicrobials effective against all potential aerobic and anaerobic pathogens. Additionally, coverage against sexually transmissible pathogens should be provided. The therapeutic regimens include doxycycline, levofloxacin or gentamicin in combination with cefoxitin, cefotetan, ampicillin–sulbactam, clindamycin or metronidazole.[82]

Skin and soft-tissue infections

Skin and soft-tissue infections that generally involve anaerobes include superficial infections, such as infected cutaneous ulcers, cellulitis, pyoderma, paronychia, hidradenitis suppurativa and a variety of secondary infected sites. These include secondary infected diaper rash, gastrostomy or tracheostomy site wounds, infected subcutaneous sebaceous or inclusion cysts, eczema, psoriasis, poison ivy, atopic dermatitis, eczema herpeticum, scabies or kerion infections, and post-surgical wound infection.[3,5,83–86]

Subcutaneous tissue infections that may also include skin involvement include cutaneous and subcutaneous abscesses, breast abscess, decubitus ulcers, infected diabetic (vascular or trophic) ulcers (Fig. 173.7), bite wound (Fig. 173.8), anaerobic cellulitis (Fig. 173.9) and gas gangrene, bacterial synergistic gangrene, infected pilonidal cyst or sinus, Meleney's ulcer and burn wound infection.[84,86] Anaerobic soft-tissue infections that occur deeper are necrotizing fasciitis (see Fig. 10.5), necrotizing synergistic cellulitis, gas gangrene and crepitus cellulitis.[87] These infections can involve the fascia and the muscle surrounded by the fascia, and can induce myositis (Fig. 173.10) and myonecrosis.

The organisms recovered from soft-tissue infections vary according to the type of infection. However, the location and the circumstances leading to the infection can influence the type of organism involved in the infection. Cultures from lesions involving anaerobes frequently contain bacterial species that are members of the 'normal flora' of the region of the infection (Fig. 173.11).

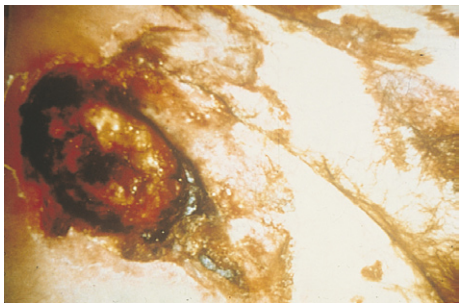


Fig. 173.9 Polymicrobial necrotizing cellulitis. The initial lesion is a reddish-brown bleb and is accompanied by local tenderness.

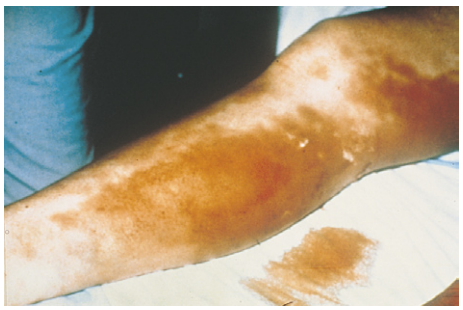


Fig. 173.10 Anaerobic streptococcal myositis involving muscle and fascial planes.



Fig. 173.7 Infected diabetic ulcer.



Fig. 173.8 Human bite wound.

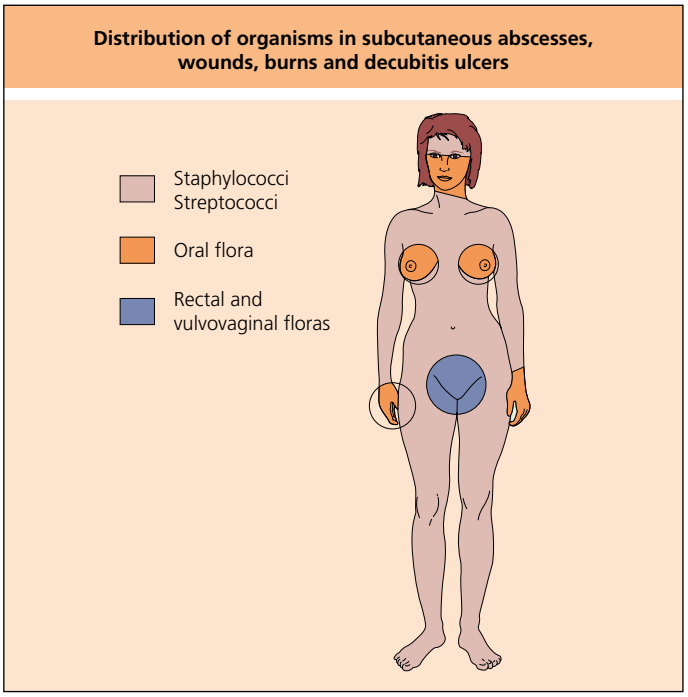


Fig. 173.11 Distribution of organisms in subcutaneous abscesses, wounds, burns and decubitus ulcers.

Aspirates from wounds and subcutaneous tissue infections and abscesses of the rectal area (decubitus ulcer, perirectal abscess) or those that originate from the gut flora (i.e. diabetic foot infection) tend to yield organisms found in the colonic flora.[3,5,84–87] These include *B. fragilis* group, *Clostridium* spp., Enterobacteriaceae and *Enterococcus* spp. In contrast, infections in and around the oropharynx, or those that originate from that site, generally contain members of the oral flora (i.e. paronychia, bites, breast abscess). These include pigmented *Prevotella* and *Porphyromonas, Fusobacterium* and *Peptostreptococcus* spp. Skin flora organisms such as *Staph. aureus* and *Streptococcus* spp. or nosocomially acquired organisms can be isolated at all body sites (see Table 173.10). In addition to oral flora, human bite infections often contain *Eikenella* spp. and animal bites harbor *Pasteurella multocida*.[88]

Infections involving anaerobes are generally polymicrobial, and in some (i.e. decubitus ulcers, diabetic foot ulcer) they are often complicated by osteomyelitis or bacteremia.[89] Deep tissue infections such as necrotizing cellulitis, fasciitis and myositis often involve *Clostridium* spp., *Strep. pyogenes* or a polymicrobic combination of aerobic and anaerobic bacteria. They often contain gas in the tissues and putrid-like pus with a gray thin quality, and are associated with a high rate of bacteremia and mortality.[87,90]

Management of deep-seated soft-tissue infection includes surgical debridement, drainage and vigorous surgical management. Improvement of oxygenation of the involved tissues through enhancement of blood supply and administration of hyperbaric oxygen, especially in clostridial infection, may be helpful, although there is no formal evidence demonstrating the benefit of hyperbaric oxygen.

Osteomyelitis and septic arthritis

Osteomyelitis caused by anaerobic bacteria is often indistinguishable from infection caused by aerobic bacteria. Anaerobes are especially notable in osteomyelitis of the long bones after trauma and fracture, osteomyelitis related to peripheral vascular disease, and decubitus ulcers and osteomyelitis of cranial and facial bones.[89,91] Most of these infections are polymicrobial.

Anaerobic osteomyelitis of cranial and facial bones is generally caused by spread from a contiguous soft-tissue source or from sinus, ear or dental infection. The high number of anaerobes within the oral flora accounts for their importance in cranial osteomyelitis. The predominance of intestinal anaerobes in pelvic osteomyelitis has been related to their spread from decubitus ulcers.[5] The anaerobes in osteomyelitis associated with peripheral vascular disease access the involved bone from adjacent soft-tissue ulcers. Osteomyelitis of long bones is generally caused by hematogenic spread, trauma or the presence of a prosthetic device.

Anaerobic streptococci and *Bacteroides* spp. are the most common organisms at all sites, including osteomyelitis associated with bites and cranial infection. Pigmented *Prevotella* and *Porphyromonas* spp. are especially prevalent in skull and bite infections, whereas members of the *B. fragilis* group are associated with vascular disease or neuropathy. Fusobacteria, which are members of the oral flora, are most frequently isolated from bites and from cranial and facial infections. Clostridia are most often found in long bones, especially in association with environmental wound contamination after trauma. Because clostridia colonize the lower gastrointestinal tract, they may contaminate compound fractures of the lower extremities.

Septic arthritis caused by anaerobic bacteria is uncommon, and is often associated with hematogenous and contiguous spread of infection, trauma and prosthetic joints.[79,82] Most cases of septic arthritis caused by anaerobes are monomicrobial. The predominant isolated anaerobes are peptostreptococci and *Prop. acnes* (often in prosthetic joint infection), *B. fragilis* and fusobacteria (often in infections of hematogenic origin), and clostridia (associated with trauma).

Bacteremia

The incidence of anaerobes in bacteremia is 5–15%.[90] However, in the 1990s the incidence was lowered to approximately 4% (0.5–12%) of all cases of bacteremia, with variation by geographic location, hospital patient demographics and especially patient age.[92] Recent studies, however, documented a resurgence in bacteremia due to anaerobic bacteria.[93] This increase is explained by the higher incidence of anaerobic bacteremia in immunosuppressed patients and patients with complex underlying disease.[93] The most prevalent blood culture isolates are *B. fragilis* group (over 75% of anaerobic isolates). Other common isolates include *Clostridium* spp. (10–20%), *Peptostreptococcus* spp. (10–15%), *Fusobacterium* spp. (10–15%) and *Prop. acnes* (2–5%).

The specific organisms involved in bacteremia largely depend on the portal of entry and underlying disease. Recovery of *B. fragilis* group and clostridia is mostly associated with a gastrointestinal source, pigmented *Prevotella* and *Porphyromonas* spp. and fusobacteria with oropharynx and pulmonary sources, fusobacteria with the female genital tract, *Prop. acnes* with foreign body, and peptostreptococci with all sources, but especially with oropharyngeal, pulmonary and female genital tract sources. The predominance of these isolates in conjunction with the specific sources is related to the origin of the primary infection and the endogenous flora at the infection site.

The predisposing factors to anaerobic bacterial bacteremia include malignant neoplasms; hematologic disorders; organ transplant; recent gastrointestinal, obstetric, or gynecologic surgery; intestinal obstruction; decubitus ulcers; dental extraction; the newborn; sickle cell disease; diabetes mellitus; postsplenectomy; and the use of cytotoxic agents or corticosteroids.[3,5]

The clinical presentation of anaerobic bacteremia is similar to that seen with aerobic infection, except for the signs of infection at the portal of entry of infection. It commonly includes fever, chills, hypotension, leukocytosis, shock, disseminated intravascular coagulation and anemia. Features typical of anaerobic infection include metastatic lesions, hyperbilirubinemia and suppurative thrombophlebitis. Mortality rate varies between 15% and 30% and is improved with early and appropriate antimicrobial therapy and resolution, when present, of the primary infection.

MANAGEMENT

The recovery from an anaerobic infection depends on prompt and proper management. The principles of managing anaerobic infections include neutralizing toxins produced by anaerobes, preventing their local proliferation by changing the environment and hampering their spread into healthy tissues.

Toxin neutralization by specific antitoxins may be employed, especially in infections caused by *Clostridium* spp. (tetanus and botulism). Controlling the environment is achieved by debriding of necrotic tissue, draining the pus, improving circulation, alleviating the obstruction and increasing the tissue oxygenation. Certain types of adjunct therapy such as hyperbaric oxygen (HBO) may also be useful. The primary role of antimicrobials is in limiting the local and systemic spread of the organism.

Hyperbaric oxygen

There is controversy regarding whether HBO should be used in infection of spore-forming Gram-positive anaerobic rods. There are several uncontrolled reports that demonstrated efficacy in individual cases.[3,5,94] However, because no well-controlled studies are available, the efficacy of HBO is unproved. Using HBO in conjunction with other therapeutic measures is not contraindicated, except when it may delay the execution of other essential procedures. Topical application of oxygen-releasing compounds may be useful as an adjunct to other procedures.

Surgical therapy

In many cases surgical therapy is the most important and sometimes the only form of treatment required, whereas in others surgical

therapy is an important adjunct to a pharmacologic approach. Surgery is important in draining abscesses, debriding necrotic tissues, decompressing closed space infections and relieving obstructions. When surgical drainage is not used, the infection may persist and serious complications can develop.

Antimicrobial therapy

Appropriate management of mixed aerobic and anaerobic infections requires the administration of antimicrobial drugs effective against both the aerobic and the anaerobic components. A number of factors should be considered when choosing appropriate antimicrobial agents. They should have efficacy against all target organisms, induce little or no resistance, achieve sufficient levels in the infected site, and have minimal toxicity and maximum stability.

Antimicrobials often fail to cure the infection. Among the reasons for this are the development of bacterial resistance, achievement of insufficient tissue levels, incompatible drug interaction and the development of an abscess. The environment of an abscess is detrimental to many antibiotics. The abscess capsule interferes with the penetration of drugs, and the low pH and the presence of binding proteins or inactivating enzymes (i.e. β-lactamase) may impair their activity. The low pH and the anaerobic environment within the abscess are especially unfavorable for the aminoglycosides and quinolones. However, an acidic pH, high osmolarity and an anaerobic environment can also develop in the absence of an abscess.

When choosing antimicrobials (see Table 173.13) for the therapy of mixed infections, their aerobic and anaerobic antibacterial spectrum (Fig. 173.12) and their availability in oral or parenteral form should be considered (Table 173.11). Some antimicrobials have a limited range of activity. For example, metronidazole is active only against anaerobes and therefore cannot be administered as a single agent for the therapy of mixed infections. Others (i.e. carbapenems) have wide spectra of activity against Enterobacteriaceae and anaerobes.

The selection of antimicrobial agents is simplified when reliable culture results are available. However, this may be difficult to achieve in anaerobic infections because of the problems in obtaining appropriate specimens. For this reason, many patients are treated empirically on the basis of suspected, rather than established, pathogens. Fortunately, the types of organism involved in many anaerobic infections and their antimicrobial susceptibility patterns tend to be predictable. However, the pattern of resistance to antimicrobials may vary in a particular hospital and resistance to antimicrobial agents may emerge while a patient is receiving therapy.

The susceptibility of the *B. fragilis* group to the frequently used antimicrobial drugs has been studied systemically over the past two decades. These surveys have failed to reveal strains resistant to chloramphenicol or metronidazole and minimal resistance (<1%) to imipenem or the combinations of a penicillin and β-lactamase inhibitors, but resistance to other agents varies. The rate differs among various medical centers and generally increases with extensive use of some antimicrobial agents (penicillins, cephalosporins and clindamycin).[95]

Aside from susceptibility patterns, other factors influencing the choice of antimicrobial therapy include the pharmacologic characteristics of the various drugs, their toxicity, their effect on the normal flora and bactericidal activity. Although identification of the infecting organisms and their antimicrobial susceptibility may be needed for selection of optimal therapy, the clinical setting and Gram-stain preparation of the specimen may indicate the types of anaerobe present in the infection as well as the nature of the infectious process.

Antimicrobial agents

Some classes of agent have poor activity against anaerobic bacteria. These include the aminoglycosides, the monobactams and the currently available quinolones. Antimicrobial agents suitable for use in controlling aerobic infections are discussed in more detail below.[96,97]

Penicillins

Penicillin G (benzylpenicillin) is still the drug of choice against most non-BLPB. These include anaerobic streptococci, *Clostridium* spp. and nonsporulating anaerobic bacilli, and most non-β-lactamase-producing Gram-negative anaerobic rods (i.e. *Bacteroides, Fusobacterium, Prevotella* and *Porphyromonas* spp.).[96] However, in addition to the *B. fragilis* group, which is known to resist the drug, many other anaerobic Gram-negative rods are showing increased resistance. These include *Fusobacterium* spp., pigmented *Prevotella* and *Porphyromonas* spp. (prevalent in orofacial infections), *Prev. bivia* and *Prev. disiens* (common in obstetric and gynecologic infections), and *Bilophila wadsworthia* and *Bacteroides splanchinus*. Resistance to penicillin of some *Clostridium* spp. through production of β-lactamase has also been noted. These included *C. ramosum, C. clostridioforme* and *C. butyricum*.

The increase in the number of penicillin-resistant bacterial strains has important implications for antimicrobial therapy. Many penicillin-resistant bacteria can produce enzymes that degrade penicillins or cephalosporins by releasing the enzyme in the area of the infection. Therefore, these organisms may protect not only themselves but also penicillin-sensitive pathogens. Penicillin therapy directed against a susceptible pathogen might therefore be rendered ineffective by the presence of BLPB.[46]

The use of combinations of β-lactamase inhibitors (such as clavulanic acid, sulbactam or tazobactam) plus a β-lactam antibiotic (ampicillin, amoxicillin, ticarcillin or piperacillin) can overcome this phenomenon in organisms that produce a β-lactamase that can be

Susceptibility of anaerobic bacteria to antimicrobial agents

Bacteria	Penicillin	A penicillin and a β-lactamase inhibitor	Ureido- and carboxy-penicillin	Cefoxitin	Chloramphenicol	Clindamycin	Macrolides	Metronidazole	Carbapenems
Peptostreptococcus spp.	Excellent	Excellent	Good	Good	Good	Good	Moderate	Moderate	Good
Fusobacterium spp.	Good	Good	Good	Good	Good	Moderate	Minimal	Good	Good
Bacteroides fragilis group	Minimal	Excellent	Moderate	Good	Good	Good	Minimal	Excellent	Excellent
Prevotella and *Porphyromonas* spp.	Minimal	Excellent	Moderate	Good	Good	Good	Moderate	Excellent	Excellent
Clostridium perfringens	Excellent	Excellent	Good	Good	Good	Good	Good	Good	Good
Clostridium spp.	Good	Good	Good	Moderate	Good	Moderate	Moderate	Good	Good
Actinomyces spp.	Excellent	Excellent	Good	Good	Good	Good	Good	Minimal	Good

Degrees of activity: Minimal, Moderate, Good, Excellent

Fig. 173.12 Susceptibility of anaerobic bacteria to antimicrobial agents.

Table 173.11 Antimicrobial drugs recommended for the therapy* of site-specific anaerobic infections

	Surgical prophylaxis	Parenteral	Oral
Intracranial	1. Penicillin 2. Vancomycin	1. Metronidazole[4] 2. Chloramphenicol	1. Metronidazole[4] 2. Chloramphenicol
Dental	1. Penicillin 2. Erythromycin	1. Clindamycin 2. Metronidazole[4], chloramphenicol	1. Clindamycin, amoxicillin + CA 2. Metronidazole[4]
Upper respiratory tract	1. Cefoxitin 2. Clindamycin	1. Clindamycin 2. Chloramphenicol, metronidazole[4]	1. Clindamycin, amoxicillin + CA 2. Metronidazole[5]
Pulmonary	NA	1. Clindamycin[5] 2. Ticarcillin + CA, ampicillin + SU[6], imipenem or meropenem	1. Clindamycin[8] 2. Metronidazole[5], amoxicillin + CA
Abdominal	1. Cefoxitin 2. Clindamycin[3]	1. Clindamycin[3], cefoxitin[3], metronidazole[3] 2. Imipenem or meropenem ertapenem, piperacillin-tazobactam, tigecycline	1. Clindamycin[8], metronidazole[8] 2. Amoxicillin + CA
Pelvic	1. Cefoxitin 2. Doxycycline	1. Cefoxitin[6], clindamycin[3] 2. Piperacillin–tazobactam[6], ampicillin + SU[6], metronidazole[6]	1. Clindamycin[6] 2. Amoxicillin + CA[6], metronidazole[6]
Skin and soft tissue	1. Cefazolin[7] 2. Vancomycin	1. Clindamycin, cefoxitin 2. Metronidazole + vancomycin 3. Tigecycline	1. Clindamycin, amoxicillin + CA 2. Metronidazole + linezolid
Bone and joint	1. Cefazolin[7] 2. Vancomycin	1. Clindamycin, imipenem or meropenem 2. Metronidazole + vancomycin, piperacillin–tazobactam	1. Clindamycin 2. Metronidazole + linezolid
Bacteremia with BLPB	NA	1. Imipenem or meropenem, metronidazole 2. Cefoxitin, ticarcillin + CA	1. Clindamycin, metronidazole 2. Chloramphenicol, amoxicillin + CA
Bacteremia with non-BLPB	NA	1. Penicillin 2. Clindamycin, metronidazole, cefoxitin	1. Penicillin 2. Metronidazole, chloramphenicol, clindamycin

* Therapies are given as drug(s) of choice (alternative drugs). BLPB, β-lactamase-producing bacteria; CA, clavulanic acid; NA, not applicable; SU, sulbactam.
1, drug(s) of choice; 2, alternative drugs; 3, plus aminoglycoside; 4, plus a penicillin; 5, plus a macrolide (i.e. erythromycin); 6, plus doxycycline; 7, in location proximal to the rectal and oral areas use cefoxitin; 8, plus a quinolone (only in adults).

bound by the inhibitor. However, if other mechanisms of resistance emerge, blockage of the enzyme β-lactamase will not prevent resistance. Other mechanisms of resistance include alteration in the porin canal through which the antimicrobial penetrates into the bacteria and changes in the penicillin-binding protein that inhibit binding of the drug into the bacterial cell wall.

The semisynthetic penicillins, carbenicillin, ticarcillin, piperacillin and mezlocillin are generally administered in large quantities to achieve high serum concentrations. These drugs have good activity against Enterobacteriaceae and most anaerobes in these concentrations. However, they are not absolutely resistant to β-lactamase produced by Gram-negative anaerobic bacilli.

Cephalosporins

The efficacy of cephalosporins varies against *Bacteroides* spp.[96,97] The activity of the first-generation cephalosporins against anaerobes is similar to that of penicillin G, although on a weight basis they are less active. Most strains of the *B. fragilis* group and many *Prevotella* and *Porphyromonas* spp. are resistant by virtue of cephalosporinase production. The second-generation cephalosporin cefoxitin is relatively resistant to this enzyme and is therefore the most effective cephalosporin against the *B. fragilis* group. However, 5–15% of *B. fragilis* group may be resistant, reflecting hospital use pattern. Because of its wide antibacterial coverage, it is often used for the therapy and prophylaxis of mixed infections. Cefoxitin is relatively inactive against most species of *Clostridium* (including *C. difficile*); *C. perfringens* is an exception. Other second-generation cephalosporins, such as cefotetan and cefmetazole, have a longer half-life than cefoxitin. These two agents are as effective as cefoxitin against *B. fragilis*, but have poor efficacy against other members of the *B. fragilis* group (i.e. *B. thetaiotaomicron*). Third-generation cephalosporins have activity against *Bacteroides* spp. that is inferior to that of second-generation cephalosporins.

Carbapenems (imipenem, meropenem, ertapenem)

The β-lactam carbapenems imipenem and meropenem have excellent activity against a broad spectrum of aerobic and anaerobic bacteria, including β-lactamase-producing *Bacteroides* spp. and Enterobacteriaceae and *Pseudomonas* spp.[97] Resistance of *B. fragilis* group is very rare (<1%). Ertapenem has similar efficacy but is not active against *Pseudomonas* spp. and *Acinetobacter* spp.[98]

Chloramphenicol

Chloramphenicol shows excellent *in-vitro* activity against most anaerobic bacteria, and resistance is rare. The drug is also effective against many Enterobacteriaceae and Gram-positive cocci. However, the experience of using this drug in intra-abdominal sepsis was disappointing. The toxicity of chloramphenicol, the rare but fatal aplastic anemia and the dose-dependent leukopenia, limit its use, especially in neutropenic patients.

Clindamycin and lincomycin

Clindamycin and lincomycin are effective against anaerobes and have good activity against aerobic Gram-positive cocci.[96] Clindamycin has broader coverage against anaerobes, including β-lactamase-producing *Bacteroides* spp. Resistance of *B. fragilis* group is 5–10%, and some *Clostridium* spp. other than *C. perfringens* are resistant. Antibiotic-associated colitis caused by *C. difficile* was first described after clindamycin therapy. However, colitis has been associated with many other antimicrobial agents, including penicillins and cephalosporins.

Metronidazole

Metronidazole has excellent activity against anaerobes. However, its antibacterial efficacy is limited to anaerobic bacteria. Microaerophilic streptococci, *Prop. acnes* and *Actinomyces* spp. are often resistant. Concern was raised about the carcinogenic and mutagenic effects of this drug; however, these effects were shown only in one species of mice and were never substantiated in other animals or humans.[3,5]

Macrolides (erythromycin, azithromycin, clarithromycin)

The macrolides erythromycin, azithromycin and clarithromycin have moderate-to-good *in-vitro* activity against anaerobic bacteria other than *B. fragilis* group and fusobacteria. They are active against *Prevotella* and *Porphyromonas* spp., microaerophilic and anaerobic streptococci, Gram-positive nonspore-forming anaerobic bacilli and certain clostridia. They show relatively good activity against *C. perfringens* and poor or inconsistent activity against Gram-negative anaerobic bacilli.[99]

Glycopeptides (vancomycin, teicoplanin)

The glycopeptides are effective against all Gram-positive anaerobes (including *C. difficile*), but are inactive against Gram-negative bacilli.

Tetracyclines

Tetracycline is of limited use because of the development of resistance by all types of anaerobe, including *B. fragilis* group. The newer tetracycline analogues doxycycline and minocycline are more active than the parent compound. Because there is significant resistance to these drugs, they can be used if the organisms are susceptible or in less severe infections in which a therapeutic trial is feasible.

Tygecycline

Tygecycline, a glycylcycline, is a direct analogue of minocycline with a 9-glycylamide moiety. It has activity against both Gram-negative and Gram-positive bacteria, anaerobes and certain drug-resistant pathogens including methicillin-resistant *Staph. aureus*.[100] It is active against *Streptococcus anginosus* group (which includes *Strep. anginosus, Strep. intermedius* and *Strep. constellatus*), *Bacteroides fragilis, Bacteroides thetaiotaomicron, Bacteroides uniformis, Bacteroides vulgatus, Clostridium perfringens* and *Peptostreptococcus micros.*[101]

Quinolones

The first generation of fluoroquinolones such as ciprofloxacin and ofloxacin are inactive against most anaerobic bacteria. However, some broad-spectrum quinolones, which have recently become clinically available or are under active development, have significant anti-anaerobic activity. Quinolones with low activity against anaerobes include ciprofloxacin, ofloxacin, levofloxacin, fleroxacin, pefloxacin, enoxacin and lomefloxacin. Compounds with intermediate anti-anaerobic activity include sparfloxacin and grepafloxacin. Trovafloxacin, gatifloxacin and moxifloxacin yield low minimum inhibitory concentrations (MICs) against most groups of anaerobes. Quinolones with the greatest *in-vitro* activity against anaerobes include clinafloxacin and sitafloxacin.[102] The use of the quinolones is restricted in growing children and during pregnancy because of their possible adverse effects on cartilage.

Table 173.12 Antimicrobial drugs of choice for anaerobic bacteria

	Drug of choice	Alternative drugs
Peptostreptococcus spp.	Penicillin	Clindamycin, chloramphenicol, cephalosporins
Clostridium spp.	Penicillin	Metronidazole, chloramphenicol, cefoxitin, clindamycin
Clostridium difficile	Vancomycin	Metronidazole, bacitracin
Fusobacterium spp.	Penicillin	Metronidazole, clindamycin, chloramphenicol
Bacteroides (BL–)	Penicillin	Metronidazole, clindamycin, chloramphenicol
Bacteroides (BL+)	Metronidazole, a carbapenem, a penicillin and β-lactamase inhibitor, clindamycin	Cefoxitin, chloramphenicol, piperacillin, tigecycline

Gram-negative bacilli include *Bacteroides fragilis* group and *Prevotella*, *Porphyromonas* and *Fusobacterium* spp.
BL, β-lactamase.

Other agents

Bacitracin is active against pigmented *Prevotella* and *Porphyromonas* spp. but is inactive against *B. fragilis* and *Fusobacterium nucleatum*.[2] Quinupristin–dalfopristin shows antibacterial activity against anaerobic organisms tested, including *C. perfringens* and *Lactobacillus* and *Peptostreptococcus* spp.[103] Linezolid is active against *F. nucleatum*, other fusobacteria and *Porphyromonas, Prevotella* and *Peptostreptococcus* spp.[104] Little clinical experience has, however, been gained in the treatment of anaerobic bacteria using these agents.

Choice of antimicrobial agents

The parenteral antimicrobials that can be used in most infectious sites (Tables 173.11, Table 173.12) are clindamycin, metronidazole, chloramphenicol, cefoxitin, a penicillin (e.g. ticarcillin, ampicillin, piperacillin) and a β-lactamase inhibitor (e.g. clavulanic acid, sulbactam, tazobactam), tigecycline, and a carbapenem (e.g. imipenem, meropenem, ertapenem).

An agent effective against Gram-negative enteric bacilli (e.g. aminoglycoside, a fluoroquinolone) or an antipseudomonal cephalosporin (e.g. cefepime) are generally added to clindamycin, metronidazole and, occasionally, cefoxitin when treating intra-abdominal infections to provide coverage for these bacteria. As failure of therapy in intra-abdominal infections has been noted more often with chloramphenicol, this drug is not recommended.

Penicillin can be added to metronidazole in the therapy of intracranial, pulmonary and dental infections to cover for microaerophilic streptococci, *Actinomyces* spp. and *Arachnia* spp. A macrolide (i.e. erythromycin) is added to metronidazole in upper respiratory infections to treat *Staph. aureus* and aerobic streptococci. Penicillin is added to clindamycin to supplement its coverage against *Peptostreptococcus* spp. and other Gram-positive anaerobic organisms.

Table 173.13 Activity of antimicrobial agents against anaerobes

Agent	Comments
Nearly always active	
Metronidazole	Inactive versus microaerophilic streptococci (e.g. *Streptococcus milleri*), *Propionibacterium* and *Actinomyces* spp.; bactericidal versus most Gram-negative anaerobic strains
Carbapenems	Resistant to most *Bacteroides* β-lactamases, although a novel β-lactamase that cleaves carbapenems was found in rare *B. fragilis* strains*
β-Lactam plus β-lactamase inhibitors	The addition of a β-lactamase inhibitor to a β-lactam dramatically increases activity against anaerobes that produce a β-lactamase
Chloramphenicol	Good activity versus virtually all clinically significant anaerobes†
Usually active	
Clindamycin	*B. fragilis* group: 10–20% of strains resistant; some clostridia other than *C. perfringens* are resistant
Cefamycins	*B. fragilis group*: 5–15% of strains resistant with considerable institutional variation at least partly reflecting use patterns; poor activity versus clostridia
Antipseudomonal	Relatively resistant to β-lactamases of *Bacteroides* spp; penicillins large doses usually employed
Variable activity	
Penicillin	Inactive versus some or most penicillinase-producing anaerobes, including most of the *B. fragilis* group and many strains of *Prevotella melaninogenica, P. intermedia, P. bivia, P. disiens* and some clostridia
Cephalosporins	Less activity *in vitro* than penicillin G versus most anaerobes and limited other than cefamycins published clinical experience to document efficacy
Tetracycline	Inactive versus many anaerobes and most strains of *B. fragilis*; doxycycline and minocycline are somewhat more active than tetracycline
Vancomycin	Active against Gram-positive anaerobes; inactive versus Gram-negative anaerobes
Macrolides	Inactive versus many *Fusobacterium* spp. and some *B. fragilis* spp.; ketolides also show reduced activity versus fusobacteria
Fluoroquinolones	'Third-generation' (gatifloxacin, moxifloxacin and gemifloxacin) show good *in-vitro* activity; limited published data
Tigecycline	Active against nearly all anaerobes including strains of *B. fragilis* that are resistant to β-lactams, clindamycin and quinolones. Minimum inhibitory concentrations are somewhat higher for clostridia‡
Penicillin	Inactive versus some or most penicillinase-producing anaerobes, including most of the *B. fragilis* group and many strains of *Prevotella melaninogenica, P. intermedia, P. bivia, P. disiens* and some clostridia
Cephalosporins	Less activity *in vitro* than penicillin G versus most anaerobes and limited other than cefamycins published clinical experience to document efficacy
Tetracycline	Inactive versus many anaerobes and most strains of *B. fragilis*; doxycycline and minocycline are somewhat more active than tetracycline
Vancomycin	Active against Gram-positive anaerobes; inactive versus Gram-negative anaerobes
Poor activity	
Aminoglycosides	
Trimethoprim–sulfamethoxazole	
Monobactams (aztreonam)	

*Edwards R, *et al*. J Antimicrob Chemother 1999;43:273–6.
†While *in-vitro* activity is excellent, clinical failures with chloramphenicol have been documented, rendering this drug less preferable than other active agents for the treatment of anaerobic infections.
‡Snydman DR, *et al*. J Antimicrob Chemother 2005;55:1024–8.

Doxycycline is added to most regimens in the treatment of pelvic infections to provide therapy for *Chlamydia* and *Mycoplasma*. Penicillin is still the drug of choice for bacteremia caused by non-BLPB. However, other agents should be used for the therapy of bacteremia caused by BLPB.

Because the duration of therapy for anaerobic infections, which are often chronic, is generally longer than for infections caused by aerobic and facultative anaerobes, oral therapy is often substituted for parenteral therapy. The agents available for oral therapy are limited and include clindamycin, amoxicillin plus clavulanic acid, chloramphenicol and metronidazole.

Clinical judgment, personal experience, safety and patient compliance should direct the physician in the choice of the appropriate antimicrobial agents. The duration of therapy generally ranges between 2 and 4 weeks, but should be individualized depending on the response. In some cases, such as lung abscesses, treatment may be required for as long as 6–8 weeks, but can often be shortened with proper surgical drainage.

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References for this chapter can be found online at http://www.expertconsult.com

Chapter 174

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Mycobacteria

INTRODUCTION

Cardinal Richelieu (1585–1642), Nicolai Paganini (1782–1840), Heinrich Heine (1797–1856), Anton Chekhov (1860–1904), Franz Kafka (1883–1924), Eleanor Roosevelt (1884–1962) and George Orwell (1903–1950) all shared a common fate. Each of these famous people died of tuberculosis. In some cases their disease was not understood, while in other cases management of the disease was inadequate, mostly because of a lack of effective therapeutic agents.

Clearly, the presence of a concurrent disease (especially AIDS) as well as the overall state of person's health and host defense mechanisms are critical determinants of the morbidity and mortality rates of tuberculosis. However, even more complex factors strongly influence the incidence of tuberculosis including poverty, ignorance and indifference about the disease, and the lack of access to adequate health care. In the World Health Organization (WHO) report on global tuberculosis control,[1] 22 countries were identified as having a 'high burden' of tuberculosis. Based on surveillance and survey data, it is estimated that there were 8.8 million new cases of tuberculosis in 2005 (136 per 100 000), including 3.9 million (60 per 100 000) new smear-positive, and thus, highly infectious cases. The goals of the WHO global tuberculosis control program are to detect 70% of tuberculosis cases in the high-burden countries and to successfully treat 85% of the identified cases.

In addition to *Mycobacterium tuberculosis*, the two most important key players in global mycobacterial disease are *M. leprae* and *M. ulcerans*. During the past years notably *M. avium* complex and the rapidly growing mycobacteria have been the subject of extensive investigations as well, largely because of the increasing incidence of human disease and awareness of disease caused by nontuberculous mycobacteria (NTM).[2–5] As a consequence, accurate laboratory diagnosis of all mycobacterial species, a major focus of this chapter, is an important element for effective treatment and control of any mycobacterial disease.

GENERAL CHARACTERISTICS OF MYCOBACTERIAL ORGANISMS

Lehmann and Neumann first introduced the genus *Mycobacterium* into the scientific literature in 1896. The subsequent history of the genus has been profoundly influenced by the fact that only very few of the more than over 100 currently recognized species have been a devastating cause of human disease and suffering, above all *M. tuberculosis*. Thus, studies of microbial physiology, structure, genetics and diagnostic tools have mainly focused on *M. tuberculosis* and secondarily on *Mycobacterium leprae*.

The genus *Mycobacterium* is the only genus in the family of the Mycobacteriaceae[6] and is related to other mycolic acid-containing genera. All mycobacteria are aerobic (though some species are able to grow under a reduced oxygen atmosphere), nonspore-forming, nonmotile, slightly curved or straight rods. The most prominent feature of mycobacteria that is uniformly present and distinctive to the genus is the lipid-rich cell envelope.[7,8] Indeed, it is the complex cell envelope of mycobacteria that confers upon these bacteria the property of 'acid-fastness' (i.e. resistance to decolorization when stained with carbolfuchsin and decolorized with dilute hydrochloric acid). Uniformly, they do not stain well with Gram stain. Mycobacteria possess a cell wall polysaccharide that resembles that of Gram-positive bacteria; however, the mycobacterial peptidoglycan contains lipids in place of proteins and polysaccharides.[9] Furthermore, the mycobacterial envelope contains a plasma membrane that is quite similar in structure and function to the plasma membrane of other bacteria, except for the presence of lipoarabinomannan (LAM), lipomannan and phosphatidylinositol mannosides (Fig. 174.1). As a whole, the cell wall component of the

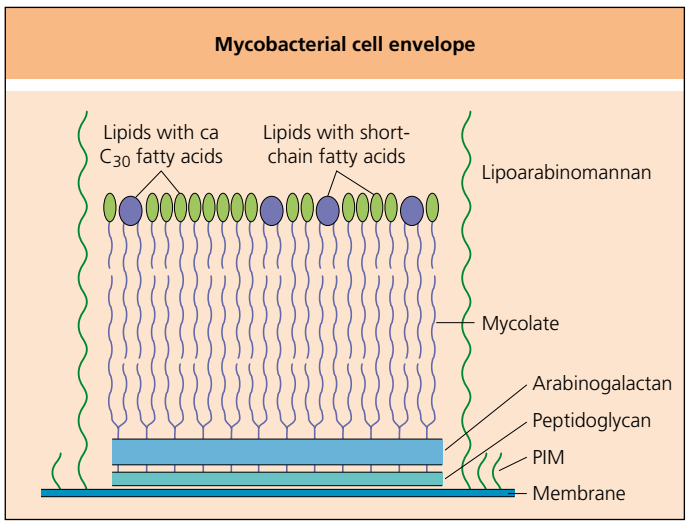


Fig. 174.1 Mycobacterial cell envelope. This model displays the asymmetric array of the structural elements extending from the plasma membrane that surrounds the cytoplasm of the mycobacterial cell. The arabinogalactan is covalently linked to the peptidoglycan, which along with the lipoarabinomannan (LAM) and phosphatidylinositol mannosides (PIM) are associated with the plasma membrane. The cell wall lipids are shown in a possible arrangement with the mycolates linked to the arabinogalactan. Two classes of polar lipids with medium and short chain fatty acids complement the varying hydrocarbon chains of the mycolates to create an even cell envelope. There is evidence for a small number of porins within the hydrophobic bilayer. Adapted from Brennan & Draper.[10]

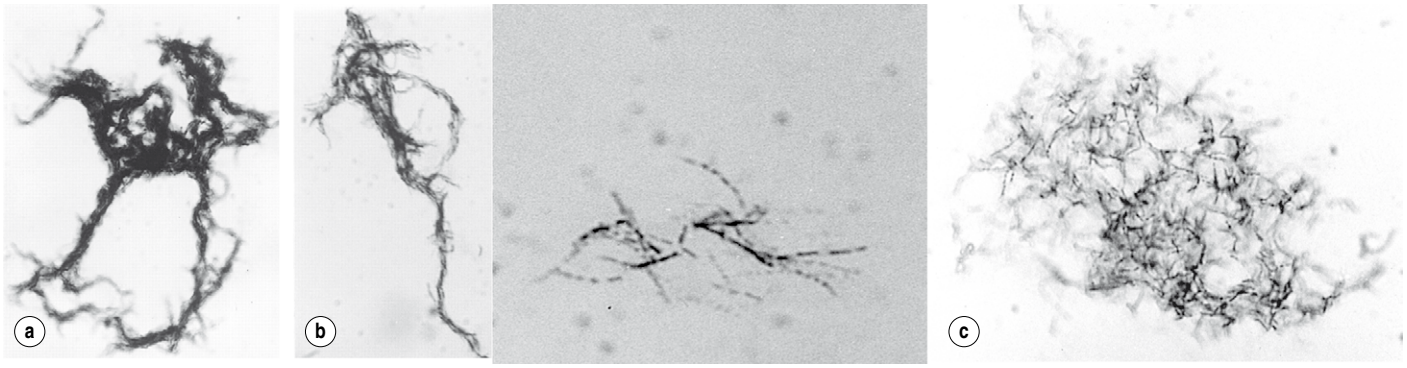


Fig. 174.2 Microscopic clusters of three different species of mycobacteria. (a) Serpentine cording of *M. tuberculosis*. (b) Cross-banding of *M. kansasii*. (c) Loose clusters of *M. avium* complex. Photomicrographs taken from Attorri *et al*.[11]

envelope confers size, shape, protection against osmotic pressure, and probably protects the plasma membrane from deleterious molecules in the environment of the cell. In summary, the peptidoglycan confers cell shape while the next layer of the envelope, arabinogalactan esterified to the mycolic acids, provides for a hydrophobic permeability barrier. Other important fatty acids are waxes, phospholipids and mycoserosic and phthienoic acids, and tuberculostearic acid (10-R-methyl-octadecanoic) acid is a unique cell component within the Actinomycetales, including the mycobacteria.[8]

In 1947, Middlebrook first described growth of tubercle bacilli in the shape of serpentine cords ('cording'). For many years cording was correlated with virulence and considered a distinctive feature of *M. tuberculosis* (Fig. 174.2). However, it is now known that several mycobacterial species display cording and the correlation with virulence, if any, is unclear. Cord factor appears to be a mixture of mycolate-containing molecules including the original cord factor associated with *M. tuberculosis*, trehalose 6,6′-dimycolate.

Many species of mycobacteria are capable of producing carotenoids (carotene and xanthophylls) and this feature can be an important aid in the presumptive identification of a mycobacterial organism. The production of carotenoids is strongly influenced by the composition of the media and growth conditions. Light has a significant effect on pigment production for some species. Scotochromogens, e.g. *M. scrofulaceum* (Fig. 174.3) or *M. szulgai* (Fig. 174.4) produce a yellow pigment in the absence of light, whereas photochromogens, e.g. *M. kansasii* (Fig. 174.5) produce pigment when stimulated by light. Nonphotochromogens, e.g. *M. tuberculosis* or *M. fortuitum* (Fig. 174.6) do not produce carotenoids. The function of mycobacterial carotenoids is largely unknown, but some evidence suggests that carotenoids provide protection against phototoxic effects.

The genome of mycobacteria displays a typical bacterial chromosomal structure, i.e. a single large circular DNA molecule with a G+C content of 60–70 mol% (except *M. leprae*; <57%). The complete

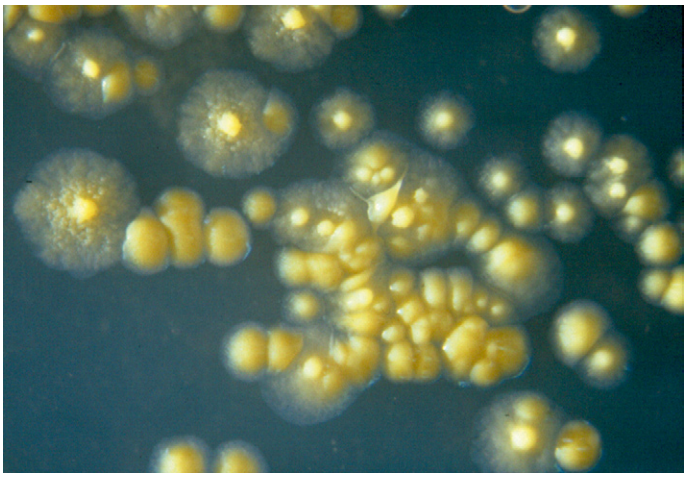


Fig. 174.4 *Mycobacterium szulgai*, a scotochromogenic mycobacterium, grown on Middlebrook 7H10 agar.

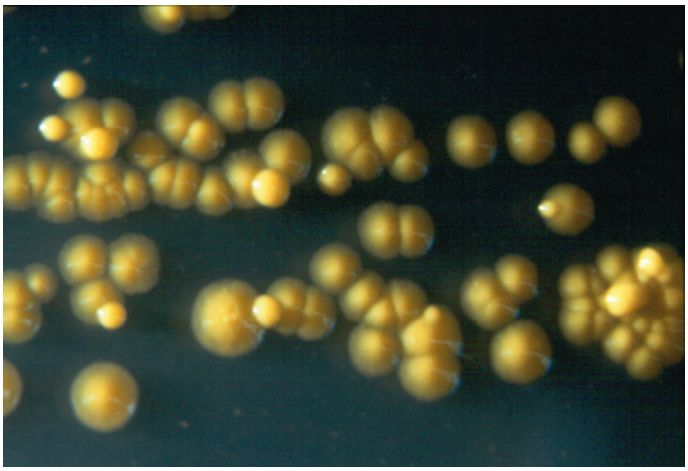


Fig. 174.3 *Mycobacterium scrofulaceum*, a scotochromogenic mycobacterium, grown on Middlebrook 7H10 agar.

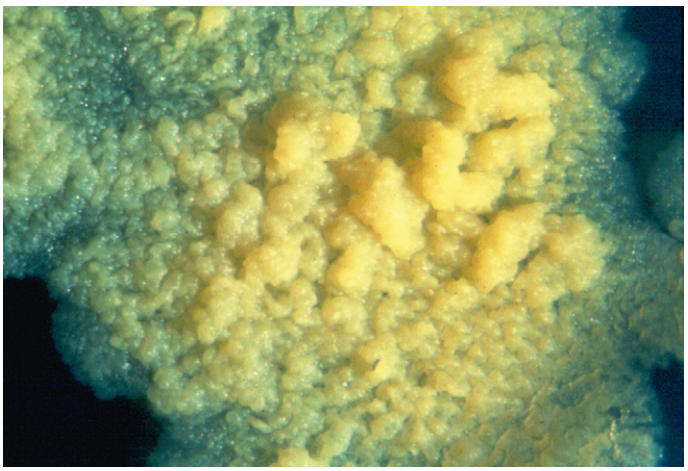


Fig. 174.5 *Mycobacterium kansasii*, a photochromogenic mycobacterium, grown on Middlebrook 7H10 agar, after exposure to light.

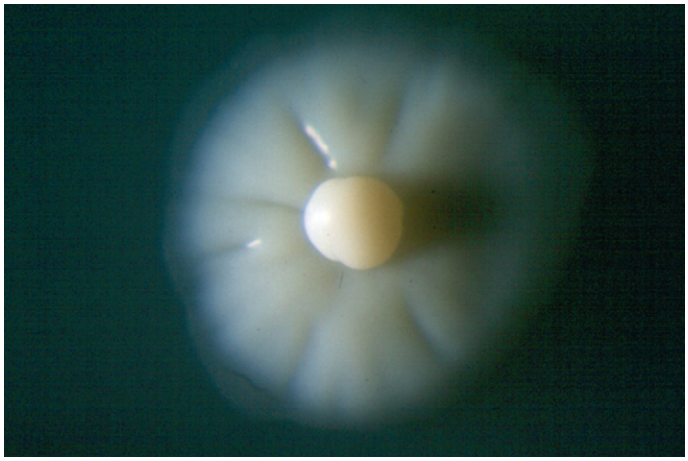


Fig. 174.6 *Mycobacterium fortuitum*, a nonchromogenic rapid grower, grown on Middlebrook 7H10 agar.

genome sequence of *M. tuberculosis* H37Rv (4.41 Mb) has recently been deciphered.[12] Most of the NTM, in particular *M. avium* complex, *M. scrofulaceum* and the rapid growers, contain plasmids. The size and number of plasmids vary; their function is largely unknown. There has been considerable speculation that plasmids in mycobacteria include DNA sequences that encode a variety of virulence factors. However, plasmids would appear to have no role in antibiotic resistance or virulence in *M. tuberculosis*, since the organism does not contain plasmids.

Overall, the analysis of genetic relatedness confirms the classic classification scheme based on pigmentation and growth rate. Genome sequence information has also revealed interesting information about differences within the *M. tuberculosis* complex. For example, comparisons of *M. tuberculosis* H37Rv (a human isolate that is also virulent in mice) with bacille Calmette–Guérin (BCG) Pasteur showed that 18 variable sequence regions are missing in BCG Pasteur. Perhaps even more intriguing is the presence of two large tandem duplications in BCG Pasteur, whereas other BCG strains lack one of these duplications and neither of the duplications is seen in *M. tuberculosis* H37Rv. Furthermore, the genome size of *M. bovis* is significantly smaller than that of *M. tuberculosis* which seems at odds with the fact that *M. bovis* has a significantly wider host range than *M. tuberculosis*.[13,14]

Very recently, sequence information has placed in doubt the longheld belief that *M. tuberculosis* evolved from an animal pathogen (i.e. *M. bovis*). Rather, it indicates that all members of the *M. tuberculosis* complex, i.e. *M. tuberculosis, M. bovis, M. bovis* BCG, *M. caprae, M. africanum, M. microti*, '*M. canettii*' and *M. pinnipedii* (Table 174.1), gradually evolved from a common ancestor and '*M. canettii*' segregated some 2.4–2.8 million years ago. *M. tuberculosis*, in its present form, is estimated to be only some 35 000 years old and was always a human pathogen.[18,19] Sequence analysis of the paralogous genes in a large set of *M. tuberculosis* strains collected worldwide confirmed the very ancient origin of '*M. canettii*'.[20,21]

Table 174.1 Major characteristics of the *Mycobacterium tuberculosis* complex

Species	Geographic distribution	Remarks	Identifying features
M. tuberculosis	Widespread, humans are the definite host; disease also in elephants and other mammals	Based on genetic analyses present day *M. tuberculosis* is relatively young (~35 000 years old)	Usually PZA susceptible, nitrate reductase activity positive, niacin positive, aerobic
M. bovis	Widespread, broad host range including nonhuman primates, cattle, goats, cats, dogs, buffalo, badgers, possums, deer and bison. Humans are viewed as a 'spillover' host	Mainly extrapulmonary manifestation in humans	PZA resistant, nitrate reductase activity negative, niacin negative, microaerophilic
M. bovis BCG	Nonvirulent vaccine strain	First reported in 1908 as a nonvirulent strain of *M. tuberculosis*; appears to be a regulatory mutant of *M. bovis*	PZA resistant, nitrate reductase activity negative, niacin negative, aerobic
M. caprae	Mainly in goats, few infections in humans		PZA susceptible, nitrate reductase activity negative, niacin negative, microaerophilic
M. africanum		Type I and type II	Mostly PZA susceptible, predominantly nitrate reductase activity negative, niacin variable, microaerophilic
M. microti	Initially considered strictly an animal pathogen (e.g. voles, llamas, cats), several human cases were reported in 1998[15]		'Croissant-like' cell morphology; does not grow well in culture; identification via molecular methods
'M. canettii'	East Africa, occurrence appears to be limited to the African continent	Based on genetic studies ancestor of all members of the *M. tuberculosis* complex, ~2.4–2.8 million years old; 'living fossil'	Smooth colonies, distinct patterns upon DNA typing
M. pinnipedii	Pinnipeds as major hosts		

Adapted and modified from van Soolingen *et al.*,[15] Vincent & Gutierrez[16] and Grange.[17]
PZA, pyrazinamide.

EPIDEMIOLOGY AND CLINICAL MANIFESTATIONS OF THE MOST IMPORTANT MYCOBACTERIAL PATHOGENS

Tuberculosis

Tuberculosis is the second most common infectious cause of adult mortality, and is ranked tenth of all causes of loss of healthy life worldwide. Of the estimated 8.8 million cases in 2005, 7.4 million were found in Asia and sub-Saharan Africa (Fig. 174.7).[1] Of these, a total of 1.6 million died of tuberculosis, including 195 000 patients infected with HIV. Before the development of effective chemotherapy, the mortality rate for tuberculosis was 50–60%. Following the discovery of isoniazid (INH) and rifampin (rifampicin; RMP), the majority of pulmonary and extrapulmonary tuberculosis became treatable. Nevertheless, treatment of tuberculosis remains problematic in many areas because of the lack of/or intermittent access to therapy, poor adherence to therapy, poor drug quality, and above all, the worldwide emergence of resistance.[22]

Multidrug-resistant *M. tuberculosis* strains (i.e. resistant to INH and RMP at least) are responsible for more than 4% of all newly and previously treated tuberculosis cases. A 'virtually untreatable form of tuberculosis' (BBC News, September 2006) has emerged recently, XDR-TB (extensively drug-resistant tuberculosis). Occurring worldwide, including highly industrialized countries such as the USA, such XDR strains are resistant against INH and RMP as well as against the two most important second-line drug classes, a fluoroquinolone (e.g. ciprofloxacin) and an injectable drug (e.g. amikacin).[23]

Tuberculosis in the most common form is a chronic pulmonary disease classified as either primary or post-primary disease.[24] Post-primary disease can be a consequence of either reactivation (endogenous infection) or re-infection (exogenous infection). By far the most common (95%) route of infection is inhalation of infectious droplet nuclei, but exposure to *M. tuberculosis* bacilli neither always leads to infection nor are all patients with disease infectious. The risk of infection is directly related to the number and distribution of tubercle bacilli in the inhaled and respired air, emphasizing the importance of infectious droplet nuclei to airborne transmission. Unless a patient receives prophylaxis, symptomatic disease eventually occurs in 5–10% of infected patients. The appearance and extent of disease varies with only one-half of infected patients developing disease within the first 2 years. Hematogenous spread of tubercle bacilli from the lung probably invariably occurs, but the bacteremia is usually occult and does not produce symptoms or disease. Nevertheless, hematogenous dissemination accounts for the occurrence of extrapulmonary involvement of lymph nodes, kidneys, reproductive organs, bones, and gastrointestinal tract (see Chapter 30).

Compared to the global incidence of tuberculosis there is a small proportion of human disease caused by other members of the *M. tuberculosis* complex such as *M. bovis* and *M. africanum*. Human infections with *M. caprae*[25] appear to be very infrequent; the same holds for *M. microti*[15] and '*M. canettii*'. The latter seems to be confined to the African continent only,[20,26] and *M. pinnipedii* appears to affect seals only.[27]

Leprosy

Leprosy (Hansen's disease) is a chronic disease of the skin, nerves and mucous membranes. The immunologic response (e.g. hypersensitivity) becomes an important component of the pathogenesis of the disease. The clinical manifestations of leprosy have been separated into six categories (Ridley–Jopling classification scheme).[28] The system is both a clinical classification based on the nature and severity of symptoms and a histopathologic classification. The classification groups are: (1) polar tuberculoid; (2) borderline tuberculoid; (3) borderline; (4) borderline lepromatous; (5) lepromatous (subpolar); and (6) lepromatous polar.

Lepromatous leprosy is the most severe form of the disease with numerous skin lesions involving face and nose. Acid-fast bacilli (AFB) are numerous and present in immature macrophages which contrasts

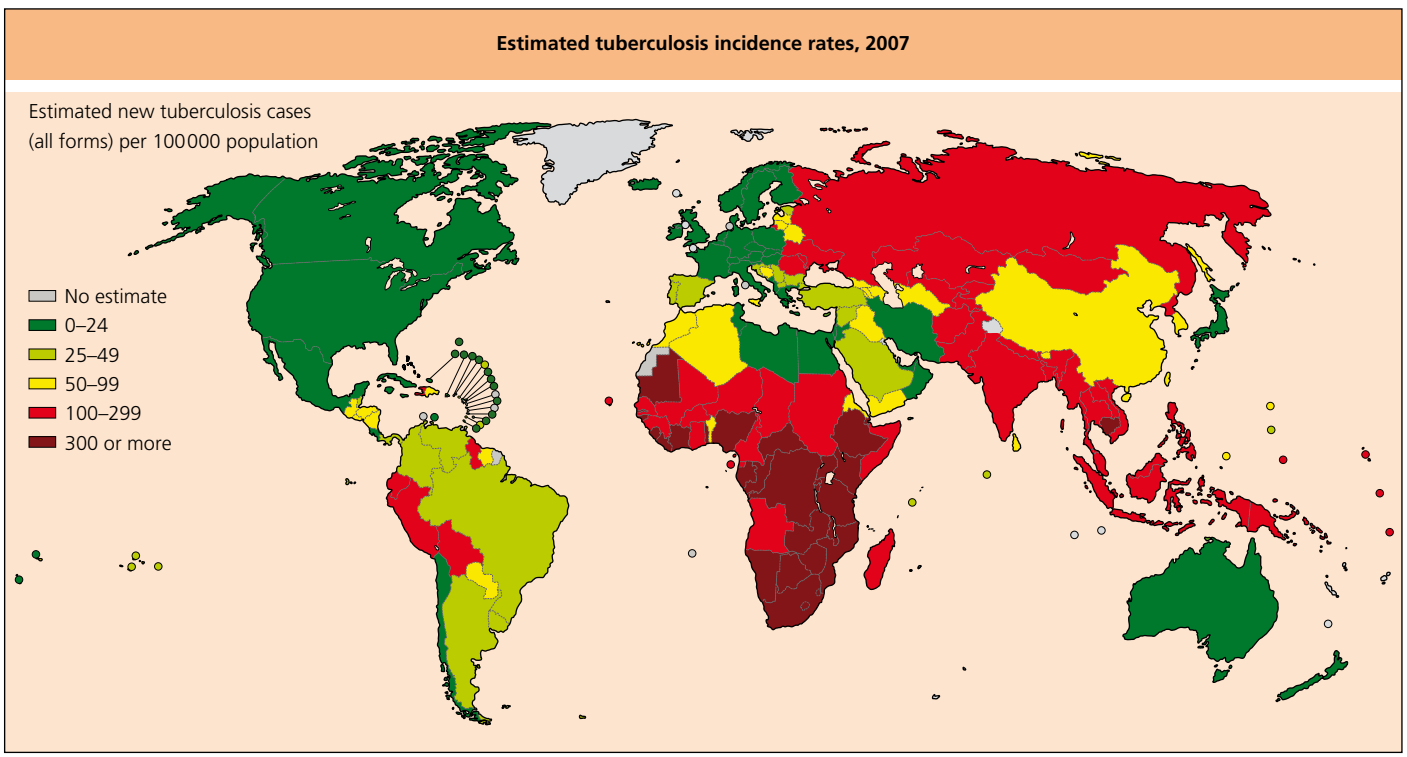


Fig. 174.7 Estimated tuberculosis incidence rates, 2007. Data and map adapted from World Health Organization. Global tuberculosis control: surveillance, planning, financing. WHO Report 2008, Geneva: WHO/HTM/TB/2008.393.

to tuberculoid leprosy, where macrophages have matured into epithelioid cells (see also Chapter 103), and AFB are hard to detect.

Epidemiology of leprosy is difficult to assess because of the lack of a diagnostic skin test, inability to cultivate the organism *in vitro* and the nature of the geographic distribution of endemic disease. Even though the disease is of great antiquity, the source for *M. leprae*, the mode of transmission and the portal of entry are not clear to date. Human-to-human transmission through nasal secretions has been accepted for a long time. However, there is evidence that the disease may also be airborne, vector-borne or vehicle-borne. The worldwide implementation of the standardized multidrug therapy has dramatically decreased the number of registered leprosy patients from 10–12 million cases in the mid 1980s to 1.15 million cases in the late 1990s.[29] Nevertheless, the annual confirmed new cases (500 000–700 000) suggest that therapy largely fails to avoid transmission.[30] The vast majority of patients are in the developing world, predominantly South East Asia and mostly in India.

Care and treatment of leprosy have two equally difficult components. The first is treatment of active disease, especially in patients with physical deformities or at risk of developing deformities. The second is care of effectively treated patients, but with disabling or debilitating deformities due to the pathology associated with the various manifestations of leprosy.

Buruli ulcer/Bairnsdale ulcer

Mycobacterium ulcerans is the etiologic agent of Buruli ulcer in Africa and Bairnsdale ulcer in Australia,[4] an emerging disease involving chronic and necrotizing skin ulcers. Occurring mostly in rural tropical regions it is the third most common mycobacterial disease in immunocompetent humans after tuberculosis and leprosy (Fig. 174.8). Disease typically begins as a lump under the skin, followed by a shallow ulcer at the site of the lump. *M. ulcerans* produces a toxin that causes necrosis. The type of disease ranges from a localized nodule or ulcer to widespread ulcerative or nonulcerative disease including, for instance, osteomyelitis.

Treatment with antimicrobial agents has widely been unsuccessful. Surgical removal of the affected tissue, often followed by skin grafting, is the method of choice. If untreated, *M. ulcerans* disease may even lead to severe sequelae such as blindness or loss of limbs. Disease control in endemic regions is limited to early case detection with surgical treatment.

Adequate surveillance data are missing for most of the endemic areas, therefore the incidence and prevalence of Buruli ulcer are not precisely defined. Ghana, an exception, has reported an overall national prevalence rate of active lesions of 20.7 per 100 000, but the rate was as high as 150.8 per 100 000 in districts where the disease was more endemic.[31] There is growing evidence that *M. ulcerans* also produces disease in reptiles and mammals. *M. ulcerans* is a heat-sensitive, fastidious organism and has an unusually long generation time. Promising molecular tests have been developed which may help to accelerate diagnosis.[32]



Fig. 174.8 Buruli ulcer on the elbow of an African girl caused by *M. ulcerans*. Courtesy of F Portaels.

Nontuberculous mycobacterial diseases

Diagnostic criteria, clinical presentation and treatment of diseases caused by more than 20 NTM have been meticulously reviewed.[3–5] The official ATS/IDSA statement covers other important aspects such as taxonomy, epidemiology, pathogenesis and laboratory aspects. In addition, the clinical significance and laboratory diagnostic aspects of NTM have been reviewed.[33–35] Therefore, the following discussion only focusses on the most prominent NTM species causing disease in humans (Table 174.2).

Mycobacterium avium complex

Mycobacterium avium complex (MAC) is common not only in soil, water and animals, but also in certain food (e.g. hard cheese). It also colonizes pools and hot water tubs. Portals of infection are probably both gastrointestinal and pulmonary by ingestion of contaminated water and food and inhalation of aerosols.

Mycobacterium avium complex causes disseminated disease usually (but not exclusively) in people with HIV infection, but may also be responsible for pulmonary disease in immunocompetent patients and cervical lymphadenitis in normal hosts, mostly children.[4,36]

Disseminated MAC disease was rarely reported before the advent of the HIV epidemics. Based on complete follow-up information, disseminated MAC disease occurred in 20–35% per year of people with HIV infection.[37] However, this dates back 10 years and MAC prophylaxis (rifabutin, azithromycin or clarithromycin) and the use of highly active antiretroviral therapy (HAART) have significantly decreased the incidence of MAC disease in people with HIV infection.

Disseminated MAC disease in people with HIV infection is a progressive illness characterized by intermittent fever, sweats, weakness, anorexia and weight loss. Patients may have nausea, diarrhea and vomiting along with abdominal pain. The microbiologic hallmark of MAC disease is a positive blood or bone marrow culture; however, duodenal, rectal, spleen or liver biopsies may be diagnostic as well (Fig. 174.9). The level of the bacteremia ranges from intermittently culture positive to 10^6 cfu/ml. The level of infection of bone marrow may be orders of magnitude higher than in blood.[38] Disseminated MAC infection in people with HIV infection clearly decreases survival. Treatment with a macrolide and another agent improves survival and the quality of life. MAC disease in people with HIV infection may also be focal, including pulmonary infection, peripheral lymphadenitis and cutaneous infection (see Chapter 91).

The majority (≥90%) of disseminated MAC disease is caused by *M. avium*. Overall, *M. intracellulare* is isolated about as frequently as *M. avium*, but the isolation of *M. avium* has been associated with a poorer prognosis. Other species should be considered for symptomatic patients as well, such as *M. triplex*, *M. genavense*, and *M. conspicuum* or, for patients with cutaneous infections, *M. haemophilum* (see Table 174.2). Disseminated MAC disease in HIV-negative patients is usually associated with congenital immunodeficiency, immunosuppression, malignancy or a specific immunodeficiency such as a deficiency in interferon gamma (IFN-γ) production or IFN-γ receptors. In children without HIV infection disseminated MAC disease with visceral involvement has been associated with a high mortality rate (82%).

Mycobacterium avium complex pulmonary disease is seen worldwide with an overall incidence of about 1/100 000 persons. The incidence of pulmonary disease appears to be increasing, but this may be due in part to increased surveillance and awareness by the medical community. MAC pulmonary disease may be associated with a history of chronic lung infection including cystic fibrosis. However, the

Table 174.2 Nontuberculous mycobacteria

Clinical disease/ specimen/site	Occurrence	Species	Growth rate	Recovered from environment	Geographic distribution	Risk factors, additional remarks	Microbiologic and diagnostic features
Cutaneous, wound	Common	*M. chelonae*	Rapid	Yes	Widespread	Contact lenses, ocular surgery (LASIK-associated), implants, catheters	
Cutaneous, wound	Common	*M. fortuitum*	Rapid	Yes	Widespread	Central catheters, postsurgical wounds, mammoplasty, nail salon furunculosis	
Cutaneous, wound	Common	*M. haemophilum*	Very slow	Yes?	Australia, Europe, North America	AIDS, organ transplant recipients, lymphoma, children; infections at the cooler sites of the body including the extremities	Fastidious, requires hemin or ferric ammonium citrate, growth at 30–32°C, growth stimulated by CO_2, may be missed by the laboratory
Cutaneous, wound, bone	Common	*M. marinum*	Rapid/slow	Yes	Widespread	Skin abrasion or trauma; in immunocompetent and immunocompromised patients; 'fish tank granuloma', 'swimming pool granuloma'	Photochromogen, optimum growth (32°C)
Cutaneous, wound	Unknown	*M. mucogenicum*	Rapid	?	Unknown	Catheters	
Cutaneous, ulcers, bones	Common (tropics)	*M. ulcerans*	Very slow	Yes	Ghana, Uganda, South East Asia, Central and South America, Australia	Normal host, third most prevalent *Mycobacterium* isolated from human specimens and continuing to emerge; Buruli ulcer (Africa), Bairnsdale ulcer (Australia)	Fastidious, vulnerable upon pretreatment, very slow growth at 30°C, microaerophilic
Cutaneous, wound	Uncommon	*M. abscessus*	Rapid	Yes	Widespread		
Cutaneous, wound	Uncommon	*M. avium* complex	Slow	Yes	Widespread		
Cutaneous	Uncommon	*M. kansasii*	Slow	Yes	Widespread		Photochromogenic
Cutaneous	Uncommon	*M. smegmatis*	Rapid	Yes	Unknown		
Cutaneous, wound	Unknown	*M. conspicuum*	Slow	No	Unknown		
Cutaneous, urine, fluids (BAL, joint), blood	Unknown	*M. immunogenicum*	Rapid	Yes	Unknown	Respiratory pseudo-outbreaks (contaminated bronchoscopy equipment), catheters	Culture at 25–30°C, *M. abscessus-chelonae*-like
Cutaneous, tenosynovitis	Unknown	*M. malmoense*	Slow			AIDS is not a predisposing factor	Microaerophilic
Cutaneous	Unknown	*M. novocastrense*	Rapid	Yes	Unknown	Potential human pathogen	Scotochromogenic *M. marinum*-like
Disseminated	Common	*M. xenopi*	Slow	Yes	Worldwide	Hot water systems	Thermophilic

Disseminated	Common	*M. avium* complex	Slow	Yes	Widespread	AIDS, hairy cell leukemia, congenital immunodeficiencies	Usually nonpigmented, pigmented colonies occur, three colony variants
Disseminated	Common	*M. chelonae*	Rapid	Yes	Widespread		
Disseminated, blood or bone marrow, joint fluid	Common	*M. haemophilum*	Very slow		Australia, Europe, North America	Common and continuing to emerge	
Disseminated	Common	*M. kansasii*	Slow	Yes	Europe, USA		Photochromogenic
Disseminated	Uncommon	*M. abscessus*	Rapid	Yes			
Disseminated, blood, bone marrow, liver, spleen	Uncommon	*M. genavense*	Slow	Yes	Widespread, isolated from pet birds	Disease in humans established, especially in AIDS patients and malnourished patients	Grows very slowly if at all, prefers slightly acidic medium (pH 6.0)
Disseminated	Uncommon	*M. malmoense*	Very slow	Yes		AIDS	
Disseminated	Unknown	*M. celatum*	Slow	No	Europe, USA	Disease in humans established	Two copies of 16S rRNA gene
Disseminated Osteomyelitis, cellulitis		*M. smegmatis*	Rapid			Catheters	
Disseminated, blood	Unknown	*M. conspicuum*	Slow	No			
Disseminated Spinal osteomyelitis Lymphadenitis	Unknown	*M. lentiflavum*	Slow	No		Disease in humans established, especially HIV and AIDS patients	
Disseminated		*M. simiae*	Slow			Infections in immunocompromised patients	
Disseminated, lymph node, spinal fluid, pericardial and peritoneal fluid	Unknown	*M. triplex*	Slow	No	Unknown	Disease in humans established	*M. simiae-avium*-like
Intestinal		*M. paratuberculosis*	Slow	Yes			Fastidious, cause of Johne's disease in cattle; association with *M. paratuberculosis* and Crohn's disease in humans (?)
Lymphadenitis	Common	*M. avium* complex	Slow	Yes	Widespread	Childhood	
Lymphadenitis	Common	*M. malmoense*	Very slow	Yes	UK, Northern Europe	Childhood	Slow growth
Lymphadenitis	Common before 1980	*M. scrofulaceum*	Slow	Yes	Widespread	Childhood	
Lymphadenitis	Uncommon	*M. abscessus*	Rapid	Yes			
Lymphadenitis	Uncommon	*M. chelonae*	Rapid	Yes			
Lymphadenitis	Uncommon	*M. fortuitum*	Rapid	Yes			

(Continued)

Table 174.2 Nontuberculous mycobacteria—cont'd

Clinical disease/ specimen/site	Occurrence	Species	Growth rate	Recovered from environment	Geographic distribution	Risk factors, additional remarks	Microbiologic and diagnostic features
Lymphadenitis	Uncommon	*M. haemophilum*	Slow	Yes?		Also in immunocompetent children	
Lymphadenitis, soft tissue	Unknown	*M. bohemicum*	Slow	Yes	Unknown	Potential pathogen	
Lymphadenitis	Unknown	*M. heidelbergense*	Slow	No	Germany	Resembles *M. malmoense*	
Lymphadenitis, pulmonary	Unknown	*M. tusciae*	Slow	Yes	Tuscany	Potential pathogen	
Lymphadenitis, disseminated	Unknown	*M. interjectum*	Slow/rapid	No		Disease in humans established	
Lymphadenitis	Unknown	*M. szulgai*	Slow				
Pulmonary	Common	*M. abscessus*	Rapid	Yes	Widespread, predominantly USA	Symptoms and radiographic presentation similar to *M. fortuitum*. Also in patients with gastroesophageal disorders with chronic vomiting	
Pulmonary	Common	*M. avium* complex	Slow	Yes	Widespread	Chronic lung disease, cystic fibrosis	
Pulmonary		*M. heidelbergense*	Slow	Yes	?	Lung tumor	
Pulmonary	Common	*M. kansasii*	Slow	Yes	Localized USA, UK, Europe, South Africa	Alcoholism, malignancy, AIDS, chronic lung diseases (COPD, pneumoconiosis); seen in gold miners	Photochromogen, intensely pigmented, large, beaded, acid-fast, elongated bacilli
Pulmonary	Common	*M. malmoense*	Very slow	Yes	Localized UK, Northern Europe	Pneumoconiosis, silicosis, industrial dust disease; common and continuing to emerge	
Pulmonary		*M. smegmatis*	Rapid			Rare, associated with lipoid pneumonia	
Pulmonary	Common	*M. xenopi*	Slow	Yes	Localized UK, Europe, Canada, infrequently in the USA	Underlying lung disease; common and continuing to emerge; associated with hospital water systems	Pigmented, optimum growth at 45°C
Pulmonary	Uncommon	*M. asiaticum*	Slow	Yes	Localized Australia, USA		
Pulmonary	Uncommon	*M. fortuitum*	Rapid	Yes		Similar to lung disease with *M. abscessus*; gastroesophageal disorders with chronic vomiting	

Pulmonary	Uncommon	*M. gordonae*	Slow	Yes	Widespread	Frequent contaminant and debatable cause of disease	'Tap-water' scotochromogen
Pulmonary	Uncommon	*M. haemophilum*	Slow		Unknown, seems localized		
Pulmonary		*M. immunogenum*	Rapid				
Pulmonary	Uncommon	*M. shimoidei*	Slow		Australia, Japan		
Pulmonary		*M. scrofulaceum*	Slow		South Africa	AIDS but also HIV-negative patients; seen in gold miners	
Pulmonary	Uncommon	*M. simiae*	Slow	Yes	Israel, Thailand, France, USA, Australia	Underlying lung disease, pneumonia; often environmental contaminant	Produces niacin
Pulmonary	Uncommon	*M. szulgai*	Slow	Yes?	Europe, Japan, USA	Clinical picture often indistinguishable from tuberculosis; underlying disease (COPD, history of tuberculosis), alcoholism, smoking	Scotochromogen, slow growth at 25–37°C, no growth at 42°C
Pulmonary	Uncommon	*M. terrae*	Slow				
Pulmonary, sputum	Unknown	*M. alvei*	Rapid	Yes	Unknown, first isolated and described in Spain	Potential human pathogen	
Pulmonary	Unknown	*M. branderi*	Slow	No	Scandinavia	Potential pathogen	
Pulmonary	Unknown	*M. brumae*	Rapid	Yes	Unknown	Potential human pathogen	
Pulmonary	Unknown	*M. celatum*	Slow	No	Europe, USA		Contains two copies of 16S rDNA
Pulmonary, sputum	Unknown	*M. confluentis*	Rapid	No	Unknown	Potential human pathogen	Brown pigment
Pulmonary	Unknown	*M. conspicuum*	Slow	No	Europe	Disease in humans established	
Pulmonary	Unknown	*M. heckeshornense*	Slow	No	Unknown	Potential pathogen	*M. xenopi*-like organism
Pulmonary	Unknown	*M. interjectum*	Slow/rapid	No		Disease in humans established	
Pulmonary	Unknown	*M. intermedium*	Slow/rapid	No	Unknown	Disease in humans established	Photochromogen
Pulmonary	Unknown	*M. kubicae*	Slow		Unknown	Potential human pathogen	
Pulmonary, sputum?	Unknown	*M. mageritense*	Rapid	No	Spain, unknown	Potential human pathogen	
Pulmonary, wound	Unknown	*M. mucogenicum*	Rapid	Yes	Unknown		
Pulmonary	Unknown	*M. triplex*	Slow	No	Unknown	Disease in humans established	*M. avium* complex-like
Urine	Unknown	*M. hassiacum*	Rapid	Yes	Unknown	Nonpathogen?	Scotochromogen
Wound	Unknown	*M. branderi*	Slow	No	Unknown	Disease in humans established	

(Continued)

Table 174.2 Nontuberculous mycobacteria—cont'd

Clinical disease/ specimen/site	Occurrence	Species	Growth rate	Recovered from environment	Geographic distribution	Risk factors, additional remarks	Microbiologic and diagnostic features
Wound, soft tissue, blood	Unknown	*M. fortuitum* (3rd biovariant)	Rapid	Yes	Unknown		
Wound, pulmonary	Unknown	*M. gondii*	Rapid	No	Unknown	Nosocomial?	Pigmented, *M. smegmatis*-like
Wound Osteomyelitis	Unknown	*M. wolinskyi*	Rapid	No	Unknown	Post-traumatic and postsurgical. wounds	*M. smegmatis*-like
Wound Tenosynovitis (hand)		*M. terrae*	Slow				
Bone	Uncommon	*M. chelonae*	Rapid	Yes	Widespread		
Bone	Uncommon	*M. fortuitum*	Rapid	Yes	Widespread		

Information compiled and adapted from a variety of sources including Falkinham,[2] Tortoli,[3] American Thoracic Society,[4] Khan,[5] Vincent & Gutierrez,[16] Grange,[17] Pfyffer & Vincent,[33] Pfyffer,[34] Brown-Elliott & Wallace.[35]
BAL, bronchoalveolar lavage.

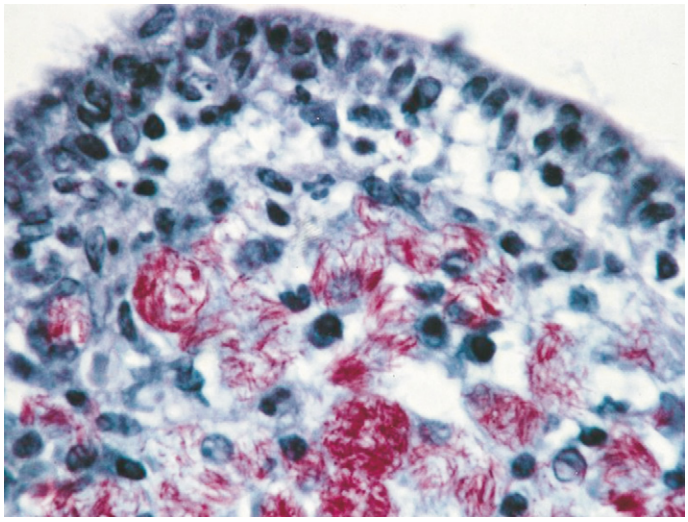


Fig. 174.9 Acid-fast stain of a section of small intestine from a patient with HIV infection and disseminated *M. avium* disease. The photomicrograph shows many acid-fast bacilli within a villus tip of the intestinal tract biopsy. The cuboidal cells at the periphery of the tip are in disarray and appear abnormal with cell nuclei not evenly distributed at the base of the cells. There is no evidence of granuloma, but the overwhelming number of mycobacteria may be partially obscuring the host's cellular response. The histopathology is consistent with the symptoms of patients with MAC gastrointestinal tract infections, including abdominal pain, diarrhea and wasting. Courtesy of LS Young.

traditionally recognized presentation of MAC pulmonary disease has been an apical fibrocavitary lung disease, sometimes showing large cavities, in males in their late forties and early fifties who have a history of smoking and, sometimes, alcohol abuse.[4] In postmenopausal, nonsmoking, white females, MAC pulmonary disease also presents with nodular and interstitial nodular infiltrates, frequently involving the right middle lobe or lingula ('Lady Windermere Syndrome').[4] In patients without underlying HIV infection, symptoms may vary and be nonspecific, including chronic productive cough, dyspnea, sweats, malaise and fatigue (see Chapter 30).

Mycobacterium avium complex cervical lymphadenitis is mostly a pediatric disease and primarily seen in children under 5 years of age. Lymphadenitis in immunocompetent children usually presents as an insidious, painless, unilateral process involving one or more lymph nodes. Mycobacteria isolated from infected lymph nodes are mostly (60–80%) MAC with the remainder being *M. scrofulaceum* and *M. tuberculosis* (see Chapter 30). *Mycobacterium avium* complex lymph node infection of children over 12 years of age is rarely simple lymphadenitis and may indicate disseminated disease and immunodeficiency.

Thus, MAC disease is frequently seen as disease in persons with and without underlying HIV infection. Although there are some similarities in the manifestation of MAC disease in both groups, the mortality rate, extent and pathologic manifestations of disease are frequently dissimilar.[36] Also, distinction between true infection with MAC and transient colonization may be difficult.

Mycobacterium kansasii

It appears that tap water is the major reservoir of *M. kansasii*. While being the second most common cause of NTM disease in the USA,[4] it is also seen in other parts of the world, i.e. in Europe (UK) and among mine workers in South Africa.[34] After MAC *M. kansasii* is the second most common NTM that causes disseminated disease in patients with AIDS.[4] Generally, the organism primarily affects middle-aged men. Risk factors are chronic obstructive pulmonary disease, pneumoconiosis, malignancy, previous mycobacterial disease and alcoholism. *Mycobacterium kansasii* pulmonary disease parallels in many ways the clinical course of tuberculosis in terms of clinical symptoms and chest X-ray.

Rapid growers

Although the number of newly discovered rapidly growing mycobacteria is increasing constantly and the pathogenic potential of many of them is well recognized,[35] the vast majority (>90%) of disease in humans is caused by three species: *M. fortuitum* (see Fig. 174.6), *M. chelonae* and *M. abscessus*. These species are important causes of cutaneous, pulmonary and postsurgical wound infections (skin, soft tissue, bone), especially following catheter placement, augmentation mammaplasty and cardiac bypass surgery. Disseminated disease is rare and almost invariably occurs in immunocompromised patients, but disease is not common in people with HIV infection.

The distribution of rapidly growing mycobacteria appears to be worldwide. The three species associated with human disease have been isolated from drinking water, natural waters, ice machines, as well as in waste water and soil. The ubiquitous nature of these microorganisms, their resistance to many disinfectants and their ability to grow on unusual substrates such as polyhalogenated phenols account for the fact that rapidly growing mycobacteria are found as contaminants of medical equipment, prosthetic valves and disinfectants.

The incidence of pulmonary disease caused by rapidly growing mycobacteria is unclear (80% by *M. abscessus*), but an increased awareness of these species as human pathogens is leading to greater insight into the true incidence of disease. Nevertheless, the isolation of rapidly growing mycobacteria from respiratory tract specimens must be interpreted with great caution because contamination and colonization are common. True infection is usually associated with chronic pulmonary disease such as cystic fibrosis, previous mycobacterial lung disease and bronchiectasis due to previous respiratory infection. Rapidly growing mycobacteria are intrinsically resistant to antituberculous agents including INH, RMP and pyrazinamide (PZA).

PATHOGENICITY

The pathogenicity of mycobacterial infections in the immunocompetent host has as much to do with the immune response of the host as with destructive virulence properties of the mycobacterial pathogen. Thus, the principal virulence factor of mycobacteria is the ability to invade and persist or replicate within macrophages. Tubercle bacilli are believed to attach to macrophages by binding to the mannose receptor on the macrophage via the mycobacterial cell envelope LAM. Alternatively, tubercle bacilli can indirectly bind via CR1/CR3 complement receptors or Fc receptors.

The bacilli enter the macrophage by phagocytosis and once internalized the bacilli are surrounded by a membrane-bound vacuole to form a nascent phagosome. With the maturation of the phagolysosome, bacilli are exposed to a variety of antimicrobial factors including reactive oxygen intermediates, hydrolytic activities and a highly acidic pH. Cytokine-activated macrophages may also produce reactive nitrogen intermediates, although this has been conclusively shown only in murine macrophages. Virulent mycobacteria obstruct the maturation of phagosomes, inhibiting acidification, and can escape from the phagolysosome into the cytoplasm. Other bacilli appear able to adapt to life within the phagolysosome.

Recent evidence indicates that the maturation of the phagolysosome varies depending on the bacterial pathogen and even between intracellular pathogens.[39,40] The composition of the phagosome membrane is determined in part by the infecting mycobacteria; for example, phagosomes containing *M. avium* do not contain the vacuolar H^+-ATPase that is needed for acidification of the phagosome. Phagosomes containing either *M. tuberculosis* or *M. avium* bacilli are frequently incompetent for fusion with endocytic organelles, suggesting a mechanism for the block in phagolysosome fusion. Inhibition of

acidification can occur as a consequence of the exclusion of a proton pump (as with *M. avium*) or fusion with a lysosome that does not contain a pump or perhaps by the release of an ammonia metabolizing enzyme such as glutamine synthetase. Once mycobacteria have modified or adapted to the intracellular environment the bacilli may proliferate, but the mechanism of intracellular replication of mycobacteria is poorly understood.

The immunopathogenesis of mycobacterial infections primarily involves a cell-mediated immune response. This response includes the activation of macrophages to identify and inhibit or kill mycobacteria and the detection and lysis of phagocytes in which mycobacteria are in a state of growth and replication (Fig. 174.10).

Mycobacterial antigens are presented by antigen-presenting cells (monocyte/macrophage lineage) resulting in secretion of interleukin (IL)-2 and clonal proliferation of $CD4^+$ and $CD8^+$ lymphocytes (α/β T cells).[42] Mycobacterial antigens arising from the phagosome are presented by major histocompatibility complex (MHC) class II molecules while antigens arising from the cytoplasm are presented by MHC class I molecules.[39] This difference determines, in part, the fate of the antigen-presenting cell, stimulation or destruction, because the presentation of antigen arising from the cytoplasm indicates that the cell has failed to control the mycobacteria. The release of IFN-γ by the clonally expanded CD4 cells, but also to a lesser extent by CD8 cells, natural killer (NK) cells and γδ cells, activates macrophages. This initiates a cascade of events including the hydrolysis of vitamin D that leads to further activation of macrophages and the release of tumor necrosis factor (TNF) by the activated macrophages. The sequence of cellular responses at the site of mycobacterial infection appears to be polymorphonuclear granulocytes (PMNs), NK cells, γδ cells and then α/β T cells; however, with time the α/β T cells become predominant in the cellular response. The sequence of recruitment is probably determined by the proximity of the cells to the site of infection; for example, γδ cells are likely to be one of the first cells recruited to the site of *M. tuberculosis* infection in the lung. The PMNs produce highly proteolytic enzymes that cause tissue liquefaction while each of the T-cell types possesses cytotoxic activity. Activated macrophages have an increased capacity for seeking out, engulfing and destroying mycobacteria, but the production of TNF by macrophages contributes to tissue necrosis and, therefore, to the immunopathology of the disease.

On the other hand, mycobacteria are equipped to resist oxidative killing by phagocytic cells. This resistance is controlled by the *katG* promoter in the mycobacterial genome. The *katG* gene encodes an enzyme with catalase, peroxidase and peroxynitritase activities.[43]

Granulomas develop as a mycobacterial infection becomes more chronic. Granulomas are aggregates of activated macrophages that take on epithelial cell-like morphology (epithelioid cells), and typically, lymphocytes are found at the periphery of the granuloma. Although the formation of a granuloma effectively contains the mycobacteria where they can be killed, bacilli can persist within giant cells (fused epithelioid cells) and the granuloma may never sterilize. With time a fibrotic wall (collagen) will encapsulate the granuloma and the center of the granuloma may become necrotic with a cheese-like appearance, which is the source of the descriptive word caseation. Hippocrates termed granulomas 'tubercles' because they were similar in morphology to the tubers of plants, hence the name tuberculosis.[17]

Host–pathogen interactions involving phagocytic uptake, intracellular survival, host immune responses and antigenic variation are highly complex and have been extensively reviewed.[41,42,44]

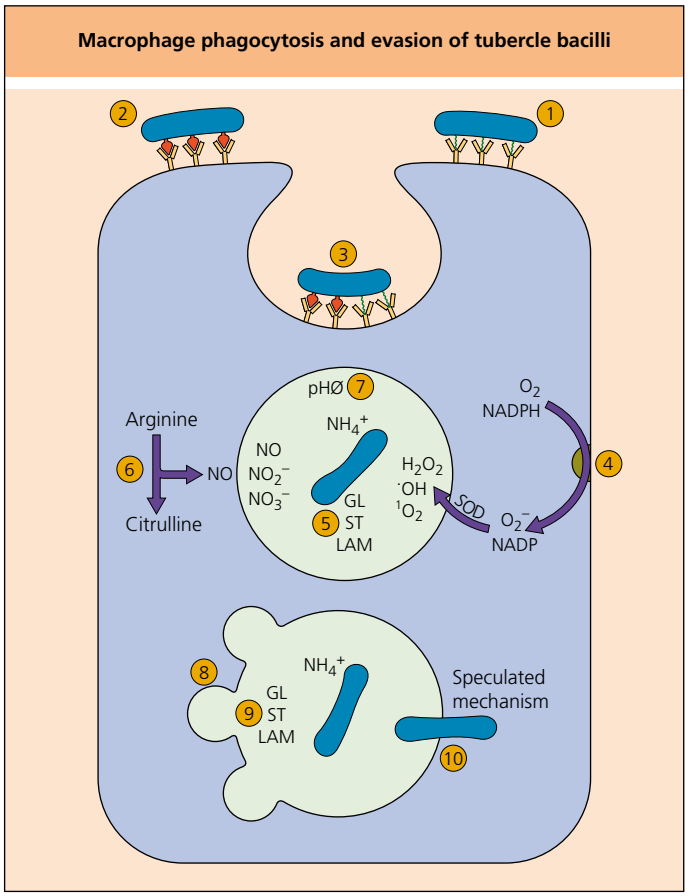


Fig. 174.10 Macrophage phagocytosis and evasion of tubercle bacilli. The tubercle bacilli bind via lipoarabinomannan (LAM) (1) or complement receptors (2); phagocytosis occurs (3) with the activation of an oxidative burst (4); glycolipids (GL), sulfatides (ST) and LAM can downregulate the oxidative burst (5); reactive nitrogen intermediates may play a role in antimycobacterial activity (6), as does the acidic pH of the phagolysosome (7). Finally, the production of ammonia by tubercle bacilli may diminish the effect of reactive nitrogen intermediates (8) and contribute to the failure to form a phagolysosome fusion (9). Tubercule bacilli may evade the antimycobacterial activities of the phagolysosome by producing a hemolysin that releases the bacilli into the cytoplasm (10). NADP, nicotinamide adenine dinucleotide phosphate; NADPH, nicotinamide adenine dinucleotide phosphate, reduced form; SOD, superoxide dismutase. Adapted from Chan & Kaufmann.[41]

LABORATORY DIAGNOSIS OF MYCOBACTERIA

General remarks

The detection and identification of mycobacteria in clinical specimens requires profound expertise and is associated with a considerable safety risk when specimens are handled improperly. Given the low infectious dose of *M. tuberculosis* for humans (50% infective dose, <10 AFB), specimens from known or suspected cases of tuberculosis must be considered potentially infectious and handled with appropriate precautions.[45] Aerosol-generating manipulations should be conducted in a biosafety cabinet class II. Level 3 facilities are required for laboratories associated with higher risk (processing specimens for culture, identification and susceptibility testing; extraction of DNA from culture). Only in complying with the generally accepted biohazard precautions will safety for both laboratory personnel and the environment be assured.

The technologies available to assist in the laboratory diagnosis of mycobacterial infections have improved remarkably.[16,34,46,47] Major achievements are:

- fully automated, nonradiometric culture systems for culture and drug susceptibility testing;
- nucleic acid amplification assays for the direct detection of *M. tuberculosis* complex in clinical specimens;

- identification at the species level by nucleic acid probes, line probe assays and gene sequencing;
- molecular assays for the rapid detection of antimicrobial resistance; and
- DNA typing methods for answering questions relating to transmission routes, to reactivation versus re-infection of disease or to the genetic diversity of strain populations.

In discussing diagnostic mycobacteriology it is important to emphasize two general principles of laboratory medicine. First, the quality of any clinical test is highly dependent on the quality of the specimen, including the time of collection in the course of the disease, the appropriateness and sufficiency of the specimen, and the prompt and proper transport of the specimen to the laboratory. Second, the intended purpose of the test must be understood by the physician who orders the test. Was the test designed to screen for disease, to provide a definitive diagnosis based on a clinical index of suspicion, to confirm another test, to monitor therapy (test of cure), or to provide epidemiologic information? Use of a test for a purpose for which it was not designed and evaluated could be misleading. For example, nucleic acid amplification assays for the direct detection of *M. tuberculosis* complex in clinical specimens were evaluated as diagnostic assays for the very first detection of disease and explicitly not for monitoring response to therapy or cure.

The Centers for Disease Control and Prevention and other expert institutions recommend that AFB smear results be available and positive results be reported within 24 hours of specimen receipt.[48] The time required for identification and susceptibility testing of *M. tuberculosis* should average 14–21 days and 15–30 days, respectively.[48,49] With the new molecular tests available this goal will easily be achieved and partial results be available to the clinician even more rapidly.

Collection of specimens

The most critical step in the laboratory diagnosis of any infectious disease often occurs before the specimen arrives at the laboratory.[34,46,50] The quality, quantity, timing, transport and appropriateness of the specimen have a greater impact on the outcome of a laboratory test than almost any other factor (Table 174.3). It is important that physicians notify the laboratory if they suspect an uncommon or fastidious mycobacterial infection (such as *M. ulcerans, M. genavense* or *M. haemophilum*) because these and other species of mycobacteria have

Table 174.3 Specimen types and requirements for the diagnosis of mycobacterial infections

Specimen type	Requirements	Collection	Note
Abscess fluid	10 ml*	Collect with syringe, submit in sterile container	Dry swab or swab submitted in transport medium unacceptable
Blood	5–10 ml (adult) 1–5 ml (child)	SPS tube, tube with heparin or citrate, Isolator tube	EDTA tube and coagulated blood unacceptable Do not refrigerate
Fluids (pleural, pericardial, peritoneal, synovial, etc.)	10–15 ml*	Collect with syringe, submit in sterile container	Swabs unacceptable
Bone	Bone chip	Sterile container, no fixative	Formalin-fixed unacceptable
Bone marrow	100 mg* or 1–3 ml aspirate	SPS tube, heparin or citrate tube, isolator tube	Aspirate volumes >5 ml may be primarily peripheral blood Do not refrigerate
Bronchoalveolar lavage or bronchial washing	≥5 ml	Sterile container	Avoid contamination of bronchoscope with tap water (possible false-positive results)
Cerebrospinal fluid	5–10 ml*	Sterile tube	
Gastric lavage	5–10 ml	Sterile container, collecting in morning before arising and eating	Neutralize with 10% sodium carbonate if delayed processing
Lymph node	Whole node or part*, caseous part	Sterile container	Formalin-fixed unacceptable
Skin biopsy	Sterile container, aspirate	Biopsy at periphery or aspirate from under margin of lesion	Note if suspicion of *M. ulcerans*, *M. haemophilum* or *M. marinum* (need special culture conditions or extended incubation)
Sputum	5–10 ml	Early morning sputum in sterile plastic container	Use sterile hypertonic saline for induced sputum, avoid exposure to tap water Do not pool sputum specimens
Stool	≥1 g	Sterile plastic container, no transport medium	Refrigerate, but do *not* freeze
Tissue biopsy	1 g*	Sterile container	Caseous portion, formalin-fixed unacceptable
Urine	50 ml*	First morning specimen, catheter urine in sterile container	24-hour urine and pooled urine specimens unacceptable Refrigerate

SPS, sodium polyanetholsulfonate.
*As much as possible (up to or in excess of the weight/volume shown).
Adapted and modified from Pfyffer,[34] CLSI,[46] Kent & Kubica[50] and Della Latta.[51]

growth requirements that are significantly different from the majority of other species of mycobacteria isolated from clinical specimens.

Respiratory specimens

Sputum is the best specimen for the diagnosis of pulmonary tuberculosis. Expectorated sputum specimen should be collected early in the morning on three occasions. The wide-mouthed, sterile, plastic container (wax free) with a tight fitting cap containing the sputum specimen should be immediately transported to the laboratory or held at 39.2°F (4°C) until processed. Prompt processing of sputum is required to minimize overgrowth of mycobacteria by the normal respiratory tract microflora. Alternative respiratory tract specimens are induced sputum, endotracheal aspiration, bronchial washings or aspirates taken during bronchoscopy, and gastric lavage. Bronchoscopes must be carefully cleaned and decontaminated after collecting specimens from patients.

Gastric lavage

Gastric lavage (for swallowed sputum) is useful for collecting specimens from patients who, for a variety of reasons, are unable to produce sputum by other means. Gastric lavage is the specimen of choice from infants and children (up to 12 years) suspected of having pulmonary tuberculosis. *M. gordonae* is frequently found in many hospital water supplies and especially in ice machines, and use of such contaminated water during the collection of a gastric aspirate may result in a false-positive smear or culture. A gastric lavage must be promptly sent to the laboratory because it must be processed as soon as possible or neutralized with 10% sodium carbonate to avoid loss of mycobacteria due to gastric acidity. As with expectorated sputum specimens, gastric lavage specimens should be collected early in the morning, before eating, and on three separate occasions.

Urine

Three early morning midstream urine specimens should be collected into a sterile plastic container with a leak-proof cap. Large volumes of urine can be concentrated by filtration, but 24-hour urine should not be used because of contamination and dilution of any mycobacteria present.

Blood and bone marrow

Blood, bone marrow aspirates or cores are ideal specimens for the diagnosis of disseminated mycobacterial infections, in particular of MAC infections. If blood has to be transported before inoculation of the medium, sodium polyanethol sulfonate (SPS), heparin or citrate may be used as an anticoagulant. Blood collected in EDTA and coagulated blood are not acceptable for culture, neither is direct inoculation of blood onto a solid medium. If blood cannot immediately be processed by the laboratory, it should be stored at room temperature.

Other fluids

Pleural, pericardial, synovial and ascitic fluids as well as pus and cerebrospinal fluid (CSF) may also be submitted for diagnostic analyses. *Mycobacterium tuberculosis* meningitis is a medical emergency and CSF specimens should be collected, transported and processed in a manner that reflects the urgent nature of such a diagnosis.

Tissues

Tissue should be submitted for both histology and mycobacteriology. As tissue is preferred over necrotic material or pus for culture, it is therefore important to have fresh tissue and not swabs submitted to the microbiology laboratory. Formalin-preserved tissue should be submitted for histologic studies. Although not suitable for culture, formalin-preserved and paraffin-embedded tissue may be submitted for polymerase chain reaction (PCR) analysis, especially if AFB are observed on microscopic examination of the tissue sections.

Feces

Feces are not a particularly useful specimen for the diagnosis of tuberculosis or other mycobacterial infections, with the exception of suspected gastrointestinal tract infection with MAC in HIV-positive patients. However, the recovery of MAC from feces is poor (low sensitivity) even in HIV-positive patients. If *M. avium* is isolated from the feces the positive predictive value of the culture is high, meaning that the patient is likely to develop disseminated disease.[52] To avoid overgrowth of normal gastrointestinal tract microflora, feces must be rather harshly decontaminated, but this is likely to also decrease the yield of mycobacteria.[53]

Processing

Contaminated specimens

Most respiratory tract specimens are expected to be contaminated with normal respiratory tract microflora. In addition, the mucin matrix both protects and traps micro-organisms and makes the specimens difficult to process. Therefore such specimens have to be decontaminated (to reduce or eliminate other micro-organisms that would be likely to overgrow the mycobacteria that might be present in the specimen) and liquefied. The most common agents used to pretreat respiratory specimens are *N*-acetyl-L-cysteine (NALC) and dithiothreitol (DTT or sputolysin). Both NALC and DTT are unstable in air and must be prepared fresh. A combination of NALC and sodium hydroxide is most commonly used for digesting and decontaminating sputum. Other agents used for digestion and decontamination are zephiran (benzalkonium chloride)-trisodium phosphate, oxalic acid, cetylpyridinium chloride (CPC)-sodium chloride, and sulfuric acid.[34,46,50,51] Finally, the pretreated specimen is centrifuged to increase sensitivity of microscopy and the recovery of mycobacteria from culture.

There is no ideal method for digesting and decontaminating respiratory tract specimens. Invariably, mycobacteria are lost during decontamination. Detailed descriptions of the various procedures for digesting and decontaminating sputum and other contaminated specimens have been well described elsewhere.[34,46,50,51]

Uncontaminated specimens

The primary concern about specimens from sterile body sites is the quantity of specimen submitted for smear and culture. Swabs usually contain insufficient material for culture, and 1 g of tissue or 10 ml of fluid are ideal. To improve recovery of mycobacteria, centrifugation should be used to concentrate large volumes of fluids. Aseptically collected tissues and fluids from normally sterile body sites usually do not require processing. These specimens can initially be inoculated on a chocolate agar plate to check for purity. If sterile, specimens are concentrated and inoculated into a liquid growth medium as well as on solid media without decontamination.

Acid-fast stain and smear microscopy

An acid-fast stain remains the most rapid and least expensive method for directly detecting mycobacteria in clinical specimens, and is highly specific. Nevertheless, the laboratory must be aware that there are organisms other than mycobacteria with various degrees of acid-fastness such as *Rhodococcus* spp., *Nocardia* spp. and *Legionella micdadei*, as well as cysts from *Cryptosporidium*, *Isospora*, *Cyclospora* and *Microsporidium* spores. The presence of AFB in a specimen from a patient with signs and symptoms of tuberculosis or another mycobacterial infection is an important guide to effective treatment and the initiation of public health measures. Furthermore, an acid-fast stain can quickly assess patient infectiousness, and is also used to confirm that a culture is acid fast. The detection limit of an acid-fast

stain has been estimated to be 5000–10 000 bacilli/ml of sputum; overall sensitivity is between 22% and 78%.[55] The importance of the acid-fast stain and the need for a prompt turnaround time cannot be overemphasized. In laboratories that do not perform cultures, an acid-fast stain can be performed using a specimen treated for a short time with 5% sodium hypochlorite. Above all, the predictive value of a positive smear for *M. tuberculosis* in expectorated sputum is very high (~90%).

The three stains that are commonly used to detect AFB are Ziehl–Neelsen, Kinyoun and auramine–rhodamine fluorochrome stains.[51] With each of these procedures, acid-fastness is defined as resistance to decolorizing with acid–alcohol (e.g. 3% hydrochloric acid in 95% ethanol). When mycobacteria are stained with Gram's crystal violet and safranin they often appear as beaded Gram-positive bacilli or fail to stain at all. It is important to note, however, that culture media, incubation conditions and the age of the culture influence the acid fastness of mycobacteria. The critical role of the mycobacterial cell wall in acid fastness is underscored by the observation that INH causes a loss of 'acid fastness' in susceptible strains of *M. tuberculosis*.

The preparation of the smear is the critical first step in performing an acid-fast stain. Using a glass slide cleaned with ethanol, make a smear of approximately 1 × 2 cm of a single specimen or isolate. For CSF specimens previously concentrated by centrifugation, three drops of the concentrate are placed on a clean glass slide, one drop at a time. The drop is allowed to air dry before the next drop is added. The slide is air dried or alternatively dried at 176°F (80°C) for 15 minutes in a biosafety cabinet class II – it is important to note that heat fixing may not kill all the mycobacteria on the slide. Mycobacteria appear brightly fluorescent against a dark background in the auramine–rhodamine stain and the increased sensitivity of the fluorescence stain compared with the other stains is used to rapidly screen slides at a lower (250–450×) magnification. However, fluorescent objects must be examined at 800–1000× magnification to confirm morphology. Positive fluorescent smears should be confirmed with either the Ziehl–Neelsen or Kinyoun stains. The Ziehl–Neelsen stain requires that the carbolfuchsin stain be heated for the stain to penetrate the mycobacterial cell. In contrast, heating is unnecessary with the Kinyoun stain because the concentration of basic fuchsin and phenol have been increased to ensure that the basic fuchsin penetrates the mycobacterial cell wall. Using a 100× oil immersion lens, 100–300 fields of a properly prepared smear and stain should be examined. It should be remembered that in examining even 100–300 fields, only 1–4% of a 1 × 2 cm smear would be examined. Mycobacteria usually appear as pink, slender rod-shaped bacilli; however, pleomorphic shapes are common and range from coccoid to long rods with curves or bends (Fig. 174.11).

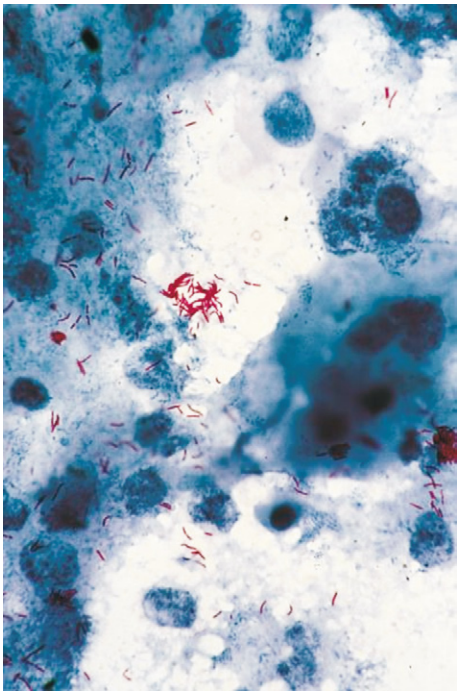


Fig. 174.11 Ziehl–Neelsen acid-fast stain of sputum containing 4+ tubercle bacilli. Courtesy of S Froman and A Gaytan.

Culture

In detecting approximately 10^2 viable organisms/ml of specimen, culture is more sensitive than smear microscopy. Also, it is the only reliable means to monitor effectiveness of therapy in tuberculosis patients.[55] A variety of solid (e.g. Löwenstein–Jensen, Ogawa, Middlebrook agar) and liquid media (see below) are available for the culture of mycobacteria from clinical specimens. It is highly recommended to use a combination of solid and liquid media to ensure good recovery for subsequent species identification and susceptibility testing. The macroscopic and microscopic characteristics, together with the rate of growth and pigmentation, are valuable in making a presumptive identification (see Fig. 174.2).

Manual culture systems used for the detection of mycobacteria are the Septi-Chek Acid-Fast Bacillus Biphasic System (Becton Dickinson Microbiology Systems, Sparks, MD), the MB Redox Tube System (Heipha Diagnostika, Eppelheim, Germany), and the Mycobacteria Growth Indicator Tube (MGIT; Becton Dickinson). The Septi-Chek AFB system consists of Middlebrook 7H9 broth and a plastic paddle with modified Löwenstein–Jensen medium, Middlebrook 7H11 agar and chocolate agar. Comparison studies with conventional culture and the BACTEC 460 TB System indicated that the Septi-Chek AFB Biphasic System is reliable and practical.[56] MB Redox tubes contain modified Kirchner medium enriched with growth supplements, antibiotic compounds and a redox indicator (tetrazolium salt) which is reduced to a pink-, red- and violet-colored formazan by mycobacteria. The accumulation of reduced dye on the surface of the bacilli results in the appearance of visible microcolonies.[57] The MGIT contains a highly enriched Middlebrook 7H9 broth in conjunction with a fluorescence quenching-based oxygen sensor (silicon rubber impregnated with a ruthenium pentahydrate). Growth is detected by exposing an inoculated MGIT to ultraviolet light and examining for fluorescence, which is an indication of growth and oxygen consumption. The performance of these manual systems in combination with conventional solid media is satisfactory.[56–59]

The radiometric, semiautomated BACTEC 460 TB System, commercially developed in the early 1980s by Becton Dickinson, became a 'gold standard' for the rapid detection of mycobacteria (Table 174.4). In the BACTEC 460 TB System growth of mycobacteria is detected by measuring the release of $^{14}CO_2$ from ^{14}C-labeled palmitate, which is the primary carbon source in the BACTEC 460 TB 12B culture medium. This technology has significantly improved recovery rates and detection time (for references, see[34]). However, as with all liquid media, neither colony morphology nor mixed cultured can be recognized. Other limitations of the BACTEC 460 TB System are the costs, the problem of radioactive waste disposal, labor intensity and the potential for needle punctures among technicians. Due to these disadvantages the BACTEC 460 TB System is gradually vanishing from clinical mycobacteriology laboratories.

The studies published to date on the nonradiometric automated systems – BACTEC MGIT 960 (Becton Dickinson), ESP Culture System II (Trek Diagnostic Systems, Cleveland, OH) and MB/BacT (bioMerieux, Marcy-L'Etoile, France) – indicate similar performance in terms of recovery rates and time to detection in comparison with conventional culture and the radiometric BACTEC.[60–62] Though costly, these new systems are convenient, labor-saving, do not require the use of radioisotope, offer continuous monitoring of the cultures and electronic data management, and address safety more appropriately.

Table 174.4 Semiautomated and fully automated systems for the detection of *Mycobacterium tuberculosis* complex and other mycobacteria in clinical specimens

Instrument/medium	Manufacturer	Detection system	SIRE/PZA/second line drugs susceptibility testing
BACTEC 460 • BACTEC 12B medium (modified 7H9 broth) • No medium available for blood and bone marrow	Becton Dickinson Microbiology Systems, Sparks, MD, USA	Detection of radioactively labeled CO_2 released during the catabolism of 14C-labeled palmitic acid in the growth medium	Yes/Yes/Yes
BACTEC 9000MB • MYCO/F medium + supplements (modified 7H9 broth) • MYCO F/LYTIC medium for blood and bone marrow	Becton Dickinson Microbiology Systems, Sparks, MD, USA	Detects changes in O_2 concentration in the growth medium using an O_2 sensor based on fluorescence quenching	No/No/No
BACTEC MGIT 960 • MGIT (modified 7H9 broth) • No medium available for blood and bone marrow	Becton Dickinson Microbiology Systems, Sparks, MD, USA	O_2 sensor based on fluorescence quenching	Yes/Yes/Yes
BACTEC 9050 • BACTEC MYCO F/LYTIC (medium for blood and bone marrow)	Becton Dickinson Microbiology Systems, Sparks, MD, USA	Fluorogenic system	No/No/No
ESP Culture System II • ESP Myco (modified 7H9 broth)	Trek Diagnostic Systems, Cleveland, OH, USA	Detects production on consumption of gas by measuring changes in partial pressure of gas phase of culture medium	Yes/No/No
MB/BacT/ALERT 3D • Bact/ALERT MP (modified 7H9 broth) • Bact/Alert ML Mycobacteria lytic (medium for blood)	BioMérieux, Marcy-L'Etoile, France	Detects changes in CO_2 concentration in gas phase of the growth medium using a colorimetric sensor and reflected light	No/No/No

PZA, pyrazinamide; SIRE, streptomycin, isoniazid, rifampin and ethambutol.

For many years the Isolator System (Wampole, Cranbury, NJ) and the radiometric BACTEC 13A blood culture medium (Becton Dickinson) were the only reliable systems for mycobacterial blood cultures. Since the Isolator Sytem is associated with a not negligible safety risk and the BACTEC 13A medium is no longer commercially available, blood and bone marrow specimens are increasingly cultivated in MYCO/F LYTIC bottles (Becton Dickinson) or the MB/BacT ALERT blood medium (bioMérieux). These two culture systems were as sensitive as and faster for the detection of MAC bacteremia than the older standard systems, Isolator and the radiometric BACTEC 13A medium.[63] Also, BacT/ALERT FA blood cultures and BACTEC MYCO/F LYTIC vials equally supported ample growth of *M. tuberculosis*.[64]

Identification

Traditional methods (Runyon groups, morphologic features and growth aspects)

In 1958, Ernest Runyon proposed that by dividing mycobacteria into four groups based on the rate of growth and pigmentation one could make a preliminary identification. Rapid growers were separated from slowly growing mycobacteria according to the time required to produce clearly visible colonies (≤7 days for rapid growers; the most accurate measure of growth is made with subcultures). Slowly growing mycobacteria were separated based on pigment production: scotochromogenic (see Figs 174.3, 174.4), photochromogenic (see Fig. 174.5) and nonphotochromogenic (see Fig. 174.6). Fifty years later, the Runyon groups remain a valuable guide for a presumptive identification of mycobacteria isolated by solid culture, and the logic of this classification has been corroborated by 16S rRNA sequence analysis. Even with biochemical identification only, the correlation between an initial classification into a Runyon group and the final identification ranges from 87% (nonphotochromogens) to 97% (rapid growers).[50]

The first challenge for the clinical mycobacteriology laboratory is to distinguish between members of the *M. tuberculosis* complex (see Table 174.1) and NTM, a taxonomically imprecise, but clinically useful distinction.

Growth of *M. tuberculosis* on Middlebrook 7H10 or 7H11 agar at 95°F (35°C) is usually detected in 2–3 weeks. The colonies are beige colored, rough, dry, corded, flat and with irregular borders (Fig. 174.12). On egg media such as Löwenstein–Jensen or Ogawa media the colonies are frequently warty and granular and with time heap into a cauliflower shape (Fig. 174.13). Although the growth of *M. tuberculosis* is quite distinct and it is not unreasonable for an experienced mycobacteriologist to make a presumptive report of *M. tuberculosis* based on the rate and appearance of growth, identification must always be confirmed by standard procedures.

Three clinically significant species of mycobacteria have lower optimal growth (86°F/30°C): *M. ulcerans, M. marinum* and *M. haemophilum*. In addition, *M. haemophilum* requires hemin for growth and, therefore, specimens suspected to harbor this species should be inoculated onto chocolate agar, Middlebrook 7H10 agar with hemolyzed blood or 7H10 agar with a hemin-containing paper strip.

Mycobacterium avium complex organisms isolated from blood or sputum appear as either glossy, whitish colonies or smaller, translucent colonies on Middlebrook 7H10 or 7H11 agar after 10–14 days (Fig. 174.14). A third, less frequently encountered morphology resembles the dry and flat colonies of *M. tuberculosis*. In older cultures colonies may even become verrucose and develop pigment. However, these colony variant types are not observed on egg-based



Fig. 174.12 Primary isolate of *M. tuberculosis* grown from sputum on Löwenstein–Jensen medium displaying characteristic beige, rough and dry-appearing growth. Courtesy of S Froman and A Gaytan.



Fig. 174.13 Primary isolate of *M. tuberculosis* grown from sputum on Löwenstein–Jensen medium displaying 'cauliflower' or verrucose colonies. These are also characteristic of other mycobacteria including MAC and rapid growers, especially as a culture ages. Courtesy of S Froman and A Gaytan.

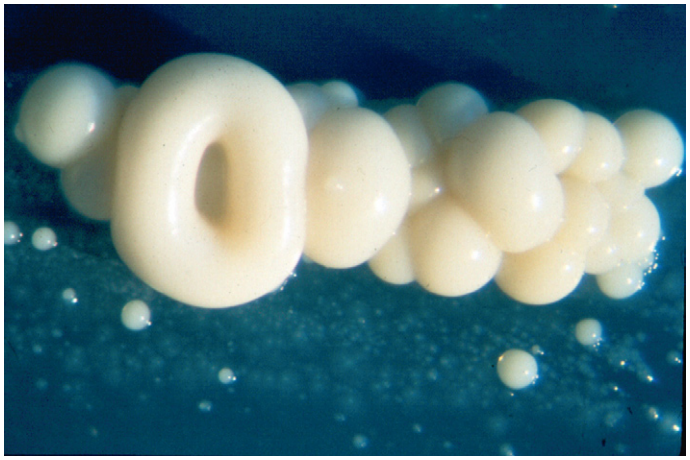


Fig. 174.14 *M. avium* grown on Middlebrook 7H10 agar. The two most frequent morphologies are shown (beige opaque colonies and smaller translucent colonies).

media. The initial distinction between colony types is important because the transparent variants tend to be more resistant to antimicrobial agents.

Conventional biochemical tests

For many decades the salient biochemical features of mycobacteria represented the 'gold standard' for identification at the species level. Details about the many biochemical tests used in the conventional identification of mycobacteria are described in a variety of sources. Wayne and Sramek[65] and Kent & Kubica[50] described strategies for the use of these tests. Their value in today's clinical mycobacteriology laboratory has, however, become limited.

For more than 10 years mycolic acids have been extracted by saponification and derivatives prepared with either a phenyl ester or a fluorescent compound, for detection by high performance liquid chromatography (HPLC). The HPLC pattern of an unknown species is then compared with a library of known patterns usually facilitated by a decision analysis system.[66] The equipment cost and the expertise required to perform HPLC analyses has restricted use of this technology mostly to large hospital, and reference and public health laboratories.[46] Alternatively, whole cell fatty acid analysis can be performed by commercially available instrumentation and software (e.g. Microbial Identification Systems, Newark, DL).

Nevertheless, with the increasing number of new mycobacterial species it is well accepted today that biochemical tests are in many instances insufficient for accurate identification. Therefore, the ultimate identification scheme of mycobacteria mainly relies on molecular methods, along with some phenotypic tests.

Molecular methods

For some time nucleic acid probes (AccuProbe; Gen-Probe, San Diego, CA) have been commercially available for the identification of *M. tuberculosis* complex, *M. intracellulare*, MAC, *M. gordonae* and *M. kansasii* from culture. The AccuProbe culture confirmation test is based on the use of acridinium ester labeled DNA probes that are complementary to species-specific rRNA which is released from the mycobacteria using a combination of a lysing reagent, sonication and heat. If a stable RNA–DNA duplex forms, the acridinium label is protected within the helix from a selection reagent while free acridinium label is inactivated. Stable duplexes are detected by the addition of an alkaline hydrogen peroxide solution, which in combination with the bound acridinium ester generates chemiluminesence. The amount of light produced is measured in a luminometer as relative light units (RLUs).

The AccuProbe test cannot be used for direct detection of mycobacteria in clinical specimens. In general, the AccuProbe tests are highly reliable and simple to perform. They are very specific and sensitive for *M. tuberculosis* complex and *M. gordonae* (100%); for the other species sensitivity is less (range 85–100%).

A more recent development is the line probe assay. In the INNO-LiPA multiplex probe assay (INNO-LiPA MYCOBACTERIA; Innogenetics, Ghent, Belgium), probes consist of the ~280 bp internal transcribed spacer (ITS) region in between the 16S and the 23S rDNAs. With this assay both *M. tuberculosis* complex as well as a number of NTM can easily be identified.[67] The GenoType kits of Hain Lifescience (Nehren, Germany) are based on similar technology and are able to identify 23 (GenoType CM) and 14 (GenoType AS) additional species, respectively. Concordant results have been obtained for 92.6% of previously sequenced mycobacterial strains with the CM Assay and 89.9% with the AS Assay.[68] Non-*Mycobacterium* species were also identified. Most attractively, the GenoType technology is able to separate most species within the *M. tuberculosis* complex.

Another way to identify mycobacteria at the species level is via PCR and PCR-restriction enzyme analysis (PRA). Telenti *et al.*[69] established a method based on the *hsp65* gene encoding for the 65 kDa heat shock protein and developed an algorithm of species identification based on the restriction profiles generated by Hae*III* and BstE*II*. Although the method is widely being used the major drawbacks of the technique are the facts that ambiguous profiles may occur and the database is very complex (http://app.chuv.ch/prasite/index.html).

Today, it is generally accepted that gene sequencing for identification purposes remains the 'gold standard' for identification. Identification of species-specific signatures within variable regions of highly conserved genes such as the 16S rDNA gene or *hsp65* gene generated PCR protocols using genus-specific primers followed by direct sequencing of the PCR products.[16,47] In addition to these genes, other targets such as the gene encoding the 32 kDa protein, the *sod* gene, the *gyrB* gene coding for the gyrase subunit B, the *rpoB* gene coding for the RNA polymerase, the *dnaJ* gene, or the ITS 16S–23S sequence may be used (for refs., see[16]). Even with the possibilities of gene sequencing the user has to be aware that each protocol has its limitations. For example, on 16S rDNA sequencing, *M. kansasii* is indistinguishable from *M. gastri*, *M. abscessus* from *M. chelonae*, and *M. ulcerans* from *M. marinum*.

Direct detection of *M. tuberculosis* complex by nucleic acid amplification

A vast number of studies have concentrated on the four commercially available nucleic acid amplification (NAA)-based kits designed to detect *M. tuberculosis* complex directly from clinical specimens:

- the Amplicor *M. tuberculosis* Test (Roche Molecular Systems, Inc., Branchburg, NJ);
- the Amplified *M. tuberculosis* Direct Test (MTD; GenProbe);
- the BDProbeTec Strand Displacement Amplification (SDA; Becton Dickinson); and
- GenoType Mycobacteria Direct (Hain Lifescience).

The Amplicor *M. tuberculosis* PCR assay is based on amplification of a 584 bp region of the 16S rRNA gene sequence. Incorporation of dUTP instead of dTTP prevents carry-over in the amplification reaction. Experience has shown that the specificity for smear-positive specimens ranges from 90% to 100% for respiratory specimens and from 87.5% to 100% for extrapulmonary specimens. Sensitivity appears to be significantly lower for smear-negative specimens, from 50% to 95% for respiratory specimens and from 17% to 70% for extrapulmonary specimens.

The amplified *M. tuberculosis* direct (MTD) test uses transcription-mediated amplification and a hybridization protection assay to qualitatively detect the *M. tuberculosis* complex. Mycobacterial rRNA is released by sonication from bacilli present in a clinical specimen, denatured with heat and transcribed into cDNA with a reverse transcriptase. One part of the oligonucleotide used in the reverse transcription step includes a promoter region for a high efficiency RNA polymerase (e.g. phage T7 polymerase); the other part of the oligonucleotide includes a 16S rRNA target-specific sequence. As a result of cycles of denaturation, reverse transcription and RNA synthesis, the mycobacterial rRNA is amplified under isothermal conditions (108°F/42°C). *Mycobacterium tuberculosis* complex-specific amplicons are detected using the GenProbe hybridization protection assay (see above). Based on published reports, overall specificity of the MTD ranges from 92% to 100%. For respiratory specimens the sensitivity is between 91% and 100% for smear-positive specimens and between 65% and 92% for smear-negative specimens. For nonrespiratory specimens, the sensitivity is between 88% and 100% for smear-positive specimens and between 63% and 100% for smear-negative specimens.

The BDProbeTec SDA is an isothermal enzymatic process that co-amplifies sequences of the IS*6110* (specific to *M. tuberculosis* complex) and the 16S rRNA gene (common to most mycobacterial species). By nicking the recognition sequence in double-stranded DNA by a restriction endonuclease and extension of that site with a polymerase that synthesizes a new strand of DNA while displacing the existing one, the assay has similarly a high specificity. Sensitivity for smear-positive specimens was between 98.5% and 100% and between 33% and 85% for smear-negative specimens.

In a large retrospective study that included 824 respiratory species, AFB smear, Cobas Amplicor MTB and BDProbeTec revealed similar specificity (99.7%, 99.9% and 99.9%, respectively), whereas overall sensitivity of the three tests was 61.5%, 78% and 86.2%, respectively (proportion of smear-positive specimens was <61%).[70]

The GenoType Mycobacteria Direct is based on the nucleic acid sequence-based amplification (NASBA) and DNA strip techniques, allowing the 23S rRNA amplification-based detection of *M. tuberculosis* complex, *M. avium*, *M. intracellulare*, *M. kansasii* and *M. malmoense* directly from specimens. Although data are still very limited, this method may turn out to be promising.[71]

From the numerous studies including both respiratory and extrapulmonary specimens it is obious that NAA-based diagnostic tests are:

- appropriate, if there is a clinical suspicion of tuberculosis;
- always performed in conjunction with culture; and
- not to be requested to monitor therapeutic success.[46,47]

Most importantly, laboratories perfoming such assays should be part of external quality control schemes.[72]

DNA fingerprinting

Molecular typing helps to differentiate relapses from exogenous re-infections in patients and to demonstrate false-positive cases due to contaminated bronchoscopes or laboratory cross-contamination of clinical specimens during workup. It also allows the definition of prevalent families of strains and elucidates the intraspecies genetic microevolution. Most importantly, it helps to identify transmission chains. Lastly, DNA typing methods are also useful in identifying members within the *M. tuberculosis* complex such as *M. microti*, *M. caprae* or *M. canettii*. For *M. tuberculosis* in particular, a number of methods have been developed to carry out DNA typing rapidly. The most important ones are as follows.[16]

IS*6110* restriction fragment length polymorphism

The IS*6110*-based analysis of *M. tuberculosis* centers on differences in fragment length and copy number of this insertion element. There are 0–20 copies of the 1355 bp IS*6110* in most strains of *M. tuberculosis*. If strains of *M. tuberculosis* contain a small number of IS*6110* only, the resulting patterns often do not discriminate between strains. The distribution of IS*6110* within the genome appears to be stable over several months to years. IS*6110*-based restriction fragment length polymorphism (RFLP) is conceptually straightforward[73] and standardized to facilitate interlaboratory comparability of patterns.[74] Basically, the method involves extraction

of genomic DNA, restriction of the DNA with an appropriate enzyme (e.g. PvuII) and electrophoretic separation of the restriction fragments (RFLP analysis). The IS*6110* pattern is revealed by Southern hybridization using a labeled fragment of the IS*6110* sequence. A computer-based method of analysis of the IS*6110* patterns was described by Heersma *et al* and software is commercially available.[75] A drawback of this method is the large amount (about 2 µg) that is required for analysis. Therefore, other PCR-based typing methods were developed that have comparable discrimination and reproducibility to RFLP analysis.

Spacer oligonucleotide typing ('spoligotyping')

This method uses PCR primers to amplify the 36 bp direct repeats (DRs) in the genomic direct-repeat region of *M. tuberculosis* complex DNA.[76] The resulting PCR products are hybridized to 43 different oligonucleotides fixed to a membrane. The 43 oligonucleotides were derived from the sequences of the spacer DNA between the DRs. This method is able to discriminate the members of the *M. tuberculosis* complex.[16] Spoligotyping also allows the identification of prevalent groups of strains, e.g. the Beijing strain, a genotype frequently encountered in China, in other regions of Asia and the former Soviet Union, or the 'W' strain, an evolutionary branch of the Beijing family which caused a large outbreak in the USA.

PCR assays targeting loci other than IS*6110*

Genotyping based on a variable number of tandem repeats (VNTRs) of different classes of interspersed genetic elements, the mycobacterial interspersed repetitive units (MIRUs), is an elegant alternative to the labor-intense classic IS*6110*-based DNA typing. It relies on:

- PCR amplification of multiple loci using primers specific for the flanking regions of each repeat locus; and
- the determination of the sites of the amplicpons which reflect the numbers of the targeted MIRU-VNTR copies.

A standardization of the method has recently been proposed.[77]

ANTIMICROBIAL RESISTANCE AND SUSCEPTIBILITY TESTING

Resistance

During bacterial multiplication resistance to antimycobacterial drugs develops spontaneously and with a defined frequency. Genetic mutations resulting in resistance of *M. tuberculosis* to RMP lead to an estimated prevalence of 1 in 10^8 bacilli in drug free environments. Antimicrobial resistance in mycobacteria is fundamentally a reflection of the large populations of mycobacteria present in infected tissues and fluids and the frequencies of individual gene mutations that result in a resistant phenotype. In pulmonary tuberculosis there are 10^7–10^9 bacilli in lung cavities, but only 10^2–10^4 bacilli in caseous lesions. The likelihood of spontaneous mutations leading to an INH- *and* RMP-resistant phenotype of *M. tuberculosis* (MDR-TB) is very low, i.e. in 10^{-14} ($10^{-6} \times 10^{-8}$) AFB. Therefore, the worldwide emergence of resistant strains cannot be explained by the phenomenon of natural mutations only. More important is the man-made impact leading to the high rate of acquired resistance,[22] mainly because of the patients' nonadherence to therapy (see above).

Antimicrobial resistance in *M. tuberculosis* is classically defined as a significant difference in the activity of an antimycobacterial drug between a wild-type strain and another strain. A wild-type strain is defined as a strain isolated from a patient before treatment and less than 1% of a population of that strain is resistant to any antimycobacterial agent. Resistance emerges as a consequence of individual mutations in mycobacterial genes that lead to a structural or functional change such that an antimycobacterial agent is no longer active against that strain. In the recent past, several resistance mechanisms at the molecular level have been elucidated (for references, see[78]). For example, there is compelling evidence that resistance to INH results from a mutation or a combination of mutations in the *katG*, *ahpC*, *inhA*, *ndh* or the *kasA* genes of *M. tuberculosis* (Table 174.5). In *M. tuberculosis*, resistance to RMP is a result of a mutation within an 81 bp (27 amino acid) sequence of the core region of the *rpoB* gene (RNA polymerase β subunit); streptomycin resistance has been attributed to mutations in either the *rrs* gene (16S rRNA gene) or the *rpsL* gene (ribosomal protein S12). Quinolone resistance has been ascribed to mutations in the *gyrA* genes and pyrazinamide resistance to mutations in the *pncA* gene that encodes pyrazinamidase/nicotinamidase activity. The targets for the major classes or types of antimycobacterial agent are shown in Figure 174.15. Antibiotic resistance does not appear to transfer between strains of mycobacteria by either plasmid exchange or resistance transfer factors. The *M. tuberculosis* MDR as well as the XDR phenotypes appear to be entirely the result of a stepwise accumulation of individual mutations.

Intrinsic resistance to antimicrobial agents is also common in both slowly and rapidly growing NTM. In most instances, this form of resistance appears to be the result of the impermeability of the mycobacterial cell envelope. For example, most MAC isolates are resistant to RMP despite the fact that the isolate has a wild-type *rpoB* gene. However, *M. avium* resistance to INH may reflect the lack of an effective antimicrobial activity rather than or in addition to a lack of permeability.[82] Both *M. chelonae* and *M. abscessus* are intrinsically resistant to quinolones, while *M. fortuitum* is susceptible.

Antimicrobial agents

There are a variety of antimicrobial agents available for the treatment of mycobacterial infections; however, until recently almost all clinical studies of these agents were focused on *M. tuberculosis* and to a lesser extent *M. leprae*. The emergence of MAC as an important opportunistic infection associated with HIV infection and increased recognition that rapidly growing mycobacteria are a significant cause of human disease led to an expansion in our knowledge about the activity of certain agents against these and other mycobacteria. Although MAC is resistant to INH and only variably susceptible to RMP, MAC infections can be successfully treated with a macrolide and other agents such as ethambutol (EMB) plus rifabutin. Infections caused by rapidly growing mycobacteria must be treated with antimicrobial agents other than the primary antituberculous agents INH, RMP, EMB and PZA. For most other species there is often only limited information about the effectiveness and efficacy of antimycobacterial agents, perhaps with the exception of *M. kansasii* and *M. marinum*. The antimicrobial agents recommended for the treatment of various mycobacterial infections are shown in Table 174.6; however, it is not always appropriate to perform susceptibility tests on all of the agents because of a lack of standardized procedures and well-established interpretive criteria[83] (see also Chapter 143).

Susceptibility testing

The susceptibility testing of mycobacteria and the definition of antimicrobial resistance have been influenced by decades of focus on *M. tuberculosis*. Thus, there is more information about antimicrobial agents and the susceptibility testing of *M. tuberculosis* than for all other species of mycobacteria. Indeed, susceptibility testing of many NTM has been largely extrapolated from experience with *M. tuberculosis*, including adoption of the same interpretive criteria. In some instances this extrapolation has proven to provide useful and reliable information, but in other situations this practice can be misleading. Therefore, with the exception of testing of *M. tuberculosis* isolates against the first-line antimycobacterial agents, susceptibility testing of mycobacteria should be performed in laboratories with extensive experience. Although the laboratory can provide valuable but limited guidance in the interpretation of results, the application of those results to the treatment of a patient with an uncommon mycobacterial infection is

Table 174.5 Mycobacterial genes with mutations associated with antimicrobial resistance

Antimicrobial agent	Species	Gene(s) involved	Proportion of resistance (%)	Gene function	Mechanisms of action
Rifampin (rifampicin)	*M. tuberculosis* *M. africanum* *M. leprae* *M. avium*	*rpoB*	>96	β-subunit of RNA polymerase	Inhibition of transcription
Isoniazid	*M. tuberculosis* *M. tuberculosis* *M. tuberculosis* *M. tuberculosis* *M. leprae* *M. tuberculosis*	*katG* *inhA* locus *ndh* *ahpC* *acpM* (*kasA*)	20–80 15–43 10 10–15	Catalase-peroxidase Enoyl ACP reductase NADH dehydrogenase II Alkyl hydroperoxidase β-ketoacyl ACP synthase	Inhibition of mycolic acid biosynthesis and multiple effects on DNA, lipids, carbohydrates, NAD metabolism
Ethambutol	*M. tuberculosis*	*embCAB*	47–65	Arabinosyltransferase	Inhibition of arabinogalactan synthesis
Streptomycin	*M. tuberculosis* *M. smegmatis* *M. tuberculosis*	*rpsL* *rrs*	52–59 8–21	S12 Ribosomal protein 16S rRNA	Inhibition of protein synthesis
Pyrazinamide	*M. tuberculosis*	*pncA*	72–97	Nicotinamidase/ prazinamidase	Acidification of cytoplasm and de-energized membrane
Fluoroquinolones	*M. tuberculosis* *M. smegmatis*	*gyrA* *gyr B*	75–94 *In vitro*	DNA gyrase subunit A and subunit B	Inhibition of DNA gyrases
Azithromycin–clarithromycin	*M. avium* *M intracellulare* *M. chelonae* *M. abscessus*	V domain 23S rRNA	95	23S rRNA	
Amikacin–kanamycin	*M. tuberculosis*	*rrs*	>6	16S rDNA	Inhibition of protein synthesis
Ethionamide	*M. tuberculosis*	*etaA/ethA* *inhA*	37 56	Flavin mono-oxygenase	Inhibition of mycolic acid biosynthesis

Proportion of resistance represents the estimated percentage of resistance that can be accounted for by mutations in the respective genes. Mutations in *katG*, *ahpC*, *inhA* and/or *kasA* collectively probably account for 90% of isoniazid resistance.
Adapted from Zhang *et al.*[78] Percentage figures taken in part from Zhang & Telenti.[79]

likely to require the involvement of a physician with experience in the management of such infections.

Drug susceptibility testing is mandatory on initial isolates of *M. tuberculosis* and related species from all patients. If culture remains positive over an extended period of time, susceptibility testing should be repeated to monitor a possible development of drug resistance. The current guidelines of the Clinical Laboratory Standards Institute (CLSI) recommend repeating susceptibility testing at least every 3 months.[83]

There are three accepted methods for drug susceptibility testing of *M. tuberculosis*: the absolute concentration method, the resistance ratio method and the agar proportion method, the latter being used most widely on the Western hemisphere.

Agar proportion method

This method is based on the concept of 'critical concentrations' of antituberculosis agents and the percentage of resistant tubercle bacilli within a test population ('critical proportion'). Critical concentrations for antituberculosis agents were established on an empiric clinical basis. Therapeutic success was unlikely if the proportion of drug-resistant mutants within a population of *M. tuberculosis* isolated from a patient exceeded a threshold of 1% at a concentration of the antituberculosis agent that was known to be therapeutically effective against a 'wild-type' or fully susceptible strain. The critical concentration may not be the same as the peak serum concentration of a drug and there might be an interest in applying the use of the minimum inhibitory

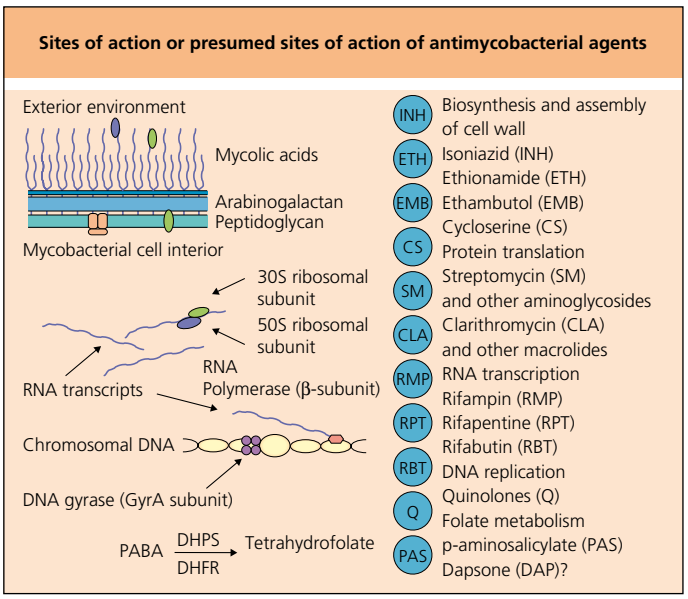


Fig. 174.15 Sites of action or presumed sites of action of antimycobacterial agents. DHFR, dihydrofolate reductase; DHPS, dihydropteroate synthase; PABA, *p*-aminobenzoic acid; PAS, *p*-aminosalicylic acid. Figure adapted from Parsons *et al.*[80] and Young.[81]

Table 174.6 Antimycobacterial agents ranked by clinical use

Species	Primary or first choice	Secondary or second choice	Notes
M. tuberculosis	INH, RMP, PZA, EMB	SM, amikacin, ciprofloxacin, linezolid	Treatment plan varies depending on incidence of INH resistance in community, if MDR or XDR, patient adherence, clinical manifestation, other factors
M. bovis	INH, RMP, EMB		Uniformly PZA resistant, usually 9–12 months' therapy recommended
M. leprae	Dapsone, RMP, clofazimine, clarithromycin	Ethionamide, prothionamide, minocycline	Treatment plan varies depending on paucibacillary or multibacillary disease
M. avium *M. intracellulare*	Azithromycin or clarithromycin, ethambutol, rifabutin Surgical excision (lymphadenitis/ children)	Amikacin, streptomycin, moxifloxacin	PZA resistant, INH resistance is usual; drugs and doses vary depending on immunocompetency, disseminated or pulmonary or other, and treatment vs prophylaxis
M. chelonae *M. fortuitum* *M. abscessus*	Amikacin, cefoxitin, ciprofloxacin, clarithromycin, doxycycline or minocycline, sulfonamides	Imipenem, levofloxacin, tobramycin (*M. chelonae* only), linezolid	All resistant to INH, PZA, RMP, SM, EMB and clofazimine. *M. chelonae* uniformly resistant to cefoxitin. EMB may be useful for treating *M. smegmatis*. Contamination with these species is common, confirm clinical significance
M. kansasii	RMP, INH, EMB	Pyridoxine, clarithromycin, rifabutin, sulfamethoxazole	PZA resistance is uniform. Only test RMP; testing of INH and EMB can be misleading. Clarithromycin resistance occurs. Treatment for 12 or more months
M. scrofulaceum	Surgical excision without chemotherapy (lymphadenitis)	Azithromycin, clarithromycin	INH and PZA resistant
M. ulcerans	Surgical excision, RMP, amikacin	EMB, trimethoprim–sulfamethoxazole, SM, ciprofloxacin, sparfloxacin	Chemotherapy rarely effective
M. marinum	Clarithromycin, minocycline, doxycycline, RMB, rifabutin, EMB, trimethoprim–sulfamethoxazole		INH and PZA resistant
M. haemophilum	Clarithromycin, rifabutin, ciprofloxacin Surgical debridement		Limited clinical experience
M. simiae	Treat like *M. avium* complex		INH, RMP, EMB and PZA resistant
M. xenopi	Azithromycin, clarithromycin, RMP	Rifabutin, SM	
M. celatum	Clarithromycin		RMP and PZA resistant
M. genavense	EMB, RMP, rifabutin, clarithromycin	Amikacin, clofazimine	PZA resistant

First-choice agents are expected to be active against wild-type isolates (i.e. from untreated patients); second-choice agents are less preferred, usually due to toxicity, expense or unclear efficacy.
EMB, ethambutol; INH, isoniazid; MDR, multidrug resistance; PZA, pyrazinamide; RMP, rifampin; SM, streptomycin; XDR, extensive drug resistance.
Adapted from American Thoracic Society[4] and Clinical Laboratory Standards Institute;[83] see also Chapter 143.

concentration (MIC) in the future. However, there are no standardized methods for MIC testing of mycobacteria and the testing of *M. tuberculosis* continues to follow the conventions of critical concentrations and the 1% growth inhibition threshold.

The proportion method can be applied as either a direct or an indirect test.[83] Inoculating several dilutions of a standardized suspension of mycobacteria onto Middlebrook 7H10 agar plates is the basis of the agar proportion method. The number of colony forming units (cfu) that grow on the drug-containing plates or quadrants are compared with the number of cfu on a drug-free plate or quandrant. If the number of cfu that grow on drug-containing medium exceeds 1% of the total number of cfu on the drug-free medium, then the isolate is considered 'resistant' to that drug at that concentration. The agar proportion method is standardized[83] against which all newer developments of culture-based drug susceptibility testing have to be validated (see below).

Modified proportion method by the BACTEC 460 TB System

The radiometric proportion method is simply an adaptation of the agar proportion method to the BACTEC 460 TB 12B medium and radiometric measurement of growth inhibition. As a consequence of this particular technique, drug susceptibitlty results become available

more rapidly, i.e. between 5 and 10 days. Critical concentrations have been established for both first- and second-line antituberculous drugs.[83,84] The isolate is inoculated into BACTEC 460 TB 12B medium with or without the addition of test drug. The concentration of mycobacteria inoculated into medium without drug is 100-fold less than the concentration inoculated into BACTEC 460 TB media with drug.

In some clinical situations the BACTEC 460 method might be best considered a screening test because the method does not allow an estimate of the percentage of resistant bacilli and is vulnerable to major errors (false susceptibility or resistance) due to mixed populations of mycobacterial species. Indeed, when an MDR isolate of *M. tuberculosis* is detected for the first time using the BACTEC 460 TB method, the identity of the isolate should be verified and the presence of a contaminant or mixed culture be ruled out before proceeding with testing secondary agents. Although the importance of promptly reporting an MDR isolate of *M. tuberculosis* cannot be overstated, the consequences of a false report of multidrug resistance must be recognized as well.

Drug testing by nonradiometric growth-based methods

Some of the new, nonradiometric methods such as the BACTEC MGIT 960 and the ESP Culture System II show excellent agreement with the agar proportion method and the BACTEC 460 TB System for drug susceptibility testing.[85–87] However, to date, only the BACTEC MGIT 960 System offers PZA testing.[88] Likewise, critical concentrations for second-line drugs for this system have been established in a large multicenter study.[89] For INH and RMP, the BACTEC MGIT 960 was also in 99–100% agreement with the resistance ratio method, and showed a concordance for EMB and PZA of 85% and 92%, respectively. Good correlation was also found for second-line drugs.[90]

Mycobacterium avium complex, *Mycobacterium kansasii* and other slowly growing NTM

In-vitro susceptibility testing of MAC and most of the other NTM, using the methods and interpretive criteria described for *M. tuberculosis*, has little value as a guide to antimicrobial treatment. One important exception is *M. kansasii*, for which *in-vitro* results based on the interpretive criteria used with *M. tuberculosis* correlate well with clinical efficacy. For many uncommon NTM (e.g. *M. simiae* and *M. szulgai*) there are few clinical cases to form a basis for interpretive criteria. As pointed out above, it is often difficult to distinguish between contamination, colonization, infection and disease with many of the NTM, especially the rapidly growing mycobacteria.

In general, the *in-vitro* susceptibility testing of MAC has limited value primarily because of the lack of a correlation with clinical response and, therefore, the lack of interpretive criteria.[83] The important exceptions are for azithromycin and clarithromycin because these macrolides have proven clinical and microbiologic efficacy in the prophylaxis and treatment of MAC disease, with interpretive criteria based, at least in part, on monotherapy trials in humans. Although wild-type MAC is uniformly susceptible to macrolides, macrolide resistance develops quickly with monotherapy. An analysis of these resistant isolates showed that over 95% of clinically significant macrolide resistance in MAC is a consequence of mutations in the V domain of the 23S rRNA gene.[82] Therefore, clinically significant macrolide resistance can be defined as an MIC for clarithromycin and azithromycin at pH 6.8 of ≥64 μg/ml and ≥512 μg/ml, respectively.[83]

Mycobacterium avium complex isolates from patients with breakthrough azithromycin or clarithromycin prophylaxis can be tested against one macrolide. Testing one drug is sufficient, since all evidence indicates that resistance crosses between these macrolides. If a patient has not received macrolide prophylaxis, it is unnecessary to perform a susceptibility test on initial MAC isolates from blood or tissue to guide treatment. However, establishing baseline MIC values for a MAC isolate may prove valuable in interpreting susceptibility test results for a subsequent isolate from the same patient weeks or months later. Susceptibility testing is also warranted if a patient relapses, if the infection is intractable or if the clinical situation is desperate. Testing may assist in deciding to add drugs; however, macrolide treatment should probably be continued even in the face of resistance.

The interpretation of *in-vitro* test results for EMB should not be attempted at this time. Ethambutol is commonly used as a 'second' agent in the treatment of MAC to prevent macrolide resistance, but EMB has little or no therapeutic activity alone against MAC.[91] The drug does, however, increase the activity of other agents, including macrolides, and this may influence the mutation frequency.[92] The CLSI now recommends that MAC be tested by establishing MICs via the BACTEC 460 TB System or via the microtiter method.[83]

Mycobacterium marinum is predictably susceptible to RMP and EMB; alternative agents are amikacin and kanamycin as well as tetracyclines, ciprofloxacin, clarithromycin and trimethoprim–sulfamethoxazole (co-trimoxazole). Susceptibility testing of *M. marinum* isolates using methods and interpretive criteria described for *M. tuberculosis* appears to be inappropriate.[83] Wild-type isolates of *M. haemophilum* are susceptible to quinolones, rifamycins, clarithromycin and azithromycin, and are likely to be resistant to INH, streptomycin, EMB and PZA.[93] *Mycobacterium simiae* is highly resistant to antimycobacterial agents; however, there are exceedingly few cases of disease on which to base any firm conclusions about susceptibility and clinical usefulness. Clarithromycin in combination with EMB and perhaps a quinolone such as ofloxacin appears to be effective.[4,94]

Rapid growers

Although four methods have been described for measuring the *in-vitro* susceptibility of rapidly growing mycobacteria, the CLSI now recommends only the broth microdilution method.[83] Broth microdilution provides a quantitative result and better supports the growth of *M. chelonae.* The broth microdilution method is essentially a modification of a standard method for nonmycobacteria that grow aerobically. Commercially prepared broth microdilution panels can be used if the appropriate drugs are available at the necessary concentrations. Alternatively, broth microdilution panels can be prepared in-house. The agents that should be tested are listed in Table 174.6. Testing rapidly growing mycobacteria should be restricted to laboratories with more extensive experience.

Practical value of molecular detection of resistance

Apart from the growth-based strategies and the numerous alternative ways to test drug susceptibility (e.g. colorimetry, flow cytometry, bioluminescence or quantitation of mycobacterial antigens) there are protocols available which detect the gene mutations involved in resistance of *M. tuberculosis* against antimicrobial agents. Although they are capable of providing the clinician with susceptibility results within 1–2 days, most of these protocols are not yet ready for the routine clinical mycobacteriology laboratory, mainly because, very often, more than a single mutation is responsible for drug resistance, making the whole issue very complex (e.g. mutations at least on four different genes are involved in INH resistance). However, since over 97% of all RMP-resistant strains carry a mutation in a specific short core region of the *rpoB* gene, molecular detection of point mutations in this case is a straightforward approach. Detection of these mutations is reliably achieved by gene sequencing or, more simply, by the use of the commercially available line probe assays.

In analyzing 360 smear-positive respiratory specimens, sensitivity and specificity of the INNO-LipA Rif. TB test (Innogenetics) were 100% and 96.6%, respectively.[95] Hillemann *et al.*[96] studied over 140 strains of *M. tuberculosis*, among them 103 MDR-TB strains, and reported 100% concordance between the GenoType MTBDR Assay

(Hain) and gene sequencing. Using the same assay, in 95.1% of RMP-resistant strains the mutations in the *rpoB* gene and in 73% of the INH-resistant strains mutations in the *katG* gene could be detected.[97] Brossier *et al.*[98] recommended the MTBDR test as a rapid method to detect RMP-resistant (concordance 100% with DNA sequencing) and high-level INH-resistant (concordance 89%) strains, but not for low-level INH-resistant strains of *M. tuberculosis* (concordance 17%). The MTBDR test has recently been modified (MTBDRplus Assay; Hain) to detect a broader variety of mutations in the *rpoB* and *inhA* genes.[99] A very promising development appears to be a line probe-based assay designed to detect mutations in the *pncA* gene by Sekiguchi *et al.*:[100] in testing some 250 strains of *M. tuberculosis*, sensitivity and specificity of the assay were both 100% compared to conventional PZA susceptibility testing.

IMMUNODIAGNOSTIC TESTS FOR TUBERCULOSIS

Historically, the first immunodiagnostic test was the tuberculin skin test (TST). Shortcomings of this test include the inability to distinguish active disease from patient sensitization, unknown predictive values and cross-reaction with NTM. None of the numerous serologic tests has found wide clinical use, mainly because of the lack of sensitivity and specificity.

In contrast, the recently developed and commercially available whole-blood IFN-γ assays are promising candidates to improve the current level of diagnostic accuracy for tuberculosis infection, particularly if skin testing remains equivocal. The two test systems – the QuantiFERON-TB Gold In-Tube (QFNG-IT; Cellestis, Victoria, Australia) and the T-SPOT.TB (Oxford Immunotec, Oxford, UK) – are not affected by BCG vaccination, do not cross-react with the majority of NTM and are less prone to variability and subjectivity associated with placing and reading of the TST (Table 174.7).

Although both tests measure T-cell INF-γ responses to similar *M. tuberculosis*-specific antigens over a 16- to 24-hour incubation period, they are based on different technologies. The T-SPOT.TB assay is based on ELISPOT methodology and requires the isolation and incubation of peripheral blood mononuclear cells (PBMC) and the standardization of 250 000 PBMC in each of its test wells. The T-SPOT.TB assay requires, overall, two working days, and may be more laborious than the QFNG-IT. Nevertheless, the use of a standardized number of washed PBMC might contribute to the greater sensitivity reported in the literature. In contrast, the QFNG-IT has technical advantages over the T-SPOT.TB assay, since the stimulation of T-cell IFN-γ response in whole blood is performed in tubes precoated with the *M. tuberculosis* antigens. Also, the enzyme-linked immunosorbent assay (ELISA) is simple and rapid to perform. Since background noise may occur, a 'Nil' control is required to adjust for this background, as well as for heterophile antibody effects and nonspecific IFN-γ in blood samples.

It is important to stress that neither of these new tests distinguishes between latent and active infection. To date, there are a large number of publications available which focus on the performance characteristics of each test; however, there are only few published head-to-head comparisons of the QFNG-IT and the T-SPOT.TB assays specifying their feasibility in different patient cohorts. In a recent systematic review of the literature Pai *et al.*[102] concluded that QFNG-IT has a specificity of 99% (T-SPOT.TB 96%) among non-BCG-vaccinated participants and a specificity of 96% (T-SPOT.TB 93%) among BCG-vaccinated participants, while the T-SPOT.TB appears to be more sensitive than QFNG-IT and TST. Diel *et al.*[103] compared both IFN-γ release assays in TST-positive persons recently exposed to pulmonary tuberculosis cases. In this study, factors independently influencing the risk of *M. tuberculosis* infection and their interactions with each other were evaluated by multivariate analysis. There were five variables which significantly predicted a positive IFN-γ release assay result, i.e. age, AFB-positivity of the source case, cough, cumulative exposure time and foreign origin of the patient. There was excellent agreement between the two assays (93.9%, kappa = 0.85), with QFNG-IT finding 30.2% of

Table 174.7 Characteristics of the commercially available tests for the diagnosis of tuberculosis infection

Variable	Tuberculin skin test	QuantiFERON-TB Gold/ QuantiFERON-TB Gold In-Tube	ELISPOT T-SPOT.TB
Administration	*In vivo* (intradermal)	*Ex vivo,* ELISA-based	*Ex vivo,* ELISPOT-based
Antigens	PPDS or RT-23	ESAT-6, CFP-10 and TB7.7	ESAT-6 and CFP-10
Standardized	Mostly	Yes	Yes
Reading prone to subjectivity	Yes	No	No
Units of measurement	Millimeters of induration	Units of IFN-γ	IFN-γ spot-forming cells
Definition of positive test results	5, 10 and 15 mm	Patient's IFN-γ ≥0.35 U/ml (after subtracting IFN-γ response in nil control)	≥6 spot-forming cells in the antigen wells, with 250 000 cells/well, and at least double-negative well
Indeterminate	If anergy (rarely tested)	Poor response to mitogen (<0.5 U/ml in positive control) or high background response (>8.0 U/ml in nil well)	Poor respone to mitogen (<20 spot-forming cells in positive control well) or high background (>10 spot-forming cells in negative well)
Time to result	48–72 h	Same day (but longer if run in batches)	16–24 h (but longer if run in batches)
Consultation(s)	2	1	1
Cost per test (US dollars)			
Materials		17.29	57.33
Labor/other		20.02	20.02
Total cost	11.58	37.31	77.35

Adapted from Menzies *et al.*[101]

contacts positive and T-SPOT.TB finding 28.7% of them. Overall, the IFN-γ release assays were more accurate indicators of the presence of latent tuberculosis than TST.

In HIV-positive asymptomatic individuals (n=286) both QFNG-IT and T-SPOT.TB assay were more sensitive than TST (20.0% and 25.2%, respectively, compared with 12.8% [TST]), but seemed, as a whole, to be less sensitive than in immunocompetent patients.[104]

The performance of IFN-γ release assays in children is less understood. Without the inconvenience and complications associated with TST they are acceptable substitutes for TST, however, the sensitivity and specificity of IFN-γ release assays compares well with the TST.[105] In latent tuberculosis the agreement between QFNG-IT and T-SPOT.TB assay was very good (92%) in children, with moderate agreement betweeen TST and QFNG-IT (77%) and TST and T-SPOT.TB (75%), respectively.[106] For culture-confirmed active tuberculosis, however, the same authors stated that the sensitivity of the TST was 83%, compared to 80% for the QFNG-IT and 58% for the T-SPOT.TB.

Basically, the problem of indeterminate results occurs with both IFN-γ release assays. In HIV-infected individuals, T-SPOT.TB provided more indeterminate results than the QFNG-IT (8 vs 1/256, p <0.01),[104] similar to that confirmed by others (14% versus 1.8%).[107] Indeterminate results appear to be dependent on the number of CD4 cells inasmuch as patients with a CD4 count ≤200 cells/ml were significantly more likely to have an indeterminate result.[104,107] In children less than 4 years of age indeterminate results were more often seen using the QFNG-IT than the T-SPOT.TB.[108] In applying the T-SPOT.TB assay after indeterminate results have been obtained from the QFNG-IT test 65% of the 40 patients yielded a valid result.[109]

Since the experience with IFN-γ release assays is still limited, longitudinal studies are needed to define their predictive values, especially in children and high-risk populations.[110] A large meta-analysis has pointed out very clearly areas of uncertainty and recommendations for research.[101] Other problems concern the phenomena of conversions, reversions and nonspecific variations in serial testing[111] and altered performance characteristics of the assays in conjunction with ethnicity (e.g. Malay and Indian race versus Chinese race).[112]

From the present state of knowledge it is obvious that application of IFN-γ release assays for tuberculosis infection should be tailored to different high-risk groups. In addition, caution should be exercised in their current use in immunosuppressed patients.

REFERENCES


References for this chapter can be found online at http://www.expertconsult.com

Chapter 175

Raul Raz

Mycoplasma and *Ureaplasma*

INTRODUCTION

Mycoplasma and *Ureaplasma* belong taxonomically to the order Mycoplasmatales in the class Mollicutes ('soft skin'). Apart from being human pathogens, some organisms of the order Mollicutes are economically important plant and animal pathogens. The initial divisions of mycoplasmas into different species were based on inhibition of growth of the organisms on agar media containing homologous antibodies. In later studies, sequencing of the genome of mycoplasmas has confirmed the relevance of the initial classification method.

Over 120 named *Mycoplasma* species exist; 13 *Mycoplasma* species, two *Acholeplasma* species and one *Ureaplasma* species have been isolated from humans. However, only four are well-established human pathogens:

- *Mycoplasma pneumoniae*;
- *Mycoplasma hominis*;
- *Mycoplasma genitalium*; and
- *Ureaplasma urealyticum*.

Apart from the species mentioned above, several other species of *Mycoplasma* occur in the human endogenous flora of the mouth and the genital tract (*M. buccale*, *M. faucium*, *M. orale* and *M. salivarum*).

NATURE

Mycoplasma and *Ureaplasma* are the smallest organisms so far identified as being capable of reproducing in broth and on agar media; on the latter they form barely visible colonies.[1] They also have the smallest known genome of any 'free living' organism. They differ from eubacteria by lacking peptidoglycan. The absence of peptidoglycan and β-lactam receptors explains the resistance to β-lactam antibiotics.

Mycoplasma pneumoniae Infection

EPIDEMIOLOGY

Mycoplasma pneumoniae is one of the most common causes of atypical pneumonia.[2,3] It is transmitted from person to person by infected respiratory droplets during close contact and particularly affects young adults. The incubation period after exposure is, on average, 2–3 weeks.[4] Infection is more frequent during the fall and winter, but can develop year round.[5] The cumulative attack rate in families approaches 90% and immunity is short.[6]

Outbreaks of *Mycoplasma* infection have been described in military recruits,[7] institutions for developmentally disabled individuals[8] and hospitals.[9]

PATHOGENESIS

Mycoplasma pneumoniae grows under both aerobic and anaerobic conditions. The organism most commonly exists in a filamentous form (Fig. 175.1) and has adherence proteins that attach to epithelial membranes with particular affinity for respiratory tract epithelium.[10–13]

Once attached, *M. pneumoniae* produces hydrogen peroxide, causing injury to epithelial cells and their associated cilia (Fig. 175.2). However, many of the pathogenic features of infection with *M. pneumoniae* are believed to be immune mediated rather than induced directly by the bacteria.[13,14]

The organism may persist in the respiratory tract for several months after initial infection and sometimes for years in hypogammaglobulinemic patients, possibly because the organism attaches strongly and invades epithelial cells.



Fig. 175.1 Electron micrograph of a *Mycoplasma* organism producing filamentous structures in a broth culture. This gives the organism the appearance of a fungal mycelium. ('Myco' in mycoplasma refers to this feature.)



Fig. 175.2 Swelling ('ballooning) of cilia in tissue cell cultures experimentally infected by *Mycoplasma hominis*. Courtesy of Per-Anders Mårdh.

CLINICAL MANIFESTATIONS (SEE TABLE 175.1)

Atypical pneumonia

Mycoplasma pneumoniae is one of the most common causes of atypical pneumonia (Fig. 175.3) and together with *Chlamydia pneumoniae* and *Legionella* spp. represents 7–20% of cases of community-acquired pneumonia (CAP) for which a pathogen can be identified.[15,16] However, the incidence may be higher in patients with milder disease. The incidence of CAP due to *M. pneumoniae* varies depending on the techniques used for diagnosis, the age of the patients and whether the series include outpatients, hospitalized patients, or both. Several prospective studies showed that the incidence of *M. pneumoniae* is between 5% and 22.8%.[17] Pneumonia can be present without any findings on chest auscultation.

Asthma[18]

Mycoplasma pneumoniae has been linked to asthma. An infection with *M. pneumoniae* may precede the onset of asthma or exacerbate asthma symptoms. Chronic infection with *M. pneumoniae* has been suspected to play a part in some patients with asthma. A role for immunoglobulin E-related hypersensitivity and induction of T-helper type 2 immune response leading to inflammatory response in *M. pneumoniae*-infected patients with asthma has also been proposed. As macrolides are both antimicrobials and anti-inflammatory drugs, their therapeutic role in reducing asthma symptoms needs further clinical studies.



Fig. 175.3 Posteroanterior chest radiograph shows poorly defined reticulonodular pattern and ground-glass opacities in the right lung and poorly defined small nodular opacities in the left lung. The patient was a 37-year-old man with *Mycoplasma pneumoniae*. Source: Image Consult, 2008.

Table 175.1 Clinical manifestations of infection due to *Mycoplasma* and *Ureaplasma*

	M. pneumoniae	*M. hominis*	*M. genitalium*	*Ureaplasma urealyticum*
Atypical pneumonia	+++	–	–	–
Asthma	+++	–	–	–
Upper respiratory tract infection	+++	+	–	+
Hemolysis	+++	–	–	–
Skin disease	+++	–	–	–
CNS involvement	+	+	–	+
Hepatitis	+	–	–	–
Arthralgia	+	++	–	–
Arthritis	+	++	–	–
Sexually transmitted disease	–	+++	+++	++
Urinary tract infection	–	+++	–	–
Postpartum	–	+++	–	–
Fever	+	+++	–	–
Pelvic inflammatory disease	–	+++	+++	+++
Chorioamnionitis	–	+++	–	–
Endocarditis	–	+	–	–
Wound infection	–	+	–	–
Preterm delivery	–	–	–	++
Chronic lung disease	–	–	–	++

Mycoplasma pneumoniae infection may worsen asthma symptoms and can produce wheezing in children who do not have asthma. On the other hand, the role of *M. pneumoniae* in patients with chronic obstructive pulmonary disease and its exacerbations has not been established.[19]

Upper respiratory tract diseases

Most patients with respiratory infections due to *M. pneumoniae* do not develop pneumonia, but can have intractable, nonproductive cough, wheezing, dyspnea and signs of upper respiratory tract infection such as pharyngitis (6–59%), rhinorrhea (2–40%) and bullous myringitis (2–35%).[14,20]

Extrapulmonary diseases[17,19]

Extrapulmonary complications of *M. pneumoniae* infections may include hemolytic anemia, mucocutaneous lesions, meningoencephalitis and other CNS manifestations, and arthritis.

An autoimmune response is thought to play a role in these manifestations; however, the bacterium can be isolated from most extrapulmonary sites.[21]

Hemolytic anemia

Antibodies (IgM) to the 'I' antigen on erythrocyte membranes appear during the course of infection and produce a cold agglutinin response in about 60% of patients. Note, however, that cold agglutinins can also occur in other respiratory infections and they are not a surrogate diagnostic marker of *Mycoplasma* infection. Although hemolysis may be severe, it is usually not clinically significant.[5]

Mucocutaneous lesions

Dermatologic manifestation may range from a mild erythema multiforme (Fig. 175.4) to Stevens–Johnson syndrome.[22–25] Antibiotics may intensify the dermatosensitive potential of *M. pneumoniae*.[25]

Meningoencephalitis and other central nervous system manifestations

Central nervous system (CNS) manifestations occur in approximately 0.1% of the patients with *M. pneumoniae* infection and in up to 7% requiring hospitalization.[21] CNS involvement occurs most frequently in children and presents with encephalitis.[26] Other manifestations include aseptic meningitis, peripheral neuropathy, transverse myelitis, cranial nerve palsies (Bell's palsy) and cerebellar ataxia.[27,28]

Although uncommon, CNS involvement is associated with 8% mortality and 23% of severe sequelae.[21]

Other

Nonspecific gastrointestinal symptoms such as hepatitis and rarely pancreatitis can occur.[29,30] Arthralgia is common, arthritis is rare.[31] Cardiac or renal involvement and uveitis are unusual.[14,32,33]

DIAGNOSIS

Although the isolation of *M. pneumoniae* on SP-4 medium is possible, culture requires 2–3 weeks (Fig. 175.5). As such, clinical laboratories do not attempt to culture *M. pneumoniae* and the specific diagnosis of *M. pneumoniae* infection relies upon nonculture techniques.

Cold agglutinins

Cold agglutinins develop in approximately 50–75% of all patients 1–2 weeks after infection.[34–36] However, other bacterial, rickettsial and viral infections can also produce cold agglutinins. Since it is neither sensitive nor specific, the test is not helpful.

Serology

Antibody titers rise 7–9 days after infection (Fig. 175.6). In the past, the most widely used approach for serodiagnosis was the complement fixation (CF) test, which measured predominantly 'early' IgM and IgG antibody. More recently, enzyme immunoassay (EIA) techniques have been used with a sensitivity of 97.8% and a specificity of 99.7%. EIAs are more sensitive for detecting acute infection and can be comparable in sensitivity to polymerase chain reaction (PCR).[37]

Polymerase chain reaction

Direct PCR detects genomic DNA and may be highly sensitive and specific in patients with respiratory tract infections. Real-time PCR may be superior.[38] PCR can also be used on respiratory secretions, cerebrospinal fluid (CSF) and other tissue samples.

TREATMENT

Empiric therapy of atypical pneumonia

Indolent onset, extrapulmonary involvement and normal white blood cell count are findings that favor the presence of atypical pneumonia. Macrolides (azithromycin, clarithromycin and erythromycin) are considered as first-line treatment.[35] Azithromycin 500 mg orally once



Fig. 175.4 The red spots on this person's back appear where blisters (bullae) caused by erythema multiforme have ruptured and the overlying skin removed (denuded). The resulting lesions are yellow-crusted ulcers (erosions). Erythema multiforme may be associated with herpes simplex infection, *Mycoplasma* pneumonia or other medical conditions such as streptococcal infection or tuberculosis, or may result from exposure to chemicals or medications.

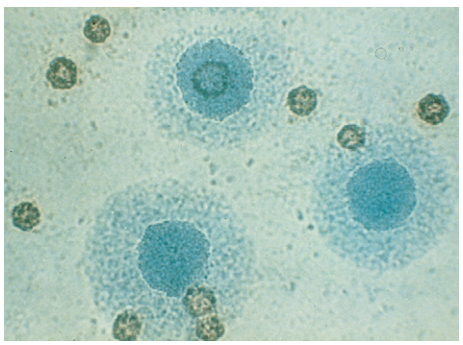


Fig. 175.5 Colonies of *Mycoplasma hominis* on PPLO agar with 'fried egg' appearance. Courtesy of Per-Anders Mårdh.

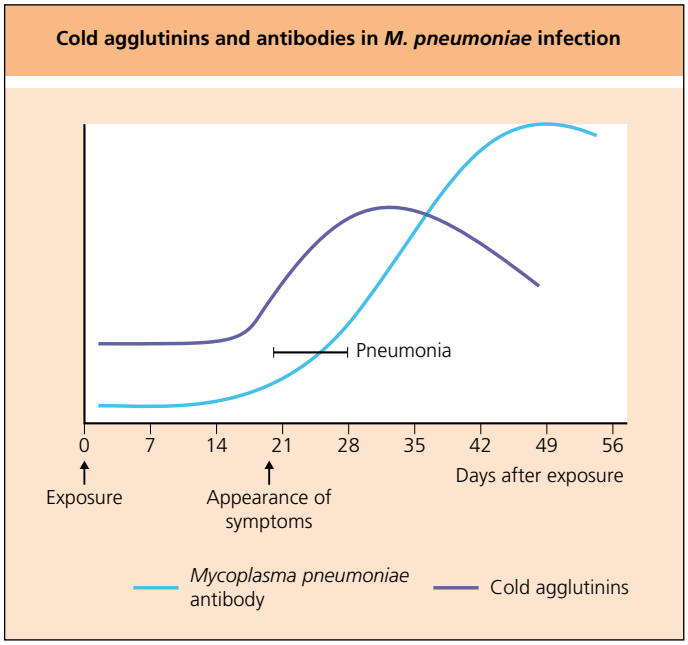


Fig. 175.6 Kinetics of appearance of cold agglutinins and specific antibodies to *Mycoplasma pneumoniae* related to time after being taken ill with *M. pneumoniae* pneumonia.

daily, followed by 250 mg orally for 4 days (or 500 mg for 3 days), is the most common regimen used. Erythromycin is less expensive but is commonly associated with gastrointestinal side-effects. Other antibiotics, such as tetracyclines (doxycycline 100 mg orally twice daily) and fluoroquinolones (levofloxacin and moxifloxacin), may be used. Although the duration of treatment for atypical pneumonia has not been firmly established, it is usual to treat for 10–14 days.[5] No studies have been performed which evaluate the treatment of patients with extrapulmonary findings of *M. pneumoniae* infection. For hemolytic anemia, some patients responded to steroids or plasmapheresis.[13]

Mycoplasma hominis

This organism has been isolated from the fallopian tubes of women with acute salpingitis.[39] The bacteria can be part of the normal genital flora for sexually experienced men and women.[40] The organism spreads through the cervical canal via the uterus and by the lymphatic vessels of the parametrium to the tubes. The percentage of women with vaginal colonization of *M. hominis* increases after puberty in direct proportion to sexual experience. In a study by McCormack[40] genital colonization with *M. hominis* was found in 1 of 91 women without a prior history of sexual activity, but in 15 of 97 sexually active women with multiple lifetime partners.[40] The rate of colonization is higher in sexually active women than in men,[41] probably because women are more susceptible to colonization.[41] Newborns are likely to become colonized during passage through the birth canal. This colonization is transient and the proportion of infants colonized decreases proportionately with age. Infants more than 3 months of age are rarely if ever colonized.[42] *Mycoplasma hominis* is common in women who lack a lactobacilli-dominated vaginal flora and have a mixture of bacterial species in the vagina. Many of these species are strictly anaerobic. Up to 75% of all women with bacterial vaginosis are carriers of *M. hominis*.[43]

IDENTIFICATION

Mycoplasma hominis is the smallest free-living bacterium. It can be detected in cultures within a few days. *Mycoplasma hominis* will escape detection using automated blood culture detection systems such as BacT/ALERT,[44] unless cultures are routinely incubated for 3–5 days using special media.[45]

To improve recovery of *M. hominis*, clinical specimens should be immediately inoculated onto culture media and not allowed to dry. After plating, cultures should be promptly incubated or kept at 39°F (4°C). The best laboratory culture medium is beef heart infusion broth (PPLO), supplemented with fresh yeast extract and horse serum. *Mycoplasma hominis* grows suboptimally *in vitro* under atmospheric conditions. Colonies develop best on agar when incubated at 95% nitrogen and 5% CO_2 atmosphere.[41]

SUSCEPTIBILITY

Susceptibility testing has not been standardized for *Mycoplasma* and there is no proven correlation between laboratory findings and clinical outcome.

Mycoplasma hominis is susceptible *in vitro* to tetracyclines and clindamycin, and is moderately susceptible to rifampin (rifampicin) and chloramphenicol. It is resistant to aminoglycosides, erythromycin and trimethoprim.[46] Fluoroquinolones are active *in vitro* but resistance may develop during treatment.[47] *Mycoplasma hominis* is susceptible to linezolid and quinupristin–dalfopristin but clinical data are not available.[48]

CLINICAL FEATURES

Mycoplasma hominis has been associated with genitourinary tract and nongenital infections. Genitourinary tract infections that have been linked to *M. hominis* include:

- pyelonephritis;
- pelvic inflammatory disease;
- chorioamnionitis; and
- postpartum and postabortal fever.

Nongenitourinary infections that have been linked to *M. hominis* include:

- septicemia;
- wound infections;
- central nervous infections;
- joint infections;
- lower respiratory tract infections; and
- endocarditis.

Urinary tract infections

Mycoplasma hominis can frequently be recovered from the lower genitourinary tract in men and women, but is not thought to cause cystitis, epididymitis or prostatitis. However, it can cause acute pyelonephritis, especially after instrumentation or if obstruction is present.[49,50]

Postpartum and postabortal fever

Ten percent of cases of postpartum and postabortal fever are related to the presence of *M. hominis*.[51] *Mycoplasma hominis* was also found in 8% of blood cultures of women with fever after abortion[51] but not in women without fever. More evidence of the pathogenicity of *M. hominis* is the fourfold rise in antibody titers in approximately 50% of women who had postabortal fever, as compared to only 2 of 53 controls who experienced abortion without fever.

Pelvic inflammatory disease

The role of *M. hominis* as a cause of pelvic inflammatory disease (PID) remains controversial.[49] *Mycoplasma hominis* has been isolated from the fallopian tubes of women with salpingitis and in whom an

antibody response could be established.[39,52] *Mycoplasma hominis* is rarely present in patients with salpingitis who do not also have evidence of concurrent chlamydial or gonococcal vaginitis or bacterial vaginosis.[53] Therefore, its role as a primary pathogen in salpingitis is still uncertain. *Mycoplasma hominis* may also increase the risk for tubal occlusion and infertility.

Chorioamnionitis

Mycoplasma hominis has been isolated from women with premature labor and chorioamnionitis and from 30% of children with birth weight below 2500 g who were born by Caesarian section with intact membranes.[54] However, the exact role of *M. hominis* in chorioamnionitis is still to be defined.

Nongenitourinary tract infections

Central nervous system disease

Infection in the CNS of neonates is probably acquired in utero or via passage through the birth canal.[55] The CNS infections related to *M. hominis* are brain abscess complicating a subdural hematoma,[56] meningitis[57] and meningoencephalitis.[55]

Arthritis

Mycoplasma hominis arthritis is characterized by the presence of fever, leukocytosis and purulent joint effusion with a negative Gram stain. A study reported 16 cases of septic arthritis due to *M. hominis*, where half of them had undergone manipulation of the urinary tract before the onset of infection.[58] *Mycoplasma hominis* arthritis was also described in patients with immune defects,[59] immunosuppression or following joint replacement or trauma.[58]

Wound infections

Mycoplasma hominis has been associated with infected pelvic hematoma[60] and infected Caesarian wounds, and is also occasionally found in other surgical wounds. Since the signs and symptoms of *M. hominis* wound infection are not specific, it can only be suspected when there are abnormal findings such as polymorphonuclear leukocytes with a negative Gram stain, negative routine cultures and a poor response to β-lactam or aminoglycoside therapy.[46,61,62]

Bacteremia

Positive blood cultures can be found after recent trauma[60] or a urologic surgical procedure. *Mycoplasma hominis* can also cause transient bacteremia after delivery, usually with no fever.[63]

Endocarditis

Mycoplasma hominis is a rare cause of native and prosthetic valve endocarditis.[64]

Respiratory tract

Mycoplasma hominis has been isolated from respiratory secretions in 1–3% of healthy persons, 8% of patients with chronic respiratory complaints and 14% of persons engaging in orogenital sex.[65] A few cases of empyema have been reported[66] but in general *M. hominis* is rarely capable of causing lower respiratory tract infections.

Prosthetic joint infection

A few cases of prosthetic joint infection have been described. Some were successfully managed with debridement followed by long courses of therapy with oral doxycycline or clindamycin.[58]

Mycoplasma genitalium

Mycoplasma genitalium (Fig. 175.7) is an emerging sexually transmitted infection.[67] Studies support a higher prevalence than gonococcus but lower prevalences than *C. trachomatis*. A role for this pathogen has also been suggested in chronic cases of nongonococcal urethritis.[68] Likewise, *M. genitalium* has been recovered from the urethra of HIV-infected men[69] and can be found in urethral cultures from men with and without discharge. In a recent study in women in the second and third trimester of pregnancy, *M. genitalium* was recovered from vaginal lavage samples in 1.6% of women who had reached the second trimester. This percentage increased in those who returned for repeat examination in the third trimester. *Mycoplasma genitalium* is a cause of cervicitis and endometritis.[70] Carriage of *M. genitalium* was associated with mucopurulent discharge, frequent vaginal douching, smoking and a history of miscarriage.[71] *Mycoplasma genitalium* was not associated with bacterial vaginosis (BV).

DIAGNOSIS

The organism is difficult to culture and most studies used PCR for identification. Antibodies were detected in 40% of women with PID with a microimmunofluorescence test.[72] Since such antibodies were detected by immunoblotting in approximately half of the women with proven tubal factor infertility, a definite diagnosis is difficult to make.

Mycoplasma genitalium has an antibiotic susceptibility pattern similar to *M. pneumoniae* and it appears to be sensitive to macrolides, especially azithromycin and tetracycline.

Ureaplasma urealyticum

Ureaplasma urealyticum (Fig. 175.8) derives its name from the fact that it possesses the enzyme urease. This organism was previously called T-strain *Mycoplasma* as it produced tiny (T) colonies in comparison to those of *Mycoplasma*. *Ureaplasma urealyticum* remains the single species in the genus *Ureaplasma*, although there have been some proposals for a differentiation based, for example, on biochemical and serology characteristics. Fourteen different serotypes of *U. urealyticum* were described.[73] One biovar of human *Ureaplasma* (biovar no. 1) has been called *Ureaplasma parvum*.[74]

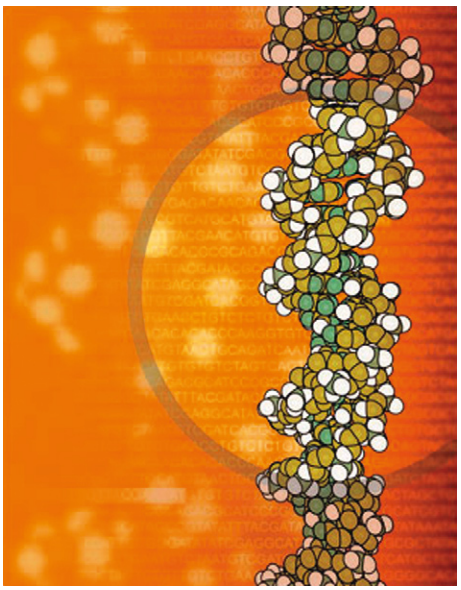


Fig. 175.7 Genome of *Mycoplasma genitalium*. With permission from *Nature*.

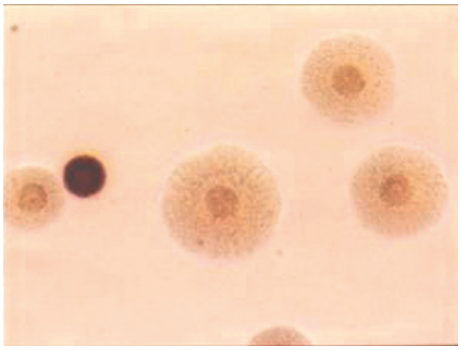


Fig. 175.8 *Ureaplasma urealyticum*.

CLINICAL MANIFESTATIONS

The presence of *U. urealyticum* in the genital tract is associated with sexual activity.[75] A large number of studies questioned the role of *U. urealyticum* in the pathogenesis of nongonococcal nonchlamydial urethritis.[73] Those studies were not consistent and are therefore difficult to interpret. Large randomized studies are needed in order to establish if *U. urealyticum* is a urethral colonizer or has a role in the sexual transmission of disease.[76,77] *Ureaplasma urealyticum* is found in the lower genital tract in up to 75% of women of reproductive age and can ascend to the fallopian tubes.[39] Treatment of infertile couples with tetracycline has provided inconclusive support to the role of *U. urealyticum* as a cause for infertility. A higher rate of recovery of *U. urealyticum* from babies with low weight and a statistical correlation of carriage of *U. urealyticum* with preterm birth has also been established. In addition, the colonization of *U. urealyticum* in the respiratory tract of premature neonates (<34 weeks) was associated with the development of chronic lung disease.[30,78] Despite high rates of colonization of the respiratory tract of newborns with *U. urealyticum* and the need for assisted ventilation, including several cases of respiratory insufficiency and death, until now there have been no studies proving the etiologic role of *U. urealyticum* in chronic lung disease in preterm births.[73] However, *U. urealyticum* can cause meningoencephalitis in newborns.

Ureaplasma urealyticum splits urea and therefore is capable of producing stones in the urinary tract.

DIAGNOSIS

Ureaplasma species can be detected in culture within 48 hours. Growth in a broth medium containing urea and an indicator causing a color change has been used to diagnose ureaplasmas.[1] Tests of the urea-splitting capability of *U. urealyticum* can also be carried out on agar media where the colonies turn dark brown after adding the test substrate. However, the use of molecular-based techniques, such as 16S rRNA gene-based PCR tests, detects *U. urealyticum* with much higher sensitivity rates than culture.[79–81] *Ureaplasma urealyticum* is known to have a similar susceptibility pattern to *Mycoplasma* spp.

THERAPY

Ureaplasma species are typically susceptible to antibiotics such as tetracyclines, macrolides and the newer fluoroquinolones.

REFERENCES

References for this chapter can be found online at http://www.expertconsult.com

Chapter 176

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Rickettsia and rickettsia-like organisms

INTRODUCTION

Rickettsia and rickettsia-like organisms are fastidious, obligate intracellular, coccobacilli that are transmitted to vertebrate hosts by arthropods. Traditional methods to classify bacteria are not useful for strict intracellular organisms as they have few phenotypes. The advent of molecular methods has enabled very accurate determinations of phylogenetic relationships between intracellular bacteria, and the order Rickettsiales has been reclassified to contain the genera *Anaplasma*, *Ehrlichia*, *Neorickettsia*, *Orientia*, *Rickettsia* and *Wolbachia*.[1] Phylogenetic studies have shown that several bacteria previously classified in the order Rickettsiales did not belong to the α subgroup of the Proteobacteria phylum;[1] the genus *Bartonella* was moved to the α_2 subgroup and the genus *Coxiella* to the γ subgroup (see Fig. 176.1).

Molecular studies have also greatly facilitated the detection and description of new organisms and the *Rickettsia* genus now contains 24 recognized species classified into three groups based on their genomic, antigenic, morphologic and ecologic patterns: the spotted fever group (SFG), the typhus group and the *R. bellii* group.[1] Similarly, the number of *Bartonella* spp. and the clinical syndromes caused by these organisms have increased rapidly. While infections with *Anaplasma* and *Ehrlichia* spp. were previously considered only of veterinary importance, studies over the past two decades have revealed several new species or strains that are pathogenic in both people and animals. In this chapter we describe the infections caused by rickettsiae and rickettsia-like organisms.

Spotted Fever Group Rickettsiae

NATURE

The SFG rickettsiae are obligate, intracellular, Gram-negative, coccobacilli (1.0–2.0 × 0.3–0.7 µm) that can infect vertebrate (endothelium, vascular smooth muscle and macrophages) and invertebrate cells (hemocytes and salivary gland epithelium). The SFG rickettsiae have a high content of lipopolysaccharides which are highly immunogenic and are responsible for the considerable serologic cross-reactivity between the different species within the group. There are, however, also species-specific antigens and these include the rickettsial outer membrane proteins A and B, *rOmpA* and *rOmpB*, respectively.[2] Until recently it appeared that there was a single pathogenic *Rickettsia* on each continent. However, between 1984 and 2004, nine more species or subspecies of tick-borne SFG rickettsiae were identified as emerging pathogens.

Rickettsia felis is also a newly described pathogenic SFG rickettsia and has pili which are probably involved in cell attachment and conjugation.[3]

The structure and the genome of *R. akari* is similar to that of other *Rickettsia* species.[4]

EPIDEMIOLOGY

Most SFG rickettsiae are found in only one or a limited number of species of ticks and hence the distribution of the various tick species determines the risk areas for SFG rickettsioses.[5] People are not considered good reservoirs for SFG rickettsiae as they are seldom infested with ticks for long periods and rickettsemia is normally of only short duration, especially with antibiotic intervention.

The reservoir and vector of *R. felis* is the cat flea (*Ctenocephalides felis*) which transmits the organism transovarially. It appears that *R. felis* is transmitted to people by flea bites.[6]

Rickettsia akari is found in the mouse mite (*Liponyssoides sanguineus*) which is the vector and reservoir of infection. Transmission might also occur with the use of contaminated needles in intravenous drug users.[6]

PATHOGENICITY

Ticks inject virulent SFG rickettsiae in their saliva while feeding and these adhere to endothelial cells, the most important target cells. Adherence is receptor mediated and results in changes to the actin cytoskeletal structure and phagocytosis of organisms into the cell.[7] The resultant phagosome lyses very rapidly and organisms are released into the cytoplasm. Some organisms escape from infected endothelial cells at the tick attachment site and spread via lymphatics to regional lymph nodes causing lymphadenomegaly. They also enter the bloodstream and infect endothelial cells throughout the body.

PREVENTION

There are no vaccines against the SFG rickettsiae and prevention depends on reducing exposure to vectors by avoiding infested areas and controlling ticks and fleas on domestic animals. People living in endemic areas and travelers to these areas should use effective tick repellants on their clothing such as DEET (*N*,*N*-diethyl-m-toluamide) or permethrins. Wearing light-colored clothes facilitates early tick detection and removal before they can attach. Prevention of rickettsialpox depends on rodent and mite control.

DIAGNOSTIC MICROBIOLOGY

The typical clinical picture of the SFG rickettsioses is high fever (103–104°F/39.5–40°C), headache and characteristic rash. Common nonspecific laboratory abnormalities include mild leukopenia, anemia and thrombocytopenia.[8]

Direct diagnosis

Rickettsia spp. can be isolated from blood or tissue samples in cell cultures (Vero, L929, HEL, XTC-2 or MRC5 cells) in biosafety level 3 laboratories.[8] Direct immunofluorescence techniques on tissues have moderate sensitivity (53–75%) but are nearly 100% specific.[9] Greater sensitivity may be achieved with polymerase chain reaction (PCR) assays amplifying sequences of several genes including 16S rDNA, *rOmpA*, *rOmpB*, *gltA* (citrate synthase) and gene D.[8]

Indirect diagnosis

Currently, the indirect microimmunofluorescence assay (IFA) is the most commonly used technique for the diagnosis of SFG rickettsioses.[10] Infection with the various SFG rickettsiae cannot always be differentiated by comparing IFA titers against the different members of the group. If differences in IFA assay titers between SFG rickettsiae are less than two serum dilutions, Western blot assays targeting two high-molecular-weight proteins (*rOmpA* and *rOmpB*)[11] and cross-absorption studies are needed to determine the SFG rickettsiae causing infection.[8]

CLINICAL MANIFESTATIONS

Currently, there are 14 SFG rickettsiae that are pathogenic in people (Table 176.1).

Rickettsia africae is the etiologic agent of African tick bite fever (ATBF) which is probably the most prevalent SFG rickettsiosis in the world. It is transmitted by *Amblyomma hebraeum* and probably also by *A. variegatum* (Fig. 176.1), with the majority of these ticks being infected and hence vectors of *R. africae* in sub-Saharan Africa. Fever is common (88%) and accompanying clinical signs are generally mild and include regional lymphadenomegaly (57%). A rash is seen in 49% of the patient and may be vesicular. To date no deaths or severe manifestations have been reported in patients with ATBF.[8]



Fig. 176.1 Female (left) and male (right) adult *Amblyomma variegatum*, vector and reservoir of *Rickettsia africae*.

Rickettsia rickettsii is the agent of Rocky Mountain spotted fever (RMSF) which is the most severe of the SFG rickettsioses. In the USA the major vectors are *Dermacentor andersonii* (wood tick) and *D. variabilis* (American dog tick) while in Central and South America the major vector is *Amblyomma cajennense* (Cayenne tick). The incubation period of RMSF is 2–14 days and initial signs include high fever (>102°F/39°C) and headache. Although a macular rash, which appears first on the wrists and ankles before generalizing, is a characteristic

Table 176.1 Prevalence of signs and prognoses for the more common spotted fever group rickettsioses

Spotted fever group	Fever	Rash	Eschar	Regional lymphadenopathy	Fatality rate without treatment
African tick bite fever	88%	49%	53–98%	49–57%	None
Rocky Mountain spotted fever	Yes	90%	Rare	None	20–25%
Mediterranean spotted fever	100%	87%	53%	Rare	1–2.5%
Israeli spotted fever	Yes	100%	90%	None	0–3.5%
Astrakhan fever	Yes	94%	23%	Rare	None
Japanese spotted fever	Yes	100%	91%	None	None
Queensland tick typhus	Yes	100%	65%	70%	Two fatal cases
Flinders Island spotted fever	Yes	100%	25–55%	55%	None
Siberian tick typhus	Yes	Common	62–77%	Uncommon	None
Lymphangitis-associated rickettsiosis	Yes	Common	75%	100%	None
TIBOLA-DEBONEL	45%	Uncommon	100%	100%	None
Far Eastern spotted fever	Yes	Common but often faint	Yes	20%	None
Flea-borne spotted fever	Common	Common	None	Yes	None
Rickettsialpox	Common	Common	Common	None	None

feature of RMSF it occurs in fewer than 50% of patients in the first 3 days of illness. In untreated patients, signs progress with the development of the vasculitis.

Rickettsia conorii has recently been shown to comprise three subspecies.[8]

- *Rickettsia conorii* subsp. *conorii* is the agent of Mediterranean spotted fever (MSF). After an incubation period of about 6 days, there is an abrupt onset of high fever (>102°F/39°C) and flu-like symptoms.[12] Typically, an eschar or tache noire (black spot) develops at the site of tick attachment. After about 4 days, a generalized maculopapular rash develops that also involves the palms and soles but spares the face (Fig. 176.2). Most patients will recover over 10 days without treatment but the rash may still be visible for 10–20 days after remission of clinical symptoms. Severe forms of MSF are seen in 5–6% of patients and the mortality rate is around 2%.
- *Rickettsia conorii* subsp. *israelensis* is transmitted by *R. sanguineus* and causes Israeli spotted fever.[8] This disease has similar clinical features to MSF. Although Israeli spotted fever is typically milder and of shorter duration than classic MSF, several fatal cases and severe forms have been described.
- *Rickettsia conorii* subsp. *caspia* causes Astrakhan fever in the Caspian Sea area in Russia and Chad.[8] The disease is similar to MSF but eschars are seen in relatively few patients (23%).

Rickettsia japonica causes Japanese or Oriental spotted fever which occurs particularly along the coast of south-western and central Japan. The rash is usually found on the palms of the hands and the soles of the feet and may become petechial after a few days before it disappears over about 2 weeks. Up to 20% of patients may have severe disease, with encephalitis, disseminated intravascular coagulopathy, multiorgan failure and acute respiratory distress syndrome having been reported.[8]

Rickettsia australis is the agent of Queensland tick typhus and is transmitted principally by *Ixodes holocyclus* in Australia.[8] An eschar at the site of tick attachment and lymphadenopathy are found in 65% and 70% of cases, respectively.

Rickettsia honei causes Flinders Island spotted fever. The organism might be the most widely distributed of the SFG rickettsiae as it has recently been identified in Thailand and Texas.[8] Patients with Flinders Island spotted fever have a sudden onset of fever, headache, myalgia, joint swelling and pain, and a slight cough in some patients.

Rickettsia sibirica subsp. *sibirica* is the agent of Siberian or North Asian tick typhus which occurs in Asiatic Russia and is transmitted by *Dermacentor* spp.[8] The disease is usually mild and there are seldom severe complications.



Fig. 176.3 Lymphangitis expanding from the inoculation eschar on the left leg to an enlarged, painful lymph node in the left groin of a patient with *Rickettsia mongolotimonae* infection. From Fournier, *et al*.[14]

Rickettsia sibirica subsp *mongolotimonae* infections have now been described in 11 patients.[13] Infections cause a 'lymphangitis-associated rickettsiosis' with signs including fever, maculopapular rash, eschar, enlarged regional lymph nodes and rope-like lymphangitis (Fig. 176.3).

Rickettsia massiliae was recently isolated from a patient with a maculopapular rash, eschar and slight hepatomegaly. The organism is transmitted by *Rhipicephalus* spp. in Europe and Central Africa.[15]

Rickettsia slovaca is transmitted by *Dermacentor marginatus* and *D. reticulatus*. Long considered nonpathogenic, *R. slovaca* has recently been found to be the agent of 'TIBOLA' (tick-borne lymphadenopathy) and 'DEBONEL' (*Dermacentor*-borne necrosis-erythema-lymphadenopathy) in Spain.[8]

Rickettsia heilongjiangensis is the agent of Far Eastern spotted fever and is found in *D. silvarum* in China and *Haemaphysalis* spp. from Siberia and the Russian Far East.[16] Infections result in fever, headache, rash, eschar, regional lymphadenopathy and conjunctivitis (Fig. 176.4).



Fig. 176.2 Maculopapular rash in a patient with Mediterranean spotted fever from Algeria. Courtesy Dr Nadjet Mouffok.



Fig. 176.4 Eschar and faint macular rash in a patient with *Rickettsia heilongjiangensis* infection. From Mediannikov, *et al*.[17]

Rickettsia aeschlimannii occurs in *Hyalomma* spp. in Africa and Europe. Two patients have been described with *R. aeschlimannii* infections but there is evidence that these may be more widespread in southern Europe.[8]

Rickettsia parkeri occurs in *Amblyomma maculatum* in the southern United States. Although the organism has been known for over 60 years, it has only recently been recognized as a pathogen in two patients.[18]

Rickettsia felis, previously known as the ELB agent, is the recently described agent of 'flea-borne spotted fever'.[19] Clinical signs are nonspecific, most commonly including fever, headache and rash. Other signs that have been reported are marked fatigue, myalgia, photophobia, conjunctivitis, abdominal pain, vomiting and diarrhea, and solitary black crusted skin lesions surrounded by a livid halo.

Rickettsia akari causes rickettsialpox which is transmitted by the mouse mite and has been reported in the USA, Ukraine, Slovenia and Korea. The incubation period is about 10 days and clinical signs include fever, headache and myalgia. There is usually an inoculation eschar and regional lymphadenopathy. About 4 days after the first clinical signs, a rash appears which has macular then papular and then vesicular lesions. The disease is usually mild and self-limiting.

MANAGEMENT

In practice, doxycycline (200 mg/day) is the drug of choice and may be used for 3–14 days, depending on the severity of the infection. It should be given for at least 3 days after clinical defervescence and resolution of signs.[8] When doxycycline cannot be used, chloramphenicol (50–75 mg/kg) can be effective but there are treatment failures and side-effects. Josamycin has been used successfully in pregnant women and children,[10] but children treated with azithromycin or clarithromycin required shorter courses of therapy and defervescence occurred in under 7 days.

Typhus Group Rickettsiae

NATURE

Both *R. prowazekii* and *R. typhi* are Gram-negative, obligate intracellular bacilli and, as with other members of the genus *Rickettsia*, their outer walls have an inner leaflet that is thicker than the outer. *Rickettsia prowazekii* is a category B bioterrorism agent and has a genome of 1.1 Mb with a single circular chromosome and a high proportion of noncoding DNA (24%).[20] The genome of *R. typhi* is nearly identical to that of *R. conorii* and other SFG rickettsiae.

EPIDEMIOLOGY

The human body louse, which lives in clothing, is the only established vector of *R. prowazekii* (Fig. 176.5). People are usually infected when feces from infected lice contaminate skin that has been scarified due to pruritus caused by the lice. Infections may also occur when conjunctivae or mucous membranes are exposed to the crushed bodies or feces of infected lice. Further, transmission might occur with inhalation of infected feces and through aerosols of feces-infected dust.

Murine typhus is one of the most prevalent rickettsial diseases throughout the world. The principal vectors are the rat flea (*Xenopsylla cheopsis*) and the rat louse (*Polyplax spinulosa*). Other possible vectors are the cat flea (*Ctenocephalides felis*) and the mouse flea (*Leptopsyllia segnis*).[21]



Fig. 176.5 The human body louse (*Pediculus humanus humanus*), the vector of *Rickettsia prowazekii*, feeding on a rabbit.

PATHOGENICITY

After local infection by *R. prowazekii* at the site of the louse bite, the organism infects the endothelial cells of capillaries and small blood vessels, producing a vasculitis. Tissue biopsies reveal perivascular infiltration with lymphocytes, plasma cells, polymorphonuclear leukocytes and histiocytes, with or without necrosis of the vessel.[22] Unlike SFG rickettsiae, *R. prowazekii* multiplies to large numbers within cells but does not cause significant injury to cells before they lyse. Infection of endothelial cells and the resultant vascular inflammation and hemostatic alterations are the principal pathogenic features of *R. typhi*.[22]

PREVENTION

Preventing epidemic typhus depends on eliminating human body lice with insecticides such as permethrin powder (1%) at doses of 30–50 g for an adult and 15–25 g for a child. Doxycycline can be used for chemoprophylaxis in visitors to high-risk areas. Vaccines using crude antigen and/or inactivated rickettsiae are partially protective against epidemic typhus but have undesirable toxic reactions and are difficult to standardize.[6]

Control of murine typhus depends on elimination of flea vectors and their mammalian reservoirs.

DIAGNOSTIC MICROBIOLOGY

Epidemic typhus should be considered in patients with body lice and in poor people living in unhygienic conditions. Murine typhus should be considered in patients who are exposed to rodents and their fleas and who have prolonged fever and rash with or without lymphadenopathy.

Direct diagnosis

Rickettsia typhi can be isolated in shell vials containing Vero or L929 cells,[9] while *R. prowazekii* can be isolated in shell vials with human embryonic lung (HEL) fibroblasts in a biosafety level 3 containment laboratory. Direct immunodetection of organisms in tissues enables diagnosis before seroconversion and facilitates early and appropriate treatment. PCR and sequencing methods are useful, sensitive and rapid tools to detect and identify typhus group rickettsiae in blood and skin biopsies. Recently a 'suicide' PCR has been reported.[23]

Indirect diagnosis

The indirect immunofluorescence antibody assay is the reference method for diagnosing typhus group rickettsioses,[9] although an

immunoperoxidase assay has been developed as an alternative for *R. typhi*.[9] Cross-adsorption followed by IFA and Western blotting can differentiate antibodies to the organisms, but the expense of the technique limits its use.

CLINICAL MANIFESTATIONS

Epidemic typhus occurs in two clinical forms: the primary febrile illness and the recrudescence of infection (Brill–Zinsser disease). After an incubation period of 10–14 days, patients may develop malaise and vague symptoms before the abrupt onset of fever (100%), headache (100%) and myalgia (70–100%). A rash is seen in 20–60% of patients[6] and typically begins in the axillary folds and upper trunk on about the fifth day of illness. In uncomplicated epidemic typhus, fever usually resolves after 2 weeks of illness if untreated, but recovery of strength usually takes 2–3 months. Without treatment, the disease is fatal in 13–30% of patients.[10] People who survive epidemic typhus remain infected with *R. prowazekii* for life. When stressed, they may experience a recrudescence, known as Brill–Zinsser disease, and they may become the source of a new epidemic if they become infested with body lice.[10]

Murine typhus is usually mild and many cases may be overlooked. When present, signs in untreated patients last for 7–14 days, when there is usually a rapid return to health. The fatality rate may reach 4%.[10]

MANAGEMENT

Doxycycline (200 mg/day) is the recommended treatment and a single oral dose of doxycycline (200 mg) usually leads to defervescence within 48–72 hours. Treatment, however, is recommended for 7–15 days, or for at least 2 days after fever resolves. Chloramphenicol (2 g/day) can be given to patients who cannot be given tetracyclines and is the drug of choice in pregnancy, except near parturition when gray syndrome may develop.[21]

Scrub Typhus

NATURE

Orientia tsutsugamushi, the causative agent of scrub typhus, is an obligate, intracellular, Gram-negative bacterium (0.5 × 1.2–3.0 µm) that has a different cell wall structure and genetic makeup to other rickettsiae.[24] Although the species is genetically stable, there is enormous genetic and antigenic variability between strains of *O. tsutsugamushi*. There are three antigenically distinct prototype strains: Karp, Kato and Gilliam originally isolated from New Guinea, Japan and Burma, respectively.[25]

EPIDEMIOLOGY

Orientia tsutsugamushi causes scrub typhus in the 'tsutsugamushi triangle' because of its vector which is bounded to the north by Siberia and the Kamchatka Peninsula, to the south by Australia, to the east by Japan and to the west by Afghanistan and India[25] (Fig. 176.6). The disease is transmitted by the bites of larval trombiculid mites (chiggers) of the genus *Leptotrombidium*. *Leptotrombidium deliense* is the most important vector species in South East Asia and southern China, whereas *L. akamushi*, *L. scutellare* and *L. pallidum* are the main vectors in Korea and Japan, and *L. chiangraiensis* is the likely vector in Thailand.[24]

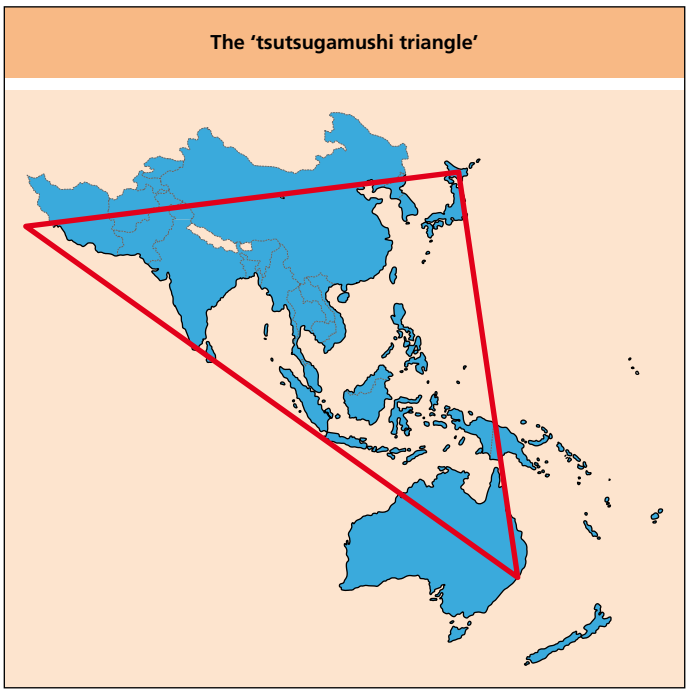


Fig. 176.6 The 'tsutsugamushi triangle'.

PATHOGENICITY

Target cells of the bacteria are mainly endothelial cells but macrophages and polymorphonuclear leukocytes are also infected. The bacteria can induce apoptosis in a variety of host cells by retarding the release of intracellular calcium.[26] In murine macrophages, *O. tsutsugamushi* produces inflammatory cytokines such as tumor necrosis factor (TNF) and interleukin (IL)-6. Tumor necrosis factor production appears to be inhibited by the production of IL-10.[24]

PREVENTION

Weekly doses of 200 mg doxycycline can prevent scrub typhus, but the efficacy of the daily 100 mg doxycycline dose regimen commonly used for malaria chemoprophylaxis has not been evaluated. Topical DEET applied to exposed skin and to the tops of boots and socks will prevent chiggers from feeding.

DIAGNOSTIC MICROBIOLOGY

The eschar is the single most useful diagnostic clue. Concurrent leptospirosis and scrub typhus should be considered in patients who are at risk and who have atypical clinical features of either disease.

Direct diagnosis

Orientia tsutsugamushi can be isolated from blood or tissues injected into white mice, chicken embryos and various cell lines such as HeLa, Vero, BHK, McCoy and L929.[27] Detection of organisms by PCR of peripheral blood and skin biopsy is a sensitive test but should be performed before antibiotic treatment or seroconversion.[24] Recently, a real-time PCR assay was described that can be used to diagnose scrub typhus by detecting major outer membrane antigen genes.[28]

Indirect diagnosis

The indirect microimmunofluorescence antibody assay has become the gold standard method for the diagnosis of scrub typhus. An immunoperoxidase assay has been developed as an alternative to the indirect microimmunofluorescence antibody assay; it has the advantages of being read with a conventional microscope and providing a permanent record of results.[9] Western blot assays differentiate true-positive from false-positive reactions with cross-reacting antibodies.

CLINICAL MANIFESTATIONS

After an incubation period of 5–20 days, infections can be mild and unapparent or fulminant and rapidly fatal (up to 30%) depending on the susceptibility of the host and/or the virulence of the strain.[6] An eschar is common but may not be seen as the chigger bite is painless and often occurs in areas not routinely examined such as the anogenital region, axilla and between skin folds. Generalized lymphadenopathy and the maculopapular rash may appear after 2–3 days. Gastrointestinal signs are common and in 30% of patients the spleen and liver are moderately enlarged.[6] Co-infection with the HIV-1 virus does not increase the severity of scrub typhus, rather HIV-1 viral replication is suppressed in both *in-vivo* and *in-vitro O. tsutsugamushi* infections.[6]

MANAGEMENT

Chloramphenicol (2 g/day) was the first recommended treatment of scrub typhus as it is a cheaper alternative to the tetracyclines. Currently, however, a 1-week course of oral doxycycline (200 mg/daily) is the treatment of choice for uncomplicated cases. Chloramphenicol- and doxycycline-resistant strains of *O. tsutsugamushi* have been reported in Thailand and azithromycin has been shown to be an alternative antimicrobial treatment in areas where doxycycline-resistant scrub typhus is prevalent.[29] Recently, a patient with leptospirosis and scrub typhus was successfully treated by early plasma exchange and a 7-day course of moxifloxacin therapy.[30]

Ehrlichioses

NATURE

Ehrlichia are obligate, intracellular, Gram-negative bacteria that survive in phagosomal vacuoles within the host cell. With Romanowsky stains they appear as mulberry-like structures, known as morulae, in monocytes or neutrophils. Two forms can be differentiated by electron microscopy, dense-cored cells and reticulate cells, both of which are capable of binary fission.[31]

EPIDEMIOLOGY

Ehrlichia chaffeensis is the agent of human monocytic ehrlichiosis while *E. ewingii* causes human granulocytic ehrlichiosis. The principal vector of both organisms is *Amblyomma americanum*, the Lone Star tick. Organisms are transmitted transtadially (larva to nymph to adult) but not transovarially (adult to larva). To date white-tailed deer (*Odocoileus virginianus*) appear to be the major reservoir of both *E. chaffeensis* and *E. ewingii*.[8]

PATHOGENICITY

Ehrlichia chaffeensis preferentially infects mononuclear leukocytes while *E. ewingii* infects neutrophils. Within the cell the organisms remain in the phagosome and inhibit its fusion with lysosomes. Ehrlichiae spread from the site of tick attachment via the lymphatics and bloodstream and infect cells around the body.[32]

PREVENTION

Avoidance of tick-infested areas is the first line of defense in preventing cases of human ehrlichiosis. If the use of protective clothing is not practical, insect repellents containing DEET can be used.

DIAGNOSTIC MICROBIOLOGY

In endemic areas, the disease should be suspected in all patients presenting with fever, headache, elevated liver transaminases, thrombocytopenia and leucopenia, even if there is no history of tick attachment.

Direct diagnosis

Occasionally, diagnoses can be made when morulae are seen in monocytes in Romanowsky stained peripheral blood smears, buffy coat preparations or cerebrospinal fluid (CSF). Various cell lines have been used for isolation, including a canine hystiocytic line (DH82), a human monocytic leukemia line (THP-1), HEL-22 cells (fibroblast-like cells), Vero cells and a human promyelocytic leukemia cell line (HL-60).[31] Detection of ehrlichial DNA/RNA in peripheral blood is probably the best method to diagnose human monocytic ehrlichiosis (HME) in the acute phase. Most often this is with primers that amplify a section of the 16S rRNA gene.[31]

Indirect diagnosis

Most diagnoses are made using the indirect microimmunofluorescence antibody assay. Many diagnoses are made retrospectively with convalescent phase sera as relatively few patients (30%) seen in the acute phase of HME have reactive antibodies.[33] Immunoblotting procedures have higher specificity than the indirect microimmunofluorescence antibody assay but are generally only available in research laboratories.

CLINICAL MANIFESTATIONS

The main clinical signs reported are fever (98%), malaise (30–80%), headache (77%), myalgias (65%), vomiting (36%), cough (25%) and neurologic signs with changes in mental status (20%).[32] A skin rash that involves the trunk and extremities, and less commonly the face, occurs in 12–36% of patients and is more common in children (67%). Although age (>60 years) is an independent risk factor for severe or fatal HME in some studies, many severe or fatal cases have been described in apparently healthy children or young adults. These also occur in immunocompromised patients.[32]

Human infections with *E. ewingii* were first reported in 1999. This organism appears to be less virulent than *E. chaffeensis* and most of the reported patients have been immunosuppressed.[34]

MANAGEMENT

Doxycycline (4 mg/kg q12h; maximum 100 mg/dose) has become the antimicrobial agent of choice, even in children.[31] In patients allergic to doxycycline, tetracycline (25 mg/kg q6h; maximum 500 mg/dose) or rifampin (rifampicin; 20 mg/kg q12h; maximum 600 mg) are acceptable alternatives.[31] Antimicrobial therapy should be continued for at least 3 days after the patient becomes afebrile with the minimum course being between 5 and 10 days.

Anaplasmosis

NATURE

Anaplasma spp. are small, often pleomorphic or coccoid, Gram-negative, nonmotile cells ranging from 0.3 to 0.4 µm in diameter. Lipopolysaccharides and lipo-oligosaccharides have not been detected in the cell wall of *Anaplasma* and it is unknown if they contain peptidoglycan.[35]

EPIDEMIOLOGY

Anaplasma phagocytophilum is the agent of human granulocytic anaplasmosis which is transmitted by *Ixodes* spp.: *I. scapularis* is the most important vector in North America, *I. ricinus* in Europe and *I. persulatus* in Eastern Europe and Asia.[35] Human granulocytic anaplasmosis is seasonal. with most cases occurring between May and October when tick vectors are most active, particularly the nymphs which are difficult to detect because of their small size.[34]

PATHOGENICITY

The bacteria presumably infect neutrophils migrating to the site of tick attachment and disseminate as these cells migrate further. Infections might also spread if the organisms replicate locally in macrophages or endothelial cells and drain to local lymph nodes and the systemic bloodstream.[36]

PREVENTION

Vaccines against *A. phagocytophilum* are not currently available. People at risk should be informed of measures to prevent exposure to vectors including the use of insect repellants such as DEET or permethrin on clothes and shoes.

DIAGNOSTIC MICROBIOLOGY

Clinical signs are not specific, as are laboratory abnormalities which can include leukopenia, a left shift, thrombocytopenia and elevated hepatic transaminases.

Direct diagnosis

The diagnosis can be confirmed by using Romanowsky stains to identify typical clusters of organisms.[37] *Anaplasma phagocytophilum* can be isolated in tissue cultures of a promyelocytic leukemia (HL-60) cell line or primary bone marrow cells. Several primer sets have been used to amplify different target sequences of *A. phagocytophilum* in clinical samples as well as in ticks.[38]

Indirect diagnosis

The indirect microimmunofluorescence antibody assay has been used most frequently. There is serologic cross-reactivity in indirect microimmunofluorescence antibody assays between *E. chaffeensis* and *A. phagocytophilum* as these organisms are related and have common antigens such as the heat shock proteins. Enzyme immunoassays have also been used to detect antibodies to *A. phagocytophilum*.[39]

CLINICAL MANIFESTATIONS

The incubation period after tick attachment is 7–10 days and, although the majority of patients recall tick attachment or exposure, other routes of transmission such as exposure to deer blood, blood transfusion or perinatal infection have been reported.[36] Clinical signs include high fever, rigors, generalized myalgias, severe headache, arthralgia, nausea and a nonproductive cough. Rash is rare (<11%) and patients may present with atypical pneumonitis and Sweet's syndrome.[36] Fatal cases have been reported in the USA and the case fatality rate is estimated to be about 1%.[40]

MANAGEMENT

The recommended therapy for adults is oral doxycycline (200 mg/day).[37] Children older than 8 years should also be treated with doxycycline given in divided doses with dosage adjusted to the patient's weight (4.4 mg/kg q24h; maximum 100 mg/dose).[37] Rifamycin has excellent activity and can be used in cases of tetracycline allergy or pregnancy.[37]

Q Fever

NATURE

Morphologically, *Coxiella burnetii* is a Gram-negative, strictly intracellular, pleomorphic coccobacillus 0.2–1.0 µm in diameter. The organism may occur as either a small-cell or large-cell variant.[41] The small-cell variant is a compact, small rod with a very electron-dense center of condensed nucleoid filaments. The large-cell variant is larger and less electron dense and is the metabolically active intracellular form of the *C. burnetii*.

Antigenic variation or phase variation is an important characteristic of *C. burnetii* and is associated with changes in the lipopolysaccharide of the outer membrane. Organisms isolated from acutely infected animals express the phase I antigen and are very infectious. After serial passage in eggs or tissue culture, the lipopolysaccharide is modified and there is an antigenic shift to phase II organisms which are minimally infectious. Phase II organisms revert to phase I following passage in a vertebrate host. There are no distinct morphologic changes associated with this phase variation.

EPIDEMIOLOGY

Coxiella burnetii has been found worldwide, with the notable exception of New Zealand. A wide variety of ticks, rodents, birds, and wild and domestic mammals can be infected, but domestic ruminants are the major reservoirs for *C. burnetii* infections in humans. Although cattle, sheep and goats may remain chronically infected for weeks to years, the majority of infections are subclinical. Infected animals can shed large numbers of organisms in birth products ($>10^9$/g), feces and urine, and a major risk factor for people developing Q fever is contact with infected domestic livestock. Most human cases occur sporadically but outbreaks occur with high-risk occupations (farmers, hunters, workers in meat, milk or hide and fleece processing plants, slaughterhouses, veterinary schools, etc.).[41]

Infections occur principally by inhalation of infected aerosols with just a single organism being sufficient to cause infection. This has led to *C. burnetii* being designated a Category B biothreat agent by the US Centers for Disease Control and Prevention. The organisms may remain infective in aerosols for up to 2 weeks and in the soil for as long as 5 months. In some outbreaks, however, there is no direct contact

with infected farms or animals and their products. In these outbreaks the source has been ascribed to windborne spread of *C. burnetii* from infected areas in the vicinity.[41]

PATHOGENICITY

The target cells of *C. burnetii* are monocytes and macrophages. Most infections occur via the respiratory route and involve the alveolar macrophages.[41] The Kupffer cells of the liver are involved in infections that take place via the digestive tract. Attachment to the host cell is followed by passive entry into the phagolysosome, where production of a potent acid phosphatase protects the organism from enzyme attack. Disturbance of cytokine regulatory processes, allowing survival and replication of *C. burnetii* within host cells, is also important in establishing infection. Subsequently, there is a bacteremia which, in overt clinical cases in humans, coincides with fever.

PREVENTION

Appropriate tick control strategies and good hygiene practices can decrease environmental contamination. Infected fetal fluids and membranes, aborted fetuses and contaminated bedding should be incinerated or buried. Vaccination of people in high-risk occupations should be considered. In Australia, a formalin-inactivated, whole cell *C. burnetii* (Henzerling strain, Phase I) vaccine (Q-Vax, CSL) has proven highly effective in reducing Q fever in abattoir workers.[42]

DIAGNOSTIC MICROBIOLOGY

While Q fever should be considered in patients who have close contact with domestic ruminants and cats, exposure to animals may not be reported as organisms persist in the environment for many years.

Direct diagnosis

Although *C. burnetii* does not grow on artificial media, it may be isolated in embryonated eggs, laboratory animals such as guinea pigs and mice, and in a number of tissue culture cell lines.[41] The shell vial assay may improve recovery of organisms from patients with endocarditis. A number of PCR assays have been described which detect various DNA sequences in *C. burnetii* and these have been shown to detect organisms in around 65% of patients with acute and chronic Q fever.[43]

Indirect diagnosis

The indirect microimmunofluorescence antibody assay has become the reference technique. Immunoglobulin M antibodies reactive with phase II *C. burnetii* appear rapidly, reach high titers within 14 days and persist for 10–12 weeks. Immunoglobulin M antibodies reactive with phase I antigens are usually at a much lower titer during acute infection. Immunoglobulin G antibodies reactive with phase II antigens reach peak titers about 8 weeks after the onset of symptoms, while those reactive with phase I antigens develop only very slowly and remain at lower titers than antibodies to phase II antigens, even after a year. In chronic Q fever, where there is persistence of organisms, the IgG titers to phase I and phase II antigens may both be high, and the presence of IgA antibody to phase I antigen is usually, although not exclusively, associated with chronic infection.

CLINICAL MANIFESTATIONS

The incubation period depends on the route of exposure, the inoculum dose and the age of the patient, but is usually about 3–4 weeks. Around 38% develop a mild, flu-like, self-limiting illness with low fever, headache, chills, sweating, cough, nausea and bradycardia relative to body temperature.[41] There is an inverse relationship between severity of disease and age, and infections in children may go unnoticed. Other frequently occurring signs include pneumonia which is found in almost 40% of patients and may be the only sign in 17%.

Women infected before they are pregnant do not have increased risks of abortion or premature delivery. When infections occur during pregnancy, however, fetal death or premature delivery is seen in almost every patient.[44] Chronic infections of the uterus develop in about 50% of patients and this may lead to abortions in subsequent pregnancies.

Chronic Q fever is seen in around 0.5% of patients and occurs months to years after infection. Endocarditis is the major clinical presentation of chronic Q fever and particularly involves the mitral and aortic valves. In other patients with chronic Q fever there may be osteoarticular infections, chronic hepatitis, chronic pulmonary infections and a chronic fatigue syndrome.

MANAGEMENT

Prompt treatment of acute Q fever with oral doxycycline, 100 mg twice a day for 14 to 21 days, is the treatment of choice. Co-trimoxazole for the duration of pregnancy is recommended for pregnant women as it might decrease the possibility of abortion and premature delivery. In patients with endocarditis, treatment with hydroxychloroquine (600 mg/day initial dose and titration to achieve a plasma level of 1 mg/ml) and doxycycline (200 mg/day) for 18 months to 3 years is required before serology shows cure.

Bartonellosis

NATURE

The *Bartonella* are Gram-negative bacilli or coccobacilli that belong in the α_2 subgroup of the class Proteobacteria.[45] The principal human pathogens are *B. bacilliformis*, *B. quintana* and *B. henselae*, but eight other species have been implicated in human disease: *B. elizabethae*, *B. koehlerae*, *B. alsatica* and *B. vinsonii* subsp. *berkhoffii* in endocarditis, *B. vinsonii* subsp. *arupensis* in bacteremia and heart valve disease, *B. grahamii* in neuroretinitis, *B. clarridgeiae* in cat-scratch disease and *B. washoensis* in myocarditis (Table 176.2).

EPIDEMIOLOGY

Each *Bartonella* species is adapted to a mammalian reservoir host where organisms occur within erythrocytes and endothelial cells and cause a long-lasting bacteremia. They are transmitted between hosts by arthropod vectors, mainly sandflies, body lice, fleas and ticks.

Bartonella bacilliformis occurs only in remote Andean valleys of Peru, Ecuador and Columbia and humans are the main reservoir of infection. The sandfly *Lutzomyia verrucarum* is the most important vector.[46]

Humans are the only known reservoir of *B. quintana* and the body louse (*Pediculus humanis corporis*) is recognized as its major vector. Recent studies have implicated *Ctenocephalides felis* in the transmission of *B. quintana*.[47]

The major vector of *B. henselae* is the cat flea (*Felis domesticus*) and large numbers of viable organisms can be found in flea feces. Contaminated cats' claws and teeth enable transmission of organisms by bites or scratches or when cats lick open wounds.[48]

Table 176.2 *Bartonella* species reported to date: epidemiologic and clinical data

***Bartonella* spp.**	**Reservoir host**	**Vector-detection in arthropods**	**Disease in humans**	**First cultivation**
B. bacilliformis	Human	Sandfly (*Lutzomia* spp.)	CD, END	1919
B. talpae	Mole	Unknown	Unknown	1911
B. peromysci	Unknown	Unknown	Unknown	1942
B. vinsonii subsp. *vinsonii*	Rodents	Unknown	Unknown	1946
B. quintana	Human, cats	Human body lice/fleas	TF, BA, BAC, END	1961
B. henselae	Cats, rats, dogs	Fleas (*Ctenocephalides felis*)	CSD, BA, BAC, LMF, END, PH, RET	1990
B. elizabethae	Rodents, dogs	Fleas	END (1 case)	1993
B. grahamii	Voles, rodents	Fleas?	RET (1 case)	1995
B. taylorii	Rats	Fleas?	Unknown	1995
B. doshiae	Voles	Fleas?	Unknown	1995
B. clarridgeiae	Cats, dogs	*Ctenocephalides felis*	Unknown	1995
B. vinsonii subsp. *berkhoffii*	Dogs, coyotes, gray foxes	Fleas and ticks	END	1995
B. vinsonii subsp. *arupensis*	Rodents, cattle	Deer ticks	BAC (1 case)	1999
B. tribocorum	Rats	Unknown	Unknown	1998
B. koehlerae	Cats	Fleas	END (1 case)	1999
B. alsatica	Rabbit	Fleas or ticks	END (1 case)	1999
B. bovis (*weissii*)	Cows, cats	Unknown	Unknown	1999
B. washoensis	Rodents, dogs	Unknown	MYOC (1 case)	2000
B. birtlesii	Rats	Unknown	Unknown	2000
B. schoenbuchensis	Wild roe deer	Unknown	Unknown	2001
B. capreoli	Wild roe deer	Unknown	Unknown	2002
B. chomelii	Cows	Unknown	Unknown	2004
B. rattimasilliensis	Rats	Unknown	Unknown	2004
B. phoceensis	Rats	Unknown	Unknown	2004

BA, bacillary angiomatosis; BAC, bacteremia; CSD, cat-scratch disease; END, endocarditis; LMF, lymphadenopathy; MYOC, myocarditis; PH, peliosis hepatitis; RET, retinitis; TF, trench fever

PATHOGENICITY

Bartonella are rapidly cleared from the blood into a primary niche, most likely endothelial cells.[49] At about 5-day intervals, further waves of erythrocyte infection occur from the primary niche, perhaps reflecting the duration of the infection cycle in these cells. *Bartonella* in erythrocytes do not cause clinical signs in most reservoir hosts; the exception is *B. bacilliformis* which is associated with severe hemolytic anemia and Oroya fever.

In endothelial cells, *B. bacilliformis*, *B. quintana* and *B. henselae* have the unique ability to cause vasculoproliferative lesions (verruga peruana, bacillary angiomatosis and bacillary peliosis) by stimulating angiogenesis and the formation of new capillaries from old ones.

PREVENTION

Control of the arthropod vectors is most important. Cat fleas live on both cats and dogs and are best controlled by fumigating areas where cats and dogs live. People infested with body lice should bathe and use a pediculicide such as permethrin lotion, shampoo or powder on the hair-covered areas of the body. Sandflies can be controlled by spraying the environment with long-acting insecticides. Individual protection depends on avoiding exposed biotopes at night and the use of insect repellents (DEET) or mosquito nets treated with permethrin/deltamethrin.

DIAGNOSTIC MICROBIOLOGY

There are no pathognomonic clinical features in patients with bartonelloses and definitive diagnoses can only be made using laboratory testing.

Direct diagnosis

Bartonella grow on most blood-enriched media when incubated at 99°F (37°C) in an atmosphere containing 5% carbon dioxide. *Bartonella* can also be isolated in various cell lines in tissue culture with a shell-vial culture technique increasing the recovery rate.[50] Recently, pre-enrichment culture in *Bartonella* α Proteobacteria growth medium before subinoculation of agar plates has been reported to improve the isolation of *Bartonella* from clinical specimens.[51] Isolates are best identified using PCR-based methods which have also been shown to be more sensitive than culture in detecting organisms in tissues and blood.[45]

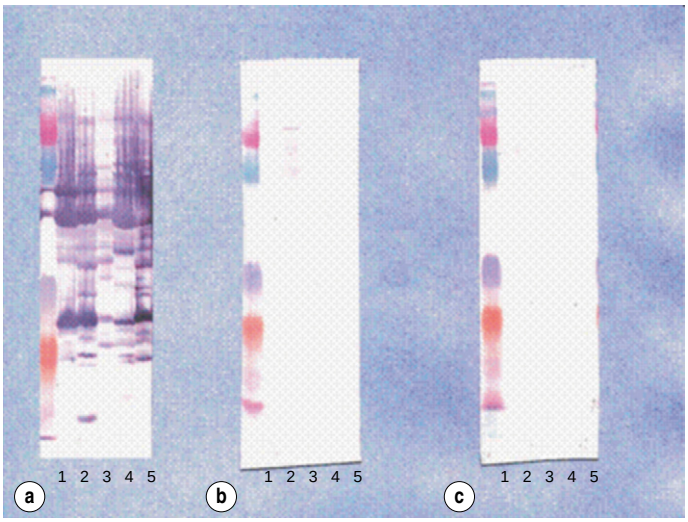


Fig. 176.7 Western blot of a patient with *Bartonella henselae* before (a) and after cross-adsorption with (b) *B. quintana* or (c) *B. henselae*. Line 1: *B. quintana*; Line 2: *B. henselae*; Line 3: *B. elizabethae*; Line 4: *B. vinsonii* subsp. *berkhoffii*; Line 5: *B. alsatica*.

Indirect diagnosis

The most commonly used technique is the indirect microimmunofluorescence antibody assay although enzyme-linked immunosorbent assays have also been described. Care should be taken in interpreting serology results from immunocompromised patients who might not mount significant antibody responses. Also, serology should be interpreted with caution in endocarditis patients as there are significant cross-reactions with other agents causing 'culture-negative' endocarditis such as *C. burnetii* and *Chlamydia* spp. Western blot analysis after cross-adsorption study has been shown to be a powerful tool for the identification of *Bartonella* at the species level in endocarditis (Fig. 176.7).

CLINICAL MANIFESTATIONS

A number of different clinical conditions have been associated with *Bartonella* infections.

Carrion's disease

Carrion's disease is caused by *B. bacilliformis*. Clinical signs are seen mostly in children and teenagers and include fever, malaise, hyporexia, nausea and vomiting, hepatomegaly, lymphadenopathy, pallor and a systolic murmur. Mortality rates can be high in untreated patients, reaching 90%, especially if infections are complicated with other diseases such as salmonellosis.[45]

Trench fever

The etiologic agent is *B. quintana*. After an incubation period of 2–3 weeks, there is a sudden onset of fever, retro-orbital headache and intense pain in the long bones of the legs, in particular the tibias.[45] While fatal cases have not been reported, the disease may persist for 4–6 weeks and result in prolonged disability.

Cat-scratch disease

Bartonella henselae is the major etiologic agent of cat-scratch disease which is now generally recognized to be the most common cause of chronic benign lymphadenopathy. In 75% of patients the adenopathy might be accompanied by mild systemic signs including fever, malaise, fatigue, headache, anorexia, weight loss and emesis that usually resolve within 2 weeks. Most cases are self-limiting, with the adenopathy resolving spontaneously in 2 weeks to 4 months.[45]

Atypical manifestations of cat-scratch disease occur in about 15% of patients. The most common is Parinaud's oculoglandular syndrome where there is unilateral conjunctivitis with preauricular lymphadenopathy that probably results from inoculation into the conjunctiva rather than the skin.

Bacillary angiomatosis

Bacillary angiomatosis is a vascular proliferative disease caused by *B. henselae* or *B. quintana*.[45] It was first described in HIV-infected patients but also occurs in organ transplant recipients and in immunocompetent patients. Lesions most often involve the skin but can occur at other sites including bone marrow, spleen, liver and lymph nodes.

Bacillary peliosis

This rare condition is caused by *B. henselae* infections of the parenchymal vasculature which results in the development of cystic, blood-filled spaces, particularly in the liver (peliosis hepatic) but also in the spleen, bone marrow or lymph nodes.[45]

Bacteremia

Persistent bacteremia is now one of the most frequently reported manifestations of re-emergent *B. quintana* infections among homeless people and alcoholics.[45] Prolonged bacteremia can also occur with *B. henselae* in HIV-infected patients. These infections may be asymptomatic or associated with an insidiously developing, prolonged symptom complex of malaise, fatigue, weight loss and recurring high-grade fevers.

Endocarditis

Bartonella endocarditis in immunocompetent and immunocompromised patients and infections with these organisms should be suspected in all patients with endocarditis that is 'culture-negative' by routine blood culture techniques.[45]

MANAGEMENT

In patients with Oroya fever, ciprofloxacin (patients <7 years, 10 mg/kg q12h; patients 7–14 years, 250 mg q12h; patients >14 years, 500 mg q12h; all for 10 days) or chloramphenicol (50 mg/kg q6h for 3 days, then 25 mg/kg/day for a further 11 days) are the drugs of choice and significantly reduce mortality.[52] The current recommendation is that *B. quintana* bacteremia be treated with intravenous gentamicin (3 mg/kg/day for 14 days) and doxycycline (200 mg/day for 28 days). While *B. henselae* is susceptible to most antibiotics *in vitro*, most typical cases of cat-scratch disease respond very poorly to antimicrobial therapy. In immunosuppressed patients the use of antibiotics is associated with rapid clinical improvement. In patients with suppurative lymph nodes, needle aspiration is an appropriate treatment.[52]

REFERENCES

References for this chapter can be found online at http://www.expertconsult.com

Chapter 177

Pekka Al Saikku

Chlamydia

INTRODUCTION

The chlamydial diseases seldom occur abruptly and they are not easily recognized; rather, chlamydial infections may run an insidious, chronic course, causing severe damage only after years of infection.[1] Their possible role in chronic diseases with an inflammatory component has led to renewed interest in these organisms. Their obligatory intracellular growth makes normal bacteriologic analysis difficult.[2] The sequencing of whole genomes of several chlamydial species[3] has provided new possibilities for studying these organisms and our concepts of the nature of chlamydiae are changing accordingly. Their small genomes as well as the wide variety of associated clinical syndromes make *Chlamydia* promising in future studies of disease pathogenesis in general.

NATURE

The current classification of chlamydial species is rapidly evolving and there are proposals to name new species and even new genera.[4] The suggested genus *Chlamydophila* has been criticized on the basis of intracellular evolution which is different from extracellular bacterial evolution in general, and the name *Chlamydia* should be conserved. *Chlamydia trachomatis* is a human pathogen with its closest relatives found in the mouse (*C. muridarum*) and pig (*C. suis*). *Chlamydia pneumoniae* is a human pathogen with strains reported in the horse, marsupials and amphibians. *Chlamydia psittaci* is a pathogen of birds, *C. abortus* and *C. pecorum* are pathogens of ruminants, *C. felis* is a pathogen of cats and *C. caviae* is found in guinea pigs. All except the last species also cause diseases in humans. Numerous *Chlamydia*-like or *Chlamydia*-related bacteria have been isolated recently, but their pathogenic potential and distribution require further studies.[5] Like *Legionella*, these new agents are able to multiply in amebae and have been called environmental *Chlamydia*-like agents.

Chlamydiae are small, Gram-negative bacteria that are obligatory intracellular parasites, the general properties of which are outlined in Table 177.1. They are not cultivable on synthetic media. They exist in nature in two forms: a nonreplicating, infectious dense particle called the elementary body (EB); and a loose, larger, intracellular form, the reticulate body (RB), which is able to multiply by binary fission but is noninfectious (Fig. 177.1).[6] The EB is a spherical particle about 300 nm in diameter; it can be stored at –94 °F (–70 °C) in sucrose-containing buffers and cultured in cell cultures.

Table 177.1 General properties of *Chlamydia*

- Obligatory intracellular Gram-negative parasites
- Elementary body–reticulate body cycle
- Lack of peptidoglycan
- Common group antigen (lipopolysaccharide)
- Tendency to persistent infections
- Host immune defense participates in the development of lesions

Chlamydiae have a double layer membrane of Gram-negative bacteria with a periplasmic space. They are usually devoid of peptidoglycan, but seem to use it when dividing.[7] The circular genomes of *C. pneumoniae* and *C. trachomatis* contain 1 230 000 and 1 039 000 base pairs, respectively, with just over 1000 potential genes.[3] Species-specific plasmid is present in 10 copies in *C. trachomatis*,[8] but is not found in the human type of *C. pneumoniae*. The major outer membrane protein (MOMP) forms the outer membrane of the particle with some minor proteins, of which two are rich in cysteine. *Chlamydia trachomatis* has nine and *C. pneumoniae* 21 genes for putative membrane proteins (PMPs). Cysteine-rich proteins apparently have replaced the peptidoglycan with their S–S bridges in maintaining the rigidity of the chlamydial membrane. On electron microscopy, the surface can be seen to consist of a hexagonal pattern with substructures made up of a few rosettes with short spikes (Fig. 177.2).[9]

The MOMP of *C. trachomatis* contains four variable regions, which divide it into about 20 immunotypes denoted alphabetically.[10] The MOMP of *C. pneumoniae* is more homogeneous and the surface structure differs from that of *C. trachomatis*. Chlamydial lipopolysaccharide (LPS) is of rough type, weakly endotoxic, and situated in the outer membrane. It contains three KDO-residues, in which the α-2-8 bond is specific for *Chlamydia*. It is 10 times less toxic than typical LPS but induces production of interleukin (IL)-8 via Toll-like receptor 4 (TLR-4) in the presence of CD14.[11]

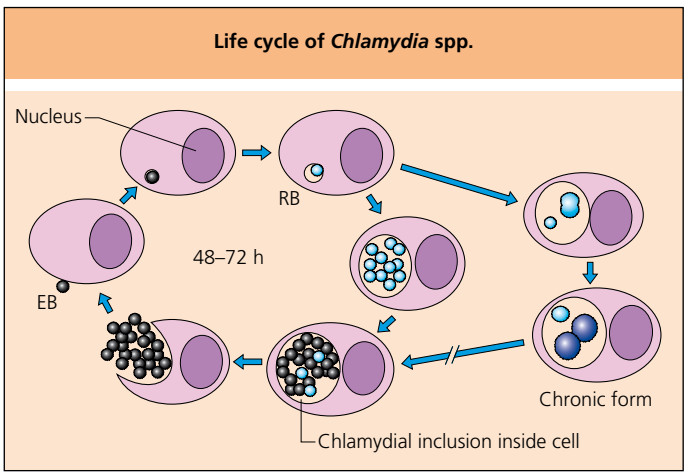


Fig. 177.1 Life cycle of *Chlamydia* spp.

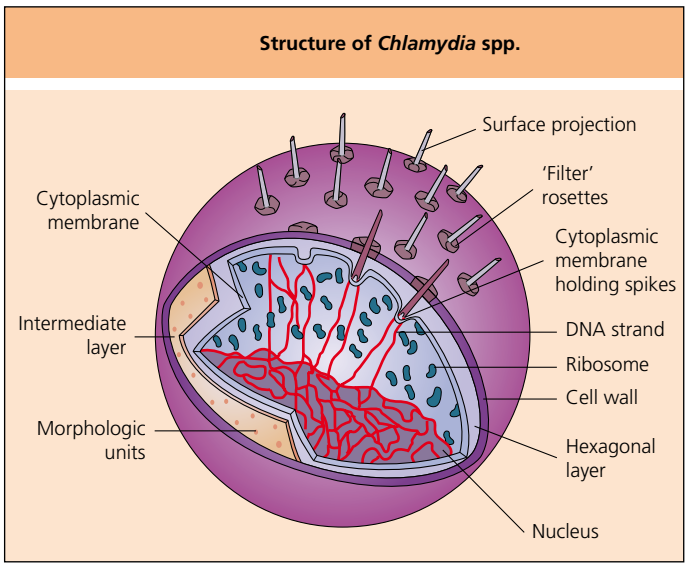


Fig. 177.2 Structure of *Chlamydia* spp. Courtesy of Dr A Matsumoto.

In the multiplication cycle, the EBs attach to the surface of susceptible cells and are passively engulfed by activity of the cell. *Chlamydia trachomatis* lymphogranuloma venereum (LGV) biotype is wrapped by heparan sulfate that is taken from the cell by the chlamydial organism, which can then use heparin receptors on the cell surfaces; *C. pneumoniae* and *C. psittaci* may also use glycosaminoglycans in the attachment but there apparently are other receptors, too.[12]

The chlamydial surface can prevent the fusion of the endocytic vacuole with lysosomes, and travels on microtubules joining to the exocytic pathway. The EB envelope changes, transforming the metabolically inactive inert EB into the metabolically active RB, which is 0.8–1 µm in diameter. In the process, the disulfide bridges are reduced and MOMP acts as a porin. DNA, RNA and proteins are synthesized within the RB, which divides successively by binary fission. The endosomal walls intercept nearby trans-Golgi vesicles that incorporate sphingomyelin and glycerophospholipids into the swelling chlamydial inclusion (Figs 177.3,177.4). Metabolically active RBs acquire nutrients and metabolic building blocks, including nucleotides and ATP ('energy' parasites) from host cells.[13] Chlamydial LPS is present in RBs and also seems to attach to host cell membranes.

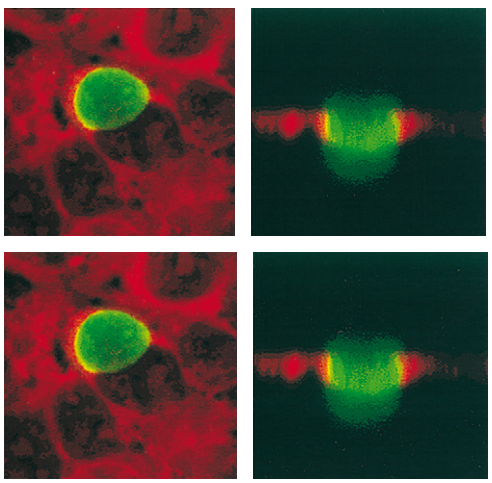


Fig. 177.3 Chlamydial inclusion in confocal stereomicroscopy. *Chlamydia pneumoniae* cultured in human line (HL) cells are shown. Fluorescein isothiocyanate (FITC)-labeled anti-LPS monoclonal antibody stain. Courtesy of Dr A Laurila.

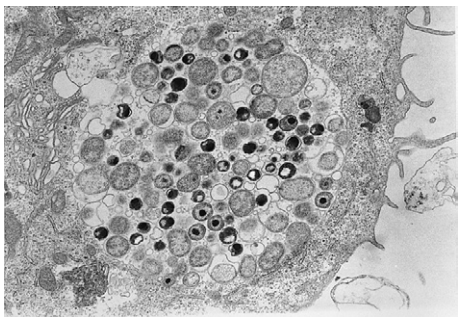


Fig. 177.4 Electron microscopy of chlamydial inclusion. Reticulate bodies and transition stages to dense elementary bodies are shown. *Chlamydia pneumoniae* cultured in HL cells. Courtesy of Dr CH von Bonsdorff.

Chlamydiae use their type III secretion system (TTSS) to gain control over the host cell and interfering cellular immune defense mechanisms by proteolytic proteins. Apoptosis is prevented during the growth period but induced in nearby inflammatory cells.[14] A chlamydial cytotoxin and proteolytic enzymes secreted through the TTSS have been demonstrated.[15] Eventually, the inclusion body may contain several thousand chlamydial RBs. At the end of the replication cycle, chlamydiae start to condense into EBs, and apoptosis and proteolysis are promoted in order to liberate the new infectious particles. Sometimes the mature inclusion is expelled intact but usually the inclusion bursts open, releasing infective chlamydial particles and the cell dies. The whole cycle takes 2–3 days. Depletion of tryptophan or iron or the presence of penicillin or interferon gamma (IFN-γ) in subinhibitory concentrations induces an aberrant, slowly metabolizing form of RB, which can persist for long periods. In chronic infections this may be the dominant form of *Chlamydia*.[13] The microscopic appearance of chlamydial inclusion varies with different strains, and the inclusions of *C. trachomatis* contain glycogen.

EPIDEMIOLOGY

Chlamydia trachomatis is transmitted by direct contact and via vectors such as flies and contaminated towels in poor sanitary conditions in trachoma-endemic areas. Newborn infants contract the infection from their mother, sometimes before birth, and can infect their siblings via excretions. In industrialized countries, *C. trachomatis* is by far the most common bacterial sexually transmitted disease (STD). The incidence is greatest in young sexually active people and varies widely among different areas, from very low levels in Scandinavian countries to 15–40% in some urban areas of other developed countries.

Chlamydia pneumoniae is a common respiratory pathogen worldwide. In tropical countries, infections are common during the first years of life, especially in urban slums. In industrialized countries, children begin to seroconvert at school age at the rate of 10% each year. The rate seems to depend on population density, but at the age of 15 years 25–50% of populations have demonstrable antibodies against the agent. The antibody prevalence continues to rise; nearly all elderly people have measurable antibodies. Because antibodies are lost by a few years after an acute infection, this steady increase points to repeated infections during life and to possible chronic infections. Spread via droplets has been proposed. Asymptomatic carriers are found but their number is disputed and there is a possibility that only some people are effective transmitters of the agent. In sparsely inhabited areas in high latitudes, *C. pneumoniae* has caused epidemics at intervals of 5–7 years. In lower latitudes, the interval between epidemics is shorter and an endemic situation can be found near the equator. In military garrisons with susceptible recruits, the slow epidemics are prolonged and can last 6 months.

Avian *C. psittaci* is contagious in dried droppings of diseased birds for several days and is typically transmitted by the inhalation of dust. Patients are usually turkey or duck farmers, plant processors or persons who have contact with diseased caged or wild birds. Person-to-person transmission occasionally occurs. Infections caused by *C. psittaci* strains from mammals are less well known. Ovine abortion strains are known to cause septic abortions in pregnant women who are in contact with affected farm cattle. Pet cats can be a source of *C. felis* infections. *Chlamydia*, like *Legionella*, are able to multiply in amebae[5] but the significance of this property in epidemiology is so far not known.

PATHOGENICITY

Owing to their unique life cycle, in which they parasitize only living, metabolically active cells, chlamydiae are not harmless commensals.[3] It is not known whether the pathogen stays dormant inside cells, protected from antibodies, for prolonged periods only to be activated later by some signal, or whether there is a special form of chronically infecting *Chlamydia* spp. In chronic persistent infections *in vitro*, it is possible that chlamydiae respond with morphologically abnormal forms, in which internal proteins such as heat shock proteins (hsps) are produced and antigens of mature EBs are diminished.[16]

Chlamydiae are potent inducers of cytokines, such as IFN-γ and IL-1, which prevent the multiplication of the agent *in vitro*.[13] In chronic infections such as trachoma, there are findings that point to diminished cell-mediated immunity and increased humoral immunity.[17] Chlamydial immunity is relatively short-lived, immunotype specific and re-infections are common. Active therapy of episodes in the community can lead to 'arrested immunity', in which re-infections are common, but chronic infections fortunately rare.[18]

In repeated chlamydial infections[19] there is a hypersensitivity component, apparently caused by common internal cross-reactive proteins. Chlamydial hsp60, a protein produced in chronic infections, has been blamed for the hypersensitivity reactions seen.[20] Moreover, it is related to the host's hsp60 and may initiate autoimmune reactions.

In chronic chlamydial infections, chlamydia can persist in noncultivable form and damage results partly from persistent inflammation. Blinding trachoma was thus named after *C. trachomatis* and long-term sequelae of lymphogranuloma venereum – rectal strictures, elephantiasis and esthiomena (genital elephantiasis) – have been known for some time, but infertility and ectopic pregnancies caused by tubal occlusion have only recently been associated with chronic *C. trachomatis* infection.

Chlamydia pneumoniae is able to disseminate inside white blood cells from lungs in the circulation[21] and thus may disseminate widely. This is obviously one reason why it has been associated with a wide variety of diseases. Most important is the association of *C. pneumoniae* with atherosclerosis.[22] Over 70 seroepidemiologic surveys have repeatedly demonstrated that the presence of elevated antibodies against *C. pneumoniae* is associated with an increased risk of having a cardiac event. The antibody findings are further supported by the presence of circulating immune complexes containing *C. pneumoniae* antibodies and antigens, pointing to an access of chlamydial components into the circulation. Human hsp60 and chlamydial hsp60 co-localize in atherosclerotic plaques and coronary angioblasty releases chlamydial components from the lesions. Final demonstration of the common presence of the agent in the atherosclerotic lesion has been obtained by immunoelectron microscopy, immunohistochemistry, nucleic acid demonstration by polymerase chain reaction (PCR), *in-situ* hybridization and even isolation of the agent from lesions.[23]

The important question is, does this presence have an effect on the atherosclerotic process? *C. pneumoniae* may simply be an innocent bystander in atherosclerotic lesions carried there in blood. However, it is an intracellular pathogen, able to multiply in macrophages, endothelial cells and smooth muscle cells. This multiplication is accompanied by induction of cytokines, adhesion molecules, growth factors and proteases. Oxidative substances appear as the host defense mechanisms against chlamydial infection. Chlamydial LPS induces foam cell formation in macrophages, the characteristic pathologic feature of the atherosclerotic plaque. Several well-known risk factors for coronary heart disease are also associated with a chronic *C. pneumoniae* infection, such as smoking, elevated cholesterol levels, lowered levels of high-density cholesterol and elevated blood pressure.[24] Animal experiments point to the possibility that it could even initiate the process[25] and that this could be prevented with antibiotics. Recently it was found that children as young as 11 years with signs of persistent *C. pneumoniae* infection show early signs of atherosclerosis including intimal thickening of their aortas.[26] *Chlamydia* can also be found in the vessel walls at the age of 14 years, decades before the complications of atherosclerosis, myocardial and cerebral infarctions start to appear.

Preliminary small-scale intervention trials with positive results encouraged pharmaceutical companies to begin intervention trials with antibiotics known to be effective in acute chlamydial infections, although animal and cell culture trials had shown them to be ineffective in chronic infections.[27,28] All the patients had advanced atherosclerosis and in this phase antibiotic treatment had no effect on the lesions formed.[29] The results mean that in advanced atherosclerosis usual antichlamydial antibiotics are ineffective and treatment of chronic infections demands further studies.[30]

The association of *C. pneumoniae* with childhood and adult asthma seems to be accepted[31] but its association with chronic neurologic diseases is debated.[32] There are reports of the association of *Chlamydia* with some forms of cancer (*C. trachomatis* with cervical cancer, *C. pneumoniae* with lung cancer, *C. psittaci* with ocular adnexal MALT lymphoma).[33] These diseases have remained mostly unstudied, although some animal models and successful intervention trials have been reported.

PREVENTION

Vaccines against *Chlamydia* spp. have been under development for 40 years without evident success. *Chlamydia trachomatis* has several immunotypes making development of a vaccine complicated, and the protective antigens of *C. pneumoniae* are not known. Knowledge of the complete genome and progress in immunology may alter this situation in the future.[34]

In 1998 the World Health Organization (WHO) started a project to eradicate trachoma. It is based on SAFE strategy (lid Surgery, Antibiotic mass treatment, Facial cleanliness and Environmental hygiene). Mass medication campaigns, washing the face every second day with clear water and wiping it with a towel that is not shared with other people is effective. Latrine hygiene is effective in controlling flies that spread trachoma. Evidence supports the effectiveness of this approach and there is a possibility that trachoma will be eradicated before 2020.[35]

The advent of nucleic acid amplification tests (NAATs) has led some countries to introduce screening in order to eradicate *C. trachomatis* from the population.

DIAGNOSTIC MICROBIOLOGY

The clinical features of chlamydial infections, such as their slow onset, the low numbers of infectious agents present and the cross-reactions seen in serology and some nucleic acid amplification tests, make usual diagnostic methods difficult.[36,37]

Chlamydia trachomatis infections

Culture

Culture was originally the gold standard in *C. trachomatis* diagnosis but due to its low sensitivity it is unsatisfactory. Although it is recommended in legal cases and in order to test antibiotic sensitivity, laboratories performing *Chlamydia* culture are nowadays rare.

Samples for culture should contain live cells from the diseased area and this can be problematic if the pathogen has invaded deeper tissues.

The sample is collected in a medium that contains sucrose, aminoglycosides, vancomycin and antifungal agents. McCoy cell lines and green monkey kidney cell lines are commonly used. Centrifugation onto cells and the addition of cytostat (usually cycloheximide) into the growth medium are needed for optimal growth. Multiwell plates are suitable for mass isolation attempts, but vials are better protected from cross-contamination. *Chlamydia trachomatis* can be stained by Lugol's iodine; immunofluorescence staining using LPS-specific monoclonal antibody gives a more clear-cut result but it stains all *Chlamydia* spp. The culture result is available in 2–3 days, but blind passage can improve the outcome.

Antigen detection

All enzyme immunoassay (EIA) kits for the diagnosis of *C. trachomatis* measure the common LPS group antigen that is present in all chlamydiae and produced in large amounts during the growth cycle. However, they lack sensitivity seen in NAATs and their use has diminished lately.

Direct fluorescent antibody (DFA) staining has been successfully used in *C. trachomatis* diagnosis. This test seems to be marginally more sensitive than EIA; moreover, the quality of the sample can be controlled in the stained smear. However, the interpretation demands expertise and is tiring to the reader, and therefore the test is often used only in the confirmation of EIA test results.

Serology

In individual *C. trachomatis* infections serologic tests are often of questionable value, although serology has been important in epidemiologic and disease association studies. The infection is often superficial without detectable seroconversion. Proper antibiotic therapy can also prevent antibody formation. The use of the complement fixation (CF) test in *C. trachomatis* infections is limited to lymphogranuloma venereum. Microimmunofluorescence (MIF) testing, if properly done, can differentiate between species and even immunotypes. It is, however, demanding and can be performed in relatively few laboratories. *Chlamydia trachomatis* antibodies start to appear at the sexually active age and peak at about 30 years of age. They are more often found in females than in males; the opposite is seen with *C. pneumoniae* antibodies, where males predominate. In *C. trachomatis* infections, MIF testing is usable in lymphogranuloma venereum, perihepatitis and infant pneumonitis, it can also give a clue to the triggering agent in reactive arthritis.

Enzyme immunoassay tests based on chlamydial EBs or chlamydial LPS (group antigen) are commercially available but have not gained wide popularity. Species-specific EIA kits based on peptides are also available. Their use in infertility studies can reveal tubal occlusions.

Nucleic acid amplification tests

The use of NAATs has altered the former concept of culture as the 'gold standard' of *C. trachomatis* diagnosis and has replaced other diagnostic methods. Several methods are commercially available. A great advantage is the possibility of using mailed morning void urine, tampons and self-collected samples for detection. To avoid leakage of urine during mailing, even self-collected dry swabs have been tried.

Nucleic acid (NA) tests are not without problems and some of the early ones have been removed from the market. In the case of *C. trachomatis* NA detection tests are usually based on its plasmids, but also on 16S RNA or chromosomal DNA. There have been reports on plasmid-free variants and, in 2006, a new plasmid variant was found to cause an epidemic in Southern Sweden, where 25% of the cases were caused by it and it was not detectable with most commonly used NAATs. The problem is now well known and diagnostic firms seem to have solved the problem.

There is no consensus as to whether a positive result should be confirmed with another test method, if the success of therapy should be monitored, and whether young females should be retested after 3–12 months. All these have been recommended but are not in general use. Cost is a major reason for this. In screening general populations, pooling of urine samples can save expense.

Chlamydia pneumoniae infections

Culture

The same principles as in *C. trachomatis* culture are used for *C. pneumoniae*, with the exception that HEp-2 or HL cells are used. *Chlamydia trachomatis* can be found in samples in amounts toxic for cells but *C. pneumoniae* cultures in primary isolation usually contain only a few inclusions. The consensus is that serology in acute infections is more sensitive than culture.[37]

Antigen detection

Direct fluorescent antibody staining from respiratory samples is still more difficult when compared to genital swabs and is used only occasionally. Since EIA detection tests are based on common chlamydial LPS they can be used in respiratory samples to obtain a presumptive diagnosis of chlamydial respiratory infection. This possibility has been used in only a few studies.

Nucleic acid amplification tests

In theory, NAATs should be as good for *C. pneumoniae* as for *C. trachomatis*. So far, most studies have been done with in-house kits and commercial kits have been slow to appear on the market. In acute infections NAATs are more sensitive than culture. One difficulty is to get representative samples from the pneumonic area because upper respiratory tract specimens can remain negative. In positive cases, a possible carriage status must be remembered.

Serology

Serology has been the method most commonly used for *C. psittaci* and *C. pneumoniae*. The need for paired samples considerably lessens the value of serology in acute situations.

Microimmunofluorescence testing seems, for the moment, to be the most convenient test for the serologic diagnosis of an acute *C. pneumoniae* infection.[37] In primary infections, the diagnosis can be obtained from the first sample that contains IgM antibodies specific for *C. pneumoniae*. The possibility of a false-positive reaction due to IgM rheumatoid factor should always be kept in mind, especially in elderly patients. In elderly patients undergoing re-infections, the rapid response can be missed if the first serum sample is not collected early enough after the onset of disease. Apart from the situation in which strong group reaction interferes with the result, an experienced reader can interpret the reaction to differentiate the species. In MIF testing, strains of *C. pneumoniae* react much more uniformly than do those of *C. trachomatis*.

The time-honored CF test has traditionally been used for the diagnosis of chlamydial respiratory tract infections. The CF test in *C. pneumoniae* infections is sensitive only in primary infections of young adults.

Enzyme immunoassay tests for species-specific serology are currently replacing MIF due to their easy laboratory performance. They seem to be more sensitive, especially in acute infections of children.

Other *Chlamydia* and *Chlamydia*-like organisms

Serology by CF test has been used traditionally in diagnosis of psittacosis. The number of different species makes MIF cumbersome and

there are few studies on the definite causative agent in chlamydioses of humans. Isolation in cell cultures can be attempted and the ability of *C. psittaci* to cause laboratory infections should be kept in mind. NAATs are not currently commercially available. Wide spectrum pan-*Chlamydia* probes which detect numerous species have been tried. Several *Chlamydia*-like agents have been associated with human diseases by isolation, serology and lately by NAATs. Environmental *Chlamydia*-like agents can be isolated in cultures of amebae. They can interfere as laboratory contaminants in *Chlamydia* NAATs.

Diagnosis of chronic chlamydial infections

Diagnosing chronic chlamydial infections is problematic. The number of infective organisms can be small and the site of infection can be difficult to reach. Culture often remains negative, especially if the sample is from a peripheral site. Antibody responses can be quite variable or even lacking. However, persistently elevated titers, especially of IgA type, have been suggested as a marker of chronic infection but their value in individual diagnosis is doubtful, especially if a single sample is collected. Enzyme immunoassay tests have been inferior when compared to the MIF test in chronic infections. Measurements of local antibodies in asthma patients and antibodies to chlamydial hsp60, with or without combination with inflammation markers such as C-reactive protein (CRP), have been used in some studies.

Antigen detection by immunohistochemistry is sensitive and diagnostic but, like nucleic acid-based methods (e.g. NAATs, *in-situ* hybridization), it is currently used in research laboratories only. The value of demonstrating circulating immune complexes that contain chlamydial antigens in blood samples has been proposed, but the technique is quite demanding. Detection of nucleic acid in circulating white blood cells is nowadays frequently used. The lack of international standards is reflected in the fact that in comparative study populations, the positivity can vary between 0% and 80%. A newly described method is to measure the presence of chlamydial LPS in the circulation, but none of these approaches indicates the site of the infection.

All these markers can also be found in otherwise healthy persons, suggesting common prevalence of chronic chlamydial infections.[21] All markers are affected by age and season so for.

CLINICAL MANIFESTATIONS

The diseases associated with chlamydial infection are listed in Table 177.2 and discussed in relevant chapters. *Chlamydia pneumoniae* has been associated with a wide variety of diseases, but in most cases these associations are highly speculative so far.

Table 177.2 Diseases associated with *Chlamydia* spp., or in which a possible association has been proposed

Species	Infection	Distribution	Disease	Incidence
Chlamydia trachomatis	Acute	Females	Cervicitis	About 30%
			Endometritis	Common
			PID	10–70%
			Perihepatitis, splenitis, appendicitis	Isolated case reports
			Bartholinitis	Rare
		Males	Urethritis	10–30%
			Epididymitis	Rare
		Both sexes	Conjunctivitis	Uncommon
			Reactive arthritis	Common cause
		Neonates	Conjunctivitis	10%
			Infant pneumonitis	By definition
	Chronic	Female	Chronic PID	Common
		Both sexes	Trachoma	By definition
Chlamydia pneumoniae	Acute	Both sexes	Pneumonia, endemic	10%
			Pneumonia, epidemic	Up to 50%
			Acute bronchitis	5%
			Sinusitis	5%
			Otitis media	Case reports
			Common cold, subclinical	10% of school children annually
			Carditis	Case reports
			Reactive arthritis	Case reports
	Chronic	Both sexes	COPD, asthma	10–50% (?)
			Atherosclerosis	50–80 % (?)
			Alzheimer's disease	?
			Multiple sclerosis	?
Chlamydia psittaci	Acute	Both sexes	Psittacosis–ornithosis	By definition
Chlamydia abortus	Acute	Female	Infectious abortion	Rare
Chlamydia felis	Acute	Both sexes	Conjunctivitis	Rare

MANAGEMENT (SEE CHAPTERS 15 AND 27)

Chlamydiae are sensitive to tetracyclines, macrolides, azalides and newer fluoroquinolones. An antimicrobial effect is also seen with rifampin (rifampicin), clindamycin and chloramphenicol. Chlamydiae are resistant to aminoglycosides, vancomycin and cephalosporins.

Chronic forms seem to be very resistant to treatment and in intervention trials even 1-year courses of monotherapy have shown no evidence of eradication.[29] Novel drugs effective against chronic chlamydial forms are currently sought.

REFERENCES



References for this chapter can be found online at http://www.expertconsult.com



Chapter **178**

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Opportunistic and systemic fungi

Opportunistic Fungi

INTRODUCTION

Fungal infections have become increasingly important clinically because of the rising incidence of immunocompromised patients as a result of infection, malignancy (especially leukemia), chemotherapy and immunosuppressive therapy, especially in transplantation medicine and in critical care. Moreover, the use of current antimicrobial prophylactic strategies has likely contributed to the changing epidemiology of invasive mycoses. Table 178.1 gives a list of medically important fungi that can cause disseminated infection in humans. Table 178.2 shows the variables that likely account for the current trends in the epidemiology of opportunistic fungal infections.

Candidiasis

NATURE

Candida spp. are yeast-like fungi that can form true hyphae as well as pseudohyphae. They are ubiquitous in soil and food and can be found as normal commensals on skin and mucosal membranes of the human gastrointestinal, genitourethral and respiratory tracts.

Candida spp. that under certain conditions are clinically important are *C. albicans*, *C. guilliermondii*, *C. krusei*, *C. parapsilosis*, *C. tropicalis*, *C. kefyr*, *C. lusitaniae*, *C. rugosa*, *C. dubliniensis* and *C. glabrata*. The fungal origin of these lesions was first proposed in 1839.[2]

EPIDEMIOLOGY

Since the introduction of antibiotics in the 1940s, there has been a sharp increase in the incidence of candidal infections. In a survey of 180 hospitals from 1980 to 1990, *Candida* spp. were the sixth most commonly isolated nosocomial pathogens, most frequently cultured from the urinary tract (46%).[3] *Candida* spp. were the fourth most common cause of bloodstream infection (8%) in US hospitals and were associated with a mortality of 29% (compared to 17% for bloodstream infections caused by nonfungal pathogens).[3,4]

Approximately 50% of invasive *Candida* infections are caused by non-*albicans Candida* spp., which appears to be stable distribution.[4] A surveillance program of bloodstream infections (BSIs) in the USA, Canada, Latin America and Europe from 1997 through 1999 detected 1184 episodes of candidemia in 71 medical centers (32 in the USA, 23 in Europe, 9 in Latin America and 7 in Canada). Overall, 55% of the yeast BSIs were due to *C. albicans*, followed by *C. glabrata* (20%), *C. parapsilosis* (15%), *C. tropicalis* (9%) and miscellaneous *Candida* spp. (6%).[5]

Changes in the frequency of invasive candidiasis are most notable in the following subgroups of patients: those hospitalized in critical care units, patients with hematologic malignancies, hematopoietic stem cell transplant (HSCT) recipients and organ transplant recipients.[1] In a review of 74 published studies,[6,7] the most important risk

Table 178.1 Fungi causing disseminated infection in humans

Fungi	Medically important species
Penicillium	*Penicillium marneffei*
Candida	*C. albicans*, *C. guilliermondii*, *C. krusei*, *C. lusitaniae*, *C. parapsilosis*, *C. pseudotropicalis*, *C. rugosa*, *C. stellatoidea*, *C. tropicalis*, *Torulopsis* (or *Candida*) *glabrata*
Cryptococcus	*C. neoformans* var. *neoformans*, *C. neoformans* var. *gattii*
Aspergillus	*A. fumigatus*, *A. flavus*, *A. niger*, *A. terreus*, *A. nidulans*
Mucorales	*Rhizopus oryzae*, *R. arrhizus*, *R. rhizopodiformis*, *Rhizomucor pusillus*, *Absidia oryzae*, *A. corymbifera*, *A. ramosa*, *Mucor circinelloides*
Histoplasma	*H. capsulatum*
Coccidioides	*C. immitis*
Blastomyces	*B. dermatitidis*
Sporothrix	*S. schenckii*
Fusarium	*F. solani*, *F. oxysporum*, *F. moniliforme*, *F. dimerum*, *F. chlamydosporum*, *F. anthophilum*
Trichosporon	*T. beigelii*, *T. capitatum*
Geotrichum	*G. candidum*
Rhodotorula	*R. rubra*
Dematiaceous molds (pheohyphomycetes)	*Pseudallescheria boydii* (*Scedosporium apiospermum*), *Bipolaris*, *Alternaria*, *Scedosporium prolificans*

Table 178.2 Variables that likely account for the current trends in the epidemiology of opportunistic fungal infections[1]

- Increasing number of susceptible hosts
- Greater laboratory expertise in the detection and identification of fungi
- Use of new transplantation modalities for hematopoietic stem cell transplantation (e.g. $CD34^+$ selected autografts and peripheral blood stem cell transplantation)
- Evolution in organ transplantation practices
- Advances in surgical technology
- Use of corticosteroid-sparing regimens and an overall conservative approach to immunosuppression
- Use of novel immunosuppressive agents
- Use of antimicrobial prophylactic practices (e.g. use of fluconazole for antifungal prophylaxis, ganciclovir for cytomegalovirus prophylaxis, quinolones for Gram-negative bacterial prophylaxis)

factors for nosocomial colonization and infection with *Candida* spp. were underlying diseases (hematologic cancer (OR 1.7–45); renal failure (OR 1.4–22); hepatic failure (OR 7–42)); invasive procedure or devices (central venous or arterial catheter (OR 5.8–26); urinary catheter (OR 13)); interhospital transfer of a patient (OR 21), prolonged use of multiple antibiotics (OR 1.7–25) and especially the usage of vancomycin (OR 275).[6] In mice and humans, vancomycin has been shown to enhance gastrointestinal colonization with *C. albicans*.[3]

Among HSCT recipients, an overall decrease has been documented in the frequency of candidal infections, as well as a shift toward isolation of non-*albicans* species of *Candida*.[8,9] It has been proposed that use of fluconazole as antifungal prophylaxis largely accounts for these trends.[8] Antifungal prophylaxis with fluconazole during neutropenia and acute graft-versus-host disease (until day 75 after transplantation) was associated with a significant reduction in the incidence of invasive candidiasis and improved survival rates.[9] However, although *C. albicans* was the most common colonizing isolate before transplantation, fluconazole-resistant species such as *C. krusei* and *C. glabrata* were isolated after transplantation and exposure to fluconazole.[9] In another study, fluconazole prophylaxis was the most important determinant for the relative increase in isolation of *C. krusei* (OR 27.07) and *C. glabrata* (OR 5).[8]

A meta-analysis of 16 randomized controlled trials, however, showed that fluconazole prophylaxis in neutropenic non-bone marrow transplant patients did not decrease fungus-related mortality or systemic fungal infections.[10] Moreover the mortality due to invasive candidiasis was significantly higher than that due to invasive aspergillosis in adult HSCT-recipients (48.9% vs 35.5%, respectively).[11]

Among organ transplant recipients, invasive *Candida* infections are most relevant for liver and pancreas transplant recipients. An overall decline in the incidence of invasive candidiasis has been noted in liver transplant recipients, even in the absence of systemic antifungal prophylaxis: many centers now report incidences of less than 10%.

Molecular typing has shown that in the majority of cases, candidemia arises from an endogenous origin after previous colonization. This holds true for *C. albicans* and most non-*albicans Candida* spp. except for infections with *C. parapsilosis*, which are thought to arise mainly from infected biomaterials, intravenous fluids or the hands of health-care workers. Also, human-to-human transmission (patient-to-patient, nurse-to-patient and between sexual partners) has become increasingly important.

Recurrence of oropharyngeal candidiasis in patients who have AIDS has been shown to be mostly due to recurrence of the same strain (relapse), although infection with a new strain also occurs.

PATHOGENICITY

Intact barrier function is an essential feature of host defense against candidiasis. The virulence of the *Candida* spp. has been shown to correlate with its ability to adhere to epithelial cells (especially *C. albicans*) or plastic polymers such as intravascular or urethral catheters (*C. tropicalis*). The fungus is capable of secreting proteinases and lipases that can assist invasion, although the clinical importance of these enzymes is not clear.[12]

After candidal invasion of the dermis or bloodstream, neutrophils constitute the first line of defense, followed by monocytes and eosinophils, which can kill *Candida* spp. via oxidative and nonoxidative pathways. Patients who have neutropenia are particularly at risk of developing candidiasis, which underscores the importance of neutrophils in host defense against this fungus. The clinical outcome of infection is primarily determined by the host defense status. Clinical and experimental data suggest that:

- an impairment of acquired cellular immunity (e.g. in HIV infection) predisposes mainly to mucocutaneous candidiasis (gastrointestinal and vaginal); and
- impaired innate immunity, especially neutrophil function, with or without impaired T-lymphocyte function, is the major risk factor for the development of systemic candidiasis.

In the rare chronic mucocutaneous candidiasis syndrome, various combinations of defect in T-lymphocyte response to *Candida* spp. have been described[13,13a,13b] (see Chapter 72).

The role of humoral acquired immunity in the body's defense against candidal infection remains to be determined,[12] although evidence is increasing that the innate immunity is an important part of the host defense against *Candida*.[14]

DIAGNOSTIC MICROBIOLOGY

In general, a diagnosis of candidiasis faces the dual problem of:

- differentiating between colonization and infection; and
- underdetection of deep-seated infection.

In culture, *Candida* spp. grow rapidly at 77–98.6°F (25–37°C) on simple media. On special culture media, hyphae or elongating pseudohyphae are formed. *Candida* spp. grow in routine ventilated blood culture flasks and on agar plates as smooth creamy white colonies. A differential culture medium (CHROMagar Candida) can distinguish between *C. albicans* and certain non-*albicans Candida* spp.

The yield from blood cultures in disseminated disease using commercial blood culture systems is approximately 80%, but low for certain *Candida* spp., such as *C. glabrata*. However, improved culture techniques such as biphasic cultures and lysis–centrifugation may greatly improve the diagnostic yield (Table 178.3).[15]

Specimens for microscopic evaluation give a better diagnostic yield after treatment with 10% potassium hydroxide, which lyses epithelial cells. The demonstration of blastoconidia (budding yeast), hyphae and pseudohyphae is highly suggestive but not diagnostic for tissue invasion. Staining with optical brighteners such as calcofluor white or Blankophor P is a sensitive method for the detection of fungi, but requires a fluorescent microscope. Although in the routine clinical microbiology laboratory the Gram stain is commonly used to detect yeast in blood culture slides (yeasts commonly stain Gram-positive), morphologic details are more difficult to detect compared to staining with an optical brightener. Spores from molds in blood culture, which might be present in invasive fusariosis or scedosporiosis, might then be misinterpreted as yeast infection. *Candida* species identification can be achieved by the germ tube test, which enables identification of *C. albicans* and *C. dubliniensis* (but not most non-*albicans Candida* spp.) by showing the formation of hyphal elements within 90 minutes. Alternatively, rapid species identification can be achieved directly on positive blood culture bottles using peptide nucleic acid fluorescent *in situ* hybridization (PNA-FISH).[16]

Table 178.3 Detection of fungal infections

Diagnostic technique	Major features	Useful	Not useful
Microscopy/histopathology	Rapid Relies on distinctive appearance of organism	Histopathologic identification of: *Cryptococcus* *Blastomyces* *Histoplasma* *Coccidioides*	Cannot give a specific species classification for: *Aspergillus* *Candida*
Culture-based methods			
• Traditional culture	Inexpensive	*Cryptococcus* grows rapidly *Aspergillus* – tissue sample	Slow growth for most endemics Poor sensitivity for *Candida* and *Aspergillus* blood samples
• Automated blood culture methods	Early detection of growth Capital expense	*Candida* and bloodstream infections *Cryptococcus* and *Histoplasma*	No value for *Aspergillus*
Nonculture methods			
• Antigen	Sensitive and specific	*Cryptococcus* and *Histoplasma* *Aspergillus*–galactomannan may be useful	No reliable tests for other mycoses
• Antibody	Moderately sensitive and specific	Endemic mycoses	No reliable tests for opportunistic fungi
• PCR	Still experimental	Potential use for *Candida* and *Aspergillus*	

Early initiation of adequate antifungal therapy was shown to have significant impact on hospital mortality, with those patients receiving very early antifungal therapy showing a significant survival benefit.[17,18] However, as the time to detection for commercial blood culture systems is too long for blood culture to be used as an early marker to initiate antifungal therapy,[15] there is a need for biologic markers that help to identify patients who require early antifungal therapy in order to benefit from this window of opportunity and to reduce hospital mortality. Biologic markers that detect *Candida* spp. and are available commercially include cell wall components such as mannan and antimannan antibody and (1,3)-β-D glucan (BG).

The diagnostic sensitivity and specificity of detection of mannan and antimannan reported in patients with candidemia was encouraging, but only if the detection of antigen and antibody was combined.[19] The cell wall component BG is present in many fungi, including *Candida* spp., and may circulate in patients with invasive candidiasis.[20] This antigen may be a useful tool for identifying patients who require antifungal therapy in the intensive care unit (ICU) setting. However, serum BG levels are not specific for fungal infection and may also be positive in bacteremic patients.[21,22]

Recent studies indicate that the performance of these biomarkers in patients with hematologic malignancy depends on the phase of the treatment of the underlying condition.[23] Circulating antibodies could be detected in patients who developed candidemia after their first episode of neutropenia and before blood cultures yielded yeast, indicating that *Candida* infection may occur long before clinical manifestation of the disease.[24] Molecular diagnosis by polymerase chain reaction (PCR) appears very promising since fungal DNA can be detected in the blood of infected patients before conventional methods. Furthermore, a broad range of yeasts and molds can be identified to species level. Automation of sample preparation and use of real-time PCR systems will help standardize the procedure and reduce false-positive results due to contamination, but commercial and clinically validated formats are not yet available.

Susceptibility testing and interpretative breakpoints

Intensive efforts in the USA and Europe to develop standardized, reproducible and clinically relevant susceptibility testing methods for the fungi have resulted in reference documents from the Clinical Standards Laboratory Institute (CLSI/NCCLS M27-A3) and from the European Committee on Antimicrobial Susceptibility Testing (EUCAST Definitive Document E.DEF 7.1).[25,26]

An important requirement for an *in-vitro* susceptibility test is that a reproducible result is obtained and that the assay is capable of separating wild-type isolates from non-wild-type isolates, i.e. those isolates that harbor a resistance mechanism. It has been shown with *Candida* that even subtle methodologic factors can individually or collectively impact on the chosen endpoint and the minimum inhibitory concentration (MIC) result.[27] Both CLSI and EUCAST methods allow reading after 24 hours of incubation which provides clinicians with useful susceptibility information sooner.

Breakpoints have been established for many antifungal agents although different approaches were followed by the CLSI and EUCAST. For breakpoint determination the EUCAST Antifungal Susceptibility Testing Subcommittee has taken into account pharmacokinetic (PK)–pharmacodynamic (PD) data of the specific drug, dosing regimens, toxicology, resistance mechanisms, wild-type MIC distributions and clinical outcome data.[28] The validation of the EUCAST method for fluconazole was performed using clinical data sets from patients with oropharyngeal candidiasis and candidemia. Using the EUCAST method the response for those infected with a strain with a MIC of 4 mg/l was 66% but reached 100% when the dose was greater than 100 mg/day, whereas the response for those infected with strains with MICs >8 mg/l was only 12%. Hence, an MIC of 2 mg/l or 4 mg/l was able to predict successful treatment.[28] Others have also shown that the fluconazole MIC of the isolate has a major impact on the response of the patient as well as on hospital mortality.[29]

Reliable and convincing interpretive breakpoints are not yet available for amphotericin B. The reference methods do not reliably identify amphotericin B-resistant isolates. However, amphotericin B resistance appears to be uncommon among isolates of *C. albicans*, *C. tropicalis* and *C. parapsilosis*.

Antifungal resistance

Resistance is relatively uncommon in patients with invasive candidiasis and the level of resistance has been stable below 3% of bloodstream isolates for many years. Resistance to fluconazole was common

in oropharyngeal candidiasis in the period prior to the introduction of highly active antiretroviral therapy (HAART) and multiple underlying resistance mechanisms were identified. In fungi, acquired resistance appears to develop primarily when a large number of micro-organisms are exposed for a relatively long period to a fungistatic drug. With the use of HAART and the greater number of alternative drugs with a different mode of action, resistance can be managed more successfully.

Elevated MIC values of echinocandins with occasional treatment failure have been reported for strains of *Candida*, and resistance was associated with amino acid substitutions in two 'hot-spot' regions of Fks1, the major subunit of glucan synthase.[30] The Fks1-mediated resistance mechanism is conserved in a wide variety of *Candida* spp. and can account for intrinsic reduced susceptibility of certain species. Fks1 mutations confer resistance in both yeasts and molds, and sporadic *Aspergillus fumigatus* isolates resistant to echinocandins have been found.

CLINICAL MANIFESTATIONS

The clinical manifestations of *Candida* infection can be divided into mucocutaneous infections and deep-seated infections. Mucocutaneous infections include thrush, candidal esophagitis, nonesophageal gastrointestinal candidiasis, candidal vaginitis and cutaneous candidiasis syndromes. Deep-seated infections include chronic disseminated candidiasis (hepatosplenic candidiasis), candidemia and candidiasis of various organ systems.

Mucocutaneous candidiasis

The most common clinical manifestation of candidal infection is oral thrush (acute pseudomembranous candidiasis), which presents as curd-like plaques on examination. It is diagnosed by the demonstration of yeast hyphae and pseudohyphae in a Gram-stained direct smear, 10% potassium hydroxide preparation, calcofluor preparation or culture of scrapings. Oral thrush should alert the physician to the possibility of an underlying disease.

Candidal esophagitis is frequently associated with AIDS, lymphoma or leukemia, although it can occur in people who are not immunocompromised. In up to 30% of cases of esophagitis there are no oral lesions on examination. Seventy-five percent of women with vulvovaginal candidiasis have no risk factors. Diagnosis is made on the basis of the combination of clinical symptoms, microscopy with 10% potassium hydroxide (include a wet preparation as well to exclude *Trichomonas vaginalis* and clue cells) and/or culture. The vaginal pH in candidiasis should be in the normal range (4.0–4.5); a pH higher than 4.7 indicates bacterial vaginosis, trichomoniasis or a mixed infection.

Chronic mucocutaneous candidiasis is a relatively rare disease characterized by protracted and persistent infections with *Candida* spp. of skin, mucosal membranes, hair and nails, and is frequently associated with endocrinopathies or autoimmune disorders. Severe disease may prove fatal, usually due to bacterial sepsis. Disseminated candidiasis is a rare complication.[13]

Deep organ infection

Disseminated candidiasis and candidemia constitute major clinical and diagnostic problems. Overall, the recovery of blood cultures depends on the *Candida* spp., with the commercial blood culture systems being positive in less than 40% of candidemia cases due to *C. glabrata*, although increased blood volume, ventilation of culture flasks and Isolator lysis–centrifugation cultivation have improved the diagnostic yield to 82%. The value of surveillance cultures is controversial, although absence of *C. albicans* in surveillance cultures has a high predictive value for the absence of disseminated candidiasis in patients who have leukemia or lymphoma or in bone marrow transplant patients.[3] However, isolation of *C. tropicalis* in surveillance cultures is highly suggestive of disseminated infection because more than 50% of these patients will develop candidemia. Skin and eye lesions are present in only 10% of cases of disseminated candidiasis.

Virtually every organ can be infected by *Candida* spp. Candidal meningitis and encephalitis usually occur as a complication of disseminated candidiasis. In disseminated candidiasis, 62% of cases developed candidal myocarditis, with electrocardiographic changes mimicking infarction and supraventricular tachycardias. *Candida* spp. are the major cause of fungal endocarditis, with up to 41% of cases caused by non-*albicans* spp. In most cases, blood cultures are positive. *Candida* pneumonia is usually associated with disseminated candidiasis. Primary candidal pneumonia is rare. Diagnosis is based on transbronchial biopsy.

In abdominal surgery, heavy growth of *Candida* spp. in the first culture (intraoperative or from abdominal drain) or an increasing fungal load in serial cultures has been shown to be highly predictive of the development of candidiasis.

Infection of the urinary tract with *Candida* spp. is difficult to discriminate from colonization or from disseminated candidiasis. Microscopic urine analysis does not discriminate, unless renal casts containing yeasts are found, and quantitation of candiduria (such as is used for bacterial urinary infections) is not a reliable indicator of deep infection. Any patient who has persistent candiduria without a recent history of urinary tract instrumentation should be evaluated for diabetes mellitus, renal insufficiency or genitourinary tract abnormalities.[31]

'*Candida* hypersensitivity syndrome'

For many years, *C. albicans* has been mentioned as the cause of a chronic syndrome called *Candida* hypersensitivity syndrome, also known as chronic candidiasis, *Candida*-related complex and 'the yeast connection'.[32] Symptoms include general malaise, fatigue and nonspecific gastrointestinal, genitourinary and neuropsychiatric complaints.

The syndrome is said to be caused by intestinal and vaginal fungal overgrowth, inflammation, production of fungal toxins and invasion of mucous membranes. Therapy usually consists of a rigorous 'yeast elimination' diet and long-term antifungal treatment. In an effort to characterize the disease, 100 individuals who had chronic fatigue syndrome were evaluated, including eight who had been given a diagnosis of 'yeast connection'. No differences in laboratory or physical findings, including candidal skin testing, could be detected between patients and control subjects.[33] A prospective randomized clinical trial comparing nystatin treatment with placebo failed to show an improvement in the systemic complaints[34] and analysis of the diet demonstrated nutritional imbalances that could lead to the development of nutritional deficiencies if the diet were followed over a prolonged period of time. The American Academy of Allergy and Immunology reviewed the literature and concluded that the *Candida* hypersensitivity syndrome is 'speculative and unproven'.[35]

MANAGEMENT

Recently the mycoses study group of the Infectious Diseases Society of America (IDSA) has published practice guidelines for the treatment of invasive candidiasis.[36] These will be followed throughout this part of the chapter. The current drugs approved for treatment of systemic fungal diseases in the USA are listed in Table 178.4. Their mechanism of action is shown in Figure 178.1.

Mucocutaneous candidiasis

Oropharyngeal candidiasis

Initial mild disease can be treated with clotrimazole troches (one 10 mg troche five times daily) or nystatin (available as a suspension of 100 000 U/ml (46 ml q6h) or as flavored 200 000 U pastilles, one

Table 178.4 Drugs approved for treatment of systemic fungal diseases

Class	Generic name	Brand name	Available formulation(s)	Year initially approved
Polyene	Amphotericin B	Fungizone (Apothecon Products, Princeton, NJ)	Intravenous, oral solution	1958
Polyene	Amphotericin B lipid complex	Abelcet (Liposome, Princeton, NJ)	Intravenous	1995
Polyene	Amphotericin B cholesteryl sulfate	Amphotec (SEQUUS Pharmaceuticals, Menlo Park, CA)	Intravenous	1996
Polyene	Amphotericin B liposomal	AmBisome (Fujisawa Healthcare, Deerfield, IL)	Intravenous	1997
Pyrimidine	Flucytosine	Ancoban (ICN Pharmaceuticals, Costa Mesa, CA)	Oral tablet, intravenous	1972
Azole	Ketoconazole	Nizoral (Janssen Pharmaceutica, Titusville, NJ)	Oral tablet	1981
Azole	Fluconazole	Diflucan (Pfizer, New York, NY)	Intravenous, oral tablet, oral suspension	1990
Azole	Itraconazole	Sporanox (Janssen Pharmaceutica, Titusvilie, NJ)	Intravenous, oral capsule, oral solution	1992
Azole	Voriconazole	Vfend (Pfizer, New York, NY)	Intravenous, oral tablet	2002
Echinocandin	Caspofungin	Cancidas (MSD, Whitehouse Station, NJ)	Intravenous	2001

or two 4–5 times daily, for 7–14 days). Oral fluconazole (100–200 mg/day for 7–14 days orally) is recommended for moderate-to-severe disease. Recently it was shown that a single dose of fluconazole of 750 mg was as efficacious as a full 2-week course of 150 mg daily for the treatment of oropharyngeal candidiasis.[37] Recommended therapy for refractory disease includes itraconazole solution (200 mg/day for 7–14 days orally); voriconazole (200 mg/day orally); posaconazole (400 mg/day orally) or amphotericin B oral suspension.[36] Intravenous echinocandin or amphotericin B deoxycholate at 0.3 mg/kg per day were also shown to be effective and may be used as last-resort therapy in patients with refractory disease.

Although suppressive therapy is effective for the prevention of recurrent infections, to reduce the likelihood of development of antifungal resistance it should be used only if the recurrences are frequent or disabling. Denture-related disease may require thorough disinfection of the denture for definitive cure.

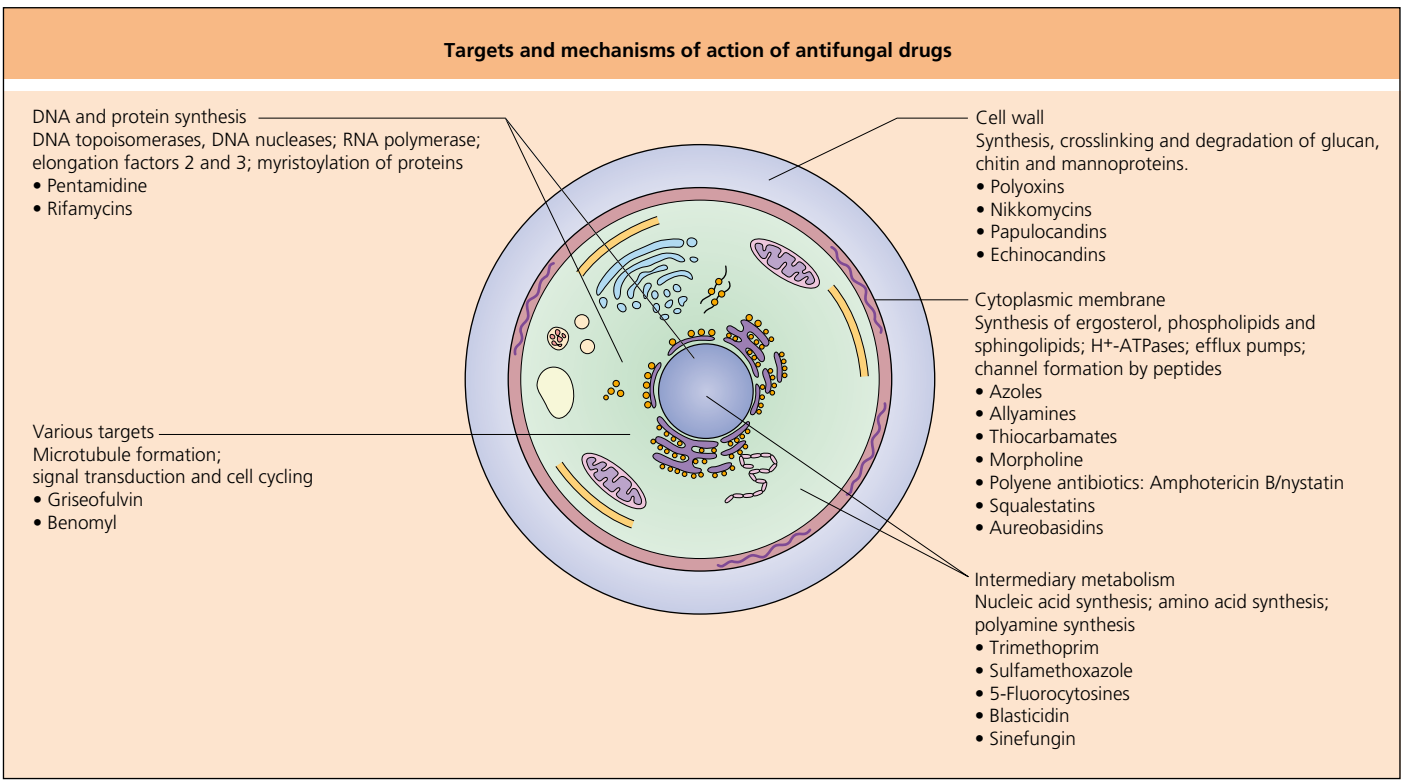


Fig. 178.1 Targets and mechanisms of action of antifungal drugs.

Esophageal candidiasis

Systemic therapy is required for effective treatment of esophageal candidiasis. Although symptoms of esophageal candidiasis may be mimicked by other pathogens, a diagnostic trial of antifungal therapy is often appropriate before endoscopy to search for other causes of esophagitis. A 14- to 21-day course of fluconazole (200–400 mg/day orally) is preferred. For patients who are unable to tolerate an oral agent, fluconazole may be administered intravenously or an echinocandin or amphotericin B deoxycholate at a dose of 0.3–0.7 mg/kg daily is considered appropriate. Suppressive therapy should be used occasionally in patients with disabling recurrent infections. Patients with refractory esophageal candidiasis should be treated with itraconazole oral solution (200 mg/day) or posaconazole 400 mg twice daily or voriconazole 200 mg twice daily. Intravenous amphotericin B (0.3–0.7 mg/kg per day as needed to produce a response) or an echinocandin may be used in patients with otherwise refractory disease.[36]

For *Candida*-associated denture stomatitis, the treatment of choice is fluconazole (50 mg/day for 2 weeks) combined with chlorhexidine.

Vulvovaginal candidiasis

This may be classified into complicated (severe, resistant species, impaired host defenses) and uncomplicated forms. Uncomplicated vaginitis is seen in 90% of patients and responds readily to short-course oral or topical treatment with any of the therapies listed above, including a single dose of fluconazole. In contrast, the recurrent complicated vaginitis seen in 10% of patients is defined as severe or recurrent disease, infection due to *Candida* spp. other than *C. albicans* and/or vulvovaginal candidiasis in an abnormal host. Complicated vulvovaginal candidiasis requires topical therapy administered intravaginally daily for 7 days or multiple doses of fluconazole (150 mg every 72 hours for three doses).[36] *Candida glabrata* may fail to respond to azole therapy, including voriconazole, and may respond to topical boric acid 600 mg/day for 14 days, topical 17% flucytosine cream alone or in combination with 3% amphotericin B cream, administered daily for 14 days. Azole-resistant *C. albicans* infections are rare.

Recurrent vaginitis is defined as four or more episodes of symptomatic vulvovaginal candidiasis within 1 year and is usually due to azole-susceptible *C. albicans*. After control of causal factors (e.g. uncontrolled diabetes), induction therapy with 2 weeks of a topical or oral azole should be followed by a maintenance regimen for 6 months. Recurrent vulvovaginal candidiasis is managed with fluconazole (150 mg orally every week). If fluconazole therapy is not feasible, topical clotrimazole (200 mg twice daily), clotrimazole (500 mg vaginal suppository once weekly) or other intermittent topical azole treatments are recommended.[36]

The persistent immunologic defect of chronic mucocutaneous candidiasis requires a long-term approach.[13] Systemic therapy is needed and all of the azole antifungal agents have been used successfully. The dosages required are similar to those used for other forms of mucocutaneous candidiasis. As with HIV-infected patients, development of resistance to these agents has also been described.

Systemic candidiasis

Candidemia and intravenous catheter-related candidal infections

Candida bloodstream infections are frequently associated with clinical evidence of the sepsis syndrome and high associated attributable mortality. In addition, hematogenous seeding may compromise the function of one or more organs.

All patients with a positive blood culture should be treated, even if the sample is drawn from a catheter. Additionally, indwelling intravascular catheters should be removed or changed, preferably without using a wire for replacement.[36] The evidence for this recommendation is strongest in the non-neutropenic patient population. In neutropenic patients, the role of the gut as a source for disseminated candidiasis is evident from autopsy studies, but in an individual patient it is difficult to determine the relative contribution of gut versus catheter as the primary source of fungemia. An exception is made for fungemia due to *C. parapsilosis*, which is very frequently associated with catheters.

The standard treatment for candidemia is fluconazole (loading dose of 12 mg/kg (800 mg), daily maintenance dose of 6 mg/kg (400 mg)) or an echinocandin (caspofungin: loading dose of 70 mg, then 50 mg/day; micafungin: 100 mg/day; anidulafungin: loading dose of 200 mg, then 100 mg/day). An echinocandin is favored for patients with moderately severe to severe illness or for patients with recent exposure to azoles.[36] Transition to fluconazole may be considered if isolates are susceptible to fluconazole, for infection due to *C. parapsilosis*, and in clinically stable patients. For infection with *C. glabrata*, an echinocandin is preferred. Voriconazole is effective for candidemia, but offers little advantage over fluconazole. The drug is recommended as step-down oral therapy for selected cases such as candidemia due to *C. krusei*. Polyenes, such as lipid-formulations of amphotericin B or amphotericin B deoxycholate, are alternatives if there is intolerance or in resource-limited situations.[36]

An echinocandin is recommended for most neutropenic patients. Fluconazole is an option in less critically ill patients and voriconazole if mold coverage is also warranted.

Antifungal treatment should be continued for at least 2 weeks after the last positive blood culture or after the clinical symptoms have subsided, and patients should be followed for 3–6 months to detect long-term sequelae due to hematogenous seeding.

Timely initiation of adequate antifungal therapy is important to improve clinical outcome[17,18] and empiric therapy should be considered in critically ill patients with risk factors for invasive candidiasis but with no known cause for the fever. For suspected candidiasis in non-neutropenic patients empiric therapy is similar to proven candidiasis with either fluconazole or an echinocandin as initial therapy. In neutropenic patients lipid-formulations of amphotericin B, echinocandins or voriconazole are recommended.

Recommended treatment of central nervous system (CNS) candidiasis is lipid formulation of amphotericin B (3–5 mg/kg) with or without flucytosine. The flucytosine dose should be adjusted to produce serum levels of 40–60 μg/ml. Fluconazole can be used as step-down therapy at a dose of 400–800 mg/day (6–12 mg/kg).

Treatment should be continued until all signs and symptoms, cerebrospinal fluid (CSF) abnormalities and radiologic abnormalities have resolved. Intraventricular devices should be removed. The occurrence of a brain abscess worsens the prognosis considerably. The indication for surgery remains to be determined but surgical drainage, if feasible, is probably advisable.[36]

Native valve *Candida* endocarditis should be treated with combined antifungal treatment (lipid formulation of amphotericin B with or without 5-fluorocytosine (5-FC)) and early valve replacement. An echinocandin may be considered as an alternative therapy. Without surgical intervention, the mortality is high (90%); with combined surgical and medical treatment, the mortality has dropped to 45%. Fluconazole therapy (400–800 mg/day) can be considered as step-down therapy in patients with susceptible isolates, who are clinically stable and who have clearance of *Candida* from the bloodstream. Treatment should continue for at least 6 weeks after valve replacement, but a longer duration is recommended in patients with perivalvular abscesses and other complications.

Because recurrences have occurred years later patients should be followed up for at least 2 years. Primary therapy with fluconazole has been successfully used on occasion, but fluconazole is more often employed as part of a long-term suppressive regimen.

In *Candida* urinary tract infection, the clinical circumstances dictate the management because candiduria can represent colonization, cystitis, pyelonephritis, disseminated candidemia or a fungus ball.

Treatment is not recommended for asymptomatic candiduria unless the patient belongs to a group at high risk of dissemination: neutropenic patients, low birth weight infants and patients who will

undergo urologic manipulations. Neutropenic patients and neonates with *Candida* urinary tract infection should be managed as described for invasive candidiasis, while those undergoing urologic procedures should receive fluconazole at a dosage of 200–400 mg/day or amphotericin B deoxycholate at a dosage of 0.3–0.6 mg/kg per day for several days before and after the procedure.[36]

For cystitis oral fluconazole at a dosage of 200 mg/day (3 mg/kg) for 2 weeks is recommended if the infection is due to a fluconazole-susceptible *Candida* spp. For fluconazole-resistant isolates amphotericin B deoxycholate (0.3–0.6 mg/kg per day) for 1–7 days or oral flucytosine (25 mg/kg four times daily) for 7–10 days is recommended. There is no role for echinocandins, voriconazole or posaconazole as these drugs do not achieve clinically useful levels in the urine. Bladder irrigation with amphotericin B (50 g/ml in sterile water or 5% glucose administered continuously over a three-way Foley catheter for 5–7 days) is not recommended but may be useful in fluconazole-resistant infection, most notably *C. glabrata*. If there is a fungus ball in the urinary tract, the treatment is surgical.

Peritonitis is usually classified based on the cause of the inflammatory process and is further differentiated into primary, secondary and tertiary peritonitis. In primary peritonitis, no breach in the gastrointestinal tract is present. Secondary peritonitis is the most frequent form of peritonitis and is the result of a local infectious process within the abdominal cavity, with or without a hollow viscous perforation, and can lead to diffuse peritonitis. Tertiary peritonitis is an ill-defined entity and is generally referred to as a persistent or recurrent peritonitis after initial adequate treatment for secondary peritonitis.[38] *Candida* is more frequently isolated in nosocomial peritonitis, especially in immunocompromised patients and those receiving broad-spectrum antibacterial agents. Upper gastrointestinal tract perforations are also commonly associated with *Candida* infection.[38]

In critically ill patients with secondary or tertiary peritonitis, the significance of the recovery of *Candida* spp. remains controversial. Therefore, the diagnosis should be based on specimens that are collected perioperatively and processing should include microscopic examination. As mortality rates of *Candida* peritonitis in critically ill patients are high, typically 50–75%, immediate antifungal therapy is recommended, preferably in combination with surgical intervention.[38] Drugs of choice include fluconazole for susceptible *Candida* isolates, and voriconazole or the echinocandins for unknown or less-susceptible species. Antifungal therapy should last for 2–3 weeks. Surgical patients with recurrent gastrointestinal perforation are at increased risk for *Candida* peritonitis and may benefit from prophylactic antifungal therapy.

Chronic disseminated candidiasis (hepatosplenic candidiasis) is difficult to treat; this syndrome is not acutely life-threatening but does require prolonged therapy to produce a cure. Fluconazole at 6 mg/kg per day is generally preferred in clinically stable patients. Lipid formulations of amphotericin B at a dosage of 3–5 mg/kg per day or amphotericin B deoxycholate at 0.5–0.7 mg/kg per day may be used in acutely ill patients or patients with refractory disease. Some but not all experts recommend an initial 1–2 week course of amphotericin B for all patients, followed by a prolonged course of fluconazole. The echinocandins are alternatives for initial therapy.[36] Therapy should be continued until calcification or resolution of lesions, particularly in patients receiving continued chemotherapy or immunosuppression. Premature discontinuation of antifungal therapy may lead to recurrent infection.

Prophylaxis

Antifungal prophylaxis is highly controversial because the relationship between candidal colonization and dissemination is often not clear and azole prophylaxis may select for *Candida* spp. that are resistant to azoles.

Postoperative prophylaxis is recommended for high-risk liver, pancreas and small bowel transplant recipients with fluconazole at a dosage of 200–400 mg (3–6 mg/kg per day) or with liposomal amphotericin B at a dosage of 1–2 mg/kg per day. For ICU patients fluconazole prophylaxis has only been shown to be beneficial in adult units with high-risk patients and a high incidence of invasive candidiasis.[39] In hematology patients with chemotherapy-induced neutropenia, fluconazole at a dosage of 400 mg (6 mg/kg) daily, posaconazole at a dosage of 200 mg three times daily or caspofungin at a dose of 50 mg daily have been shown to prevent invasive fungal infection when administered for the duration of the neutropenia.[36] Itraconazole oral solution at a dosage of 200 mg/day is considered an alternative but is commonly less well tolerated. For HSCT recipients with neutropenia fluconazole at a dosage of 400 mg (6 mg/kg) daily, posaconazole at a dosage of 200 mg three times daily or micafungin at a dosage of 50 mg daily is recommended during the period of risk of neutropenia.[36] Besides pharmacologic prophylaxis, simple measures such as hand disinfection by medical personnel are of paramount importance, especially in preventing the spread of *C. parapsilosis*.[4]

Aspergillosis

NATURE

Aspergillus spp. can cause disease in humans by:

- colonization and subsequent allergic reactions;
- colonization of pre-existing cavities (fungus ball or aspergilloma); or
- tissue invasion.

Aspergilli are found ubiquitously in organic debris such as hay, decaying vegetation (compost piles), soil, potted plants, in pepper and spices and in construction sites. The genus *Aspergillus* contains over 250 species, although only a few are associated with disease. Recently the use of polyphasic taxonomy, which combines phenotypic features of the fungi with molecular sequence analysis, has significantly changed the classification of aspergilli. New cryptogenic species have been identified that cannot be differentiated from conventional species by microscopic morphologic features. For instance, within *Aspergillus* section *Fumigati* 30 new species have been described.[40] The clinical implications of the new species are unknown, although *in vitro* several new species are less susceptible to licensed antifungal agents compared to the conventional species.

EPIDEMIOLOGY

Worldwide, aspergillosis is the most common invasive mold infection.[41]

In a large cohort of 11 802 patients with hematologic malignancies it was shown that the overall incidence of invasive fungal infection was 4.6% with 69% of cases occurring in patients with acute myeloid leukemia.[42] Mold infection was observed in 2.9% of patients, with *Aspergillus* spp. being responsible for almost 90% of cases. Autopsy studies indicate that approximately 15% of patients have a mixed fungal infection which is not diagnosed during life in the majority of patients. In hematology patients with invasive aspergillosis the survival at 12 weeks was 52.2%.[43] Receipt of an allogeneic HSCT or solid-organ transplant, progression of underlying disease, prior respiratory disease, receipt of corticosteroid therapy, renal impairment, low monocyte counts, disseminated aspergillosis, diffuse pulmonary lesions, pleural effusion, and proven or probable (as opposed to possible) aspergillosis were predictors of increased overall mortality.[43] In most centers *Aspergillus fumigatus* is the most prevalent species and the lungs and the brain are the most common sites of infection.

The reported prevalence of allergic bronchopulmonary aspergillosis (ABPA) in patients who have asthma varies from 6% to 28%. In cystic fibrosis, 6–25% of patients are reported to have ABPA.[44]

PATHOGENICITY

The main route of infection in aspergillosis is airborne, via inhalation of the 2.5–3.0 µm conidia (spores), which settle in lungs, nose or paranasal sinuses. However, most studies suffer from a lack of correlation between air spore counts and the rate of infection. Hospital water supply has also been recognized as a source of infection, but transmission via water appears to play a modest role compared to the airborne route.[45] Other routes of infection are traumatized skin, especially due to burns, insertion openings of indwelling intravenous catheters and intravenous drug use. Patient-to-patient transmission is very uncommon, but has been reported via transplantation of an infected organ and through a patient with skin aspergillosis.

The main risk factors for invasive aspergillosis include prolonged profound granulocytopenia ($<0.5 \times 10^9$/l), especially in allogeneic HSCT recipients, high-dose corticosteroid therapy, chronic granulomatous disease, AIDS with a $CD4^+$ lymphocyte count below 50/l, and treatment with cytotoxic drugs such as ciclosporin. Less commonly encountered risk factors are diabetes mellitus, alcohol excess, influenza, prematurity and exposure to aspergilli in large quantities.[46]

In chronic granulomatous disease, phagocytes are unable to generate the respiratory burst to kill micro-organisms. The fact that these patients and patients who have neutropenia are at risk of developing invasive aspergillosis underscores the importance of phagocytes in host defense. Alveolar macrophages and neutrophils play a major role in the defense against aspergilli.[47] Alveolar macrophages form the first line of defense by ingesting and killing conidia, even in the absence of opsonins. When conidia escape and germinate to form hyphae, granulocytes will adhere to and kill the fungus. The conidia that have not been cleared may germinate in the alveolar spaces and hyphal forms invade the pulmonary tissue and vasculature, leading to hematogenous dissemination to other organs (brain). However, blood cultures are rarely positive, except for *A. terreus*. Conidia that stay in the dormant state can survive for many days in the reticuloendothelial system. Killing of aspergilli by phagocytes is impaired by corticosteroids.

The role of humoral immunity is double edged. In ABPA, aspergilli elicit an anti-*Aspergillus* IgE antibody response, which evokes a type I immediate hypersensitivity reaction. Anti-*Aspergillus* IgG antibodies have been demonstrated in ABPA, aspergilloma and in the convalescence phase of invasive aspergillosis. These antibodies do not seem to play a significant role in host defense.

The fungus secretes various metabolic products (gliotoxin) and enzymes, such as phospholipases, hemolysin and elastase, which may play a role in virulence.[46] However, the biologic significance of these agents is not clear, since molecular studies in which gene-deletion mutants have been constructed do not show diminished virulence.

DIAGNOSTIC MICROBIOLOGY

Aspergillus is a mold that grows in tissue with dichotomously branching and regular septate hyphae (Fig. 178.2). Optical brighteners, such as Blankophor P, can be used to detect fungal elements directly in clinical specimens. However, the microscopic characteristics are not specific for *Aspergillus* spp., as other molds such as *Fusarium* or *Scedosporium* have similar microscopic morphology. At best the *Aspergillus*-like microscopic morphology can be differentiated from zygomycetes, which present as nonseptate, irregular hyphae that commonly branch at 90°. Therefore, identification to the species level can be achieved only by culture or molecular tests. *Aspergillus* spp. readily grow on standard bacteriologic media, although mycologic media will give a higher yield and are therefore recommended. Identification to the species level should always be performed with clinically relevant isolates, as *Aspergillus* spp. differ in their susceptibility to antifungal agents.

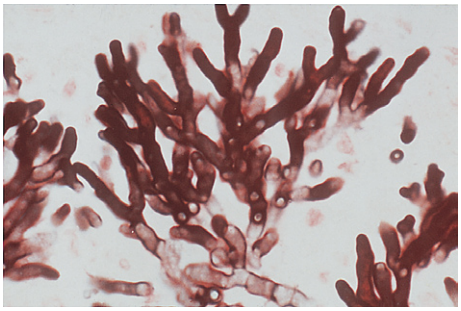


Fig. 178.2 *Aspergillus* hyphae.

Aspergilli can be isolated from the sputum of 1–6% of healthy individuals. Also, *Aspergillus* colonization is found in patients who have chronic lung disease, cigarette smokers and those who have HIV infection. In these patient groups a positive culture is often not associated with invasive disease.[48] However, for high-risk patients, such as allogeneic bone marrow transplant recipients (60%), persons with hematologic cancer (50%), and those with signs of neutropenia (60%) or malnutrition (30%), a positive culture result is associated with invasive disease.[48]

In the high-risk patients the diagnostic yield of cultures is low and a negative culture does not rule out the presence of invasive disease. Therefore, other tests and procedures, such as nonculture-based methods and high-resolution CT scan, are used to diagnose invasive aspergillosis. The detection of circulating galactomannan (GM) by Enzyme Immuno Assay (Platelia Aspergillus, BioRad Laboratories, France) has become an important tool in the management of neutropenic patients with hematologic malignancy, although significant heterogeneity has been reported between centers using this assay.[49] Often GM is detected in serial serum samples although the antigen can also be detected in bronchoalveolar lavage (BAL) fluid or CSF.[50] Serum reactivity has been reported in patients treated with certain antibiotics such as piperacillin–tazobactam and the diagnostic sensitivity is significantly reduced in patients receiving mold-active antifungal agents.[51]

Other nonculture-based methods include (1,3)-β-D glucan (BG) antigen which is released by a variety of fungi and circulates in patients with invasive fungal infection. BG is released by *Candida*, *Aspergillus* and *Fusarium*, but infections due to zygomycetes and *Cryptococcus* will not be detected. In addition, extensive research has been performed to investigate the utility of PCR for the diagnosis of invasive aspergillosis. Although numerous studies have shown that circulating *Aspergillus* DNA can be detected early in high-risk patients, a clinically validated commercial assay is currently not available.[52]

To facilitate the validation of diagnostic tools and the performance of clinical trials, consensus definitions have been proposed. These definitions are based on host factors, clinical features (including radiology) and mycology and were recently revised.[53] In addition to culture, GM and BG are accepted as mycologic evidence for invasive aspergillosis, but not PCR.

CLINICAL MANIFESTATIONS

Aspergillosis can manifest as either fungal colonization, which can cause allergic disease and saprophytic disease (aspergilloma), or invasive disease.

The spectrum of disease caused by *Aspergillus* spp. includes:

- noninvasive aspergillosis – sinotracheobronchial colonization, allergic (bronchopulmonary) aspergillosis, aspergilloma (i.e. secondary saprophytic colonization of pre-existing cavities) and obstructing bronchial aspergillosis (mainly in patients who have AIDS); and
- invasive aspergillosis with pulmonary involvement such as acute bronchopneumonia, cavitation or slowly progressive bronchopneumonia, or extrapulmonary involvement of paranasal sinuses, CNS, skin, bone and heart.

Allergic bronchopulmonary aspergillosis

Allergic aspergillosis can either manifest as an extrinsic allergic alveolitis, causing a hypersensitivity pneumonitis in nonatopic patients, or it can cause ABPA in atopic individuals.

The major criteria for the diagnosis of ABPA are:[44]

- asthma;
- immediate cutaneous reaction to *A. fumigatus*;
- total serum IgE concentration (>1000 ng/ml);
- elevated *A. fumigatus*-specific serum IgE levels;
- precipitating antibodies to *A. fumigatus* in the serum;
- peripheral blood eosinophilia (not essential for diagnosis);
- chest roentgenographic infiltrates (not essential for diagnosis); and
- central bronchiectasis.

Eight criteria for ABPA diagnosis were initially identified, but only some of them are essential. The nonessential criteria (e.g. pulmonary infiltrates or blood eosinophilia) may be present only at the time of exacerbation or during the acute phase of the disease. The presence of bronchiectases in ABPA signifies more permanent lung damage.[44]

Aspergilloma

Aspergillomas are fungus balls that colonize pre-existing cavities in the lungs and occasionally in the sinuses and the nose. They can cause massive hemoptysis, which is fatal in 10% of cases.[46] Other symptoms can include fever, malaise and weight loss. A pulmonary CT scan may show a cavitary mass surrounded by air. Microscopic and cultural identification of *Aspergillus* spp. in a sputum smear and high antibody titers to *Aspergillus* support the diagnosis. Over 95% of patients have detectable IgG antibodies to *Aspergillus*, and antibody levels usually decrease after successful surgical removal of the aspergilloma.

Aspergillomas have been subdivided into simple and complex according to the radiologic appearance. Other chronic forms of *Aspergillus* infection of the lung that have systemic symptoms have been described. These entities include chronic necrotizing pulmonary aspergillosis (CNPA), semi-invasive aspergillosis, chronic invasive pulmonary aspergillosis, symptomatic pulmonary aspergilloma and *Aspergillus* pseudotuberculosis.[54] Diagnostic criteria for chronic pulmonary aspergillosis with response parameters have been proposed.[54]

Invasive aspergillosis

Invasive aspergillosis is a life-threatening aggressive disease that affects immunocompromised patients. High-resolution CT has proved to be a more sensitive and specific tool than conventional chest X-ray in neutropenic patients to diagnosis invasive aspergillosis. Lesions associated with invasive aspergillosis include nodular infiltrates, the halo sign and the air crescent sign, but these may be due to other micro-organisms such as other molds or bacteria.[55] In the absence of specific clinical symptoms and sensitive diagnostic tools, high-risk patients commonly received antifungal therapy empirically if their fever persisted for more than 3–5 days despite broad-spectrum antibacterial therapy. However, the availability of GM and high-resolution CT has enabled an alternative approach in which antifungal therapy is started pre-emptively if circulating antigen is detected and CT lesions consistent with fungal disease are observed.[56] Prospective studies that compare the benefits of management strategies, i.e. empiric versus pre-emptive treatment strategies, are currently underway.

Although HSCT recipients and other patients with hematologic malignancy are at highest risk for invasive aspergillosis, the disease is increasingly reported in critically ill patients.[57] The diagnosis of invasive aspergillosis in this patient group is challenging as circulating GM is detected in only 42% of patients. Culture and GM detection in BAL fluid appeared to be most useful, with a sensitivity of 88% for GM detection.[57]

Invasive aspergillosis is classified according to the revised consensus definitions in the categories proven, probable or possible disease.[53] For definite proof of invasive aspergillosis, a biopsy is required which shows tissue invasion by hyphae in combination with identification of an *Aspergillus* species. CT-guided lung biopsy might be a feasible tool, even in thrombocytopenic patients, with a promising diagnostic yield.[58]

Although *in-vitro* susceptibility testing is not routinely performed in invasive aspergillosis, the emergence of azole-resistant aspergillosis was recently described.[59] The *A. fumigatus* isolates were cross-resistant against azoles and the presence of resistance was associated with treatment failure.[59] In patients failing azole therapy it seems appropriate to determine the *in-vitro* activity of antifungal agents to help guide the treatment.

Extrapulmonary aspergillosis

Extrapulmonary dissemination of pulmonary aspergillosis, especially to the brain, is a well-known complication in the immunocompromised host. Meningitis due to *Aspergillus* spp. can occur, but is rare. A multivariate discriminant analysis of autopsy-proven fungal infections of the CNS demonstrated that a combination of pulmonary infiltrates and focal neurologic disease in an immunocompromised patient is more likely to be caused by *Aspergillus* spp. than by either *Candida* spp. or *Cryptococcus neoformans*. Definitive diagnosis is made by biopsy,[53] although the detection of GM in CSF is also highly indicative of CNS aspergillosis.

Virtually any organ can be infected by *Aspergillus* spp. The fungus may cause local infection in the ear or eye (endophthalmitis, keratomycosis). Direct bony invasion or hematogenous spread can occur, causing osteomyelitis. In the gastrointestinal tract, aspergillosis can lead to fatal perforation. Black necrotizing skin lesions can be a sign of disseminated aspergillosis. As in candidiasis, aspergilli can form fungal balls in the urinary tract, which present as renal colic.

Fungal sinusitis

Fungal sinusitis should be considered in all patients who have chronic sinusitis.[60] Clinical features and predisposing conditions are listed in Table 178.5. It is important to distinguish between noninvasive and invasive sinusitis because the latter can progress through invasion into soft tissue, cartilage and bone into the palate and nose or it can invade cerebral blood vessels, resulting in ischemic infarction and direct infection of the brain.

Chronic indolent invasive sinonasal infections occur in immunocompetent hosts in regions with high levels of environmental spores, such as the Sudan, Saudi Arabia and other tropical or desert areas, and occasionally in patients with diabetes in other locales. *Aspergillus flavus* is the most common causative agent of these infections, in contrast to the frequent isolation of *A. fumigatus* from sites of infection in immunocompromised hosts. These infections have a progressive clinical course over months to years, with invasion of the surrounding tissues: the ethmoid sinuses, orbit and subsequent cranial bone osteomyelitis and intracranial extension.

Radiologic findings associated with fungal sinusitis are calcifications and loss of bony sinus margins, as well as features that are common in bacterial sinusitis, such as air–fluid levels of more than 8 mm of mucoperiosteal thickening.

To distinguish between invasive and noninvasive fungal sinusitis, adequate tissue biopsies as described in Table 178.5 are necessary. The presence of hyphae excludes chronic bacterial sinusitis. In superficial fungal disease, hyphae are found only in mucopurulent material within the sinus. In invasive fungal infection, hyphae penetrate into the sinus submucosa, blood vessels or bone. The main differential diagnosis is zygomycosis.

Aspergillosis and AIDS

Aspergillosis and AIDS is discussed in Chapter 91.

Table 178.5 Key clinical points in the diagnosis and treatment of fungal sinusitis

Type	Clinical clues	Most common causes	Diagnosis	Initial management
Noninvasive fungal sinusitis	Immunocompetent patient; intractable symptoms despite adequate treatment for bacterial sinusitis; allergic rhinitis, asthma, nasal polyps. Calcifications in sinus on CT; proptosis in children	Hyaline molds; *Aspergillus* spp.; *Fusarium* spp.; dematiaceous molds; *Bipolaris* spp.; *Curvularia lunata*; *Pseudallescheria boydii*	Aspiration of sinus contents should be followed by silver impregnation staining and culture of aspirate; sinus contents often have the consistency of peanut butter or cottage cheese; in patients who have diabetes mellitus or other conditions involving immunocompromise, biopsy of healthy and diseased mucosa and bone should be considered to rule out tissue invasion	Surgery is necessary to establish drainage and to remove impacted mucus, polyps or fungus ball
Invasive fungal sinusitis	Fever, headache, epistaxis and cough in an immunocompromised patient; diabetes mellitus; hemochromatosis; protein-calorie malnutrition; leukemia; neutropenia. Nasal mucosal ulcer or eschar; calcifications in sinus on CT; orbital apex syndrome; proptosis in adults	Hyaline molds, zygomycetes; *Rhizopus oryzae*; *Cunninghamella bertholetiae*; *Aspergillus* spp.; *Fusarium* spp.; dematiaceous molds; *P. boydii*	Early endoscopic evaluation should be followed by biopsy of healthy and diseased mucosa and bone; sinus contents should be followed by silver impregnation staining culture of aspirate; if the results of endoscopic evaluation are negative, open biopsy should be performed immediately	Emergency surgery is necessary to remove necrotic and devitalized tissue; treatment with amphotericin B should be initiated on demonstration of tissue invasion and before culture results become available; immunosuppression should be reversed including discontinuation of corticosteroids and treatment of iatrogenic neutropenia

Adapted from DeShazo *et al.*[60]

MANAGEMENT[61]

In ABPA, the treatment of choice is a combination of systemic corticosteroids and itraconazole. Two double-blind, randomized, placebo-controlled trials have shown a benefit of adding itraconazole to corticosteroid therapy, which is the mainstay of therapy for ABPA. The mechanism of this effect is to eradicate *Aspergillus* species from the lung and diminish the antigenic stimulus for bronchial inflammation. The trials demonstrated that adding itraconazole had a significant ability to ameliorate disease, as assessed by the reduction in corticosteroid dose, increased interval between corticosteroid courses, eosinophilic inflammatory parameters and IgE concentration, as well as improvement in exercise tolerance and pulmonary function.[44,62] Itraconazole may be useful as a corticosteroid-sparing agent. Detailed therapeutic guidelines are provided by Walsh *et al.*[61]

For aspergillomas, the natural history is variable and therapy must be individualized. A conservative approach is prudent. Surgical resection (or where available, selective radiologic embolization of the feeding vessels) is indicated for hemoptysis. Spontaneous resolution of pulmonary aspergillomas has been reported. In patients treated with azoles resistance can emerge, which is commonly directed against multiple compounds of the azole class depending on the underlying resistance mechanism.[63]

The medical treatment options for invasive pulmonary aspergillosis are listed in Table 178.6. For neutropenic patients, the isolation of *Aspergillus* spp. from any sterile site or from respiratory specimens warrants immediate diagnostic work-up. Early initiation of antifungal therapy in patients with strongly suspected invasive aspergillosis is warranted while a diagnostic evaluation is conducted. The same is true for any sudden intracranial event in this group, especially in the presence of pulmonary infiltrates, with or without fever.[65]

Although significant experience exists with amphotericin B deoxycholate, because of significantly better survival and improved responses of initial therapy with voriconazole, primary therapy with amphotericin B deoxycholate is not recommended. At week 12, there were successful outcomes in 52.8% of the patients treated with voriconazole compared to 31.6% of those receiving amphotericin B.[66] Two issues with voriconazole must be taken into account: the occurrence of transient visual disturbances in about 30% of patients, and the considerable number of drugs showing important interactions with voriconazole, most notably drugs that are metabolized by the cytochrome p450 enzymes such as ciclosporin A. Another problem remains the intravenous administration of voriconazole in patients with renal insufficiency. As the cyclodextrin is known to accumulate in patients with kidney failure, the dose should be reduced which also reduces the exposure of the active drug. Especially in the ICU setting this restricts the use of voriconazole. In addition, evidence is accumulating that therapeutic drug monitoring is necessary to guide adequate drug exposure in the patient.

Based on the results of a randomized trial comparing two initial doses of liposomal amphotericin B (3 mg/kg per day and 10 mg/kg per day) which showed comparable efficacy with voriconazole, liposomal amphotericin B may be considered an alternative for primary therapy. The echinocandin caspofungin was investigated in a phase II trial for primary therapy of invasive aspergillosis in patients with hematologic malignancy, but the response rate was only 31%, thereby providing insufficient evidence for a recommendation for first-line therapy. Caspofungin, micafungin, liposomal amphotericin B, itraconazole and posaconazole are recommended for salvage therapy. In that setting the diagnosis should be confirmed and a change of class appears appropriate.

There is insufficient evidence to support the use of a combination of antifungal agents for primary therapy of invasive aspergillosis. A study compared the efficacy of voriconazole (31 patients) to voriconazole

Table 178.6 First- and second-line therapy for invasive aspergillosis

Generic (trade) name	Dosage	Comments
Amphotericin B deoxycholate	0.8–1.25 mg/kg iv q24h	High doses in neutropenia; significant interaction with ciclosporin
Itraconazole	Intravenous form, recently available 200 mg iv q24h; 200 mg q8h for 4 days, then 200 mg po q12h (for cerebral disease 400 mg q12h)	If patient can eat and does not take hepatic cytochrome P450-inducing co-medication; significant interaction with ciclosporin; measure itraconazole serum levels; new itraconazole cyclodextrin oral solution has better bioavailability; limited data on iv use >14 days
Voriconazole	Loading 6 mg/kg q12h, thereafter 3 mg/kg q12h or 200 mg po q12h	At least as effective as liposomal amphotericin B for empiric antifungal therapy in febrile neutropenia[64] More effective than D-AmB for invasive aspergillosis[64]
Caspofungin	70 mg iv on day 1, 50 mg iv thereafter	FDA approved for refractory aspergillosis
Lipid-formulations of amphotericin (all iv)		
Liposomal amphotericin (AmBisome)	1–5 mg/kg q24h	Less toxic than amphotericin B deoxycholate, but higher dosage needed to be equally effective
Amphotericin B colloidal dispersion (ABCD, Amphotec)	4–6 mg/kg q24h	Less toxic than amphotericin B deoxycholate, but higher dosage needed to be equally effective; expensive
Amphotericin B lipid complex (ABLC, Abelcet)	5 mg/kg q24h	Less toxic than amphotericin B but higher dosage needed to be equally effective; expensive

Adapted from Denning.[41]

and caspofungin (16 patients) in patients with invasive aspergillosis who had failed to amphotericin B therapy. Salvage therapy with the combination of voriconazole and caspofungin was associated with reduced mortality compared with therapy with voriconazole, independent of other prognostic variables.[67] However, the patients who had received voriconazole alone had been treated between 1997 and 2001, while those who received the combination had been treated between 2001 and 2004, which might bias the results due to other improvements in supportive care that could have had a positive impact on the survival of patients.

Another issue that remains unresolved is the choice of antifungal in patients who have breakthrough infection during mold-active prophylaxis. Azoles, especially posaconazole, are increasingly used to prevent invasive fungal infection in high-risk patients. There are no studies that address the optimal choice of antifungal drug although it seems appropriate to change to another drug class.

The optimal duration of therapy is unknown and dependent on the extent of invasive aspergillosis, the response to therapy and the patient's underlying disease(s) or immune status. A reasonable course would be to continue therapy to treat microfoci, after clinical and radiographic abnormalities are resolving, cultures (if they can be readily obtained) are negative, and reversible underlying predispositions have abated. Duration of therapy should be guided by clinical response and radiologic follow-up. Continuation of antifungal therapy through re-induction cancer chemotherapy, or resumption of antifungal therapy in patients with apparently resolved fungal infection who are about to receive re-induction chemotherapy, is worthy of consideration. The ultimate response of these patients to antifungal therapy is largely related to host factors, such as the resolution of neutropenia and the return of neutrophil function, lessening immunosuppression and the return of graft function from a bone marrow or organ transplant, as well as the extent of aspergillosis when diagnosed.

The administration of antifungal agents is one of the three cornerstones of treating invasive fungal infections. The other two, surgical interventions in order to reduce the fungal burden, and resolution or reduction of the immune suppression, should be considered in every patient with invasive aspergillosis.

PREVENTION

If untreated, the mortality of invasive aspergillosis reaches 100%.[41]

Preventive measures include reduction of environmental exposure of patients from sources of infection and antifungal prophylaxis. Specialized air-handling systems capable of excluding *Aspergillus* spores, such as high-efficiency particulate air (HEPA) filtration with or without laminar air flow ventilation, have proven to be very effective in reducing spore counts. However, there is evidence that 50–70% of patients acquire *Aspergillus* infection in the community and go on to develop clinical disease during admission. In those patients air-handling measures will not be effective. The alternative approach is targeted antifungal prophylaxis for hematologic patients who are at high risk for developing invasive fungal infections.

Two recent randomized studies in neutropenic patients and patients with graft-versus-host disease have shown that prophylaxis with posaconazole was more effective than fluconazole or itraconazole in preventing invasive fungal infection.[68,69] Significantly fewer patients with neutropenia in the posaconazole treatment group developed invasive aspergillosis (1% vs 7%, p <0.001) and survival was significantly longer among recipients of posaconazole than among recipients of fluconazole or itraconazole (p=0.04). In these high-risk populations prophylaxis might be a useful management strategy.

Cryptococcosis

NATURE

Cryptococcosis is a systemic infection caused by the encapsulated yeast-like fungus *Cryptococcus neoformans*. As early as 1894, Otto Busse described 'corpuscular' tumor-like lesions caused by 'coccidia species'. Since then *C. neoformans* has been known by a variety of names, including *Saccharomyces neoformans* and *Torula histolytica*.

Table 178.7 Names in current and former use in the C. *neoformans*/*C. gattii* species complex and with reference to the different typing nomenclatures in current use

***Cryptococcus neoformans*, type strain CBS 132 (teleomorph *Filobasidiella neoformans*)**
Cryptococcus neoformans var. *neoformans* (serotype D). Synonyms: *Saccharomyces neoformans* and *Torula nasalis*
Cryptococcus neoformans var. *grubii* (serotype A). Synonyms: *Candida psicrophylicus* and *Saccharomyces hominis*
Intervarietal *grubii* × *neoformans* hybrids
Serotype AD hybrids. CBS 132, the type strain of *C. neoformans* var. *neoformans* is a serotype AD hybrid
***Cryptococcus gattii* (teleomorph *Filobasidiella bacillispora*)**
No infraspecific taxa are described, but four different genotypes do occur: • AFLP genotype 4 = serotype B (or C). Synonyms: *Cryptococcus neoformans* var. *gattii*, *Candida hondurianus*, *Torulopsis neoformans* var. *sheppei*, *Cryptococcus neoformans* var. *shanghaiensis* and *Saccharomyces subcutaneous tumefaciens* • AFLP genotype 5 = serotype B or C. Synonym: *Cryptococcus bacillisporus* • AFLP genotype 6 = serotype B (or C) • AFLP genotype 7 = serotype B or C
Interspecific *gattii* × *neoformans* hybrids
Serotype BD hybrid = AFLP genotype 8 Serotype AB hybrid = AFLP genotype 9
Adapted from Bovers *et al*.[70]

An overview of names in current and former use in the *C. neoformans*/*C. gattii* species complex and the different typing nomenclatures was given by Bovers *et al*.[70] (Table 178.7).

Cryptococcus neoformans or *C. gattii* commonly cause pulmonary infection, which most likely starts with inhalation of infectious basidiospores, although this transmission route is not yet fully understood. Inhaled basidiospores may cause infection in mice and are more effective than yeast cells in causing cryptococcosis. Humans probably come into contact with *C. neoformans* frequently as the majority of children have been exposed to *C. neoformans* before they reach the age of 5 years,[71] and antibodies are present in adults without a history of disease. In immunocompetent individuals *C. neoformans* infection is either cleared or remains dormant. In immunocompromised individuals, however, *C. neoformans* can disseminate, most notably to the CNS causing meningoencephalitis. *Cryptococcus gattii* primarily infects otherwise healthy individuals, with exception of serotype C isolates of *C. gattii* that were found to be implicated in HIV-associated infections.[70]

EPIDEMIOLOGY

Cryptococcus neoformans var. *grubii* occurs throughout the world and causes 99% of the cryptococcal infections in persons with HIV. *Cryptococcus neoformans* var. *neoformans* also occurs worldwide, but appears to be a more common cause of infection in Europe.[70] *Cryptococcus gattii* infections have been geographically restricted to tropical and subtropical climates. However, recently an outbreak was detected in Vancouver Island (British Columbia, Canada), which has a temperate climate, with an incidence of *C. gattii* infection between 8.5 and 37 cases per million residents per year between 1999 and 2003.[72] This incidence is significantly higher than areas where *C. gattii* is typically observed such as Australia.[72] The peak incidence of disease coincides with the flowering season of the eucalyptus tree, namely November through February.

Cryptococcal meningitis is the most common life-threatening fungal infection in people who have HIV infection. Recently the global burden of disease was estimated using published studies.[73] The incidence ranged from 0.04% to 12% per year among persons with HIV. Sub-Saharan Africa had the highest yearly burden estimate, with a median incidence of 3.2% which is equivalent to 720 000 cases (range: 144 000–1.3 million). The median incidence was lowest in Western and Central Europe and Oceania (approximately 0.1% each). Globally, approximately 957 900 cases (range: 371 700–1 544 000) of cryptococcal meningitis occur each year, resulting in 624 700 deaths by 3 months after infection.[73]

The use of novel immunosuppressive agents may also have a role in the changing frequency, spectrum and clinical presentation of opportunistic mycoses. With the declining incidence of *C. neoformans* infection in HIV-infected patients treated with HAART, organ transplant recipients have re-emerged as one of the leading groups of immunocompromised patients at risk for cryptococcal infections.[74] Cryptococcosis is the third most commonly occurring invasive fungal infection in solid-organ transplant (SOT) recipients, representing approximately 8% of invasive fungal infections.[74]

PATHOGENICITY

Cryptococcus neoformans is a facultative intracellular organism. Its pathogenicity depends upon the immune status of the host, virulence factors of the *C. neoformans* strain and the size of the inoculum.

Risk factors for cryptococcosis include (in decreasing order of frequency):

- HIV infection (especially with a low $CD4^+$ lymphocyte count);
- corticosteroid treatment;
- organ transplantation;
- chronic leukemia;
- lymphoma; and
- sarcoid, even without corticosteroid treatment.

The main fungal virulence factors consist of its:

- ability to grow at body temperature;
- polysaccharide capsule;
- production of melanin and mannitol;
- alpha mating type; and
- mannoprotein-4.[75]

The polysaccharide capsule is known to interfere with phagocytosis, antigen presentation and leukocyte migration, and it can activate immunosuppressive T lymphocytes.[76] Most clinical cryptococcal strains develop large capsules during infection.[76] The cryptococcal metabolic products melanin and mannitol can function as antioxidants that can protect the yeast against oxidative attacks of phagocytes.

The first line of host defense consists of alveolar macrophages, followed by infiltration of neutrophils and later T lymphocytes, natural killer lymphocytes and monocytes. Neutrophils can kill opsonized cryptococci *in vitro*. There is evidence that the capsular polysaccharide of *C. neoformans* plays an active role in interfering with neutrophil migration into the subarachnoid space during inflammation,[77] despite the presence of the neutrophil attractant interleukin (IL)-8 in the CSF.

However, the role of neutrophils in host defense against *C. neoformans* is not clear because patients who have severe neutropenia are not at risk of developing cryptococcosis. Experimental data have shown that functional T lymphocytes are essential in restricting cryptococcal spread. With intact T-lymphocyte function, confinement of yeasts and granuloma formation has been observed. Occasionally, *C. neoformans* can be found in granulomatous pulmonary lesions and reactivates upon decreasing host resistance and appears analogous to the primary complex in tuberculosis.

DIAGNOSTIC MICROBIOLOGY

Cryptococcus neoformans grows on routine laboratory media such as Sabouraud's agar, producing white-to-cream colored mucoid colonies, which develop within 36–72 hours. Unlike nonpathogenic cryptococci, *C. neoformans* replicates at 98.6°F (37°C). For blood cultures, the lysis–centrifugation (Isolator) method has been recommended. Because *C. neoformans* is susceptible to cycloheximide, media containing cycloheximide should not be used. A rapid test to identify *C. neoformans* is the urease test because cryptococci produce large amounts of urease in the presence of urea (as do all basidiomycetous yeasts). To distinguish *C. gattii* from *C. neoformans* var. *neoformans*, glycine-L-canavanine-bromothymol blue agar can be used.

Microscopically, *C. neoformans* can be distinguished from other yeasts by its capsule, which is visualized by an India ink preparation (Fig. 178.3). The preparation is made by mixing equal volumes of sample fluid and ink, and ideally one should be barely able to read a newspaper through the preparation. The preparation typically shows budding yeast with a double refractive wall, distinctly outlined capsule and refractive inclusions in the cytoplasm. Occasionally, short hyphal yeast forms can be seen.

The laboratory diagnosis of cryptococcosis is rather straightforward compared with that of other fungal infections (see Table 178.3).

Latex agglutination tests (or enzyme-linked immunosorbent assay, ELISA) for cryptococcal antigen in body fluids are sensitive and specific and detect all serotypes. Due to differences between various commercial kits, absolute titer values are not interchangeable. In general, however, a positive test at a dilution of greater than 1:4 is highly suggestive of cryptococcal infection. False-positive results can be caused by rheumatoid factor or (rarely) through cross-reaction with other micro-organisms such as *Trichosporon beigelii* and the bacterium *Capnocytophaga canimorsus* or contamination of the specimen with agar or agarose in the laboratory. In CSF, false-negative results can be caused by either low or high antigen titers (prozone effect, especially in patients who have AIDS) or because of immune complex formation. Pronase pre-treatment of the sample reduces both prozone reactions and rheumatoid factor interactions. Antigen testing in CSF is more sensitive than India ink preparation or culture. In patients who have pulmonary cryptococcosis without dissemination, serum samples may test negative for cryptococcal antigen. However, in these cases, *C. neoformans* antigen is likely to be positive in BAL fluid. Cryptococci are occasionally isolated from the sputum. In patients who are not immunocompromised it is safe to keep the patient under close observation without starting treatment. In all other cases, a careful search for infection of other sites should be made (including CSF examination) and there should be a low threshold for initiating therapy.

Antibodies to *C. neoformans* have no diagnostic value and are found in healthy people as well as in those with cryptococcosis. Presumably, antibodies have a favorable prognostic value when they become positive during convalescence in patients who do not have AIDS. In pulmonary cryptococcosis, antibodies may interfere with antigen testing due to the formation of complexes, necessitating pronase pre-treatment of the samples.

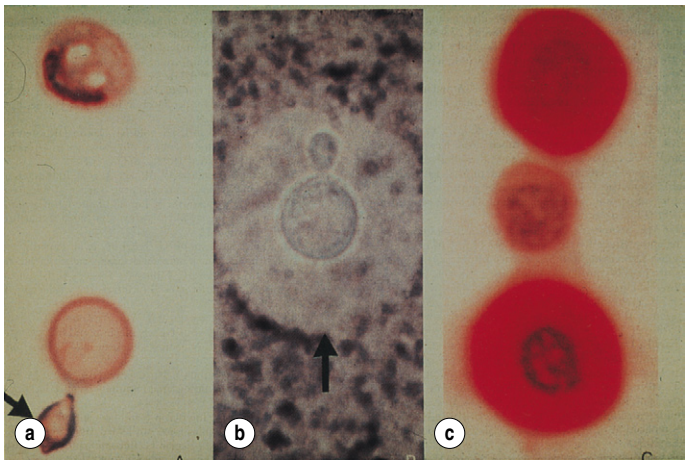


Fig. 178.3 *Cryptococcus neoformans.* (a) Cytologic preparation of CSF, (b) India ink preparation, (c) Mayer's mucicarmine stain. With permission from Jaster and Malecha. Cryptococcal meningitis, New England Journal of Medicine, Dec 1996. Copyright 1996 Massachusetts Medical Society. All rights reserved.

CLINICAL MANIFESTATIONS

Although *C. neoformans* usually enters the body through the lungs, the main site of infection is the CNS. However, any other organ can be involved, mainly skin, bone, prostate and eye. The clinical picture of cryptococcosis in patients who have AIDS resembles that in severely immunocompromised patients who do not have HIV infection. In SOT recipients the use of calcineurin inhibitors, which is the mainstay of immunosuppression in this patient group, may affect the clinical presentation of cryptococcal disease, but appears to have no influence on the incidence.[74]

In the immunocompetent host, disease is usually more focal, with cryptococcoma formation (especially with *C. gattii*), and disease is more often confined to the lungs.[78] Although survival is higher for infection with *C. gattii*, it causes more neurologic complications, residual disease and relapses than *C. neoformans* var. *neoformans* and *grubii*.[78]

Pulmonary involvement

The clinical picture of pulmonary cryptococcosis depends upon the immune status of the host. In the immunocompetent host, one-third of patients are asymptomatic and in some cases isolation of *C. neoformans* may represent colonization. The majority of patients present with pulmonary symptoms such as cough (54%), chest pain (46%) and sputum production (32%). If the cryptococcosis is confined to the lungs, cultures and antigen titers in CSF, blood and urine can be negative.

Compared with the immunocompetent host, cryptococcosis in the immunocompromised patient who does not have HIV infection has a more rapid course with early dissemination. In SOT recipients 54% of patients had pulmonary disease. Patients receiving a calcineurin inhibitor-based regimen were less likely to have disseminated disease and more likely to have cryptococcosis limited to the lungs.[79] This is thought to be due to the anticryptococcal activity of these compounds.[79] In SOT recipients pulmonary cryptococcosis may be detected in asymptomatic patients, but may also present as acute respiratory failure, which is associated with a grave prognosis.[74]

Patients who have HIV infection and pulmonary cryptococcosis are almost invariably symptomatic, presenting with fever (84%), cough (63%), dyspnea (50%), weight loss (47%) and headache (41%). They have disseminated disease, as shown by positive cultures and antigen testing in CSF, blood and urine. Most patients have low $CD4^+$ lymphocyte counts, often less than 100 000/ml.

Pulmonary cryptococcosis is diagnosed through antigen detection or culture of expectorated sputum, BAL, transbronchial lung biopsy (Fig. 178.4) or needle aspiration. In all cases, serum and CSF analysis for antigen and cryptococcal culture should be performed to assess dissemination.

Central nervous system involvement

If the CNS is involved in cryptococcosis, both the brain and the meninges are usually diffusely affected. In the immunocompromised patient, cryptococcomas and focal signs of disease are rare. Approximately

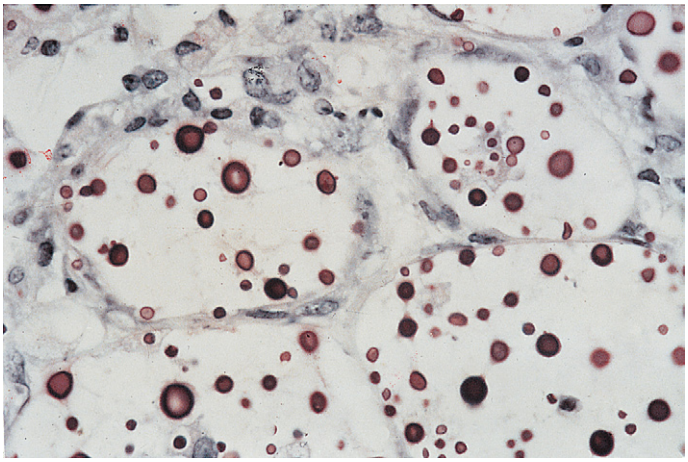


Fig. 178.4 Histology of pulmonary cryptococcosis.

70–90% of patients present with signs of subacute meningitis or meningoencephalitis: headache, fever, irritability, dizziness, memory loss, personality change, somnolence, confusion or obtundation.

Classic signs of meningitis such as nuchal rigidity are often absent. Papilledema is seen in one-third and cranial nerve palsies in one-fifth of cases. Symptoms may wax and wane over weeks to months and are often nonspecific.

Cryptococcal meningoencephalitis in immunocompromised patients who do not have HIV infection resembles the clinical picture in patients who do have HIV infection, with three exceptions:

- the duration of symptoms is usually shorter in patients who have AIDS, due to paucity of the inflammatory response and high fungal burden;
- patients who have AIDS present more often earlier in the disease with a second site of infection such as lungs, skin or blood; and
- concomitant illness such as infections (e.g. due to *Toxoplasma gondii*) or neoplasms is more likely.

Because focal neurologic symptoms due to cryptococcosis occur in only 10% of patients who have HIV infection, these symptoms should alert the physician to concomitant pathology. Patients who are successfully treated for cryptococcosis but who present with insidious mental impairment, ataxia or other neurologic signs should be evaluated for hydrocephalus by CT. Shunting is indicated for hydrocephalus.

Analysis of CSF in cryptococcal meningitis will typically yield the following findings:

- high opening pressure (reflecting raised intracranial pressure);
- CSF glucose level that may be either low or normal; and
- elevated or normal protein levels.

Usually, the CSF contains remarkably few cells with mononuclear predominance, especially in patients who have AIDS. Cryptococci can be cultured or identified directly through staining with India ink (see Diagnostic microbiology, above); cryptococcal antigen levels in CSF and serum are almost always positive. Semiquantitative CSF cultures showed baseline CSF colony-forming units to be an important prognostic factor, with a high burden associated with high mortality.[80]

Cerebral CT findings can be normal in 50% of patients who have cryptococcal meningitis, regardless of whether they have HIV infection. In patients who do not have AIDS, the CT may reveal hydrocephalus, gyral enhancement and/or multiple focal nodules with or without contrast enhancement. In patients who have AIDS, diffuse cortical atrophy is more common. Cerebral MRI appears to be more sensitive than CT in cryptococcal meningoencephalitis. The finding of pseudocysts and choroidal ependymal granulomas (cryptococcomas) is thought to be relatively specific for cryptococcosis. Lesions due to *C. neoformans* var. *gattii* tend to be fewer in number, bigger in size and surrounded by edema as compared to those caused by *C. neoformans* var. *neoformans* and *grubii*.

Other sites of infection

About 10–15% of patients will develop skin manifestations, which can present in many forms, including papular, nodular or ulcerative lesions, or as cellulitis. In patients who do not have AIDS, skin lesions can be the sole site of infection; however, in patients who have AIDS, cryptococcal skin lesions are almost always a sign of disseminated disease. A diagnosis of cryptococcal skin disease is confirmed by biopsy.

The eye is a frequent site of infection and any part of the eye may be involved. Visual loss is a distinct threat, especially in cases of endophthalmitis, although some patients have visual loss without signs of endophthalmitis, possibly due to optic neuritis or elevated intracranial pressure. Early diagnosis and rapid treatment are essential to preserve sight.

From 5% to 10% of patients have bone lesions, which are mostly osteolytic and have to be distinguished from cold abscesses (e.g. due to tuberculosis) or neoplasms.

Many other body sites can be affected by *C. neoformans*. In men, the prostate gland is thought to serve as an extraneural reservoir and source of relapse.

MANAGEMENT[81]

In isolated pulmonary cryptococcosis and other non-CNS disease in patients who do not have HIV infection, patients can be treated according to their risk and disease severity. In the immunocompetent asymptomatic patient who has minor lesions on the chest radiograph and no extrapulmonary dissemination, careful observation or fluconazole monotherapy (200–400 mg/day for 3–6 months) is justified because many undergo spontaneous regression. Every patient who has symptomatic or disseminated disease or a compromised immune system should be treated with antifungal medication. In all cases, lumbar puncture should be performed to exclude meningeal involvement.

There is no consensus about the treatment schedule for pulmonary cryptococcosis and other non-CNS disease. For patients who have extensive lobar consolidation or mass lesions (more frequent with *C. gattii*), surgical resection of the lesions can be warranted.

All patients who have cryptococcal meningitis should be treated, regardless of their immune status, because 10–20% will either die or develop serious neurologic sequelae. For patients who have cryptococcal meningitis and AIDS, the antifungal regimen of choice is amphotericin B (0.7 mg/kg/day) plus 5-FC (100 mg/kg/day) for 2 weeks, followed by consolidation therapy with fluconazole 400 mg/day for 8–10 weeks, and subsequent lifelong maintenance therapy at 200 mg/day. This regimen has been shown to reduce the mortality from about 15% to 6% in the first 2 weeks in a double-blind, randomized, multicenter trial.[82] Combination therapy of amphotericin B and flucytosine will sterilize CSF within 2 weeks of treatment in 60–90% of patients. The addition of flucytosine to amphotericin B was independently associated with earlier CSF sterilization,[80] and 5-FC is thought to be important in preventing early relapse. In patients treated with amphotericin B and 5-FC, factors independently associated with mycologic failure at 2 weeks were high serum antigen titer and abnormal brain imaging at baseline.[83] Hematologic malignancy, abnormal neurology at baseline and prescription of 5-FC for less than 14 days were independently associated with treatment failure at 3 months.[83] Recently a randomized, open-label, phase II trial showed that amphotericin B combined with fluconazole at a dosage of 400 mg/day or 800 mg/day was safe and effective.[84] There was a trend towards better outcomes in the combination therapy arms at days 42 and 70.[84]

For consolidation therapy, it is unlikely that there is any added benefit to routine substitution of fluconazole with extended-spectrum azoles, such as itraconazole, voriconazole or posaconazole.

Itraconazole (400 mg/day) was inferior to fluconazole during the consolidation and clearance phases.

It has been shown that maintenance therapy can be safely discontinued if the CD4 cell count is ≥200 for 3 months.[85]

For patients who have cryptococcal meningitis (Table 178.8), but who do not have AIDS, the regimen of choice is less well defined. In SOT recipients the use of the lipid formulations of amphotericin B (liposomal amphotericin B at a dosage of 3–4 mg/kg/day or amphotericin B lipid complex at a dosage of 5 mg/kg/day) is favored over amphotericin B deoxycholate, because many transplant recipients receiving calcineurin inhibitors have renal function impairment and any further worsening of organ dysfunction should be avoided.[74] A total treatment duration of 4–6 weeks has been successful, but the relapse rate may be lower with longer treatment courses or azole consolidation therapy.

It is advisable to follow patients closely over the first 6–12 months because most relapses occur in the first year after treatment. In male patients who do not have AIDS, positive urine culture after prostatic massage can predict a high risk for recurrence and prolonged oral treatment is advisable in these cases.

For patients with elevated baseline opening pressure, lumbar drainage should remove enough CSF to reduce the opening pressure by 50%. Patients should initially undergo daily lumbar punctures to maintain CSF opening pressure in the normal range. When the CSF pressure is normal for several days, the procedure can be suspended. Occasionally patients who present with extremely high opening pressures (≥400 mmH_2O) may require a lumbar drain, especially when frequent lumbar punctures are required to or fail to control symptoms of elevated intracranial pressure. In cases where repeated lumbar punctures or use of a lumbar drain fail to control elevated pressure symptoms, or when persistent or progressive neurologic deficits are present, a ventriculoperitoneal shunt is indicated. There are no data to support the routine use of direct intraventricular therapy (e.g. via an Ommaya reservoir).

Despite wide use of triazole (and other antifungal) drugs, drug resistance has not yet been a serious concern in cryptococcal infections.

Table 178.8 Preferred treatment options for cryptococcal disease in HIV-negative patients

Pulmonary and other non-CNS disease
Mild-to-moderate symptoms or culture-positive specimen from this site • Fluconazole, 200–400 mg q24h for 6–12 months • Itraconazole, 200–400 mg q24h for 6–12 months • Amphotericin B, 0.5–1 mg/kg q24h (total 1000–2000 mg)
Severe symptoms and immunocompromised hosts
Treat like CNS disease
CNS
Induction/consolidation: amphotericin B 0.7–1 mg/kg q24h plus flucytosine 100 mg/kg q24h for 2 weeks, then fluconazole 400 mg q24h for a minimum of 10 weeks
Amphotericin B 0.7–1 mg/kg q24h plus flucytosine 100 mg/kg q24h for 6–10 weeks
Amphotericin B 0.7–1 mg/kg q24h for 6–10 weeks
Lipid formulation of amphotericin B 3–6 mg/kg q24h for 6–10 weeks
The clinician must determine whether to follow lung therapeutic regimen or CNS (disseminated) regimen for treatment of infection in other body sites (e.g. skin). When other disseminated sites of infection are noted or the patient is at risk for disseminated infection it is important to rule out CNS disease. Duration of therapy is based on resolution of disease. Adapted from Saag *et al.*[81]

PROGNOSIS

For patients who do not have AIDS or cancer, the mortality rate due to cryptococcal infections is about 25–30%. After initial curative treatment, 20–25% of patients who do not have AIDS relapse. Among cured patients, 40% have significant neurologic deficits such as visual loss, cranial nerve palsy, motor dysfunction, personality change and decreased mental function due to chronic increased intracranial pressure or hydrocephalus. Mortality in patients after SOT is 33–49%.[74]

For patients who have AIDS, the mortality rate during initial therapy has been 10–25%, and 30–60% of patients die within 12 months. The relapse rate without maintenance treatment is 50–60%. Currently, the prognosis is mainly determined by the response to HAART. The prognosis for patients who have a malignancy is worse than for patients who have AIDS, but this probably reflects the course of the underlying disease rather than the cryptococcosis.

Adverse prognostic clinical features in patients who do not have HIV infection are listed in Table 178.9.

Immune reconstitution inflammatory syndrome

Immune reconstitution inflammatory syndrome (IRIS) has emerged as a major complication in patients with AIDS receiving HAART, with an estimated incidence of 4–16 cases per 100 person-years in AIDS-associated cryptococcosis.[86] IRIS has also been reported in SOT-related cryptococcosis, with a prevalence of 4.8%. IRIS comprises a constellation of clinical manifestations due to an inflammatory tissue response in patients experiencing improvement in cellular immunity after reduction or cessation of immunosuppressive therapy. Fungemia, an extremely low CD4 cell count, cryptococcosis as an AIDS-defining illness, lack of CSF sterilization at week 2, introduction of HAART within 1–2 months after the diagnosis of cryptococcosis, and a rapid decrease in HIV load after HAART have been recognized as risk factors for IRIS in HIV patients.[87]

There is no evidence-based treatment for IRIS but anti-inflammatory drugs may be considered for major complications related to inflammation in the CNS and other severe manifestations. It is important to distinguish IRIS from worsening of the cryptococcosis.

Table 178.9 Adverse prognostic clinical features in cryptococcal meningitis in patients who do not have HIV infection

• Initial positive India ink examination of CSF • High CSF opening pressure • Low CSF glucose • Low CSF leukocyte count (<20/μl) • Cryptococci isolated from extraneural sites • Initial CSF (or serum) antigen titer >32 • Corticosteroid treatment or lymphoreticular malignancy
Recurrent cryptococcal disease • Abnormal CSF glucose concentration after ≥4 weeks of therapy • Absence of anticryptococcal antibodies • Post-treatment CSF (or serum) cryptococcal antigen titer of ≥8 • No decrease in antigen titers during therapy • Daily corticosteroid treatment ≥20 mg prednisone after completion of antifungal therapy
A retrospective study of patients who had AIDS demonstrated only a prognostic value of antigen levels in the CSF, not in serum. There is substantial variability in titers with the different antigen detection kits used.

Mucormycosis and Infections by Other Zygomycetes

NATURE

Mucormycosis refers to disease caused by fungi belonging to the order Mucorales. Other names for the disease include phycomycosis and zygomycosis, the latter including diseases caused by Entomophthorales. Both Mucorales and Entomophthorales belong to the class Zygomycetes.

The major forms of zygomycosis include rhinocerebral, pulmonary, cutaneous, gastrointestinal and disseminated diseases. *Rhizopus*, *Mucor*, *Rhizomucor* and *Absidia* are the most common organisms that cause zygomycosis in humans. The taxonomy of the genus *Absidia* has recently been revised and the thermotolerant *Absidia* species have been renamed *Mycocladus* species.[88]

EPIDEMIOLOGY

Although these organisms are ubiquitous and grow in decaying organic material, mucormycosis is an uncommon disease and occurs almost exclusively in patients who have an underlying disease, with the exception of *Apophysomyces elegans*, which has been reported as a causative agent of zygomycosis, especially in immunocompetent patients. Some investigators report an increase in the prevalence of zygomycosis, which might be associated with the increased use of voriconazole that has no activity against zygomycetes.[89] A comprehensive review of 926 cases of zygomycosis, however, indicated that the increase in cases occurred before the clinical use of voriconazole, especially in bone marrow transplant recipients.[90]

Risk factors for mucormycosis are listed in Table 178.10. Diabetes mellitus and neutropenia are the most commonly encountered risk factors.

PATHOGENICITY

Infection with Mucorales usually occurs through inhalation of spores or deposition of spores in the nasal turbinates. Cutaneous mucormycosis is the primary presentation in immunocompetent patients, and results from direct inoculation of abraded skin.[93]

After pulmonary infection, the first line of defense is provided by alveolar macrophages. In animal studies, alveolar macrophages from healthy mice have been shown to inhibit germination of *Rhizopus oryzae* spores. In contrast, alveolar macrophages from corticosteroid-treated mice or diabetic mice fail to inhibit spore germination and the mice are rapidly killed by pulmonary and disseminated disease.

Table 178.10 Risk factors for mucormycosis

- Diabetes mellitus, especially with ketoacidosis
- Immunosuppression, especially corticosteroid treatment
- Iron overload with or without deferoxamine treatment (e.g. hemodialysis, hemochromatosis)
- Hematologic disease, especially neutropenia
- Intravenous drug use (CNS mucormycosis)
- Sustained skin trauma (cutaneous mucormycosis)
- Kwashiorkor (gastrointestinal mucormycosis)

From Sugar[91] and Yohai *et al.*[92]

Neutrophils play an important role in the second line of host defense. It is not known how diabetes mellitus or corticosteroids interfere with elimination of the fungi. Normal human serum can inhibit the growth of *Rhizopus* spp., unlike serum from patients who have diabetic ketoacidosis, which enhances fungal growth. The presence of elevated available serum iron predisposes the host to mucormycosis, and treatment with the iron chelator deferasirox (desferrioxamine) was shown to be as effective as liposomal amphotericin B in an experimental model of invasive zygomycosis.[94] Deferasirox treatment also enhanced the host inflammatory response to mucormycosis and showed synergistic interaction with liposomal amphotericin B for improving survival and reducing tissue fungal burden.[94] This opens new perspectives for the treatment of invasive zygomycosis, but the experience with deferasirox in a limited number of clinical cases is variable.

As in aspergillosis, a hallmark of mucormycosis is angioinvasion, resulting in thrombosis and tissue necrosis. The fungus has a predilection for veins over arteries.

DIAGNOSTIC MICROBIOLOGY

Mucorales grow at temperatures of 77–131°F (25–55°C) with an optimal temperature of 82.4–86°F (30°C). Clinical isolates will grow at 98.6°F (37°C) in the laboratory 2–5 days after incubation under aerobic conditions.[93] Cycloheximide inhibits their growth, so culture media containing cycloheximide should not be used. The recovery of zygomycetes in culture of clinical specimens is reduced by refrigeration of the sample, vigorous homogenization of the specimen and exposure to antifungal drugs.

On light microscopy, Mucorales have irregularly shaped, nonseptate broad (10–20 mm in diameter) hyphae with right-angle branching, visualized with hematoxylin and eosin staining, periodic acid–Schiff (PAS) reaction or Grocott–Gomori methenamine silver nitrate stains.

There are currently no biologic markers that can be detected in patients with invasive zygomycosis. Zygomycete antigens are not detected with the galactomannan and (1,3)-β-D-glucan assay.

CLINICAL MANIFESTATIONS

The clinical manifestations of mucormycosis can be divided into seven syndromes:

- rhinocerebral;
- pulmonary;
- cutaneous;
- gastrointestinal;
- CNS;
- disseminated; and
- miscellaneous (e.g. bones, kidney, heart or mediastinum).

The clinical manifestations depend upon the underlying disease. In patients who have diabetic ketoacidosis, rhinocerebral disease is the most common manifestation. Leukemic patients who have neutropenia are susceptible to rhinocerebral, pulmonary and disseminated disease. Children who have kwashiorkor (protein–calorie malnutrition) are especially at risk of developing gastrointestinal mucormycosis. Patients treated with deferoxamine because of iron or aluminum overload mainly present with disseminated mucormycosis.

In general, clues to the diagnosis of mucormycosis are signs of vasculitis with tissue necrosis, such as a black discharge or eschar on the skin, palate or nasal mucosa. Also, any radiographic imaging that reveals a lesion that surrounds vessels without a mass effect in an immunocompromised patient is suggestive of mucormycosis.

A biopsy with culture and microscopic evaluation is needed in order to discriminate between mucormycosis, other invasive fungal infections including aspergillosis and infection caused by Gram-negative bacteria such as *Pseudomonas* spp.

About 60% of rhinocerebral mucormycosis cases occur in diabetic patients. It is a rapidly fulminant disease, presenting with fever, nasal mucosal ulceration or necrosis, sinusitis (in 26% as an early sign), headache and facial pain or orbital involvement.

Typically, when a ketoacidotic diabetic patient who has decreased consciousness does not wake up within 24–48 hours after correction of serum glucose and electrolytes, (rhino)cerebral mucormycosis should be considered and the palate should be inspected for a black necrotic eschar resulting from extension of the disease towards the oropharynx. In rare cases, rhinocerebral disease may follow a chronic course.

The pulmonary form of mucormycosis occurs especially in severely neutropenic hematologic patients or those who have diabetes mellitus. It presents with cough, fever, chest pain and dyspnea and progresses rapidly. The diagnosis of pulmonary zygomycosis is difficult to make as the clinical presentation is very similar to that of invasive aspergillosis in patients with cancer. However, concomitant sinusitis and voriconazole prophylaxis were significantly associated with zygomycosis.[95] CT images such as masses, halo sign, air crescent sign and cavities did not discriminate between aspergillosis and zygomycosis. Only the presence of 10 or more nodules and pleural effusion on the initial CT were associated with zygomycosis.[95]

Cutaneous mucormycosis usually occurs in people who have sustained trauma (including indwelling postoperative catheters) or who have an underlying illness such as diabetes mellitus. Occasionally, cutaneous mucormycosis is a manifestation of systemic disease. Gastrointestinal mucormycosis is primarily found in patients who have extreme malnutrition. All segments of the gastrointestinal tract can be involved. The clinical symptomatology mimics an intra-abdominal abscess.

Central nervous system mucormycosis is rare and occurs most frequently as a direct extension from the nose or paranasal sinuses, or is associated with intravenous drug use. Cerebral lesions appear on CT scanning as low-density masses with variable peripheral enhancement. Gadolinium-contrast MRI can suggest cavernous sinus thrombosis and thrombosis of other vessels as indirect signs of infection, or ocular muscle involvement may be demonstrated.

MANAGEMENT

Mucormycosis is a rapidly progressive disease that warrants immediate aggressive combined surgical and medical treatment. All devitalized tissue should be removed, if necessary repeatedly, followed by reconstructive surgery in a later phase. Optimal treatment of the underlying disease is vital, including rapid correction of diabetic ketoacidosis. Intravenous lipid formulation of amphotericin B in high (initial) doses of 5 mg/kg per day is the treatment of choice. Recently, in a case series of 41 patients, it was shown that the combination of a polyene with caspofungin was more effective than amphotericin B alone for the treatment of invasive zygomycosis.[96] Patients treated with polyene–caspofungin therapy (six evaluable patients) had superior success (100% vs 45%, p=0.02) and Kaplan–Meier survival time (p=0.02), compared with patients treated with polyene monotherapy. Patients treated with amphotericin B lipid complex had inferior success (37% vs 72%, p=0.03) and a higher clinical failure rate (45% vs 21%, p=0.04), compared with patients who received other polyenes.[96]

PROGNOSIS

The prognosis of mucormycosis depends largely upon the underlying disease and in general the prognosis is better for those who have diabetes mellitus. In the review of 929 cases of invasive zygomycosis, analysis of survival by decade revealed that overall mortality improved from 84% in the 1950s to 47% in the 1990s.[90] However, mortality due to zygomycosis has remained essentially unchanged since the 1960s, when amphotericin B deoxycholate was widely introduced. Survival was 3% for cases that were not treated, 61% for cases treated with amphotericin B deoxycholate, 57% for cases treated with surgery alone and 70% for cases treated with antifungal therapy and surgery. By multivariate analysis, infection due to *Cunninghamella* species and disseminated disease were independently associated with increased rates of death.[90] Adverse prognostic factors in rhinocerebral disease include hemiparesis or hemiplegia, bilateral infections, nondiabetic co-morbidity and extensive facial necrosis.

Gastrointestinal mucormycosis has a high mortality and is usually diagnosed at autopsy.

Penicilliosis

NATURE

Penicilliosis is caused by the fungus *Penicillium marneffei*, a dimorphic mold with yeast-like growth in tissue.

The fungus is endemic in South East Asia and was originally isolated from the bamboo rat *Rhizomys sinensis*. It causes deep-seated infections in humans and rodents.

EPIDEMIOLOGY

Before the AIDS era, most patients in endemic regions (tropical Asia, especially Thailand, north-eastern India, China, Hong Kong, Vietnam and Taiwan) affected with penicilliosis had no known underlying disease. Now, the infection mainly affects patients who have HIV infection and is recognized as an AIDS-defining opportunistic infection. In Thailand's Chiang Mai province it is the third most common HIV-related opportunistic infection (after tuberculosis and cryptococcosis).[97]

PATHOGENICITY

Penicillium marneffei is a facultative intracellular organism that can survive and replicate in macrophages. It was also shown that deficiency of $CD4^+$ T-cell-dependent immunity contributes to the development of fatal disseminated penicilliosis in AIDS patients. Whether infection occurs as a consequence of zoonotic (animal) or sapronotic (environmental) transmission remains unknown to date.[98] The fungus can be cultured from the internal organs of four species of rodents but has also been recovered from soil specimens.

Penicillium marneffei evokes three patterns of tissue response:

- in the immunocompetent host, granuloma formation with central necrosis;
- suppurative abscesses are found in various organs; and
- in the immunocompromised host, an anergic necrotizing reaction in lung, liver and skin is seen, with diffuse infiltration of macrophages in tissues with proliferating yeast.

Antibody-mediated immunity does not seem to play a major role in host defense, although the host–fungus interaction is poorly understood.[98]

Penicilliosis affects all ages and both sexes, although 90% of the cases reported in the English literature are male.[99]

DIAGNOSTIC MICROBIOLOGY

In culture, *P. marneffei* is the only known *Penicillium* spp. that exhibits temperature-dependent dimorphic growth. The fungus grows as a mold at 77°F (25°C) and looks grayish and downy. It produces a distinctive red diffusible pigment, which is visible on agar media. At 98.6°F (37°C) it grows as a yeast on Sabouraud's glucose agar with cerebriform colonies that do not produce the red pigment.

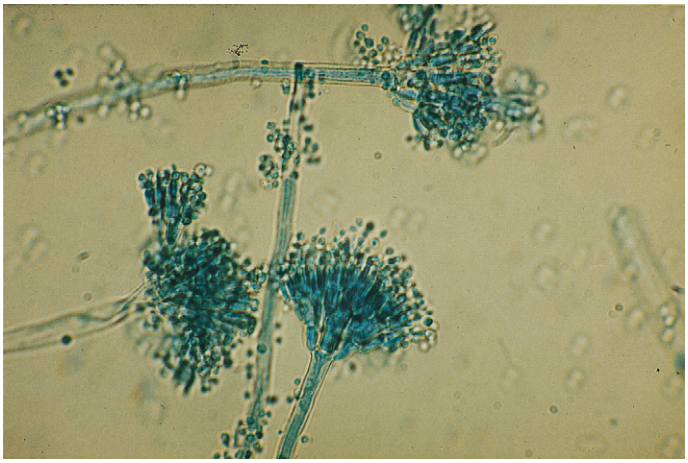


Fig. 178.5 Lactophenol cotton blue preparation of *Penicillium brevi compactum.*



Fig. 178.6 HIV-infected patient with *Penicillium marneffei* infection with molluscum contagiosum-like lesions.

On microscopic examination (Fig. 178.5) *Penicillium* spp. appear as short, septate, branching hyphae as well as sausage-shaped cells that divide by fission instead of budding, and may show a central septum, which distinguishes it from *Histoplasma capsulatum*. *Penicillium* spp. can be identified both inside macrophages and extracellularly when tissue preparations are stained with PAS, Wright or Giemsa stain. Clinical specimens that are commonly used for microscopy and culture include bone marrow aspirate, blood, lymph node biopsies, skin biopsies, skin scrapings, sputum, BAL pellet, pleural fluid, liver biopsies, CSF, pharyngeal ulcer scrapings, palatal papule scrapings, urine, stool samples, and kidney, pericardium, stomach or intestine specimens.[98] Several nonculture-based methods have been developed and evaluated for rapid diagnosis of penicilliosis using the detection of circulating fungal antigens and fungal DNA.[98] Assays that detect *Aspergillus* galactomannan cross-react with *P. marneffei* antigen.

CLINICAL MANIFESTATIONS

In patients who are not immunocompromised, the clinical picture may strongly resemble that of tuberculosis or histoplasmosis (e.g. generalized lymphadenopathy, fever, weight loss, anemia and a nonproductive cough). In patients who have HIV infection, the disease is usually disseminated, affecting skin, reticuloendothelial system, lung and gut. Other tissues that may be involved are liver and spleen, kidney, bone, joints and pericardium. In contrast to histoplasmosis and tuberculosis, adrenal involvement and CNS infections are rare. The molluscum contagiosum-like lesions of skin and mucosa indicate disseminated disease (Fig. 178.6)[99] Most patients acquire skin lesions on the face and neck.

Chest radiographs show patchy infiltration and sometimes abscess formation. Abdominal CT scanning often demonstrates hepatomegaly or hepatosplenomegaly, but the diffuse microabscesses that cause the hepatomegaly are usually indistinguishable.

The diagnosis is made by culturing *P. marneffei* from blood, bone marrow, skin scrapings or liver biopsy specimen or by identifying the organism microscopically in a touch smear of a skin biopsy or bone marrow aspirate.

MANAGEMENT

In-vitro susceptibility testing shows that the fungus is highly susceptible to miconazole, itraconazole, ketoconazole and flucytosine, moderately susceptible to amphotericin B and the least susceptible to fluconazole.[98] Recommended treatment of penicilliosis is amphotericin B (0.5–1.0 mg/kg/day for 2 weeks), followed by itraconazole 200–400 mg/day for 6 weeks for people who do not have HIV infection, and indefinitely for patients who have HIV infection.[100] If treated appropriately, the reported response rate ranges between 59%[69] and 75%.[100]

Prevention with itraconazole in advanced HIV disease is successful, but does not provide survival benefit.

Fusariosis

Fusarium spp. are found in soil with a worldwide distribution and are important plant pathogens. Infections with *Fusarium* spp. are uncommon and are most frequently caused by *Fusarium solani* (50%) followed by *F. oxysporum* (approximately 20% of cases). In humans *Fusarium* spp. may cause a wide range of infections, including superficial (keratitis and onychomycosis), locally invasive and disseminated infections. *Fusarium* spp. may also cause allergic diseases (sinusitis) in immunocompetent individuals and mycotoxicosis in humans and animals following ingestion of food contaminated by toxin-producing *Fusarium* spp.[101] Immunocompromised patients at high risk for fusariosis are those with prolonged and profound neutropenia and/or severe T-cell immunodeficiency. Infections in these patient groups are characterized by rapid dissemination. Fusariosis may be acquired through inhalation of airborne conidia, but skin breakdown (trauma, burns, onychomycosis or cellulitis at toes and fingers) has also been shown to lead to disseminated infection. *Fusarium* spp. may also be recovered from hospital water, and water-related activities and showering appear to be a mechanism for transmission to the immunocompromised host.[102] Most patients have prolonged and severe (<100 neutrophils/ml) neutropenia. The disease mimics aspergillosis and presents with an abrupt onset of fever, often combined with sinusitis, painful ecthyma gangrenosum-like skin lesions, pulmonary involvement and myalgia.

Histopathologic tissue examination with PAS or Gomori methenamine silver staining reveals vascular invasion by hyphae with infarction and necrosis. Contrary to aspergillosis, 50–70% of cases with disseminated fusariosis have positive blood cultures.[101] This may be due to the formation of yeast-like structures during invasive infection, which might be misinterpreted as yeast infection on microscopic examination of positive blood cultures.

Fusarium spp. grow rapidly on agar media but growth is inhibited by cycloheximide. Localized infection is likely to benefit from surgical debridement, while systemic antifungal agents are required in disseminated disease. Different species may exhibit different susceptibility to antifungal agents, with *F. solani* commonly being resistant to azoles and with high MICs of amphotericin B, and *F. oxysporum* showing susceptibility to voriconazole and posaconazole.[101] High-dose

amphotericin B is therefore recommended for *F. solani* and *F. verticilloides*, and high-dose amphotericin B or voriconazole for other species. Performing *in-vitro* susceptibility testing may help to guide antifungal therapy. Response rates are below 50% and depend on the underlying condition of the patient and the possibility of immune reconstitution. Successfully treated disseminated fusariosis tends to recur with repeated bone marrow suppression.

Systemic Fungi

Infections with systemic fungi are acquired by inhaling the airborne conidia (asexual spores) of dimorphic, exogenous fungi. In the lungs, these fungi convert to tissue forms that may disseminate to other internal organs as well as the skin. The etiologic agents, and consequently the prevalence and geographic distribution of their respective mycoses, tend to be restricted to certain geographic areas. The classification as systemic or endemic mycoses is not exclusive, as many opportunistic and subcutaneous mycoses often exhibit systemic clinical manifestations as well as discrete areas of endemicity.

This section will focus on four common systemic and endemic mycoses: blastomycosis, coccidioidomycosis, histoplasmosis and paracoccidioidomycosis. All are caused by dimorphic fungi. The fungi that cause coccidioidomycosis and histoplasmosis exist in nature, where they are associated with dry soil or soil mixed with guano. The agents of blastomycosis and paracoccidioidomycosis undoubtedly exist in nature, but their habitats have not been clearly defined. Inhalation of conidia of any of these fungi can lead to pulmonary infection, which may or may not be symptomatic. If symptoms are not transient, self-resolving or otherwise immediately manifested, the organism may have become dormant and entered a latent phase with the potential to produce active disease in the future. Alternatively, progressive pulmonary disease may develop with possible dissemination to other parts of the body. Except for a few extremely rare cases, these infections are not contagious, and there is scant evidence of transmission among humans or animals. Table 178.11 compares some of the epidemiologic features of these systemic mycoses.

Although most symptomatic cases of blastomycosis, coccidioidomycosis, histoplasmosis and paracoccidioidomycosis occur in patients without significant pre-existing and predisposing disease, individuals with defects of cell-mediated immunity have long been recognized to be at risk for these mycoses if they are exposed by residence or travel in the appropriate endemic areas. In recent years, these infections have increasingly emerged as opportunistic mycoses in patients with HIV/AIDS, either due to new exposure or reactivation of previously latent infections. As described earlier in this chapter, the AIDS pandemic has revealed another endemic and systemic, dimorphic, opportunistic pathogen, *Penicillium marneffei*, which is prevalent in South East Asia.

Blastomycosis, coccidioidomycosis, histoplasmosis and paracoccidioidomycosis are caused respectively by the following dimorphic fungi: *Blastomyces dermatitidis*, *Coccidioides immitis* or *Coccidioides posadasii*, *Histoplasma capsulatum* and *Paracoccidioides brasiliensis*. Each species grows as a mold in nature or the laboratory at temperatures below 98.6°F (37°C). On routine fungal culture media at 77–86°F (25–30°C), they produce mycelial colonies that vary in texture, pigment and growth rate, but may be indistinguishable from each other or many saprophytic molds. The ecology and geographic distribution of these fungi are summarized in Table 178.12. Microscopically, the hyphae are uniform in width, hyaline (not pigmented), branched, septate and similar in appearance except for the production of asexual spores or conidia, which are helpful aids to their identification. In the host, or under the appropriate growth conditions *in vitro*, they convert to a distinctive form of growth that is found in tissue: *B. dermatitidis*, *H. capsulatum* and *P. brasiliensis* produce characteristic budding yeast cells, and *C. immitis* and *C. posadasii* produce spherules.

Genetic and molecular research on these fungi in recent years has identified specific genes and signal transduction pathways that trigger host recognition and morphogenetic conversion, which are essential for their pathogenicity.[104] Molecular phylogenetic studies have determined that these pathogenic fungi are closely related and classified within the same family of the Phylum Ascomycota. All four of these infections begin in the lungs and Chapter 31 provides detailed descriptions of pulmonary blastomycosis, coccidioidomycosis, histoplasmosis and paracoccidioidomycosis.

Table 178.11 Epidemiologic features of fungal infections

Feature	Blastomycosis	Coccidioidomycosis	Histoplasmosis	Paracoccidioidomycosis
Geographic areas of high endemicity	Yes	Yes	Yes	Yes
Saprophytic form (<95°F): hyaline, septate hyphae	Yes	Yes	Yes	Yes
Predominant tissue forms	Yeasts	Spherules	Yeasts	Yeasts
High infection rate in endemic areas	?	Yes	Yes	Yes
At least 90% of infections are initiated by inhalation	Yes	Yes	Yes	Yes
Approximate percentage of infections that are asymptomatic	?	≥60	>95	>95
At least 90% of infections are self-limited	?	Yes	Yes	Yes
Approximate percentage of immunocompetent patients	>90	>90	>90	>90
Approximate percentage of males among patients with disease	50–90	75–90	80–90	95
Relative frequency of disease among AIDS patients in the endemic areas	Rare	Common	Common	Infrequent

From Larone *et al.*[103]

Table 178.12 Summary of systemic mycoses

Mycosis	Etiology	Ecology	Geographic distribution	Mycelial form*	Tissue form
Blastomycosis	*Blastomyces dermatitidis*	Unknown (riverbanks?)	Endemic along Mississippi, Ohio and St Lawrence River valleys and south-eastern USA	Slow to moderate growth rate. Colonies are white to tan, flat, velvety or cottony. Hyaline septate hyphae and short conidiophores bearing single globose to pyriform conidia, 2–10 µm	Thick-walled yeasts with broad-based, usually single, buds, 8–15 µm
Coccidioidomycosis	*Coccidioides immitis* and *C. posadasii*	Soil	Semiarid regions of south-western USA, Mexico, Central and South America	Moderate to rapid growth rate. Colonies are white to brown, flat to wooly. Hyaline septate hyphae and arthroconidia, 3 × 6 µm	Spherules, 10–80 µm or larger, containing endospores, 2–4 µm
Histoplasmosis	*Histoplasma capsulatum* var. *capsulatum* and *H. capsulatum* var. *duboisii*	Bat and avian habitats (guano); alkaline soil	Global; endemic in Ohio, Missouri and Mississippi River valleys, central Africa (var. *duboisii*)	Slow growth rate. Colonies are white to brown, flat to wooly. Hyaline septate hyphae and arthroconidia, 3 × 6 µm	Oval yeasts, 2 × 4 µm, intracellular in macrophages
Paracoccidioidomycosis	*Paracoccidioides brasiliensis*	Unknown (soil?)	Central and South America	Slow growth rate. Colonies are flat to velvety, white to brown. Hyaline septate hyphae and rare globose conidia and chlamydospores	Large, multiple budding yeasts, 15–30 µm or larger

*Colony descriptions are for typical isolates grown at 77°F (25°C) on Sabouraud's glucose agar.

Blastomycosis

Blastomycosis is a chronic infection characterized by granulomatous and suppurative lesions, but the clinical manifestations may be protean. Following inhalation of airborne conidia, dissemination may occur to any organ, but preferentially to the skin and bones. Although the prevalence of blastomycosis is greatest on the North American continent, autochthonous cases have been documented in Africa, South America and Asia. It is endemic for humans and dogs in the eastern United States and south-eastern Canada. Early histopathologic evidence confirmed that both cutaneous and systemic manifestations originated in the lung; however, the respiratory episode may be completely asymptomatic.

Table 178.13 Species-specific exoantigens* for the identification of systemic dimorphic fungal pathogens

Fungus	Exoantigens
Coccidioides immitis	HS, F, HL
Histoplasma capsulatum	h, m
Blastomyces dermatitidis	A
Paracoccidioides brasiliensis	1, 2, 3

*Exoantigens are detected by precipitin lines of identity in immunodiffusion tests of concentrated culture supernatant fluids versus reference antigens and antisera.

MYCOLOGY

At temperatures below 95°F (35°C), *B. dermatitidis* grows as a mold, producing a colony of uniform, hyaline, septate hyphae and conidia. Isolates vary in their rate of growth, colony appearance, and degree and type of conidiation. Many strains produce a white, cottony mycelium that becomes tan to brown with age (Table 178.13). On enriched media at 98.6°F (37°C), *B. dermatitidis* converts to growth in the yeast form and produces colonies that are folded, pasty and moist.

The mycelia produce abundant conidia from short lateral conidiophores on the aerial hyphae. The conidia are spherical, ovoid or pyriform in shape, smooth walled and up to 10 µm in diameter. Because the colony and conidia of *B. dermatitidis* may be confused with many other fungi, identification must be confirmed by conversion to the characteristic yeast form. This conversion can be accomplished by *in-vitro* cultivation on rich medium (e.g. brain–heart infusion, chocolate agar or Kelly's medium) at 98.6°F (37°C). Under these conditions or in tissue, *B. dermatitidis* grows as a thick-walled spherical yeast that usually produces single buds (Fig. 178.7). The bud and parent yeast have a characteristically wide base of attachment and the bud often enlarges to a size equal to that of the parent cell before they separate. Yeast cells are multinucleated and normally range in size from 8 µm to 15 µm, although some cells reach a diameter of 30 µm.

The sexual or teleomorphic form is *Ajellomyces dermatitidis*. Sexual reproduction may be stimulated *in vitro* with appropriate tester mating strains, but patients are only infected with haploid strains of one mating type, which reproduce asexually.

ECOLOGY AND EPIDEMIOLOGY

The natural habitat of *B. dermatitidis* has not been resolved. Over the years, *B. dermatitidis* has been isolated, but never repeatedly, from soil samples collected in rural environments, including a chicken house, a

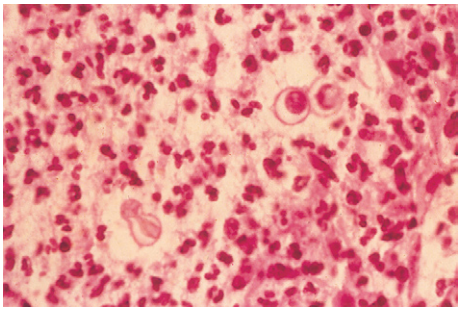


Fig. 178.7 *Blastomyces dermatitidis*, yeast form in hematoxylin- and eosin-stained section of microabscess from a cutaneous lesion. This shows large spherical yeasts characterized by thick, highly refractile cell walls, single budding and a broad attachment between the parent yeast cell and bud.

cattle crossing and several river banks. Positive samples were collected in Wisconsin from a beaver dam associated with a large outbreak of blastomycosis and from a fishing site involved in another outbreak. Several clusters of cases, with or without recovery of *B. dermatitidis* from environmental samples, have implicated river banks, fresh water and soil.[105]

Dogs are commonly infected and there are numerous reports of isolated cases in other animal species, but there is no evidence that an animal reservoir perpetuates *B. dermatitidis*. It probably resides in nature in a protected and dormant condition during most of the year, until it is stimulated by a suitable climatic or other specific but transient event to propagate and produce conidia that become airborne and infectious.

Because *B. dermatitidis* is not readily recoverable from nature and an adequate skin test antigen is not available for conducting population surveys of exposure, the geographic distribution of blastomycosis has been estimated from reports of human and canine cases. The endemic area extends broadly eastward from states that border the Mississippi River. Blastomycosis is endemic in southern Canada east of Manitoba and, in the USA, in Illinois, Wisconsin, Minnesota, Ohio, the Atlantic Coastal states, and the south-eastern states with the exception of Florida. Blastomycosis is rare in the New England and western states. The highest incidence occurs in Arkansas, Kentucky, Illinois, Louisiana, Mississippi, North Carolina, Tennessee and Wisconsin. Within these areas, local pockets of high endemicity have been identified. Canine cases exhibit the same endemic pattern as those of humans. Indeed, blastomycosis may occur more frequently in dogs than in humans. Clinical reports have also documented autochthonous cases in both northern and southern Africa, as well as India, Israel, Mexico and Venezuela. Reports of the infection occurring elsewhere are dubious because the diagnosis was questionable or the patients had a history of travel to an endemic area.

The data from large clinical reviews indicate that blastomycosis occurs more frequently during middle age and in males. Although the disease can occur at any age, 60% of patients were between the ages of 30 and 60 years. Among the hundreds of cases compiled in several reviews, less than 4% of the patients were under 20 years of age and blastomycosis was rarely reported in children. However, in more recent studies, especially involving outbreaks, blastomycosis occurred equally in both sexes and two-thirds of the patients associated with outbreaks have been children (<16 years old).[105]

The male to female ratios reported in several surveys involving hundreds of patients vary from 6:1 to 15:1, but lower ratios have been reported in smaller studies and the overall sex ratio of outbreak cases is approximately 1. Perhaps both sexes are equally susceptible to acute blastomycosis, but males are more inclined to develop chronic or disseminated disease. The other systemic mycoses also occur more often in males (Table 178.14), and male animals are more susceptible (or less resistant) than females to challenge with *B. dermatitidis*. Blastomycosis may be similar to histoplasmosis in that children are equally susceptible to infection and disease, but the sex-related differences are manifested after puberty.

Genetic or racial differences in prevalence have not been confirmed. Socioeconomic and occupational data have associated blastomycosis with poverty, malnutrition, manual labor, agriculture, construction work and exposure to dust and wood. The incidence of blastomycosis among immunocompromised patients, including patients with AIDS, is comparatively lower than that observed with other systemic mycoses.

PATHOGENICITY

Evidence has linked experimental virulence of strains of *B. dermatitidis* with the amount of α-1,3-glucan in the cell walls. This polysaccharide has been shown to vary in strains of *H. capsulatum* and *P. brasiliensis* that differ in virulence.[109] However, the best characterized virulence factor is BAD1, a cell wall adhesin and immunodominant antigen. This protein contains multiple copies of a 25-amino acid tandem repeat, which binds to CR3 complement receptors and elicits both antibodies and cell-mediated immune responses.[110,111] Compared with less virulent mutants, the wild type contains less surface-bound BAD1. Although the mechanisms whereby BAD1, as well as α-1,3-glucan, contribute to virulence have not been fully elucidated, Klein and associates have postulated that shedding of BAD1 by virulent strains may neutralize macrophage defenses by occupying receptors, diminishing their ability to bind yeast cells.[111] In the alveoli, *B. dermatitidis* induces an inflammatory response characterized by the infiltration of both macrophages and neutrophils and leading to the subsequent formation of granulomata. Both conidia and yeast cells are susceptible to the killing mechanisms of neutrophils and macrophages.[110,111]

If the pathogenesis of blastomycosis resembles that of histoplasmosis and coccidioidomycosis, most infections may be subclinical and resolve spontaneously. However, because specific and sensitive skin test antigens are lacking, the extent of exposure to *B. dermatitidis* in the general population has not been determined. In addition, calcification is uncommon and there is little radiologic or histopathologic evidence of residual blastomycotic lesions. The best evidence for the existence of subclinical blastomycosis derives from the reported outbreaks, where specific serologic and skin test reactions documented exposure to *B. dermatitidis* in the absence of symptoms.[105]

PREVENTION

There are no clear guidelines for the prevention of blastomycosis. However, the BAD1 antigen may be an excellent vaccine candidate for future study.[112]

DIAGNOSTIC MICROBIOLOGY

Microscopic examination

Blastomycosis is best diagnosed by direct examination or positive culture of sputa, skin lesions or other specimens. In calcofluor white or KOH preparations of pus, exudate, sputum, BAL fluid or other specimens, a diagnosis can be established detecting the characteristic yeast cells of *B. dermatitidis* (see Table 178.12 and description above). In tissue stained with hematoxylin and eosin, the yeast cytoplasm stains darkly and the cell wall appears colorless (Fig. 178.7). The multinucleated yeast cells are often abundant in cutaneous lesions. If the yeasts are sparse, fungal cell wall stains, such as PAS or methenamine silver, are helpful.

Table 178.14 Summary of serologic tests for antibodies to systemic dimorphic fungal pathogens in patients without AIDS

Mycosis	SENSITIVITY AND VALUE				Limitation/specificity
	Test	Antigen	Diagnosis	Prognosis	
Blastomycosis	CF	By	Less than 50% of cases positive; reaction to homologous antigen only is diagnostic	Fourfold change in titer	Highly cross-reactive
	ID	Bcf	Up to 80% of cases positive, i.e. A band	Loss of A band	More specific than CF test
	EIA	A	Up to 90% of cases positive (titer ≥1:16)	Change in titer	92% specificity
Coccidioidomycosis	TP	C	Early primary infection; 90% cases positive	None	None
	CF	C	Titer ≥1:32 5 secondary disease	Titer reflects severity (except in meningeal disease)	Rarely cross-reactive with H
	ID	C	More than 90% cases positive, i.e. F and/or HL band		More specific than CF test
Histoplasmosis	CF	H	Up to 83% of cases positive (titer ≥1:8)	Fourfold change in titer	Cross-reactions in patients with blastomycosis, coccidioidomycosis, cryptococcosis, aspergillosis; titer may be boosted by skin test with H
		Y	Up to 94% of cases positive (titer ≥1:8)	Fourfold change in titer	Less cross-reactivity than with H
	ID	H(10X)	Up to 85% of cases positive, i.e. m or m and h bands	Loss of h	Skin test with H may boost m band; more specific than CF test
Paracoccidioidomycosis	CF	P	80–95% of cases positive (titer ≥1:8)	Fourfold change in titer	Some cross-reactions at low titer with aspergillosis and candidiasis sera
	ID	P	98% of cases positive (bands 1, 2 and/or 3)	Loss of bands	Band 3 and band m (to H) are identical

A, antigen A of *B. dermatitidis*; Bcf, culture filtrate of *B. dermatitidis* yeast phase; By, yeast phase of *B. dermatitidis*; C, coccidioidin; CF, complement fixation; EIA, enzyme immunoassay; H, histoplasmin; ID, immunodiffusion; P, culture filtrate of *P. brasiliensis* yeast phase; TP, tube precipitin; Y, yeast phase of *H. capsulatum*.[106–108]

Culture

On most routine culture media, *B. dermatitidis* produces a mold with variable macroscopic features and conidia. Cultures should be incubated at 86°F (30°C) or room temperature for at least 4 weeks. Their identification is confirmed by growth on a rich medium at 98.6°F (37°C) and conversion to the characteristic yeast form. Alternatively, the identity of a culture may be established by an immunodiffusion test to detect a *B. dermatitidis*-specific antigen (antigen A). As indicated in Table 178.13, the technique has also been expanded to identify other dimorphic pathogens. In addition, DNA probes are commercially available to identify cultures of *B. dermatitidis*, as well as other agents of systemic mycoses.[113]

Serology

Since the tests for complement fixing antibodies and delayed-type skin reactivity lack specificity and sensitivity, they are not helpful unless the patient is negative to heterologous fungal antigens; even then, positive, monospecific serologic tests to *B. dermatitidis* may not indicate active infection. Serologic tests for antibodies to the specific antigen

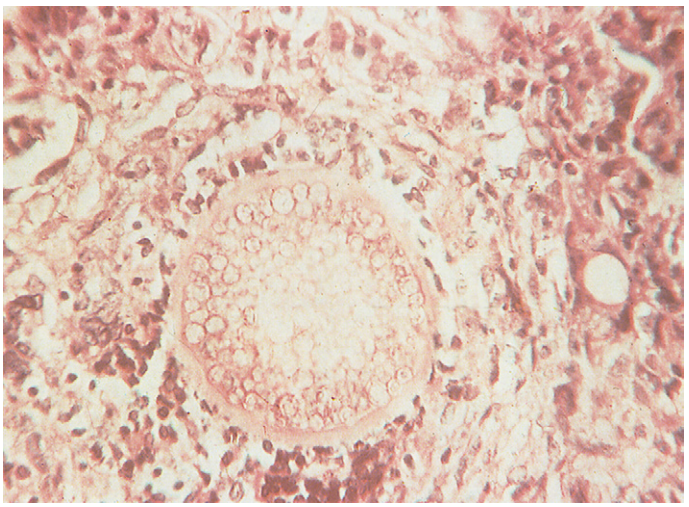


Fig. 178.8 *Coccidioides immitis*, spherule in hematoxylin- and eosin-stained section of lung lesion. This shows refractile cell wall and internal endospores.

A, which can be detected by the immunodiffusion test or an enzyme immunoassay (EIA), are more suggestive of infection, but negative tests do not exclude blastomycosis. Table 178.14 summarizes the conventional serologic tests for antibodies to *B. dermatitidis* and other systemic fungi. The most useful serologic procedure is an EIA for antibodies to antigen A, and EIA titers reflected the severity of disease (see Table 178.14). False-positive results may occur with sera from patients with histoplasmosis

CLINICAL MANIFESTATIONS

Two classic forms of blastomycosis are recognized: pulmonary, often with dissemination, and chronic cutaneous blastomycosis. A wide variety of symptoms, pathology and radiographic appearances may be observed in blastomycosis. The most common symptoms include cough, weight loss, chest pain, skin lesions, fever, hemoptysis and localized swelling. After the lung, the most frequently involved organs are the skin, bones and genitourinary tract, followed by the central nervous system, liver or spleen.

Primary pulmonary blastomycosis may be asymptomatic or present as acute or subacute pneumonia, ranging from mild to severe (see Chapter 31 for a detailed clinical description). In chronic cutaneous blastomycosis, lesions initially occur as one or more subcutaneous nodules that eventually ulcerate. Lesions are more common on exposed skin surfaces, such as the face or extremities. Spread may occur by extension to contiguous areas and require weeks or months for the ulcerative process to evolve. If untreated, elevated granulomatous lesions with advancing borders will develop. The yeast cells can be observed in microabscesses near the dermis. Extensive, often verrucous, epithelial hyperplasia overlying the abscesses may develop and resemble carcinoma. These extensive cutaneous lesions are characteristically discolored and crusty, and tend to heal and scar in the central, older areas. The active microabscesses found at the leading edge of the lesion can be aspirated or biopsied, and the typical yeast cells of *B. dermatitidis* can be observed on direct microscopic examination.

Dissemination may be widespread and unpredictable in blastomycosis and include the genitourinary tract, CNS and spleen, as well as the skin and bones. Less frequently, the liver, lymph nodes, heart and other viscera are infected. The progressive systemic form of blastomycosis develops in patients with unresolving pulmonary infection, but the degree of pulmonary involvement is not related to the extent of dissemination. This infection may be chronic, with few organisms present, or multiple pulmonary foci may be demonstrable at the time generalized systemic disease develops.

In fulminant cases, the dermal lesions may be more severe than those of chronic cutaneous blastomycosis and are seen in about 75% of cases. Overall, skeletal involvement occurs in approximately a third of patients. Osteomyelitis and, in some cases, draining sinuses to the skin develop, and should be examined for the presence of characteristic yeast cells. Because of the frequency of bone involvement and because almost any bone can be affected, whenever blastomycosis is diagnosed a complete radiographic examination is advisable. Arthritis may develop by extension from infected bone or by direct dissemination from the lung without bone infection. In up to 22% of patients the urogenital tract is involved, especially the prostate, male genitalia, kidney and adrenals. Metastasis to the CNS, resulting in meningitis or brain abscess, occurs in up to 10% of patients.

Primary cutaneous blastomycosis is initiated by traumatic autoinoculation or contamination of an open wound with infectious material. The lymphatics and regional lymph nodes are involved, but the infection remains localized and often resolves without treatment. The natural history and pathology are similar to subcutaneous mycoses (see Chapter 179) and systemic dissemination is rare in the immunocompetent host.

There are well-documented series of patients with HIV/AIDS and blastomycosis. However, the incidence of blastomycosis in HIV-positive patients is not as high as co-infection with other endemic mycoses. Because of the presumed rural reservoir of *B. dermatitidis* or its low census in nature, patients with AIDS may be exposed less often than similar patients in the endemic areas for coccidioidomycosis or histoplasmosis. Alternatively, the host defenses against *B. dermatitidis* may be less dependent upon cell-mediated immunity. Cases of blastomycosis and AIDS have presented with acute miliary pneumonia and a relatively higher frequency of CNS disease. The management of blastomycosis in immunocompromised patients is more difficult and the prognosis is worse.

MANAGEMENT

As demonstrated by several outbreak cases, primary blastomycosis in immunocompetent individuals may not require therapy (see Chapter 31). Patients with protracted, severe or progressive primary infection, chronic pulmonary or disseminated blastomycosis require treatment. Depending on the manifestations of disease and the integrity of the underlying host defenses, chemotherapeutic success rates with amphotericin B, ketoconazole or itraconazole currently vary between 70% and 95%. Mortality is higher in patients with AIDS and other immunocompromising conditions. Recent guidelines recommend itraconazole for mild to moderate disease, and a lipid formulation of amphotericin B (AMB) followed by itraconazole for more severe forms.[114]

Fluconazole can be an effective alternative for the treatment of non-life-threatening cases of blastomycosis, especially in patients who may not have responded to another drug. Its only advantage over itraconazole may be in patients with involvement of the CNS, which is penetrated well by fluconazole.

Blastomyces dermatitidis is quite susceptible to amphotericin B, which is the recommended treatment for patients with life-threatening or severe disease (e.g. involvement of multiple organs), those with meningitis, or blastomycosis in immunocompromised patients, as well as patients who do not respond to an azole.[114]

The protocol for administration of conventional amphotericin B or a lipid formulation and monitoring renal function are the same as its application for other mycoses. Most patients experience adverse side-effects, including renal dysfunction, fever, anorexia, phlebitis, hypokalemia, nausea, chills, headache and anemia.

Lipid emulsions of amphotericin B have the advantage of delivering higher doses with diminished toxicity. Adult respiratory distress syndrome due to overwhelming pulmonary blastomycosis requires aggressive treatment. Blastomycosis in patients with AIDS may require suppressive therapy following the initial course of treatment with amphotericin B, similar to the management of cryptococcosis and histoplasmosis in patients with AIDS. Suppressive treatment in AIDS patients would be daily itraconazole or possibly fluconazole for CNS disease.[114]

In addition, voriconazole appears to be effective. In a retrospective review of 24 patients with endemic mycoses who were treated with voriconazole (often as salvage cases), 20 patients showed improvement or stabilization of their disease after 2 months and at subsequent follow-up.[115]

Corrective surgery may be necessary as an adjunct to antibiotic treatment. Because of the occurrence of relapse or reactivation blastomycosis, patients should be observed for years after treatment and resolution of disease.

Coccidioidomycosis

Coccidioidomycosis is caused by either of two phenotypically indistinguishable species, *Coccidioides immitis* or *Coccidioides posadasii*, dimorphic fungi that normally live in well-defined geographic areas. Both the agents and coccidioidomycosis are almost entirely limited to these endemic regions. It was recognized quite early that coccidioidomycosis is confined to the south-western USA, contiguous regions of

northern Mexico and specific areas of Central and South America. The natural reservoir was established by isolating *Coccidioides* species from soil samples collected throughout the endemic areas, and the environmental conditions under which the fungus was propagated have been described. From mycelial culture filtrates, the skin test antigen coccidioidin was developed to detect exposure to *C. immitis* or *C. posadasii* and used to conduct population surveys of skin reactivity. The more common primary form is a mild respiratory ailment, also called valley fever or San Joaquin Valley fever.

MYCOLOGY

Coccidioides posadasii was described by Fisher *et al*.[116] They compared the genotypes of isolates of '*C. immitis*' from different geographic regions and, based on their phylogenetic analyses of these populations, determined that the majority of isolates outside California belong to a different species, which they named *C. posadasii* to honor Dr Posadas who described the first case. Although the two species differ in their geographic distribution and genotype, differences in their phenotypes or pathogenicity have yet to be delineated. Because they cannot be distinguished by simple laboratory tests, the more familiar name, *C. immitis*, will be used here.

The life cycle of *C. immitis* (or *C. posadasii*) encompasses several distinct morphologic structures that are produced under different conditions. In nature and in the laboratory, either species grows as a mold, producing hyaline, branching, septate hyphae. As the culture ages, characteristic arthroconidia are formed, usually, but not invariably, in alternate hyphal cells. With time, the arthroconidial chains fragment to release the unicellular, barrel-shaped arthroconidia, which are approximately 3 µm × 6 µm in size, easily airborne and small enough to be inhaled into the alveoli. They are highly resistant to desiccation, temperature extremes and deprivation of nutrients, and may remain viable for years. Under appropriate growth conditions the arthroconidia will germinate to recycle the saprophytic mycelial form.

Following their inhalation, the arthroconidia become spherical. In the infected host, *C. immitis* exists as spherules (spherical thick-walled structures, 15–80 µm in diameter) that are filled with a few to several hundred endospores (see Fig. 178.8). As a spherule enlarges, the nuclei undergo mitosis, the cytoplasm condenses around these nuclei and a cell wall forms around each developing endospore. At maturation, the spherule ruptures to release its endospores (2–5 µm in size), which may enlarge to form mature spherules. Hyphae as well as spherules may be observed in the tissues and sputum of patients with coccidioidal cavities of the lungs.

On routine mycologic media, such as inhibitory mold or Sabouraud's agar, at the usual incubation temperature of 77–86°F (25–30°C), different isolates of *C. immitis* produce a wide variety of colony types. Because numerous infectious arthroconidia are produced in culture and can be readily aerosolized in the dry state, cultures should be examined only under a safety cabinet that protects both the laboratory worker and the environment. Spherules can be produced in the laboratory on a complex medium at 104°F (40°C) under 20% CO_2.

ECOLOGY AND EPIDEMIOLOGY

In the USA, the geographic areas endemic for coccidioidomycosis and from which *C. immitis* can be isolated from the environment correspond to the Lower Sonoran life zone. These areas are characterized by a semiarid climate, alkaline soil and characteristic indigenous desert plants and rodents. The endemic foci in Mexico, Argentina and other areas of Central and South America are associated with ecologically similar environments. The mycelia, which can be found several inches beneath the soil surface, can be recovered at the surface after the spring rains. As the weather becomes hot and dry, the mycelia convert to infectious arthroconidia, accounting for the peak infection rate during the summer. In the endemic areas, natural infections also occur among indigenous fauna, such as desert rodents, dogs and cattle.

Table 178.15 Risk factors for disseminated coccidioidomycosis

Age	Infants and elderly
Sex	Male
Genetics	Filipino > African-American > Native American > Hispanic > Asian
Serum CF antibody titer	>1:32
Pregnancy	Late pregnancy and postpartum
Delayed-type hypersensitivity skin test	Negative
Depressed cell-mediated immunity	Malignancy, chemotherapy, steroid treatment, HIV infection

Inhalation of the arthroconidia of *C. immitis* leads to infection and acquisition of a positive delayed-type hypersensitivity response to coccidioidin. More than half of these infections are benign and most of the others are symptomatic but self-limited (see Table 178.11). Approximately 1% of these cases will develop progressive pulmonary disease, dissemination, or both. Some individuals have an increased risk of developing disseminated disease following primary infection (Table 178.15). These include persons in certain ethnic groups: Filipinos, African-Americans, Latin Americans and Native Americans. This ethnic predilection for severe disease clearly indicates the importance of the genetic background of the host in mounting an effective immune response to infection.

In addition to ethnicity, males, women in the third trimester of pregnancy, persons with a cellular immunodeficiency (including AIDS) and individuals at the extremes of age are more susceptible to severe disease (Table 178.15). In addition, risks for severe pulmonary disease include diabetes, cigarette smoking, low income and old age.

The areas of endemicity defined by case reports and by isolation of *C. immitis* from soil have been confirmed by skin test surveys with coccidioidin. Within the endemic areas, which include portions of the south-western USA (California, Arizona, New Mexico, Nevada, Utah and Texas) and north-western Mexico, the percent reactivity varies; some of the highest rates are found in Phoenix and Tucson, Arizona, and Kern County, California. Isolated cases of coccidioidomycosis occurring outside the established areas of endemicity have been attributed to fomite transmission of the arthroconidia or to patient travel through the endemic area.

Numerous outbreaks of primary infection have been reported among individuals simultaneously exposed to a heavy aerosol of arthroconidia. Coccidioidomycosis is therefore an occupational hazard for construction workers, archeology students and others who disrupt the soil in the endemic areas.

PATHOGENICITY

In vivo, potent cell wall antigens are released when the arthroconidia develop into spherules. Although arthroconidia and endospores are readily engulfed by alveolar macrophages, killing is enhanced by activation of macrophages with the appropriate T cells or cytokines.[117] When stimulated by spherules, leukocytes from both patients and skin test-positive subjects secrete potentially protective cytokines, such as interferon gamma (IFN-γ) and IL-12.

Investigators have identified several potential virulence factors. For example, *C. immitis* produces a serine proteinase with broad specificity for host substrates, such as elastin, collagen, IgG and IgA. Other proteinases have also been detected and are thought to contribute to the development of spherules and release of endospores.[117]

Many of the patients who develop disseminated coccidioidomycosis have depressed cell-mediated immunity. There is a marked inverse relationship between the antibody titer (see below) and specific cell-mediated immunity, as measured by skin test and, *in vitro*, by the numbers of $CD4^+$ and $CD8^+$ T lymphocytes, the responsiveness of T cells to mitogens or antigens, and the production of cytokines. In severe coccidioidomycosis, patients have elevated antibody titers, circulating immune complexes and depressed cellular immunity. This condition has been related to increased antigen burden, populations of suppressor cells, immune complexes and impaired lymphocyte circulation. Immune complexes are detected in serum of patients with coccidioidomycosis and correlate with the severity of disease.

In the mouse model of experimental coccidioidomycosis, specific anergy is correlated with the amount of coccidioidal antigen present. Recovery often leads to restoration of immune functions. However, the impaired cellular immune responses are likely governed by whether T-helper 1 (Th1) or Th2 responses predominate early in infection.[117] In strains of mice that differ in susceptibility to *C. immitis*, protective responses are correlated with the secretion of IFN-γ, which is a Th1-associated cytokine and potent activator of macrophages. Conversely, much of the immunopathology may be attributable to excess production of tumor necrosis factor (TNF). Perhaps the genetic predisposition to disseminated coccidioidomycosis (see Table 178.15) is related to genetic control of the T-cell response to *C. immitis*.

PREVENTION

Coccidioides immitis cannot be eliminated from the soil, but public health efforts to reduce the dust associated with dispersion of the arthroconidia are helpful in areas of high endemicity. Another approach has focused on the development of a vaccine for persons at risk. In mice and humans, cell-mediated immunity to *C. immitis* confers excellent protection against disease. Spherule-derived vaccines in the past were not successful, but several new approaches to identify specific candidate epitopes are currently under investigation.[117]

DIAGNOSTIC MICROBIOLOGY

Microscopic examination

A definitive diagnosis of coccidioidomycosis requires the finding of spherules of *C. immitis* in sputum, draining sinuses or tissue specimens (see Fig. 178.8). Clinical exudates should be examined directly in 10% or 20% KOH, with or without calcofluor white, and tissue obtained from biopsy can be stained with hematoxylin and eosin or special fungal stains, such as Gomori methenamine silver or the PAS stain, which stain fungal cell walls black or reddish, respectively. Direct microscopic examination of cutaneous or deep tissue specimens, either in calcofluor/KOH preparations or histologic sections, yields positive results in approximately 85% of proved cases. However, sputum specimens are positive by direct examination or culture in less than half of the cases.

Culture

Clinical specimens are cultured on inhibitory mold, Sabouraud's agar or other routine fungal medium. Colonies of *C. immitis* develop within 1 or 2 weeks and are examined for the production of characteristic arthroconidia. Microscopic preparations of mycelia should always be prepared under a biosafety hood. The identification of *C. immitis* can be readily confirmed by demonstrating the production of exoantigen F (see Table 178.13). Alternatively, DNA-based identification is available with commercial kits.

With the exception of tissue scrapings, biopsies and surgical specimens, cultures are more often positive than microscopic examinations of clinical material. However, use of both procedures will optimize the opportunity to establish a diagnosis. Between 25% and 50% of sputa, bronchial washes, spinal fluids and urine specimens yield positive cultures. Positive blood cultures are infrequent but significantly associated with acute, disseminated coccidioidomycosis and a poor prognosis.

Skin tests

As noted above, coccidioidin, a standardized toluene extract of mycelial culture filtrate, is used for skin testing, and a positive test for delayed-type hypersensitivity is denoted by induration exceeding 5 mm in diameter. Another *C. immitis* antigen, prepared from cultured spherules and termed spherulin, is more sensitive but less specific than coccidioidin. Skin testing with either antigen does not induce or boost an immune response. The skin test becomes positive within 2 weeks after the onset of symptoms and before the appearance of antibodies and often remains positive indefinitely. A positive reaction has no diagnostic significance without a history of conversion, but a negative test can be used to exclude coccidioidomycosis, except in patients with severe disseminated coccidioidomycosis who may have become anergic. Indeed, a negative skin test in confirmed cases is associated with a grave prognosis. Conversely, a positive skin test in healthy subjects implies immunity to symptomatic reinfection.

Serology

As indicated in Table 178.14, tube precipitins or latex agglutinins measure specific IgM antibodies. They assist in the diagnosis of primary infections because these antibodies are detected in 90% of patients within 2 weeks after the appearance of symptoms and disappear in most cases by 4 months.

The CF test, which measures IgG antibodies to coccidioidin, is a useful diagnostic and prognostic tool. The CF titer correlates with the severity of disease. Most patients with secondary coccidioidomycosis develop a titer of 1:16 or higher, whereas in nondisseminated cases the titer is almost invariably lower. Therefore, a critical titer of 1:32 or higher reflects active, disseminated disease. However, a lower titer does not exclude disseminated disease, because many patients, such as those with single extrapulmonary lesions, notably coccidioidal meningitis, do not develop high titers. Multiple serum specimens are helpful because a change in the CF titer reflects the prognosis. The CF titer declines with recovery and eventually disappears, while a rising titer indicates active, uncontrolled infection and a poor prognosis.

The immunodiffusion (ID) method can be used to detect both TP and CF antibodies by using reference antisera and heated (TP only) and unheated antigen. Antibodies to two specific heat-labile antigens, termed F (or CF) and HL, may be detected.

CLINICAL MANIFESTATIONS

Following inhalation of arthroconidia, the primary infection in most individuals is asymptomatic. Others may develop flu-like symptoms: fever, chest pain, cough or weight loss. Radiographic examination often reveals discrete nodules in the lower lobes. Primary pulmonary coccidioidomycosis is detailed in Chapter 31.

Only about 1% of primary cases progress to secondary or disseminated coccidioidomycosis. The numerous manifestations of secondary coccidioidomycosis include chronic and progressive pulmonary disease, single or multiple extrapulmonary dissemination or generalized systemic infection. Chronic pulmonary coccidioidomycosis usually involves a single, thin-walled cavity, but patients may develop enlarging or multiplying nodules or cavities. Dissemination may be fulminant or chronic, with periods of remission and exacerbation.

Extrapulmonary lesions most frequently involve the meninges, skin or bone. Chronic cutaneous coccidioidomycosis develops from initial lesions that usually appear on the face or neck and that, over a period of years, evolve into thick, raised, verrucous lesions with extensive epithelial hyperplasia. Bone involvement may accompany generalized systemic disease. Both osteomyelitis of long bones, vertebrae and other bones and arthritis may occur. Draining sinus tracts may evolve from subcutaneous and osseous lesions.

Coccidioidomycosis is the AIDS-defining illness for many patients, who commonly present with fever and chills, weight loss and night sweats. After pulmonary disease, coccidioidal meningitis is a frequent complication. Serologies are often negative in AIDS patients and the mortality rate is high. Diffuse pulmonary disease and a low $CD4^+$ lymphocyte count (<50/μl) carry a poor prognosis.

MANAGEMENT

Symptomatic treatment is usually adequate for the patient with primary coccidioidomycosis, although ketoconazole may reduce the symptoms. However, if the primary infection is persistent or severe, or if there is evidence of dissemination, fluconazole or itraconazole should be administered for 2–6 months.

The chronicity of the disseminated disease usually requires prolonged therapy. Successful treatment may require continuous administration for more than a year. Chronic coccidioidal meningitis is often treated with fluconazole, which has excellent penetration across the blood–brain barrier. Some cases may require the intrathecal administration of chemotherapy. Grave disease is treated with amphotericin B in the usual formulation or as a lipid preparation to provide a higher dosage with reduced toxicity. For patients with AIDS, the total dose of amphotericin B should be at least 1.0 g, after which a maintenance regimen of itraconazole or fluconazole may be started. Patients treated with amphotericin B or azoles are more likely to relapse if the delayed skin test becomes negative and the CF antibody titers equal or exceed 1:256.

Histoplasmosis

Histoplasmosis is caused by the thermally dimorphic fungus, *Histoplasma capsulatum*. The infection occurs worldwide and is initiated by inhalation of microconidia produced by the mold form (see Table 178.12). The incidence varies considerably, being negligible in many parts of the world but significant in local regions of endemicity where most of the native population has been infected. Ninety-five percent of these infections are unapparent and detected only by the manifestation of residual lung calcification(s), a delayed hypersensitivity skin test reaction to histoplasmin, or both. The skin test antigen, histoplasmin, is a standardized culture extract similar to coccidioidin.

MYCOLOGY

There are three varieties of *H. capsulatum*, but most human cases are caused by *H. capsulatum* var. *capsulatum*, while *H. capsulatum* var. *duboisii* is found in Africa (see below), and *H. capsulatum* var. *farciminosum* causes infection in horses. Although *H. capsulatum* can undergo sexual reproduction with a compatible mating strain, clinical isolates reproduce asexually, being haploid and clonal. *Histoplasma capsulatum* and *B. dermatitidis* are closely related ascomycetous fungi and members of the same teleomorphic or sexual genus, *Ajellomyces*.

Histoplasma capsulatum is a thermally dimorphic fungus. At temperatures below 95°F (35°C) it grows as a mold, often white or brown in color, and at 98.6°F (37°C) it grows as a yeast with small, heaped and pasty colonies. *Histoplasma capsulatum* characteristically grows slowly. Under optimal conditions, the mold colony develops after 1 or 2 weeks and conidia are produced shortly thereafter. However, cultures of clinical specimens sometimes require incubation periods of 8–12 weeks before there is detectable growth. Primary isolates are often brown and become white with prolonged cultivation.[113]

Microconidia and macroconidia are produced directly by the aerial hyphae at temperatures below 95°F (35°C). The microconidia are globose and 1–5 μm in diameter. Upon dehydration, they are easily dislodged by the wind and become airborne. Their small size enables the microconidia to be inhaled into the alveoli. The macroconidia are larger and more distinctive. At 98.6°F (37°C) the mold converts to growth as a small, budding yeast. Conversion is often difficult to effect *in vitro* but is enhanced by a rich, complex medium, such as brain–heart infusion agar. The yeast cells are small, ellipsoidal, approximately 1–3 μm × 3–5 μm in size, and virtually identical to the yeasts observed *in vivo* within phagocytes (see Table 178.12 and Fig. 178.9).

ECOLOGY AND EPIDEMIOLOGY

In nature, *H. capsulatum* lives in soil with a high nitrogen content and is associated with bat and avian habitats. *Histoplasma capsulatum* has been isolated many times from bird roosts, chicken houses, bat caves and similar environments. Conidia, when dry, are easily airborne and spread by wind currents, as well as by birds and bats. *Histoplasma capsulatum* is most prevalent in the environment where the infection is most endemic, namely in the Ohio–Mississippi Valley: in Missouri, Kentucky, Tennessee, Indiana, Ohio and southern Illinois. This region also has the highest population of starlings, which tend to congregate in large numbers. The excrement from these birds provides an ideal medium for the enrichment of *H. capsulatum*. In South America, the chief reservoir appears to be chicken coops and bat caves.

In cases of histoplasmosis from Africa, both *H. capsulatum* var. *capsulatum* and *H. capsulatum* var. *duboisii* have been isolated. Infections elsewhere are due to the global variety, *H. capsulatum* var. *capsulatum*. *Histoplasma capsulatum* var. *duboisii* causes African histoplasmosis, which is distinguished from the usual infection by:

- a greater frequency of skin and bone lesions;
- diminished pulmonary involvement;
- pronounced giant cell formation; and
- larger, thick-walled yeast cells in tissue.

Although these clinical features are characteristic and reproducible, *H. capsulatum* var. *duboisii* cannot be reliably differentiated *in vitro* from the type species on the basis of morphology, physiology and antigenic composition.

Much of the knowledge concerning the prevalence of histoplasmosis has been derived from global skin test surveys with histoplasmin. The region with the highest level of reactivity is the central USA, along the river valleys of Ohio, Mississippi, St Lawrence and Rio Grande,

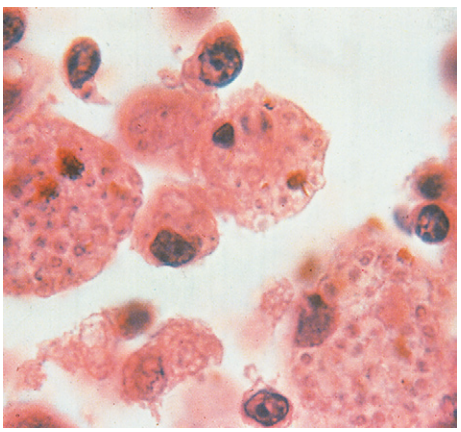


Fig. 178.9 *Histoplasma capsulatum*. Small yeast cells packed inside macrophages in a Giemsa-stained smear of bone marrow aspirate.

where in some locales 80–90% of the population may be skin test positive by the age of 20 years. Foci of high reactivity exist elsewhere in the world, such as southern Mexico, Indonesia, the Philippines and Turkey. In the USA alone, projections based on skin test surveys have led to estimations that more than 40 million people have been exposed with 500 000 new infections every year. Of these, perhaps 55 000–200 000 cases will be symptomatic, 1500–4000 will require hospitalization annually and 25–100 deaths will occur. These projections were made prior to 1980 and do not reflect the increasing incidence of opportunistic histoplasmosis in patients with AIDS.

Outbreaks or epidemics of acute respiratory histoplasmosis result from the simultaneous exposure of a large number of people. These epidemics are not caused by direct spread among humans or animals. Rather, the sudden release leads to multiple exposure of a heavy inoculum that has accumulated in a dormant environment. Silos, air-conditioning units contaminated with bird droppings, and accumulations of guano in caves, attics or parks have all been implicated as reservoirs for *H. capsulatum* in outbreaks of this type.

Males develop symptomatic histoplasmosis more often than females and approximately 75% of cases occur in males. Before puberty, the attack rate for males and females is identical and the percentage of positive skin test reactors is the same for both sexes at all ages. These epidemiologic data suggest that either adult males are inherently more susceptible to the disease or females are more resistant. Severity of disease and mortality are greater at the age extremes, in infancy and after the age of 50.

In addition to humans, many wild and domestic animals are susceptible to histoplasmosis. Some animals, including the bat, may act as vectors to disseminate the organism in nature.

PATHOGENICITY

All clinical forms are believed to evolve from the same natural history. Microconidia are inhaled from an exogenous source and penetrate to the alveoli, where they convert to small, budding yeast cells. The conversion of *H. capsulatum* to the yeast form at 98.6°F (37°C) is essential for pathogenicity, and expression of yeast-specific genes has been correlated with virulence and thermal tolerance.[118] The yeast cells are readily phagocytized by alveolar macrophages. At this stage, the yeast-laden macrophages may be cleared through the upper respiratory tract. They may also disseminate through the circulation, spreading the yeasts to other reticuloendothelial organs, and/or they may invoke a tissue response *in situ*. The tissue reaction may involve an early influx of neutrophils and lymphocytes, but the pyogenic inflammatory response gives way to epithelioid cell tubercle formation. In the course of these various possible reactions, the intracellular yeasts may or may not be inactivated by the phagocytes, depending upon the immune status of the host.

The phagocytized yeast cells of *H. capsulatum* are able to survive intracellularly by a calcium-dependent process, block acidification within the phagolysosome and multiply within macrophages.[119] However, macrophages from immunized animals, as well as normal macrophages activated by immune lymphocytes or cytokines, restrict the growth of intracellular yeasts. In experimental, self-limited murine histoplasmosis, various parameters of cell-mediated immunity are depressed during the height of antigen (yeast) burden, suppressor T cells and macrophage-like suppressor cells are detected, and production of IL-1 and IL-2 is impaired. Concomitant with resolution of the infection, the number of suppressor cells in the spleen diminishes and T helper cells increase.[110] These correlations of competent cell-mediated immune responses with resistance to infection are supported by the clinical data. Similar to coccidioidomycosis, there is an inverse relationship between the magnitude of the cell-mediated immune response, as measured by delayed-type hypersensitivity or *in-vitro* lymphoblastogenesis and high levels of specific antibody, which correlate with the severity of the disease. Additional evidence suggests that TNF may be crucial in the induction of protective T-cell responses.[110]

DIAGNOSTIC MICROBIOLOGY

Microscopic examination

Histoplasmosis can be diagnosed on finding the yeast cells in clinical material. Suitable specimens include sputa, tissue from biopsy or surgical specimens, spinal fluid and blood. The buffy coat of a blood specimen may reveal yeast-filled macrophages. Bone marrow obtained when patients are febrile may contain yeast cells. Smears of infected sputum, blood, marrow or tissue that have been fixed with methanol and stained with the Wright or Giemsa stain will reveal the characteristically small, ellipsoidal yeast cells (approximately 2 × 4 µm) inside macrophages. With either stain, the larger end of the yeast cell contains an eccentric, red-staining mass (see Fig. 178.9).

Culture

Sputum specimens should be collected early in the morning and purulent or sanguineous portions should be selected for culture. A bronchial wash is even more likely to be positive. Because *H. capsulatum* may grow very slowly, cultures should be incubated for up to 12 weeks, if possible, before discarding as negative. If a sporulating mold develops, *H. capsulatum* can be identified by the presence of its characteristic macroconidia (see Table 178.12) and by conversion to the yeast form by growth on an enriched medium at 98.6°F (37°C), by the detection of *H. capsulatum*-specific exoantigens (see Table 178.13) or by using a specific DNA probe.

Lysis-centrifugation is the most sensitive and rapid method to recover dimorphic fungi from blood, especially *H. capsulatum*.[113]

Skin test

The antigen, histoplasmin, is a standardized culture filtrate preparation and 0.1 ml is injected intradermally. A positive reaction is indicated by induration of >5 mm diameter after 48 hours. As with coccidioidin, a positive test, if specific, denotes previous sensitization to *H. capsulatum*. Without a history of prior negativity, the positive test has no diagnostic significance. Histoplasmin is a polyvalent mixture of antigens, only some of which are specific for *H. capsulatum*. Because some antigenic determinants are shared by other pathogenic fungi, cross-reactions can occur. For example, some individuals sensitive to *B. dermatitidis* or *C. immitis* will give a false-positive reaction to histoplasmin. Therefore, along with histoplasmin, it is routine to administer coccidioidin in the USA or coccidioidin and paracoccidioidin in South America. A reaction to a single antigen is generally considered specific. Reactions to two antigens may be caused by sensitization to one or both, although the larger reaction is often considered more specific.

Serology

Specific antibodies to *H. capsulatum* antigens can be detected during infection. Two serologic tests are now widely accepted because of their convenience, availability and utility: the measurement of antibodies by complement fixation (CF) and the immunodiffusion (ID) test for precipitins. Both tests may be helpful in the diagnosis and prognosis of histoplasmosis, provided the results are properly interpreted (see Table 178.14).

CF tests detect antibodies to two antigens of *H. capsulatum*: histoplasmin and a standardized suspension of killed yeast cells. Because of the possibility of cross-reactivity, patient sera are tested concomitantly against other fungal antigens, such as coccidioidin, spherulin, *B. dermatitidis* or *Paracoccidioides brasiliensis*. Serum antibodies specific for *H. capsulatum* antigens can be detected by the CF test 2–4 weeks following exposure. Most laboratories perform the CF test on twofold dilutions of patient serum, beginning with a dilution of 1:8. With resolution of the

infection, the antibody titer gradually declines and disappears (i.e. titer <1:8), in most cases by 9 months. The CF test with either *H. capsulatum* yeast or mycelial (histoplasmin) antigen is very sensitive and 90% of patients are positive (i.e. titer >1:8). A titer of 1:32 that persists or rises over the course of several weeks indicates active disease in patients with an established diagnosis of histoplasmosis. Unfortunately, in sensitive patients, the skin test antigen may boost the CF antibody titer to histoplasmin and the elevated titer may remain for as long as 3 months. If a patient's serum is reactive to more than one antigen or if it is anticomplementary, the ID test should be conducted.

Precipitins can be detected by immunodiffusion (ID) of serum and antigen in agarose. The ID test becomes positive in up to 80% of patients with histoplasmosis by the third or fourth week of infection. This test, while less sensitive and requiring a longer time to become positive, is more specific than the CF test. There are two specific precipitin bands, m and h (see Table 178.13). The m line, which is observed more frequently, appears soon after infection and may persist in the serum up to 3 years following recovery. The h band is more transient. Because it disappears soon after the disease, the presence of serum antibodies to the h antigen is better correlated with active infection. As with the CF titer, the m band may be boosted by the administration of the histoplasmin skin test.

An excellent radioimmunoassay (RIA) test for antigenemia and antigenuria has been developed by MiraVista Diagnostics (Indianapolis, IN, USA).[120] Tests for polysaccharide antigen in the serum have been detected in 79% of patients with disseminated histoplasmosis and 97% had a positive test for antigen in the urine. After treatment with amphotericin B, serum titers dropped in all the patients and urine titers dropped in 91% of patients. Testing either serum or urine had prognostic value, detecting relapses in AIDS patients. The antigen can also be measured in CSF or BAL fluid specimens. However, the test lacks optimal specificity because patients with other systemic mycoses have been shown to yield positive tests for the antigen.

PREVENTION

Several approaches are currently underway to develop an effective vaccine for histoplasmosis.[112]

CLINICAL MANIFESTATIONS

The manifestations of infection with *H. capsulatum* are numerous. The initial pulmonary episode may be acute or chronic, or dissemination may occur by hematogenous or lymphatic spread from the lungs to other organs. Most normal individuals are able to contain the infection. The granulomata that form may undergo fibrosis and residual scars may remain in the lungs or spleen. Resolution appears to confer some immunity to re-infection. This process occurs without symptoms in 95% of all persons with acute, primary histoplasmosis, whether disseminated or confined to the lung. For a description of the pneumonic forms of histoplasmosis, see Chapter 31.

The gamut of clinical forms and pathology observed in pulmonary histoplasmosis can also occur in any other part of the body. The yeast cells are probably disseminated throughout the body while inside macrophages. The most common sites of involvement, after the lung, are the reticuloendothelial tissues of the spleen, liver, lymph nodes and bone marrow. However, lesions have been documented in almost every organ. Dissemination may be completely benign and unapparent except for the presence of calcified lesions, usually in organs of the reticuloendothelial system.

Alternatively, disseminated histoplasmosis may be acute and progressive. In such cases, the pulmonary symptoms are insignificant and patients may have splenomegaly and hepatomegaly, weight loss, anemia and leukopenia. Granulomatous lesions and macrophages packed with yeast cells can be observed throughout the liver, spleen, marrow and, quite often, the adrenals. Acute progressive histoplasmosis is often fulminant and rapidly fatal; ultimately every organ can become diseased. This form of histoplasmosis is an opportunistic disease associated with compromised cell-mediated immunity, such as patients with AIDS or those receiving immunosuppressive drugs and those with underlying lymphomatous neoplasia. In most cases, the compromising condition served to reactivate a quiescent lesion that was originally acquired years earlier. Within the endemic area, infants with histiocytosis may develop disseminated histoplasmosis that is characteristically fulminant. Chronic disseminated histoplasmosis may evolve from protraction of the acute disease. This form is progressive, with eventual involvement of every organ, especially the mucocutaneous areas around the eye, tongue and anus.

Within the vast endemic regions, histoplasmosis is often the initial AIDS-defining condition among HIV-infected patients. The survival time for patients with AIDS is significantly shorter for those with disseminated histoplasmosis. Important risk factors for the development of histoplasmosis in AIDS patients include environmental exposure to likely sources of *H. capsulatum* (chicken coops), positive CF antibodies to histoplasmin, chronic medical condition(s), herpes simplex infection and a $CD4^+$ lymphocyte count of <150 000/ml.[121]

MANAGEMENT

Most primary pulmonary infections with *H. capsulatum* go undetected and require no treatment. Symptomatic cases in immunocompetent persons are treated with itraconazole. This recommendation applies to patients with progressive pulmonary or disseminated disease, as well as mild to moderate or stable disease.[120] Severe cases of any form are treated initially with amphotericin B followed by itraconazole. The regimen of amphotericin B is similar to that applied to other systemic mycoses. Recovery following treatment with amphotericin B is generally faster and fewer relapses occur than are experienced with blastomycosis and coccidioidomycosis. Arrested pulmonary lesions are often removed surgically. Less severe cases in non-immunocompromised patients may be treated with itraconazole. AIDS patients with histoplasmosis may be treated initially with a lipid formulation of AMB and then placed on maintenance therapy with itraconazole. Cases of meningitis due to *H. capsulatum* are treated with an amphotericin B preparation followed by fluconazole. As noted earlier, voriconazole has been used successfully in a limited number of patients with endemic mycoses.[115]

Paracoccidioidomycosis

Paracoccidioidomycosis (South American blastomycosis) is caused by the thermally dimorphic fungus, *Paracoccidioides brasiliensis*. The infection is restricted to endemic areas of Central and South America. It is a chronic, granulomatous disease that begins with a primary, pulmonary, usually inapparent infection that disseminates to produce ulcerative granulomata in the mucosal surfaces of the nose, mouth and gastrointestinal tract. In addition to the skin and lymph nodes, the infection may spread to the internal organs.

MYCOLOGY

Colonies of *P. brasiliensis* grow very slowly and the macroscopic features of the mold colony are variable and nonspecific. Various asexual reproductive structures are produced by *P. brasiliensis*, including chlamydospores, arthroconidia and singly borne conidia. In the absence of conidia, which may not be produced for 10 weeks in culture, the mycelia and colony may be indistinguishable from *B. dermatitidis* or many saprophytic molds. The yeast cells, which can be induced by cultivation on a rich medium at 95–98.6°F (35–37°C), are readily identified by their unique appearance. As shown in Figure 178.10, the yeasts produce multiple buds, each attached to the parent yeast by a narrow base. The yeast cells are large, up to 30 µm in diameter, and

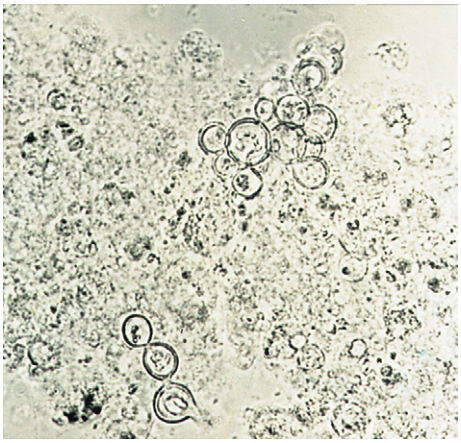


Fig. 178.10 *Paracoccidioides brasiliensis*. Large multiply budding yeast cells in a potassium hydroxide preparation of a scraping of cutaneous paracoccidioidomycosis.

have thinner walls than the yeasts of *B. dermatitidis*. These forms have been described as 'pilot wheels'.

ECOLOGY AND EPIDEMIOLOGY

The natural habitat of *P. brasiliensis* has not been proven, but is presumed to be soil. The organism has been recovered only sporadically and not repeatedly from soil in Venezuela and Argentina. Like *B. dermatitidis*, its environmental niche and life cycle in nature are unknown. There is no evidence of an animal vector or transmission of the infection. Human infections are presumed to follow exposure to the organisms from an exogenous source. Most patients are males (>90%), agricultural workers, often malnourished, and usually 30–60 years of age.

Thousands of cases of paracoccidioidomycosis have been reported from Brazil, Venezuela and Colombia, and fewer cases from Argentina, Ecuador and other South and Central American countries, with the exception of Chile and the Caribbean nations. Discrete endemic foci exist within this broad area of geographic distribution. However, all cases are isolated and outbreaks have not been observed. The endemic zones are associated with moderate temperatures (57–86°F/14–30°C) and rainfall, elevation of 500–6500 feet, subtropical forests and river valleys, but not all areas fitting this description have paracoccidioidomycosis.

Skin test surveys have been conducted with various antigens derived from *P. brasiliensis*. These paracoccidioidins exhibit cross-reactivity with histoplasmin and it is difficult to interpret double reactions of equal size in the same individual. As with the skin test antigens of the other dimorphic, systemic pathogens, paracoccidioidin elicits a delayed, indurative reaction that indicates previous exposure. The percentage of reactivity in the endemic areas varies up to 75% and occurs equally in both men and women. Significant risk factors for infection (i.e. positive skin test) include agricultural occupations, association with certain aquatic environments and contact with bats. Many patients with paracoccidioidomycosis are malnourished and exhibit depressed cell-mediated immune responses.

PATHOGENICITY

Paracoccidioides brasiliensis, like the other systemic fungi, causes disease in males more frequently than females (see Table 178.11), although skin test surveys have revealed comparable reactivity between the sexes, implying equal exposure. Sex-linked differences may be associated with the generally more potent cellular immunity of females. Limited studies indicate that physiologic concentrations of sex hormones do not directly inhibit these fungi. However, a protein from the mycelial cytosol of *P. brasiliensis* has been shown to bind estrogen but not testosterone or other hormones. Binding blocks conversion of the mycelium to yeast at 98.6°F (37°C) and may explain the resistance of females to paracoccidioidomycosis.[122] Once yeast cells of *P. brasiliensis* have developed in the lung, yeast cell wall polysaccharides, such as α-glucan, are associated with virulence and the ability to stimulate granulomata.

DIAGNOSTIC MICROBIOLOGY

Sputum, tissue or scrapings of mucocutaneous lesions may reveal the multiply budding yeast cells that are pathognomonic for *P. brasiliensis* (see Fig. 178.10). Specimens should be cultured at 77–86°F (25–30°C) on mycologic media. The yeast form often grows better at 95°F (35°C) or 96.8°F (36°C) than 98.6°F (37°C).

The ID test is extremely useful. As indicated in Table 178.14, nearly all patients have at least one of three specific precipitin lines, which are detected by identity with reference serum. The ID test also has prognostic value, as the bands disappear with clearing of the infection, and the number of bands is somewhat correlated with the severity of the disease. The CF test is quantitative and useful for assessing prognosis, but cross-reactions occur with other fungi.

CLINICAL MANIFESTATIONS

The initial contact with *P. brasiliensis* occurs by inhalation. This episode is unapparent and the organism becomes quiescent for an indefinite period or the lesion may resolve. This asymptomatic infection results in the acquisition of a positive delayed skin test reaction to paracoccidioidin. The eventual development of symptomatic disease depends upon the host–fungal interaction, i.e. the integrity of the cell-mediated immune responses of the individual, environmental conditions (e.g. temperature, nutrients), host conditions (e.g. age, sex, state of nutrition) and the virulence of the strain of *P. brasiliensis*.

Acute or subacute disease

Patients under 30 years of age may develop an acute, progressive infection characterized by lymphonodular lesions in the lung. This 'juvenile' form is rare. The yeasts may disseminate to the reticuloendothelial tissue, lymph nodes, liver, spleen, skin, bone, joints or other organs. The severity and duration of the illness depend upon the extent of organ involvement and it may be fatal within a period of several weeks or months.

Chronic disease

More than 90% of cases are the chronic 'adult' type and develop from activation of latent cells, usually after several years. Lesions may be localized in the lung or metastasis may occur from the lung to other organs, particularly the skin and mucocutaneous tissue, lymph nodes, spleen, liver, adrenals and combinations thereof. Mucocutaneous, often petechial, lesions frequently develop on the corners of the mouth, lips, gingiva or tongue. Pulmonary lesions are granulomatous nodules that may cavitate but rarely calcify.

MANAGEMENT

Patients should be assessed for malnourishment, alcoholism and extensive tobacco use, all of which exacerbate the disease. Since many of the antifungal drugs are effective against *P. brasiliensis*, the initial treatment choice may reflect the expense and local availability

of antifungal agents. Sulfonamide derivatives are among the earliest but less effective drugs. Trimethoprim–sulfamethoxazole (TMP–SMZ) is the currently recommended formulation of this type. The azoles are much more effective. Itraconazole is currently the drug of choice and a clinical cure rate approaching 100% is achievable with a daily dose of 100 mg for 6 months. Relapses are rare. Fluconazole is also effective, producing cures in about 90% of patients who received 200–400 mg/day for 6 months. Although amphotericin B is highly effective against paracoccidioidomycosis, it should be reserved for patients who fail to respond or cannot tolerate one of the azoles. After initiating therapy, serologies are checked every few months to monitor the effectiveness of treatment. Some clinicians recommend a maintenance regimen of TMP–SMZ or itraconazole for up to 1 year after serologic tests become negative or indefinitely for patients with AIDS.

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Chapter 179

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Superficial and subcutaneous fungal pathogens

Superficial Fungal Pathogens

The majority of superficial fungal infections are caused by three groups of fungi: dermatophytes, *Candida albicans* and *Malassezia* spp.[1–3]

NATURE

Candida albicans is the principal agent of candidiasis. *Malassezia* spp. are lipophilic (lipid-requiring) yeasts found on the body surface as commensals and are agents of the skin disease tinea versicolor (pityriasis versicolor). In addition, they may be causal in the allergically mediated condition seborrheic dermatitis.

Other fungi cause less common types of superficial disease. Yeasts in the genus *Trichosporon* may cause white piedra, superficial colonizations of the scalp, and axillary and pubic hair shafts. The melanized yeast-like organism *Hortaea werneckii* (also known by several older names all ending in '*werneckii*') causes the dark skin lesions of tinea nigra. The rare hair shaft colonization black piedra is caused by *Piedraia hortae*. Finally, approximately 35 nondermatophytic filamentous fungi and a few yeasts have been authenticated as causing onychomycosis (i.e. fungal nail disease). Most prominent among these are members of the genera *Scytalidium*, *Aspergillus*, *Scopulariopsis*, *Fusarium* and *Onychocola*.

EPIDEMIOLOGY

Dermatophytosis is among the most common of all communicable diseases. Because many cases are never brought to medical attention, fully reliable incidence figures do not exist. Onychomycosis, in which the great majority of cases are dermatophytic, may account for 10–30% of all superficial mycoses. In the USA Medicare insurance for seniors (≥65 years old) in 1 year paid for consultations involving 662 000 patients, or 2.7% of the senior population, at a cost of over US$43 000 000. Less than 10% of those aged under 30 years and more than 20% of those aged over 75 years had tinea unguium (dermatophytic onychomycosis; Table 179.1) in population studies.[4] Tinea capitis in children is common in some areas. In an urban center in the USA up to 4% of children presenting with nondermatologic complaints at a clinic were infected by *Trichophyton tonsurans*, whereas up to 30% of asymptomatic adults accompanying infected children were carriers.[5] A study of 224 children in a Philadelphia school reported 3% symptomatic and 14% asymptomatic, infected children.[6] Tinea pedis has been found to affect 1.5% of pediatric patients, 5.9% of 11–15 year olds[5] and up to 45% of adult marathon runners.[7] There is a significant age-dependent association between sporting activities and pedal dermatophytosis.[8] Many of the most prevalent dermatophytes are cosmopolitan, but certain species, especially agents of tinea capitis, have defined endemic regions (see Table 179.1).

Cutaneous candidiasis is particularly common in infants, at least 10% of whom have candidal skin colonizations. Fifty per cent of this colonized group go on to become symptomatic, usually with candidal diaper dermatitis. Many adults harbor an indigenous strain of *C. albicans*; up to 30% of healthy women, for example, are culture positive for this species in vaginal swab samples, at least when pregnant or taking oral contraceptives. The lower gut may serve as a reservoir when other body sites are free of *C. albicans*. Normal skin only rarely yields *C. albicans* but the yeast may rapidly colonize chronically moist or damaged skin, including moist dermatophyte lesions.

Malassezia spp. have been found as skin commensals in over 90% of humans surveyed.[1,2] In the temperate zone they only occasionally proliferate to the point of causing the finely scaly maculae of tinea versicolor, but in the tropics the prevalence may reach 50% of the population. *Malassezia* spp. in patients who have HIV and other immunocompromised patients may cause pustular hair follicle inflammations referred to as *Malassezia* folliculitis, and may be associated with an increased prevalence of seborrheic dermatitis.[9] In atopic individuals, *Malassezia* spp. growing commensally on skin may serve as a triggering allergen in atopic dermatitis.[9] A possible association between *Malassezia* and neonatal acne or cephalic pustulosis is under investigation.

White piedra is found worldwide, but is now uncommon because of modern hygiene. Black piedra is native to South East Asia, the Pacific and South America, and affects other primates in addition to humans. It is uncommon when discouraged but is cultivated in some cultures. Tinea nigra is of tropical or subtropical origin; about 150 indigenous US cases were reported between 1950 and 1988, in addition to many more cases acquired by travelers, mostly in the Caribbean. *Scytalidium* infections of nails, soles or palms are usually acquired in the tropics. *Scytalidium dimidiatum*, the most common infectious species, has rapidly growing pantropical forms as well as a distinctive slow-growing Indian subcontinent form that may be transmitted among family members in the Indian Diaspora. Most other agents of nondermatophytic onychomycosis are cosmopolitan saprobic molds and, despite their opportunistic potential, are more frequently seen as insignificant contaminants of body surfaces than as etiologic agents.[10]

PATHOGENICITY

Dermatophytes normally infect only the keratinized stratum corneum of the epithelial skin layers. They degrade keratin and other skin proteins. They are restricted to the stratum corneum by cellular immune components, and by the iron limitation and other effects mediated by transferrin. A twofold higher prevalence in males than females may reflect

Table 179.1 Definition and distribution of dermatophytoses

Area of infection	Disease name	Prevalent agents	Geographic area
Scalp	Tinea capitis	*Trichophyton tonsurans*	Cosmopolitan, especially urban Americas, Latin America
		Microsporum canis	Cosmopolitan; predominant dermatophyte in parts of south and east Europe
		Trichophyton violaceum	Endemic to Middle East, North Africa
		Trichophyton soudanense	Endemic to sub-Saharan Africa
Beard area	Tinea barbae	*Trichophyton rubrum*, *Trichophyton verrucosum*	Cosmopolitan
Glabrous skin (trunk, limbs, face)	Tinea corporis	*Trichophyton rubrum*, *Trichophyton mentagrophytes* complex, *Microsporum canis*, *Epidermophyton floccosum*	Cosmopolitan
		Trichophyton megninii	Endemic to Portugal, Sardinia
Scalp, glabrous skin	Favus	*Trichophyton schoenleinii*	Relict in rural Africa, central Asia; formerly widespread
Palms	Tinea manuum	*Trichophyton rubrum*, *Trichophyton mentagrophytes* complex	Cosmopolitan
Groin	Tinea cruris	*Trichophyton rubrum*, *Trichophyton mentagrophytes* complex, *Epidermophyton floccosum*	Cosmopolitan
Soles, toe webs	Tinea pedis	*Trichophyton rubrum*, *Trichophyton mentagrophytes* complex, *Epidermophyton floccosum*	Cosmopolitan
Nails	Tinea unguium (dermatophytic onychomycosis)	*Trichophyton rubrum*, *Trichophyton mentagrophytes* complex	Cosmopolitan

inhibition by progesterone. Involvement of an autosomal dominant susceptibility allele has been proposed in the common chronic form of *Trichophyton rubrum* infection.[11] An indication of the relative importance of lymphocytes in host defense is seen in HIV infection, in which helper T-cell counts below 100 cells/ml correlate with a marked increase in onychomycosis,[12] including the unusual 'proximal white' form.

Dermatophytes differ in their host interactions. Anthropophilic dermatophytes (Table 179.2), specific to human disease, are distinguished from zoophilic dermatophytes, which have specific animal associations but may be transmitted to humans, and from geophilic dermatophytes, which are occasionally pathogenic to humans or animals but primarily grow on decaying keratinous material. The *Trichophyton mentagrophytes* complex, containing four sibling species distinguishable by molecular study, has both anthropophilic and zoophilic variants. Infection of humans by zoophilic dermatophytes usually elicits a pronounced inflammatory response.[14] Such inflamed lesions may resolve spontaneously, unlike the often chronic lesions of anthropophilic dermatophytoses.

The common anthropophilic dermatophytes include lower body dermatophytes associated with sites other than the scalp, and dermatophytes strongly adapted for tinea capitis, less commonly causing other tineas. *Trichophyton rubrum*, *T. mentagrophytes* complex and *Epidermophyton floccosum* are the common lower body species. Tinea capitis dermatophytes consist of two major groups distinguished by their colonization of hair (see Table 179.2). In the 'ectothrix' group, hair shafts are externally ensheathed with masses of arthroconidia (spores). In the 'endothrix' group, the hair shafts are heavily invested internally by arthroconidia. An anomalous scalp infection, favus, caused by the now rare *Trichophyton schoenleinii*, is distinguished by the presence of follicles transformed into scutula, that is, pits lined with fungal mycelium.

Tinea capitis agents primarily cause new infections in children, and may cause dramatic outbreaks. *Microsporum audouinii* infections spontaneously resolve at 15–19 years of age, but most endothrix agents cause lifelong asymptomatic infections in some adult carriers[6] and occasionally cause new tinea corporis or other infections in adults. New anthropophilic tinea capitis infections are usually acquired via shared headgear, bedding or grooming and haircutting instruments. Adults who acquire new infections caused by endothrix species usually have intimate contact with infected children. Anthropophilic lower body dermatophytoses are often acquired via the feet, either from family members or in communal aquatic or exercise facilities. After infecting the feet, these fungi may go on to infect other body sites.

Zoophilic dermatophytes (see Table 179.2) usually cause tinea corporis or tinea capitis in humans. They may be transmitted directly from infected animals or from fomites, such as fence posts in cattle yards. *Microsporum canis* may cause limited outbreaks among humans before virulence is attenuated.[15]

Candida albicans is often acquired in the birth canal or in infancy from caregivers. Generally, an individual harbors only one or two strains. Cutaneous candidiasis is predisposed to by warm, moist conditions with abrasion, especially in the diaper rash of infancy but also in adult occupations that involve wet hands. In the latter cases, paronychia or interdigital erosion frequently results. Intertriginous candidiasis occurs in moist body folds and is exacerbated by diabetes mellitus or obesity. Chronic mucocutaneous candidiasis (CMC), in which skin

Table 179.2 Common and formerly common dermatophytes and their infectivity

Anthropophiles (type of scalp infection if characteristic)	Zoophiles (type of human scalp infection) [principal hosts]	Geophiles (type of human scalp infection)
Epidermophyton floccosum	*Microsporum canis* (ectothrix) [cat, dog]	*Microsporum gypseum* (ectothrix)
Microsporum audouinii (ectothrix)	*Microsporum gallinae* [fowl]	*Microsporum praecox*
Microsporum ferrugineum (ectothrix)	*Microsporum nanum* [swine]	*Microsporum racemosum*
Trichophyton concentricum	*Microsporum persicolor* [vole]	*Microsporum vanbreuseghemii*
Trichophyton megninii (ectothrix)	*Trichophyton equinum* [horse]	The listing of geophiles does not include species that have not been well substantiated to cause human disease. These species are not properly considered dermatophytes according to Ajello[13]
Trichophyton mentagrophytes complex (phylogenetic species *Trichophyton interdigitale*)	*Trichophyton mentagrophytes* complex (phylogenetic species *Trichophyton interdigitale*, *Trichophyton mentagrophytes*, *Trichophyton erinacei*) [rodents, rabbits, hedgehogs]	
Trichophyton rubrum	*Trichophyton simii* [monkey]	
Trichophyton schoenleinii (favus)	*Trichophyton verrucosum* [cattle]	
Trichophyton soudanense (endothrix)		
Trichophyton tonsurans (endothrix)		
Trichophyton violaceum (endothrix)		

and mucosa are extensively colonized by *C. albicans*, results from inherited defects in cellular immunity.[16] Particular associated conditions include congenital thymus dysplasias, polyendocrine deficiencies and leukocyte dysfunctions such as chronic granulomatous disease. In this latter disease, the impaired production of toxic oxygen species prevents killing of *Candida* spp. within phagocytes.

Malassezia spp. are also generally acquired as commensal surface flora in early infancy. They primarily use fatty acids secreted by the skin. Corticosteroid use, Cushing's disease, malnutrition and immunosuppression may contribute to an increased frequency of tinea versicolor; HIV infection and miscellaneous immunosuppressions predispose to folliculitis.

Nondermatophytic onychomycosis caused by genera other than *Scytalidium* is strongly predisposed to by advanced age.

Details regarding the prevention of infection caused by agents of superficial fungal infection, diagnosis, clinical manifestations and management can be found in Chapter 13.

DIAGNOSTIC MICROBIOLOGY

Superficial mycotic infections are best tested by the combination of direct microscopy for fungal elements in hydroxide (KOH, NaOH) slides, and fungal culture. Direct microscopy may be facilitated by the use of fluorescent fungal cell wall dyes such as calcofluor or Congo red. Histopathology performed with fungal stains has been suggested as an alternative to 'KOH and culture' for onychomycosis, but the ability of this technique to distinguish dermatophyte from nondermatophyte infections has received only preliminary investigation.

Dermatophytes in direct microscopy show cylindrical fungal filaments that may include chains of rounded arthroconidia. At times filaments may be converted entirely into arthroconidia, such as within or around hair shafts in tinea capitis. In superficial white onychomycosis caused by *T. mentagrophytes* complex members, microscopy may show 'frondose branching', an appressed, palmate spreading of fungal hyphae between keratin lamellae. In nails, soles and palms, all morphologies of dermatophyte filaments overlap with those of other potentially infectious fungi, especially *Scytalidium* spp.

Generally, dermatophyte filaments from the growing fronts of infection, such as the peripheral 'ring' in classic ringworm and adjacent to the nailbed in tinea unguium, will be alive and will stain with vital stains such as neutral red.[17] Those from prior growth fronts, such as centers of ringworm lesions or extruded, distal nail, tend to be senescent and will not yield a culture. For this reason, sampling tinea at its extending margin, and subungual onychomycosis at the juncture of nail and nailbed, is recommended. Tinea corporis is sampled by using a scalpel to remove skin scale from the advancing lesion margin after light disinfection with an ethanol swab. In subungual onychomycosis, heavily contaminated debris is scraped from the undersurface of the nail and discarded; then, after swabbing with ethanol, friable material is scraped from the nail undersurface as close as possible to the nailbed. Clippings may also be taken. In superficial white onychomycosis, white, opaque areas on the upper nail surface are scraped. As with subungual onychomycosis, superficial debris is scraped and discarded before sample collection. In tinea pedis of the sole and tinea manuum, in which growth fronts may not be apparent, the affected area should be scraped broadly so that the whole is represented in the sample.

Even with expert sampling approximately 10–25% of nail samples positive for fungal filaments in direct microscopy fail to yield a culture.[17] That such a finding accurately reflects the active infection can be confirmed by vital staining,[18] and should not be mistaken for a technical 'false-negative' culture result. Also, approximately 45–50% of toenails and 60% of fingernails clinically suggestive of onychomycosis fail to yield any evidence of fungal infection.[19] Dermatophyte colonization in very degraded nails may be partially supplanted by secondary invaders such as *Pseudomonas* bacteria and *Scopulariopsis* mold (the latter may also appear as a sole agent of onychomycosis or as a contaminant), and the original etiologic agent may be difficult to trace.

Hair from tinea capitis or barbae is plucked so that the hair root, the best source of inoculum, is included. In 'black dot' tinea capitis, in which hairs are fragile, a few hair roots may be extracted with the tip of a scalpel and included along with surface scrapings of the lesion. *Microsporum* scalp lesions typically show strong yellow–green fluorescence under a Wood's light and can be presumptively diagnosed in this

manner. In zoophilic tinea corporis and other lesions with vesicles or bullae, the apices of these may be clipped and included in the sample. Moist, suppurating lesions may be sampled with a swab. Swab samples may make direct microscopy difficult and are best evaluated by culturing.

For culture, scrapings or fragments from clippings are usually planted on Sabouraud's agar supplemented with antibacterials and the selective antifungal cycloheximide. Common commercial equivalents include Mycosel, Dermasel and Mycobiotic. Dermatophytes and *C. albicans* are among the small minority of fungi able to grow well on these media. Potato glucose or potato flake agar with cycloheximide is preferred by some laboratories because the distinctive pigmentation of some species is enhanced. A cycloheximide-free medium is generally used for nails, and also recommended for soles and palms because nondermatophytic etiologic agents such as *Scytalidium* and *Fusarium* spp. must also be isolated from at least some of these sites. The most common of such media are plain Sabouraud's agar supplemented with antibacterials only, and two restrictive media: Littman oxgall agar and inhibitory mold agar. These media, unlike plain Sabouraud's agar, inhibit fast-growing contaminant molds from overgrowing the slower growing etiologic agents.

The characteristics and criteria used in identifying dermatophytes have been summarized.[20–22] Since most dermatophyte species are susceptible to similar therapies (duration of therapy may vary, e.g. between *Microsporum* spp. and the more rapidly inhibited endothrix *Trichophyton* spp. in tinea capitis), species identification is salient primarily to recognize situations in which animal hosts or familial or institutional carriers constitute potential sources of re-infection and continuation of outbreaks. Zoophilic dermatophyte species and the endothrix tinea capitis agents are most notorious in these situations, but unusual outbreaks of anthropophilic lower body dermatophytoses may also be detected and controlled through species identification.

Candidiasis in skin and nails is recognized in direct microscopy by the presence of budding yeast cells and candidal-type filaments; the yeast cells bud through a narrow constriction, unlike *Malassezia* yeasts, and the filaments give rise to budding cells on side branches, unlike dermatophyte filaments. Although filaments indicate an invasive condition in cutaneous candidiasis, and are usually found in any genuine case of infection, masses of budding cells alone may be seen in some nail specimens. In fingernails, this often indicates nearby paronychia that was not sampled directly. In some cases, however, especially with toenails, yeast cells merely indicate harmless growth of *Candida* spp. (other than *C. albicans*) of the normal skin flora in crevices in onychomycotic or traumatized nails. *Candida albicans* should normally be identified when isolated from skin; there are a number of inexpensive, specific, rapid tests for this species such as rice-tween, cornmeal-tween or oxgall medium tests for formation of chlamydospores under a cover slip. *Candida* spp. other than *C. albicans* need not be identified to species level from superficial sites except in rare cases in which they are isolated from material with conclusive evidence of invasive yeast infection (e.g. formation of filaments with lateral budding cells) in direct microscopy. Because *C. albicans* commonly contaminates moist dermatophyte lesions without significantly exacerbating symptoms, and because normal flora yeasts may proliferate harmlessly in nail crevices, the laboratory gold standard for any diagnosis of cutaneous yeast infection is the specific presence of yeast-type filamentous elements within cutaneous tissue in direct microscopy.

Tinea versicolor is recognized in direct microscopy by the rounded yeast cells and short hyphal fragments ('spaghetti and meat balls') of *Malassezia* spp. Culture is normally unnecessary and may be problematic when attempted. Although there is considerable research interest in quantitative cultural analysis of *Malassezia* spp. in seborrheic dermatitis, clinical diagnosis based on symptoms remains the gold standard.

Other purely microscopic diagnoses not requiring culture include black and white piedra. Tinea nigra evinces distinctive dark filaments in direct microscopy, but is also readily cultured to yield the etiologic agent, *H. werneckii*. No other organism shows melanized, two-celled yeasts budding from annellidic apertures heavily fringed with collarette remnants. Mycology laboratories must also recognize the microscopically distinctive corynebacterial and actinomycetous elements found in erythrasma, trichomycosis axillaris and pitted keratolysis.[1] All intertriginous skin samples should be stained with methylene blue preparations to detect erythrasma. In specimens from this infection, methylene blue deeply stains delicate branching filaments less than 1 mm in diameter, often seen breaking up into smaller bacillary or coccoid forms. The poorly understood 'dermatophilus-like' agent of pitted keratolysis[1] also stains in methylene blue, and shows a mixture of coccoid cells and short filaments, at least some of which appear tapered and, in broader regions, subdivide to give rise to a second or third longitudinal series of cells. In trichomycosis axillaris, hair is heavily ensheathed in corynebacterial cells (gram-positive rod and coccus forms less than 1 mm in diameter). These may be readily distinguished in crushed preparation in hydroxide from the over 2 mm in diameter arthroconidia and yeast cells seen in the macroscopically similar white piedra.

The correct diagnosis of onychomycosis caused by nondermatophyte molds can be especially challenging. These nondermatophyte filamentous fungi can easily be ascertained as causing onychomycosis if distinctive morphologic elements such as conidiophores are seen in addition to filaments in direct microscopy of nail specimen, or if a fungus from warm latitudes, such as a *Scytalidium*, is isolated in an area in which only such fungi occur in infected patients. Most cases are more ambiguous. A fungal species such as *Aspergillus sydowii* may be isolated either as a contaminant or as an etiologic agent, and filaments seen in direct microscopy may be either nonviable dermatophyte elements or genuine nondermatophyte elements. Therefore, even exclusive and heavy isolation of such a nondermatophyte from a specimen positive for fungal filaments does not guarantee that the nail is infected by the same nondermatophyte.[10] The current gold standard, which may not be easy to attain in practice, is:

- first to demonstrate fungal elements in direct microscopy compatible with the suspected agent; and
- second, in culture, to show, through correlating the results of two nail samples collected at least 1 week apart, that the nondermatophyte in question consistently grows from the diseased nail.

True mixed infection by a dermatophyte and a nondermatophyte may occur, but again can only be demonstrated scientifically by showing a consistent presence of the latter in more than one serial sample.

Subcutaneous Fungal Pathogens

Although subcutaneous fungal infections exhibit extraordinary heterogeneity, they have certain features in common – infection is usually acquired from nature and not from infected humans or animals, and the endemic areas are delineated by an ecosystem that consists of altitude, temperature, rainfall, type of soil and type of vegetation. Most patients belong to low socioeconomic groups or live in rural areas. Subcutaneous mycoses arise from inoculation of soil or vegetation into the skin by minor trauma, and most patients have an occupation connected either with agriculture or an outdoor activity and do not use appropriate footwear.

The group of fungi that cause the majority of subcutaneous infections in humans are termed black molds.[2,23–25] Black molds are a heterogeneous group of darkly pigmented (dematiaceous) fungi, widely distributed in the environment, that occasionally cause infection in humans. The taxonomy and terminology of dematiaceous fungal infections is baffling. The term chromoblastomycosis was introduced in 1922 and was later modified in 1935 to a broader term 'chromomycosis'. More recently, the term 'phaeohyphomycosis' was proposed to cover 'all infections of cutaneous, subcutaneous and systemic nature caused by hyphomycetous fungi that develop in the host tissues in the form of dark walled dematiaceous septate mycelial

elements'. In 1981, the term was further expanded to include deuteromycota and ascomycota whose tissue forms are filamentous and dematiaceous. This certainly excludes infections by fungi that produce thick-walled 'sclerotic bodies' in the tissues and are classically labeled as chromoblastomycosis. The line of demarcation is, however, only histopathologic and very thin because some of the fungi (e.g. *Exophiala dermatidis*), in addition to mycelial forms, produce rounded structures closely resembling sclerotic bodies. Thus, there has been plenty of overlap in the nomenclature of these cases, especially during the 1970s and 1980s.

The clinical spectrum of infection includes mycetomas, chromoblastomycosis, sinusitis and superficial, cutaneous, subcutaneous and systemic phaeohyphomycosis. During the past few years, there have been reports of infections caused by black molds in previously healthy individuals and in immunocompromised patients. Molecular studies have contributed to our understanding of the epidemiology of these infections. In addition, data on antifungal susceptibility tests have become available. Surgical excision and antifungal therapy (usually itraconazole) remain the standard treatment for these infections.

There are many extensive reviews of all the infections described here.[1–3,18,26,27] Specific references are cited here where new information, particularly concerning diagnosis and therapy, supersedes that found in the general texts. A special issue of *Medical Mycology* entitled: 'The dark world of black fungi – a major area of concern' reviews many topics outlined here.

NATURE

Chromoblastomycosis

Chromoblastomycosis (also known as chromomycosis, Carrión mycosis, Lane–Pedroso mycosis, verucoid dermatitis and black blastomycosis) is a term that describes a group of chronic localized infections of the skin and subcutaneous tissue, most often involving the limbs.[19,29,30] It is characterized by raised crusted lesions. It is most often caused by several species of brown-pigmented (dematiaceous) fungi.

Entomophthoramycosis

Rhinofacial conidiobolomycosis is a chronic mycosis affecting the subcutaneous tissues. It originates in the nasal sinuses and spreads to the adjacent subcutaneous tissue of the face, causing disfigurement. Basidiobolomycosis is a chronic subcutaneous infection of the trunk and limbs. For an exhaustive review of entomophthoramycosis, refer to Ribes *et al*.[20]

Lobomycosis

Lobomycosis, also known as keloidal blastomycosis or Lobo disease, is an uncommon and chronic subcutaneous mycosis. It is characterized by slowly developing, variably sized cutaneous nodules after a traumatic event. The dermal nodules manifest as smooth, verrucose or ulcerated surfaces that can attain the size of a small cauliflower-like keloid. The onset of the disease is generally insidious. The increase in size or number of lesions is a slow process, progressing over a period of 40–50 years. The lesions are composed of granulomatous inflammatory tissue containing numerous globose or subglobose to lemon-shaped, yeast-like fungal cells singly or in simple and branched chains.

Lobomycosis is a chronic dermal infection that presents a wide spectrum of clinical–dermatologic manifestations, mainly characterized by the development of keloid lesions as well as nodular, verrucoid and sometimes ulcerous forms. The etiologic agent at an international level, according to the consensus nomenclature, has been called *Loboa loboi*, even though recently it has been accommodated as *Lacazia loboi*.

Mycetoma

Mycetoma is a chronic suppurative infection of the skin, subcutaneous tissue and bone.[21] It usually affects the hand or foot and may be caused by various fungi (eumycetoma) or actinomycetes (actinomycetoma). The micro-organisms are inoculated into subcutaneous tissue by minor trauma. A characteristic feature of mycetoma is the production of grains in the infected tissue; these grains are compact masses of fungal or actinomycete elements and they discharge to the outside through sinus tracts.

Phaeohyphomycosis

Phaeohyphomycosis is a rare infection caused by dematiaceous fungi, involving the skin and subcutis, paranasal sinuses or central nervous system (CNS).[22] Phaeohyphomycosis refers to subcutaneous and deep-seated infections caused by brown-pigmented (dematiaceous) molds that adopt a septate mycelial form in tissue. This term was also created to separate various clinical infections caused by dematiaceous molds from the distinct subcutaneous infection known as chromoblastomycosis. Unlike mycetoma and chromoblastomycosis, phaeohyphomycosis is not limited to the skin or subcutaneous tissues, and elicits a wider variety of inflammatory responses.

Phaeohyphomycosis is characterized by a nodule, cyst or pyogranuloma. Histopathologically, the lesions show brown-walled hyphae in the dermis, subcutis or sometimes in the epidermis.

Rhinosporidiosis

Rhinosporidiosis is a chronic granulomatous infection of the mucous membranes caused by *Rhinosporidium seeberi*. The disease is characterized by the chronic and benign evolution of polyps that primarily affect the mucous membranes, especially the nostrils and ocular conjunctiva; visceral dissemination is rare.

Sporotrichosis

Sporotrichosis is a subacute or chronic infection caused by the dimorphic fungus *Sporothrix schenckii*. After implantation, this organism can cause cutaneous or subcutaneous infection, which commonly shows lymphatic spread. Occasionally, widespread disseminated infection also occurs.

EPIDEMIOLOGY

Chromoblastomycosis

Chromoblastomycosis is encountered mainly in arid parts of tropical and subtropical regions. Most cases occur in Central and South America, but chromoblastomycosis has also been reported in South Africa, Asia and Australia. Another major focus appears to be Madagascar.[17,29] Although common in rural areas, the disease lacks epidemic potential.

Chromoblastomycosis is caused by various dematiaceous fungi, to which a number of names have been given. There is therefore a great deal of confusion in the nomenclature used by various authors. The most frequently involved etiologic agents, beginning with the most common, are *Phialophora verrucosa*, *Fonsecaea pedrosoi*, *Fonsecaea compacta*, *Cladosporium carrionii* and *Rhinocladiella aquaspersa* (*Ramichloridium cerophilum*). Sporadic cases of chromoblastomycosis can also be caused by other dematiaceous molds. These organisms form characteristic thick-walled, dark brown muriform sclerotic cells in tissue.

The etiologic agents of chromoblastomycosis are widespread in the environment, being found in soil, wood and decomposing plant matter. Human infection usually follows the traumatic inoculation of the

fungus into the skin. Minor trauma, such as cuts or wounds due to thorns or wood splinters, is often sufficient. The disease is most prevalent in rural parts of warmer climates where people go barefoot. There is no human-to-human transmission.

Chromoblastomycosis is unusual in children and adolescents. Except in Japan, men contract the disease much more frequently than women, reflecting the importance of occupational exposure. Men have a greater opportunity for soil contact and predisposition to injury while working in the fields. The majority are aged 30–50 years. The rarity of the disease in children exposed to the same environmental conditions as adults suggests a long period of latency.

Entomophthoramycosis

Entomophthoramycosis occurs mainly in the tropical rain forests of East and West Africa, South and Central America, and South East Asia.

Conidiobolus coronatus (*Entomophthora coronata*), the causative organism of rhinofacial conidiobolomycosis, lives as a saprophyte in soil and on decomposing plant matter in moist, warm climates. It can also parasitize certain insects.

A few cases of conidiobolomycosis have been caused by *Conidiobolus incongruus* which has been reported to affect the lower extremities.[30] This species rarely produces infections in humans but reported cases have involved deep structures such as lung, mediastinum, heart, liver and gastrointestinal tract.[30]

The most widely held view is that *Basidiobolus ranarum* is the sole agent causing basidiobolomycosis, and that *B. meristosporus* and *B. haptosporus* are synonyms of the former; not all authors are of this opinion, however. *Basidiobolus ranarum* has been recovered from soil and decaying vegetation; it has also been isolated from the gut of frogs, toads and lizards that had apparently swallowed infected insects. It is still uncertain how the disease is acquired and what is the length of incubation. Inoculation through a thorn prick or an insect bite has been suggested, as has contamination of a wound or other abrasion. The infection is most common in children. More detailed aspects of entomophthoramycosis caused by *B. ranarum* can be found in the review by Gugnani.[31]

For an exhaustive review of the epidemiology of entophthoramycosis, refer to Ribes *et al*.[20]

Lobomycosis

In lobomycosis, the onset of the disease is generally insidious and difficult to document. The increase in size and number of lesions is a slow process; it can take 40–50 years. This latency period often makes it important to note the patient's history of travel or stay in areas of endemicity to arrive at a proper diagnosis. The history often reveals the cause being a trauma, for example an arthropod sting, a snake bite, a cut from an instrument, or a wound acquired while cutting vegetation. The causal agent of lobomycosis appears to be saprobic in aquatic environments, which probably plays an extremely significant part in its life cycle. Recent reports have substantiated the Amazon basin as an endemic area for the disease.[32]

The human disease is endemic in the tropical zone of the New World. There have been isolated cases reported in Holland and a doubtful case in Bangladesh. Identification of the disease in dolphins widened the geographic distribution of the disease. Seven cases of lobomycosis involving two species of dolphins, namely marine dolphins (*Tursiops truncatus*) and marine freshwater dolphins (*Sotalia fluviatilis*), have been reported in Florida, the Texas coast, the Spanish–French coast, the South Brazilian coast and the Surinam River estuary. Although lobomycosis in dolphins has been reported in the USA, only one human case has been reported from the USA.[33] Although identification of the disease in dolphins has widened its known geographic distribution, the source of the organism is still unknown

All attempts to isolate the fungus from lesions of infected people have failed. In the dermis it appears as spheric or elliptic budding cells. Although it is accepted that the infection is exogenous in origin, the natural habitat of the causal fungus remains unknown.

The organism gains entry through the skin; it develops *in situ* for an unspecified period (several years) and then reaches the subcutaneous tissue. The disease is most prevalent in men aged 30–40 years; it is much less common in women and children.

Mycetoma

Mycetomas are most common in arid tropical and subtropical regions of Africa and Central America, particularly those areas bordering the great deserts.[2] However, sporadic cases have been reported from many parts of the world. The countries surrounding the Saharan and Arabian deserts form the most important endemic area, not only because of the number of new cases occurring each year, but also because of the diversity of causal organisms. Mycetoma is also endemic in certain regions of India and in Central and South America.

Mycetomas are caused by various actinomycetes and fungi that occur as saprophytes in the soil or on vegetation. Individual species of fungi or actinomycetes are often associated with particular geographic areas. About six species of fungi are common causes of eumycetoma and five aerobic actinomycetes are common etiologic agents of actinomycetoma. The geographic distribution of these environmental organisms is influenced by climate.

In the arid regions of the tropics and subtropics, the most frequent etiologic agents are *Madurella mycetomatis*, *Actinomadura madurae*, *Actinomadura pelletieri* and *Streptomyces somaliensis*. These organisms are encountered in the great deserts of Africa and Asia and on their fringes, as well as in south-eastern Europe. In the relatively humid mountain regions of Latin America, *Nocardia brasiliensis* is the predominant organism while *Madurella grisea* is a less prominent cause of infection.

In the occasional cases of mycetoma that occur in temperate regions, the principal isolates have been *Pseudallescheria boydii*, *A. madurae* and *M. mycetomatis*. Mycetomas occur more frequently in men than in women. Adults aged between 20 and 50 years are the most commonly affected, although cases in children have also been reported. Most patients come from rural districts in the tropics and subtropics, but cases often occur in some countries with a temperate climate, such as Romania.

Trauma is a critical factor in acquisition of the infection. The organisms may be implanted at the time of injury, or later as a result of secondary contamination of the wound. Traumas are often due to vegetable matter (grasses, wisps of straw, hay). In the tropics and subtropics thorny trees such as the acacia are abundant and are often used for fuel. Wounds caused by the thorns may facilitate the entry of soil organisms, or the causative agents may grow on the thorns and be implanted directly into the subcutaneous tissue. It is not surprising, therefore, that mycetomas affect mainly the feet of country-dwellers who walk barefoot.

The vast majority of organisms causing mycetomas are saprophytes of the external environment: *Nocardia* spp. exist in the soil; other species are encountered not only in the soil, but also on living and dead plants. However, little is known about their behavior outside the human host.

Phaeohyphomycosis

Black molds are widely encountered in soil and wood. In addition, some organisms can produce yeast-like synanamorphs that adapt to aqueous environments. Typically, the infection is acquired by the inoculation of the fungus through a penetrating injury. In addition, other possible portals of entry have been suggested, including the inhalation of spores with lung or sinus invasion, the ingestion of contaminated food or water with subsequent penetration through the gastrointestinal tract, contamination of the skin at the insertion of a vascular catheter, and contamination of the catheter itself. Some cases of systemic infection have no apparent portal of entry.

Phaeohyphomycosis has a worldwide distribution, but subcutaneous infection is most often seen in the rural population of tropical parts of Central and South America. Most cases of cerebral or paranasal sinus

infection have been reported from the USA. There is little information on the incidence of phaeohyphomycosis. The number of organisms implicated as etiologic agents of phaeohyphomycosis is increasing. More than 80 different molds, classified in 40 different genera, have been incriminated. These fungi have often been given different names at different times, and there is therefore a great deal of confusion in the nomenclature used in different reports.

Among the more important etiologic agents, *Alternaria*, *Bipolaris*, *Curvularia*, *Exophiala*, *Exserohilum* and *Phialophora* spp. and *Xylohypha bantiana* can be included. Many of these organisms are found in soil or decomposing plant debris; others are plant pathogens.[34] The most important predisposing factor for cutaneous and subcutaneous infection is exposure to contaminated material present in the environment (decaying wood, plants).

Human infection follows inhalation or traumatic implantation of the fungus. In addition to these agents of phaeohyphomycosis, others are being reported. For example, *Colletotrichum* spp., which are common plant pathogens, have been reported as a cause of subcutaneous phaeohyphomycosis in patients undergoing chemotherapy for hematologic malignancies and may cause life-threatening phaeohyphomycosis in immunosuppressed patients.[35]

Rhinosporidiosis

Rhinosporidiosis is endemic in India[36] and Sri Lanka, where the incidence is estimated at 1.4% of the pediatric population, as well as in South America and Africa. Occasional cases have been reported from the USA, South East Asia and other parts of the world. Some arid countries of the Middle East also show a high incidence of the disease. The etiologic agent is an endosporulating organism, *Rhinosporidium seeberi*. So far, all attempts to isolate this fungus from lesions have failed. The life cycle of the parasite is complicated. In tissue, large, thick-walled sporangia (spherules) are formed. Large numbers of spores are produced within the sporangia and, when the spores are mature, they are released through a pore in the wall. Each spore may develop to form a new sporangium. Little is known about the natural habitat of *R. seeberi*, but it is believed that stagnant pools of water may be the source of human infection. The most prevalent location of the disease is the nasal cavity

The disease is most prevalent in rural districts, particularly among people working or bathing in stagnant water (such as rice fields). Men are more commonly affected than women. In arid countries most infections are ocular and dust is postulated to be a vector.

The disease affects mostly males (70–90%) and the incidence is greater in those aged between 20 and 40 years. Ocular infection is more prevalent in women, while nasal and nasopharyngeal infection preferentially affects males.

Sporotrichosis

Sporotrichosis is worldwide in distribution, but occurs most frequently in temperate humid climatic regions.[37] At present, the largest number of reported cases comes from the North American continent.[38] Other regions where the infection is endemic include South America,[38] South Africa and South East Asia.

The causative agent is a dimorphic fungus, *Sporothrix schenckii*, which is found in the soil and on plants and sphagnum moss. It grows in nature as a mycelium, but in tissue it forms small, budding cells.

It is not clear whether the infection is more common among men than women. Incidence in the different age groups is also variously assessed, but children are less often affected than adults.

Sporotrichosis caused by the dimorphic fungus *Sporothrix schenckii* is widely distributed throughout the world, especially in tropical and subtropical regions. Classically, infection is caused by traumatic inoculation of soil, plants and organic matter contaminated with the fungus, occasionally by inhalation. Some leisure and occupational activities such as agriculture and floriculture have been associated with transmission of the disease. To date, the largest epidemic of sporotrichosis occurred in Witwatersrand, South Africa, in the 1940s when about 3000 miners were infected from wood timbers in the mines. However, the literature about epidemics is scant and usually related to a common source of infection.[37,38]

Since 1998, the Infectious Diseases and Zoonosis Services of the Evandro Chagas Clinical Research Institute (IPEC), Oswaldo Cruz Foundation (Fiocruz), Brazil, have been diagnosing a growing number of cases of human and feline sporotrichosis emanating from the city of Rio de Janeiro and its surroundings.

Although the main clinical characteristics of human and feline sporotrichosis have been described elsewhere,[39] many questions related to the mechanism of zoonotic transmission and to the context in which this transmission occurs remain unanswered.

Recently, the role of felines in the transmission of sporotrichosis to humans has gained importance.[38] In Rio de Janeiro from 1998 to 2004, 759 humans, 64 dogs and 1503 cats were diagnosed with sporotrichosis. Of them, 85% of dogs and 83.4% of patients were reported to have had contact with cats with sporotrichosis, and 55.8% of the latter reported cat bites or scratches. Unusual manifestations were diagnosed in humans. Canine sporotrichosis presented as a self-limited mycosis. Feline sporotrichosis varied from subclinical infection to severe systemic disease with hematogenous dissemination of *Sporothrix schenckii*. The zoonotic potential of cats was demonstrated by the isolation of *S. schenckii* from skin lesion fragments and from material collected from their nasal and oral cavities. An accompanying editorial commentary on this report has been published by Hay & Morris-Jones.[37]

PATHOGENICITY

Chromoblastomycosis

The causative fungi require implantation through the skin into subcutaneous tissue. The lesion appears at the site of skin trauma or puncture wound. However, the inoculation may have occurred so long before that no history of injury can be elicited. In general, the disease remains localized to the area surrounding the initial infection. In rare cases, hematogenous spread to the brain, lymph nodes, liver, lungs and other organs is observed.

Entomophthoramycosis

Pathogenicity of the causal organisms is a reflection of inoculum size and frequency of exposure in endemic areas. For an exhaustive review of the pathogenicity of the agents causing entomophthoramycosis, refer to Ribes *et al*.[20]

Lobomycosis

Lobomycosis develops following trauma to the skin, but in most clinical histories the event is so minimal that it is not remembered. The disease runs an extremely slow course and years may elapse before the patient seeks medical advice. The organism has been transmitted successfully to an armadillo and to tortoises. In addition, the infection has been maintained through nine generations in the footpads of mice. Most of our knowledge of the etiologic agent of lobomycosis is derived from histopathologic and electron microscopy studies.[27]

The fungus is abundant in lobomycotic skin lesions. It is a remarkably homogenous, spherical intracellular yeast, 5–12 μm in diameter. *L. loboi* is predominantly an intracellular pathogen. Organisms, singly or in chains, reside predominantly in macrophage vacuoles. They probably reproduce by budding; linear or radiating chains of as many as 20 organisms linked by tubules have been observed.[27]

Mycetoma and phaeohyphomycosis

The organisms causing these conditions are not regarded as being pathogenic. Typically, the infection is acquired by the inoculation of the fungus through a penetrating injury

Rhinosporidiosis

Studies on the virulence of *R. seeberi* have not been carried out. Nothing is known about the mode of infection. It is most likely that trauma is an essential factor in the initiation of disease. Spores of *R. seeberi* are not able to penetrate intact epithelium. Because the nose and eyes are the most common sites of the disease it is suggested that the organisms are transmitted in dust and water.

Sporotrichosis

Sporothrix schenckii usually enters the body through traumatic implantation, but occasionally the fungus is introduced through inhalation of the conidia. Because the infection can also be hematogenously disseminated, it may be that the yeast cells are able to resist phagocytosis and intracellular killing by host effector cells, although *in-vitro* data suggest that the yeast cells are readily killed in the presence of human serum. Host defense mechanisms in response to *S. schenckii* have not been extensively studied.

For many years it has been known that the epidemiology of *S. schenckii* may change with time. The observation has also been made that the human population is now more resistant through better nutrition and living standards or reduced contact with environmental spores. There is also some evidence that phenotypic changes may occur that could affect the ability of *Sporothrix* to infect. Findlay & Vismer[40] showed that when natural isolates of *Sporothrix* were grown in culture media containing a wood source, the fungus became a much more prolific producer of conidia. It has been suggested that wood may enhance the invasive potential of *Sporothrix*. An additional view is that the production of melanin by *Sporothrix* may enhance the ability of the fungus to scavenge free oxidative radicals from host phagocytic cells.[41] *Sporothrix schenckii* conidia and yeast forms can synthesize melanin in the external environment.[41]

PREVENTION

Avoidance of skin penetration is the best means of preventing chromoblastomycosis, entomophthoramycosis and phaeohyphomycosis. Suitable footwear will help to prevent chromoblastomycosis.

Very little is known about the ecology of *Loboa loboi*. However, the agent is probably introduced directly into the dermis through a penetrating injury, such as a thorn prick or an insect bite, or close, abrasive contact with a dolphin. In areas where infections have been reported it would be advisable to avoid penetrating injuries.

The causative agents of mycetoma normally live as saprophytes in the soil. Because the most common site for mycetoma is the foot it is reasonable to assume that the wearing of appropriate footwear would prevent infection. Avoidance of trauma to the hands and other areas is difficult to encourage because most infections seem to be related to outdoor activities.

Rhinosporidiosis can be prevented by avoiding eye and nose contact with contaminated dust and water.

Occupations that predispose persons to sporotrichosis include gardening, farming, masonry, floral work, outdoor labor and other activities involving exposure to contaminated soil or vegetation such as sphagnum moss or roses. Wearing gloves and protective clothing while carrying out these activities may therefore prevent traumatic implantation of the fungus through the skin.

DIAGNOSTIC MICROBIOLOGY

Chromoblastomycosis

Microscopic examination of wet preparations of pus, scrapings or crusts from lesions can permit the diagnosis of chromoblastomycosis if clusters of the characteristic small, round, thick-walled, brown-pigmented sclerotic cells are seen (Fig. 179.1). These cells are often divided by longitudinal and transverse septa.

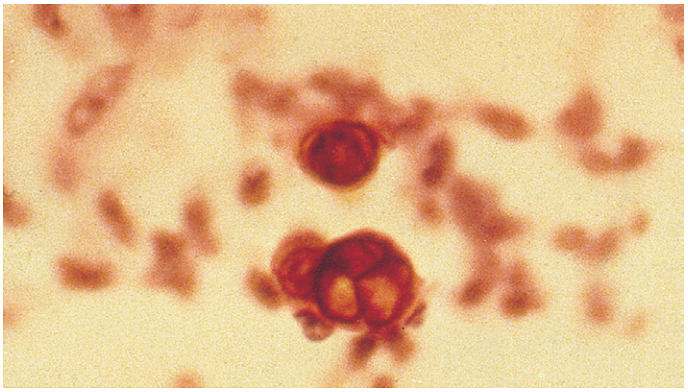


Fig. 179.1 Chromoblastomycosis. Thick-walled, septate, dematiaceous muriform cells. With permission from Richardson MD, *et al*. Slide atlas of fungal infection: subcutaneous and unusual fungal infections, Oxford: Blackwell, 1995.

The definitive diagnosis of chromoblastomycosis depends on the isolation of the etiologic agent in culture. Identification of the individual etiologic agents is difficult.

Entomophthoramycosis

Microscopic examination of smears or tissue from the nasal mucosa will reveal broad, nonseptate, thin-walled mycelial filaments.

The culture of causal organisms of entomophthoramycosis is difficult. To optimize the recovery of fungus from clinical material, specimens must be inoculated on the largest possible number of media.

For an exhaustive review of the diagnosis of entomophthoramycosis, refer to Ribes *et al*.[20]

Lobomycosis

The etiologic agent of lobomycosis is an obligate pathogen of humans and lower mammals that has yet to be isolated and grown *in vitro*; therefore, nothing is known of its basic cultural characteristics and growth. Diagnosis is based on demonstrating the presence of globose, thick-walled, yeast-like cells ranging from 5 to 12 µm in diameter in lesion exudate or tissue sections. The organism multiplies by budding and thus mother cells with single buds are often observed. However, characteristic sequential budding leads to the production of chains of cells that are linked to each other by a tubular connection, or isthmus. Budding may occur at more than one point on a cell, giving rise to branched or radiating chains of cells. These thick-walled, hyaline, spherical cells with chains of cells interconnected by tubular connections are the basis on which a diagnosis of lobomycosis rests. The thick-walled, budding hyaline cells with catenate chains of conidia can be readily observed in tissue smears or exudates mounted in 10% potassium hydroxide or in calcofluor white preparations. Tissue sections can be stained with the use of periodic acid–Schiff digest, Grocott–Gomori methenamine-silver nitrate or Gram stains.

Microscopic examination of specimens of pathologic material will reveal numerous hyaline, round or ovoid cells with an average diameter of 9 µm (Fig. 179.2). These cells closely resemble the yeast forms of *Paracoccidioides brasiliensis* or *Histoplasma duboisii*. The cells are enclosed in a double-contoured membrane and are capable of budding. They often form chains and appear to be joined together by bridge-like structures within the chain. If the individual elements show multiple budding, the chains are divided into branches.

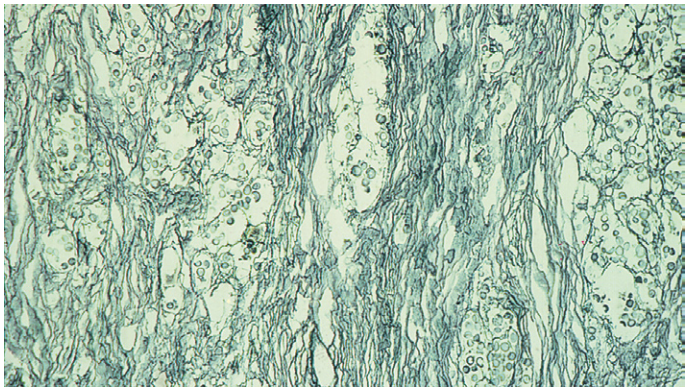


Fig. 179.2 Lobomycosis. Yeast cells are attached to each other in short chains. Nonbudding and single-budding cells are also present.

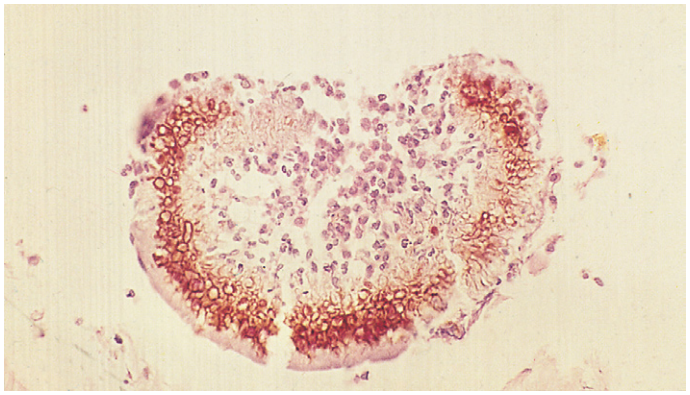


Fig. 179.3 Granule of *Madurella mycetomatis*. The granules have a deeply pigmented periphery of compact hyphae. Randomly oriented, poorly pigmented fungal elements in the interior of the granule are less compact.

Loboa loboi has never been successfully cultured. This distinguishes it from *P. brasiliensis*, which it closely resembles morphologically. The globose and subglobose budding cells of *L. loboi* resemble budding cells of *P. brasiliensis* in tissue. However, the central mother cells of *P. brasiliensis* become large and thick-walled compared to the daughter cells, which remain smaller. In contrast, yeast cells of *L. loboi* remain consistent in diameter, giving rise to branching chains of blastoconidia. In addition, the cell wall of *L. loboi* contains constitutive melanin,[42] which can be detected by the use of the Fontana–Masson histologic stain. The walls of cells of *P. brasiliensis* are not known to contain melanin. *Loboa loboi* has never been cultured *in vitro*. On the other hand, *P. brasiliensis* can be grown in artificial culture and is known to be a dimorphic pathogen.

Serologic tests have high sensitivity but lack specificity because of antigenic cross-reactivity with fungi from the genus *Paracoccidioides*.[27]

Molecular methods have been used in an attempt to characterize the causative agent of lobomycosis.[42,43] Fungal-specific primers targeted for highly conserved genomic nucleic acid sequences were used in a polymerase chain reaction (PCR) to amplify DNA from lobomycosis lesions in a bottlenose dolphin. Sequence alignments of this DNA possessed high homology to fungal ribosomal DNA sequences found in the genus *Cladosporium*. When used for *in-situ* hybridization, the riboprobe transcribed from a cloned PCR-generated fragment bound to *L. loboi* cells. These results support the hypothesis that *L. loboi* in dolphin tissue is a fungus.

A new monotypic genus, *Lacazia*, with *Lacazia loboi* as the type species, was recently proposed[42,43] to accommodate the obligate etiologic agent of lobomycosis in mammals. The continued placement of *L. loboi* in the genus *Paracoccidioides* as *Paracoccidioides loboi* O.M. Fonseca et Lacaz was found to be taxonomically inappropriate. The older name *Loboa loboi* Ciferri *et al*. was considered to be a synonym of *P. brasiliensis*.

Mycetoma

The diagnosis of mycetoma depends on the identification of grains. These should, if possible, be obtained by puncture from a softened, but not ulcerated, nodule with a syringe. Failing this, grains can be obtained with a dissecting needle or by aspiration from the secretion flowing from a sinus.[44] If there is no pus flowing from the lesion, small fragments of tissue should be removed. If possible, between 20 and 30 grains should be obtained; these should be rinsed in sterile saline before being cultured.

Gross examination of the grains may give a clue to the etiologic diagnosis. Black grains suggest a fungal infection, minute white grains often indicate a *Nocardia* infection, and larger white grains the size of a pinhead may be of either fungal or actinomycotic origin. Small, red grains are specific to *Actinomadura pelletieri*, but yellowish-white grains may be actinomycotic or fungal in origin. Their shape, consistency and structure must be carefully determined.

Direct microscopic examination will confirm the diagnosis of mycetoma and will also reveal whether the causative organism is a fungus or an actinomycete.[44] Actinomycotic grains contain very fine filaments (<1 µm diameter), whereas fungal grains contain short hyphae (2–4 µm diameter), which are sometimes swollen. This can be seen by direct microscopic examination of crushed grains in potassium hydroxide, but it is much more readily observed in stained histologic sections (Fig. 179.3).

Although the identification of the causal agents of mycetoma can often be deduced from the morphologic characteristics of the grains, it is also important to isolate the organism in culture. Agar plates should be inoculated with several grains (or with secretion or tissue fragments). Cultures should be retained for up to 6 weeks before being discarded. The actinomycetes grow much more slowly than the fungi.

Madurella mycetomatis is the commonest cause of eumycetoma in Sudan and other countries in tropical Africa. Currently, the early diagnosis of mycetoma is difficult. In attempting to improve the identification of *M. mycetomatis* and, consequently, the diagnosis of mycetoma, a specific oligonucleotide primer based on the sequence of the internal transcribed spacer (ITS) regions spacing the genes encoding the fungal ribosomal RNAs has been described.[45] More recently, the molecular identification of black-grain mycetomas has been reported.[46]

Phaeohyphomycosis

One common factor among these fungi is their melanin formation in the cell wall in culture and, in most cases, in human tissue. Microscopic examination of stained histopathologic sections or wet preparations of clinical material, such as pus or skin scrapings, can permit the diagnosis of phaeohyphomycosis if brown-pigmented septate mycelium with occasional branching is seen (Fig. 179.4).

Identification of the etiologic agent is essential for correct management, and this depends on its isolation in culture.[34] No serologic tests are available.

Rhinosporidiosis

Microscopy of clinical material reveals round or ovoid organisms that, depending on age, vary in diameter with a prominent wall.[47–49] These mature forms are known as sporangia. Sporangia measure up to 350 mm in diameter and have a cell wall measuring about 5 mm. The sporangia may be filled with up to 12 000 endospores, 7–15 µm in diameter (Fig. 179.5). The immature forms of the organism are known as trophocytes; they are smaller than sporangia, have a relatively thinner wall and do not contain endospores. Trophocytes mature into sporangia. Endospores develop within sporangia. Endospores are released

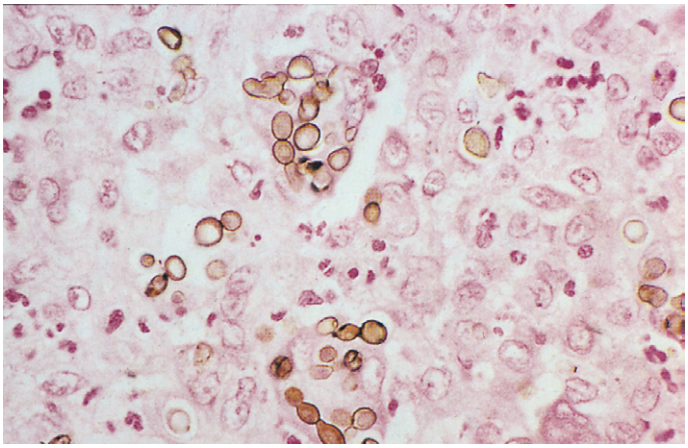


Fig. 179.4 Subcutaneous phaeohyphomycosis caused by *Bipolaris spicifera*. The fungal elements are stained with Fontana–Masson, which accentuates and confirms the presence of melanin or melanin-like pigment in the fungal cell walls.

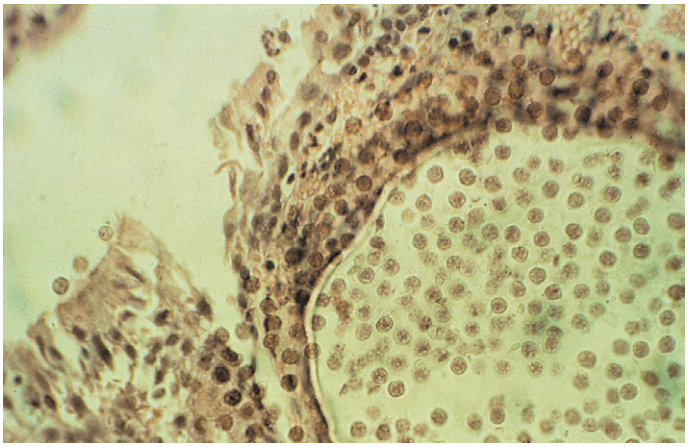


Fig. 179.5 Rhinosporidiosis.

upon maturity and thereafter develop into trophocytes. The organisms are abundant and appear in various sizes and stages of development. It is not necessary to perform special staining because of the size of the agent.

Rhinosporidium seeberi has traditionally been classified as a fungus on the basis of morphologic and histochemical characteristics. Recent molecular studies have generated conflicting results.[27,50] The organism may be either a cyanobacterium or a protist. Using consensus PCR, a portion of the *R. seeberi* 18S rRNA gene directly from infected tissue has been amplified.[51] Analysis of the aligned sequence and inference of phylogenetic relationships showed that *R. seeberi* is a protist from a novel clade of parasites that infect fish and amphibians. Fluorescent *in-situ* hybridization and *R. seeberi*-specific PCR showed that this unique 18S rRNA sequence is also present in other tissues infected with *R. seeberi*. These data support the *R. seeberi* phylogeny recently suggested by others. *Rhinosporidium seeberi* is not a classic fungus, but rather the first known human pathogen on the basis of a previously aligned dataset of the DRIPs clade (named after the organisms *Dermocystidium*, the rosette agent, *Ichthyophonus* and *Psorospermium*), a novel clade of aquatic protistan parasites (*Ichthyosporea*).

Immunologic methods have been used to identify the causal agent of rhinosporidiosis *in situ* where the immunolocalization of *R. seeberi* antigens using sera from individuals infected with *R. seeberi* and tissue from Sri Lankan patients with rhinosporidiosis was determined by electron microscopy.[52] This study found that the expression of this antigen occurs only in the final developmental stages of *R. seeberi* mature sporangia. The data may explain why circulating antibodies to *R. seeberi* were not detected previously in studies that used endospores as antigen in immunoassays. This appears to be the first report in which an antigenic material with a potential role in the immunology of rhinosporidiosis has been described.

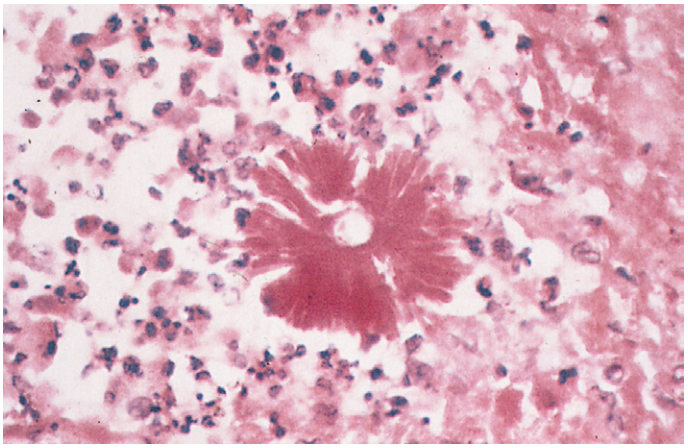


Fig. 179.6 Asteroid body in cutaneous sporotrichosis. A yeast-like cell of *Sporothrix schenckii* with faintly basophilic, retracted cytoplasm is intimately surrounded by elongated spicules of Splendore–Hoeppli material.

Sporotrichosis

Direct examination of clinical material, such as pus or tissue, is often disappointing because the organism is seldom abundant. However, it can be of value if conducted with painstaking care. The detection of typical ovoid or cigar-shaped cells or asteroid bodies of *S. schenckii* will confirm the diagnosis (Fig. 179.6).

The definitive diagnosis of sporotrichosis depends on the isolation of the etiologic agent in culture. Identifiable mycelial colonies will appear in 2–5 days. The color usually changes from cream or light brown to dark brown or black with age. Confirmation of the identification depends on the morphologic characteristics of the mycelial form and its conversion to the yeast form.

CLINICAL MANIFESTATIONS

Chromoblastomycosis

The lesions of chromoblastomycosis are usually unilateral and occur mainly on the exposed parts of the body, particularly the feet and lower legs.[17,29,53] Other less common sites include the hands, arms, shoulders and neck. The initial lesion is a painless papule or nodule on an erythematous and occasionally verrucous base. The condition may also present as an abscess surrounded by infiltration or as a psoriasiform lesion with erythema and scaling. As the disease develops, the affected limb becomes enlarged. Small satellite nodules may occur at the edge of the original lesion. Itching often occurs and may be severe.

The primary lesion develops very slowly, its diameter increasing by only 1–2 mm per year. It is firm and elastic in consistency and colored red or violet verging on gray. There is a warty, papillomatous margin surrounding a center that may be flat, smooth or scaly, with areas of scarring.

Later in the disease, the lesion may become pedunculated or ulcerated (if bacterial superinfection occurs). However, the lesions usually retain a warty, dry character.

Secondary lesions may appear, especially along the lymphatics draining the site of infection; here again, development is slow and symptoms are few. The lymph nodes are only involved if there is superimposed bacterial infection.

In endemic areas the unilateral development of vegetative, atrophic and scarred lesions on a lower limb is suggestive of chromoblastomycosis. The condition must be distinguished from a number of other fungal infections including blastomycosis, lobomycosis, paracoccidioidomycosis, phaeohyphomycosis, rhinosporidiosis and sporotrichosis. It must also be differentiated from protothecosis, leishmaniasis, verrucous tuberculosis, certain leprous lesions and syphilis. On the upper limbs the erythematosquamous lesions can be confused with psoriasis or subacute or discoid lupus erythematosus. Mycologic and histologic investigations are indispensable for confirmation of the diagnosis.

Entomophthoramycosis

Entomophthoramycosis is a chronic subcutaneous infection of the trunk and limbs. The subcutaneous swelling that characterizes this disease is usually localized to the buttock and thighs, but may also be found on the arm, leg or shoulder.

The initial swelling may be rapid or slow in onset and is hard and painless. The spread is slow but relentless, and a large mass is formed that is attached to the skin but not to the underlying tissue (unlike *Conidiobolus* infection). This is a disfiguring infection, but the skin covering the lesions does not ulcerate. Lymphatic obstruction may occur and can result in massive lymphedema. There is no functional impairment as long as the joints are not blocked by the volume of the swellings. The underlying bone and joints are not affected by the disease.

The disease is most common among adult males, particularly those living or working in tropical rain forests. Infection is acquired through inhalation of spores or their introduction into the nasal cavities by soiled hands.

Very rarely, *B. ranarum* can cause gastrointestinal basidiobolomycosis.[31]

Conidiobolus infection generally begins with unilateral involvement of the nasal mucosa. The most common nasal symptom is obstruction, but frequent nose bleeding can occur and is evidence of the development of a nasal polyp in the anterior region of the inferior turbinate. Subcutaneous nodules then develop in the nasal and perinasal regions and may be associated with epidermal lesions.

The spread of the infection is slow but relentless. It is usually confined to the face, and the development of gross facial swelling involving the forehead, periorbital region and upper lip is very distinctive. As a rule, the lesions are firmly attached to the underlying tissue, although the bone is spared. The skin remains intact. Spread to the lymph nodes has been reported. Even if, in advanced cases, the diagnosis is obvious from the appearance, mycologic and histologic examinations are essential for its confirmation.

The disease can be diagnosed with confidence on the basis of appearance and the results of the mycologic and (in particular) the histologic examination. Specimens must be taken from the subcutaneous tissue where the infection develops.

For an exhaustive review of the clinical manifestations of entomophthoramycosis, refer to Ribes *et al*.[20]

Lobomycosis

Lobomycosis is an indolent infection that first manifests as a papule or small nodule of normal pink skin color or with a grayish tinge. The nodule then proliferates and, by partial or total coalescence, may form extensive multilobar lesions. The disease spreads by peripheral extension or autoinoculation from scratching, or it may follow the draining lymphatics, especially in elderly people. Because of the slow growth of lobomycosis lesions, patients do not present themselves for treatment until many years have passed and usually after the lesions have become large.

The lesions are located in the dermis and subcutis and may form massive tumors, which are firm and resistant to pressure at the outset, but which later become hard and fibrous and resemble a keloid. The typical keloid-like skin lesions appear only after several months. If there is ulceration, depressed scars may remain; their surface is smooth and shiny in places, owing to atrophy of the underlying epidermis, and wrinkled and fissured elsewhere.

The disease may be symptomless or cause itching and burning, and trauma to the affected area may be especially painful. The most common sites of infection are the coolest parts of the body – the limbs, face, ears and buttocks. Differentiation of keloidiform lesions on the ears from lepromatous leprosy is difficult, but in lobomycosis the presentation is unilateral. The lesions may cover a whole limb. If the head is involved, the patient may be so grossly disfigured as to be completely excluded from social life. With a few exceptions there are no adenopathies. No deaths from lobomycosis have been reported.

Lesions may be keloid scars or irregular fibrous changes of the skin without secretion. Leprosy, leishmaniasis and chromoblastomycosis can produce similar lesions. Mycologic and histologic examination will confirm the diagnosis.

Mycetoma

Mycetoma is a chronic, suppurative infection of the subcutaneous tissue and contiguous bones. The lesion appears to begin at a site of minor trauma and continues to spread locally over the ensuing months and years.

The clinical features of the disease are fairly uniform, regardless of the type of organism causing it. Eumycetoma follows a slower and generally less destructive course than actinomycetoma. Spread to the internal organs and involvement of the regional lymph nodes is rare, occurring in no more than 2–5% of cases.

The feet are by far the most common site of involvement and account for two-thirds or more of cases. Other sites include the lower legs, hands, head, neck, chest, shoulder, arms and abdomen.

In most cases, the first sign of the disease is a small, hard, usually painless, subcutaneous nodule that is not attached to the underlying tissue. It is covered by taut thinned skin, which is reddish-violet in color. A number of small nodules may coalesce to form a larger and frequently multilobar nodule.

Over the ensuing months the nodule begins to soften on the surface, caves in, ulcerates and partly empties, discharging a viscous, purulent fluid containing grains. If there is little fluid, the grains may not escape. The lesion then broadens out at the surface and also spreads inward to infect muscles and bones. The lesions, which are covered with depigmented and scarred skin, present as swellings, which are often covered with a crust. Later, the lesions develop sinus tracts that discharge pus and blood containing the characteristic grains.

The infection slowly spreads to adjacent tissue, including bone; this often causes considerable deformity. Mycetomas of the feet make the arches convex, thus preventing the toes from touching the ground. However, the general health of the patient is not affected. Pain, burning and pruritus may occur but are usually mild. Depending on the location and size of the lesion, and also on whether there is any bone involvement, limb function may be impaired.

Radiologic examination is useful in determining the extent of bone destruction. Abnormalities include periosteal reactions, sclerosis, endosteal reactions, cortical erosions and joint destruction. CT scanning is also helpful in delineating the extent of lesions.

Bacterial superinfection is not uncommon and is largely responsible for adenopathies and impairment of the general health. Visceral and especially cerebral metastases are the most serious complications; they cause cachexia and are often fatal. Fortunately, they are rare.

In most cases, the diagnosis of mycetoma of the feet presents no problems, but it may be difficult if other body sites are involved, particularly in regions where the disease is not endemic and if no grains have been discharged at the time of examination.

The characteristic feature of mycetoma is the presence in a fistulated swelling of grains that are found to contain actinomycotic or fungal filaments. This finding distinguishes mycetoma from chromoblastomycosis, cutaneous tuberculosis, certain syphilitic or leprous lesions, botryomycosis and other conditions.

Phaeohyphomycosis

Phaeohyphomycosis can be divided into a number of distinct clinical forms, including cutaneous and subcutaneous infection, paranasal sinus infection, cerebral infection and invasive and systemic disease.[54] The disease spectrum of noncutaneous phaeohyphomycosis includes sinusitis, pulmonary disease, CNS infection, ocular disease, arthritis, osteomyelitis, fungemia, endocarditis, peritonitis and gastrointestinal disease.

Subcutaneous phaeohyphomycosis

Subcutaneous phaeohyphomycosis usually follows the traumatic implantation of the fungus into the subcutaneous tissue. Minor trauma, such as cuts or wounds from thorns or wood splinters, is often sufficient. The principal etiologic agents include *E. jeanselmei*, *Exophiala spinifera*, *Exophiala dermatitidis* (*Wangiella dermatitidis*), *Phialophora richardsiae* and *Phialophora parasitica*.[54]

The lesions occur mainly on the arms and legs. Other less common sites include the buttocks, neck and face. The initial lesion is a firm, sometimes tender, subcutaneous nodule that may enlarge slowly to form a painless cystic abscess. Lesions are attached to the skin but not to the underlying tissue or bone. Unless the cyst ruptures, the overlying skin remains unaffected. In immunosuppressed patients with subcutaneous phaeohyphomycosis, the lesions are more likely to drain through sinuses.

Phaeohyphomycosis in transplant recipients

Infection of subcutaneous tissue by black fungi has only been reported in six transplant patients, all of whom were solid organ recipients.[55] These patients presented with indolent, localized infections at least 1 year after transplant, while on maintenance immunosuppressive regimens. They were cured by surgical resection, either alone or in conjunction with antifungal agents. A further case report illustrates the features of subcutaneous phaeohyphomycosis occurring in a bone marrow transplant recipient receiving high doses of immunosuppressive agents, in whom widespread subcutaneous infection due to *E. jeanselmei* was not eradicated by repeated resections and therapy with amphotericin B and flucytosine.[56] The infection was eventually cured after the addition of itraconazole to the therapeutic regimen. Results of *in-vitro* testing of the isolate for susceptibility to a combination of amphotericin B, flucytosine and itraconazole confirmed the potential role of combination antifungal therapy in the setting of refractory infection.

Local recurrence of subcutaneous phaeohyphomycosis in transplant recipients after medication or surgical treatment is also seen.

Paranasal sinus infection

This form of phaeohyphomycosis is becoming more common. The principal etiologic agents include *Alternaria* spp., *Bipolaris spicifera*, *Bipolaris hawaiiensis*, *Curvularia lunata* and *Exserohilum rostratum*.

It is a slowly progressive condition that may remain confined to the sinuses or spread to contiguous structures. Affected people usually complain of longstanding symptoms of allergic rhinitis, nasal polyps or intermittent sinus pain. Patients present with nasal obstruction and facial pain, with or without proptosis. The sinuses are filled with a thick, dark, tenacious, inspissated mucus.

CT scanning is the best method for evaluating the extent of the infection. The typical finding is a large mass filling one or more of the sinuses.

Alternaria and *Curvularia* spp. occasionally cause necrotic lesions of the nasal septum in patients with leukemia or AIDS.

Cerebral phaeohyphomycosis

This form of phaeohyphomycosis may follow hematogenous dissemination of infection from the lungs, or it may result from direct spread from the nasal sinuses. Involvement of the CNS carries a poor prognosis. The routes of infection have not been clearly established, but the most likely port of entry seems to be the respiratory tract, although direct inoculation into the brain and extension from the sinuses, ear and pulmonary infections have also been reported. Most cases are due to *X. bantiana* (*Cladosporium trichoides*). Other etiologic agents include *Bipolaris* spp. and *E. dermatitidis*. Many cases of cerebral infection with *X. bantiana* have occurred in people with no obvious predisposing factors.

The symptoms of cerebral phaeohyphomycosis are gradual in onset. Persistent headache is the most common presenting symptom. The most frequent clinical findings include focal neurologic signs, hemiparesis and fits. Fever is minimal or absent. Chest radiographs are normal.

CT scans of the head will often reveal a unilateral, well-circumscribed lesion, with the frontal lobes of the brain being the most common location. Cerebrospinal fluid (CSF) findings are varied. The opening pressure may be raised, the protein concentration may be increased, the glucose concentration may be reduced, and pleocytosis may be present. It is most unusual to recover the fungus from the CSF. The diagnosis is seldom established until neurologic resection is performed.

Cutaneous infection

Alternaria spp. and *Exophiala spinifera* have been seen in and isolated from crusted, ulcerated or scaling skin lesions.[54] Many of these infections have followed traumatic implantation and a substantial proportion have occurred in leukemic patients or transplant recipients. The arms and legs are the more common sites of infection.

Other forms of phaeohyphomycosis

Dematiaceous molds have caused endocarditis after valve insertion or replacement, and peritonitis in patients on continuous peritoneal dialysis.[2] Fungemia due to black fungi is unusual.[22] Fever without a clear source of infection is the most frequent presentation. In a series of 23 cases occurring in a tertiary hospital, fever was the most frequent clinical manifestation, and only one patient developed signs of deep-seated infection, with a clinical picture of necrotizing pneumonia similar to that caused by *Aspergillus* spp.[22]

The lesions of subcutaneous phaeohyphomycosis can be confused with the small initial lesions of chromoblastomycosis, sporotrichosis, blastomycosis, coccidioidomycosis and paracoccidioidomycosis, as well as with cutaneous leishmaniasis. Lymphangitic spread of sporotrichosis and the development of verrucous lesions in the other conditions makes the distinction easier.

In people who are not immunosuppressed, the clinical presentation of phaeohyphomycosis of the paranasal sinuses cannot be distinguished from that of *Aspergillus* infection. In immunosuppressed patients, *Aspergillus* sinusitis is a fulminant and often lethal condition, unlike phaeohyphomycotic sinusitis. However, both groups of organisms can cause black necrotic lesions of the nasal septum.

Bacterial brain abscess is the most common initial diagnosis in patients with cerebral phaeohyphomycosis. In occasional patients, the diagnosis of cryptococcosis, histoplasmosis, coccidioidomycosis or sporotrichosis must be excluded.

Rhinosporidiosis

Rhinosporidium seeberi causes the production of large polyps or wart-like lesions that occur predominantly on the mucous membranes. The nasal mucosa is affected in more than 70% of cases. The onset of the disease in the nose is insidious and the patient remains unaware of its presence until symptoms of obstruction develop. In some cases, the patient complains of itching and unilateral coryza. Rhinoscopic examination will reveal papular or nodular smooth-surfaced lesions that gradually become pedunculated and acquire a papillomatous or proliferative appearance. They are pink, red or violet in color. The polyps

may obstruct the nasal passages, particularly in the event of even slight trauma. If located low in the nostril, they may protrude and hang onto the upper lips. If they are sited in the posterior part of the fossa, they may partially obstruct the pharynx or larynx and cause dysphagia or dysphonia and dyspnea.

In some cases the eyes are affected, the lesions being located on the conjunctiva. Initially these are small, flat granulations that may grow to form multilobed polyps of a pale pink color. At the same time there is diffuse vascular dilatation, photophobia and lacrimation, which is often due to involvement of the lacrimal sac and duct.

The ears may also become involved; depending on their size and location, these polyps may impair hearing.

Lesions may also develop on the male genital organs (the penis and, in exceptional cases, the urethra) and on the vulva and vagina in women. They may resemble flat or acuminate condylomas; lesions in the anus present as polyps and may sometimes be mistaken for hemorrhoids.

Cutaneous rhinosporidiosis, which is very rare, is generally due to spread from a neighboring mucosal lesion. It presents initially as minute papillomas; these gradually become larger and pedunculated. The surface is irregular, verrucous and polypous.

Cutaneous lesions are usually asymptomatic, but depending on their location (especially if they are on the sole of the foot) or their size they may cause pain. The surface of ulcerated rhinosporidiosis lesions is dotted with white spots, which are more readily discerned when depressed with a glass spatula; on microscopy these are seen to be sporangia.

Dissemination to the internal organs or bones is rare. In most cases the general health of the patient is unimpaired.

The appearance of pedunculated or unpedunculated polyps or nodules covered with white dots on the nasal mucosa or the conjunctiva should suggest the diagnosis of rhinosporidiosis. The condition must be distinguished from cryptococcosis, cutaneous tuberculosis, leprous lesions, leishmaniasis, treponematoses, and myospherulosis, an iatrogenic condition related to the application of nasal substances.

Sporotrichosis

The clinical manifestations of sporotrichosis are rather variable, which helps to explain the large number of different classification schemes that have been proposed.[57] The most common clinical presentation is a localized cutaneous or subcutaneous lesion. Lymphatic spread may then lead to the development of further cutaneous lesions. Much less commonly, the fungus may cause infection of the lungs, joints, bones, eyes and meninges. Widespread disseminated infection has been reported in patients with diabetes, alcoholics, drug abusers and patients with AIDS.

Cutaneous sporotrichosis

Cutaneous sporotrichosis tends to affect exposed sites, mainly the limbs and especially the hands and fingers. The right hand is affected more frequently than the left. The initial lesion develops at the site of implantation of the fungus. It is a painless nodule that is movable at first but that later becomes attached to the neighboring tissue. The skin turns red then violaceous, and the nodule breaks down to form an ulcer, which discharges a serous or purulent fluid. The edge of the ulcer is often irregular and it may become edematous, vegetative and crusted.

After a period of a few days to several weeks, the primary lesion may become surrounded by satellite lesions, or further nodules along the course of the draining lymphatics may develop. These soon become palpable and ulcerate through to the skin. In most cases, however, the lymphangitis heals or remains static for a long time without ulcers forming. In most cases the regional lymph nodes are not involved. However, this is not an invariable rule. Any involvement of these lymph nodes is evidence of a superimposed bacterial infection and they may ulcerate in turn.

Apart from these very typical lesions, sporotrichosis may present a different clinical picture. Extension over large areas of skin, often described as the disseminated cutaneous form, may occur. Flat, infiltrated or papulopustular or nodulopustular lesions may develop. Whether oozing, proliferative, papillomatous or verrucous, the lesions of sporotrichosis are generally painless but often pruritic. Several ulcers may be interconnected by subcutaneous fistular passages. Confluent lesions may form a purulent and warty plaque with a continually expanding margin, whereas the center becomes atrophied, smooth and shiny.

Primary cutaneous lesions may heal spontaneously, leaving behind unsightly and even disfiguring scars, which may be a functional impediment. However, secondary lesions may persist for several years.

Extracutaneous sporotrichosis

Pulmonary sporotrichosis is a rare but well-recognized condition. It may be primary, following the inhalation of spores, and may be accompanied by enlargement of the hilar or tracheobronchial lymph nodes. It may, however, also be of a secondary character, caused by hematogenous dissemination. The symptoms are nonspecific and include a productive cough, fever and weight loss. Hemoptysis may occur and can be massive and fatal. The course may be chronic. The typical radiologic finding is a single, nodular upper lobe lesion, which may or may not cavitate. The natural course of the lung lesion is gradual progression to death.

Most patients with osteoarticular sporotrichosis also have preceding cutaneous lesions. This condition presents as stiffness and pain in a large joint. The onset is indolent. In almost all cases of arthritis, the knee, elbow, ankle or wrist is involved. Osteomyelitis seldom occurs without arthritis; the lesions are usually confined to the long bones nearby affected joints.

Endophthalmitis, although very rare, may result in blindness; chorioretinitis has also been reported. Cases of meningitis have also been seen.

The development of a cutaneous lesion on the limbs following trauma is suggestive of sporotrichosis, particularly if the patient is resident in an endemic region. The development of multiple ulcers along lymphatics is also suspicious. At a later stage of development sporotrichosis must be distinguished from mycoses such as blastomycosis, chromoblastomycosis and paracoccidioidomycosis, and from leishmaniasis, verrucous tuberculosis and tertiary syphilis. The diagnosis ultimately depends on mycologic and histologic examination.

MANAGEMENT

There are no trials comparing different strategies for the treatment of infection caused by black fungi. Treatment depends on the clinical form of the disease and is reviewed by Silveira & Nucci.[23] Cutaneous and subcutaneous phaeohyphomycosis are usually treated with complete surgical excision of the lesion, resulting in complete cure in the majority of cases. In addition, various antifungal agents have been used. Itraconazole is considered the drug of choice, and the dose has ranged from 200 to 600 mg daily. The duration of treatment is not established. Although unusual, progression or recurrence of disease, even with adequate itraconazole serum levels, has been observed. Whenever possible, surgical resection is also recommended for lesions in other organs, in association with an antifungal agent.

A number of reviews expanding many of the guidelines given here have been published.[24,58]

Chromoblastomycosis

Chromoblastomycosis is a difficult condition to treat.[17,29] Surgical excision should be reserved for small lesions; it carries a high risk of local dissemination and should only be attempted in conjunction with antifungal treatment.

There is no ideal antifungal treatment for chromoblastomycosis.[19] The most commonly used drug is flucytosine (150–200 mg/kg per day given as four divided doses), but resistance is a frequent problem during long-term treatment. Amphotericin B is not effective as monotherapy but appears to be effective in combination with 5-fluorocytosine (5-FC). Ketoconazole is effective in combination with 5-FC. Fluconazole is reported to be successful.

Much better results have been obtained when 5-FC (4 g/day given as four divided doses) is combined with oral thiabendazole (1 g/day given as two divided doses). Treatment should be continued for at least 1 month after clinical cure is obtained. Itraconazole (400 mg/day) has given promising results in a few patients. Itraconazole is particularly effective when combined with liquid nitrogen cryotherapy.[19] Recent trials have shown terbinafine (50 mg/day) to be effective, even in chronic cases.[19] The local application of heat to the lesions may be beneficial.

Entomophthoramycosis

Conidiobolomycosis

There is no standard treatment for all forms of this disease. Treatment has so far been disappointing. Surgical resection of infected tissue is seldom successful and may hasten the spread of infection. The condition can be treated with saturated potassium iodide solution (up to 10 ml q8h as tolerated) or amphotericin B. Long-term results are poor.

Patients with rhinofacial conidiobolomycosis treated with fluconazole (200 mg/day for 4 months) have been completely cured or have exhibited considerable improvement. Some patients have responded to combination treatment with amphotericin B and terbinafine,[59] or a combination of itraconazole and fluconazole.[60]

Trimethoprim–sulfamethoxazole, amphotericin B and fluconazole are useful in the treatment of *C. incongruus* infection[61] but clinical failure has been reported during treatment with amphotericin B and flucytosine.

Basidiobolomycosis

The therapy of choice still appears to be saturated potassium iodide solution (30 mg/kg per day) which should be given for 6–12 months. Oral ketoconazole (400 mg/day) has sometimes been successful, but amphotericin B has seldom been helpful. Surgical resection is not curative.

Ribes *et al*.[20] provides an exhaustive review of the treatment of entomophthoramycosis.

Lobomycosis

Antifungal drugs are ineffective. Amphotericin B, griseofulvin, sulfonamides and ketoconazole have been employed without adequate clinical responses.

Cure can only be achieved by surgical excision, the extent of the lesions permitting. Care must be taken during surgery of lobomycosis to avoid contaminating healthy tissue. Cryosurgery has also been used. Unfortunately, however, recurrence after excision is common. In advanced cases, the extensive excision required to remove the lesion may not be justified if the infection is not life-threatening. In cases involving larger areas of infection, treatment with clofazimine (300 mg/day) is recommended. The drug must be used, after initial clinical improvement, for at least 2 years at 100 mg/day. At present, the disease does not have a satisfactory medical treatment. The course of the disease is slow and chronic and the prognosis is poor. Lobomycosis never heals spontaneously and is never fatal, but it may be a very serious impediment.

Mycetoma

Early actinomycetomas (and some late and advanced cases) respond well to treatment.[62,63] The drug of choice is streptomycin sulfate (1000 mg/day intramuscularly). This should be combined with trimethoprim–sulfamethoxazole (co-trimoxazole) 960 mg q12h in cases caused by *S. somaliensis*, *A. pelletieri* or *N. brasiliensis*. Other regimens include trimethoprim–sulfamethoxazole and amikacin, and streptomycin combined with either dapsone or trimethoprim–sulfamethoxazole. If no response is seen after 3 weeks of treatment, other regimens can be substituted. These include streptomycin and rifampin (rifampicin), or streptomycin and sulfadoxine plus pyrimethamine. Therapy must be continued for months or even years. In favorable cases, edema and tenderness regress, discharge of secretion and grains diminishes, and sinuses dry up and close.

Even after symptoms and clinical signs have disappeared, the disease has become clinically silent and laboratory tests have become normal, it is recommended that treatment be continued for the same period of time as was required to achieve these results.

The response of eumycetoma to antifungal treatment is disappointing.[62,63] *Madurella mycetomatis* and *P. boydii* infections have been known to respond to ketoconazole (400 mg/day), but it is essential to test liver function before starting and during treatment with this drug. Long-term treatment with itraconazole has resulted in improvement in *M. grisea* mycetoma. The fungal agents causing mycetoma are resistant to amphotericin B and 5-FC. Terbinafine has been successful in the treatment of maduromycetoma.

Mycetoma due to *Acremonium* spp. and *P. boydii* appears to respond to fluconazole (200 mg daily for 10–12 weeks).

Surgical excision is the method of choice if the eumycotic lesions are small enough for total removal to be possible. Amputation is often required in advanced cases with bone involvement, particularly when there is no response to drug treatment. Prostheses and rehabilitation are indispensable in every case of mutilating surgery.

Phaeohyphomycosis

Subcutaneous phaeohyphomycosis

Incision and drainage of subcutaneous lesions is seldom successful. Surgical resection is required. Treatment with amphotericin B has cured or improved unresectable lesions, but later relapse has been common. Itraconazole and terbinafine have been successful in the treatment of phaeohyphomycosis.

Paranasal sinus infection

Complete surgical debridement combined with amphotericin B treatment is essential to halt the progression of this form of phaeohyphomycosis. Even so, it is not uncommon for the condition to recur. The need for repeated debridement is most evident in patients with disabling symptoms or erosion of the bone separating the paranasal sinus from the brain.

Oral treatment with itraconazole (100–400 mg/day) appears promising, although the optimum dosage and duration of treatment have not been defined.

Necrotic nasal septum lesions due to *Alternaria* spp. or *Curvularia* spp. have been cured after surgical excision.

Cerebral phaeohyphomycosis

In no case has a patient survived without surgical resection of the lesion. Treatment with amphotericin B on its own is ineffective. Lesions that have not been completely removed have usually proved fatal. A single case of cerebral phaeohyphomycosis due to *Cladosporium* spp. responded partially to fluconazole (300 mg/day for 5 weeks).

Cutaneous infection

Surgical debridement of cutaneous lesions combined with parenteral amphotericin B is the most effective method of treatment. Topical antifungal treatment is seldom helpful.

Other forms of phaeohyphomycosis

Too few patients have been treated for firm recommendations to be possible. However, the response to amphotericin B has been partial at best. Surgical resection of lesions or oral treatment with itraconazole should be considered.

In *in-vitro* tests the new azole antifungals, including voriconazole, appear to be as active as itraconazole against a number of agents of phaeohyphomycosis.

Rhinosporidiosis

The treatment of choice is surgical excision. No drug treatment has proved effective. If left untreated, the polyps will continue to enlarge slowly. Local injection of amphotericin B may be used as an adjunct treatment to prevent reinfection and spread of the disease.[27] In very rare cases, widely disseminated or deep-seated visceral lesions may develop. Spontaneous remission is unusual.

Sporotrichosis

Saturated potassium iodide solution remains the treatment of choice for patients in developing countries who contract cutaneous sporotrichosis, owing to its ease of administration and low cost.[64] However, new drugs are needed, especially for disseminated sporotrichosis.[64] The starting dose is 1 ml q8h, and this is increased to 4–6 ml q8h. Treatment should be continued for at least 1 month after clinical cure is obtained, which may take 2–4 months. Intolerance (iodism) is frequent and consequently therapy is often stopped.

If the patient cannot tolerate potassium iodide, oral itraconazole (100–200 mg/day) can be used.[65] Treatment should be continued for up to 6 months. The most appropriate therapeutic regimen appears to be 200 mg/day during the entire treatment period, or 200 mg/day at the beginning of treatment, reduced to 100 mg/day after improvement. Many cases treated in this way are cured relatively quickly. Cutaneous sporotrichosis has also been successfully treated with terbinafine (125 mg/day).[66]

Local heat, on its own or in combination with drug treatment, has been shown to improve cutaneous lesions. Besides thermotherapy, the simple warming of diseased limbs in winter months has proved helpful.

Amphotericin B has cured some patients with extracutaneous forms of sporotrichosis, but failures are common. In cases of arthritis or osteomyelitis, better results have been obtained when the drug has been combined with surgical debridement.

Itraconazole (400 mg/day) has given good results in patients with extracutaneous infection, especially in those who have not responded to fluconazole.[66] The Mycoses Study Group of the Infectious Diseases Society of America has published detailed practice guidelines for sporotrichosis.[67] Their recommendations were derived primarily from multicenter, nonrandomized treatment trials, small retrospective series and case reports. The treatment of choice for fixed cutaneous or lymphocutaneous sporotrichosis is itraconazole 100–200 mg/day for 3–6 months.

REFERENCES



References for this chapter can be found online at http://www.expertconsult.com

Chapter 180

Lynne S Garcia

Protozoa: intestinal and urogenital amebae, flagellates and ciliates

This chapter discusses the amebae, flagellates and ciliates that parasitize the intestinal and urogenital systems of humans (Table 180.1). With the exception of *Trichomonas vaginalis*, all of the organisms live in the intestinal tract. Intestinal protozoa vary in pathogenicity and prevalence, but, with rare exceptions, all have a worldwide distribution.

NATURE

Amebae, flagellates and ciliates are single-celled eukaryotic organisms belonging to the subkingdom or phylum Protozoa. In general, the organisms related to human infection change their form and function from the active, feeding trophozoites to the resting cyst form. All protozoa contain a nucleus, often with a karyosome near its center. The cytoplasm is composed of the endoplasm (which immediately surrounds the nucleus) and the ectoplasm (which functions as the locomotion apparatus). Reproduction is relatively simple and is accomplished through repeated asexual multiplication by binary fission. Although not completely clear, *Blastocystis hominis* is currently classified with the protozoa and will be discussed in this chapter.

Table 180.1 Amebae, flagellates and ciliate that parasitize the intestinal and urogenital systems of humans

Type	Species	Pathogenicity*
Amebae	*Entamoeba histolytica*	1
	Entamoeba dispar	2
	Entamoeba hartmanni	2
	Entamoeba coli	2
	Entamoeba polecki	2
	Entamoeba moshkovskii	1
	Entamoeba gingivalis†	2
	Endolimax nana	2
	Iodamoeba bütschlii	2
	Blastocystis hominis	6
Flagellates	*Dientamoeba fragilis*	1
	Giardia lamblia	1
	Trichomonas vaginalis‡	1
	Pentatrichomonas (Trichomonas) hominis	2
	Trichomonas tenax†	2
	Chilomastix mesnili	2
	Enteromonas hominis	2
	Retortamonas intestinalis	2
Ciliate	*Balantidium coli*	1

*Pathogenic = 1; nonpathogenic = 2; pathogenicity controversial = 6.
†Body site: mouth.
‡Body site: urogenital system.

EPIDEMIOLOGY

Intestinal amebae, flagellates and ciliates are transmitted through fecally contaminated food, water or other materials. Prevalence is correlated with socioeconomic conditions, and higher infection rates occur in people who have poor personal hygiene, who live in areas with poor sanitation, or men who have sex with men.[1] Contaminated water supplies are a particular problem because the usual levels of chlorination may not kill cysts.[2] Filtration is required. Endemic and epidemic disease has been traced to water supplies that use surface water which either is not filtered or has been improperly filtered.

These organisms generally have a cystic stage that develops when conditions in the environment are unfavorable for continued multiplication. The cyst wall is thicker than the trophozoite membrane and thus provides protection. Once these cysts have been transferred to a new host, usually by fecal–oral contamination from person to person, the organisms excyst. Excystation factors include osmotic changes in the environment, enzymatic action of the enclosed organism on the inner surface of the cyst wall and, among the parasitic protozoa, favorable pH and enzymatic action of the host tissues. Transmission of those protozoa with no identified cyst stage has not been totally explained.

Entamoeba histolytica

Infections with *E. histolytica* are seen worldwide and are more prevalent in the tropics. In 2003, over 500 million people were estimated to be infected with *E. histolytica*, 50 million had extensive symptoms, including colitis or extraintestinal abscesses, and there were 110 000 deaths.[3] For every case of invasive disease diagnosed, there are at least 10–20 asymptomatic individuals excreting infective cysts. Population groups with a higher incidence of amebiasis include people from the developing world or recent immigrants from there to the developed nations.

Social changes seen in the late 1960s – open expression of homosexuality, increased sexual contacts, increased frequency of sexual activities and anonymity of sexual partners – contributed to dramatic increases in sexually transmitted organisms, including *E. histolytica*. Clinical presentations within the homosexual community often differ from those seen in the heterosexual population and almost 50% of all homosexual men found to be infected with *E. histolytica* are asymptomatic.[4] However, it is an accepted fact that the ameba

morphologically identified as *E. histolytica* is actually two separate and distinct species. *E. histolytica* is the pathogenic species and is considered the etiologic agent of amebic colitis and extraintestinal amebiasis, while *E. dispar* is the nonpathogenic species and does not invade tissue or cause intestinal symptoms. Therefore, in epidemiologic studies prior to the development of specific reagents for the identification of true *E. histolytica*, those organisms identified as *E. histolytica* were actually in the *E. histolytica*/*E. dispar* group.

Entamoeba moshkovskii, which is morphologically identical to *E. histolytica* and *E. dispar* but biochemically and genetically different, has been considered until recently to be primarily a free-living (nonpathogenic) ameba. However, human isolates have now been detected in North America, Italy, South Africa, Bangladesh, India, Iran and Australia.[1] Recent studies suggest that infection with this species can cause diarrhea and other intestinal disorders.[1] Since *E. moshkovskii* is indistinguishable in its cyst and trophozoite forms from *E. histolytica* and *E. dispar,* it is not possible to differentiate the three species on the basis of traditional microscopic examination. Therefore, past studies on the prevalence of *E. histolytica* may be flawed if the possible presence of *E. dispar* and *E. moshkovskii* was not considered.

Epidemiologic evidence of sexual transmission of *E. histolytica* has grown significantly since the early 1970s, particularly in New York City and San Francisco. In San Francisco, the incidence of reported symptomatic intestinal amebiasis increased by over 1000% from 1978 to 1988 among homosexual men between 20 and 39 years of age.[4] Direct oral–anal contact (anilingus) leads to fecal exposure and oral contact with a variety of intestinal pathogens. Transmission can also occur during oral–genital sex after anal intercourse has occurred. Active heterosexuals can acquire infection with *E. histolytica* through sexual activities that provide an opportunity for fecal–oral contamination. The key factor is not necessarily homosexuality, but the frequency of sexual activity and potential for fecal–oral contact. In a study from Japan, symptomatic amebiasis in the east-southeast area of Tokyo is a disease that predominantly afflicts males, and the high rates of patients who engaged in male homosexual or bisexual practices suggest that amebiasis is likely to be a sexually transmitted disease in homosexual or bisexual men. These infections were confirmed to be true *E. histolytica*, primarily zymodeme II.[5]

With the advent of AIDS, and the subsequent modifications in sexual practices within homosexual communities, the incidence of *E. histolytica* infection has decreased. In recent years, the increased incidence of disease caused by coccidian parasites, *Cystoisospora belli* and *Cryptosporidium* spp. and the microsporidia, *Enterocytozoon bieneusi* and *Encephalitozoon intestinalis* has become much more of a problem in patients who have AIDS.

In certain urban areas (Mexico City, Mexico; Medellin, Colombia; and Durban, South Africa), the incidence of invasive disease is considerably higher than in the rest of the world. Contributing factors may include poor nutrition, tropical climate, decreased immunologic competence of the host, stress, altered bacterial flora in the colon, traumatic injuries to the colonic mucosa, alcoholism and genetic factors.

The human is the reservoir host for *E. histolytica*/*E. dispar* and can transmit the infection to other humans, primates, dogs, cats and possibly pigs. The cyst stages are very resistant to environmental conditions and remain viable in the soil for days. The asymptomatic cyst passer who is a food handler is thought to play the most important role in transmission.

It has been postulated that a colonization–blocking vaccine could eliminate *E. histolytica* as a cause of human disease, particularly as humans serve as the only significant reservoir host, and a number of potential protective antigens are being investigated.[6]

Blastocystis hominis

Blastocystis hominis is transmitted via the fecal–oral route through contaminated food or water; the cysts survive in water for up to 19 days at normal temperatures.[2,7,8] Although other modes of transmission are not defined, the incidence and apparent worldwide distribution suggest the traditional route of infection. When genotypic results from animal isolates were compared with the diversity of genotypes of 102 human *Blastocystis hominis* isolates, 67.4 % (62/92) of isolates from mammals and birds were identical to human *B. hominis* genotypes. Thus, the possibility of zoonotic transmission appears to be very likely.[9] Prevention would involve improved personal hygiene and sanitary conditions.

Giardia lamblia (intestinalis, duodenalis)

Various peer-reviewed journal publications through 2007 use different terms for the species of *Giardia* that infect humans; however, we have decided to maintain *G. lamblia* for this chapter. Transmission is by ingestion of viable cysts, and contaminated food and drink are the usual sources. These zoonotic protozoa are responsible for disease in a broad range of hosts, including humans, have a low infectious dose enhancing the possibility of transmission, have transmission stages that are small and environmentally resistant, and are insensitive to the disinfectants commonly used in the water industry.[10] This infection is found in children or in groups that live in close quarters.[11,12] Also there may be outbreaks caused by poor sanitation facilities or breakdowns; travelers and campers often experience such outbreaks.[13,14] There is also an increase in the prevalence of giardiasis in the male homosexual population, probably as a result of anal and/or oral sexual practices.[15]

Decreased gastric acid production may predispose people to infection with *G. lamblia*. Normal gastric acidity may act as a barrier to infection; patients who have had a gastrectomy are prone to infection with *G. lamblia*. Reduction in gastric acid also occurs as a result of malnutrition and both factors may, as a group, increase susceptibility to infection with this organism. Impairment of the host's immune system may also play a role.

Lower incidence of giardiasis in infants up to 6 months of age may be associated with breast-feeding and some protection against infection via secretory IgA; however, lower incidence may also be related to a decreased exposure to *G. lamblia* in breast-fed infants.

Giardiasis is one of the common causes of travelers' diarrhea and is worldwide in distribution. Visitors in areas endemic for *Giardia* spp. are more likely to become symptomatic than the inhabitants of that area; this is probably because the latter have developed immunity from previous, and possibly continued, exposure to the organism. A number of outbreaks in the USA have been attributed to resort or municipal water supplies, such as Oregon, Colorado, Utah, Washington, New Hampshire and New York.[16–18] High infection rates were also reported from hikers and campers who drank stream water. Because some of these areas were remote from human habitation, infected wild animals, especially the beaver, were suspected of being the source.[13,17,19–21] Surveys show human infection rates of 2–15% in various parts of the world.

Because of potential wild animal reservoirs and possibly domestic animal reservoir hosts, measures in additional to personal hygiene and improved sanitation have to be considered.[22,23] Iodine is recommended as an effective disinfectant for drinking water, but it must be used according to directions.[24,25] Filtration systems have also been recommended, although they have certain drawbacks, such as clogging.

Isoenzyme studies, used for parasite identification and classification, have provided information related to organism pathogenicity, possible implication in water-borne outbreaks, and the potential cause of human disease. The examination of isoenzyme patterns of *G. duodenalis* (*G. lamblia*) obtained from humans and animals showed no obvious correlation between clinical symptoms and isoenzyme patterns. These studies also demonstrated significant differences between isolates from within a single region and those from other distant geographic locations.[26,27]

Dientamoeba fragilis

Dientamoeba fragilis infection is commonly associated with enterobiasis, and it has been suggested that *D. fragilis* may infect *Enterobius* spp. eggs and thus bypass gastric acidity. Although clinical infections with

D. fragilis occur, they are not often reported. This is probably because the infection is self-limiting, stool examination is not requested and laboratory identification is difficult. However, there exists a growing body of case reports from numerous countries around the world that have linked this protozoal parasite to clinical manifestations such as diarrhea, abdominal pain, flatulence and anorexia. A number of studies have even incriminated *D. fragilis* as a cause of irritable bowel syndrome, allergic colitis, and diarrhea in human immunodeficiency virus patients.[28–30] The incubation period for clinical disease is not clearly defined.

Trichomonas vaginalis

Infection with *T. vaginalis* is acquired primarily through sexual intercourse; asymptomatic men therefore need to be diagnosed and treated. The organism can survive for some time in a moist environment such as damp towels and underclothes; however, this mode of transmission is thought to be very rare.

Balantidium coli

Domestic pigs are probably the most important reservoir host for human infection with *B. coli*. In areas in which pigs are the main domestic animal, the incidence of human infection can be quite high (e.g. New Guinea). Human infection is fairly rare in temperate areas; however, once the infection is established, it can develop into an epidemic, particularly when environmental sanitation and personal hygiene are poor. This situation has been seen in mental hospitals in the USA. Preventive measures involve increased attention to personal hygiene and sanitation, as the mode of transmission is ingestion of infective cysts through contaminated food or water.

PREVENTION

Transmission of the majority of the intestinal protozoa occurs through ingestion of infective cysts, which can be acquired from food, water and person to person by the fecal–oral route. These infections tend to be found more frequently in groups that live in close quarters or in certain population groups. There may be outbreaks due to poor sanitation facilities or breakdowns as evidenced by infections in travelers and campers; certainly this has been found for giardiasis. Although amebiasis is usually associated with poor sanitation and underdeveloped areas of the world, sexual transmission has also been documented, mainly among urban homosexual men. Prevention in this group is directly related to limiting sexual practices that provide an opportunity for fecal–oral contamination. The single most effective practice that prevents the spread of infections with intestinal protozoa, particularly in the childcare setting, is thorough hand washing by the children, staff and visitors. In the case of *D. fragilis*, it has been suggested that the trophozoites may infect *Enterobius* spp. eggs, thus allowing protection from gastric acidity; however, under most circumstances, total prevention of enterobiasis and/or infection with *D. fragilis* is neither realistic nor possible. *T. vaginalis* is acquired primarily though sexual intercourse, hence the need to diagnose and treat asymptomatic males. The organism can also survive for some time in a moist environment such as damp towels and underclothes; however, this manner of transmission is considered rare.

Although incomplete epidemiologic investigation and reporting make it difficult to determine the significance of the water-borne transmission of giardiasis accurately, the water-borne route seems to be more important for this protozoan than for other more commonly recognized water-borne pathogens, with the possible exception of *Cryptosporidium* spp. Iodine has been recommended as an effective disinfectant for drinking water, but it must be used according to directions. Because of the potential for wild animal and possibly domestic animal reservoir hosts related to *Giardia* spp., measures in addition to personal hygiene and improved sanitary measures have to be considered and implemented. If appropriate procedures are followed, conventional water filtration should trap most protozoan parasites, including *Giardia*. One should avoid swallowing water when in lakes, rivers, pools or hot tubs; also do not drink directly from lakes, rivers, streams, or springs. Filtration devices for hikers and campers are also available; however, one should look for 'reverse osmosis', 'absolute 1 micron', 'Standard 53' and the words 'cyst reduction' or 'cyst removal'. Boiling water, at a rolling boil, for 1 minute is sufficient to kill organisms, including *Giardia* spp. and *Cryptosporidium* spp. It is also important to thoroughly wash all fruits and vegetables if eating uncooked, use safe water for washing food, peel fruit, and avoid unpasteurized milk or dairy products.

PATHOGENICITY

Some of the intestinal protozoa are nonpathogenic and produce no disease; however, microscopists must be able to distinguish pathogenic from nonpathogenic species. The presence of nonpathogenic species indicates that the person has been exposed to fecal contamination. Several species can cause mild to severe gastrointestinal symptoms, and *E. histolytica* may produce extraintestinal lesions. However, pathogenic or potentially pathogenic protozoa do not always produce symptoms or they may remain after symptoms have resolved. Asymptomatic individuals may serve as reservoirs for the infection. Detection of a potentially pathogenic protozoan does not necessarily prove that the organism is causing the illness. Patients may have diarrhea caused by other organisms such as *Salmonella* spp., *Shigella* spp., *Escherichia coli* or rotavirus. Current intestinal protozoan pathogens included in this chapter are: *Entamoeba histolytica*, *Blastocystis hominis*, *Giardia lamblia*, *Dientamoeba fragilis* and *Balantidium coli*. *Trichomonas vaginalis*, a urogenital flagellate, is also considered pathogenic, and may cause mild to severe vaginitis and other urogenital problems.

Entamoeba histolytica

Although many people worldwide are infected with *E. histolytica*, only a small percentage develops clinical symptoms. Morbidity and mortality caused by *E. histolytica* vary, depending on geographic area, organism species (*E. histolytica* vs *E. dispar*) and the immune status of the patient.

During the 1980s and 1990s several publications reviewed the issues regarding pathogenic *E. histolytica* vs nonpathogenic *E. dispar*. On the basis of current knowledge, pathogenic *E. histolytica* is considered to be the etiologic agent of amebic colitis and extraintestinal disease, whereas nonpathogenic *E. dispar* produces no intestinal symptoms and is not invasive in humans.[31–33](See also Chapter 110.) Diamond & Clark[34] redescribed the two species as *E. histolytica* (Schaudinn 1903), which is the invasive human pathogen, and *E. dispar* (Brumpt 1925), which is noninvasive and which does not cause disease. During the past several years, extensive work has been published related to the pathogenesis of *E. histolytica*.[35,36]

Blastocystis hominis

The true role of this organism in terms of colonization or disease and the relevance of organism numbers require additional clarification. In studies of patients with irritable bowel syndrome, there are patients in whom the presence of *B. hominis* did not appear to be incidental.[37,38] The first report of a possible relationship between intestinal obstruction and a concomitant *B. hominis* infection has also been published.[39] In patients with other underlying conditions, the symptoms may be more pronounced.[40] There is evidence to indicate there are several ribodeme types, and there may be a relationship between ribodeme type and pathogenicity, only some of which will be responsible for increased intestinal permeability and symptoms.[41,42]

Giardia lamblia

The majority of individuals infected with *G. lamblia* are asymptomatic. Preliminary studies indicate that there may be two different strains of *G. lamblia*, Group A and Group B, associated with different degrees of virulence. Group A appears to be more pathogenic and is associated with symptomatic infection. Isoenzyme and molecular studies also support the differences between these two groups.[43–47]

Dientamoeba fragilis

During the past several years, the pathogenicity of *D. fragilis* has been associated with a wide range of symptoms.[28–30] However, it appears that there may be both pathogenic and nonpathogenic variants. Evidence for two genetically distinct forms has been obtained using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis of ribosomal genes.[48,49]

Trichomonas vaginalis

Trichomonas vaginalis is site specific and usually cannot survive outside the urogenital system. After introduction, proliferation begins, with resulting inflammation and large numbers of trophozoites in the tissues and the secretions. Nutrient acquisition and cytoadherence, immune system evasion and regulation of virulence genes are virulence factors associated with pathogenesis.[50] It appears that interference with trichomonads, mucin receptors and proteinases may form a strategy to prevent colonization with this pathogenic flagellate.[51]

Balantidium coli

Some infections with *B. coli* produce no symptoms, while others cause symptoms with severe dysentery similar to that seen in patients with amebiasis. Symptoms usually include diarrhea or dysentery, tenesmus, nausea, vomiting, anorexia and headache. Insomnia, muscular weakness and weight loss have also been reported. Diarrhea may persist for weeks to months prior to the onset of dysentery. There may be tremendous fluid loss, with a type of diarrhea similar to that seen in cholera or in some coccidial or microsporidial infections.

On contact with the mucosa, *B. coli* may penetrate the mucosa with cellular infiltration in the area of the developing ulcer, which may extend to the muscular layer. The ulcers may vary in shape, and the ulcer bed may be full of pus and necrotic debris. Although the number of cases is small, extraintestinal disease has been reported (peritonitis, urinary tract infection, inflammatory vaginitis).[52]

DIAGNOSTIC MICROBIOLOGY

Because intestinal symptoms are nonspecific, diagnosis requires laboratory identification of the organisms present. Immunodiagnostic methods for antibody detection are useful for the diagnosis of extraintestinal amebiasis, but their utility is limited for intestinal disease.

Organism morphology varies and species characteristics often overlap. For reliable identification, microscopists must be able to differentiate all species regardless of their potential for causing disease. Special attention will be given to the clinically significant intestinal pathogens, especially *E. histolytica*, *B. hominis*, *G. lamblia*, *D. fragilis* and *B. coli*.

Identification of amebae in fecal specimens

Trophozoites and cysts are diagnostic stages of the amebae, and either or both stages can be detected in feces. Microscopists must be able to distinguish trophozoites and cysts from epithelial cells, polymorphonuclear leukocytes and macrophages, as well as from pus cells, yeasts, pollen, molds, and vegetable and crystalline artifacts.

Trophozoite motility in physiologic saline mounts of fresh material and cytoplasmic inclusions, such as erythrocytes in trophozoites and chromatoid bodies in cysts, can be observed. Iodine solutions are used for temporary cyst stains of fresh or fixed specimens. Cyst characteristics are less variable than those of trophozoites, and species of cysts can often be identified in iodine-stained wet mounts. However, examination of permanent stained smears using oil immersion (×1000) is recommended for definitive identification of trophozoites and cysts. Size is not a reliable feature for species differentiation of either trophozoites or cysts except when separating the *E. histolytica*/*E. dispar* group from the nonpathogen *Entamoeba hartmanni*.

The microscopist must observe the cytoplasmic and the nuclear characteristics of several organisms before making a final identification. Although cysts are more easily identified than trophozoites, several cysts (particularly if they are immature) should be observed to ensure that the identification is reliable. If two species are identified, there should be distinct populations of each.

Sometimes, although amebic organisms are seen, species cannot be identified. In these instances, the laboratory should report 'unidentified ameba trophozoites (or cysts)'; if the genus can be determined but the species cannot, 'unidentified *Entamoeba* trophozoites or cysts' should be reported, and another specimen should be requested.

Entamoeba histolytica

Intestinal infection is usually diagnosed by the microscopic identification of organisms in feces or in sigmoidoscopic material from ulcerations (Fig. 180.1). Nonpathogenic amebae can be confused with pathogens (Figs 180.2–180.5). Only trophozoites are found in tissue lesions, but both trophozoites and cysts may be found in the intestinal lumen. Some patients who have invasive disease may have only trophozoites in fecal specimens, and examination of permanent stained smears may be required to establish the diagnosis.

Suspected amebic abscesses are often diagnosed by positive serologic tests. Aspirates of abscesses or intestinal lesions may contain amebic trophozoites, sometimes with ingested erythrocytes seen in direct wet mounts or in permanent stains such as trichrome or iron-hematoxylin stains.[52,53] Other species of intestinal amebae are not pathogenic but must be differentiated from *E. histolytica*. *Entamoeba polecki* is seen occasionally in refugees from South East Asia and may be confused with *E. histolytica*, as can *E. moshkovskii*.[52] *Entamoeba gingivalis* is a common inhabitant of the oral cavity, particularly in patients who have poor oral hygiene. It resembles *E. histolytica* but has no known cyst stage. As a result, trophozoites of *E. gingivalis* may lead to the misdiagnosis of amebic lung abscess by morphologic examination of pulmonary material, especially sputum.

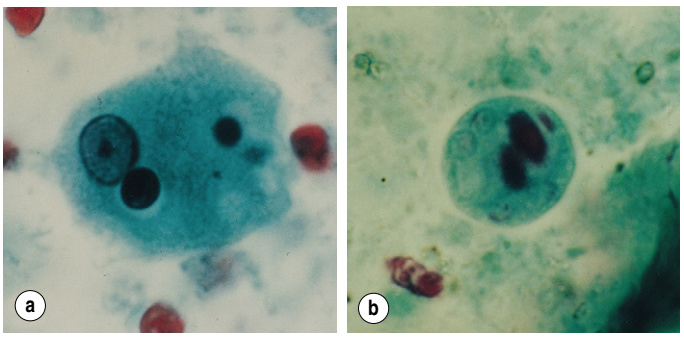


Fig. 180.1 (a) *Entamoeba histolytica*, trophozoite containing ingested red blood cells (the presence of red blood cells confirms the organism is the true pathogen, *E. histolytica*). (b) *Entamoeba histolytica*/*E. dispar*, cyst containing four nuclei and chromatoidal bars with smooth, rounded edges (trichrome stain). Note: from the cyst morphology, it is not possible to differentiate pathogenic *E. histolytica* from nonpathogenic *E. dispar*.

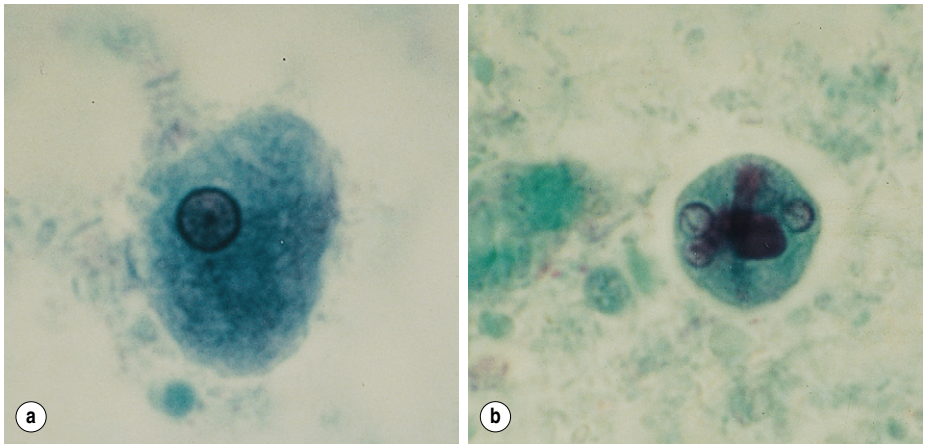


Fig. 180.2 *Entamoeba hartmanni*. (a) Trophozoite. (b) Cyst containing up to four nuclei and chromatoidal bars with smooth, rounded edges (trichrome stain). Note: *E. hartmanni* measures smaller than *E. histolytica/E. dispar*; the trophozoite is <12 μm and the cyst is <10 μm.

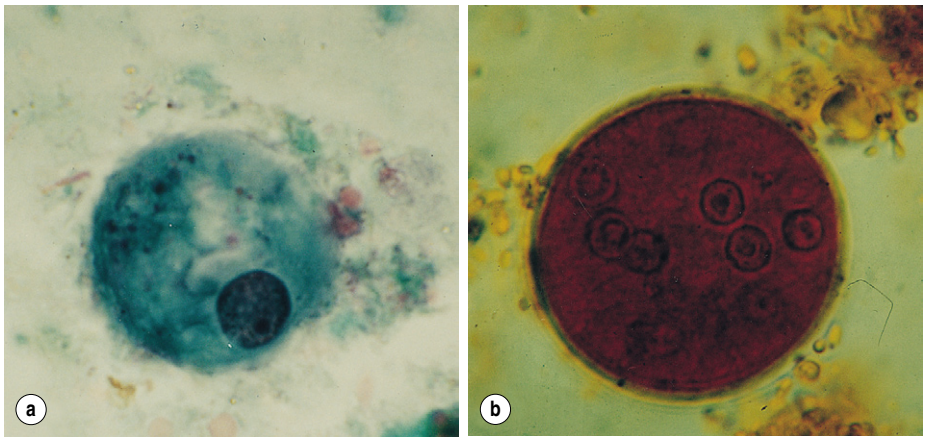


Fig. 180.3 *Entamoeba coli*. (a) Trophozoite containing a single nucleus in which the karyosome is eccentric (tends to be centrally located in *E. histolytica/E. dispar*). (b) Cyst contains more than five nuclei (d'Antoni's iodine).

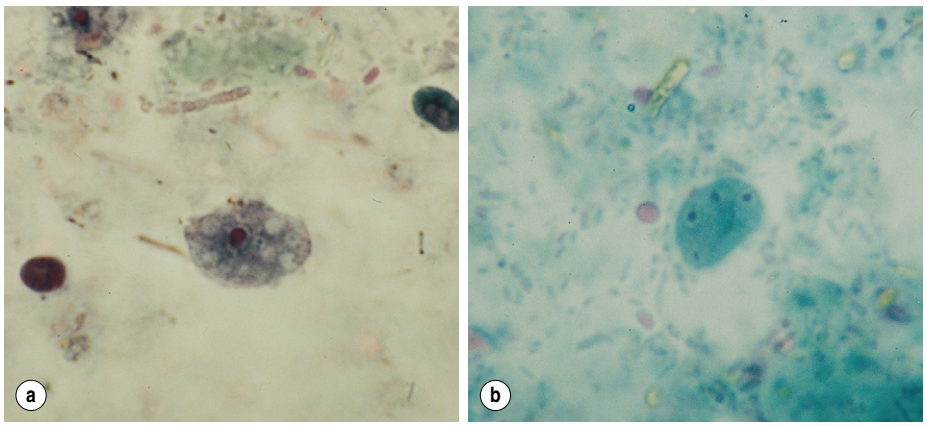


Fig. 180.4 *Endolimax nana*. (a) Trophozoite containing a single nucleus with no peripheral chromatin (large karyosome only) and vacuolated cytoplasm. (b) Cyst containing four nuclei (three easily visible) (trichrome stain).

Currently, there are several immunoassays that can be used to identify organisms in the genus *Entamoeba* (*E. histolytica/E. dispar* group) and other reagents that can differentiate pathogenic *E. histolytica* from nonpathogenic *E. dispar*. The antigen detection enzyme-linked immunosorbent assay (ELISA) kits are based on specific amebic adhesin molecules found in the feces of people infected by either *E. histolytica* or *E. dispar*. The second ELISA reagent is able to detect the adhesin produced by *E. histolytica* in feces. Another immunoassay product is available for the detection of *E. histolytica* and *E. dispar* in fecal specimens; however, this kit does not differentiate between the two organisms, but is specific for the *E. histolytica/E. dispar* group.[54] These procedures are simple, sensitive and specific. It is important to remember that these kits require fresh stool specimens; specimens preserved in any of the routine stool collection fixatives are not acceptable. Polymerase chain reaction methods are also being developed for the differentiation of *E. histolytica* from *E. dispar*.[55]

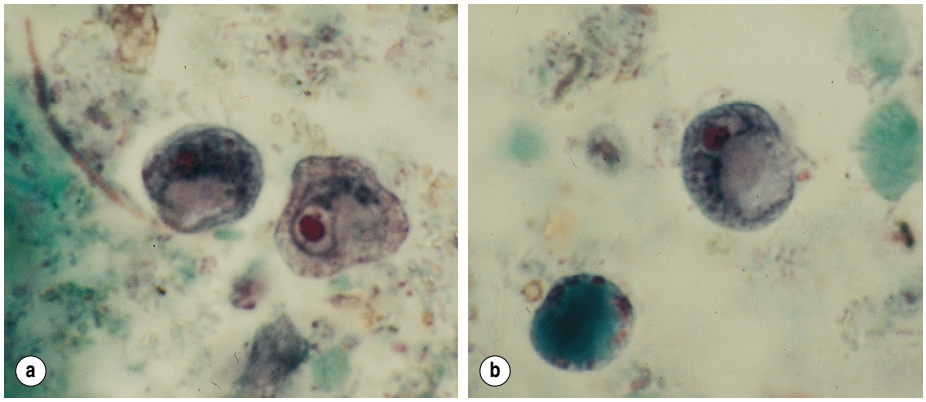


Fig. 180.5 *Iodamoeba bütschlii*. (a) Trophozoite containing a single nucleus with a large karyosome and cyst containing a single nucleus and a large glycogen vacuole. (b) Cyst containing a single nucleus and a large glycogen vacuole – note the size of the karyosome (trichrome stain). There is also a *Blastocystis hominis* central body form present at the lower left of the image.

Serologic testing for intestinal disease is not recommended unless the patient has true dysentery; even in these patients, the titer (e.g. indirect hemagglutination) may be low and difficult to interpret. The definitive diagnosis of intestinal amebiasis should not be made without demonstrating the organisms. When extraintestinal disease is suspected, serologic tests are much more relevant. Indirect hemagglutination and indirect fluorescent antibody tests (FAs) have been reported positive with titers of ≥1:256 and ≥1:200, respectively, in almost 100% of cases of amebic liver abscess.[52] Positive serologic results, in addition to clinical findings, make the diagnosis highly probable. In the absence of rapid serologic tests for amebiasis (tests with very rapid turnaround times for results), the decision as to causative agent often must be made on clinical grounds and on results of other diagnostic tests such as scans.

Histologic diagnosis of amebiasis can be made when trophozoites within the tissue are identified and differentiated from host cells, particularly histiocytes and ganglion cells. Periodic acid–Schiff staining is often used; the organisms will appear bright pink with a green–blue background (depending on the counterstain used). Hematoxylin and eosin staining will also allow typical morphology to be seen. As a result of sectioning, some organisms will exhibit the evenly arranged nuclear chromatin with the central karyosome, and some will no longer contain the nucleus.

Blastocystis hominis

The characteristic form of *B. hominis* that is usually seen in human fecal specimens varies in size from 6 to 40 µm and contains a large central body resembling a vacuole, which may be involved with carbohydrate and lipid storage. The amebic form can occasionally be seen in diarrheal fluid but may be difficult to recognize.[56] Generally, *B. hominis* will be identified on the basis of the typical round form containing the central body.[52] Routine stool examinations are very effective in recovering and identifying *B. hominis* (Fig. 180.6), although the permanent stained smear is the procedure of choice because the examination of wet preparations may not easily reveal the organism. The organisms should be quantitated on the report form, that is, as rare, few, moderate, or many. It is also important to remember that other possible pathogens may be present and should be ruled out before a patient is treated for *B. hominis*.

Identification of flagellates in fecal specimens

The flagellates are a more diverse group than the amebae. The type of motility, shape, number of nuclei and other characteristics, such as an undulating membrane, sucking disk, cytostome, spiral groove, and the number and location of flagellae, are important characteristics used to identify flagellate trophozoites. The organism shape and size, number and position of nuclei, and absence or arrangement of fibrils are used to identify flagellate cysts.

In some cases, species can be determined by the examination of either direct or concentrated wet mounts. Species of cysts may be identified in iodine-stained mounts. However, permanent stains are always recommended for every stool specimen submitted; organisms identified in wet preparations may not represent all types of organism present.

Giardia lamblia

Diagnosis is usually established by the demonstration of cysts or, occasionally, trophozoites in feces (Fig. 180.7). Nonpathogenic

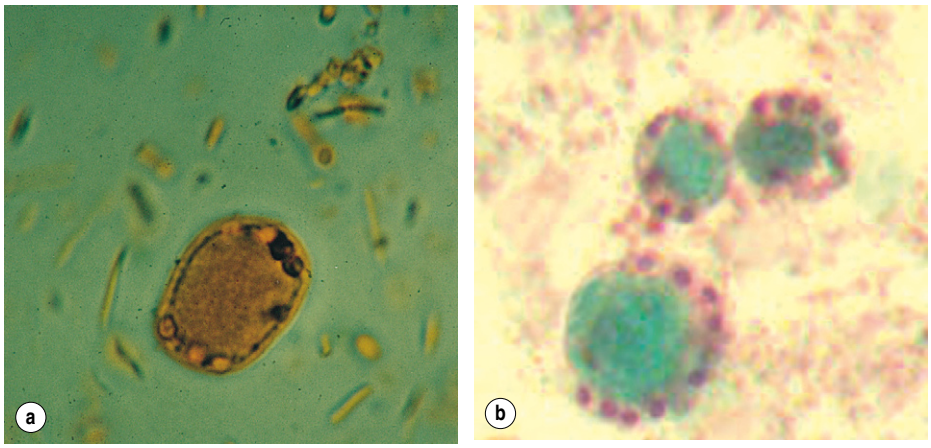


Fig. 180.6 *Blastocystis hominis*. (a) Central body form with large 'empty' area (appears like a vacuole) with multiple nuclei around the edges (d'Antoni's iodine). (b) Three central body forms with the large empty area surrounded by nuclei (trichrome stain).

flagellates can be seen in Figure 180.8. Because of the variable shedding of organisms, several stool specimens should be examined before the infection is ruled out. In some cases, a series of stools can be examined and be negative, and yet the patient still has giardiasis. It is important for the laboratory and the clinician to recognize this fact. Permanent stains are recommended for the definitive diagnosis of this infection.

When *G. lamblia* organisms are not found in stool specimens, duodenal aspirates, string test mucus or biopsied mucosal tissue can be examined. The string test[52] is used to collect mucus from the duodenal area and its use may be less traumatic for the patient than other methods. Materials obtained by drainage, aspiration or the string test can be examined by simple, direct wet mounts. Biopsy tissue may be processed and stained by the usual histopathologic methods; however, before preservation, a fresh imprint smear of the mucosal surface on a slide can be made and stained with trichrome or Giemsa stain.

Immunoassay methods (enzyme immunoassay, FA, immunochromatographic assay) are available and may be appropriate in test ordering situations. Education of the medical staff will be mandatory to ensure that tests are appropriately ordered and that there is complete understanding of the limits of the information generated (test results limited to absence or presence of *G. lamblia*). Recently, industrial companies and municipalities have shown a great deal of interest in these reagents. This is particularly relevant when the water sources are used for drinking and/or recreational purposes.[52]

Dientamoeba fragilis

Permanent stains are required to diagnose this infection, and multiple specimens may be required because shedding varies from day to day. The delicately staining trophozoites are usually binucleate, although the nuclei may be in different planes of focus (Fig. 180.9). The nuclei tend to be fragmented and can often be seen in a 'tetrad' formation. They must be differentiated from the trophozoites of *Endolimax nana*, *Iodamoeba bütschlii* and *Entamoeba hartmanni*. Nuclear characteristics, the presence of binucleate forms, tremendous size variation and the absence of cysts will aid in identification of this organism.

A permanent stained smear should be examined for every stool specimen submitted to the laboratory for an ova and parasite examination.[52,53] If this approach is not used, then many infections with this organism can be missed. If a laboratory periodically finds and identifies *G. lamblia*, but never sees *D. fragilis*, then collection and diagnostic methods should be reviewed.

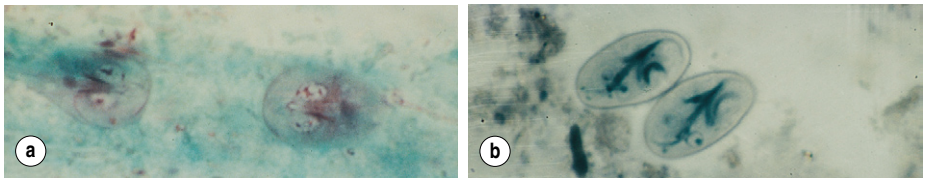


Fig. 180.7 *Giardia lamblia*. (a) Trophozoites in mucus – note the sucking disk area, linear axonemes, curved median bodies and two nuclei (trichrome stain). (b) Cysts containing multiple nuclei, linear axonemes and curved median bodies (iron-hematoxylin stain).

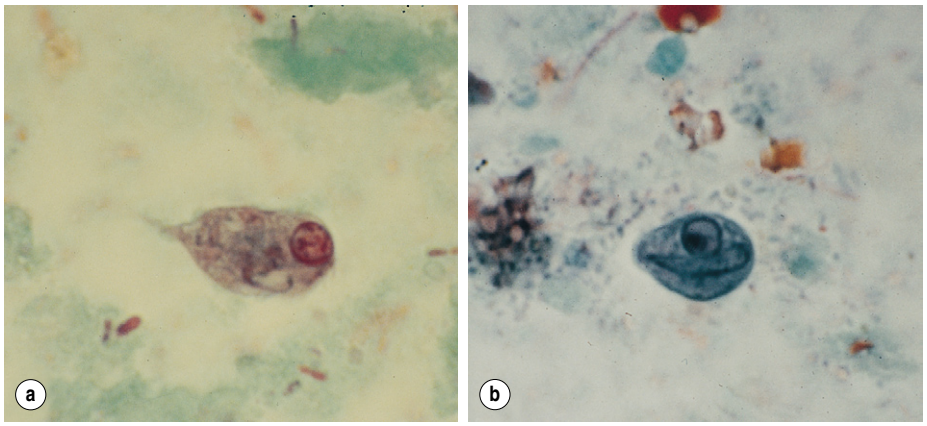


Fig. 180.8 *Chilomastix mesnili*. (a) Trophozoite with single nucleus and clear feeding groove/cytostome. (b) Cyst containing single nucleus and curved fibril called the 'shepherd's crook' (trichrome stain).

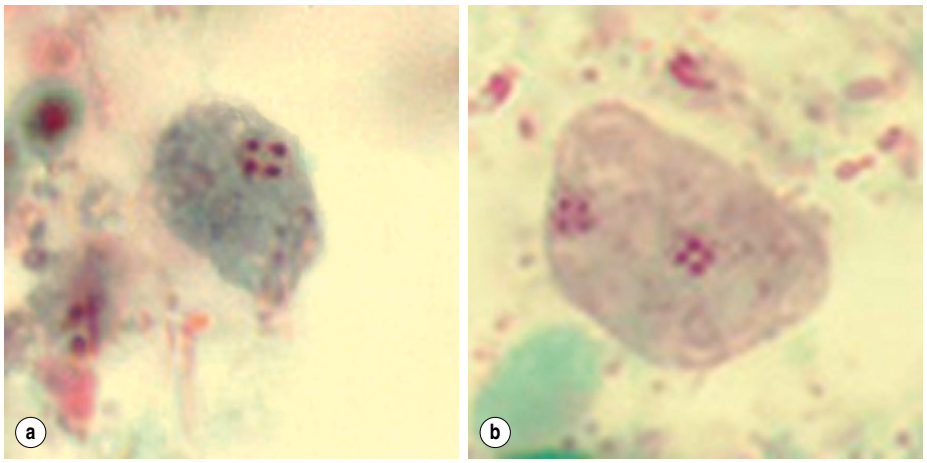


Fig. 180.9 *Dientamoeba fragilis*. (a) Trophozoite with single nucleus fragmented into a 'tetrad' configuration. (b) Trophozoite containing two nuclei, each showing fragmented chromatin (trichrome stain). There is no known cyst form for this organism.

Identification of flagellates in urogenital specimens

Trichomonas vaginalis

Infections with *T. vaginalis* are usually detected by finding the motile trophozoites in wet mounts of vaginal fluid, prostatic fluid or sediments of freshly passed urine. In wet mounts, the trophozoites move with a nervous, jerky motion and possess an undulating membrane, which extends only one-half the length of the organism (Fig. 180.10). In old urine specimens, the organisms may be dead or badly distorted and thus cannot be identified or may be confused with host cells.

Specimens include vaginal fluid, scrapings or washings. They may be examined in a saline wet mount or as a stained smear, or the material can be cultured. Although some consider that wet-mount examinations are as efficient as cultures in revealing infections, current evidence suggests that cultivation methods are superior.[57,58] Immunofluorescent and ELISA methods have also been described. Organisms may be difficult to recognize in permanent stains; however, if a dry smear is submitted to the laboratory, Giemsa or Papanicolaou stain can be used. Chronic *T. vaginalis* infections may cause atypical cellular changes that can be misinterpreted, particularly on the Papanicolaou smear. Organisms are routinely missed on Gram stains. The number of false-positive and false-negative results reported on the basis of stained smears strongly suggests that identification should be confirmed by observation of motile organisms, either from the direct wet mount or from appropriate culture media.



Fig. 180.10 *Trichomonas vaginalis*. Trophozoite showing axostyle, flagella, and part of the undulating membrane (Giemsa stain). There is no known cyst form for this organism.

For culture, it is mandatory that the specimen be collected correctly, immediately inoculated into the proper medium and properly incubated. Excellent methods are available using plastic envelopes containing appropriate media. This envelope approach allows both immediate examination and culture in one self-contained envelope. These systems are commercially available and serve as the specimen transport container, the growth chamber during incubation and the 'slide' during microscopy.[52,59]

Monoclonal antibodies and DNA probe procedures for the detection of *T. vaginalis* have been reported as being very effective.[60,61] An enzyme immunoassay has been developed for the detection of the *T. vaginalis* antigen from vaginal swabs.[62,63] The predictive value of a positive test was 82% and that of a negative test was 99.3%. Commercial products based on these methodologies should be very helpful in diagnosing this infection. Serologic tests have been tried; however, none is commercially available.

Identification of ciliates in fecal specimens

Balantidium coli

In human feces, *B. coli* trophozoites are readily recognized by their large size, their shape and their rapid, rotating motion. Cysts are less easily identified, but they usually cause few diagnostic problems. The morphology of trophozoites and cysts is seen in Figure 180.11.

Examination of direct saline mounts is the most practical method of detecting these protozoa. Cysts can be recovered by concentration, but in human infections trophozoites are usually more numerous than cysts. Iodine-stained mounts and permanent stains are of little value because the organisms tend to overstain and may resemble helminth eggs and/or debris.[52]

CLINICAL MANIFESTATIONS AND MANAGEMENT

The clinical manifestations and management of infections caused by these organisms is discussed in Chapters 49, 108 and 110.

REFERENCES

References for this chapter can be found online at http://www.expertconsult.com

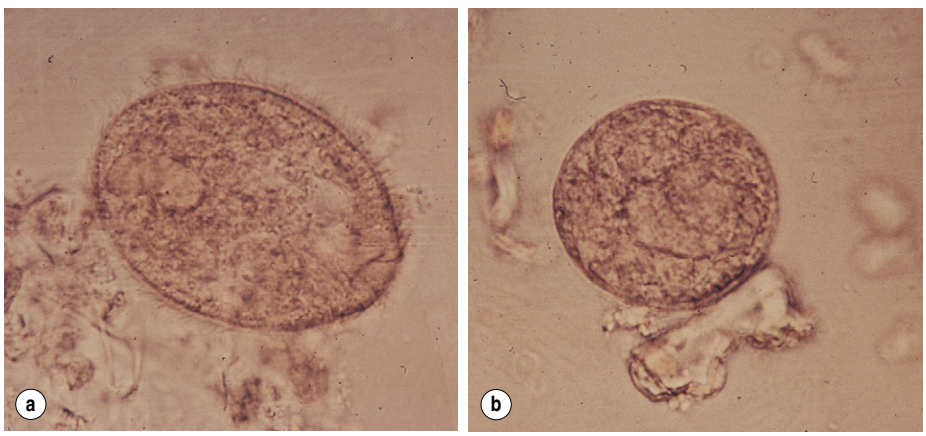


Fig. 180.11 *Balantidium coli*. (a) Trophozoite – note the cilia around the edges ('fuzzy football'). (b) Cyst – note the cilia are difficult to see within the cyst wall (d'Antoni's iodine – light preparation, pale color).

Chapter 181

Rainer Weber

Protozoa: intestinal coccidia and microsporidia

Intestinal coccidia and microsporidia have primarily gained attention as etiologic agents of HIV-associated diarrhea and intestinal disease among persons with other cellular immunodeficiency, including organ transplant recipients or patients undergoing chemotherapy. These organisms, however, are not only opportunistic pathogens, but are also the cause of common, worldwide intestinal infections in immunocompetent children and adults. Also, newly described microsporidia are recognized as causing a wide range of organ or systemic infections in immunocompromised patients.

NATURE

Intestinal coccidia

The intestinal coccidia are obligate intracellular protozoa that belong to the phylum Apicomplexa, subphylum Sporozoa, and infect small intestinal enterocytes. Species of four genera (*Cryptosporidium*, *Cyclospora*, *Isospora*, *Sarcocystis*) are pathogenic in humans.[1–3]

The most frequent agents of human cryptosporidiosis are the bovine *Cryptosporidium* species, *C. parvum*, and *C. hominis*, of which humans and rarely domestic animals are the natural reservoir.[1,4,5] Also, other zoonotic cryptosporidia, including *C. meleagridis*, *C. felis*, *C. canis*, *C. muris* and *C. suis*, have been identified in stools of patients with HIV infection and in HIV-seronegative children living in resource-poor countries.[5–7] *Isospora belli* has been identified as the only accepted cause of human isosporiasis.[3] Intestinal organisms that were previously termed blue–green algae, cyanobacterium-like bodies or coccidia-like bodies were characterized as belonging to the coccidians, and are named *Cyclospora cayetanensis*.[2]

Microsporidia

The term 'microsporidia' is a nontaxonomic designation commonly used to describe a group of obligate intracellular protist-like parasites belonging to the phylum Microsporidia. The nomenclatural status of Microsporidia is ambiguous at present.[8] Microsporidia are considered specialized parasites that evolutionarily seem to be derived from an endoparasitic chytrid ancestor on the earliest diverging branch of the fungal phylogenetic tree,[9] but their exact relationship to the fungi remains to be determined.[10] Approximately 1200 species have been identified that are parasitic in every major animal group, and 14 or these can infect humans (see Table 181.1). To date, seven genera (*Enterocytozoon*, *Encephalitozoon*, *Anncaliia*, *Nosema*, *Pleistophora*, *Trachipleistophora* and *Vittaforma*) and unclassified microsporidia (referred to collectively as *Microsporidium*) have been implicated in human infections.[11,12]

EPIDEMIOLOGY

Cryptosporidium species

Cryptosporidial infections have been detected on all continents. Cumulative prevalence rates are between 1% and 3% in industrialized nations and between 5% and 10% in developing countries.[13] Children, particularly those less than 2 years of age, have a higher prevalence of infection than adults. Seroepidemiologic studies indicate that cryptosporidiosis may be more common than is estimated based upon surveys of fecal oocyst shedding. Seroprevalence rates in developed countries range between 25% and 35%, and in developing countries they are up to 65%. In severely immunodeficient patients with HIV infection, cryptosporidiosis is among the most important causes of chronic diarrhea, accounting for 10–20% of diarrheal episodes.[14]

Sources of infectious cryptosporidial oocysts are humans and animals. Oocysts are transmitted by the fecal–oral route and infection may be acquired from contaminated surfaces, ground and recreational water, pets and farm animals (particularly cattle and sheep), contaminated foods and person-to-person contact, including transmission between household members, sexual partners, children in day-care centers and nosocomial infections involving both medical care staff and patients. An increasing number of outbreaks of cryptosporidial infections attributed to drinking water have been reported, including an outbreak in Milwaukee, USA in 1993 that affected over 400 000 persons.[15] Ingestion of as few as 10 oocysts may cause diarrhea.

Cyclospora species

Cyclospora spp. have been identified worldwide in stool specimens of immunocompromised and immunocompetent patients, including travelers, but the source of infection appears to be restricted to tropical and subtropical areas. The parasite is transmitted by the fecal–oral route and infection is most probably acquired from contaminated water or food (particularly raspberries and green leafy vegetables) from tropical or subtropical countries.[16] It is not known whether animals can be infected and serve as sources for human infection. Direct person-to-person transmission is unlikely because excreted oocysts require days to weeks to become infectious. Warm temperatures and high humidity facilitate sporulation.[17]

Isospora species

Isospora belli is endemic in many parts of Africa, Asia and South America, and is particularly common in patients from developing countries who have AIDS and chronic diarrhea; for example, it occurs in 10–20% of such patients in Haiti or Africa. Modes of transmission are not known but it is assumed that they comprise water and/or food that contains oocysts.

Microsporidia

Reported human infections are globally dispersed. Although microsporidiosis appears to occur most frequently in persons infected with HIV, it is emerging as an infection in otherwise immunocompromised hosts, such as organ transplant recipients or patients undergoing chemotherapy, and in immunocompetent individuals, including travelers, the elderly and children.[10,11]

The sources of microsporidia infecting humans and modes of transmission are uncertain. Because microsporidial spores are released into the environment via stool, urine and respiratory secretions, possible sources of infection may be persons or animals infected with microsporidia as well as contaminated water and food.[10,12] Ingestion of microsporidial spores is the most probable mode of transmission. Transmission by the aerosol route has also been considered because spores have been found in respiratory specimens of patients who have *Encephalitozoon* spp. infection.[18] Epidemiologic and experimental studies in mammals suggest that *Encephalitozoon* spp. can be transmitted transplacentally from mother to offspring, but no congenitally acquired human infections have been reported.

PATHOGENICITY

Cryptosporidium species

Cryptosporidium spp. develop intracellularly at the microvillous border of enterocytes (Fig. 181.1). Infected cells lack microvilli at the site of parasite attachment and the mucosal surface appears disrupted. In immunocompetent individuals, infection is usually limited to the intestine. In immunocompromised patients, organisms are found throughout the entire gastrointestinal tract and within epithelial cells of the biliary tree, the pancreatic ducts and the airways. In the intestines, cryptosporidial infection induces atrophy, blunting or loss of villi, crypt hyperplasia, and infiltration of lymphocytes, neutrophils, plasma cells and macrophages into the lamina propria. Cryptosporidial infection has been associated with marked reduction in the brush border enzyme activities, including sucrase, lactase and maltase deficiency, with impaired absorption of vitamin B12 and D-xylose, and with increased permeability of the intestinal epithelium to organic molecules. Malabsorption and intestinal injury appear to correlate with the number of organisms infecting the intestine. No specific virulence determinants of the parasite have been clearly linked to direct or indirect damage of intestinal host tissues. Putative virulence factors include molecules that are involved in parasite attachment to host cells and host cell membrane disruption.

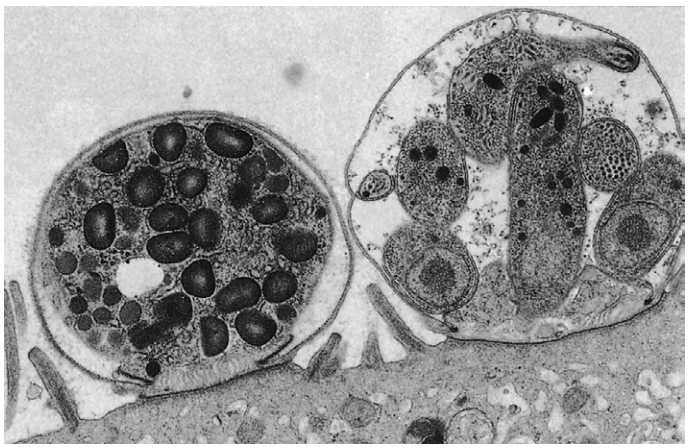


Fig. 181.1 Intestinal cryptosporidial infection. Transmission electron micrograph of duodenal tissue of a patient with HIV infection showing two different developmental stages of *Cryptosporidium* spp. on the brush border of the mucosal surface: mature schizont with merozoites (right), undifferentiated zygote (left). *Cryptosporidium* spp. develop intracellularly just under the plasma membrane of the host cell. Courtesy of MA Spycher.

The immune responses to cryptosporidial infection involves both innate and adaptive immune responses. $CD4^+$ lymphocytes and interferon gamma (IFN-γ) play key roles in the memory response.[19] Severe or persistent cryptosporidiosis has primarily been observed in HIV-infected patients with marked $CD4^+$ cell immunodeficiency,[14] and rarely in persons with IFN-γ deficiency[20] or with X-linked hyper-IgM syndrome which leads to secondary hypogammaglobulinemia and impaired cellular immune function.[21]

Isospora species

Isospora belli develops within parasitophorous vacuoles deep in the cytoplasm of the enterocyte. Histologic abnormalities associated with isosporiasis range from minimal changes of the small intestinal architecture to marked villous atrophy, crypt hyperplasia and inflammatory infiltrates in the lamina propria consisting of eosinophils, neutrophils, lymphocytes and plasma cells. The mechanisms by which these changes occur are unknown. As a result of the intestinal injury, malabsorption and steatorrhea have been documented.[3,17]

Cyclospora species

Cyclosporiasis is associated with villous atrophy, crypt hyperplasia and inflammatory infiltrates. The mechanisms that lead to the clinical features are unknown.

Microsporidia

Microsporidiosis has been associated with abnormalities in structure and function of infected organs but how the different microsporidial species cause disease is not sufficiently understood.[10,11]

Enterocytozoon bieneusi infection generally appears to be limited to intestinal enterocytes (Fig. 181.2) and biliary epithelium. Patients who have severe cellular immunodeficiency appear at highest risk of developing microsporidial disease but little is known about immunity to *E. bieneusi* infection due to the lack of tissue culture or small animal models.[10] It is not understood whether microsporidial infection in these patients is primarily a reactivation of latent infection acquired before the state of suppressed immunity or whether microsporidial disease is caused by recently acquired infection.

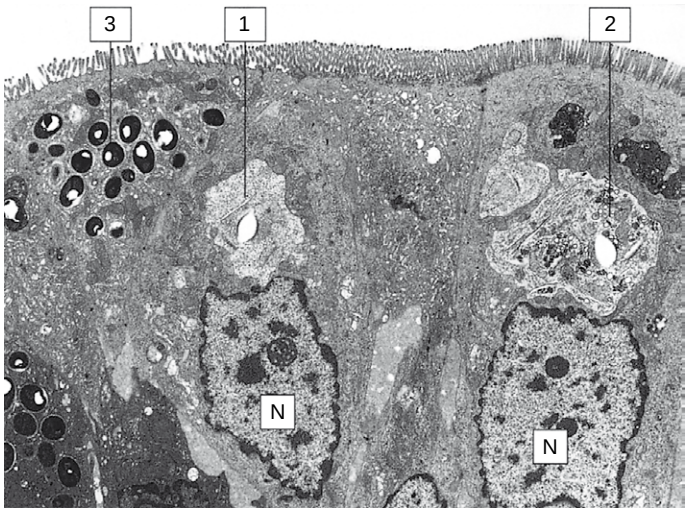


Fig. 181.2 Intestinal *Enterocytozoon bieneusi* infection. Transmission electron micrograph showing duodenal epithelium of a patient with HIV infection who has *Enterocytozoon bieneusi* infection. The different developmental stages between the enterocyte nuclei (N) and the microvillous border include: (1) a proliferative plasmodium; (2) late sporogonial plasmodia; and (3) mature spores. Courtesy of MA Spycher.

Encephalitozoon cuniculi and *E. hellem* infect a variety of cells including epithelial and endothelial cells, fibroblasts, kidney tubule cells, macrophages and possibly other cell types in numerous mammalian hosts, for example rabbits, rodents, carnivores, monkeys and humans.[12,22] In mammals, they usually cause latent asymptomatic or chronic mildly symptomatic infection, but interstitial nephritis and severe neurologic disease caused by central nervous system vasculitis and granulomatous encephalitis may occur. The parasites are able to persist in their animal hosts despite an active immune response. Microsporidial infection activates antibody production, although antibodies alone do not appear to yield protection. The role of a competent cellular immune response in suppressing microsporidial multiplication is suggested by epidemiologic studies in humans, and has been established experimentally.[23] *In-vitro* studies demonstrated the importance of IFN-γ and tumor necrosis factor in resistance to *Encephalitozoon* spp.[23,24] The pathogenesis of human *Encephalitozoon* infection has yet to be defined. Rare histologic and clinical investigations in immunodeficient patients have indicated that *E. cuniculi* and *E. hellem* usually cause disseminated infection in this patient group.[11,12,18,22]

PREVENTION

Cryptosporidiosis

Cryptosporidial oocysts are remarkably resistant to many common disinfectants, including chlorine-based compounds.[25] Therefore, the water industry studied alternative methods to inactivate water-borne oocysts, including a water disinfection device delivering germicidal ultraviolet (UV) light which yielded promising results.[26] However, control of surface-water contamination and adequate filter systems are required to guarantee the complete removal of cryptosporidia from water supplies.

Cyclosporiasis, isosporiasis

Avoiding food or water that may be contaminated with feces may prevent cyclosporiasis and isosporiasis, but details of the sources of infection and the modes of transmission are unknown.

Microsporidiosis

Laboratory experiments indicate that the thick-walled spores may survive in the environment for months or years depending on the temperature and humidity. Exposure to recommended working concentrations of most disinfectants, boiling and autoclaving seem to kill *Encephalitozoon* spp. spores but no data are available for *Enterocytozoon* spp. Recent studies demonstrated successful disinfection of *Encephalitozoon* spp. in water using chlorine and ozone disinfection.[10,27]

DIAGNOSTIC MICROBIOLOGY

Stool examination by light microscopy is the most important test to diagnose intestinal coccidia and intestinal microsporidia. In many laboratories, tests for *Cryptosporidium* spp., *Isospora* spp., *Cyclospora* spp. and microsporidia must be specifically requested because the general request of 'stool for O & P' (ova and parasites) often does not mean that the specific methods to detect these organisms are applied. Microsporidial species that cause systemic infection are best detected in urine sediments.

Cryptosporidium species

Examination of stool specimens by light microscopy

To visualize the *Cryptosporidium* spp. oocysts in fecal smears, acid-fast staining (e.g. modified cold Kinyoun technique; Fig. 181.3) and the immunofluorescence (IF) technique are among the most sensitive, specific and widely used methods. The IF detection procedure is more sensitive than the acid-fast staining but the difference in sensitivity may not be of clinical relevance when watery stools of patients with HIV infection are examined. These patients usually excrete an amount of oocysts that can easily be detected using acid-fast staining. The oocysts should be measured in order to distinguish *Cryptosporidium* spp. (4–6 µm in diameter) from *Cyclospora* spp. (8–10 µm). Enhanced sensitivity of stool examination can be obtained by concentrating oocysts, preferably with the formalin-ethyl acetate (FEA) technique.

The exact sensitivity of the coprodiagnostic techniques is not known but some data raise questions about the widely held belief that these techniques are sufficient to meet the needs of clinicians and epidemiologists.[28] The minimum number of oocysts in human stool specimens that can be detected by the FEA stool concentration technique and the IF staining procedure was found to be unexpectedly high: 5000–10 000 oocysts per gram of stool in watery stool specimens and 10 000–50 000 oocysts per gram of stool in formed stool. Examination of multiple specimens may be necessary, because clinical studies have shown that examination of single stool specimens may have an insufficient diagnostic yield. Furthermore, in a prospective analysis of jejunal biopsies in patients who have AIDS, *Cryptosporidium* spp. were present in more than 10% of patients whose stool examinations were negative. These results, however, were in contrast to another study that only identified organisms on biopsy in one-third of patients shedding *Cryptosporidium* spp. in their feces.

Stool antigen detection techniques have been developed but the sensitivity of currently available tests is not better than microscopic techniques.[29] Molecular diagnostic methods have improved diagnostic yields but currently are not ready to use in routine laboratories.[30]

Cytologic diagnosis

Aspiration of duodenal fluid or small intestinal brushing can be used for diagnosis when upper endoscopy is performed. Examination of centrifuged duodenal aspirates under the microscope may be the most sensitive diagnostic procedure.

Histologic examinations

Cryptosporidium spp. appear basophilic by examination under the light microscope of small intestinal tissue sections stained with hematoxylin and eosin. The intracellular organisms seem to project into the intestinal lumen because of their apical extracytoplasmic localization. Under electron microscopy the unique ultrastructural features of different developmental stages of the parasite can be seen (see Fig. 181.1), but this is rarely necessary for diagnostic purposes.

Serology

Specific IgM or IgG antibodies to *Cryptosporidium* spp. can be detected within 2 weeks of onset of symptoms in most patients. In the majority of patients IgG titers may persist for long periods. Serologic testing is mainly used as an epidemiologic tool and has no diagnostic application, particularly because antibody persistence limits its use in the diagnosis of acute infection.

Cyclospora species

Diagnosis of *Cyclospora* spp. is dependent on detection by light microscopy of the refractile oocysts, 8–10 µm in diameter, in wet mounts of fresh stool specimens or in acid-fast stained smears prepared from stool concentrated by the FEA sedimentation.[17,31] Acid-fast stained oocysts vary in appearance from faint pink to deep red, and many organisms remain as unstained spheres (see Fig. 181.3). The sensitivity of the coprodiagnostic techniques is unknown. In many patients' specimens, however, the number of oocysts detected per slide is low, indicating that not all symptomatic patients excrete a large enough number of oocysts for laboratory detection to be assured. Examination of small intestinal tissue of patients who have *Cyclospora* infection

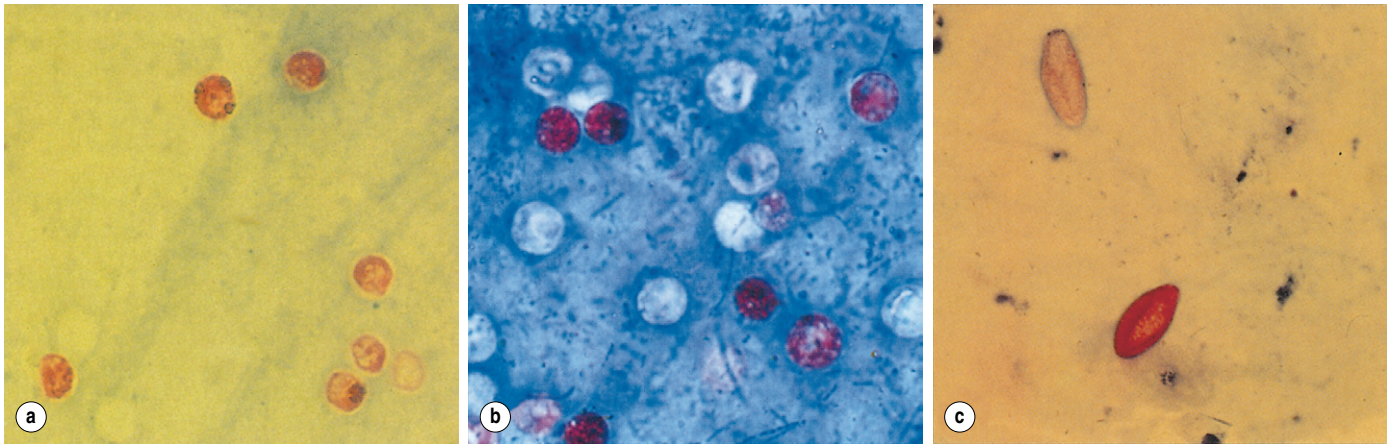


Fig. 181.3 Acid-fast stained smears of fecal specimens showing intestinal coccidia. (a) *Cryptosporidium* spp., round, 4–6 µm diameter. (b) *Cyclospora* spp., round, 8–10 µm diameter. Courtesy of EG Long. (c) *Isospora belli*, elliptical, 23–33 µm long and 10–19 µm wide. Modified Kinyoun stain.

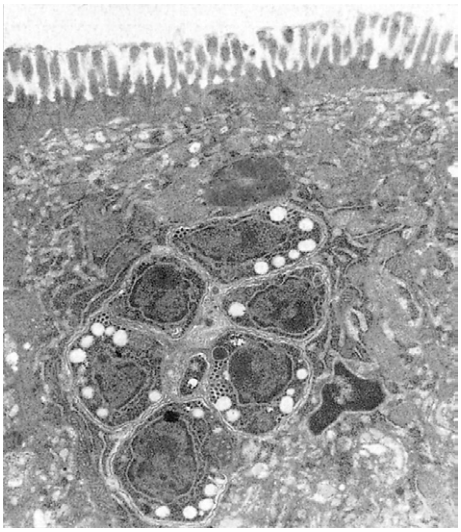


Fig. 181.4 Intestinal *Cyclospora* infection. Transmission electron micrograph of duodenal epithelium obtained from a patient with HIV infection who has *Cyclospora cayetanensis* infection. A mature schizont filled with numerous merozoites is shown. Courtesy of AM Deloul and FP Chatelet.

often did not reveal any parasites. The presence of intracellular parasites has rarely been documented in aspirated duodenal or jejunal fluid and on duodenal and jejunal biopsy (Fig. 181.4).

Isospora species

Diagnosis of *I. belli* is usually achieved by detection under the light microscope of the parasite oocysts in wet preparations or acid-fast stained smears of concentrated stool specimens (see Fig. 181.3). Repetitive stool examinations may be necessary because the parasite may be excreted intermittently or in low numbers. Histologic examination of small intestinal tissue sections may reveal the parasite within enterocytes.

Microsporidia

Diagnosis of microsporidial infection is dependent on morphologic demonstration of the organisms themselves. This can be difficult because of the organisms' small size and staining properties hamper visualization of the spores and developing stages using routine staining techniques. The spores, the stages by which microsporidia are usually identified, are small, ranging in size from 1 to 3.5 µm in diameter.

Evaluation of patients who have suspected microsporidiosis should begin with examination of body fluids by light microscopy using special staining techniques. Definitive species identification of microsporidia is made using electron microscopy, antigenic analysis, and molecular analysis. Collection of fresh material (without fixative) may be useful for cell culture and for future molecular analysis.[11]

Examination of stool specimens

In patients who have suspected enteric microsporidiosis, examination of stool specimens by light microscopy is the first step. It is at least as sensitive as examination of biopsy specimens. Detection of microsporidial spores requires adequate illumination and magnification [i.e. ×630 or ×1000 magnification (oil immersion)], and special staining methods. The two most commonly used stains are the chromotrope stain, which appears to be the most specific (Fig. 181.5),[32] and chemofluorescent agents, which might be more sensitive but may produce false-positive results.[33] An epidemiologic comparison of these techniques resulted in the recommendation to screen specimens with chemofluorescent agents and to confirm the results with the chromotrope stain.

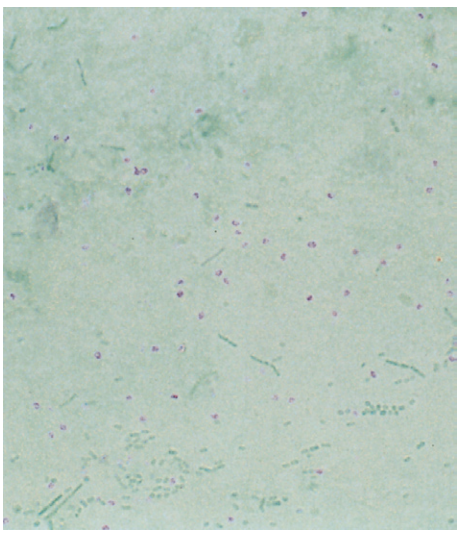


Fig. 181.5 Detection of microsporidia in stool samples. Smear of a formalin-fixed, unconcentrated stool specimen of a patient who has AIDS and chronic diarrhea, showing pinkish-red-stained spores of *Enterocytozoon bieneusi*. Chromotrope staining (oil immersion).

The differences in spore size between *Enterocytozoon* (1–1.5 µm in diameter) and *Encephalitozoon* spp. (2–3 µm in diameter) often permit a tentative diagnosis of the genus from examination of stool under the light microscope. The microsporidian should be identified to the level of genus by electron microscopy or molecular analysis because *Encephalitozoon* spp. have a propensity for dissemination, and have a different drug sensitivity pattern compared with *Enterocytozoon bieneusi*.

Immunofluorescent procedures for diagnosis of *Encephalitozoon*-like microsporidial spores are promising but not widely available. Diagnostic application of polyclonal antibodies in fecal samples has been hampered by background staining, cross-reactions with yeast and bacteria, and low sensitivity. Monoclonal antibodies against *Encephalitozoon* spp.[34] and against spores of *Enterocytozoon bieneusi* have been generated.[35]

Histologic examination

Among patients with HIV infection who suffered from chronic diarrhea, stool examinations proved as sensitive as endoscopic evaluation for all pathogens except cytomegalovirus and *Leishmania*.[14] Examination of duodenal and terminal ileal tissue has resulted in detection of microsporidia but the parasites are rarely found in colonic tissue sections.

Only highly experienced pathologists have reliably and consistently identified microsporidia in tissue sections using routine techniques such as hematoxylin and eosin stain. Ultra-thin plastic sections stained with methylene blue-azure II–basic fuchsin or with toluidine blue may facilitate detection but these techniques are not routinely used. In our experience, tissue Gram stains such as Brown–Brenn or Brown–Hopps have proved to be the most useful for the rapid and reliable identification of HIV-associated microsporidia in routine paraffin-embedded tissue sections (Fig. 181.6). Others prefer a silver stain (Warthin–Starry stain) or the chromotrope-based staining technique.

Cytologic diagnosis

Microsporidial spores have been detected in sediments of duodenal aspirate, bile or biliary aspirates, urine (Fig. 181.7), bronchoalveolar lavage fluid, cerebrospinal fluid (CSF) and in smears of conjunctival swabs, sputum and nasal discharge.[11] Microscopic examination of centrifuged duodenal aspirate obtained during endoscopy appears to be the most sensitive technique for diagnosis of intestinal microsporidiosis. Because microsporidial infection often involves multiple organs, detection of microsporidia in virtually any tissue or bodily fluid should prompt a thorough search of other sites. In particular, urine specimens of patients suspected of having disseminated microsporidiosis should be examined.

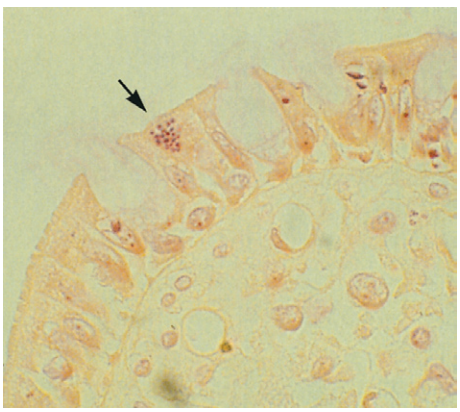


Fig. 181.6 Intestinal *Enterocytozoon bieneusi* infection. Terminal ileal tissue obtained by ileocolonoscopy in a patient who has AIDS and chronic diarrhea caused by *Enterocytozoon bieneusi* infection. Gram-positive or Gram-labile microsporidial spores (arrow) are found in supranuclear location within small intestinal enterocytes. Brown–Brenn stain.

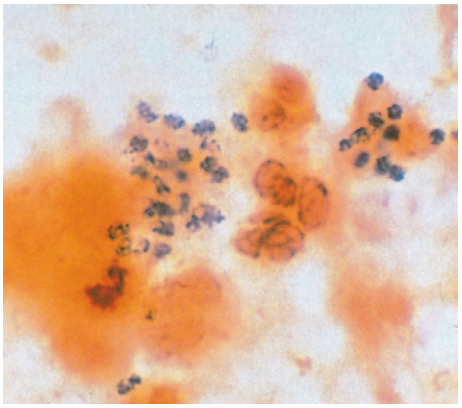


Fig. 181.7 Detection of microsporidia in urine sediment. Urine sediment from a patient who has AIDS and disseminated *Encephalitozoon hellem* infection, showing Gram-labile intracellular and extracellular microsporidial spores. Gram stain (oil immersion).



Fig. 181.8 *Encephalitozoon intestinalis* (formerly *Septata intestinalis*): developing spores within enterocytes separated by a fibrillar matrix. *Encephalitozoon intestinalis* develop within parasitophorous vacuoles, unlike *E. bieneusi*, which develop in direct contact with enterocyte cytoplasm. Courtesy of MA Spycher.

Electron microscopy

Microsporidial ultrastructure is unique and pathognomonic for the phylum and, with rare exceptions, ultrastructural features can distinguish between most genera of microsporidia (Fig. 181.8; see Fig. 181.2).[36,37]

Serology

Serologic assays (including carbon immunoassay, indirect IF test, enzyme-linked immunosorbent assay and Western blot immunodetection) have been useful in detecting antibodies to *E. cuniculi* in several species of animals, but reliable serologic tests for diagnosis of human microsporidiosis are lacking. This lack of availability is partly because *Enterocytozoon* spp. have not been continuously propagated in cell culture or laboratory animals.

Cell culture

Encephalitozoon spp., *Trachipleistophora* spp., *Vittaforma corneae* and *Anncaliia algerae* have been isolated using cell culture systems, but these tests are fastidious and costly, and the most common human species, *E. bieneusi*, has not been continuously propagated.

Molecular techniques

Universal panmicrosporidian and species-specific primer pairs that amplify the short regions of the small subunit rRNA gene have been developed,[22,38] but, at present, these techniques are confined to research laboratories. Recently, simultaneous detection of four human pathogenic microsporidia from patients' specimens by oligonucleotide microarray has been described.[39] Diagnosis and identification of *Enterocytozoon bieneusi* and the different *Encephalitozoon* spp. have been successfully performed with fresh stool specimens, formalin-fixed stool specimens, intestinal tissue obtained by endoscopic biopsy, urine specimens and other body fluids.[10] *In-situ* hybridization to visualize *E. bieneusi* in tissue sections has been developed.

CLINICAL MANIFESTATIONS

Cryptosporidiosis

For cryptosporidiosis the mean incubation period between infection and onset of symptoms is approximately 7–14 days (range 5–28 days). The severity and duration of illness vary depending on the immune status of the hosts. Children, the elderly and individuals with nutritional deficiencies may suffer from a more severe and prolonged disease, as is observed in immunodeficient patients. Whether cryptosporidiosis in immunocompromised patients is mainly a reactivation of latent infection or whether clinical illness is caused by recently acquired infection is not known.

Immunocompetent patients

In immunocompetent patients, cryptosporidia cause a self-limiting, usually watery, diarrhea lasting 10–14 days (range 2–28 days), but the clinical presentation varies from asymptomatic shedding of oocysts to severe disease that may last up to 3 months.[15,40] Patients often complain of abdominal pain, flatulence, loss of appetite, nausea and vomiting, and may suffer from low-grade fever, anorexia, malaise, weakness, fatigue, myalgias and headaches. The diarrhea and abdominal pain are often made worse by eating. Cough appears significantly more frequent in children with cryptosporidiosis than in children with diarrhea of another etiology, but the parasite has rarely been documented in the airways of immunocompetent individuals. Single case reports of pancreatitis associated with cryptosporidiosis and reactive arthritis have been described. Poorly understood lasting adverse effects of infection in children may include deficits of linear growth and cognitive sequelae, even if cryptosporidial infection is otherwise symptomless.[41,42]

Immunocompromised patients

In immunocompromised patients, particularly individuals with HIV infection, cryptosporidiosis is a more severe, often chronic and incurable illness that can be life-threatening. The main clinical presentation is watery diarrhea that can lead to severe dehydration, electrolyte depletion, malnutrition and weight loss. In addition, infection of the biliary tract and the gallbladder, resulting in acalculous cholecystitis, sclerosing cholangitis ('AIDS cholangiopathy') and stenosis of the papilla of Vater, frequently occurs. Infection of the epithelial cells of the respiratory tract, including sinuses, is increasingly described, but it is not clear whether this finding is of clinical relevance because most of these patients have a concomitant respiratory infection. Systemic cryptosporidiosis has not been described.

The clinical course of HIV-associated cryptosporidiosis is highly variable. Four clinical patterns of disease have been identified:

- asymptomatic shedding of oocysts;
- transient diarrhea with transient or chronic shedding of oocysts;
- chronic diarrhea; and
- fulminant disease that leads to cachexia and death within months.

Most patients who have severe illness have $CD4^+$ lymphocyte counts below 50 cells/μl. Spontaneous clinical recovery may occur and is mainly correlated with higher $CD4^+$ lymphocyte counts, but a benign course of the diarrhea may also occur in severely immunodeficient patients. Clinical observations suggest that cryptosporidial disease is more severe if co-infections caused by other enteropathogens are present. Concurrent dual or multiple intestinal infection is found in up to 50% of patients who have HIV-associated cryptosporidiosis.

Cyclosporiasis

The spectrum of *Cyclospora*-associated illness is not yet fully defined but it may range from asymptomatic carriage of the organism to severe and prolonged diarrhea in immunocompetent and immunocompromised patients. Patients who have AIDS tend to have a more prolonged and severe illness, and *Cyclospora*-associated cholangiopathy has been described in this group.[16,17]

The incubation period between infection and onset of symptoms ranges between 2 and 11 days and is usually about 7 days. In symptomatic infections the main clinical manifestation is mild to severe watery diarrhea, accompanied by abdominal cramps, bloating, increased flatus, nausea, anorexia, substantial weight loss and fatigue. Vomiting is less common and about 25% of the patients report fever and myalgias.

If not treated, diarrhea is self-limiting but may last for several weeks (range 2–107 days), and remissions and relapses may occur during gradual resolution of the illness. *Cyclospora* infection does not appear to provide lasting immunity.

Isosporiasis

In immunocompetent persons, *I. belli* infection causes self-limited watery diarrhea accompanied by malaise, anorexia, cramping abdominal pain, weight loss and, less frequently, low-grade fever. In immunocompromised patients, the illness is more severe and prolonged, or chronic if untreated. Also, acalculous cholecystitis and two single cases of disseminated extraintestinal isosporiasis in patients with AIDS have been reported.

Microsporidiosis in immunocompromised patients

The spectrum of clinically manifest microsporidial infection is diverse and includes intestinal, ocular, muscular, cerebral, respiratory and urinary tract disease (Table 181.1).[10,11] The most prevalent microsporidial disease is HIV-associated chronic diarrhea.[14] Disseminated microsporidial infections are being increasingly recognized in patients who have AIDS.

Diarrhea, cholangitis, acalculous cholecystitis

Two microsporidial species – *Enterocytozoon bieneusi* and *Encephalitozoon intestinalis* – cause chronic diarrhea and wasting, cholangiopathy and acalculous cholecystitis in patients who have HIV infection or who are otherwise immunodeficient, particularly when $CD4^+$ lymphocyte counts drop below 50–100/μl.

Enterocytozoon bieneusi is estimated to be one of the most important HIV-associated intestinal pathogens, present in 5–30% of those with otherwise unexplained diarrhea. The main symptoms are chronic nonbloody diarrhea, anorexia, weight loss and bloating. Some patients experience intermittent diarrhea and a few excrete microsporidial spores without having diarrhea. The stool is watery or soft, and diarrhea seems to be worsened by most foods. Some of the patients report abdominal pain or nausea and vomiting. Laboratory evidence for intestinal malabsorption is common. *E. bieneusi* itself is not immediately life-threatening, but diarrhea is debilitating, and weight loss may lead to cachexia, which is a significant cause or cofactor in the deaths of many patients. Up to one-third of patients who have intestinal microsporidiosis have

Table 181.1 Microsporidial species pathogenic in humans, and clinical manifestations

Microsporidial species	Clinical manifestations
Enterocytozoon bieneusi	Diarrhea, wasting syndrome Cholangiopathy, cholangitis, acalculous cholecystitis Sinusitis, bronchitis, pneumonitis
Encephalitozoon intestinalis (formerly *Septata intestinalis*)	Diarrhea Cholangiopathy, cholangitis, acalculous cholecystitis Sinusitis, bronchitis, pneumonitis Urinary tract infection, nephritis Bone lesions; nodular cutaneous lesions
Encephalitozoon cuniculi	Systemic infection Keratoconjunctivitis Sinusitis, pneumonitis Urinary tract infection, nephritis Hepatitis Peritonitis Symptomatic and asymptomatic intestinal infections Seizures, encephalitis
Encephalitozoon hellem	Systemic infection Keratoconjunctivitis Sinusitis, bronchitis, pneumonia Nephritis, ureteritis, cystitis, prostatitis, urethritis
Anncaliia connori (formerly *Brachiola connori* and *Nosema connori*)	Systemic infection
Trachipleistophora anthropophthera	Systemic infection including brain, heart, kidney, eye
Pleistophora ronneafiei	Myositis
Trachipleistophora hominis	Myositis, keratoconjunctivitis, sinusitis
Anncaliia vesicularum (formerly *Brachiola vesicularum*)	Myositis
Anncaliia algerae (formerly *Brachiola algerae* and *Nosema algerae*)	Keratitis, myositis
Vittaforma corneae (formerly *Nosema corneum*)	Keratitis Urinary tract infection
Nosema ocularum	Keratitis
Microsporidium ceylonensis	Corneal ulcer
Microsporidium africanum	Corneal ulcer

dual or multiple co-infection with other intestinal pathogens. The parasite has also been detected in the biliary tree and/or gallbladder of patients who have cholangitis and acalculous cholecystitis. Imaging procedures often reveal dilatation of both intrahepatic and common bile ducts, irregularities of the bile duct wall and gallbladder abnormalities such as wall thickening, distention or the presence of sludge.[11,14]

Encephalitozoon intestinalis primarily causes diarrhea, and the parasite may also spread into the biliary tract and gallbladder, causing cholangitis and cholecystitis. In contrast to *Enterocytozoon bieneusi*, systemic dissemination to kidneys and other sites may occur.[43]

Systemic microsporidiosis

There are three microsporidial genera, *Encephalitozoon* spp., *Trachipleistophora* spp. and *Vittaforma corneae*, which have been found to disseminate in severely immunodeficient patients who have HIV infection.[22,44–46] Disseminated infection caused by *Anncaliia* (formerly *Brachiola connori* or *Nosema connori*) was found at autopsy in a 4-month-old athymic boy reported in 1973.

Encephalitozoon spp. were initially identified in patients who had AIDS and keratoconjunctivitis.[47] Subsequently the spectrum of recognized *Encephalitozoon*-associated disease has expanded to include keratoconjunctivitis, bronchiolitis, sinusitis, pneumonitis, nephritis, ureteritis, cystitis, prostatitis, hepatitis, peritonitis, diarrhea and encephalitis.[18,22] Clinical manifestations may vary substantially, ranging from an asymptomatic carrier state to organ failure.

Trachipleistophora anthropophthera was identified at autopsy in cerebral, cardiac, renal, pancreatic, thyroid, hepatic, splenic, lymphoid and bone marrow tissue of two patients who had AIDS and initially presented with seizures.[45]

Urinary tract infection

Predominant genitourinary signs and symptoms caused by *Encephalitozoon* infections have been observed. Clinical manifestations included asymptomatic microhematuria, urethritis, prostatitis, acute cystitis and interstitial nephritis associated with dysuria, gross hematuria and progressive renal insufficiency.[18]

Respiratory tract infection

In most patients who have respiratory tract microsporidial infection, including bronchiolitis, pneumonia and progressive respiratory failure, intestinal or systemic microsporidiosis was also present. Single cases of patients have been reported in whom sinusitis causing nasal obstruction and persistent mucopurulent nasal discharge caused by *E. bieneusi* or *Encephalitozoon* spp. was a predominant manifestation of systemic microsporidiosis.

Keratoconjunctivitis

HIV-associated ocular microsporidiosis caused by *Encephalitozoon* spp. is restricted to the superficial epithelium of the cornea and conjunctiva. Most patients exhibit bilateral coarse punctate epithelial keratopathy (Fig. 181.9), conjunctival inflammation resulting in redness and foreign body sensation, decreased visual acuity and photophobia. In patients who initially present with symptomatic keratoconjunctival microsporidiosis, dissemination of the parasite may be common, but clinical manifestations other than keratoconjunctivitis may be mild or absent.[48]

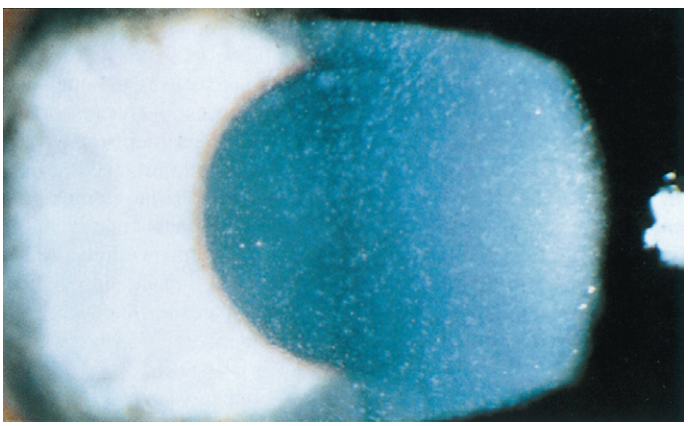


Fig. 181.9 Keratopathy caused by *Encephalitozoon hellem*. Slit-lamp demonstration of punctate epithelial keratopathy in a patient who has AIDS and keratoconjunctivitis caused by *Encephalitozoon hellem*. Ocular microsporidiosis can often be diagnosed by examination under the light microscope of a smear obtained by a nontraumatic conjunctival swab. Courtesy of M Diesenhouse and DA Schwartz.

Cerebral microsporidiosis

Microsporidia was first accepted as the etiologic agent of a neurologic disorder in two children, reported in 1959 and 1984. Both presented with seizures and might have had impaired immune responses. *Encephalitozoon cuniculi* and *Trachipleistophora anthropophthera* were detected in CSF or brain tissue of patients with HIV infection who presented with fever and somnolence or seizures and mental decline. MRI disclosed multiple small, contrast-enhancing, mostly ring-like lesions localized to the hippocampus, mesencephalon and cerebral cortex (Fig. 181.10).[45,49]

Myositis

Myositis caused by *Pleistophora* spp. has been described in an HIV-seronegative patient and in a patient with HIV infection, both of whom had severe cellular immunodeficiency. Newly characterized microsporidia, *Trachipleistophora hominis* and *Anncaliia* (formerly *Brachiola vesicularum*), were detected in muscle biopsies of patients who had AIDS and presented with myositis.[44,50]

Microsporidiosis in immunocompetent persons

Enterocytozoon bieneusi and *Encephalitozoon* spp. are associated with self-limiting watery diarrhea in immunocompetent adults as well as in children, particularly among persons who reside or have traveled in tropical countries.[51] An unexpectedly high prevalence (17%) of intestinal microsporidiosis due to *Enterocytozoon bieneusi* was found in HIV-seronegative elderly patients in Spain.[52]

Deep stromal infections of the cornea caused by different microsporidial species have been described in otherwise seemingly healthy persons who presented with severe keratitis or a corneal ulcer or who were contact lens wearers (see Table 181.1).

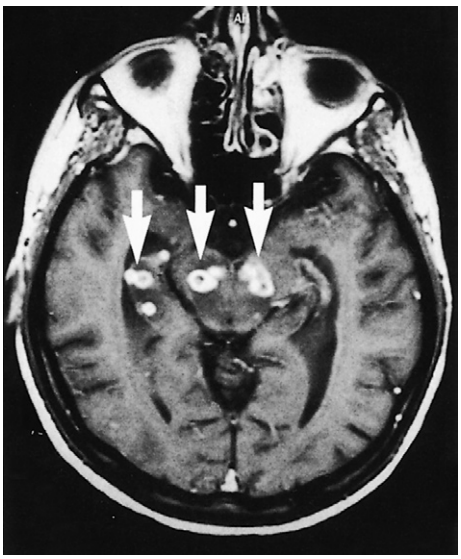


Fig. 181.10 Cerebral microsporidiosis caused by *Encephalitozoon cuniculi* in a patient with HIV infection. The MRI shows multiple small contrast-enhancing, mostly ring-like, partly micronodular, lesions in hippocampal, mesencephal and intracortical regions (arrows), partly accompanied by slight edema, and congestion of the right ethmoid sinus. *Encephalitozoon cuniculi* was isolated from CSF. From Weber *et al.*,[49] with permission from Massachusetts Medical Society.

MANAGEMENT

Cryptosporidiosis

The efficacy of antiparasitic therapy of cryptosporidiosis remains controversial.[53] Among immunocompetent patients, diarrhea due to cryptosporidial infection is usually self-limiting but chronic infection may cause malnutrition in children in developing countries. Nitazoxanide may be useful in immunocompetent individuals with cryptosporidiosis. In a randomized, placebo-controlled trial in apparently immunocompetent children and adults in Egypt, nitazoxanide (500 mg q12h for 3 days in adults, 100–200 mg q12h in children) decreased the duration of diarrhea and cryptosporidial oocyst shedding.[54]

In contrast, in patients who have AIDS no agent has proven effective in the absence of treatment of HIV infection.[53] A randomized trial in HIV-infected persons with cryptosporidiosis in Mexico suggested a shorter duration of diarrhea and oocyst clearance with nitazoxanide treatment among patients with $CD4^+$ lymphocyte counts above 50/μl but no effect was found among patients with $CD4^+$ cell counts below 50/μl.[55] Because the latter group of patients is mainly in need of effective antiparasitic therapy, and because diarrhea in HIV-infected persons with $CD4^+$ cell counts above 50/μl frequently improves due to improved immune function, the usefulness of nitazoxanide in patients with AIDS is doubtful. Case series and a randomized, controlled trial suggested that treatment with oral paromomycin (500 mg q6h for at least 4 weeks) may result in decreased oocyst shedding and improved intestinal function and morphology,[56] but other investigators have not found any clinical benefit.[57] *In-vitro* studies have indicated that nitazoxanide activity may be enhanced by the co-administration of azithromycin or rifabutin,[58] but clinical experience of such combinations is lacking. Hyperimmune bovine colostrum has been found in *in-vitro* experiments in animal studies and in single patients to reduce or eradicate cryptosporidia, but these results could not be reproduced in larger human studies.

Symptomatic treatment of diarrhea may include drugs that affect gut motility such as loperamide, diphenoxylate, opiates, somatostatin and octreotide. Immune reconstitution following initiation of potent antiretroviral therapy of patients who have HIV infection results in cessation of oocyst shedding and diarrhea, but cryptosporidial infection is controlled rather than cured because failure of antiretroviral therapy often results in relapse of cryptosporidiosis.[59]

Cyclosporiasis

A placebo-controlled trial showed that trimethoprim–sulfamethoxazole (TMP–SMX [co-trimoxazole]; double-strength TMP 160 mg/SMX 800 mg q12h for 7 days) was clinically successful and shortened oocyst shedding in immunocompetent patients who have cyclosporiasis.[60] In this study, 3 days of treatment with TMP–SMX was not sufficient to eradicate *Cyclospora*. Trimethoprim 160 mg/SMX 800 mg q6h for 10 days cured HIV-associated cyclosporiasis but the relapse rate was high (43%). Maintenance therapy with double-strength TMP–SMX three times per week did prevent relapses in these patients. For patients who cannot tolerate sulfonamides, a 1-week course of ciprofloxacin (500 mg q12h) may be an alternative although it was not as effective as TMP–SMX in a randomized trial.[61]

Isosporiasis

Immunocompetent and immunocompromised patients respond promptly to therapy with TMP–SMX (double-strength TMP 160 mg/SMX 800 mg q6h for 10 days).[62] In patients with sulfonamide allergies, pyrimethamine 75 mg/day plus folinic acid 10 mg/day may be successful. To prevent the high rate of relapses in patients who have AIDS, maintenance therapy with double-strength TMP–SMX, three times per week, or pyrimethamine 25 mg/day plus folinic acid 5 mg/day, is recommended. In case of sulfonamide intolerance, a 1-week

course of ciprofloxacin (500 mg q12h) may be an alternative although it was not as effective as TMP–SMX in a randomized trial among HIV-infected Haitians.[61]

Microsporidiosis

Albendazole, fumagillin, its analog TNP-470, and nikkomycin Z have been found to inhibit completely or partially the replication or spore germination of *Encephalitozoon* spp. and *Vittaforma corneae* propagated in cell cultures, but did not destroy mature microsporidial spores, so that these may sustain infection.[63–65] Numerous other antiprotozoal drugs and antibiotics have been tested *in vitro*, with negative findings. *In-vitro* systems to investigate *Enterocytozoon bieneusi* are not available.

Among HIV-infected persons with access to antiretroviral therapy and immune reconstitution, the occurrence of microsporidiosis has been substantially reduced.[59] Little information on clinical experience in the therapy of human microsporidiosis is available, and only two controlled treatment trials have been conducted,[64,65] confirming previous case observations which indicated that albendazole can result in clinical cure of HIV-associated encephalitozoonosis in parallel with the cessation of spore excretion. In contrast, albendazole is not effective for the treatment of *Enterocytozoon bieneusi* infection and does not reduce the parasite load although previous observations had suggested that clinical improvement may occur in some patients. Oral purified fumagillin was used in a pilot study and subsequently in a small randomized trial to treat HIV-associated diarrhea due to *E. bieneusi*. Fumagillin appeared to eradicate the parasite transiently in many patients but serious adverse events and parasitic relapse were observed.[65,66]

REFERENCES



References for this chapter can be found online at http://www.expertconsult.com

Chapter 182

Govinda S Visvesvara
Augusto Julio Martinez
(deceased)

Protozoa: free-living amebae

NATURE

The concept that small free-living amebae, particularly *Acanthamoeba* spp. have the potential to cause disease in humans was developed by CG Culbertson of the Indiana University School of Medicine and the Eli Lilly Laboratories in 1958. The basis of this observation was a chance discovery of an ameba growing in a batch of monkey kidney cell cultures that were to be used for growing polio virus for the development of polio vaccine. This ameba, described as *Acanthamoeba* sp. (Lilly A-1), was found to produce, on intracerebral, intraspinal and intranasal inoculations, meningoencephalitis in cortisone-treated monkeys and mice.[1–3] This isolate is now called *Acanthamoeba culbertsoni*.

The first case of amebic meningoencephalitis in humans, attributed initially to *Acanthamoeba* although later the causative organism was identified as a species of *Naegleria*, was described in Australia in 1962. It is now known that besides *Acanthamoeba* spp., two other free-living amebae, *Naegleria fowleri* and *Balamuthia mandrillaris*, also cause central nervous system (CNS) disease in humans and other animals.[1–4] Recently, however, *Sappinia diploidea*, now reidentified as *Sappinia pedata*, a saprophytic ameba that has been previously isolated from the fecal specimens of lizards, elks and bisons, was identified in a brain biopsy specimen of a previously healthy 38-year-old man who developed visual disturbances, headache and a seizure.[5-6] This suggests that there are cases of human infections caused by free-living amebae other than *Acanthamoeba*, *Balamuthia* and *Naegleria* spp. that may have been either misdiagnosed or unrecognized.

Taxonomy

The free-living amebae, according to the classic taxonomic system, were classified under the subphylum Sarcodina and superclass Rhizopodea.[3] The International Society of Protistologists abandoned the older hierarchical system and recently replaced it with a new classification system emphasizing modern morphologic approaches, biochemical pathways and molecular phylogenetics. According to this new schema the Eukaryotes have been classified into six clusters or 'Super Groups', namely Amoebozoa, Opisthokonta, Rhizaria, Archaeplastida, Chromalveolata and Excavata. *Acanthamoeba* and *Balamuthia* are included under Super Group Amoebozoa (Acanthamoebidae), along with *Sappinia* (Flabellinea: Thecamoebidae), and *Naegleria fowleri* under Super Group Excavata (Heterolobosia: Vahlkampfiidae).[7]

The free-living amebae (*Acanthamoeba*, *Naegleria*, *Balamuthia*, *Hartmannella* and *Vahlkampfia* spp.) and the parasitic amebae (e.g. *Entamoeba histolytica*) move by producing cytoplasmic bulges, the lobopodia, from the surface of the body. In contrast to *E. histolytica*, which is a mitochondria-lacking ameba that causes gastrointestinal disease, *Naegleria*, *Acanthamoeba* and *Balamuthia* are mitochondria-bearing amebae that cause diseases of the CNS of humans and animals, which almost always lead to death. The term amphizoic amebae indicates the ability of these amebae to exist as free-living in nature and as parasites within host tissue; this differentiates them from the truly parasitic *E. histolytica*.[8]

Although several species of *Naegleria* have been described, so far only one species, *N. fowleri* (*Naegleria aerobia* and *Naegleria invadens* are non-valid synonyms), is known to infect the human CNS. Several of the more than 20 species of *Acanthamoeba* that have been described so far cause not only a chronic granulomatous CNS disease in humans and other animals, but also infect the cornea (*Acanthamoeba* keratitis), the skin, the nasal sinuses and pulmonary tissues. The disease caused by *Acanthamoeba* spp. has been described as granulomatous amebic encephalitis (GAE). *Balamuthia mandrillaris*, the only known species of *Balamuthia*, causes GAE and skin infections in humans and other animals.[1–3]

Naegleria fowleri

Naegleria fowleri is also described as an ameboflagellate because it has a transient flagellate stage in its life cycle in addition to a feeding and dividing form, the trophozoite, and a resistant cyst stage (Fig. 182.1). The trophozoite, measuring 10–25 µm, normally feeds on bacteria and multiplies by binary fission. However, it is able to differentiate into a pear-shaped biflagellate stage in response to sudden changes in the ionic concentration of its environment. When the conditions become unfavorable the trophozoite differentiates into a resistant cyst stage. The trophozoite is usually uninucleate; the nucleus is spherical and contains a large, centrally placed, dense nucleolus. Additionally, numerous dumbbell-shaped mitochondria, vacuoles, lysosomes and ribosomes are present within the cytoplasm. Cysts are round and contain a single nucleus with a central dense nucleolus; the dense cyst walls are plugged with one or more flat pores. The cysts are 7–14 µm in diameter, with a mean of 10 µm.[3,8]

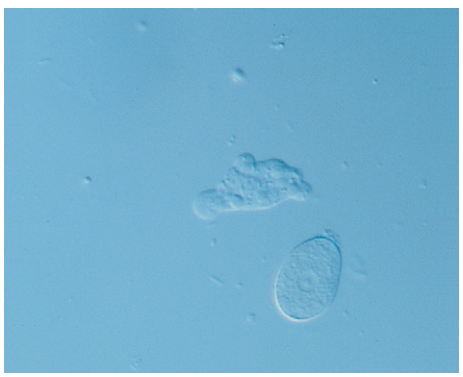


Fig. 182.1 *Naegleria fowleri*. The trophozoite can be differentiated from the cyst by its characteristic lobopodial locomotion; both are taken from culture. Differential interference contrast.

Acanthamoeba spp.

In 1930 Aldo Castellani isolated an ameba from a yeast culture; it was later named as *Acanthamoeba castellanii*.[1–3] Currently, more than 24 species have been identifed in the genus *Acanthamoeba* based on morphologic criteria such as size of the trophozoites and cysts as well as differences in the cyst wall configuration. The various species are included in three different groups:

- Group I comprises those species that have large trophozoites with cysts that range in size from 16 to 30 µm (e.g. *A. astronyxis*, *A. comandoni*, *A. tubiashi*);
- Group II includes by far the largest number of species with cysts measuring around 18 µm or less (e.g. *A. castellanii*, *A. polyphaga*, *A. rhysodes*, *A. hatchetti*); and
- Group III consists of species with subtle differences in cyst morphology, also measuring 18 µm or less (e.g. *A. culbertsoni*, *A. royreba*, *A. lenticulata*).

Currently, identification of species based on morphologic criteria such as size are considered unreliable and hence sequencing of the 18S rDNA is being used not only to differentiate isolates but also to understand the phylogeny of *Acanthamoeba*. Based on sequence differences, 15 genotypes (T1–T15) of *Acanthamoeba* have been established.[1–4] The acanthamebae are also called opportunistic amebae because they produce disease principally in immunodeficient individuals.

The life cycle of *Acanthamoeba* spp. consists of two stages: a feeding and reproducing trophozoite stage and a resistant cyst stage (Fig. 182.2). The trophozoites feed on bacteria and detritus present in the milieu and multiply by binary fission. They are uninucleate and are 15–45 µm in size. The nucleus has a centrally placed, large, densely staining nucleolus. The cytoplasm is finely granular and contains numerous mitochondria, ribosomes and lysosomes. Cysts are 10–25 µm in size and are double walled: the outer wall (the ectocyst) is wrinkled and contains protein; the inner wall (the endocyst) is usually stellate, polygonal, oval or spherical and contains cellulose. Pores covered by opercula are present at the point of contact between the ectocyst and the endocyst. Cysts are uninucleate.

Balamuthia mandrillaris

Balamuthia mandrillaris, previously called leptomyxid ameba, is the only species included under the genus *Balamuthia*. Based on molecular analysis, all isolates studied so far appear to be homogeneous and belong to one genotype. *Balamuthia mandrillaris* has two stages in its life cycle (Fig. 182.3). The trophozoite is irregular in shape and measures from 12 to 60 µm with a mean size of about 30 µm. Although usually uninucleate, binucleate forms are occasionally seen. The nucleus contains a large centrally placed nucleolus. Occasionally, in infected human tissues, trophic stages containing a large nucleus with two or three nucleolar bodies have been observed. Cysts are also uninucleate, more or less spherical, and range in size from 12 to 30 µm with a mean of 15 µm. Cysts appear to be double walled with a wavy ectocyst and a spherical endocyst when viewed under the light microscope. However, ultrastructurally the cysts are tripartite with an outer thin and irregular ectocyst, an inner thick endocyst and a middle amorphous fibrillar mesocyst.[1,2]

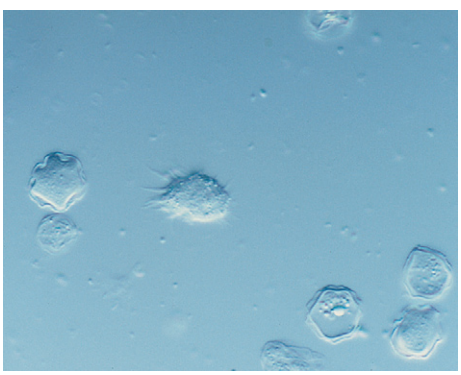


Fig. 182.2 *Acanthamoeba castellanii*. The trophozoite has spiny acanthopodia whereas the cyst has an outer wrinkled ectocyst and a stellate endocyst; both are taken from culture. Differential interference contrast.

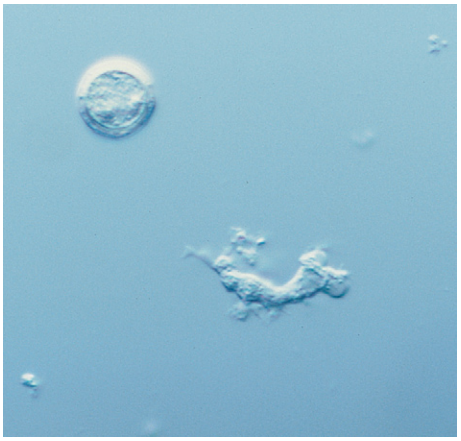


Fig. 182.3 *Balamuthia mandrillaris*. The trophozoite is irregularly shaped whereas the cyst is spherical; both are taken from culture. Differential interference contrast.

Cultivation

Acanthamoeba spp. and *N. fowleri*, but not *B. mandrillaris*, can be cultivated on non-nutrient agar plates coated with suitable Gram-negative bacteria such as *Escherichia coli* or *Enterobacter aerogenes*. The amebae will feed on the bacteria, multiply and differentiate into cysts within a few days. They can be easily subcultured by transplanting a small piece of agar containing trophozoites and/or cysts onto a fresh agar plate coated with bacteria as before. *Naegleria fowleri* and *Acanthamoeba* spp. can also be grown successfully on mammalian cell cultures. *Balamuthia mandrillaris* will not grow on bacteria-coated agar plates. However, it can be isolated from infected human or animal tissue by inoculating monkey kidney or human lung fibroblasts with the triturated tissue and from which a continuous culture can be established by periodic transfers.

Naegleria fowleri and *Acanthamoeba* spp. can be grown axenically (bacteria free) in a complex chemical medium. Although several different formulations are available, in our laboratory we use a modified version of Nelson's medium that contains a 0.5% solution of liver digest, 0.5% glucose and a low osmolarity buffered salt solution supplemented with 3–5% fetal bovine serum. *Acanthamoeba* spp. can also be easily grown in a medium composed of 2% proteose peptone, 0.5% yeast extract and 0.1% glucose made up in a low osmotic buffered salt solution with or without serum. Additionally, *N. fowleri* and several species of *Acanthamoeba* have also been grown in a chemically defined medium consisting of several different amino acids, vitamins, hemin and salts. *Balamuthia mandrillaris* has also been grown in an axenic medium, thus facilitating the screening of various pharmaceutical agents.[9]

EPIDEMIOLOGY

Naegleria fowleri is widely distributed throughout the world and has been isolated from fresh water, thermal discharges of power plants, heated swimming pools, hot springs, aquariums, soil, dust in air, sewage and even from nasal passages of children. *Naegleria fowleri* is thermophilic and grows well, even at temperatures of up to 113°F (45 °C). It is therefore not surprising that primary amebic meningoencephalitis (PAM) cases have occurred in the hot summer months when many people engage in aquatic activities in lakes, ponds and swimming pools that may harbor these amebae in large numbers.

In colder climates these amebae will probably encyst and remain dormant in the sediments of fresh water lakes, ponds, rivers and swimming pools.[1–3,10]

Acanthamoeba spp. have been isolated from soil, sewage, fresh, brackish and sea water, bottled mineral water, cooling towers of electric and nuclear power plants, physiotherapy pools, jacuzzis, heating and ventilating and air conditioning units, eye wash stations, dialysis machines, dust in the air, bacterial, fungal and mammalian cell cultures, contact lens paraphernalia, the nose and throat of patients who have respiratory complaints and healthy individuals, and biopsy or autopsy specimens of cornea, lungs, nasal sinuses, skin and CNS tissue of humans and animals. Acanthamoebae have generated considerable interest in recent years because they act, under laboratory conditions, as hosts for pathogenic bacteria such as *Legionella* spp, *Mycobacterium avium*, *Listeria monocytogenes*, *Burkholderia pseudomallei*, *Vibrio cholerae*, *Escherichia coli* serotype O157, *Francisella tularensis*, *Helicobacter pylori* and *Afipia felis*. Recently, *Mycobacterium massiliense* has been isolated from human clinical specimens such as sputum, liver, lung abscesses and even human feces. Additionally, a virus (mimivirus) about the size of a small bacterium with a 1.2 megabase genome has been discovered in *A. polyphaga*. *Acanthamoeba* is therefore of great public health importance.[1–3]

Cases of GAE may occur at any time of the year and therefore transmission has no relation to climatologic changes.

Balamuthia mandrillaris has only recently been isolated from soil and it may probably occur in water also. It is, therefore, believed to have the same habitat as *Acanthamoeba* spp. and *N. fowleri*. *Balamuthia mandrillaris* has also been isolated from biopsy and autopsy specimens of humans and other animals.[1,3]

PATHOGENICITY

Naegleria fowleri

Primary amebic meningoencephalitis occurs in active healthy children and young adults with no known history of immune disorder. Most patients who have PAM have a history of swimming, diving or playing under water; the ameba enters through the nasal passages and the nasal mucosa is the initial site of the primary lesions. The route of invasion into the brain is through the fila olfactoria of the olfactory nerves. The trophozoites cross the cribriform plate and reach the subarachnoid space, which is richly vascularized and bathed in the cerebrospinal fluid (CSF), thus constituting an ideal medium for their proliferation and subsequent dissemination into the brain parenchyma and other areas of the CNS.[1–3]

Naegleria fowleri is a highly pathogenic and virulent ameba. However, the minimum number of amebae required to cause infection and death in humans is not known. Experiments with infection have shown that mice, when just a few amebae are instilled intranasally, die of PAM in a similar fashion to humans. However, different isolates of *N. fowleri* vary in their degree of virulence: some are highly virulent whereas other isolates are only moderately virulent. Furthermore, any isolate can become avirulent on prolonged cultivation in an axenic medium. It is believed that the probability of infection in nature may depend upon the number and virulence of the amebae.[3,11]

The trophozoite of *N. fowleri* is highly phagocytic and induces necrosis of the CNS tissue. It is believed to ingest human tissue directly by producing a food cup or amebostome and by producing lysosomal hydrolases and phospholipases that degrade myelin. It has also been shown experimentally that the amebae exert a contact-dependent cytolysis mediated possibly by a multicomponent system that consists of a heat-stable hemolytic protein, heat-labile cytolysin and/or phospholipase A enzyme.[1–3,11,12]

The incubation period of PAM varies from 2 to 15 days depending on the size of the inoculum and the virulence of the amebae. In experimental infections with a mildly virulent *N. fowleri*, the incubation period has been as long as 3–4 weeks.[1–3]

Acanthamoeba spp.

Granulomatous amebic encephalitis, caused by *Acanthamoeba* spp., usually occurs in chronically ill, debilitated individuals, in immunosuppressed patients including those who have AIDS, or in those who have received broad-spectrum antibiotics or chemotherapeutic medications.[1–3] *Balamuthia mandrillaris* causes infection in both immunodeficient and immunocompetent individuals. The pathogenesis of GAE is complex and poorly understood. It is believed that the immunity is predominantly T-cell mediated and therefore depletion of $CD4^+$ and T-helper lymphocytes permits the growth and development of the amebae. The incubation period is unknown and several weeks or months may elapse before the disease becomes apparent. The respiratory tract or the skin may act as the portal of entry. One hypothesis is that ulceration of the skin may enable the amebae to enter.[1] The route of invasion to the brain must be via the bloodstream because there are no lymphatic channels within the brain.[1,13] Furthermore, trophozoites and cysts are often seen around blood vessels and within necrotic CNS tissue. The acanthamoebae are known to secrete enzymes such as lysosomal hydrolases, aminopeptidases and phospholipases, which may contribute to CNS damage.[1,2]

According to more recent studies the initial process of invasion, in case of *Acanthamoeba*, occurs when a 136-kDa mannose-binding protein (MBP), a lectin, expressed on the surface of the ameba, adheres to mannose glycoproteins on the surface of the epithelial cells and causes destruction of the epithelial cells. *Acanthamoeba* may also produce food cups on its surface and ingests the epithelial cells.[14] *Balamuthia*, like *Acanthamoeba*, probably invades human tissue by interacting with the host connective tissue and ingesting bits and pieces of host tissue as well as by producing enzymes that will degrade the tissue, thus helping *Balamuthia* to ingest.[15]

Acanthamoeba keratitis

Acanthamoeba keratitis (AK) is inflammation of the cornea caused by ocular trauma or by contact lens wear and the use of nonsterile homemade saline solution that has been contaminated with bacteria and fungi, which support the growth and multiplication of *Acanthamoeba* spp. The latter may adhere to the corneal epithelial cells as well as secrete proteolytic and collagenolytic enzymes that may damage the corneal epithelium and thus contribute to the pathogenesis of AK. If proper treatment is not provided, AK may lead to a vascularized scar within a thin cornea, causing impaired vision or perforation of the cornea and loss of the eye.[1–4,16]

PREVENTION

Primary amebic meningoencephalitis

The trophic and the cyst forms of *N. fowleri* are susceptible to chlorine and are killed at 1 mg/l, provided the water temperature is 78.8 °F (26 °C) or below. If, however, the water temperature is above 78.8 °F the chlorine concentration may need to be increased to 2 mg/l. It is therefore important for swimming pools to be maintained properly with adequate chlorination at all times. As it is not possible to disinfect natural bodies of waters such as lakes and ponds in which *N. fowleri* may be found, appropriate warnings should be posted, particularly during the hot summer months.[1,2]

Granulomatous amebic encephalitis

New and creative preventive measures need to be formulated because GAE produced by *Acanthamoeba* spp. and *B. mandrillaris* occurs principally in immunocompromised individuals. As *Acanthamoeba* spp. can grow and colonize hot water tanks, jacuzzis, filters used in heating, ventilating and air conditioning units, and in-line filters used for purifying portable water supplies and eye wash stations, periodic inspection of these systems is recommended.[1–3]

Acanthamoeba keratitis

It is recommended that eye care professionals educate patients about the proper care and use of contact lenses. Contact lenses and contact lens paraphernalia, particularly the solutions, should be kept meticulously clean. Contact lens wearers should follow the directions and recommendations of the manufacturers and eye care professionals. Additionally, contact lenses should not be used during swimming or other water sports.[16]

DIAGNOSTIC MICROBIOLOGY

Primary amebic meningoencephalitis

In individuals who have PAM, the CSF is characterized by pleocytosis, with a predominance of polymorphonuclear leukocytes but without bacteria. The CSF pressure is elevated (100–600 mmHg). Glucose concentration may be slightly reduced or normal, but the protein content is elevated, ranging from 100 mg/100 ml to 1000 mg/100 ml. CT scans show obliteration of the cisterns around the midbrain and the subarachnoid space over the cerebral hemispheres. Differential diagnosis of PAM from acute pyogenic or bacterial meningoencephalitis is dependent upon the detection of the ameba in the CSF *in situ* under a microscope. Smears of CSF should also be stained with Giemsa or trichrome stains for the delineation of the characteristic nuclear morphology. Gram stain is not useful. Care must be taken to differentiate amebic trophozoites from macrophages. Many cases have been diagnosed retrospectively based on examination of hematoxylin and eosin-stained sections or immunohistochemical tests.[1–3]

Recently, a real time multiplex polymerase chain reaction (PCR) assay has been developed which identifies *N. fowleri* DNA in the CSF within 5 hours of receipt of the specimen, thus greatly facilitating a quick diagnosis so that anti-*N. fowleri* therapy can be initiated promptly.[17] Serologic tests are of no value in the diagnosis of PAM because most patients die too early (within 3–7 days) in the disease process to mount a detectable immune response.[1–3]

Granulomatous amebic encephalitis

Examination of the CSF in patients who have GAE reveals lymphocytic pleocytosis with mild elevation of proteins and normal levels of glucose. Visual detection of *Acanthamoeba* spp. trophozoites in the CSF has rarely been reported. However, *Acanthamoeba* spp. have been identified in brain biopsies from several patients. Molecular techniques such as PCR and real-time PCR have also been used recently to identify *Acanthameba* in the CSF, brain and corneal tissue as well as in tear fluid.[1–3,18] CT scans and MRI are nonspecific and of limited value in the diagnosis. Single or multiple heterogeneous, hypodense, non-enhancing, space-occupying lesions that involve the basal ganglia, cerebral cortex, subcortical white matter, cerebellum and pons may be encountered, suggesting a brain abscess, brain tumor or intracerebral hematoma.

Brain and skin biopsies are important diagnostic procedures. *Acanthamoeba* spp. can be easily cultured from the brain, skin, lung and corneal tissue by placing a portion of the tissue that has been minced on non-nutrient agar plates coated with a layer of Gram-negative bacteria. Specimens for culture should be processed as quickly as possible. The incubation temperature depends upon the source of the samples. The agar plate should be incubated at 86°F (30°C) if the specimens originate from cornea or skin, but at 98.6°F (37°C) if the specimens are from the brain, the lung or any other internal organ. Amebae, if present in the samples, will feed on bacteria and multiply by binary fission. If the plates are examined under the light microscope, distinctive track marks with an ameba at the end of each track may be seen. The amebae will differentiate into cysts after a few days of incubation. Amebae can be identified to the level of genus on the basis of the characteristic morphology of trophozoites and cysts. Additionally, *Naegleria* spp. can be identified if flagellates appear within 2–4 hours of a loopful of amebae from the agar plate being suspended in distilled water. Identification to the species level, however, is difficult on the basis of morphology alone; nonmorphologic methods such as serology, isoenzyme analysis or DNA profiles therefore need to be used.[1–3,18]

Balamuthia mandrillaris will not grow on bacteria-coated agar plates. However, *B. mandrillaris* can be isolated from the CNS by inoculating monkey kidney cell culture with brain extract and subsequently growing the amebae in an axenic medium.[9]

Acanthamoeba keratitis

For the diagnosis of AK, deep corneal scraping and biopsy is recommended. Unfixed specimens should be processed for culture. Smears should also be prepared and fixed with methanol, Schaudinn's fixative or a spray-on fixative, and stained with Giemsa–Wright or Hemacolor, or with Wheatly's or Masson's trichrome stain. Hemacolor staining is quick and stains the distinctive cyst wall pinkish-red. The trichrome stains the nucleolus of the trophozoite reddish-pink and the cytoplasm greenish-purple and is therefore useful in differentiating trophozoites of *Acanthamoeba* spp. from the host cells.[1–3] Confocal microscopy has also been used recently to diagnose AK.[19]

CLINICAL MANIFESTATIONS

Primary amebic meningoencephalitis

The first case of PAM was reported in 1962.[1–3] Although at that time the case was thought to be caused by *Acanthamoeba* sp., it is now considered to be caused by *N. fowleri*. It is believed that more than 200 cases of PAM have occurred worldwide as of March 2008. Further, as many as 121 cases have been reported in the USA alone. Primary amebic meningoencephalitis has also been described in a South American tapir and cattle.[1]

Primary amebic meningoencephalitis is an acute, fulminating and usually fatal CNS disease that occurs mainly in healthy young adults and children with a recent history of watersport activities. Primary amebic meningoencephalitis has a rapid onset and a short incubation period that lasts from 3 to 7 days. It is characterized by the sudden onset of bifrontal or bitemporal headaches, fever, nausea, vomiting and stiff neck. Nuchal rigidity usually occurs with positive Kernig's and Brudzinski's signs. Abnormalities in taste or smell and cerebellar ataxia may be seen early but photophobia occurs late in the clinical course. An increase in intracranial pressure has been reported in most patients. Generalized seizures leading to lethargy, confusion, coma and death within 48–72 hours have been reported in a number of patients.[1] Focal myocardial necrosis has also been reported.

Pathologic features

At autopsy the cerebral hemispheres are swollen and edematous. The olfactory bulbs and the orbitofrontal cortices are necrotic and hemorrhagic. Because of increased intracranial pressure, uncal and cerebellar tonsillar herniations are usually seen. The arachnoid membrane is severely congested with scant purulent exudate. Amebic trophozoites are usually seen within the Virchow–Robin spaces with minimal or no inflammatory reaction (Fig. 182.4). Cysts are not seen within the CNS lesions. Necrotizing angiitis may occasionally be seen.[1,2]

Granulomatous amebic encephalitis produced by *Acanthamoeba* spp. and *Balamuthia mandrillaris*

Granulomatous amebic encephalitis caused by *Acanthamoeba* spp. occurs principally in individuals who are immunosuppressed (either iatrogenically or because of AIDS) and GAE caused by *B. mandrillaris*

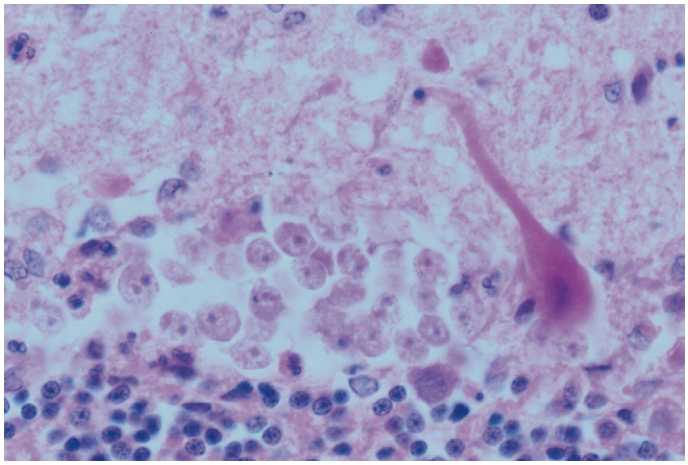


Fig. 182.4 CNS section demonstrating numerous trophozoites of *Naegleria fowleri*. Note the absence of cysts. (H & E.)

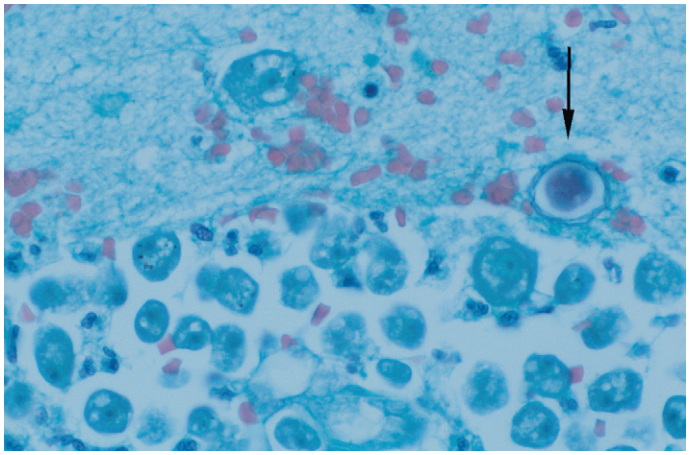


Fig. 182.5 Brain section showing numerous trophozoites and a cyst (arrow) with typical features of *Acanthamoeba culbertsoni*. (Masson's trichrome.)

occurs in either the very young or very old and in individuals who have AIDS. In the USA as many as 120 cases attributable to *Acanthamoeba* spp. (70 in people with AIDS) and 101 attributable to *B. mandrillaris* have occurred. Additionally, several cases of GAE caused by *Acanthamoeba* spp. and *B. mandrillaris* have been described in gorillas, a baboon, monkeys, dogs, sheep and cows.[1,3,20]

The clinical manifestations and pathologic features of GAE are similar regardless of which of these two organisms is the cause. It is an insidious disease and has a long and protracted clinical course. Clinical signs include personality changes, headache, low-grade fever, nausea, vomiting, lethargy, hemiparesis, seizures, depressed levels of consciousness and coma. Third and sixth cranial nerve palsies may be seen in some patients. Clinically, GAE may mimic bacterial leptomeningitis, tuberculous or viral meningitis, or single or multiple space-occupying lesions. Cerebellar ataxia and diplopia have been described in some patients. Pneumonitis with the presence of trophozoites and cysts within pulmonary alveoli has also been described.[1] In most cases of GAE, however, final diagnosis is made at autopsy.

Pathologic features

The cerebral hemispheres are edematous. Encephalomalacia with multifocal areas of cortical softening and hemorrhagic necrosis may be seen. Multifocal necrotic lesions may also be seen in the posterior fossa structures, midbrain, thalamus, brain stem and cerebellum. Trophozoites and cysts of the infecting organisms are seen, most often in the necrotic lesions in basal ganglia, midbrain, brain stem and cerebral hemispheres (Fig. 182.5). Microglial nodules may be seen within the necrotic tissues. Occasionally, angiitis may be seen with perivascular cuffing by inflammatory cells, chiefly lymphocytes, a few plasma cells and macrophages. In patients who have advanced AIDS there is very little inflammation. Trophozoites and cysts can easily be identified by light microscopic examination of the tissue sections. Also in patients who have AIDS, multiple ulcerations of the skin with acute and chronic inflammation may be seen and the ulcers may contain trophozoites and cysts of the infecting ameba (Fig. 182.6). The kidneys, prostate gland, adrenal glands, lungs and liver may also be involved, suggesting hematogenous dissemination. The ulcerated skin may serve as a portal of entry for the amebae in some patients. Several cases of skin and nasal infection without dissemination into the CNS have also been reported.[1,21–24]

In general, the trophic and cyst stages of *Acanthamoeba* spp. and *B. mandrillaris* look similar in formalin-fixed and hematoxylin and eosin-stained sections under the light microscope. In some patients, however, differential identification of *B. mandrillaris* can be made if the nuclei of the amebae in the sections possess two or three nucleolar

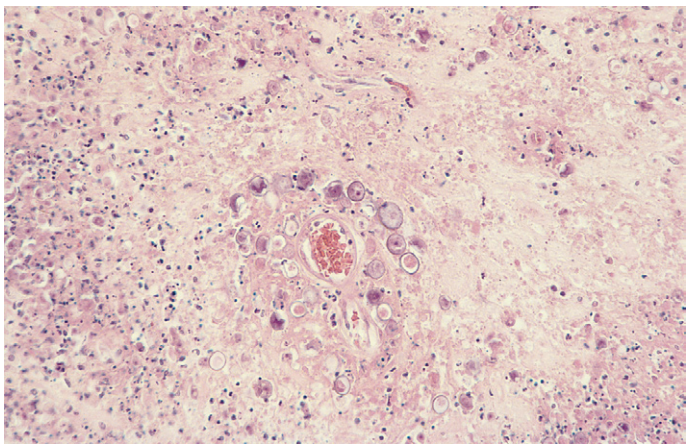


Fig. 182.6 Trophozoites and cysts of *Acanthamoeba* sp. surrounding a blood vessel in a skin biopsy of a patient who has AIDS. A diffuse but modest inflammatory reaction is seen.

elements because these are not seen in *Acanthamoeba* spp. (Fig. 182.7). A definitive identification may be arrived at by carrying out immunohistochemical analysis of the tissue sections using rabbit anti-*Acanthamoeba* spp. or anti-*B. mandrillaris* sera. The cyst wall of *B. mandrillaris* is characteristically tripartite and hence can be identified definitively by electron microscopy analysis of brain sections.[1–3]

Acanthamoeba keratitis

Acanthamoeba keratitis is a sight-threatening chronic inflammation of the cornea. It is associated with contact lens wear and the use of homemade nonsterile saline solution, corneal abrasion or trauma. Although the first case of AK in the USA was reported in a farmer from south Texas with ocular trauma of the right eye, wearing contact lenses and using nonsterile homemade saline was found to be a risk factor for AK in 1985 because most of the 24 patients reported in the previous 2 years were contact lens wearers.[1–3,16] By December 1996, more than 700 cases of AK were estimated to have occurred worldwide. Recently, an increase in the AK cases in the Chicago (Illinois) area was reported during May 1, 2003 and September 15, 2006.[25] An in depth epidemiologic survey conducted by CDC because of a multistate outbreak of *Acanthamoeba* keratitis revealed that the national increase in the number of *Acanthamoeba* keratitis cases was associated

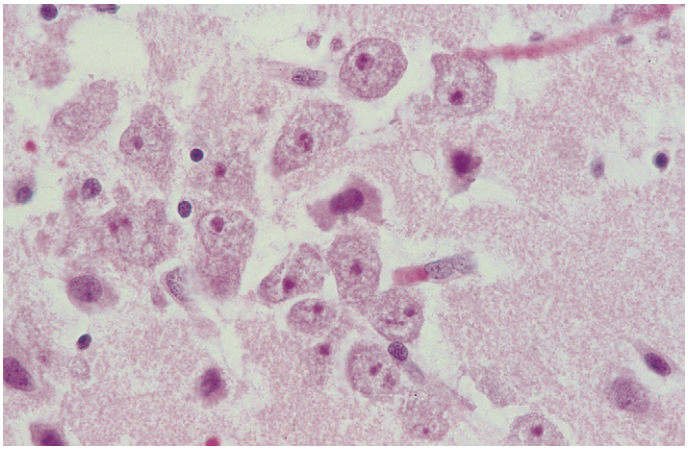


Fig. 182.7 CNS section showing many trophozoites of *Balamuthia mandrillaris*. More than one nucleoli can be seen within the nuclei of the trophozoite.

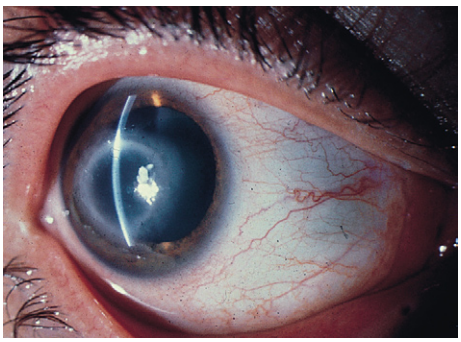


Fig. 182.8 *Acanthamoeba keratitis*. Note the typical central or paracentral ring infiltrate. Courtesy of Dr Theodore.

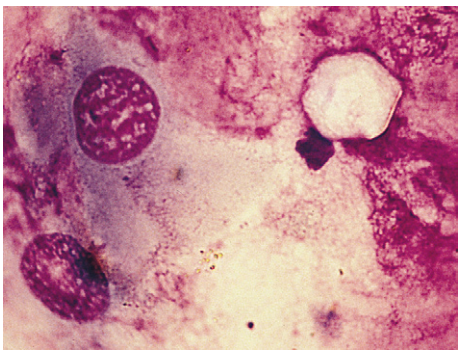


Fig. 182.9 Characteristic star-shaped morphology of the cyst of *Acanthamoeba* sp. demonstrated by a corneal scraping stained with Hemacolor. Courtesy of Dr Theodore.

with the use of Advanced Medical Optics Complete® MoisturePlus™ multipurpose contact lens solution leading to an international recall by the manufacturer.[26] Additionally, another study revealed that most contact lens solutions marketed in the US do not have sufficient disinfection activity against *Acanthamoeba* spp.[27]

The hallmark of AK is severe ocular pain, photophobia, a central or paracentral 360° stromal ring infiltrate (Fig. 182.8), recurrent breakdown of corneal epithelium with a waxing and waning clinical course and a corneal lesion refractory to the usual antibacterial, antiviral and antimycotic medications.[1–3]

When examined under the light microscope, corneal scrapings and/or sections of biopsied corneal tissue reveal trophozoites and cysts of *Acanthamoeba* spp. infiltrated between the lamellae of the cornea (Fig. 182.9). Polymorphonuclear leukocytes, eosinophils, lymphocytes, macrophages and plasma cells have also been seen occasionally. Ulceration, descemetocele formation and perforation are often seen in the later stages of AK.[1–3]

MANAGEMENT

Primary amebic meningoencephalitis

Primary amebic meningoencephalitis has almost always resulted in death. A few patients, however, have survived, probably because of early diagnosis and aggressive treatment with amphotericin B, miconazole (not available in the USA) and rifampin (rifampicin).[28] Amphotericin B and miconazole can be administered intrathecally or intravenously, alone or in combination, and rifampin can be given orally. A 9-year-old girl from California, one of the patients who survived this disease in the USA, was treated as follows:

- intravenous amphotericin B 1.5 mg/kg body weight per day in two divided doses for 3 days and then 1 mg/kg for 3 days;
- intrathecal amphotericin B 1.5 mg/day for 2 days followed by 1 mg/day for 8 days;
- intravenous miconazole 350 mg/m^2 body surface area per day in three divided doses for 9 days;
- intrathecal miconazole 10 mg/day for 1 day followed by 10 mg every other day for 8 days; and
- oral rifampin 10 mg/kg per day in three divided doses for 9 days.

The patient was also given sulfisoxazole (sulphafurazole) q6h for 3 days, but this was discontinued once *N. fowleri* was identified as the cause of the disease. Dexamethasone and phenytoin were given to lower the intracranial pressure and seizure activity, respectively. Amebae disappeared from the CSF after 3 days of treatment. Although cell counts and biochemistry of the CSF were abnormal for several months afterwards, the patient recovered completely without any neurologic sequelae.[28] It has also been reported that a boy from Texas, USA, recovered from PAM after intravenous amphotericin B and treatment in a hyperbaric chamber.[29]

Granulomatous amebic encephalitis

Although it is known from *in-vitro* experiments that several drugs, including pentamidine, propamidine, dibromopropamidine, miconazole, paromomycin, neomycin, ketoconazole and 5-fluorocytosine, have inhibitory effects on several isolates of *Acanthamoeba*, there is as yet no effective treatment for GAE and therefore the prognosis is poor.[1–3] This is partly due to the difficulty in the diagnosis of GAE because of a lack of clear-cut symptoms, lack of a reliable noninvasive diagnostic test and lack of knowledge of the care givers; diagnosis is often made at the time of postmortem. The disease has been identified in only a very few patients before death. Several patients who were diagnosed premortem, however, have reportedly recovered from this disease. *Acanthamoeba rhysodes* was isolated from the CSF of a Nigerian patient with neurologic disease who showed clinical improvement after treatment with sulfamethazine. He was discharged from the hospital but was lost to follow-up.[1–3]

Acanthamoeba culbertsoni was isolated from the CSF of an Indian patient with CNS impairment who recovered after treatment with chloramphenicol.[2,3] A 7-year-old girl from Barbados with a 'brain tumor' recovered without any neurologic sequelae after surgical excision. *Acanthamoeba healyi* was isolated from the 'tumor' and also demonstrated in the hematoxylin and eosin-stained, formalin-fixed sections. The patient also received oral ketoconazole treatment for 8 months postoperatively.[30,31] Prognosis of patients who have disseminated skin infections without CNS involvement is, however, good. In an immunocompromised (without HIV/AIDS) patient with *A. rhysodes* skin infection, skin ulcers healed after the following treatment regimen: cleansing of skin ulcers twice daily with chlorhexidine gluconate solution followed by the application of 2% ketoconazole cream. The patient was also given 4 mg/kg intravenous pentamidine isethionate for 1 day. Because of pentamidine toxicity the dosage was cut to 2700 mg and given over a 4-week period. Thereafter, he was given itraconazole orally at a dose of 200 mg daily for 8 months, resulting in

the total healing of the skin lesions.[21] However, several patients with GAE caused by *Acanthamoeba* spp., as well as *Acanthamoeba* cutaneous infections without CNS involvement, have been successfully treated with a combination of pharmaceuticals including pentamidine isethionate, sulfadiazine, flucytosine, and fluconazole or itraconazole.[21–24,29] Another drug, miltefosine, a hexadecylphosphocholine, has also been shown to have amebicidal potential and miltefosine has been successfully used to treat GAE patients in Austria.[33,34] For *Acanthamoeba* cutaneous infection without CNS involvement, topical applications of chlorhexidine gluconate and ketoconazole cream in addition to the above have resulted in therapeutic success.[1–3]

Recently, voriconazole, a triazole compound, was used to clear cutaneous infection in a patient recovering from lung transplantation.[24] Another recent case with a cadeveric liver transplant was initially treated empirically with a combination of drugs including sulfadiazine, pyrimethamine, cefotaxime, chloramphenicol, gentamicin, isoniazid, rifampin and pyrazinamide to cover *Toxoplasma*, bacterial and tuberculous infections. Since the patient did not show any improvement a lobectomy was performed and *Acanthamoeba* was identified in the tissue sections. Pyrimethamine was stopped and rifampin was continued at 600 mg q8h and co-trimoxazole at 960 mg q8h was added to the regimen. The patient improved and is alive 11 years after *Acanthamoeba* infection was diagnosed.[35]

Although the majority of patients with *Balamuthia* GAE have died, a few patients have survived the infection. For example, two patients, a 60-year-old man and a 6-year-old girl from California, and a 70-year-old woman from New York have survived balamuthiasis after treatment with a combination of pentamidine isethionate, sulfadiazine, macrolide antibiotics (azithromycin–clarithromycin), fluconazole an d flucytosine (5-fluorocytosine).[36,37] Two Peruvian patients with cutaneous lesions became well after prolonged therapy with albendazole and itraconazole. One of the Peruvian patients had a large lesion on the chest wall that was surgically removed. Surgical excision of the lesion may have reduced the parasite load, thus helping in the recovery process.[38]

Acanthamoeba keratitis

Treatment of AK with topical application of polyhexamethylene biguanide or chlorhexidine gluconate in combination with propamidine isethionate appears to be the treatment of choice, as several patients have been treated successfully in this way.[1–3] Many patients have also been treated with topical application of propamidine isethionate 0.1% and dibromopropamidine, in conjunction with neosporin. Ketoconazole and clotrimazole appear to be effective *in vitro* and *in vivo*. Other reports of successful treatment with 0.1% hexamidine di-isethionate eye drops have been reported. Some patients have also been treated successfully with combinations of topical propamidine and miconazole and systemic ketoconazole, or topical clotrimazole or oral itraconazole with topical miconazole.[39] Medical cure has been achieved with the administration topically of polyhexamethylene biguanide[40] or chlorhexidine with or without propamidine.[41,42] Debridement of the cornea, penetrating keratoplasty and corneal grafting have also been performed with good results in some patients. Recurrence of AK has been reported after corneal transplantation, probably caused by cysts of *Acanthamoeba* spp. still present in the corneal stroma.

Disclaimer

The findings and conclusions in this report are those of the authors and do not necessarily represent the views of the Centers for Disease Control and Prevention.

REFERENCES

References for this chapter can be found online at http://www.expertconsult.com

Chapter 183

Pablo Martín-Rabadán
Emilio Bouza

Blood and tissue protozoa

Organisms of the genera *Plasmodium, Babesia, Toxoplasma, Leishmania* and *Trypanosoma* are the most prevalent protozoa able to invade blood and human tissues. They cause diseases of immense socioeconomic impact, most of them having a limited (although extensive) geographic distribution.

Malaria is considered separately in Chapter 111.

Babesiosis

Human babesiosis is an uncommon tick-borne parasitic zoonotic disease that was first described by Babes in Rumanian cattle in 1888. It was not reported as a human disease until 1957.

NATURE

The genus *Babesia* belongs to the family Piroplasmida, closely related to *Plasmodium* and *Theileria* genus, and comprises over 70 species that parasitize mammals and birds. Infection in cattle is widespread and it is an economically important disease. Most human cases in Europe are caused by *Babesia divergens* and by *Babesia microti* in the USA. A number of *Babesia*-like piroplasms are being increasingly found infecting humans. Among them, former *Babesia* WA1 and CA5, endemic of western North American states, have been recently renamed *Babesia duncani*.[1]

EPIDEMIOLOGY AND PATHOGENICITY

Babesiosis in humans occurs predominantly in the USA. It is highly endemic in the coastal areas of Massachusetts, Connecticut and New York State, where seroprevalence rates reach 4–6%, indicating that subclinical infections are common. In these areas rodents are the main reservoir and humans are usually infected by the bite of an infected nymph of *Ixodes scapularis* tick. Transfusions of blood or blood products have caused a few cases. Subclinical infections are common in endemic areas. Serosurveys in the USA have found 3–8% of samples positive for *B. microti* and up to 16% for *B. duncani*.

In Europe *B. divergens* is the main causative agent of bovine babesiosis. The seroprevalence for this species in exposure-selected human populations reaches 1.5–11.5% but only a few clinical cases have been reported, with splenectomized patients being at highest risk.[2–4]

Suspected or confirmed cases of babesiosis have also been reported from Colombia, Mexico, India, China, Taiwan, Japan, Egypt and South Africa.

PREVENTION

General recommendations include avoidance of known endemic areas, especially in the warm months of the year. Clothes should be sprayed with tick repellents before going on outdoor activities and on return the skin should be thoroughly examined for the presence of ticks.

DIAGNOSTIC MICROBIOLOGY

Babesia spp. are intraerythrocytic parasites that may resemble plasmodium trophozoites, but the hemozoin pigment present in the late stages of malarial trophozoite development is never present in babesiosis. Schizonts and gametocytes are not formed. Multiple erythrocyte parasitization is common and organisms are occasionally arranged in a characteristic Maltese cross shape (Fig. 183.1).

Parasitemia usually persists, demonstrable by light microscopy, for 3–12 weeks.

Serologic diagnosis can be made using indirect immunofluorescent antibody (IFA) assay. A serum titer of at least 1/256 is diagnostic of recent *B. microti* infection. Within a few weeks, most patients who have active infection have serum titers equal to or higher than 1/1024, falling over a few months to levels under 1/256. Due to the lack of cross-reactivity,

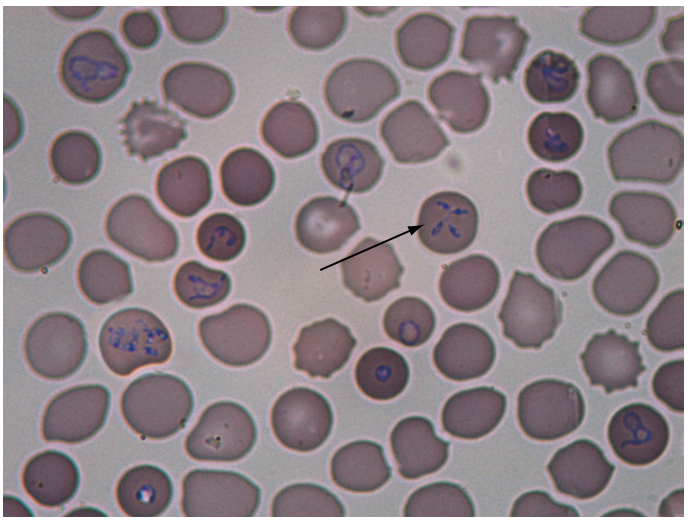


Fig. 183.1 *Babesia* spp. heavy infection. Single and multiple intraerythrocytic parasites can be seen. The arrow marks a typical Maltese cross arrangement.

individuals exposed to the infection on the west coast of the USA should also be tested for antibodies to the *Babesia* WA1 species.[5] *B. microti*, WA1 and *B. divergens* have limited serologic cross-reactivity.

CLINICAL MANIFESTATIONS

The clinical forms of babesiosis vary widely depending upon the immune status of the host and the species of *Babesia* involved.

In the American Northwest, the symptoms of *B. microti* infection (Nantucket fever) range from a nonspecific flu-like self-limited febrile illness to severe haemolytic anemia. It is most severe in those who are older or asplenic. The usual incubation period is 1–3 weeks. The onset is gradual with nonspecific symptoms such as malaise, fatigue, anorexia, headache, emotional lability, myalgias and arthralgias. Blood leukocytes are not elevated and platelets are usually decreased. There may be a haemolytic anemia. The mortality is less than 10% of symptomatic cases.

The European clinical cases diagnosed occur mainly in asplenic patients who present with a high fever and severe haemolytic anemia, and the infection is usually fatal.

MANAGEMENT

Early treatment is recommended for all diagnosed cases to prevent long-term sequelae and potential transmission through blood donation.

The standard treatment is clindamycin, 1200–2400 mg/day q6–8h plus 650 mg of quinine q6–8h; both drugs are given for 5–10 days. Combination of atovaquone (750 mg q12h) and azithromycin (500 mg on day 1 and 250 mg per day thereafter) for 7 days is also effective and is associated with fewer adverse reactions.[6] Chloroquine is not recommended. Blood transfusion and general supportive treatment may be needed for severe disease. Exchange transfusion has been used in an attempt to decrease parasite load, but its usefulness has not been demonstrated. It is recommended to rule out co-infection by *Borrelia burgdorferi* and other tick-transmitted micro-organisms.

Toxoplasmosis

Toxoplasmosis is a widespread zoonosis, caused by the coccidian parasite *Toxoplasma gondii*, first recognized in 1908. *Toxoplasma* (from the Greek, meaning 'shaped as a bow') is a one-species genus.

Humans are usually infected orally, developing few, if any, symptoms of infection. The infection persists for life without signs of disease. However, during immunosuppression quiescent parasites multiply, resulting in neurologic disease or, more rarely, other organ manifestations. Congenital transmission is a cause of major concern as it is frequently associated with severe disease in the newborn and retinochoroiditis later in life.

NATURE

Toxoplasma gondii is a parasite of many species of mammals and birds. The main forms of the parasite lifecycle are oocysts, tachyzoites and bradyzoites.

The oocysts result from the sexual multiplication that takes place exclusively in the intestinal epithelium of cats and other felines. They are present in feline feces, and after maturing in the environment become the source of infection for noncarnivorous animals.

In the secondary host (all hosts but felines) *T. gondii* cells have two forms:

- rapidly multiplying tachyzoites (*tachy*, rapid); and
- 'dormant' bradyzoites (*brady*, slow).

When the host is infected by oocysts, tachyzoites or bradyzoites, a disseminated infection by tachyzoite forms takes place. Tachyzoites multiply inside the host cells, which rupture when 8–32 tachyzoites are produced. The tachyzoites released infect new cells. When the immune response controls the infection, some surviving parasites persist for many years in tissue cysts, which are sacs containing hundreds or thousands of *T. gondii* bradyzoites. They represent the parasite reservoir present in secondary hosts. Tissue cysts can be found in any tissue, but are most common in muscle and brain.

Cats become infected from eating birds, small rodents or other sources of raw meat. Humans and other animals become infected from either oocysts in soil and contaminated food or tissue cysts in raw meat. When acute infection occurs during pregnancy, tachyzoites are able to infect the placenta and, in a second step, the fetus. The parasite can also be acquired by organ transplantation or blood transfusion.

EPIDEMIOLOGY

Seroprevalence to *Toxoplasma gondii* in sheep and cattle varies widely but has been frequently found above 25%; however, it is lower in pigs and poultry and is declining in intensive reared animals. The prevalence of oocyst-shedding cats in Europe has been found to be less than 0.3%.[7]

Infection rates in humans are very variable and depend upon eating habits and environmental factors. It has been estimated that one-third of mankind is infected.[8,9]

Patients with schizophrenia and other psychiatric disorders have been found to have higher seroprevalence of *T. gondii* than matched controls but an etiologic link has not been proved.[10]

Human disease caused by *T. gondii* is much more restricted than the infection itself, and is generally a consequence of either intrauterine acquisition or immunocompromise.

PATHOGENICITY AND IMMUNITY

Toxoplasma gondii tachyzoites invade cells from nearly every organ, where they survive inside parasitophorous vacuoles. Resistance to the infection has been found to be enhanced by interferon gamma (IFN-γ) and diminished by IL-6. Extracellular tachyzoites are lysed by complement combined to specific antibody, and $CD4^+$ and $CD8^+$ T lymphocytes, lymphokine-activated killer and natural killer cells play a major role in infection control.

PREVENTION

Toxoplasmosis can be prevented at three different levels:

- prevention of primary infection;
- prevention of vertical transmission and congenital disease; and
- prevention of disease in infected immunocompromised individuals.

To prevent primary infection, the exposure to parasite can be reduced by health education (Table 183.1). No vaccine is presently available. Maternal immunity due to toxoplasmosis passed before conception protects the fetus from the infection. Immunodeficient patients receiving trimethoprim–sulfamethoxazole (co-trimoxazole) as prophylaxis for *Pneumocystis* infection are substantially protected from toxoplasmosis.

Toxoplasma gondii IgG serology should be included in the initial workup of pre-transplant patients and transplantation of a solid organ from a seropositive donor to a seronegative recipient should be avoided if possible. If such a transplant is performed, then the recipient should have anti-*T. gondii* treatment for at least 2 months.

Individuals who have HIV infection and are seronegative for *T. gondii* should avoid being exposed to the parasite. For those patients who have HIV infection and are seropositive for *T. gondii*, further serologic tests do not provide useful information.

Table 183.1 Toxoplasmosis primary preventive measures

- Do not eat undercooked or raw red meat or eggs. Cook meat until it is no longer pink in the center
- While handling raw meat avoid touching your mouth or contaminating other food. Normal hygienic washing of hands and utensils will suffice
- Cured or smoked meat is generally considered safe from *Toxoplasma*. Freeze it at –4°F (–20°C) for at least 24 hours as an additional precaution
- Consume only pasteurized or ultra-heat-treated (UHT) sterilized milk and dairy products
- Wash or peel all fruits and vegetables to be eaten uncooked
- Control insect pests and their access to foodstuffs
- Avoid living with cats or kittens. If unfeasible take special care: change the cat litter box daily and rinse it with nearly boiling water. Wear gloves and wash your hands well with soap and water immediately after. Prevent your cat from hunting birds and rodents or eating raw meat
- Use gloves and wash your hands well after gardening and other outdoor activities with soil contact.

Routine serologic screening during pregnancy remains controversial. It is aimed at detecting acute maternal infections. It is, however, sometimes difficult to establish an early and reliable diagnosis of acute maternal infection. When acute infection is diagnosed in a pregnant woman, anti-*T. gondii* treatment, further assessment of fetal infection and abortion may be offered.

DIAGNOSTIC MICROBIOLOGY

The diagnosis of *T. gondii* past infection is usually made by reactive IgG testing. The suspicion of acute or recent infection may rely on a single serum sample but a reliable serologic diagnosis requires paired sera. A typical acute infection would have rising titers of positive IgM with the IgG negative or positive with low avidity. Nevertheless, in some cases, reactive IgM may be undetectable or may persist for more than 12 months.[11,12] The functional affinity of specific IgG antibodies (avidity) is initially low, and usually increases during the subsequent 3–6 months, although in some cases it may take much longer periods.[13]

Congenital infection in the newborn by IgM or IgA testing detects only one half of infected infants.[14]

Parasite demonstration by polymerase chain reaction (PCR) of tissue or body fluids is the most efficient diagnostic tool for active infection. Examination of stained tissue or fluid smears, growth in tissue cultures and experimental animal inoculation may be used.[15,16] The most sensitive method for chorioretinitis is PCR plus relative concentration measures of antitoxoplasma antibodies in serum and aqueous humor.[17]

CLINICAL MANIFESTATIONS

There are three different forms of toxoplasmosis: congenital infection, acute extrauterine infection and toxoplasmosis of the immunocompromised host.

Congenital toxoplasmosis

Toxoplasmosis acquired in utero can be asymptomatic or may produce signs of disease that can be present at birth or later in life.

The risk of congenital toxoplasmosis depends upon the time of acquisition of acute maternal infection.[18] Vertical transmission of *T. gondii* increases with gestation age (15–25% in the first trimester of pregnancy, 30–54% in the second and 60–65% in the third). Conversely, the severity of congenital disease is increased when infection occurs in early pregnancy. Signs of infection at delivery are present in 21–28% of those infected in the second trimester and ≤11% of those infected in the third trimester. Overall, 10% are born with severe disease.

Clinical manifestations of congenital toxoplasmosis include strabismus, chorioretinitis, encephalitis, microcephaly, hydrocephalus, psychomotor retardation and convulsions, as well as nonspecific manifestations such as anemia, jaundice, hypothermia, thrombocytopenia, diarrhea and pneumonitis. The characteristic triad of hydrocephalus, cerebral calcifications and chorioretinitis resulting in mental retardation, epilepsy and impaired vision is the most severe and extreme form of the disease. Cerebral lesions may calcify, providing retrospective evidence of congenital toxoplasmosis.

Toxoplasma gondii chorioretinitis in immunocompetent patients is frequently secondary to congenitally acquired infection (Fig. 183.2). The proportion of congenitally infected asymptomatic individuals who develop chorioretinitis later in life (usually in the first four decades) is unknown, but may well be over two-thirds. Up to 30% of patients relapse after treatment.

Acquired toxoplasmosis

In 90% of cases, no clinical symptoms are apparent during acute infection. Most symptomatic patients present with enlarged lymph nodes that are located mainly in the head or neck area. Malaise and low-grade fever are present in less than 50% of symptomatic cases. Although *T. gondii* organisms are not usually seen, the histologic picture of acute *T. gondii* lymphadenopathy is usually diagnostic. Occasionally the lymph node histopathology can be mistaken for that of cat-scratch disease, lymphoma and other infections.[19]

Rare manifestations of acute infection include chorioretinitis, myositis and heart, lung, liver or central nervous system (CNS) symptomatic involvement.

Toxoplasmosis in the immunocompromised host

Toxoplasmosis is a serious disease in patients who have profound immunosuppression such as those who have had a transplant or who have AIDS.

In transplant patients, the incidence and severity of the disease depends upon previous exposure to the parasite of donor and recipient, the type of organ transplanted and the degree of immunosuppression induced. The disease can be due to reactivation of a chronic silent infection or an acute primary infection acquired from the transplanted organ. The risk of developing toxoplasmosis for seronegative recipients of an organ from a seropositive donor is 57% for heart, 20% for kidney and minimal for liver transplants, reflecting the tropism of tissue cysts for striated muscle.[20] Incidence in bone marrow transplants is usually under 5%.[21] The disease manifests as a systemic disease with diverse



Fig. 183.2 Fundoscopic image of toxoplasmic chorioretinitis. Most cases presenting in adults represent a late manifestation of congenitally acquired toxoplasmosis.

degrees of multiorgan involvement including pneumonitis, carditis, hepatitis, myositis and encephalitis.

In patients who have AIDS, the clinical manifestations are usually related to CNS dysfunction or ocular lesions. Myocarditis is frequently found at autopsy, but is rarely clinically apparent. Infections of lung and other organs have also been reported.

Patients who become infected with *T. gondii* risk developing toxoplasmosis when their $CD4^+$ T-lymphocyte count is less than 100 000/ml. The onset of *T. gondii* encephalitis is usually subacute. Fever and malaise usually precede the first neurologic symptoms. Headache, confusion, neuropsychiatric manifestations, seizures or other focal signs strongly suggest the diagnosis. Diagnosis is based upon clinical data, neuroradiology (an MRI scan is the procedure of choice) and response to therapy. *T. gondii* encephalitis in patients who have AIDS usually presents as multiple abscesses in the basal ganglia or the grey–white matter junction with a contrast-enhanced ring (Fig. 183.3). Diffuse *T. gondii* encephalitis is an uncommon rapidly fatal form.

In immunocompromised patients there is no good serologic marker of active toxoplasmosis. The presence of anti-*T. gondii* antibody means previous exposure and therefore present infection, but not necessarily disease. The absence of specific antibodies makes toxoplasmosis unlikely.

MANAGEMENT

Congenital toxoplasmosis

In gestational toxoplasmosis, the drug therapy may be intended to treat the mother, the fetus or the newborn (Fig. 183.4). Spiramycin is a macrolide antibiotic that concentrates in the placenta, and is used early after maternal infection aiming to decrease vertical transmission. Spiramycin 3 g/day q8h is given to pregnant women who have an acute infection from diagnosis to delivery unless fetal infection is proved.

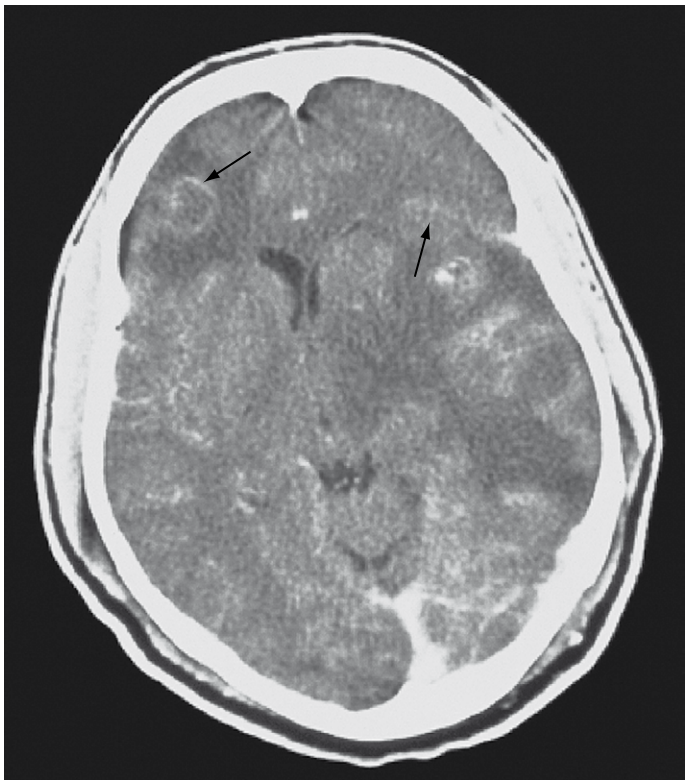


Fig. 183.3 Toxoplasmic encephalitis in a person who has AIDS. A cranial CT scan shows bilateral contrast-enhanced ring lesions with peripheral edema and mass effect.

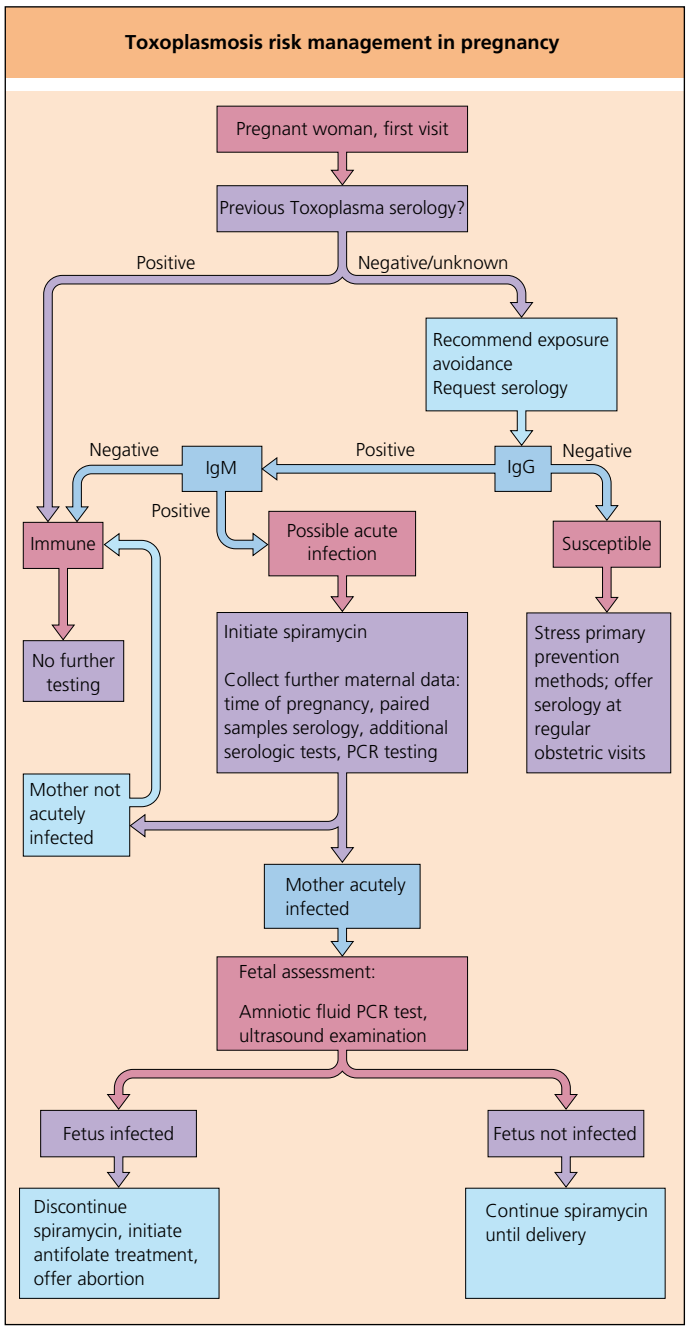


Fig. 183.4 Toxoplasmosis risk management in pregnancy.

In such a case, the maternal regimen is switched to sulfadiazine 4 g plus pyrimethamine 25 mg and folinic acid 15 mg a day until delivery, although no study has proved the benefit of such treatment.[22,23] All infected newborns should have anti-*T. gondii* treatment (sulfadiazine 50 mg/kg q12h plus pyrimethamine 1 mg/kg/day and folinic acid 5 mg/kg/day for at least 6 months). No treatment regimen seems to decrease the rate of chorioretinitis.[24]

To confirm fetal infection, ultrasound fetal examination and sampling the amniotic fluid for *T. gondii* PCR and culture are recommended. Fetal blood sampling obtained by cordocentesis has been commonly used to detect fetal antibodies and for *T. gondii* culture. Cordocentesis had an inherent risk of fetal loss of 2–3% and has been replaced by amniotic fluid PCR testing.

Termination of pregnancy is commonly offered to women who seroconvert in the first 8 weeks of pregnancy and to those infected in the first 22 weeks of pregnancy when fetal infection is confirmed.

A more conservative approach recommends abortion only if there is ultrasonographic evidence of hydrocephalus, although a small percentage of cases with neurologic disease will then be born.[25]

Acquired toxoplasmosis

Symptomatic *T. gondii* infection in nonpregnant immunocompetent individuals does not need to be treated unless there is visceral involvement or the patient has been infected in a laboratory accident.

Toxoplasmosis in the immunocompromised host

Standard anti-*T. gondii* treatment is sulfadiazine 4 g/day q6h plus pyrimethamine as a first 100 mg loading dose followed by 50 mg/day and folinic acid 15 mg/day for 6–8 weeks. If brain edema is of relevance corticosteroids should be given, but the clinical response to anti-*T. gondii* therapy would be masked by the unspecific improvement due to the reduction of edema. If the patient has not improved in the first 2 weeks of therapy a control CT or MRI scan should be repeated to review the empiric diagnosis. A repeat scan should be carried out after completing treatment. With this regimen 80% of patients survive, although 50% of the survivors will have neurologic sequelae. Serious adverse effects to the medication can be expected in 40% of patients. In cases of intolerance to sulfadiazine, 75–100 mg/day of pyrimethamine alone or with clindamycin has been used. Azithromycin has been used as a reserve agent in some cases.

Secondary prevention

Approximately 80% of AIDS patients relapse after an initial episode of toxoplasmosis. Daily treatment with pyrimethamine 50 mg plus sulfadiazine 1 g and folinic acid 10–15 mg should be given to reduce the relapse rate to 5–10%. Preventive treatment can be safely discontinued if immune system function is restored.[26]

Leishmaniasis

Leishmaniasis is the term used to describe a group of chronic parasitic diseases caused by a number of species of the genus *Leishmania*. The infection can be localized in the skin or mucous membranes or disseminated in the reticuloendothelial system. It is usually a zoonosis transmitted by *Phlebotominae* sandflies, which are endemic in areas of Southern Europe, Asia, Africa and the Americas.

NATURE

The genus *Leishmania*, along with the genus *Trypanosoma*, belongs to the order Kinetoplastida, which comprises flagellated protozoa that possess a characteristic extranuclear DNA mass called the kinetoplast.

Leishmania parasites are classified into two broad groups:

- New World *Leishmania* spp.; and
- Old World *Leishmania* spp.

Closely related species are grouped into complexes (Table 183.2). Members of *Leishmania braziliensis* complex have been renamed *Leishmania* subgenus *viannia*.

Sandflies are small (3–4 mm), hairy mosquito-like insects, which transmit the disease. Old World sandflies belong to the genus *Phlebotomus*; New World species belong to the genera *Lutzomyia* and *Psychodopygus*.

Sandflies become infected when taking a blood meal containing infected macrophages, which are present in blood or skin. *Leishmania* live in the digestive tract of the insect as flagellated parasites (the

Table 183.2 Summary of epidemiology of leishmaniasis

Complex	Main pathogenic species	Main reservoir	Geographic distribution (maps)	Clinical disease (rarely)
Leishmania donovani	*L. donovani*	Humans	India, Bangladesh, Burma	VL, PKDL, CL
		Canids	Sudan, Kenya, Somalia	VL (PKDL, CL)
	L. infantum	Dogs; also found in rats	Extending from the Mediterranean to China	VL, CL
	L. chagasi (*infantum*)	Foxes, dogs, opossums	South of Mexico to northern South America	VL, CL
Not included in a complex	*L. major*	Gerbils, girds and other rodents	Arid areas north of the Sahara, Arabia and central Asia	CL (LR)
		Rodents	Sub-Saharan savannah	CL
	L. tropica	Humans	Towns in East Mediterranean countries, Middle East and central Asia	CL (LR)
	L. aethiopica	Hyraxes	Highlands of Ethiopia and Kenya	CL, DCL (MCL)
	Leishmania sp.	Hyraxes?	Namibia	CL
Leishmania mexicana	*L. mexicana*	Forest rodents	Tropical forests of southern Mexico, Belize and Guatemala	CL
	L. amazonensis	Forest rodents	Tropical forests of South America	CL, DCL
	L. venezuelensis, L. garnhami, L. pifanoi	Undetermined	Venezuela	CL
Leishmania braziliensis	*L. braziliensis*	Forest rodents, peridomestic animals	Tropical forests of South and Central America	CL, MCL (VL)
	L. guyanensis	Sloths, arboreal ant-eaters	Guyana, Surinam, areas of Brazil.	CL, MCL
	L. panamensis	Sloths	Panama, Costa Rica, Colombia	CL MCL
	L. peruviana	Dogs	Areas of Peru and Argentina	CL

CL, cutaneous leishmaniasis; DCL, diffuse cutaneous leishmaniasis; LR, leishmaniasis recidivans (chronic lupoid leishmaniasis); MCL, mucocutaneous leishmaniasis; PKDL, post-kala-azar dermal leishmaniasis; VL, visceral leishmaniasis. Common names are in parentheses.

promastigote form). The parasites divide in the midgut of the insect except for the *viannia* group, which multiply in the hindgut. The highly motile promastigotes migrate to the buccal cavity of the insect, which regurgitates them on biting, thereby infecting a vertebrate host. Promastigotes rapidly penetrate macrophages and transform into an aflagellated oval body measuring 2–3.5 mm across and called an amastigote (literally, without whip). Amastigotes live as obligate intracellular parasites inside the macrophages where they multiply and are phagocytosed by other mononuclear cells when the host cell ruptures. The infection may disseminate to internal organs, spread locally to mucous membranes or persist as a chronic skin lesion.

EPIDEMIOLOGY

Leishmaniasis is endemic in 88 countries (see Chapter 117, Fig. 117.1). The world prevalence and annual incidence have been estimated to be 12 and 1.5–2 million people, respectively. The incidence of all forms of the disease is steadily increasing.[27] Some individuals may be asymptomatic Leishmania carriers.[28]

The vast majority of patients are infected by sandflies, but rare cases in which infection is transmitted by blood transfusion have been reported. Transmission by sharing needles among intravenous drug abusers may occur.

PATHOGENICITY

The internalization of *Leishmania* in the macrophage results from the interaction of complement CR3 and fucose–mannose receptors with the parasite major surface protein (Gp63) cysteine proteases and membrane lipophosphoglycan. Parasite survival inside the parasitophorous vacuole is facilitated by iron superoxide dismutase and acidic phosphatase secreted by the parasite. In animal models, susceptibility to infection is mediated by two subsets of $CD4^+$ T lymphocytes, named T-helper Th1 and Th2.

The $CD8^+$ T lymphocytes also play a major protective role. The host's immune system, parasite species and strain-dependent factors interact to create the full spectrum of clinical disease.

DIAGNOSTIC MICROBIOLOGY

The laboratory diagnosis can be made by demonstrating *Leishmania* by microscopy or culture, detecting parasite DNA by PCR techniques or demonstrating immune response by serology or leishmanin cutaneous tests.

To study skin lesion, smears can be prepared from the tissue juice of the raised edge of an ulcer. The sample can be aspirated with a needle or scraped with a blade. Parasites can be detected by microscopy or culture in only 30% of cases of leishmaniasis recidivans, 50% of cases of mucocutaneous leishmaniasis (MCL) and 70% of cases of cutaneous leishmaniasis (CL).

In cases of visceral leishmaniasis (VL) sternal bone marrow aspirate is the sample of choice for parasitologic diagnosis (62–100% sensitivity). Splenic fluid aspirate has a higher diagnostic rate (95–100%), but splenic rupture may complicate the procedure. Other samples such as blood, lymph nodes or liver tissue may be of value. To increase positive yield of microscopic examination, samples should be routinely cultivated in special media. The use of a microcapillary culture method has been found more sensitive and rapid than traditional tube cultures.[29,30] Most PCR-based tests show higher sensitivity than standard parasitologic methods and, in some cases, species identification can be accomplished by PCR testing. Post-treatment-positive PCR on bone marrow is associated with a higher risk of relapse.[31,32]

Serologic testing is useful in the diagnosis of VL and can be of value in cutaneous forms of leishmaniasis. The sensitivities and specificities of the tests vary depending on the manufacturer, the area of acquisition, proportion of HIV co-infected patients and controls used. Indirect fluorescent antibody test (IFAT) is sensitive and group specific, and its sensitivity is 80–100% in VL patients who do not have HIV infection and 77–89% in MCL. In CL, low titers can occasionally be found. Cross-reactivity has been found in the sera of patients who have leprosy, tuberculosis, Chagas disease, African trypanosomiasis, malaria and schistosomiasis. Newer direct agglutination test and tests based on the detection of antibodies against recombinant kinesin protein of 39 kDa (rK39) are more specific with similar sensitivity. An rK39 based dip-strip test is suitable for field conditions. A proportion of individuals who have HIV infection and VL have persistently negative serology; rK39 dip tests in co-infected European patients had a sensitivity of 20–22% while in Sudan sensitivities of 98–95% have been reported.[33–36]

CLINICAL MANIFESTATIONS

Most cases of leishmanial infection are probably subclinical. The disease produced can be classified in two groups: VL (also called kala-azar) and cutaneous leishmaniasis. Cutaneous disease can be divided into Old World cutaneous leishmaniasis, New World cutaneous leishmaniasis and MCL. Less frequent forms include diffuse cutaneous leishmaniasis (DCL), cutaneous recidivans leishmaniasis and post-kala-azar dermal leishmaniasis (PKDL).

Visceral leishmaniasis

The population at higher risk includes children under 5 years of age, immunocompromised patients and those who are malnourished. The incubation period is usually 2–8 months. Most patients do not recall having a suggestive skin lesion during the previous months. The onset is usually as an insidious febrile illness, but can be sudden with a high fever and chills. An enlarged spleen and moderately enlarged liver and lymph nodes can be detected on physical examination. As the disease progresses, signs of anemia, ecchymoses and abdominal discomfort become apparent. Anemia is due to splenic sequestration, hemodilution, bone marrow infiltration, hemolysis and bleeding. Thrombocytopenia and leukopenia are also common. In advanced disease, massive spleen enlargement, emaciation, ascites, subcutaneous edema, diarrhea, bleeding and severe anemia develop.

The mortality of untreated disease is 85–95%. Intercurrent infections are the cause of death in most cases.

Visceral leishmaniasis in immunocompromised patients

Patients who have HIV infection and immunosuppressed transplant recipients have an increased risk of developing the disease.[37] In Southern Europe, the prevalence of leishmaniasis among AIDS patients is 2–9%. This represents 10 000 times the rate in the general population. Several years may elapse from exposure to development of the disease. Immunodepressed patients may develop VL when infected with strains that in immunocompetent individuals cause only cutaneous disease. Atypical manifestations of VL encountered in these patients include localized lesions on bony prominences (Fig. 183.5), tongue, larynx, intestinal tract and lung, and Kaposi's sarcoma. Most of these atypical manifestations have also been recognized in non-AIDS patients who have advanced disease. In immunocompromised patients the disease almost invariably relapses after treatment.[38] Some patients may develop manifestations in atypical locations or localized visceral leishmaniasis mostly in areas where parasitized leukocytes were gathered by an inflammatory response.



Fig. 183.5 Cutaneous manifestation of kala-azar in AIDS.

Old World cutaneous leishmaniasis

There are four cutaneous forms of leishmaniasis with distinctive clinical and immunologic features.

Oriental sore or localized CL appears on the site of the sandfly bite after approximately 2–4 weeks. The lesion begins as an asymptomatic papule, which enlarges and forms a well-circumscribed ulcer with a raised violaceous border. The lesion spontaneously resolves over a period of months, leaving a depressed scar.

Leishmaniasis recidivans presents as a chronic skin lesion that appears on the skin areas where an oriental sore has been. It has also been called 'chronic relapsing CL' and 'chronic lupoid leishmaniasis' because it closely resembles lupus vulgaris both clinically and histologically.[39]

Diffuse cutaneous leishmaniasis is a rare form of diffuse skin infection characterized by heavy parasitization of skin macrophages and absence of an effective cellular response. The lesions do not ulcerate and spread locally and at distant sites.

Post-kala-azar dermal leishmaniasis consists of symmetric macules or nodules on the face and sometimes on the trunk and extremities of patients, months to years after having VL.[40]

New World cutaneous leishmaniasis

Cutaneous leishmaniasis and MCL in the Americas take several clinical forms. The most notorious are chiclero ulcer, uta, cutaneous diseases caused by *Leishmania* (*Viannia*) *braziliensis* and MCL.

Chiclero ulcer caused by *Leishmania mexicana* was originally seen in latex (chicle) collectors who entered the forest. The lesion appears as a destructive ulcer of the pinna of the ear or the face and progresses over months (Fig. 183.6).

In uta, a disease of young children caused by *Leishmania peruviana*, the skin ulcer heals in 3–6 months leaving a disfiguring scar, usually on the face.

The cutaneous diseases caused by *Leishmania* (*Viannia*) *braziliensis* complex consist usually of multiple shallow ulcers that have a tendency to metastasize along the lymphatic vessels. They evolve to MCL in 5% of cases. Untreated, such diseases persist for years. Relapses after treatment are common.

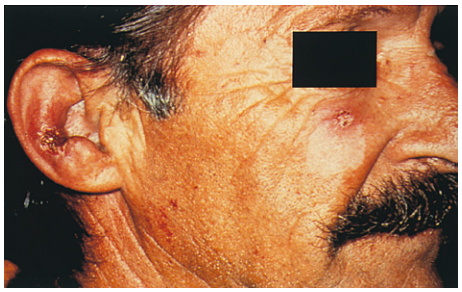


Fig. 183.6 New World cutaneous leishmaniasis (chiclero ulcer).

Mucocutaneous leishmaniasis, a markedly disfiguring, life-threatening disease, is typically caused by *L. braziliensis* and appears during the first 10 years after resolution of cutaneous disease. It consists of a pseudotumoral destructive lesion. It usually starts in the nasal mucosa and spreads to oronasopharyngeal mucosa, larynx and skin of the lips and nose. Death may be due to pneumonia, laryngeal obstruction, secondary sepsis or starvation. Spontaneous cure is rare and response to therapy is poor. Differential diagnoses include carcinoma, syphilis, yaws, rhinoscleroma and rhinosporidiasis.

MANAGEMENT

Treatment schemes can be divided in two groups – those applied to localized CL and those for extensive disease (VL, MCL and DCL).

Most cutaneous sores will slowly resolve spontaneously providing lifelong immunity. Indications for treatment are imported disease infection by *L. braziliensis* complex and sores in potentially disabling sites. Surgical excision and cryotherapy are suitable for small lesions. For larger lesions infiltration of the sore with antimonials is used: 0.5–1.5 ml of parenteral solution is carefully injected into the edges and base of the ulcer and three doses are given 3 days apart. Systemic treatment is recommended for all forms of *L. v. braziliensis* complex infections.

Alternative treatments include topical aminosidine (paromomycin) ointment (once daily for 1 month), and oral allopurinol (5 mg/kg q6h) plus probenecid (500 mg q6h) for 1 month and oral miltefosine (2.5 mg/kg/day for 28 days).

For VL, amphotericin B, antimonials, aminosidine or miltefosine may be used.

The membrane of *Leishmania* amastigotes contains ergosterol. For this reason, antifungals that interfere with the ergosterol pathway can be used in leishmaniasis. New lipid-associated amphotericin compounds are theoretically targeted against *Leishmania* because the amphotericin B–lipid complexes are cleared from blood by the monocellular/macrophagic system where the parasite persists. Liposomal amphotericin at total doses of up to 21 mg/kg are used following different dosage regimens. In Europe is common to use 3 mg/kg during the firsts 5 days followed by one or two more doses at day 10 or at days 14 and 21.

Antimonials, either meglumine antimoniate or stibogluconate sodium, are given at an antimony dose of 20 mg/kg/day. The duration of treatment is 1 month for VL and 2–3 months for DCL and PKDL. If the patient is not thrombocytopenic, intramuscular administration is recommended. Intravenous drug should be infused over 2 hours to prevent cardiac arrhythmia. Other common side-effects are pancreatitis, arthralgia, myalgia, exfoliative rash and abdominal cramps. In areas of India were antimonial resistance is widespread, parenteral administration of aminosidine (11 mg/kg/day intramuscularly for 3 weeks), an aminoglycoside with parasiticidal activity has been used successfully.[41]

Miltephosin is an orally administered drug that given at 2.5 mg/kg/day for 28 days cured 94% of Indian VL cases.[42]

Continuous suppressive treatment with one dose of antimonials every 2–4 weeks is indicated for patients who have HIV infection and leishmaniasis after a full course of treatment. Amphotericin every 2–4 weeks is also being used. Pentamidine might prevent relapses when used as prophylaxis against *Pneumocystis jirovecii* infection. When suppressive therapy was discontinued after successful antiretroviral therapy, relapses were observed only in patients with CD4 counts under 350.[43]

Trypanosomiasis

Trypanosomes produce two clinically and epidemiologically different diseases that are presented separately:

- American trypanosomiasis or Chagas disease; and
- African trypanosomiasis or sleeping sickness.

Table 183.3 Features of human trypanosomes

Species	*T. brucei gambiense* *T. brucei rhodesiense*	*T. cruzi*
Geographic distribution	Sub-Saharan Africa	Central and South America
Name of disease produced	Sleeping sickness	Chagas disease
Main affected organ	Brain	Heart, colon, esophagus
Vector (common name)	*Glossina* (tsetse fly)	*Reduvidae* (kissing bugs)
Mode of transmission	Bite (insect saliva)	Insect feces
Multiplying form in man	Trypomastigote	Amastigote

When a human host is infected, the organisms multiply in the inoculation area, then pass into the blood; in a third phase they localize in some internal organs causing chronic disease.

Trypanosomes pass through the morphologic stages of amastigote and trypomastigote. The differences between the main species that can be found in humans are summarized in Table 183.3.

CHAGAS DISEASE (AMERICAN TRYPANOSOMIASIS)

Chagas disease is named after Carlos Chagas, who discovered *Trypanosoma cruzi* and described its lifecycle and the clinical features of the disease.

The disease starts as an acute febrile illness followed by a long asymptomatic period and a chronic symptomatic stage characterized by distention and abnormal function of the heart and gastrointestinal tract.

Epidemiology

Chagas disease is endemic in most Latin American countries. Low income families of rural areas are most frequently affected and up to 30% of the population have the infection in the most affected regions. The World Health Organization estimates that there are 7 694 500 infected persons.

Hemipterous insects of the Reduviidae family (kissing bugs) are responsible for an estimated 41 200 new cases annually. The parasite has a sylvatic cycle maintained by reduviids that feed on wild animals, and which occasionally bite humans. It may also persist in a domestic cycle maintained by reduviids that live in the cracks of mud huts, feeding on humans, house rodents and domestic animals. In some areas, these two cycles overlap. As asymptomatic individuals may harbour a low number of parasites in their blood for many years, transmission by blood product transfusion, especially platelets, is a major cause for concern.

Vertical transmission occurs in approximately 1–10% of infected mothers, causing more than 14 000 new cases per year. Oral transmission through contaminated food is a less common route of infection.

Pathogenicity

Triatomid bugs infect when taking a blood meal from a host infected by *T. cruzi*. The parasite develops in the gut of the insect and infective trypomastigotes are released with insect feces. *Triatoma* spp. bite humans at night and defecate soon after they are engorged, contaminating the skin with trypomastigotes that penetrate through skin erosions or mucosal surfaces (typically the conjunctiva). The parasite multiplies inside cells as amastigotes. Some amastigotes differentiate into trypomastigotes, which disseminate through the circulation and may eventually be eaten by a reduviid bug.

Although the histopathology of Chagas disease is characteristic, the pathophysiologic mechanism that leads to these changes is not clearly understood. As a result of an inflammatory process (and perhaps a neurotoxin) the conducting system of the heart and the myenteric plexus are damaged. The mononuclear cell response gradually controls the infection, but does not usually eradicate it. In the late stages of the disease, only small numbers of parasites are found in cardiac muscle and the gastrointestinal tract. Neuronal depletion of the myenteric plexus leads to asynchronous peristaltic movement and, in the most severe cases, the esophagus, colon or other internal ducts dilate to enormous size.

Prevention

General improvement in housing standards or at least the use of non-cracking plaster and the regular use of residual insecticides is recommended to eliminate domestic vectors. Organ or blood donors at risk should be serologically screened for trypanosome infection. Maternal screening is aimed to detect newborns at risk.

Diagnostic microbiology

Direct parasitologic diagnosis can be obtained by visualizing the parasite in blood or tissue. Serology is especially useful for screening and for patients who have undetectable parasitemia. Xenodiagnosis is a sensitive method but is slow and cumbersome.

Parasitemia is usually detectable in the acute phase of disease by staining the buffy coat. Needle aspiration or slit-skin smear of inoculation lesions may be positive.

Serology is a reliable sensitive method of diagnosis. It usually becomes positive 1 month after infection and, in untreated individuals, frequently remains positive for life. Cross-reactivity exists with leishmaniasis, malaria, toxoplasmosis, pemphigus foliaceus, infectious mononucleosis, *Trypanosoma rangeli* and mycobacterial infections.

PCR techniques may be especially useful in early detection of infection in newborns, and in assessing suspected cross-reacting serology or combined infections. The potential of PCR in defining a parasitologic cure is a subject of current research.

Clinical manifestations

The disease evolves through three different clinical stages: acute infection, asymptomatic period and chronic disease.

Acute infection is symptomatic in only one-third of those infected. The severity and intensity of signs are greatest in younger patients, but the prevalence of infection increases with age. Symptomatic patients may develop an inflammatory lesion at the site of inoculation and an enlarged satellite lymph node. Manifestations of acute disease are fever, generalized lymphadenopathy, moderate hepatosplenomegaly, erythematous rash and myocarditis. Most patients survive the acute stage and gradually become asymptomatic within a few months. Congenitally infected newborns may be asymptomatic or present with enlarged liver and spleen. Most severe cases develop myocarditis or meningoencephalitis.

The indeterminate form is the asymptomatic period during which the infection can only be detected by serology or PCR, although some degree of esophageal dysfunction or cardiac disease may be found when appropriate diagnostic methods are used.

The chronic symptomatic stage develops in one-third of those infected, usually 5–15 years after infection (Figs 183.7, 183.8). Chronic cardiomyopathy is eventually present in 27%, digestive manifestations in 6% and neurologic disorders in 3% of infected individuals.

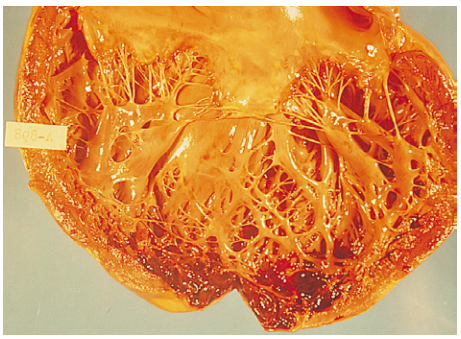


Fig. 183.7 A dilated heart from a fatal case of chagasic cardiomyopathy. Chronic heart disease is the commonest cause of death in Chagas disease. Courtesy of Dr J Cohen, Cordoba, Argentina.

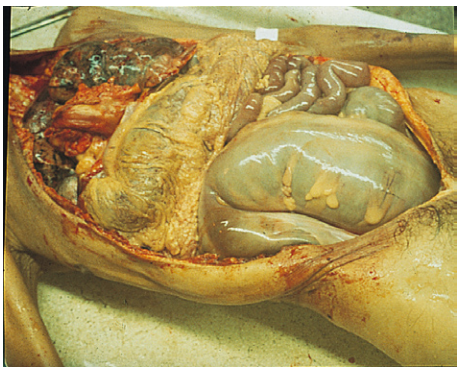


Fig. 183.8 Post-mortem of a patient with chagasic megacolon. Grossly enlarged colon as shown here or other duct dilation is characteristic of chronic Chagas disease. Courtesy of Dr J Cohen, Cordoba, Argentina.

Congestive heart failure, arrhythmias and embolic disease characterize chagasic cardiomyopathy, which is the most common cause of death in Chagas disease.[44] The presence of right bundle branch block or any of these conditions with suggestive epidemiologic data strongly suggest *T. cruzi* infection.

Esophageal disorders include achalasia, cardiospasm and enlargement. Megacolon results in constipation and abdominal pain. Associated complications include esophagitis, esophageal hemorrhage, esophageal cancer, volvulus and obstructive intestinal disease.

Latent disease reactivates in immunocompromised patients. In one series of AIDS patients who had Chagas disease, 87% developed encephalitis and 21% acute myocarditis.

Management

Antiparasitic therapy is recommended at least for the acute and recently acquired forms and parasitemic immunocompromised patients. Recent data suggest that it may also be beneficial in later stages.[45,46] Nifurtimox and benznidazole are the only two parasiticidal drugs routinely being used against *T. cruzi*.

Nifurtimox treatment is seldom available. Benznidazole (5–7 mg/kg/day in two divided doses for 2 months) seems slightly more active and better tolerated.

Pre-emptive benznidazole therapy is recommended for chagasic patients undergoing bone marrow or organ transplantation.[47] Cardiac complications may need a pacemaker or anticoagulation.

Esophageal disease is treated with cardiac dilatation in early cases. Surgery is reserved for severe esophageal or colonic dilatation.

AFRICAN TRYPANOSOMIASIS

Two African trypanosomes of the *brucei* complex infect humans:

- *Trypanosoma brucei gambiense*, which usually produces chronic infections; and
- *Trypanosoma brucei rhodesiense*, which tends to produce a more acute disease.

After an initial stage of parasitization restricted to the bloodstream, some individuals develop encephalitis. One of its most striking symptoms is a somnolence/insomnia disorder after which the disease was named 'sleeping sickness'.

Epidemiology

The vector of *T. brucei* subspecies is a fly of the genus *Glossina* (tsetse fly), which is geographically confined to tropical Africa; 80% of cases occur in Congo. It is estimated that at least 300 000 people are presently infected and 45 000 new cases occur annually.

Several *Trypanosoma* spp. infect wild and domestic animals and the infection is an important economic problem.

Trypanosoma b. rhodesiense is transmitted by *Glossina morsitans*. This fly lives in the dry savannas of East Africa, feeding mainly on the wild ungulates, which represent the main reservoir. *Trypanosoma b. gambiense* is endemic in West Africa. Humans are considered to be the main reservoir. Transmission usually occurs in the shaded riverine areas where *Glossina palpalis* and *Glossina tachinoides* live and breed.

Nature

The vector fly is infected when taking a blood meal containing trypanosomes. The ingested parasites transform and multiply in the insect midgut and migrate to the salivary glands from where new hosts are infected by biting. In the human host the parasite multiplies asexually in the interstitial space and spreads through the lymphatic vessels to the lymph nodes and blood (Fig. 183.9).

Pathogenicity

The parasite can produce hundreds of antigenically different surface glycoproteins serially. This process enables it to elude the host immune

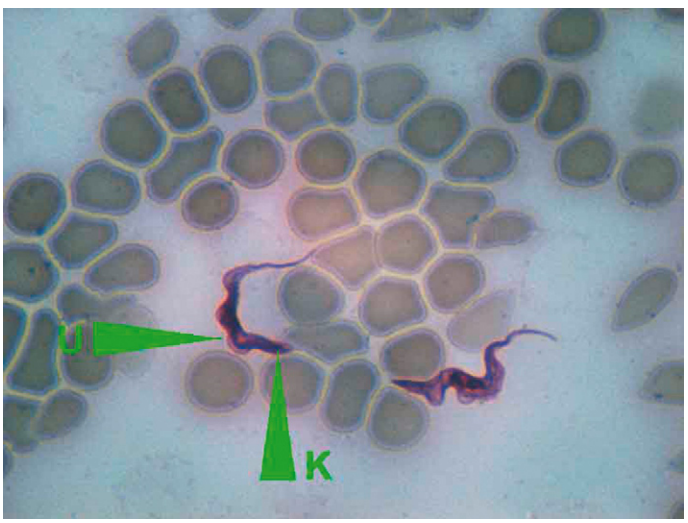


Fig. 183.9 *Trypanosoma brucei* in a Giemsa-stained blood smear. Note the central nucleus on an undulating (U) membrane and a clump of mitochondrial DNA called a kinetoplast (K).

response, resulting in a characteristic fluctuating parasitemia pattern throughout the course of infection. After a variable period, the parasite reaches the CNS, causing meningitis and choroid plexus breakdown. Large amounts of IgM are produced in the serum and cerebrospinal fluid (CSF). Characteristic plasmacytic morular cells can be seen in tissues, blood, bone marrow and CSF. Autoimmune phenomena, circulating immune complex deposition and increased vessel permeability play an important role in pathogenesis.

Prevention

Massive clearance of *Glossina* spp. habitats, insecticide spraying, destruction of wild game, periodic mass treatment and enforced relocation of entire populations were successfully used in the past. A more recent development for control is the use of *Glossina* spp.-attracting traps. Active surveillance is recommended periodically in areas of *T. b. gambiense* and during epidemics in *T. b. rhodesiense* areas. Travelers visiting endemic areas should be warned of the types of activity that may increase the chances of being bitten by tsetse flies and report any possible exposure. Mosquito repellents may be useful.

Diagnostic microbiology

The diagnosis of the disease essentially depends upon the careful repeated microscopic examination of blood or CSF samples. Increased intrathecal synthesis of IgM is a sensitive marker of CNS involvement.[48] Lymph node and bone marrow aspiration may be positive in cases with a low parasitemia. *In-vitro* culture and a miniature anion exchange column test are also feasible. Serologic screening can be carried out with the card agglutination test for trypanosomiasis (CATT) but confirmatory tests are needed.[49] PCR testing is available in some reference laboratories.

Clinical manifestations

In African trypanosomiasis, the disease develops in two stages:

- a hemolymphatic stage that spares the nervous system; and
- a second stage defined by CNS involvement.

The disease produced by *T. b. gambiense* usually develops chronically whereas in *T. b. rhodesiense* infection the disease tend to be more acute, without a clear distinction between stages.

An infective bite by a *Glossina* spp. fly may produce an inoculation chancre. Within days or weeks, the parasite disseminates to the blood. In the Gambian form of the disease, an enlarged neck lymph node may be present (Winterbottom's sign) and the patient may remain asymptomatic for years. In the hemolymphatic stage, the symptoms may be few and nonspecific: undulant fever, headache, malaise, weight loss, anemia, edema, arthralgia, diarrhea and pruritus.

On physical examination there may be erythematous circinate papules on the trunk, disproportionate pain to soft tissue pressure (Kerandel's sign) and discrete enlargement of lymph nodes, liver and spleen. Pancarditis is common in the more acute forms, when it constitutes a major cause of death. Some degree of anemia, thrombocytopenia, leukocytosis, hypogonadism, renal disease and thymus atrophy may be seen.

The CNS manifestation that gave the disease its name is the disappearance of the circadian distribution of sleep and wakefulness, which are therefore fragmented throughout the day and night. Other CNS manifestations include altered reflexes, paresthesiae, pareses, dyskinesia, choreoathetosis, epilepsy, slurred speech, mood changes, lethargy, delirium and psychosis. Without treatment, nearly all patients will develop neural involvement and die.

Management

The choice of the antiparasitic drug will depend upon whether the CNS is infected. Pentamidine or suramin can be used when the CNS is spared from infection. Melarsoprol and eflornithine are effective for both hemolymphatic and neural stages. Suramin dosage is 20 mg/kg/day (maximum 1500 mg), one intravenous injection every 5 days for 25 days. Pentamidine dosage is 4 mg/kg/day, one dose every second day for 20 days. *Trypanosoma b. rhodesiense* and some strains of *T. b. gambiense* do not respond to pentamidine.

Melarsoprol, a toxic trivalent arsenical derivative, has been the treatment of choice for patients who have had CNS involvement for five decades. Relapse after treatment occurs in up to 6% of cases and is reduced by nifurtimox combination therapy.[50] Frequently fatal arsenic-related encephalopathy occurs in 10% of cases and prednisolone treatment may reduce its incidence.

Being much less toxic, intravenous eflornithine (400 mg/kg/day in two divided doses for 7 days) plus nifurtimox (15 mg/kg/day given orally q8h for 10 days) appears to be a promising first-line therapy for second-stage sleeping sickness.[51]

General supportive treatment, anticonvulsant preventive therapy and early recognition and treatment of associated parasitic and bacterial infections are essential.

INFECTION BY OTHER *TRYPANOSOMA* SPECIES

Trypanosoma rangeli is an American parasite transmitted by triatomids. In some areas of Central America, human parasitization by *T. rangeli* is more frequent than that by *T. cruzi*. Although regarded as nonpathogenic to humans, it is medically important as a source of misdiagnosis of Chagas disease.

Animal pathogenic trypanosomes are lysed by apolipoprotein L-I, a component of human high-density lipoprotein (HDL). Patients with very low levels of high-density lipoproteins (such as those with Tangier's disease) may be susceptible to those trypanosomes. Two human cases by *Trypanosoma evansi* in India and *Trypanosoma lewisi* from Thailand have been recently reported.

REFERENCES

References for this chapter can be found online at http://www.expertconsult.com

Chapter 184

HD Alan Lindquist
John H Cross

Helminths

Helminths are the most common parasites infecting humans. The world population is over six billion and there are probably a similar number of helminthic infections occurring in humans. They are transmitted to humans by food, water, soil and arthropod and molluscan vectors. Once established, they can be found in all organs, particularly intestines but also in liver, lungs, blood and occasionally in brain and other organs. This chapter describes some of these worms, their biology, epidemiology, pathogenicity, clinical aspects, diagnosis and prevention.

NATURE

The word helminth comes from the Greek meaning worm and refers to all types of worm, both free-living and parasitic. The major parasitic worms are found primarily in two major phyla:

- the Nematoda or roundworms; and
- the Platyhelminthes or flatworms, which are divided into the Trematoda or flukes and the Cestoda or tapeworms (Table 184.1).

Structurally the helminths have an outer covering called a cuticle or tegument. It may be tough or delicate and essentially protects the organism, especially from digestion, while in the host intestinal tract. This structure may also possess spines, hooks, cutting plates and stylets used for attachment or to aid in penetration. There are also suckers or acetabula used by the flatworms for attachment. Some species have lytic glands near the mouth and the secretions digest host tissue for food or lyse tissues during migration.

The helminths have digestive systems of various types, excretory systems and massive reproductive systems. Most of the trematodes are hermaphroditic, one worm possessing both male and female reproductive organs; the schistosomes, on the other hand, have separate sexes. Each segment of a tapeworm has both male and female sex organs, whereas the nematodes have separate sexes. Some worms produce larvae, but most produce eggs, which pass in the host's excrement. Worms in the circulatory system produce larvae, which are carried in the blood, are ingested by blood-sucking arthropods and are then transmitted to the next host via the bite. The life cycles of the parasites are highly variable, from simple direct egg transmission to complex cycles involving one or more intermediate hosts (Figs 184.1–184.4).

EPIDEMIOLOGY

Helminthic infections are the most common parasites of humans. The highest prevalence occurs in tropical countries with poor or inadequate food supplies, and where insect, molluscan and other invertebrate vectors abound and sanitary conditions are poor.

Nematodes

Intestinal nematodes

Over one billion people, primarily children, are infected with *Ascaris lumbricoides*. In some areas of Indonesia infection rates are as high as 90%.[1] Nearly one billion people are infected with hookworm, *Necator americanus*, *Ancylostoma duodenale* or both. The former has a worldwide distribution, whereas the latter is found in certain parts of Africa, China, India and Japan. Hookworm prevalences are highly variable, being 35% in the Philippines and 90% in Indonesia, with infection rates highest in adult males.[1] Up to 800 million people are infected with *Trichuris trichiura*; both sexes and all age groups are infected, and in the Cameroons 3.1–97.3% of 22 000 children were found to be infected.[2]

Strongyloides stercoralis, like other soil-transmitted helminths, is endemic in areas with high humidity, warm temperatures and poor sanitation. The prevalence of this parasite varies from 10% to 20% in areas of Africa, South America and Asia. In surveys conducted in Indonesia and the Philippines using the Harada–Mori filter paper test-tube technique on single-stool specimens the prevalence rates were usually less than 1%.[1,3] There are reports, however, of rates as high as 25% in areas with concomitant high prevalence of human T-lymphocyte leukemia/lymphoma virus-1 virus infections.[4]

Pinworm or *Enterobius vermicularis* is a common nematode in temperate regions with an estimated 200 million infections worldwide. It is a parasite that most individuals acquire in childhood, but it is not uncommon in adults in a family with infected children. It is a common nematode infection in the USA. A high prevalence of pinworm is also reported in male homosexuals.[5]

Tissue nematodes

Larva migrans is a syndrome caused primarily by nematode parasites of lower animals. Larvae of dog hookworms such as *Ancylostoma braziliensis* and *A. caninum* penetrate human skin and migrate through this tissue causing a creeping eruption or cutaneous larva migrans. This occurs worldwide, but mostly in areas with warm, moist climates. *Ancylostoma caninum* can also cause an enteritis in humans, especially in Australia.[6] The larvae of dog and cat ascarids or *Toxocara* spp. cause visceral larva migrans in children when embryonated eggs are ingested. The larvae provoke granuloma formation, often in the central nervous system (CNS) and eye. Seroprevalence rates for *Toxocara* antibodies are high in England and France.

Although there are now seven recognized species of *Trichinella*, the only species that is important to humans is *T. spiralis*. This species has a worldwide distribution in the temperate regions,[7] whereas the other species are reported from animals in Africa (*T. nelsoni*), Arctic regions (*T. nativa*) and Palearctic and Nearctic regions (*T. pseudospiralis*, *T. britovi*, *T. murrelli*) and the Australian region (*T. papuae*). *Trichinella* spp.

Table 184.1 The major parasitic worms

Nematodes (roundworms)	Intestinal *Ascaris lumbricoides* *Enterobius vermicularis* *Ancylostoma duodenale*	*Necator americanus* *Strongyloides stercoralis* *Trichostrongylus* spp.	*Trichuris trichiura* *Capillaria philippinensis*
	Tissue *Trichinella spiralis* *Visceral larva migrans (Toxocara canis* or *Toxocara cati)* *Ocular larva migrans (Toxocara canis* or *Toxocara cati)* *Cutaneous larva migrans (Ancylostoma braziliensis* or *Ancylostoma caninum)* *Dracunculus medinensis*	*Angiostrongylus cantonensis* *Angiostrongylus costaricensis* *Gnathostoma spinigerum* *Anisakis* spp. (larvae from saltwater fish)	*Phocanema* spp. (larvae from saltwater fish) *Contracaecum* spp. (larvae from saltwater fish) *Capillaria hepatica* *Thelazia* spp.
	Blood and tissues (filarial worms) *Wuchereria bancrofti* *Brugia malayi* *Brugia timori* *Loa loa*	*Onchocerca volvulus* *Mansonella ozzardi* *Mansonella streptocerca* *Mansonella perstans*	*Dirofilaria immitis* (usually lung lesion; in dogs, heartworm) *Dirofilaria* spp. (may be found in subcutaneous nodules)
Platyhelminthes (flatworms) Cestodes (tapeworms)	Intestinal *Diphyllobothrium latum* *Dipylidium caninum*	*Hymenolepis nana* *Hymenolepis diminuta*	*Taenia solium* *Taenia saginata*
	Tissue (larval forms) *Taenia solium* *Echinococcus granulosus*	*Echinococcus multilocularis* *Multiceps multiceps*	*Spirometra mansonoides* *Diphyllobothrium* spp.
Trematodes (flukes)	Intestinal *Fasciolopsis buski* *Echinostoma ilocanum*	*Heterophyes heterophyes* *Metagonimus yokogawai*	
	Liver/lung *Clonorchis (opisthorchis) sinensis* *Opisthorchis viverrini* *Fasciola hepatica*	*Paragonimus westermani* *Paragonimus mexicanus* *Paragonimus heterotremus*	*Paragonimus skrjabini* *Paragonimus* spp.
	Blood *Schistosoma mansoni* *Schistosoma haematobium*	*Schistosoma japonicum* *Schistosoma intercalatum*	*Schistosoma mekongi*

Adapted from Garcia LS. Classification of human parasites. Clin Infect Dis 1997;25:21–3.

are ubiquitous and found in a wide variety of animal life. Transmission occurs among animals by the ingestion of *Trichinella*-infected meat. All types of carnivous mammals and birds are susceptible to infections. Most human infections are obtained by eating infected pigs and wild animals. Reports of trichinosis are sporadic, usually among groups who have participated in a communal meal. An epidemic in France reported several thousand with some deaths after the consumption of 'steak tartare' prepared from imported horse meat.[8]

The 'firey serpent of Medina' or *Dracunculus medinensis* (also known as the guinea worm) is reported from 17 African countries, Yemen, Saudi Arabia, India and Pakistan. Ten million people may be infected, and in some areas 50% of a population may have the infection every year. Sex prevalences are variable, but most infection occurs in those aged 15–40 years. Transmission is seasonal and closely related to rainfall, when the population of the major copepod vectors (*Cyclops* spp.) are abundant. Education programs are underway to eradicate the parasite.[9]

Angiostrongyliasis, or parastrongyliasis, is caused by the molluscan-borne nematodes *Angiostrongylus cantonensis*, the rat lung worm and *A. costaricensis*. The former is endemic in rats (*Rattus* spp.) in Asia, the Pacific Islands, Australia, India, Africa, the Caribbean and Louisiana and Florida in the USA. *Angiostrongylus costaricensis* is reported in Latin America and Texas, but most human infections are reported from Costa Rica. Human infection with *A. cantonensis* is reported primarily from Taiwan and Thailand, with infection acquired from eating the snails *Achatina fulica* in Taiwan and *Pila ampullacae* in Thailand. In Taiwan most infections are in children of both sexes while in Thailand the parasitosis is seen mostly in adult males. Two human infections have been reported in Louisiana.[10–12] *Angiostrongylus costaricensis* is usually seen in children who accidently ingest the slug *Vaginulus plebeius* on vegetation. Rats (*Rattus* spp.), cotton rats (*Sigmodon* spp.) and rice rats (*Oryzomys* spp.) are natural hosts for *A. costaricensis*.

Anisakiasis is caused by third-stage larvae of *Anisakis simplex* and *Pseudoterranova decipiens*, which are acquired by eating species of raw marine fish and squid. The adult worms, related to *Ascaris*, are found in marine mammals worldwide, but most infections in humans have occurred in Japan and other countries where people eat uncooked marine fish and squid.

Gnathostoma spp. larvae can invade human tissue and most infections are reported from Thailand and Japan, and occasionally Mexico. *Gnathostoma spinigerum* is the most common species reported. *Gnathostoma hispidium* has also been found in humans. Dogs and cats are the natural hosts for adult worms. Copepods serve as the first intermediate host, and fish, frogs, snakes and chicken are the second intermediate hosts. Infections are more common in adults of both sexes.

Blood and tissue nematodes (see Chapter 115)

The filarids are a group of nematodes located in the lymphatics, subcutaneous and cutaneous tissues. There are seven major filarids of humans transmitted by arthropods in tropical and subtropical parts of the world.

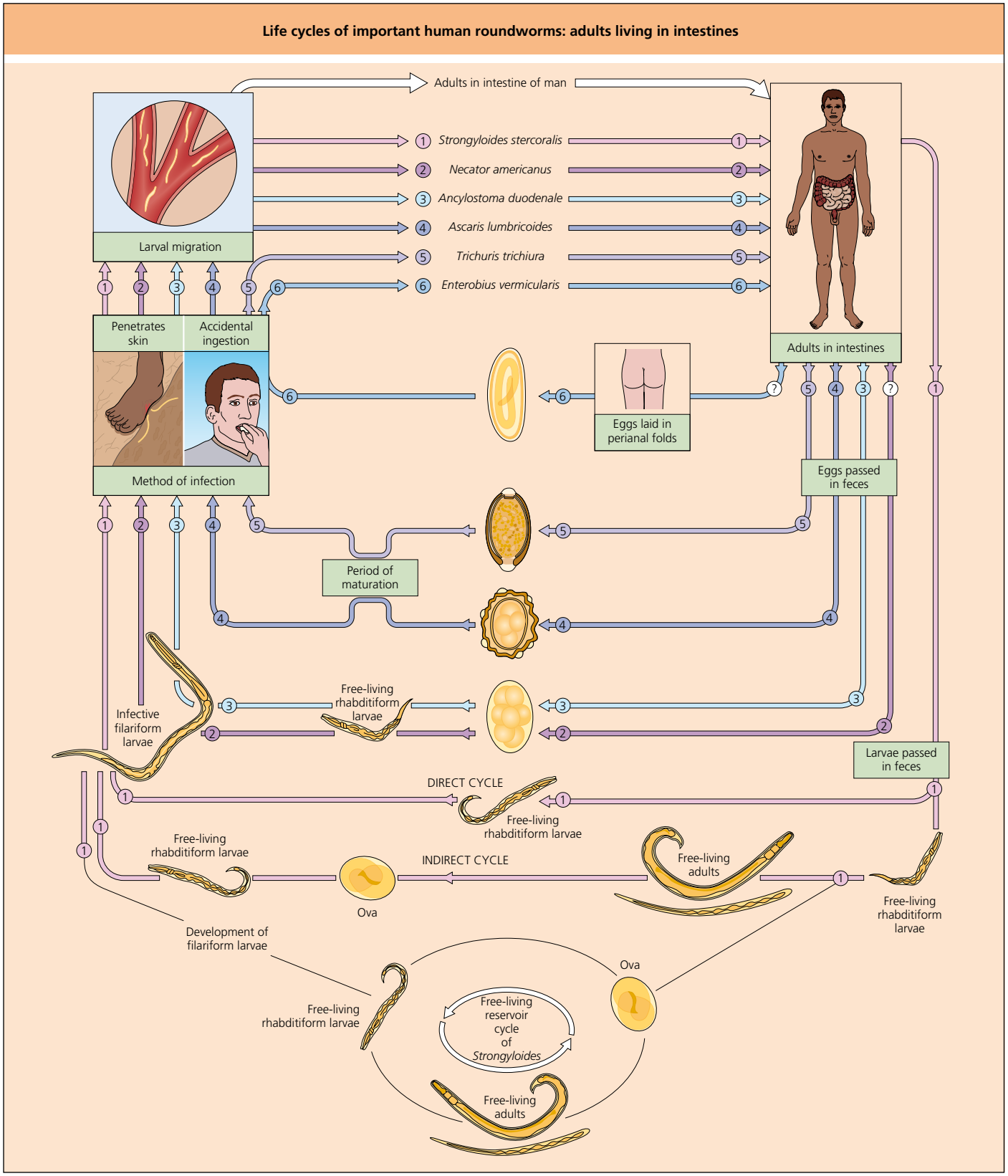


Fig. 184.1 Life cycles of important human roundworms: adults living in the intestines.

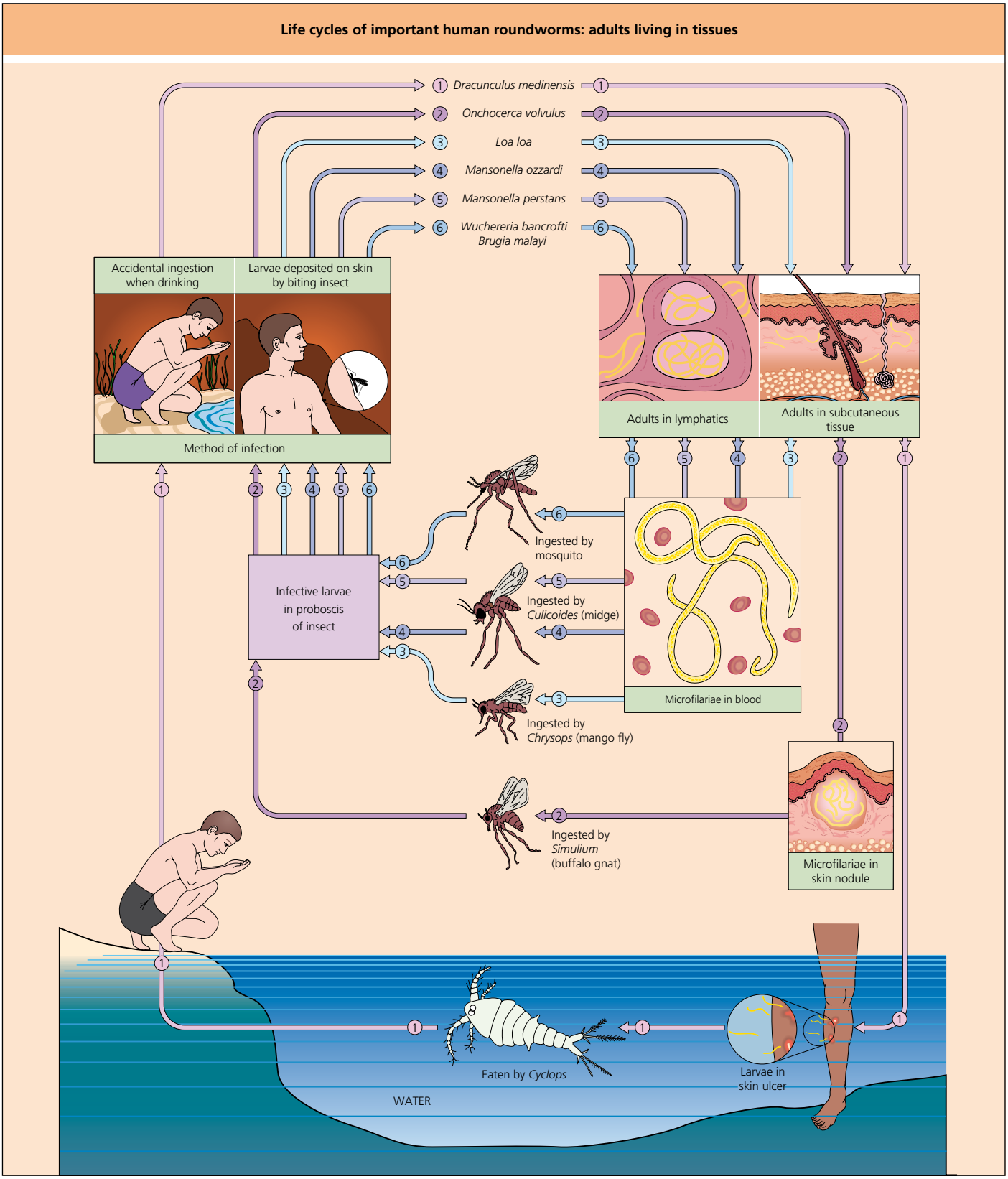


Fig. 184.2 Life cycles of important human roundworms: adults living in tissues.

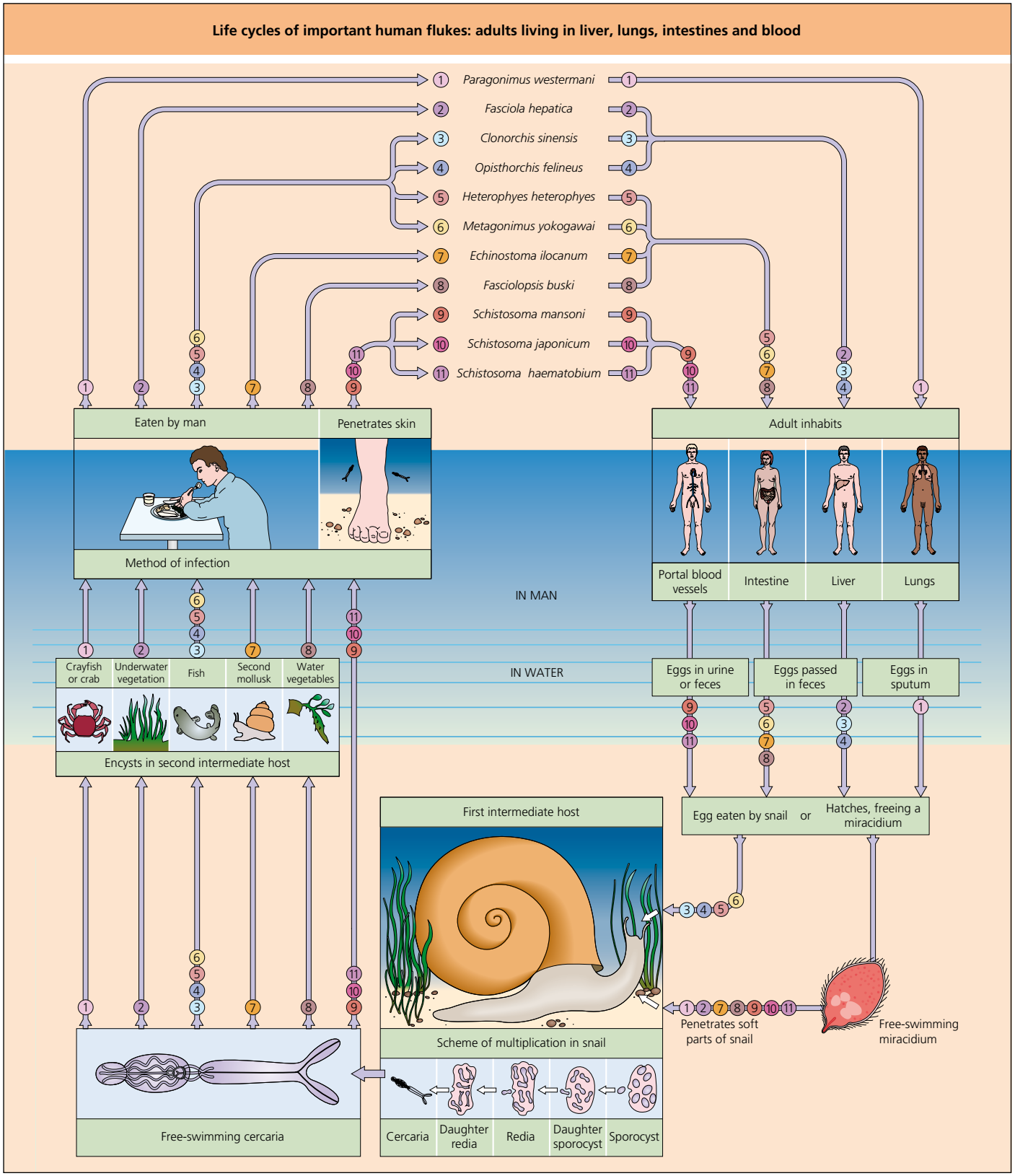


Fig. 184.3 Life cycles of important human flukes: adults living in the liver, lungs, intestines and blood.

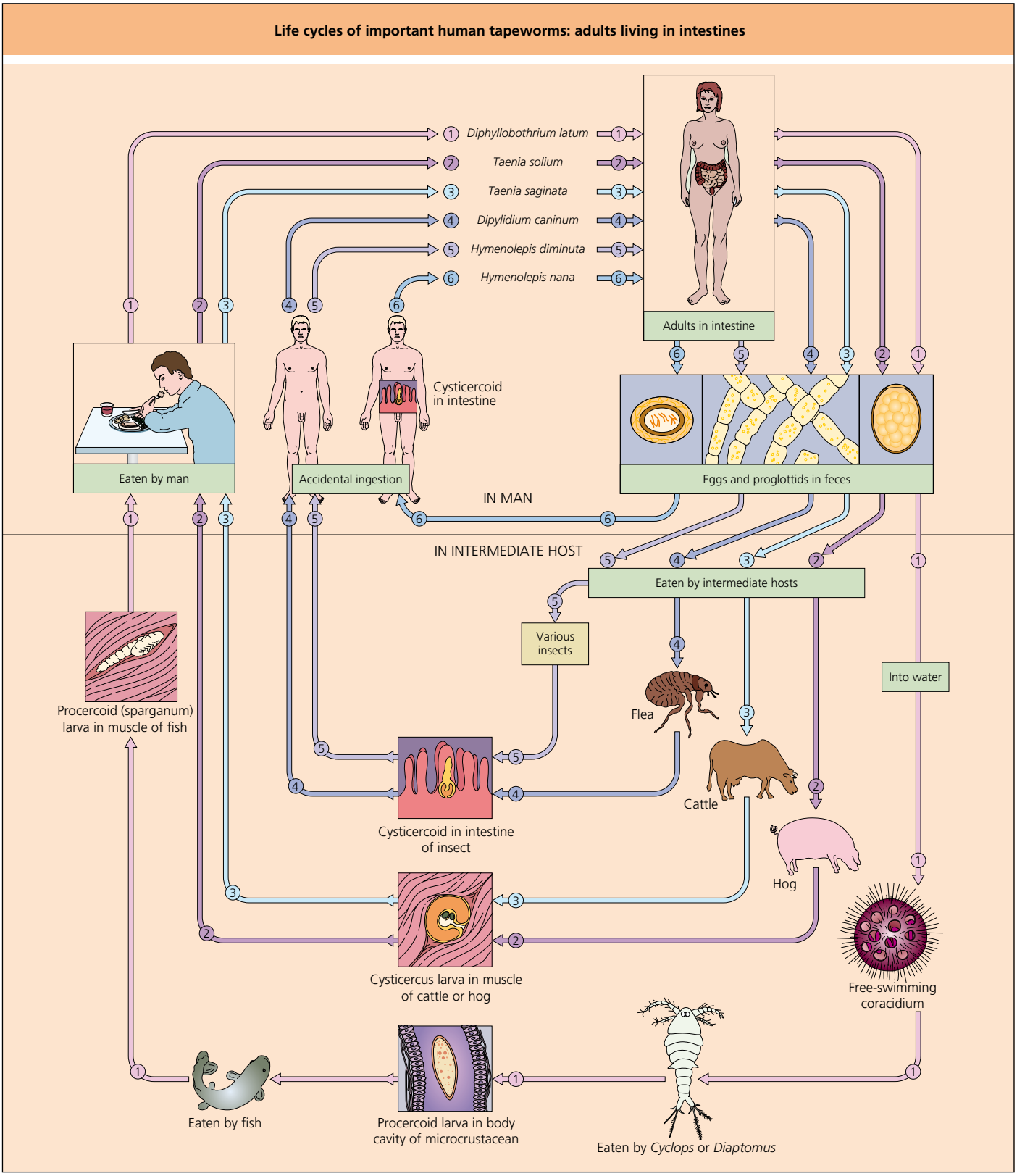


Fig. 184.4 Life cycles of important human tapeworms: adults living in intestines.

Wuchereria bancrofti is the most widespread, being endemic in Asia, Africa, Central and South America and the Pacific Islands. Prevalence rates are highly variable depending upon the vector mosquito population, temperature, humidity, a susceptible human population and environmental sanitation. An estimated 115 million people may be infected worldwide, with 50 million in Africa, 62 million in Asia, 2 million in the Pacific Islands and less than 1 million in Latin America.[13] There are different vectors in different endemic areas; the most extensive is *Culex quinquefasciatus*, whereas *Aedes polynesiensis* is the important vector in the Pacific Islands. There is a nocturnal periodicity associated with the feeding habits of the mosquitoes, except in the Pacific where there is a diurnal periodicity of microfilariae and the mosquito vector feeds in the daytime. Although a limited number of monkeys have been experimentally infected with *W. bancrofti*,[14] no naturally infected animal reservoirs have been documented.

Brugia malayi is found only in rural Asia and infects approximately 13 million people.[13] Many of the islands of Indonesia are endemic, with prevalence rates from less than 1% to over 30%.[15] The major mosquito vectors are *Mansonia*, *Anopheles* and *Aedes* spp. and cats, and *Macaca* and *Presbytis* spp. monkeys are known reservoir hosts.

Brugia timori is found only in the Lesser Sunda Islands of Indonesia. A prevalence rate of 25% was reported in a population on the island of Flores.[16] *Anopheles barbirostris* is a major mosquito vector and, although no naturally infected reservoirs have been found, cats and Mongolian gerbils have been experimentally infected.[17]

Onchocerca volvulus is endemic in 26 countries in Africa and six in Central and South America. Approximately 18 million people are infected and as a result 270 000 are blind and 500 000 severely disabled.[18] Infections are more common in males than females, reflecting male occupations and their higher exposure to vector flies. Prevalences are also higher in areas close to running water where the blackfly *Simulium damnosum* and related species breed. In the Americas infections are more common in the highlands 1000–4000 feet above sea level, whereas in Africa infection is common below 1000 feet. Infections decrease with distance from the rivers and streams. There are no animal reservoirs and there is no microfilarial periodicity.

Loa loa, the African eye worm, is endemic in West and Central Africa where *Chrysops* spp. are present. Prevalence rates vary from 8% to 40%, with infections increasing with age; 20–30 million people live in endemic areas. Transmission by the day-biting female deerflies is highest during wet seasons. Monkeys serve as reservoir hosts. The microfilariae are diurnal.

A unique feature of filarids is the dependency upon *Wolbacter* spp., a intracellular bacterial symbiont associated with pathogenesis and fertility.[19,20]

Trematodes (see Chapter 113)

Over 40 million people worldwide are infected with food-borne trematodes; the most important occur in the liver, lungs, blood and intestines.

Liver/lung trematodes

The Chinese liver fluke, *Clonorchis sinensis*, is reported from China, Japan, Korea, Taiwan and Vietnam. Infections are highest in older males and females and there is a tendency toward familial aggregations. An estimated 7 million people are infected, with prevalence rates in China of 15–24.6%, in Korea 11.7–30.8% and in Japan 2.9%. Infections are highest in the snail and fish intermediate hosts in the warmer months. Snails in the families Hydrobiidae, Melaniidae, Assimineidae and Thiaridae are first intermediate hosts, and fish, primarily of the family Cyprinidae, are second intermediate hosts. *Opisthorchis viverrini* is endemic in Thailand, Laos and Vietnam. The infection is found in an estimated 10 million people in all age groups, increasing from childhood to adulthood, and in both sexes. Prevalences vary from 7.3% to 53.2% in Thailand. *Bithynia* spp. are important snail hosts and *Cyclocheilichthys* spp. freshwater fish are important second intermediate hosts. Dogs, cats and other fish-eating mammals are reservoir hosts. Over 10 million people are infected with *O. felineus* in Europe and Siberia.[21]

Fasciola hepatica, the sheep liver fluke, is endemic in most sheep-raising areas of the world. Human infections are increasing and are most common in parts of Europe, South America and the Middle East. Approximately 2.4 million people are infected. Sheep, goats and cattle are the natural hosts, with infection rates varying from 25% to 92%. The prevalence in humans reaches 65% in Bolivia, 24–53% in Ecuador, 10% in Peru and 2–17% in Egypt.[21] Infections are more common in adults who acquire infection from eating watercress salads.

Although over 40 species of *Paragonimus* are described, only a few infect humans and the Oriental lung fluke, *Paragonimus westermani*, is responsible for the most serious disease. It is reported from China, Taiwan, Japan, Korea, the Philippines and Thailand. Other species occur in China, Thailand, Mexico, Ecuador, Peru and parts of Africa. The parasitosis is acquired by eating metacercariae-laden crabs and crayfish. The juice from crabs is used for seasoning and traditional medicine. The juice may contain metacercariae. There are 20 million suspected infections in China, and over 600 000 elsewhere.[21] There are many snail hosts, with *Semisulcospira* spp. being major vectors in China and Korea. The crabs *Eriocheir* and *Potamon* spp. and the crayfish *Cambaroides* spp. are important second intermediate hosts. Dogs, cats and other carnivorous mammals are reservoir hosts.

Intestinal trematodes

There is a very large number (about 70) of intestinal flukes reported mostly in Asians who acquire infections because of eating raw food. *Fasciolopsis buski*, the giant intestinal fluke, is reported from China, Taiwan, Thailand, Bangladesh and India and occasionally from Indonesia. Over 200 000 infections have been reported in China and 10 000 in Thailand.[21] Pigs are the reservoir host, and snails such as *Segmentina*, *Hippeutis* and *Gyraulus* spp. are important vectors. Cercariae from snails encyst on water plants such as water caltrop, watercress, water bamboo and water chestnut which are eaten uncooked.

Other important intestinal flukes include *Echinostoma*, *Heterophyes* and *Metagonimus* spp. There are an estimated 150 000 cases of echinostomiasis, 240 000 cases of heterophyiasis and 650 000 cases of metagonimiasis, most in China, Korea and Japan. Echinostome metacercariae are acquired from snails, fish and other aquatic animals, whereas *Heterophyes* and *Metagonimus* infective stages occur in fish. All ages and both sexes are infected as a result of eating the second intermediate hosts uncooked.

Blood trematodes (see Chapter 112)

Schistosomiasis is endemic to many tropical areas of the world:

- *Schistosoma japonicum* is found in Asia;
- *S. haematobium* and *S. mansoni* in Africa and the Middle East; and
- *S. mansoni* in South America and some islands in the Caribbean.

Other species of less importance are *S. mekongi* and *S. intercalatum*, which are focally located in South East Asia and Africa, respectively.

It is estimated that there are over 200 million infections, with the prevalence and intensity of infections being highest in children aged 5–15 years. Infection rates depend upon water contact in endemic areas. Despite the widespread distribution of snails and frequent opportunities for water contact, high transmission only occurs at a few sites. It is important to identify these sites.[22] Snail vectors are members of the genera *Bulinus* spp. for *S. haematobium*, *Biomphalaria* spp. for *S. mansoni* and *Oncomelania* spp. for *S. japonicum*. *Bulinus* and *Biomphalaria* spp. are strictly aquatic whereas the *Oncomelania* are amphibious snails. There are some animal hosts for *S. mansoni*, but humans are the most important source of infection. Humans are also the most important host for *S. haematobium*, whereas many domestic and wild animals are a reservoir host for *S. japonicum*. Although *S. japonicum* is endemic on Taiwan, the strain of parasite on the island will not infect humans. It is considered to be a zoophilic strain.[23]

Cestodes (see Chapter 113)

Cestode or tapeworm infections are acquired by ingestion of intermediate hosts containing the infective larval stages:

- the beef tapeworm *Taenia saginata* and pork tapeworm *T. solium* are acquired by ingesting the cysticercus stage in beef or pork;
- the fish tapeworm *Diphyllobothrium latum* is acquired by eating raw fish infected with the plerocercoid or sparganum stage; and
- *Hymenolepis nana* and *H. diminuta* are rodent tapeworms that use fleas and beetles as intermediate hosts.

Taenia saginata is reported worldwide, with high infection rates in Africa and Asia. Most infections occur in adults. The cysticercus is found only in bovids; however, in parts of Asia, especially in Taiwan, pigs are a recognized source of infection for a strain of the parasite, namely *T. saginata* (*asiatica*).[24]

Taenia solium is found sporadically in pigs worldwide, with many human cases reported from Mexico and Central and South America, South West Asia and Africa. Human cysticercosis is also present in these areas. Certain areas of Irian Jaya in Indonesia report many cases of cysticercosis because of the habit of eating uncooked pork.

Hymenolepis nana is the most common tapeworm in North America and is particularly reported in children worldwide. *Hymenolepis diminuta* is not common but reported occasionally in South East Asia.

There is a high prevalence of *D. latum* in humans in Scandinavia, Finland, Alaska, Canada and Northern USA. Other species of *Diphyllobothrium* are reported from Japan and South America.

Sparganosis or infections with the larval stage of *Spirometra* spp. is reported occasionally from many parts of the world. Infection is acquired by ingesting copepods or second intermediate host (frogs, toads or other aquatic animals). Infections in southern Asia are attributed to the use of fresh animal tissues as poultices.

Echinococcus granulosus and *E. multilocularis* are associated with human hydatid disease, whereas *E. vogeli* and *E. oligarthrus* rarely cause disease in humans. *Echinococcus granulosus* is the most important and found primarily in sheep-raising areas. Strains of the parasite are also found in goats, swine, cattle, horses and camels.

The parasitoses are found worldwide, with canines serving as the definitive host and animals such as sheep as intermediate hosts. Humans acquire infection by ingesting eggs from dogs, whereas dogs acquire the infection by eating sheep liver and other organs containing cysts with large numbers of scolices. Infections usually occur in the young and the cysts develop over a period of years.

Echinococcus multilocularis has a limited distribution in dogs, foxes, wolves and cats as the reservoir hosts and larval stages in wild rodents, especially voles. The parasite, however, is reported to be moving southward in the USA. Rare human infections are acquired by the ingestion of eggs passed by the canines. Infection with any of the *Echinococcus* spp., however, is related to poor sanitary conditions in populations with a low level of education and in people closely associated with canine reservoir hosts.

PATHOGENICITY

Helminths seem to live in peaceful coexistence with their hosts and usually cause few problems. However, if there are many worms, there may be severe disease. The parasites, especially in the intestines, can cause obstruction and possibly perforation. The worms also secrete or excrete toxic substances, which affect the tissues. Lytic substances, such as those secreted by hookworms to obtain blood, can induce inflammation and there may be changes due to malabsorption and competition for nutrients. Larval stages may be more pathogenic, secreting antigenic substances that cause hypersensitivity, while the antigens promote antibody production and cellular immune responses. Worms in the wrong place can be highly pathogenic.

Helminths have intricate life cycles and disease is usually associated with the larval migratory pathways and the final habitat of adult worms in the host. The amount of pathology and disease varies with the parasitosis.

Nematodes living in the intestine (see Chapter 108)

Enterobius vermicularis

The life cycle of *Enterobius vermicularis* is presented in Figure 184.1. Pinworm resides in the large bowel, where mating occurs. Gravid females migrate out of the anus and deposit eggs on the perianal skin. Eggs under the fingernails in children lead to re-infection when the fingers are placed in the mouth. Eggs can be released onto the bedsheets and become disseminated throughout the household when the sheets are shaken. The eggs will survive for 2 weeks in a humid and cool environment. When eggs are ingested they hatch in the intestine and the larvae migrate to the large intestine to mature in 6 weeks.

Ascaris lumbricoides

Infection with *Ascaris lumbricoides* can occasionally be highly pathogenic. The life cycle is presented in Figure 184.1. The adult male worm may measure 15–31 cm by 2–4 mm and the female 20–35 cm by 3–6 mm. The female can live for a year or more and produce 240 000 eggs/day. The eggs pass in the feces and embryonate in the soil in 10–14 days. Upon ingestion the egg hatches in the intestine and the liberated larva penetrates the mucosa and passes to the liver via the portal vessels and then to the lungs. After a few weeks the larva penetrates the alveolar air sac, passes up the pulmonary tree, is coughed up and swallowed and becomes sexually mature in the small intestines in 60–75 days.

Trichuris trichiuria

Trichuris trichiuria has a direct life cycle (see Fig. 184.1). Adult females in the large intestine deposit eggs in the fecal stream and after a few weeks in the soil they become embryonated. When eggs are swallowed they hatch in the intestine and the larva moves down the bowel to the colon. The worms bury their narrow anterior end into the mucosa with the wider posterior end extended freely into the lumen of the colon. The females lay eggs after 3 months and produce 3000–10 000/day. The females measure 35–55 mm and the males 30–45 mm.

Hookworms

A number of hookworm species can enter the human body, but most are natural parasites of animals other than humans (i.e. zoonotic parasites). The species from lower animals usually cause a creeping eruption or cutaneous larva migrans. Only two hookworms are considered to be important human species:

- *Necator americanus*; and
- *Ancylostoma duodenale*.

Both species measure about 10–13 mm for females and 6–11 mm for males and can be 0.4–0.6 mm in width. The anterior mouths have cutting plates or teeth and the males have bell-shaped bursal rays at the posterior end that are used for holding females during copulation. The vulva is mid-central and eggs pass from the females into the fecal stream and out with host feces onto the soil. Female worms pass 5000–10 000 (*N. americanus*) and 10 000–20 000 (*A. duodenale*) eggs/day. In warm, moist climates the eggs embryonate in the soil and rhabditform larvae are released. Within a few days the larvae molt and develop into filariform larvae or the infective stage. The larvae climb to the top of the soil or grass, cling together and wait for a human host to come by. They usually penetrate the skin between the toes or other body surfaces. Some *A. duodenale* larvae may be ingested in water and enter the oral mucosa. The larvae are carried by the blood to the heart and then the lungs where they remain for a period, break out into the alveoli, pass up the pulmonary tree and are swallowed. The parasite matures in the small intestine, copulates and produces eggs in about 5 weeks (see Fig. 184.1).

The infections can remain active for as long as 13 years. The attachment of adult worms can cause small erosive lesions, hemorrhage, tissue cytolysis and neutrophilic infiltration. The worms change

attachment sites regularly, leaving old sites oozing blood and plasma. Iron deficiency anemia and blood loss result from long-term infection with a large number of worms.

Pathologic changes occur in the bone marrow due to blood loss. Liver function may change as a result of anemia, as well as reduced capacity for albumen synthesis. Hookworm disease with iron deficiency anemia, hypoproteinemia and hepatosplenomegaly may contribute to thousands of deaths each year.

Strongyloides stercoralis

Strongyloides stercoralis is a unique nematode having a parasitic life cycle and a free-living cycle, with males and females present in the free-living cycle (see Fig. 184.1) and only females present in the parasitic life cycle. The parasitic females measure 1.5–2.5 mm and have a long cylindric esophagus. The vulva is in the posterior third of the body and the paired uteri contain thin-shelled eggs.

The eggs are deposited in the small intestinal epithelium and hatch soon after release. The first-stage or rhabditiform larva passes in the feces and develops into a second-stage and then a third or infective-stage filariform larva after a few days. The third-stage larvae penetrate the skin and migrate through the body and via the blood to the lungs; after several days they migrate up the respiratory tree and are swallowed. The larvae enter the intestinal mucosa and mature, and the female worms produce eggs parthenogenetically. Some larvae transform into infective forms in the bowel, penetrate the mucosa, migrate and develop into adults. This is internal autoinfection and can lead to hyperinfection in the immunocompromised host. Some rhabditiform larvae transform into rhabditoid free-living male and female adults in the soil and reproduce. The eggs hatch, releasing rhabditiform larvae, which develop into filariform larvae that can enter the skin of a host.

Cellular immunity is responsible for keeping infections under control, but when immunity is affected by disease or immunosuppression, the parasite multiplies unabated and this leads to hyperinfection and dissemination.

Capillaria philippinensis

Intestinal capillariasis is a disease in which the parasite can multiply in the digestive tract. *Capillaria philippinensis* is a tiny worm; females measure 2.5–5.3 mm and males 1.3–3.9 mm. The anterior body is narrow and the posterior is slightly wider and contains reproductive organs and digestive tract. The females deposit eggs, which must reach water where they embryonate. When these are eaten by small freshwater fish the eggs hatch and the larvae develop into infective stages in 3 weeks. When humans eat the fish the larvae mature in 2 weeks. After mating the females first produce larvae that will mature in the gut, whereas the second-generation worms produce thick-shelled eggs, which pass out in the feces. The parasites can multiply rapidly, producing thousands of progeny.[25] If the patient is not treated the disease can be fatal. Over 200 000 worms were recovered from 1 liter of bowel fluid at one autopsy. The worms enter the crypts and cause atrophy, the villi are flattened and denuded, the mucosal glands are denuded and the lamina propria is infiltrated with inflammatory cells. Other organs are also affected by malnutrition and hypokalemia. Most of the pathology is in the jejunum.[26]

Nematodes living in tissues

Trichinella spiralis

Trichinella spiralis is related to other trichiurids in having a slender anterior and a wider posterior end. Female worms measure 2–4 mm and males 1–1.5 mm. Infections are acquired by eating uncooked muscle containing encysted larvae from infected animals, usually pigs.

The larvae are digested from the cyst, pass to the small intestines and burrow beneath the epithelium where they develop into adults, re-enter the gut lumen and reproduce. The larvae produced enter the gut wall, are picked up by the blood and carried throughout the body to striated muscle where they become encysted. The larvae entering the muscle cell cause alterations in morphology, which results in the characteristic 'nurse cell'. Morphologic and molecular changes occur in the cell until it eventually becomes calcified. The larvae can remain alive for many years in the cell. Chronic inflammatory cells may surround the parasitized muscle cell.

Filarial nematodes

The filarial nematodes are long and slender and are found in the lymphatics, tissues and body cavities. The life cycles of some of the filarids are presented in Figure 184.2. Microfilariae are produced by female worms and arthropods are the vectors (see Chapter 115).

Wuchereria bancrofti, Brugia malayi *and* Brugia timori

The lymphatic filarids *W. bancrofti*, *B. malayi* and *B. timori* produce microfilariae that usually appear in the blood between 2200 and 0200 hours (nocturnal periodicity). *Wuchereria bancrofti*, found in some of the Pacific Islands, produces microfilariae that appear in the blood in the daytime (diurnal periodicity). Mosquitoes obtain blood at night and the larvae develop into the infective stage in 10–14 days. At the next feeding the infective larvae migrate from the thoracic muscles of the mosquito to the proboscis and crawl into the hole made by the bite. The larvae migrate in the host until they reach the definitive habitat and develop into adults. The worms copulate and the females produce microfilariae; the life cycle of *W. bancrofti* is a few months longer than that of *B. malayi*. Infections may persist for several years.

The pathogenesis of lymphatic filariasis has been a matter of debate and a model integrating various aspects of the parasitosis has been proposed.[27] It has also been suggested that *Wolbachia*, an endosymbiont of filarids, may be associated with the pathogenesis.[19,20] On the other hand, the pathology of lymphatic filariasis may be associated with immunologic responsiveness. Some people who have the infection are microfilaremic but without antibodies or disease, whereas other are amicrofilaremic and have antibodies. There is an inflammatory stage with lymph channel irritation. Adult worms can be found in the lymph vessels, primarily the axillary, epitrochlear, inguinal and pelvic nodes as well as those in the testis, epididymis and spermatic cord.

Onchocerca volvulus

Onchocerca volvulus adults are coiled in fibrous tissue nodules in the subcutaneous tissue. The females may reach 50 cm in length and 0.5 mm in diameter and the microfilariae produced are released into the interstitium of the skin. Males are smaller, being 5 cm in length. Microfilariae are picked up by biting flies or black flies of the genus *Simulium* and after developing to the infective stage the larvae are introduced into a host at the next feeding. The larvae migrate through the tissue and after many months settle down, usually in pairs, and become encapsulated (see Fig. 184.2).

The microfilariae leave the nodule and migrate through the dermis, provoking a dermatitis and blindness.

Loa loa

The African eye worm *Loa loa* actually moves through subcutaneous tissue and often traverses the conjunctiva. The males are 3–4 cm and the females 4–7 cm in length and 3–5 mm wide. Microfilariae have a diurnal periodicity and are ingested by species of *Chrysops* at the time of a blood meal. The larvae develop to the infective stage in 10–13 days and enter the host through the hole made by the bite at the next feeding (see Fig. 184.2). The adult worms cause little damage as they migrate quickly through the tissue.

Dracunculus medinensis

Dracunculus medinensis is similar to the filarids. The females measure up to 120 cm long and 2 mm wide and the males 2 cm. The worms mature in the connective tissue; gravid females then migrate to the

subcutaneous tissue where they cause ulcers through which larvae are released when the lesion is immersed in water. The larvae are ingested by copepods and develop into infective larvae in 2 weeks. When the copepods are ingested in drinking water the larvae are liberated, penetrate the digestive tract and develop into adults in the connective tissue in a year (see Fig. 184.2).

Angiostrongylus cantonensis

Angiostrongylus cantonensis is found naturally in the lungs of rats. The larvae produced by the females move up the pulmonary tree, are swallowed and pass out in the rat feces. Mollusks (snails and slugs) serve as intermediate hosts and, when eaten by rats, the larvae are digested out of the snail tissue, migrate from the gut to the brain, and after 3 weeks migrate to the lungs. Larvae are produced by adult females a few weeks later. Humans who ingest snails or slugs acquire the infective larvae, which migrate to the CNS and cause eosinophilic meningitis. The females reach 2–3 cm and the males 1–2 cm in length and 0.2–0.3 mm in width. In most human infections the larvae die in the brain and cord and elicit an inflammatory reaction in the meninges. Eosinophils, monocytes and foreign body giant cells infiltrate around the dead worms. Tissue necrosis is also evident. Larvae are reported in the eye, and are often recovered from cerebrospinal fluid (CSF). Adult worms have been found on a few occasions in the pulmonary artery.[10]

Anisakis simplex and *Pseudoterronova decipiens*

Anisakis simplex larvae measure 2–3 cm by 0.3–0.6 mm and *Pseudoterranova decipiens* 2–3 cm by 0.3–1.2 mm. They are found in the muscle and body cavity of marine fish and squid. When eaten by marine mammals (e.g. whales, seals, porpoises) the worms mature in the stomach and the females deposit eggs in the feces. In the ocean, a larva hatches from the egg and is eaten by a small marine crustacean. When the crustacean with a third-stage larva is eaten by fish, the freed larva migrates to the abdominal cavity. When infected fish are eaten raw by humans the liberated larvae penetrate the stomach or small intestine and provoke a foreign body reaction around the worm.

Gnathostoma spinigerum

Gnathostoma spinigerum is a robust nematode with a large globose head surrounded by rows of spines. The females measure 1–1.5 cm by 1–2.5 mm and the males 1–2.5 cm by 1–2 mm. The adults are coiled in the wall of the digestive tract of dogs and cats. Eggs pass in the feces, embryonate in fresh water and are ingested by copepods. The larvae hatch and develop into the second larval stage. When the copepod is eaten by a second intermediate host, the third-stage larva develops, and when this is eaten by a dog or cat the worm penetrates the gut wall and matures. Humans who eat infected fish, frogs or other aquatic food, raw or fermented, acquire the infection and the larvae migrate through the tissue in a fashion similar to that of cutaneous larva migrans. The CNS may be invaded, resulting in an eosinophilic myeloencephalitis with eosinophilic pleocytosis and bloody and xanthochromic CSF. The eye may also be invaded, causing palpebral edema, exophthalmos and subconjunctival hemorrhage.

Larva migrans

Cutaneous larva migrans is caused by dog or cat hookworm larvae that enter the skin and are unable to complete the life cycle. They either migrate through subcutaneous tissue or encyst in the tissue and the larvae may cause serpiginous erythematous tracts. The tracts eventually become dry and encrusted.

Visceral larva migrans is caused by the dog and cat ascarid larvae (*Toxocara* spp.) that escape from eggs that are accidentally swallowed. The larvae migrate through the tissue and encyst as second-stage larvae. The migrating larvae produce tracts with hemorrhagic necrosis.

Trematodes living in liver, lungs, intestines and blood

Although many trematodes infect humans, only a few are considered to be important pathogens. These flat worms are found in all organs, especially the intestines, and a few are found in the liver, lungs and blood. The life cycles of the important trematodes are presented in Figure 184.3.

The opisthorchid liver flukes

Clonorchis sinensis, *Opisthorchis viverrini* and *Opisthorchis felineus* are found in the bile ducts. The worms are hermaphroditic and about 1.5 cm long and tapered at both ends. Eggs produced pass down the bile ducts to the intestine and out with the feces. In water, snails of a number of species (*Parafossarulus*, *Thiara* and *Bithynia*) serve as the first intermediate host; they ingest the egg and the miracidium is released from the egg. After reproducing by polyembryony, cercariae are produced that leave the snail and encyst as metacecariae in freshwater fish (cyprinids or carp). When the fish is eaten raw or improperly cooked the metacercariae are digested out of the fish and migrate down the bowel and then into the bile passages. Cholangiocarcinoma is a possible complication and DNA carcinogens may be associated with infection. There may also be an association with nitrosamines, which are commonly found in Asian foods.[28]

Fasciolopsis buski

Fasciolopsis buski is the largest intestinal trematode of humans, measuring 5–7 cm by 8–20 mm by 1–3 mm. It has an oral and ventral sucker, two large ceca, two branched testes and a central and coiled uterus. Eggs pass in the feces into water and the hatched miracidium enters a specific planorbid snail. Cercariae released from the snail attach to aquatic vegetation and form into metacercariae. When eaten the metacercariae excyst and attach to the mucosa of the small intestine.

Fasciolia hepatica

Fasciolia hepatica is acquired by eating aquatic vegetation on which the metacercariae are attached. Upon ingestion the metacercariae are released and penetrate the gut wall, traverse the peritoneal cavity, pass through the liver capsule into the liver parenchyma and into the bile duct. Worms may also re-enter the liver parenchyma. The worms are large, 4 cm in length and 1.5 cm wide, with a large cephalic cone at the anterior end. The egg passes down the bile duct to the intestines and out with the feces. It hatches in the water and the miracidium enters into snails of the genus *Lymnea*. Cercariae are released and encyst on all varieties of aquatic vegetations.

Paragonimus westermani

Paragonimus westermani usually resides in pairs in the lung. They are reddish brown, plump bodied and shaped like coffee beans, and measure about 1.2 cm long, 0.6 cm wide and 0.4 cm thick. They have two large branching testes in the posterior half of the body. Eggs enter the alveoli, are coughed up and swallowed, and pass out in the feces or in sputum. The egg hatches in water, enters a specific snail and multiplies; released cercariae enter crabs and crayfish where the metacercariae encyst. When the infected crustacean is eaten the metacercariae are released from the cyst, penetrate the gut wall and enter into the peritoneum, diaphragm and lung.

Young migrating worms produce local hemorrhage and cellular infiltration. These worms may settle in ectopic locations and evoke a pronounced tissue reaction. In the lung and other locations a leukocytic infiltration develops around the worm and fibrous tissue infiltrates to form a cyst wall. Eggs may migrate into pulmonary tissue and other locations and evoke granulomatous reactions. Cerebral paragonimiasis often develops, with hemorrhage, eosinophils, a yellowish exudate and Charcot–Leyden crystals. Some lesions may eventually calcify.

Other species of *Paragonimus* can also infect humans and invade subcutaneous tissue and the abdominal cavity.

Other intestinal fluke infections

Most intestinal fluke infections are innocuous unless there is a large number of worms. The eggs of the heterophyids are tiny and may be carried by the lymphatics and venules to ectopic locations such as the heart and evoke granulomas. Eggs of the intestinal flukes pass in the feces and hatch in water and the miracidia enter snails. Cercariae emerging from the snails enter fish and other aquatic animal life; when these animals are eaten the metacercariae excyst and develop into adults in the small intestine.

Human schistosomes

Human schistosomes are found in the venous bloodstream. Eggs are passed into water and the miracidia released search out specific snails in which to continue the life cycle. Cercariae released from the snails penetrate the skin and the larval schistosomulae migrate to the lungs and then to the mesenteric and vesical veins. Eggs carried back through the mesenteric veins to the liver are responsible for granuloma formation.

The schistosomes are the only trematodes with separate sexes. They have oral and ventral suckers. The females are 1.5–2.5 cm in length and 0.2–0.3 mm in diameter. The male is 0.5–2.0 cm and, although flat, usually curls up to form the gynecophoral canal in which the female lies. The female leaves the male, crawls to the small venules close to the lumen of the gut or urinary bladder and deposits eggs. The miracidium releases enzymes to work with the spines on the *S. mansoni* and *S. haematobium* eggs to digest the tissue and enter the lumen of the intestine and bladder. Some eggs are carried back via the venules and veins to the liver. Infections are known to persist for many years.[29] The life cycles of the human schistosomes are presented in Figure 184.3.

Cestodes living in the intestines and tissues

Adult tapeworms residing in the intestines do not cause serious disease, but larval stages of two species are highly pathogenic. The adults in the intestines pass eggs, which are taken up by an intermediate host (see Fig. 184.4).

Eggs of *T. saginata* and *T. solium* are ingested by bovids and swine and the larval or cysticercus stages develop in muscles. When the meat is eaten insufficiently cooked the cysticerci are released and develop into adults in the small intestine.

The life cycles of the fish tapeworm *D. latum* and related species involves two intermediate hosts. The egg hatches in water and the coricidium is taken in by a copepod and develops into a procercoid larva. When eaten by a fish the larva develops into a pleroceroid larva and when the fish is eaten the larva becomes an adult in the intestine.

Hymenolepid cestodes often require an insect (beetles and fleas) as an intermediate host. *Hymenolepis nana* can also be acquired by ingestion of the egg. Some strains of *H. nana* are also capable of internal autoinfection.

Morphologically, all tapeworms have a scolex and neck, and immature, mature and gravid proglottids. The scolex of *D. latum* has a sucking groove as a hold-fast organ, whereas the other tapeworms have a scolex with four suckers, and *T. solium* and *H. nana* have a rostellum on the scolex with rows of hooklets. Each mature proglottid is hermaphroditic with both male and female sex organs. The length and width of each species varies with the longest being *D. latum* (2–15 m), followed by the taeniids (1–4 m), *H. nana* (1.5–4 cm) and *H. diminuta* (1–6 cm).

Larval stages of tapeworms also infect humans (Fig. 184.5). Infection with the plerocercoid larva of diphyllobothriid species such as *Spirometra* is the cause of sparganosis. The larvae acquired from eating aquatic animals or drinking copepods in water penetrate the gut wall and migrate through the tissue. Animal poultices used in Asia are another source of infection. The larvae cause painful inflammatory swellings or transient lesions. The parasite may invade the eye causing periorbital edema.

Humans who become infected with the eggs of *T. solium* may develop cysticercosis. The larvae invade the muscles, brain, eye and skin. Although the cyst may remain intact and dormant for years, it may eventually break and the released fluids cause granulomas and calcification.

Echinococcosis or hyatid disease develops in humans who accidentally ingest the eggs of the dog tapeworms *E. granulosus* or *E. multilocularis*. The egg hatches in the duodenum and the oncosphere penetrates the gut and becomes established in the liver and other organs. A cyst develops brood capsules and protoscolices proliferate from the inner germinal epithelium of the brood capsule. An outer laminated acellular limited membrane forms and the cyst develops over many years. Unilocular cysts develop with *E. granulosus* and multilocular alveolar cysts with *E. multilocularis*. Cysts develop in many organs. The adult worms are parasites of canines and the intermediate host can be any animal, but most often are sheep for *E. granulosus* and rodents for *E. multilocularis*.

PREVENTION

Most parasitic infections can be prevented if good sanitary practices are followed. The soil-transmitted nematodes can be controlled and possibly eradicated with proper disposal of feces. This is true for most intestinal parasites, even those that require an intermediate host. The disposal of fecal matter into water bodies containing molluscan intermediate hosts perpetuates trematode infections. The construction and use of sanitary privies will go a long way toward preventing infections of all types, but in most endemic areas these practices are usually not followed and indiscriminate defecation is the trend. Infection with skin-penetrating helminths such as hookworm and *Strongyloides* spp. can be prevented by wearing shoes and protective clothing.

Arthropod-borne helminthiasis, such as filariasis, can be prevented by the use of insecticides in houses. Breeding areas of the vectors can also be treated with insecticides. The use of repellents will also help, as will sleeping under insecticide-impregnated bed nets.

The avoidance of water known to harbor snail vectors will prevent schistosome infections. The destruction of snail breeding areas and the use of molluscicides will help to control infections.

The ingestion of raw foods should be restricted in endemic areas. The eating of raw fish is a practice in many countries, and if this is discouraged certain trematode and cestode infections could be prevented. The cooking of marine fish would prevent anisakiasis. Similarly the cooking of aquatic vegetation would prevent trematode infection such as fascioliasis and fasciolopsiasis. Undercooked beef and pork are responsible for trichinosis and tapeworm infections and thorough cooking will destroy the parasites. Freezing foods will kill most parasites and in the future irradiation of foods will make food safe.[30] Microwave treatment, however, is not considered adequate for destroying parasites.

The use of anthelmintics can be used to eliminate helminthic infections. Mebendazole, albendazole and praziquantel have been used in mass treatment campaigns for some nematode and trematode infections. Antifilarial drugs have been used in mass treatment programs and have even been included in table salt distributed to a population group. Reduction of infections in a population will be of significant benefit and will augument control and eventual eradication programs (see Chapter 115). These methods along with education programs would be a definite adjunct to prevention and control.

DIAGNOSIS

The diagnosis of helminthic infections depends upon the location of the parasite in the body. The majority of worms are in the digestive tract and the diagnosis is made by stool examination. Blood is examined for

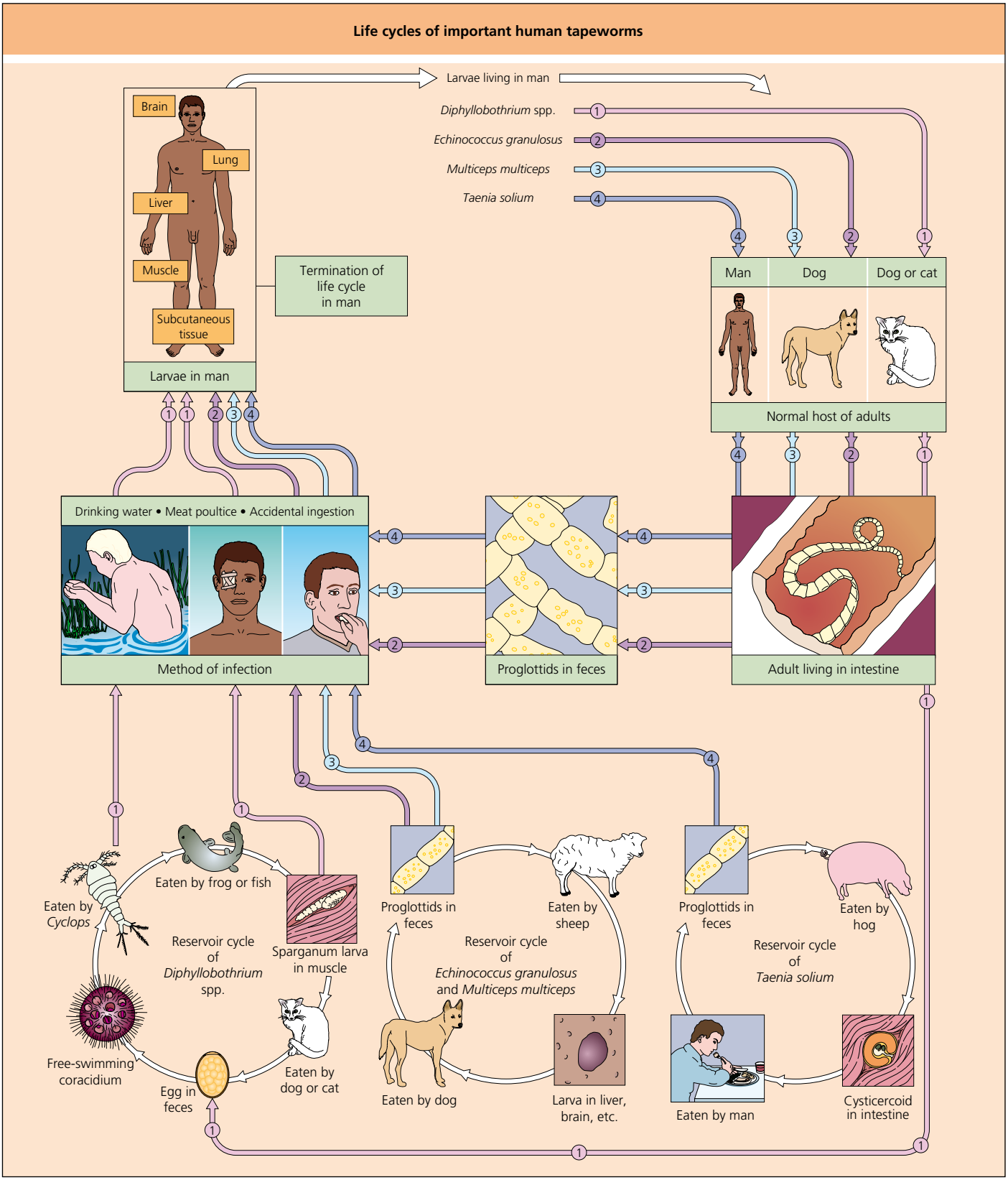


Fig. 184.5 Life cycles of important human tapeworms; adults living in tissues and intestines and blood: humans are accidental hosts.

parasites in the circulatory system, and urine and sputum are examined for worms in the genitourinary systems and respiratory passages. Tissue parasites are diagnosed by biopsies and by immunologic methods. Many techniques have been developed to aid in the diagnosis and most of these have been presented in detail.[31]

Stool specimens

Intestinal parasites are detected by microscopic examination of one or preferably more than one stool specimen.[32] The stools should be collected in a clean dry container and examined as soon as possible after being passed. A direct examination is made by making wet films on a clean microscope slide. A small portion of feces is mixed with a drop of saline on the slide and examined under low-power (3100) and high-power (3450) magnification, if needed. The preparation should not be too thick; it should be thin enough to read newsprint through. Iodine stain preparations can also be used, especially for protozoan infections, but do not add a great deal to the observation of helminth eggs and larvae. If a stool specimen cannot be examined within a few hours after passage it should be placed into 10% formalin or another preservative. The examination can then be done when it is more convenient.

Concentration techniques are also available. The simplest is sedimentation of feces in a tube or sedimentation flask and the sediment is then examined microscopically. The formalin–ethyl acetate technique, in which the stools are mixed with saline and passed through gauze or a screen, is widely used. The material collected is centrifuged several times in saline and finally formalin. Ethyl acetate is added and the preparation is centrifuged slowly. The detritus layer and supernatant are poured off and the sediment examined microscopically. The zinc sulfate flotation method is another simple technique in which small samples of feces are mixed thoroughly with zinc sulfate (1.18 sp gr or 1.2 sp gr for formalized stools). This may be centrifuged or permitted to stand for a period and the surface fluids examined for eggs. This method is unsatisfactory for operculated eggs, however. Eggs from various helminths as well as larvae of hookworm and *Strongyloides* are depicted in Figures 184.6 and 184.7.

The Kato method to examine stools has been modified into the Kato–Katz technique for estimating the number of eggs in the feces. Screened feces are placed into a small hole in a template, which delivers 41.7 mg feces onto a microscope slide. A Cellophane square soaked in a glycerine–malachite green is placed onto the feces and the preparation is permitted to stand for 30–60 minutes. The glycerine clears the fecal material and eggs can be seen against a green background. This technique is especially good for thick-shelled eggs such as those of *A. lumbricoides*, *T. trichiura* and *S. mansoni*. The preparation should be examined within 30 minutes for thin-shelled eggs such as hookworm, which may dissolve if they stand for too long. The number of eggs observed is multiplied by 24 to obtain the number of eggs/g of feces. The Kato method uses only the glycerine-soaked Cellophane and is not quantitative.

Fecal cultures can be used to recover nematode larvae, especially of the hookworms and *Strongyloides* spp. The feces are mixed with charcoal with a small amount of water and placed into a Petri dish with moist filter paper lining the bottom of the dish. This is kept at room temperature for several days and the larvae will migrate to the surface of the charcoal. The culture is placed into a Baermann apparatus consisting of a funnel with rubber tubing attached to the stem and a pinch-clamp closing the tube. A sieve is placed into the top of the funnel and lined with gauze. The funnel is filled with warm water and the culture placed onto the gauze. The apparatus stands at room temperature for 10–12 hours and the fluid is drawn off through the tubing into a glass flask. Larvae can be collected from the flask and examined microscopically to determine the species.

The Harada–Mori technique is also used to culture feces and recover larvae. Filter paper strips are coated on one side with feces and placed into a tube containing a small amount of water. The tube is kept upright so that the filter paper is kept moist by capillary action. After 4–10 days the filter paper is removed and the water examined for larvae.

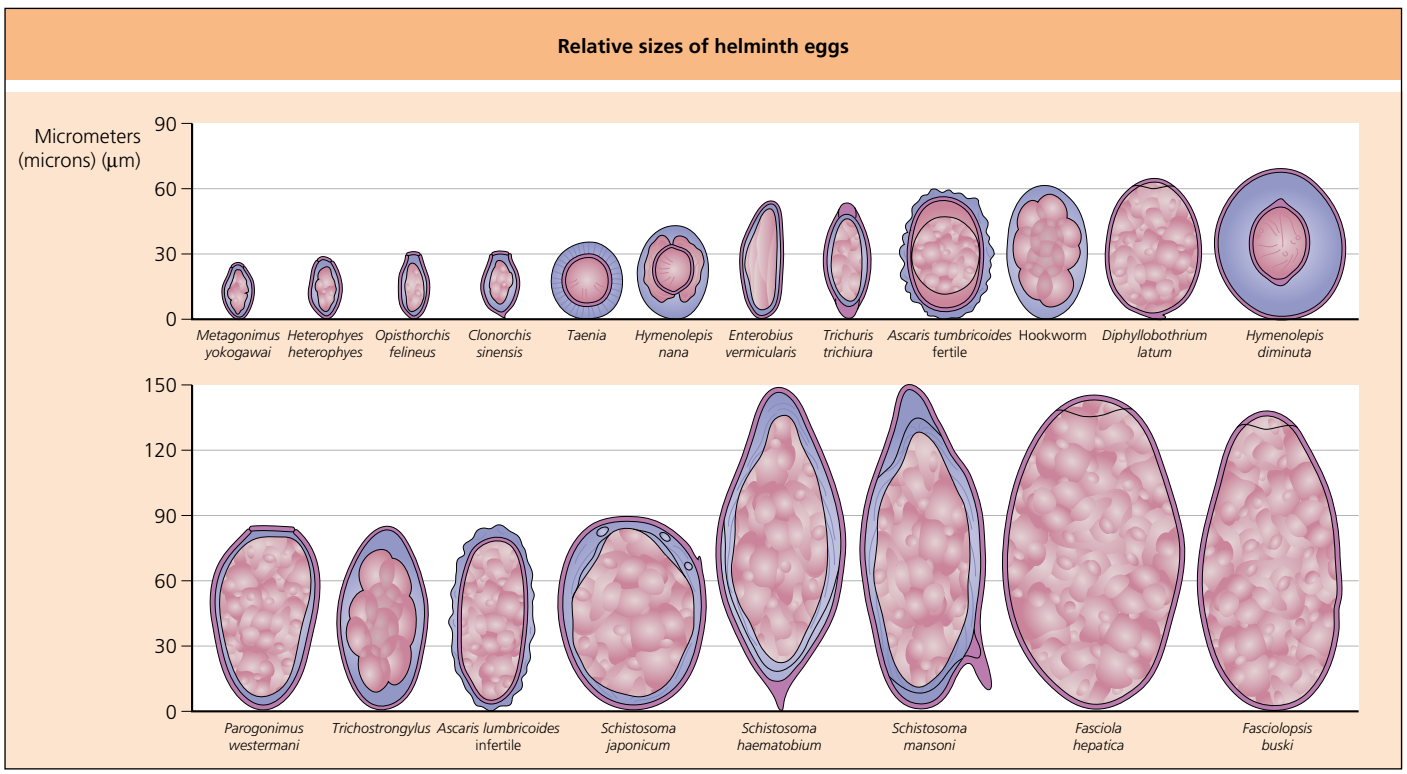


Fig. 184.6 Relative size of helminth eggs. HHS Publication No. (CDC 89–8116)

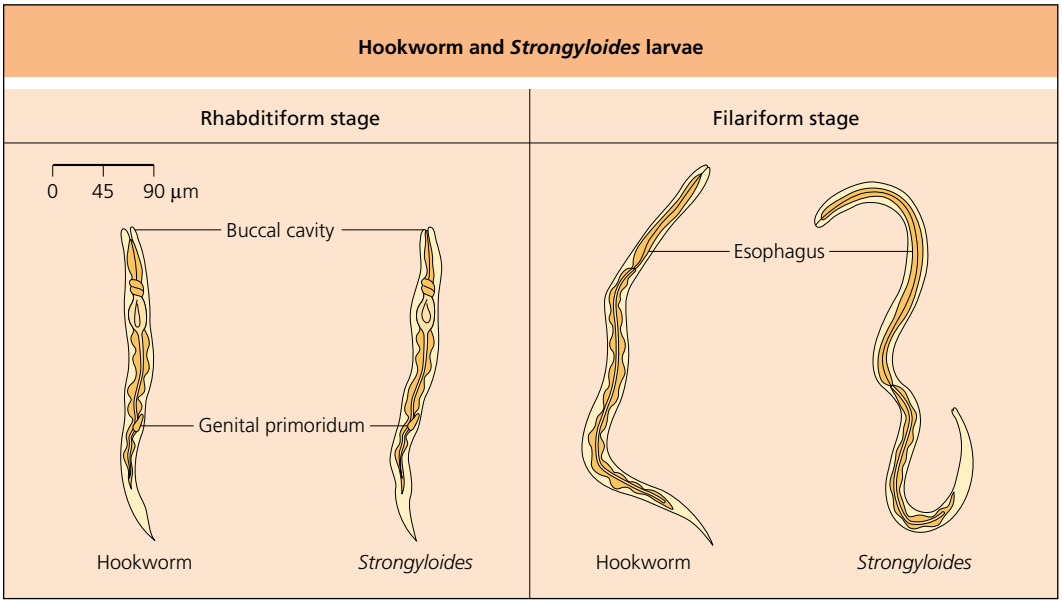


Fig. 184.7 Hookworm and *Strongyloides* larvae. HHS Publication No. (CDC 89–8116)

An agar plate culture for *Strongyloides* spp. has been developed.[33] A few grams of feces are placed into the center of the agar and the plate is incubated at room temperature for 48 hours. Tracks are made by the parasite in the agar and the larvae can be recovered by a Pasteur pipette and placed onto a microscope slide for identification.

Eggs of some parasites can be picked up on perianal swabs. Swabs such as scotch tape or paddle swabs are placed on the perianal area and then placed under a microscope for examination. Pinworm, tapeworm and *A. lumbricoides* eggs can be recovered.

Eggs of *Schistosoma* spp. can be found in feces using some of the above-mentioned examinations. An egg-hatching technique can also be used in which feces are placed into a sidearm flask and water added until it reaches a level just below the sidearm. When the suspension settles more water is added until the level is above the sidearm. A dark cloth or bag is placed over the flask without covering the sidearm. The sidearm is then placed into the light and hatching miracidia from the schistosome eggs will be attracted to the light. They can be examined microscopically.

Specimens can be collected directly from the duodenum and examined for helminthic infections. Material collected by duodenal aspiration can be examined microscopically for *Strongyloides* and *Ascaris* spp. and flukes.

The Entero-Test or string capsule is also available for examining duodenal specimens. A gelatin capsule containing a string is swallowed and the end of the string taped to the cheek. The string in the capsule contains a small weight that carries the string down the duodenum after the capsule dissolves. After 4 hours the string is pulled up and the mucus adhering to the string is placed onto a microscope slide and examined.

Tapeworm proglottids are at times found in stool specimens. These segments should be washed in water and placed between two microscope slides. The slides are than tied together with string and the specimens placed into a fixative – alcohol, formalin, acetic acid (AFA). After fixation the worms can be stained and examined for specific identification. If the proglottids have rosette-shaped uterine branches the worm can be identified as a diphyllabothriid. The number of uterine branches of *Taenia* spp. can be counted and the specimen identified as *T. solium* (6–11) or *T. saginata* (15–20). India ink can be injected into the uterine pore and the branches are then more easily counted.

Adult trematodes found in feces should be washed and placed between two microscope slides that are then tied together with string. The specimens are than placed into a fixative such as AFA. After fixation the worms can be stained and examined for speciation.

Sputum specimens

Sputum to be examined for eggs or larvae should be mixed with 3% sodium hydroxide, centrifuged and the sediment examined.

Urine specimens

Urine specimens will sometimes help in the diagnosis of *S. haematobium* eggs or microfilariae of *W. bancrofti* and *O. volvulus*. The urine specimen is centrifuged and the sediment examined.

Blood samples

Several of the filarial infections can be diagnosed by the examination of drops of blood from finger or ear lobe pricks. The blood is placed onto a microscope slide and covered with a coverglass and examined under low power for thrashing microfilariae. For specific identification thick blood smears are made with several drops of blood, the smear is permitted to dry for several hours and then stained with Giemsa stain. Identifications are based upon, for example, microfilarial sizes, cephalic space and tail nuclei. Microfilariae of *W. bancrofti*, *B. malayi*, *B. timori* and *L. loa* are present in the blood. *Onchocerca volvulus* microfilariae are found in skin snips.

Blood should be obtained at night for most lymphatic filariae and in the daytime for *L. loa*. Microfilariae can also be recovered from blood by membrane filtration. In some cases of lymphatic filariasis adult worms may be seen by ultrasound and the worms can be seen 'dancing' in the lymph channels.[34]

Cerebrospinal fluid

Cerebrospinal fluid is examined for evidence of helminthic infections. Eosinophilic pleocytosis is suggestive of angiostrongyliasis, paragonimiasis, cysticercosis, gnathostomiasis and schistosomiasis. Larvae of *A. cantonensis*

are reported in CSF, especially in children, and *T. spiralis* larvae can be found in the CSF in very heavy infections. The CSF is centrifuged and the sediment examined for evidence of a helminth infection.

Tissue specimens

Tissue specimens may be taken and examined for helminthic infections. Muscle biopsies can be sectioned, stained and examined microscopically for *T. spiralis*, or the muscle can be pressed between two glass slides and examined for trichina larvae.

Rectal and bladder biopsy specimens can be examined for schistosome eggs. Skin biopsies may reveal the presence of microfilariae of *O. volvulus*. Fluid obtained from suspected hydatid cysts, although dangerous to obtain, may reveal scolices of *E. granulosus*. Biopsies can also be made when anticipating a diagnosis of gnathostomiasis, sparganosis or infection with another migrating helminth.

Immunodiagnostic tests

There are myriad immunodiagnostic tests available to aid in the diagnosis of helminthic infections. Immunologic tests, however, only offer a presumptive diagnosis, and the finding of the worms, eggs or larvae provides the definitive diagnosis. Immunologic tests are useful when the parasite is not demonstrated such as in cases of toxocariasis and in some cases of cysticercosis and echinococcosis. Immunodiagnostic tests have been developed for most parasitic diseases to detect antibodies as well as antigens. Techniques in molecular biology have also been emerging and may eventually become valuable tools in detecting helminthic infections.[35]

Detailed information on immunologic and molecular methods in the diagnosis of parasitic diseases has been presented elsewhere.[36] Older tests such as complement fixation are still of value in the diagnosis of cysticercosis, paragonimiasis and schistosomiasis, especially for indicating active infections. Gel diffusion has been used in fascioliasis and the circumoval precipitation test (COPT) is used to detect antibodies in cases of schistosomiasis. In the latter, eggs of schistosomes develop precipitates around the shell in the presence of antibody-positive sera.

The enzyme-linked immunosorbent assay (ELISA) is currently one of the most widely used tests for many parasitoses. Indirect fluorescent antibody is also used, especially on tissue and parasite sections, to detect antigenic material. The indirect hemagglutination test along with EIA (enzyme immunoassay) is used for hydatid disease and EIA for cysticercosis. An EIA test is now recommended for strongyloidiasis, toxocariasis and other larval migrans. It is also of value in the diagnosis of paragonimiasis, schistosomiasis, trichinosis, angiostrongyliasis, gnathostomiasis and filariasis. *Taenia*-specific antigens can be detected in human feces by ELISA.[37]

The immunoblot or Western blot is being applied to the diagnosis of most helminthiases, especially fascioliasis, schistosomiasis, paragonimiasis and cysticercosis. Molecular diagnostics have been reported for most species of parasites, and mostly applied to research laboratories; the detection of DNA by the polymerase chain reaction (PCR) is reported for the most important human helminths. For example, a PCR assay has been developed to detect *W. bancrofti* DNA in blood[38] and a PCR to determine the presence of *W. bancrofti* DNA in vector mosquitoes.[39]

CLINICAL MANIFESTATIONS

An intestinal helminthiasis is usually unnoticed unless a large worm is found by the patient in the feces.

Pinworms

Pinworms generally cause little disease, but in children infections lead to perianal itching, eczema, nose-picking, pica and loss of sleep. Worms may migrate into the vagina, uterus and fallopian tubes and peritoneal cavity. Pinworms can be responsible for recurrent urinary tract infections in young girls.

Ascariasis

Ascariasis is innocuous unless the worms become erratic and migrate into vital organs or pass out of one of the orifices – nose, mouth or anus. Erratic ascariasis may be caused by anesthesia, trauma or some medications. A bolus of a large number of worms can cause volvulus or intussusception, blockage of the bile ducts, cholangitis, intestinal perforation, abdominal distress, pneumonitis and cough. Infection is often associated with malnutrition and decreased growth.

Trichuriasis

Severe trichuriasis can lead to abdominal discomfort, dysentery, colitis, anemia and bloody stools. There may be nutritional loss, weight loss and malnutrition. Rectal prolapse occurs with some infections.

Hookworm

Hookworm larvae entering the skin may provoke a ground itch and secondary bacterial infections. Migrating larvae can cause pneumonitis and eosinophilia, and worms in the intestine can cause abdominal pain, nausea, vomiting and diarrhea. Large numbers of worms sucking blood cause iron deficiency anemia, hypoproteinemia and hepatosplenomegaly, and children will have impaired mental, physical and sexual development. Severe malnutrition develops, especially with concomitant infections with *A. lumbricoides* and *T. trichiura*.

Toxocariasis

Toxocariasis (visceral larva migrans) causes hypereosinophilia, hepatomegaly and symptoms of chronic pulmonary inflammation with cough. There may also be visual difficulties due to retinochoroiditis or peripheral retinitis.

Strongyloides stercoralis

Early infections with *S. stercoralis* are similar to hookworm infections, causing ground itch and pneumonitis. Worms in the intestines cause inflammation, irritation, diarrhea, intestinal bleeding and melena. Sprue-like symptoms may also develop, along with malabsorption, weight loss and eosinophilia. Dermal lesions may occur with urticarial eruptions on the buttocks, termed larval currens. Immunocompromised people, especially those with human T-cell leukemia virus 1 (HTLV-1) associated lymphoma, are at risk of developing disseminated strongyloidiasis and present with severe enteritis, a protein-losing enteropathy, bronchitis, pneumonia, pleural effusion, cough and a blood-tinged sputum with rhabditiform larvae; this is often referred to as the 'hyperinfection syndrome'. Eosinophilia is usually absent in these cases. Death may result. Disseminated strongyloidiasis may also present as Gram-negative pneumonia or meningitis, or as a polymicrobial bacteremia, with bacteria being spread by worms migrating from the bowel.

Capillariasis philippinensis

Capillariasis philippinensis leads to a protein-losing enteropathy, malabsorption, electrolyte imbalance, weight loss, wasting and death. Patients initially present with abdominal pain, borborygmi and diarrhea. Untreated infections usually lead to death.[25]

Trichinosis

Trichinosis is asymptomatic except when a large number of trichina larvae are ingested. Adult worms in the small intestines cause symptoms of gastroenteritis, nausea, abdominal pain, anorexia, diarrhea,

fever and weight loss. During the migratory phase, muscle and joint pain develop, followed by periorbital edema and eosinophilia. Larvae can cause cell destruction, acute inflammatory changes and interstitial myocarditis. The infection can also cause neurologic and pulmonary complications.

Lymphatic filariasis (see Chapter 115)

Symptoms associated with lymphatic filariasis are associated with the host's immune status; little immunity is associated with more severe disease. Early symptoms are fever, lymphangitis, lymphedema and lymphadenitis, which may be transitory and occur periodically. Scrotal involvement leads to orchitis, hydrocele and chyluria. Worms may block the lymph flow leading to lymphatic varicoses and eventual skin fibrosis, thickening and elephantiasis. Renal lesions may also occur, with microfilariae in the urine and chyluria. There may be enlargement of the legs, arms, scrotum, mammary glands and vulva. Tropical pulmonary eosinophilia with fever, splenomegaly, pulmonary infiltrates and hypereosinophilia is associated with *W. bancrofti* and *B. malayi* infections. Microfilariae are absent and are probably destroyed by host immune mechanisms in chronic infections.

Subcutaneous migration of *L. loa* may cause transient swelling known as Calabar swellings, which are a local reaction to the worm and its products. It is characterized by fever, eosinophilia and the urticarial swellings, which are more common in Caucasians. Migrating adult worms may pass over the bridge of the nose or through the conjunctiva across the eyeball. Migration of the worms in abnormal locations can cause symptoms in the scrotum, bowel, kidney and heart, but not usually in natives living in the African endemic areas. Visitors suffer more than indigenous populations.

Oncocercomas due to *O. volvulus* develop over bony prominences. Some nodules develop in deeper tissue and are unnoticeable. Dermatitis and blindness are caused by the microfilariae. Recent investigations suggest that the inflammatory response in the eye is induced by the endosymbiotic *Wolbachia* bacteria released by dead microfilariae.[20] Lymph nodes in the groin may show lymphocyte depletion and fibrosis, followed by 'hanging groin', especially in Africans. In the Americas nodules commonly develop on the upper part of the body, whereas in Africa the nodules develop on lower parts of the body.

Guinea worm

Guinea worm in the subcutaneous tissue elicits allergic manifestations such as itching. When the female worms get close to the skin there is a localized erythema followed by pruritus, nausea, vomiting, diarrhea or asthmatic symptoms. The worm will secrete a toxic substance that causes a blister, which breaks when the area comes in contact with water, and the female releases larvae when the lesion opens up. The area becomes painful. The worm will eventually die and is resorbed or becomes calcified.

Angiostrongylus cantonensis and *Angiostrongylus costaricensis*

Migrating third-stage larvae of *A. cantonensis* may cause vague symptoms of gastroenteritis, vomiting, headache and fever. Once the worms reach the CNS they cause headache, nausea, vomiting, stiff neck, myalgia, pain and paresthesia. Coma may be a feature of heavy infections. Worms have been seen in the eyes of some patients. There may be paralysis of eye muscles. Frequent signs are abnormal tendon reflexes including abnormal Achilles' reflex, a positive Kernig's sign and impaired sensorium and vision. Infections with a large number of worms may be fatal.[10]

Abdominal angiostrongyliasis due to *A. costaricensis* resembles acute appendicitis. A tumor-like mass is palpable in the right lower quadrant. There may be fever, diarrhea or vomiting, along with eosinophilia.[40]

Gnathastome and anisakid larvae

Shortly after ingestion of gnathostome larvae there are symptoms of nausea, vomiting, pruritus and urticaria, and at times upper abdominal pain. Larval invasion into the liver will cause right upper quadrant tenderness and changes in liver function. In the lung there is a pulmonary infiltration and pneumothorax in patients with pleural infusion. Larvae migrating through the subcutaneous tissue and skin cause a rash along with red pruritic painless swellings. Invasion of the CNS often leads to death.

Anisakid larvae in the throat may lead to a 'tickle-throat', causing cough, but when they invade the mucosa of the intestinal tract they provoke eosinophilic granuloma formation, severe abdominal pain, nausea, vomiting and diarrhea. Usually, only a single worm is involved and the symptoms disappear when it is removed.

Trematodes

The trematodes, like the nematodes, cause little disease unless a large number of worms are involved. The liver flukes (*C. sinensis*, *O. viverrini*, *O. felineus*) in the bile ducts may cause symptoms early in the infection such as hepatomegaly, jaundice, diarrhea, anorexia, epigastric pain and fever. Repeated infection over a period of years may lead to ductal fibrosis, obstruction, cholangitis, cholecystitis and cirrhosis, and in some patients cholangiocarcinoma.

The sheep liver fluke *F. hepatica*, because of its large size, may block the bile ducts and cause cholangitis. Toxic secretions cause fever, chills, jaundice, an enlarged tender liver, cough, vomiting, abdominal symptoms and eosinophilia. Young worms may attack the pharyngeal mucosa, causing bleeding and edema. This occurs after eating raw sheep liver containing the parasite. The condition has been called halzoun.

The large intestinal fluke *F. buski* may cause intestinal blockage and toxemia when there is a large number of worms. Eosinophilia is common and ulcers may develop, which often hemorrhage. It causes abdominal distention, hunger pains and increased appetite, diarrhea and a foul-smelling yellowish stool. Allergic manifestations, nausea, vomiting, ascites and cachexia develop as a result of toxins secreted by the worms. Other intestinal flukes cause little disease, but tiny eggs of the heterophyids may enter the mucosa and are carried to ectopic locations such as the brain and heart, provoking granuloma formation. Echinostomes may produce inflammation and ulceration with diarrhea and abdominal pain.

The young lung fluke of *P. westermani* produces little disease during migration, but once established in the lung may cause fever, dyspnea, cough, chest pain and the production of a rusty sputum. At first the disease is often thought to be tuberculosis. The worms may enter the cranial cavity and invade the brain causing fever, headache, nausea, vomiting and visual disturbances, convulsions and meningeal signs.

Schistosome infections (see Chapter 112)

There may be petechial hemorrhages at the site of penetration where schistosome cercariae enter the skin. There will be localized edema and pruritus. After a few weeks there are toxic or allergic reactions and symptoms include fever, nausea, abdominal pain, rigor, urticarial rashes and eosinophilia. This acute stage is known as Katayama syndrome. In the chronic stage granulomas have formed around the eggs in tissue and hepatomegaly develops and the spleen becomes enlarged. This is followed by esophageal varices and finally ascites. Intestinal disease, usually with *S. mansoni* and *S. japonicum*, may involve the entire intestine or more often the large bowel. There may be abdominal cramps, tenderness and bloody mucoid stools. A protein-losing enteropathy, weight loss and anemia may also develop.

Features of *S. haematobium* infection are dysuria, urinary frequency and hematuria. Eosinophils in the urine are not uncommon. Heavy infections, with the deposition of many eggs in the bladder tissue, can lead to squamous cell carcinoma.

Pulmonary involvement can be a feature of all schistosome infections, and causes cor pulmonale with dyspnea, cough and hemoptysis.

Cerebral manifestations are common in oriental schistosomiasis, with symptoms of lethargy and confusion followed by speech difficulties and optical field defects. *Schistosoma mansoni* occasionally causes the spinal cord symptoms of a transverse myelitis, usually in the lumbar region. Flaccid paralysis of the lower limbs is also reported.

Tapeworm (see Chapter 113)

Tapeworm, even the larger ones, in the small intestines provoke few symptoms. Patients usually become aware of being infected only when worm segments are passed in the feces. If *D. latum* attaches to the proximal portion of the jejunum vitamin B12 deficiency results, with the development of pernicious anemia. This was seen in Finland but is now very rare. Sparganosis associated with other diphyllobothriid species causes painful inflammatory swellings, which may be transient. Spargana in the eyes cause intense reactions and periorbital edema.

Taenia spp. in the intestine are asymptomatic in most patients, but some have hunger pains, abdominal discomfort and indigestion. Patients usually become symptomatic when proglottids are found in the feces or passing from the anus. Eosinophilia is not uncommon. Cysticercosis or infection with larvae of *T. solium* is a serious disease, especially if there are cysticerci in the CNS. Cysticerci in the muscle are asymptomatic, but can give rise to myositis, fever and eosinophilia. The cysticerci usually die and become calcified. Cysticerci in the eye cause visual symptoms such as a decrease in visual acuity, retinal edema and hemorrhage. Neurocysticercosis results in arachnoiditis with CSF pleocytosis and an increase in CSF pressure. Obstructive hydrocephalus, cerebral infarction, epilepsy, papilledema, vomiting, headache, a toxic gait and intellectual deterioration may also develop. Dead or dying parasites may exacerbate symptoms, and location of the parasite in the CNS is responsible for a variety of CNS symptoms.

Hyperinfections with *H. nana* may cause diarrhea, loss of appetite, abdominal pain, headache, weakness, and at times epileptoid convulsion, dizziness and eosinophilia. *Hymenolepis diminuta* may cause diarrhea.

Hydatid disease (see Chapter 114)

There are usually no symptoms with hydatid disease until the cysts are large and in vital organs. Many years are usually required for a cyst to reach a significant size. Cysts in the liver may put pressure on the bile ducts and blood vessels. Large cysts in the lung can cause cough, shortness of breath and chest pains. Involvement of the CNS results in different symptoms depending upon the location of the cyst. Leaks of hydatid fluid sensitize the patient and can cause anaphylactic shock. A cyst in the eye causes proptosis.

MANAGEMENT (SEE CHAPTERS 113, 115 AND 150)

Management of helminthic infection is variable and depends upon the specific parasite, the location in the host and the number of worms involved.

REFERENCES


References for this chapter can be found online at http://www.expertconsult.com

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Note: Page numbers in **bold** refer to major discussions. Page numbers suffixed by (i) refer to illustrations and page number suffixed by (t) refer to tables. Page numbers suffixed by (PP) refer to Practice Points.

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